



Society of Green World for Sustainable Environment

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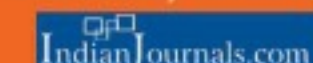
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Society of Green World for Sustainable Environment (SGWSE)

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Aims and Objectives

The Society (SGWSE) has been functioning with following aims and objectives:

- To constitute a forum at international and national level for bringing together individuals and organization involved in agriculture and biological science activities.
- To develop international research/development linkages and disseminate up-to-date technologies in the field of agriculture and biological science.
- To promote and undertake research and development and extension service in the field of agriculture and biological science.
- To explore new areas in agriculture, biological research, biotechnology crop cultivation technologies, development activity and logistics management.
- To propagate utilization on non-conventional and renewable sources in agriculture and biological research.
- To develop purely organic package for growing and cultivation of crops.
- To develop technology of in vivo propagation of important crops.
- To offer recognition and awards to professional groups and individual for attainment of excellence in the field of agriculture, biological science and Biotechnology.
- To interact with government agencies, scientific organization and NGOs to promote and protect interest of agriculture and biological science researcher.

To organize symposia, seminar, and workshops and bring out timely publication(s) to meet the objectives of the society. An International Journal of Biological Sciences “Biotech today” is the official journal of Society of Green World for have been receiving research articles from all over the world. Concerned libraries from all over the World have been subscribing Biotech Today has come to be a leading international journal with its unique quality and standard. We have been covering current references on different crops or biological sciences activities and technologies along with addresses of corresponding authors, so that interested person could contact them as per their own interest biotech Today is being indexed in Google Scholar, Summon Proquest and CNKI Scholar, EBSCO Discovery. The society requests al the cientists/industrialists/technologists engaged in biological science and agriculture to contribute their research findings to Biotech Today and also to become members of the society with their active participation so that the aims and objectives of the society could be fulfilled in their true perspectives.

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Evaluation of biochemical, Proximate composition of some Quality germplasm of Indian Mustard

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Abstract

Indian mustard belongs to family Brassicaceae that covers large number of species exhibiting great economic, nutritional and pharmaceutical applications. The present study was conducted to investigate various phytochemical and proximate constituents in the five genotypes of Indian mustard *viz.*, PDZ-14, PDZ-15, PDZ-16, PDZ-17 and PDZ-M-31. After analysis of bioactive principles among these, PDZ-14 was found to have maximum content of α -carotene (6.49ppm), methionine (1.95g/100g of protein), PDZ-15 had highest content of micro-minerals, PDZ-16 had maximum phytic acid (3.42mg/100g), tryptophan (1.21g/100g of protein), sinapine (2.99%) content, PDZ-17 had maximum antioxidants (20.19mg/g), PDZ-M-31 had maximum glucosinolates (23.64 μ mol/g), phenols (6.28mg GAE/g) and ortho-dihydric phenols (0.848mg/g). Proximate analysis revealed presence of crude protein, crude fiber, oil and carbohydrates in the ranges of 28.83-33.96%, 8.65-9.55%, 36-40% and 20.80-27.99%, respectively. Minerals analysis showed Fe (13.38 ppm), Zn (0.343-0.957 ppm), Cu (0.227-0.600 ppm) and Mn (0.171-0.458 ppm) in respective ranges. The results en-made presence of anti-nutrients like phytic acid and

glucosinolates, but glucosinolates were found to be in range of globally acceptable canola standards showing they are double-zero genotypes. To add to their benefits, edible mustard possesses enormous bioactive phyto-constituents whose use greatly benefits human health.

Keywords: Anti-nutrients, Brassicaceae, Indian mustard, Phytate, Seed Storage Proteins.

Introduction

With 338 genera, confining to around 3700 species, Brassicaceae family inputs the biological world with significant therapeutic and nutritional potential favored for humans as well as with in-diet potential for cattle, poultry and fishes. As the pressure on world is rising with increasing population and while, 80% of world's people relying on native plant sources, as per World Health Organisation (WHO), the beneficial roles of mustard cannot be ruled out (Biondi *et al.*, 2021). The presence of biochemicals possessing nutritive potential is not only confined to mustard seeds, but stem, leaves and roots are also enriched with potential bioactive phytochemicals. All these plant parts of mustard are utilized in different aspects of food, fodder and feed. The cultivation of mustard therefore becomes essential and it comes even with better suitability to environmental conditions as the plant is enriched with disease and pest resistance producing bioactive compounds as well as the same providing hardiness and sturdiness to growing plant of mustard (an oilseed crops) unlike cereal crops like rice, wheat and maize (Bora *et al.*, 2021).

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The morphological and anatomical features of Indian mustard seed elucidate out the seed size as 1.5 to 2.5 mm, with colors of seed varying from dark black to light yellow, white and dark brown to light red also. Meanwhile, the variation in the oil content of seed is attributable to its physical characteristics it also depends on the presence of anti-nutritional factors concentrations like those of glucosinolates, phytic acid, sinapic and erucic acid content. The higher crude protein content of (36-41) % makes mustard attractive food alternative to soybean (Favela-González *et al.*, 2020). However, the presence of anti-nutritional factors limits its consumption to a limited scale. The overall ill effects of higher doses of mustard consumption in diet relates to allergenicity, goiter and other gastro-intestinal apathies (Gupta *et al.*, 2019).

The oil of mustard is of high global and economic value, the extraction of which is carried out by finely grounding mustard seeds and their subsequent subjection to the soxhlet assembly by usage of an appropriate non-polar solvent, gives out oil with potential bioactive compounds. Of the important noticed ones are phenolics, carotenes, sterols, terpenes and sulfated glycosides. The industrialization of oil extraction at large scale includes the screening of potential toxic effects carried out by glucosinolates in mustard (Kwon *et al.*, 2020; Li *et al.*, 2018; Nguyen *et al.*, 2020; Ogidi *et al.*, 2019). In contrast to the toxic effects of indole-glucosinolates, they also possess free-radical scavenging activity. The similar effects of anti-oxidant potential are also noticed in other bioactive compounds of mustard like polyphenols and terpenoids. These are also attributed to certain stone fruit crops like nuts and monocotyledonous cereal plants (Papola *et al.*, 2015) Research from long-lasting decades have assured the beneficial effects of mustard biochemicals against life threatening diseases like,

atherosclerosis, tumors and long-term indigestion. Moreover, *in vitro* effects of these compounds have been screened to elucidate their role in protection against oxidfative protein, lipid and DNA (Deoxyribose Nucleic Acid) damage (Mawlong *et al.*, 2017; Salehi *et al.*, 2021). To other beneficial roles of mustard include anti viral-, bacterial-, ulcerative- and anti-hypercholestermic effects. Some cases suggested it boosted fertility and possessed hypotensive inducing potential. To other contributions of therapeutic value includes anti protozoal-, fungal- and anti-diabetic effects (Shankar *et al.*, 2019; Sies *et al.*, 1995; Thakur *et al.*, 2019). Nonetheless, the culinary usage of mustard has been of immense scale from time immemorial for treatment of indigestion, rheumatism and inflammation (Salehi *et al.*, 2021).

The large concentrated protein pool of mustard is diversified by the presence of cruciferins (80 %) and napins (10-15 %). The core seed structure studies have elucidated the phytate hindering the bioavailability of these mustard proteins in diet (Sehwag and Das 2015).

To account for the role of diversified phytochemical compounds in Indian mustard seed, the present study has been conducted in order to assess the in-diet potential of mustard against screened anti-nutritional factors and mineral composition here. The presence of important phyto-nutrients suggests the health-boosting role of Indian mustard for humans and proximate composition elucidates the food functional characteristics of Indian mustard.

Materials and Methods

Collection of Plant Material

Seeds of five genotypes of yellow Indian mustard were procured from DRMR, Bharatpur. Seeds were oven dried, crushed, defatted and stored in sealed containers prior to biochemical and proximate analysis.

Quantitative Phytochemical Analysis

Estimation of Total Antioxidant Content

The phosphomolybdenum method given by (Prieto *et al.*, 1999) was used to estimate the total antioxidant content. Absorbance was measured at 695 nm against a reagent blank. Ascorbic acid solution of 1mg/mL concentration was used for standard curve.

-Carotene Content Determination

The β -Carotene was quantified using water-saturated butanol (8:2) as per the method reported (Sies and Stahl 1995). Absorbance at 440 nm was measured. β -Carotene solution dissolved in water-saturated butanol was used for standard curve formation.

Determination of Total phenol Content

The Folin-Ciocalteu colorimetric method developed by (Sharma *et al.*, 2019) was used. Absorbance at 765 nm was measured. For standard curve preparation gallic acid was in concentrations ranging from 20-100 μ g/mL.

Determination of Ortho-dihydric Phenols

Ortho-dihydric Phenol (ODP) was determined using Arnou's technique as proposed by (Sharma *et al.*, 2019). Absorbance at 515 nm was measured. 10 mg of catechol was dissolved in 10 mL of distilled water yielding a concentration of 1 mg/mL which was used as a standard solution.

Total Flavonoid Content Estimation

The method proposed by (Chang *et al.*, 2002) was applied for estimation the total flavonoid content and was modified as necessary. Absorbance at 415 nm was measured. Quercetin was used as a standard which was prepared at different concentrations (20-100 μ g/mL) in methanol.

Determination of Sinapine Content

The estimation of sinapine determination in the sample was carried out using the procedure outlined by (Kolodziejczyk *et al.*, 2019). Absorbance at 330 nm was read.

Methanol as a blank was used. The following formula was used to calculate the sinapine content present in the meal:

$$\% \text{ sinapine} = \frac{2184 \text{ Absorbance } 10}{\text{weight of sample taken}(g)}$$

Quantification of Essential Amino Acids Methionine

Methionine present in the samples was quantified using the procedure outlined by (Horn *et al.*, 1946). Absorbance was measured with a spectrophotometer set at 520 nm against blank. 0.5-3.0 mg of methionine was used to prepare standard curves.

Tryptophan

To quantify tryptophan in the test samples, method proposed by (Spies and Chambers 1949) was applied. The absorbance at 454 nm was measured. Tryptophan concentrations of 40–200 μ g/mL were used to prepare blank.

Determination of Total Glucosinolates

The method developed by (Mawlong *et al.*, 2017) was used to perform spectrophotometric estimation of methanolic extracts by using sodium tetrachloropalladate. Absorbance at 425 nm was measured. Blank contained all but extract.

Determination of Phytic Acid Content

The method established by (Haug and Lantzsich 1983) was used for estimation of phytic acid in freshly ground seed samples. Absorbance at 519 nm was taken while 0.2 N HCl as a blank. Standard curve prepared by sodium phytate solutions containing 20–100 μ g of phytic acid.

3. Proximate Analysis

Using the standard methods of the Association of Official Analytical Chemists (AOAC) (Wang *et al.*, 2016) reported moisture, ash, crude fat, crude protein and crude fiber contents were analyzed in powdered Indian mustard seed samples. Based on the net difference between the other nutrients and the overall percentage composition, the total carbohydrate content was calculated.

Mineral analysis using Atomic Absorption Spectroscopy (AAS)

1 g of dried sample was weighed and transferred in a conical flask of 150 mL and 10 mL of diacid (Nitric acid: Perchloric acid :: 3 : 1) was added to and was allowed to stand overnight. Next day, the mixture was heated until a clear solution formed and volume was reduced to 3 to 5 mL. This mixture was allowed stand overnight. Next day, it was filtered in a volumetric flask. Volume of the solution was made up to 100 mL with distilled water and was used for elemental analysis through AAS.

Results and Discussion

The biochemical and proximate composition of five double-zero genotypes of Indian mustard were examined in the current study. The study revealed the presence of anti-oxidative secondary metabolites (Table 1) in Indian mustard *viz.*, phenols, ortho-dihydroxy phenols, carotenoids (α -carotene), and anti-oxidants. Total phenol content ranged from 3.22 ± 0.131 mg GAE/g to 6.28 ± 0.163 mg GAE/g with highest in PDZ-M-31 (6.28 ± 0.163 mg GAE/g). Ortho-dihydroxy phenol (ODP) content ranged from 0.687 ± 0.015 mg/g to 0.848 ± 0.028 mg/g with highest in PDZ-M-31 (0.848 ± 0.028 mg/g). Total flavonoid content ranged from 0.637 ± 0.006 mg QE/g to 0.957 ± 0.049 mg QE/g with highest in PDZ-M-31 (0.957 ± 0.049 mg QE/g). Antioxidant content ranged from 12.63 ± 0.341 mg/g to 20.19 ± 0.393 mg/g with highest in PDZ-17 (20.19 ± 0.393 mg/g). α -carotene content ranged from 4.04 ± 0.047 ppm to 6.49 ± 0.043 ppm with highest in genotype PDZ-14 (6.49 ± 0.043 ppm). Sinapine content was ranged from 2.14% to 2.99%.

Table-1 Anti-oxidative secondary metabolites in Indian mustard

Sr. No.	Genotypes	Total antioxidant content (mg/g)	α -carotene content (ppm)	Total Phenol content (mg GAE/g)	ODP content (mg/g)	Flavonoid content (mg QE/g dry weight)	Sinapine content (%)
1.	PDZ-16	17.72 ± 0.196	4.04 ± 0.047	4.90 ± 0.053	0.781 ± 0.014	0.637 ± 0.006	2.99
2.	PDZ-17	20.19 ± 0.393	5.15 ± 0.038	3.22 ± 0.131	0.687 ± 0.015	0.763 ± 0.018	2.41
3.	PDZ-14	14.87 ± 0.196	6.49 ± 0.043	5.33 ± 0.020	0.821 ± 0.010	0.877 ± 0.003	2.22
4.	PDZ-15	12.63 ± 0.341	4.48 ± 0.055	4.30 ± 0.065	0.736 ± 0.014	0.675 ± 0.026	2.19
5.	PDZ-M-31	13.26 ± 0.413	4.62 ± 0.061	6.28 ± 0.163	0.848 ± 0.028	0.957 ± 0.049	2.14

The results also showed the presence of anti-nutrient compounds (Table 2) like phytic acid ranging from 2.11 ± 0.035 mg/100g to 3.42 ± 0.036 mg/100g and glucosinolates from 14.95 ± 0.080 μ mol/g to 23.64 ± 0.355 μ mol/g, but the amount of glucosinolates was found to be below the globally acceptable canola standards, that is 30 μ mol/g, showing that they are double-zero genotypes with improved oil and seed meal quality.

Table-2 Anti-nutritional components present in Indian mustard

Sr. No.	Genotypes	Phytic acid content (mg/100g)	Glucosinolate content (μ mol/g)
1.	PDZ-16	3.42 ± 0.036	15.04 ± 0.093
2.	PDZ-17	2.73 ± 0.009	14.95 ± 0.080
3.	PDZ-14	2.99 ± 0.012	15.19 ± 0.382
4.	PDZ-15	2.11 ± 0.035	16.97 ± 0.526
5.	PDZ-M-31	2.21 ± 0.018	23.64 ± 0.355

The proximate Composition of Indian mustard genotypes is mentioned in Table 3. Moisture and ash content in Indian mustard genotypes ranged from 1.50-2.70% and 3.50-4.45% respectively. Given the low moisture content in mustard seeds, it was predicted that microbial activity would be reduced, resulting in a longer shelf life of seed. Additionally,

mustard seeds were found to have high levels of protein (28.83-33.96%), oil (36-40%), carbohydrates (20.80-27.99%) and fibers (8.65-9.55%). Due to high percentage of oil, mustard seeds have the potential to be a lucrative source of premium vegetable oil for both domestic and commercial applications. These results also imply that mustard seeds might be effective as supplement of fiber and protein in low-nutrient diets. Consuming fiber-rich foods help avoid digestive and intestinal issues like constipation and also lower blood sugar and cholesterol levels.

Table-3 Proximate Composition of Indian mustard genotypes

Sr. No.	Genotypes	Moisture (%)	Ash (%)	Dry matter (%)	Oil Content (%)	Crude Protein (%)	Crude Fiber (%)	Total carbohydrate (%)
1.	PDZ-16	2.16	4.05	97.84	39.03 ±0.23 9	33.96 ±0.15 5	8.65± 0.077	20.80 ±0.15 7
2.	PDZ-17	1.50	4.20	98.50	36.17 ±0.35 2	32.91 ±0.04 3	9.35± 0.046	25.22 ±0.14 7
3.	PDZ-14	2.47	3.50	97.53	36.08 ±0.05 7	30.86 ±0.09 3	9.55± 0.038	27.09 ±0.06 2
4.	PDZ-15	2.12	3.60	97.88	37.46 ±0.51 0	28.83 ±0.25 3	9.45± 0.072	27.99 ±0.27 8
5.	PDZ-M-31	2.70	4.45	97.30	38.39 ±0.36 9	30.78 ±0.04 6	8.80± 0.056	23.68 ±0.15 7

Table 4 provides the results of mineral analysis for Zinc (Zn), Manganese (Mn), Copper (Cu), Iron (Fe) and % N content. The findings of a mineral analysis show that mustard meal has a high concentration of Fe and only trace amounts of other micro-minerals, viz., Cu, Zn and Mn. These metals, like Cu and Mn, serve as co-factors of anti-oxidant enzymes to shield the organism from free radicals generated during oxidative stress.

Table-4 Mineral Composition of Indian mustard genotypes

Genotypes	%N	Cu (ppm)	Mn (ppm)	Fe (ppm)	Zn (ppm)
PDZ-16	5.43	0.447	0.272	6.407	0.694
PDZ-17	5.26	0.227	0.171	4.448	0.343
PDZ-14	4.93	0.464	0.254	7.105	0.557
PDZ-15	4.61	0.600	0.458	13.38	0.957
PDZ-M-31	4.92	0.306	0.455	12.25	0.595

The sample values discussed above represent high-quality double-zero genotypes of Indian mustard. Based on the aforementioned findings, the potential use of these double-zero genotypes in the food industry and as nutraceutical supplements appears promising. Having improved oil and seed meal quality, they will be advantageous to agricultural producers, marketers, and consumers and due to the existence of various bioactive phytoconstituents, their use will greatly benefit human health and may lower the risk of chronic diseases. The *in silico* study might prove useful in understanding the synthesis and accumulation of seed storage protein (SSPs) in the crop species and their interaction with other biochemical compounds like phytates, so that we can control the quality and amount of seed proteins in mustard and manipulate them for our benefits.

Declaration

The authors have declared that no competing interest exists.

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Studies on varietal response of finger millet (*Elusine Coracana* (L.) Gaertn.) production in Bhatwari Block of district Uttarkashi, Uttarakhand

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Abstract

The present studies have been carried out to find out the location specific suitable varieties in terms of biomass and productivity in district Uttarkashi. Three varieties of Finger Millet (VL-149, VL-315, VL-146) with local check had been grown in village Rathal of district Uttarkashi. The data related to the growth and productivity of millet crop was observed. The result obtained revealed that all the three improved varieties were more productive as compared to the local cultivars. The productivity of improved varieties of finger millet was about three times more than the local variety. The improved varieties i.e. VL-149, VL-315 and VL-146 showed significant improvement in grain and straw yields. Apart from this, seed treatments, line sowing, and timely weeding enhanced the yield of local variety too. As compared to the local variety, the improved varieties VL-149, VL-315 and VL-146 produced much higher grain yields, amounting 17.31, 13.30 and 14.93 Q/ha, respectively.

Introduction

Himalayan region has been endowed with a wide variety of nutritional crops like Finger Millet, Grain Amaranth, Foxtail Millet, Buck

Wheat, Rice Bean, Barnyard Millet, etc. Finger Millet is a highly nutritive crop, which is commonly known as life support crop. However, with the advancement of new scientific era, no much attention had been paid to these nutritional crops by the researchers, as the result of which, these crops were neglected from the cropping pattern in some places of Himalayan region. However, some work had been conducted by Shobana and Krishnaswamy (2013). One of the ancient Indian millets dated to 2300 B.C. are finger millet also known as ragi. This study revolves around exploring the historical significance, consumption patterns, nutritional content, processing methods, and the positive impact on health that finger millet offers. Among all cereals and millets, finger millet stands out with the most abundant calcium (344 mg %) and potassium (408 mg %) content. Furthermore, it surpasses white rice, which is currently the primary staple food in India, in terms of dietary fiber, essential minerals, and amino acids containing sulfur.

Bala (2002) reported that eight minor millet crops grown in different regions of Africa, Asia and Eurasia are Finger millet (*Eleusine coracana*), Proso millet (*Panicum miliaceum*), Little millet (*Panicum sumatrense*), Foxtail or Italian millet. (*Setaria italica*), Barnyard millet (*Echinochloa crus-galli* and *E. colona*), Kodo millet (*Paspalum scrobiculatum*), Teff (*Eragrostis tef*) and Fonio (*Digitaria iburua*). Out of these, Finger millet is although a native of Ethiopia, but it spread out most to Asia few thousand years ago. Most of the world's millet

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production is shared by South and East Asia i.e. about 60%. Out of the others Eurasia and Central Asia share 14%, Africa 16% and rest of the world only about 10%. India is the leading producer of millet contributing about 38% of the production of the world. Finger millet constitutes about 81% part of the minor millets produced in India. Other 19% millets produced in India include Kodo millet, Foxtail millet and Little millet.

Iren Leder (2002) revealed that the principal use of millet grain is for food (85%), while about 9% is used for feed (Kent, 1975). Millet is a high-energy, nutritious food, especially recommended for children, convalescents and elderly persons. Several food preparations are made from millet, they differ between countries and even between different parts of a country.

Negi Biju (2010) reported that Uttarakhand people mainly grow Mandua in Pauri and Almora, and Jhangora in Almora, Chamoli, Rudraprayag, Pauri, Tehri and Uttarkashi. In the case of Mandua, the national average yield of 1226 kg/ha (base year 2006-07) is only marginally higher than the average yields in Pauri (1068 kg/ha) and Almora (1200 kg/ha). But in the case of jhangora, against the national average of 475 kg/ha, Almora already yields 996 kg/ha, Chamoli 1372 kg/ha, Rudraprayag 1146 kg/ha, Pauri 1072 kg/ha, Tehri 1250 kg/ha and Uttarkashi 1245 kg/ha.

The present study was undertaken to find out the location specific suitable variety to be grown in Uttarkashi area of Uttarakhand.

Research Problem

Present research work has been designed to investigate the scope of the empowerment of rural community for enhanced household food and nutritional security coupled with income generation through participatory approach in cultivation, processing and value addition of

life support crops. The main location specific crops are finger millet (*Elusine coracana*) barnyard millet (*Echinochloa crus-galli*) and grain amaranth (*Amaranth sp*), which are considered as life support and climatic resilient crops due to their enhanced nutritional importance. Traditionally, these crops were grown in common practices but during Green Revolution era, they were not given much attention, as the result their cultivation area reduced at an alarming rate, and the nutritional security also decreased. The over exploitation of natural resources has also caused the erosion of fertile soil from the fields of the farmers. The change in the biodiversity of Himalayan region has also shifted the agricultural practices in the area.

Material and Methods

Experimental Design

The different varieties of Finger Millet were sourced from different institutes i.e. ICAR-VPKAS, Almora and G.B Pant University of Agriculture and Technology (GBPUAT), Pantnagar. For the germination studies, morphological similar selected seeds were rinsed with 0.1 % mercuric chloride for 1 minute and thoroughly washed with distilled water and finally placed in petri dishes for germination studies under lab condition. The growth and productivity trials were conducted under field conditions. The trials were conducted at farmer's field in different locations using Randomize Block Designed (RBD) method. The seeds were sown in lines with a line to line distance of 4-6 cm. The sowing was done by line sowing marker method. The data of growth and yield were collected from the fields at a regular time intervals and analyzed statistically. The detail of methods has been given in table 1 and table 2.

Table 1: General features of crop cycle, location and methods adopted of Finger millet varietal trials.

S. No.	Common Name of the Crop	Botanical Name	Variety	Location of Field Trial	Row to Row Distance (cm)	Sowing Method
1.	Finger Millet	Elusine coracana	V-149	Rathal	7.5-10	Line Sowing
			V-146	Rathal	7.5-10	Line Sowing
			V-315	Rathal	7.5-10	Line Sowing
			Local	Rathal	—	Broadcasting

Table 2: Frequency and area of Finger Millet varietal trials performed at village Raithal, District Uttarkashi (U.K.)

S. No.	Varieties Used	No. of Demos	Range of Plot Size (ha)	Total Area of the Plot (ha)
1.	VL-149	12	0.1-0.5	0.32
2.	VL-146	9	0.1-0.5	0.1
3.	VL-315	10	0.1-0.5	0.29
4.	Local	4	0.1	0.06

Results and Discussions

Table 3: Germination percentage in different varieties of Finger Millet

S. No.	Common Name of the Crop	Botanical Name	Variety	Seed germination (%)
1.	Finger Millet	Elusine coracana	V-149	96 ± 2.5
			V-146	98 ± 1.9
			V-315	95 ± 1.4
			Local	72 ± 1.7

The germination percentage was 96%, 98%, 95%, and 72% in VL-149, VL-146, VL-315, and local respectively (Table 3). The results shows that VL-149 have the highest germination capacity while local variety shows lowest germination capacity.

Yield performance of different genotypes in demonstrations:

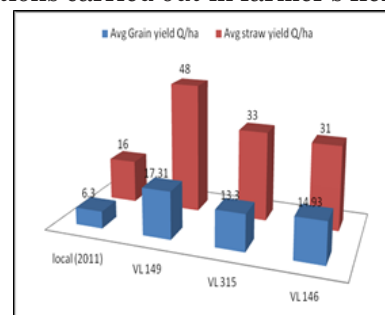
A total of 35 finger millet demonstrations were performed at Raithal village of District Uttarkashi involving marginal farmers. The demonstrations covered an area of 0.77 ha during all the three varieties of Finger Millet crop trials. The results of productivity as grain and straw yield both have been presented in Table-4. The plant height and days of maturity varies with the locations of cultivation. The productivity of improved varieties of finger millet was found almost three times more than the local variety. The criterion of higher yield encouraged the famers to take an initiative for

growing the improved varieties like VL149, VL315 and VL146. Apart from this the agronomic practices like seed treatment, line sowing, bed preparation, irrigation and timely weeding had also been done in the farms at appropriate stage of crop cycle.

Table 4: Performance of different varieties of Finger Millet.

Location of Field Trials	Geographical Location (Latitude Longitude)	Varieties of Grain Amaranth	Plant Height (cm)	Days of Maturity (Days)	Yield (q/ha)	
					Grain Yield	Straw Yield
Village Raithal, District Uttarkashi (2151 mamsl*)	30.8196° N 78.6016° E	VL-149	82 ± 1.2	82 ± 2.1	21 ± 1.7	58 ± 1.9
		VL-146	84 ± 1.7	95 ± 1.3	17 ± 1.4	46 ± 1.7
		VL-315	97 ± 2.3	108 ± 1.2	18 ± 1.2	48 ± 1.5
		Local	102 ± 1.1	145 ± 2.4	7 ± 1.2	18 ± 1.2

*± Standard Deviation. The values are mean of 35 demonstrations carried out in farmer's field.

**Figure 2: Vegetative and reproductive yields of different varieties of Finger Millet****Figure 2 (a-d): Vegetative and reproductive crop pattern of grain Elusine Coracana in Bhatwari block of district Uttarkashi.**

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Physico-Chemical Study of Ecology and Water quality in Eastern, South-Eastern part of Central Indian Himalayas as a result of Magnesite Mining

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Abstract

Water is an inorganic transparent, tasteless, odorless, and nearly colorless chemical substance. It is a main constituent of earth's hydrosphere and protoplasm and is vital for all known forms of life. The study area is located in Kumaun which lies along the eastern and south-eastern part of the Central Indian Himalaya. The magnesite mines under study are located in Kumaun Lesser Himalaya of Central Indian Himalaya at Jhiroli of district Almora of Uttarakhand amidst latitude $29^{\circ} 45' 30''$ N and $29^{\circ} 47' 30''$ N and longitude $79^{\circ} 44'E$ and $79^{\circ} 46'E$. The mining is being carried out by an open cast method with a semi-mechanized system of overburden removal with manual sorting, dressing, and loading of magnesite. The magnesite mines are located in the region representing the hilly nature of topography. The hill slopes of the area are generally concave and convex with slope inclination ranging between 34° to 70° . The mining operation can't be fully mechanized as the magnesite mines have varying length and thicker layer of magnesite deposits. Therefore, the recourse is taken to selective mining and hand sorting. The mining of raw magnesite involves drilling, blasting, crude collection, and spoil removal. The parameters with respect to water analysis discussed, are turbidity, colour, odour, pH, total dissolved solids (TDS), total solids (TS),

total suspended solids (TSS), dissolved Oxygen, acidity, alkalinity, ammonical nitrogen, hardness in water due to sulphates, chlorides, fluorides, magnesium, calcium, sodium, potassium etc.

Keywords: Magnesite, Mining, Turbidity, pH, Total Dissolved Solids (TDS), Total Solids (TS), Dissolved-Oxygen, Acidity, Alkalinity, Hardness, Nitrogen, Nitrite, Sulphates, Calcium, Potassium, Sodium, Fluoride content, Chloride content and Magnesium.

Introduction

The drainage pattern of the mining area comprises of first and second order streams and is controlled by Billori Nala on the south-western part of mining lease area. The first order streams are prominent in limestones and salty rocks. Both first and second order streams merge into Billori Nala, a natural perennial stream flowing through the footwall of a mineralized belt and joining the Karas Nala to form a perennial Jaigangad. The Jaigangad after moving in the southeasterly direction finally merges with the Saryu river. The drainage system is marked by dendrite pattern and is of trellis type. The seasonal water flowing through upper reaches attains sharp erosional gully downwards till it joins the main drainage system at foothills. There are no springs in the mining area and the only natural perennial spring is on the Northwest corner of the Jhiroli village.

This water source is unaffected because it is beyond the pale of mining leasehold area. So, perennial sources of water or watercourse are intact. However, it has been noticed that the use of explosives for blasting of rocks has destabilized hill slopes by opening out joints, fissures and cracks. It has diverted ground water resulting in drying up of rain-fed seasonal springs in villages of Jhiroli, Matela and Billori. The spring of village Joshigaon, according to the local populace has also dried up. The springs are controlled by litho-structural setting. The disturbance in hydrological balance brought about by widespread damages to slopes due to mining activities is responsible for the reduction in discharge of springs, besides, the scree originating from mining and uncompact dumps easily roll down the slopes and cause sedimentation of the springs and reduction in their capacity. Since the surface and groundwater hydrology of the mined area has undergone considerable change, therefore, infiltration rate is very poor influencing discharge rate and finally erosion and sedimentation of agricultural land and degradation of the watershed. The run-offs from hill and discharge of industrial effluents from the Dead Burnt Magnesite plant ultimately find its way in the Jaigangad at the foothills. Studies were carried out to determine the water quality of this source.

Water Sampling

Water samples were collected from the Karas (western) and Matela (northern) downstream and the Jaigan stream one sample was collected from upstream of Karas stream which is drinking water source of the plant. Collection of second sample was done from upstream of Karas stream, Billori upstream and plant effluents. In all, a total of eight samples were collected. The water source, sites, time of their collection and identification of samples are given in the Table 1. The location of water sampling sites is given in fig1. The sites of magnesium concentration in

the soil affecting water quality in given Fig.2. All the samples were collected twice in a day.

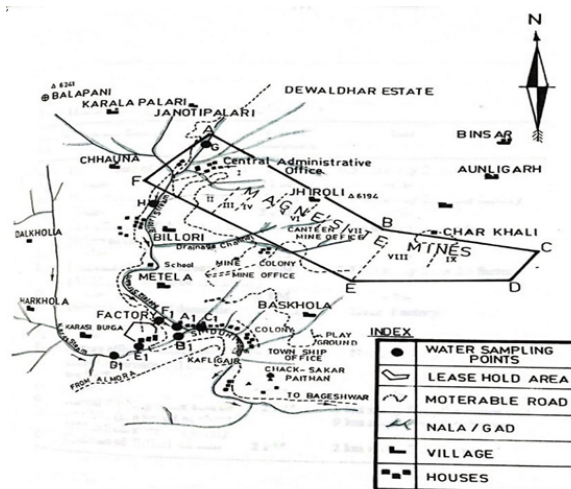


Fig.1. Locations of Water Sampling Sites

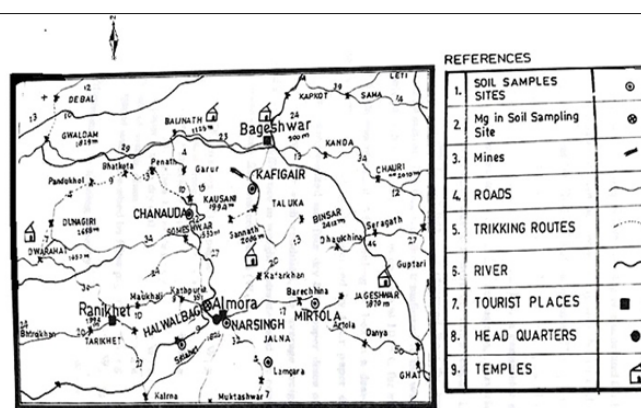


Fig.2. Sites of Mg concentration in Soil affecting Water Quality.

Table 1

Description of water sampling sites			
Sampl e No	Water sources	Time of Sampling	Site
A1	Downstream of Matela stream	5:00 AM	0.5 km away from the plant
A2	-do-	2:00 PM	-do-
B1	Downstream of Karas stream	5:00 AM	0.5 km away from the plant
B2	-do-	2:00 PM	-do-
C1	At the confluence of above streams(Jaigan stream)	5:00 AM	1 km away from the plant
C2	-do-	2:00 PM	-do-
D1	Upstream of Karas stream(drinking water source of the plant)	5:00 AM	0.5 km away from the plant
D2	-do-	2:00 PM	-do-

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E1	Plant effluents of domestic and automobile workshop	10:00 AM	Near plant
E2	-do-	5:00 PM	-do-
F1	Plant effluents mixed with domestic, furnace oil storage, wash offs	10:00 AM	Near plant
F2	-do-	5:00 PM	-do-
G	Natural drinking water source for mines (at a height of about 1600 m from mean sea level)	2:00 PM	1 km away from the mines and 9 km away from the plant.
H	Upstream of Billori stream	2:00 PM	2 km away from mines

Samples were grabbed i.e. were drawn from a particular place representing the whole water quality. The water sample was collected from a depth of 0.5 m in boro-silicates glass bottles of 2.5 liters. Prior to the collection, acid cleaned sample bottles were rinsed thoroughly with the sample water. The samples were collected up to the top without leaving any space so as to prevent the premature release of dissolved gases during transit period. Immediately after collection, each sample bottle was labeled with waterproof ink and relevant details of each sample were recorded. Since most of the parameters change with time, preservatives as referred to in the "Standard Methods for the examination of water and wastewater (18th edition, 1992) APHA, AWWA and WEF (1980) and methods for chemical analysis of water and wastes, EPA 625/6-74-003 a, 1976 were used. Dissolved Oxygen and pH change quickly with time, hence their estimation was carried out in the field itself. Preservation techniques of the water samples for Chemical Analyses are given in table2.

Table 2 Preservation techniques of the water samples for Chemical Analyses

Parameters	Container	Optimum holding time	Method of preservation
Acidity	P, G	24 hrs.	Refrigerate
Alkalinity	P, G	24 hrs.	Refrigerate
Chloride	P, G	7 days	Not required
Colour	P, G	24 hrs.	Refrigerate

Dissolved Oxygen Winkler	G	Analysed immediately	Determined on the site
Hardness	P, G	7 days	Refrigerate
Metals	P, G	6 months	HNO ₃ to pH < 2
Ammonia	P, G	24 hrs.	Refrigerate
Nitrite	P, G	24 hrs	Refrigerate
pH	P, G	Analysed immediately	Determined on the site
Phosphate	P, G	24 hrs	Refrigerate
Solids (total, dissolved)	P, G	7days	Refrigerate
Sulphate	P, G	7days	Refrigerate
Turbidity	P, G	24 hrs	Analysed same day stored in dark up to 24 hrs refrigerated

P - Plastic (Polyethylene or equivalent)

G - Borosilicate glass

Refrigerated - Storage at 4° C in the dark

Though there are many parameters to determine water quality, however, in the present study those physicochemical attributes were studied which were significant from the point of view of health hazards. Unless stated otherwise, the procedures described by "Standard Methods for the Examination of Water and Waste Water", 18th Edition, 1992 (APHA. AWWA-WEF), course manual, NEERI, 1979 and "The examination of waters and water supplies", (Taylor, 1958) have been followed. For each analysis, three replicates were taken into account and the chemicals used were of A.R. grade.

Materials and Methods

Turbidity: Turbidity was determined in Nephelometric turbidity units (NTU) by Systronics turbidly meter (Turbidimeter).

Colour: Colour was determined by the Platinum-Cobalt method .

Odour: Prepared the odour free water by filtering through a bed of Activated Charcoal. Determined the approximate range of Threshold Odour Number (TON) by diluting 200ml, 50 ml, 12ml and 2.8 ml of sample in 50 ml glass stopper Erlenmeyer flasks to 200 ml with odour free water.

pH: pH was determined by the Electrometric method.

Total Dissolved Solids (TDS): 100 ml sample filtered through Whatman filter paper No. 42, taken in a pre-weighed 100 ml beaker and evaporated to dryness in the oven at 103°C – 105°C. When completely dried, cooled in a desiccator and weighed again.

Total Solids (TS): Dried empty 100 ml. beaker was weighed, then 100 ml sample was taken in that beaker, the sample was evaporated to dryness on water bath, then was dried in hot air oven at 103°C- 105°C, cooled in a desiccator and weighed

Total Suspended Solids (TSS): After determining the total solids and total dissolved solids, the total dissolved solids were subtracted from the total solids.

$TSS = TS - TDS$ (All values are in mg/l)

Dissolved Oxygen: Dissolved Oxygen was determined by the Dissolved Oxygen content in water sample in mg/l by using Azide modification of the Winkler's iodometric Method, as per the APHA-AWWA-WPCF (1980).

Acidity: Hydrogen ions present in a sample as a result of dissociation or hydrolysis of solutes are neutralised by titration with standard alkali.

Total Alkalinity and Phenolphthalein alkalinity as $CaCO_3$: The alkalinity of water sample was determined by titrating the sample with a standard solution of strong acid using phenolphthalein and methyl orange as an indicator, expressed as phenolphthalein, methyl orange, total hydroxyl, bicarbonate and carbonate alkalinities, as prescribed in APHA-AWWA-WPCF (1980).

Ammonical Nitrogen: Ammonical nitrogen was determined using Nesslerization method following APHA-AWWA-WPCF (1980). In this method, Nessler reagent combines with the ammonical nitrogen of water sample and formed yellow-brown colour.

Nitrite-N: The Nitrite-N was determined in mg/l by Diazotization method. In this determination reddish purple azo dye was formed at pH 2.5 by the coupling of diazotized

sulfanilic acid with N – 1 Naphthyl ethylenediamine dihydrochloride (NED) and formed a pink colour

Hardness: Total hardness was determined by EDTA titrimetric method following APHA-AWWA-WPCF (1980). At a pH of 10.0 EDTA combines with both calcium and magnesium to form a blue colour at the endpoint.

Sulphate content: Sulfate was determined by using Turbidimetric method following APHA-AWWA-WPCF (1980). Sulfate ions are precipitated in HCl acid medium with barium chloride so as to form barium sulfate crystals of uniform size.

Chloride content: Chloride content was determined in mg/l by Argentometric titrimetric method following APHA-AWWA-WPCF (1980).

Fluoride content: Fluoride in water sample was determined through SPADNS colorimetric method.

Magnesium content: Magnesium content was calculated in mg/l by deducting the value of calcium hardness in total hardness value and was multiplied by a conversion factor as given in APHA-AWWA-WPCF (1980).

Sodium content: Sodium was determined by employing the flame emission photometric method as prescribed in APHA-AWWA-WPCF (1980).

Potassium content: Potassium was determined in by using the flame emission photometric method.

Calcium content: Calcium content was determined in mg/l by titrimetric method following APHA - AWWA - WPCF (1980).

Phosphate as total Phosphorous content: Total phosphorous content includes both the soluble and insoluble forms of phosphate. It was determined by digesting the sample more rigorously with H_2SO_4 and HNO_3 , and then the estimation was done by following molybdophosphoric acid method following APHA-AWWA-WPCF (1980). The spectronic

20 was employed in the measurement of color intensity of the blue solutions. The absorbance concentration of phosphate was measured photo metrically at 690 nm.

Iron content: Colorimetric method was used for the determination of trace amounts of iron which involves the complexation of Fe^{2+} with 1, 10-phenanthroline (phen) to produce an intensely red-orange colored complex. The determination of the iron-phen complex was performed with a spectrophotometer at a fixed wave length of 508 nm using external calibration based on iron standard solutions.

Table 3 Downstream Quality

Sample						
Parameters	A ₁	A ₂	B ₁	B ₂	C ₁	C ₂
Turbidity	8.14	8.85	7.4	7.82	8.3	8.5
Colour	Colour -less	Colour -less	Colour -less	Colour -less	Colour -less	Colour -less
Odour	Odour less	Odour less	Odour less	Odour less	Odour less	Odour less
Total Dissolved Solids	280	230	255	280	235	204
Total Suspend Solids	30	32.6	25	38	30	26
pH	7.8	7.2	6.2	7.8	6.8	7.2
Dissolved Oxygen	6.6	7.0	6.0	7.2	7.5	6.4
Acidity	-	-	-	-	-	-
Phenolphthalei n Alkalinity	-	-	-	-	-	-
Total Alkalinity	385	398	302	210	232.14	256.45
Ammonical Nitrogen	0.06	Traces	0.02	0.02	0.05	0.06
Nitrite	Traces	0.005	0.003	0.003	Traces	Traces
Hardness as CaCO ₃	70.50	72.18	51.25	54.40	98.72	99.15
sulphate	3.41	4.21	22.20	23.25	24.32	25.96
Chloride	18.15	10.20	30.26	35.40	32.14	36.75
Fluoride	0.40	0.52	0.27	0.35	0.49	0.42
Magnesium	23.16	28.56	6.08	7.05	14.76	13.42
Sodium	2.6	2.0	2.2	2.05	2.2	2.35
Potassium	1.65	1.85	1.95	1.7	1.35	1.20
Calcium	12.3	12.0	30.2	35.17	16.79	19.36
Phosphate as total phosphorous	0.092	0.15	0.16	0.18	0.074	0.98
Iron	0.08	0.076	0.04	0.15	0.06	0.098

All values are in mg/l except pH.

Table 4 Upstream Water Quality

Samples				
Parameters	D ₁	D ₂	G	H
Turbidity	6.6	6.45	3.2	4.2
Colour	Colourles s	Colourles s	Colourles s	Colourles s
Odour	Odourles s	Odourles s	Odourles s	Odourles s
Total dissolved Solid	156	174	108	112

Total Solids	Suspended	18	15	11	13
pH		7.0	7.2	7.4	7.3
Dissolved Oxygen		8.0	8.2	8.4	8.1
Acidity		-	-	-	-
Phenolphthalein Alkalinity		-	-	-	-
Total Alkalinity		210.45	230.15	190.17	165.85
Ammonical Nitrogen		0.26	0.18	0.32	0.29
Nitrite		0.01	0.02	0.19	0.16
Hardness as CaCO ₃		50.12	50.17	160.18	152.36
Sulphate		3.2	3.6	2.5	2.7
Chloride		17.86	12.46	8.26	9.6
Fluoride		0.17	0.02	0.15	0.13
Magnesium		27.35	52.3	28.45	24.2
Sodium		2.45	2.52	2	2.4
Potassium		1.74	2	1.49	1.54
Calcium		10.35	11.24	65.23	52.47
Phosphate as total phosphorous		0.04	0.175	0.045	0.13
Iron		0.12	0.04	0.17	0.165

All values are in mg/l except pH.

Table 5 Plant effluent Water Quality

Samples				
Parameters	E ₁	E ₂	F ₁	F ₂
Turbidity	9.0	9.5	8.2	8.8
Colour	Colourless	Colourless	Colourless	Colourless
Odour	Bad	Bad	Odourless	Odourless
Total Dissolved Solid	350	280	275	360
Total Suspended Solids	105	90	210	190
pH	7.2	7	7.4	7.6
Dissolved Oxygen	5.0	5.6	5.5	5.3
Acidity	-	-	-	-
Phenolphthalein Alkalinity	-	-	-	-
Total Alkalinity	400.25	370.82	392.42	385.45
Ammonical Nitrogen	0.1	1.18	0.14	0.078
Nitrite	0.19	0.17	0.078	0.082
Hardness as CaCO ₃	101.5	103.6	118.17	127.23
Sulphate	10.2	9.8	8.2	7.25
Chloride	24.52	22.16	20.45	26.16
Fluoride	0.12	0.89	..19	0.98
Magnesium	40.2	44.5	36.5	39.28
Sodium	3.6	3.9	2.8	3.4
Potassium	2.01	2.5	1.95	2.35
Calcium	10.2	10.5	9.6	10.1
Phosphate as total phosphorous	0.199	0.172	0.17	0.1
Total phosphorous	0.09	0.03	0.125	0.15
Iron				

All values are in mg/l except pH.

Results

All the water samples were colourless and odourless except the samples E₁ and E₂ of plant effluents which had bad odour. The turbidity values of Samples varied from 3.2 to 9.5. The minimum turbidity was observed in sample G taken from natural drinking water

source. The maximum turbidity was recorded in the samples of plant effluent. The values of total dissolved solids ranged from 108 to 360 mg/l and thus were within the prescribed limits (500 mg/l). The total suspended solids were found to vary between 11 to 210 mg/l. The maximum total dissolved solids (360) were found in the plant effluent water, the minimum (108) value was observed in the natural drinking water source. As regards the suspended solids, the higher values (210) were recorded in the samples of plant effluents and minimum total suspended solids (11) were found to be present in natural drinking water source (sample G). The pH value of the water samples was normal and was within the range of 6.2 to 7.8. The dissolved oxygen contents in water samples were observed to be varying between 5.0 and 8.4. The lower concentration was found in plant effluent, while higher concentration was recorded in drinking water sources. By and large, the dissolved oxygen was more than the prescribed limit (more than 5 mg/l). Acidity was not recorded at any of the study sites. Phenophthalein Alkalinity was not recorded at any of the study sites. Alkalinity at these sites was entirely due to carbonates and bicarbonates.

The total alkalinity was noted to be higher in the samples of plant effluents. The ammoniacal nitrogen was noticed in very little concentration varying from 0.078 to 1.18 mg/l, while nitrite was detected to be in traces. Hardness values fluctuated between 51.25 to 260.18 mg/l. However, it was within the prescribed limits (300 mg/l). The highest values of Hardness were recorded from natural drinking water source and lowest from upstream of Karas stream water samples. The concentration of sulphates was found in the range of 2.5 to 25.96 mg/l. Thus, sulphates were also less than the drinking water standard (200 mg/l). The Jaigan stream contained a higher amount of sulphates, while natural drinking water source for mines and upstream of Billori stream contained lower concentration. The chloride was observed in

lower degree in natural drinking water source and in a greater degree in Jaigan stream. However, the chloride was less than the drinking water standard (250 mg/l). Fluoride was also less than standard drinking water limit (1.0 mg/l).

Magnesium exceeded the prescribed limit (30mg/l) in water samples E₁, E₂, F₁ and F₂ of plant effluents samples. The sodium concentration was found to be varying between 2.0 and 3.9 mg/l. The sodium was slightly higher in plant effluent samples. The potassium was found to be present between 1.20 and 2.35 mg/l. Potassium concentration was also marginally higher in samples obtained from plant effluent source. Though the calcium was below the prescribed standard limit (75 mg/l) in all the samples, except samples G and H which contained comparatively more calcium than other water samples. The phosphate and iron have been observed to be present in some amount. Iron was more in samples G and H, but it was less than the maximum permissible limit of (0.3 mg/l) of WHO, 1971 and phosphates were normal. According to USEPA (1976), the concentration of phosphorous should not exceed 50 in any tributary to a river or a lake and 25 /l within these main sources.

Discussion

Odour : The samples of plant effluents containing domestic and plant wastes had shown bad odour which was essentially due to automobile discharges.

Turbidity: The maximum turbidity was recorded in samples collected from plant effluents which may be ascribed to oil and grease of automobile discharges. Oil and grease in natural waters are not of autochthonous origin but enter as a result of pollution. Being immiscible in water and low in density, they float and form a film on the surface thereby precluding gaseous transactions through water surface and penetration of light to a more or less extent (Saxena, 1987). Turbidity in natural water is

caused by clay, slit, organic, matter, phytoplankton and other microscopic organism.

Total Dissolved Solids and Total Suspended Solids: The total dissolved solids and total suspended solids have recorded a similar trend to their increase-decrease pattern and positive correlation was observed with turbidity. Tripathi and Pandey (1990) had observed that pond water was affected by eutrophication due to increased concentrations of total dissolved solids, total suspended solids and total solids. Singh (1965) noted that the total phytoplankton shows a striking parallelism with suspended solids. In a most general limnological sense this limiting nature of dissolved solids is essentially two-fold (Reid and wood, 1976; Shastree 1991, Singh, 1991): the chemical density of the environment of aquatic biota is a function of total dissolved solids, and dissolved solids influence the nature of the community by regulating the supply of nutrients and otherwise important materials. The role of human activities such as farming, construction, logging and channelization works on rivers is a dominant factor in increased erosion rates resulting in an increased sedimentation (Leopold *et al.*, 1964 ; Goswami, 1985).

pH: The pH values of all the water samples were found normal. Bhatt and Pathak (1992) have recorded alkaline pH in mountainous streams throughout water column with a mean maxima ($X = 7.83$; $N = 140$) in the Saryu basin. Gupta *et al.* (1990) have recorded pH to be between 6-6.5 for various water sources in Almora town.

Dissolved Oxygen: Among the chemical parameters, dissolved oxygen is one of the most important factors as its deficiency directly affects the ecosystem of a river due to bioaccumulation and bio-magnification. The high concentration of dissolved oxygen observed in the sample of natural drinking water source may be due to low water temperature obtained at a height of 1600 m

and growth of algae which release an appreciable amount of dissolved oxygen as a result of photosynthetic activities. Bhatt and Pathak (1992) have observed that for most of the winter season the dissolved oxygen in the rivers of Kumaon remains high as the solubility of gas is found to be inversely proportional to temperature. Similarly Chakraborty *et al.* (1959), Pahwa and Mehrotra (1966), Ray *et al.* (1977), Sunder (1988), Sharma *et al.* (1990), Trivedi *et al.* (1990) have reported that low turbidity and low water temperature may cause a rise in oxygen during winter season. In the present study, the low values of dissolved oxygen of other samples may be due to comparatively high turbidity, total dissolved solids, total suspended solids and their location at lower elevation resulting in higher temperature. Besides, lower values of dissolved oxygen may also be due to the discharge of plant effluents and domestic wastes. Organisms have a specific requirement of oxygen, for example, game fish requires at least 5 mg/l and coarse fish 2 mg/l of minimum dissolved oxygen in water (Trivedy and Goel, 1984).

Alkalinity: In the present study, phenolphthalein alkalinity was not observed at any of these sites indicating that alkalinity to these sites was entirely due to carbonates and bicarbonates. Higher values of total alkalinity have been recorded in the samples of plant effluents which is in agreement with observations of Philipose (1959) who had recorded higher alkalinity from polluted parts of river Sone in Bihar. The accumulation of large quantities of bicarbonates, and hence, high alkalinity may be due to surplus free carbon dioxide produced in the process of decomposition of bottom deposits. In Kumaon, Bhatt and Pathak (1992) have recorded moderately high values of alkalinity in all watersheds except river Gomti which could be accounted for a comparatively high H_2CO_3 content.

Hardness: The hardness of water is objectionable from the viewpoint of water use for laundry and domestic purposes since it

consumes a large quantity of soap. Ordinarily, salts of Ca and Mg contribute to total hardness (course manual, NEERI, 1979). Higher values of hardness as CaCO_3 have been recorded in the sample of natural drinking water source which may be due to the water passing through the calcareous zone. Precipitation of bicarbonates of Ca and Mg as CaCO_3 is thus, due to magnesite mining area being rich in MgO and CaO. Hardness has no known adverse effects on health, however, some evidence has been given to indicate its role in heart disease (Peter, 1974).

Sulphate Content: Present studies showed a very little concentration of SO_4^{2-} ion. However, a slightly higher presence of SO_4^{2-} ion was observed in Jaigan stream. Golterman, 1975 has shown that SO_2 resulting from human activity brings about the formation of sodium sulphate in the rainwater and volatile acid (HCl). Bhatt and Pathak (1992) have recorded that in Kumaon rivers, Sulphur (as sulphate) occurs in varying quantities depending upon the composition of substrata, the catchment area, the precipitation rates and geomorphological course of channels.

Chloride content: A low chloride concentration has been recorded from all the water samples under study. Bhatt and Pande (1991) have observed fairly low chloride concentration, an important ion required by photosynthesizing cell, in water bodies of Kumaun. In Alaknanda and Nandakini the snow-fed rivers of Garhwal Himalaya, a low range of chloride from 1.2 to 2.18 mg/l against the permissible limit 250 mg/l has been recorded (Tewari *et al.*, 1990).

Fluoride content: The fluoride values were found to be within the range of permissible limits of 1.00- 1.50 mg/l (BIS, 1991) which indicated that water is safe for drinking purposes.

Magnesium content: The plant effluents were found to contain Magnesium exceeding the permissible limit (30 mg/l). Obviously, this is due to the effluents of magnesite plant. Bhatt and Pathak (1992) have reported magnesium

content of Rivers; Ram Ganga, Saryu, Kosi and Gomati varying from 3.91 to 11.84 mg/l.

Sodium and Potassium content: The sodium and Potassium level in the effluents were found high in plant effluents. The Sodium is reported to be in more proportion than Potassium. It is an accepted phenomenon that where sodium and potassium occur in low concentration, the proportion of sodium is usually slightly greater than that of potassium, and as the total content of both increases, the concentration of sodium greatly exceeds that of potassium (Reid and Wood, 1976). Bhatt and Pathak (1992) have noticed a low concentration of Na during storm runoff period indicating its dilution by surface and subsurface flows. Potassium is most likely to be absorbed by soil colloids (Hem, 1970), its concentration was low in all water basins.

Calcium content: The calcium was observed below the permissible limit (75 mg/l). According to Bhatt and Pathak (1992), it is lesser in Himalayan streams. Igneous rocks (such as headwater reaches of Saryu) contain comparatively little calcium when compared to those of the sedimentary formations. The waters of Rivers Panar were highly calcareous followed by Ram Ganga, Saryu, Kosi and Gomati in decreasing order. Calcium, as such, has no hazardous effects on human health. In fact it is one of the important nutrients required by the organisms. Concentrations up to 180 mg/l have been found not to impair any physiological reactions in men (Lehr *et al.*, 1980).

Conclusion

Water quality analysis has revealed that samples of plant effluents have shown water pollution. However, all the values except that of Magnesium are within permissible limits as prescribed by Bureau of Indian Standards. The values of downstream water are slightly higher in few of the parameters which suggest that plant effluents have made an impact, though not appreciable, on the water quality of downstream water. The natural drinking

water source contains more calcium. The sample of plant effluents has reflected higher turbidity, higher total dissolved solids, and total suspended solids. The higher total alkalinity in the samples of plant effluents was due to carbonates and bicarbonates. The plant effluents are higher in Magnesium which could be ascribed to industrial wash offs. The acidity and phenolphthalein alkalinity were not recorded in any of the samples. On the whole, the plant effluents have a slight influence on the downstream water quality, as there was little difference between water quality of confluence of northern and western side stream and individual streams itself.

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Optimization of process parameters for the production of nanobioplastics using agricultural waste

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Abstract

Agricultural waste has become a major issue to be solved especially in regions where stubble burning has created drastic figures, hence it is inevitable to strategically manage these waste. Biodegradable and renewable polymers derived from the cellulosic biomass of these agricultural wastes are quite attractive to replace non-biodegradable conventional types of petrochemical plastics. Moreover, these developments with effective optimization could be very cost effective, economic and feasible. Furthermore, the future market of bioplastics will be increase owing to its sustainability. Recent studies and researches are playing a crucial support to commercialize and demonstrate the biopolymer based plastics. But, Nevertheless, these biopolymers are mixed with nanoparticles to achieve better strength and mechanical properties.

Keywords: Biopolymer, Bioplastic, Nanobioplastic, Nanomaterials, Eco friendly, Sustainable, Non-conventional and Degradable.

Introduction

The decrement in fossil fuel resources and the resulting deteriorative effects on the environment are of major concern globally, hence it is very necessary to create a peculiar method for the production of various plastics that when synthesized creates pollution (Puscaselu *et al.*, 2019). One alternative way is to develop some novel chemicals and a series of

nobel processes to create the plastic products which uses renewable feedstock, typically biomass from various sources and chemicals derived from biomass (Xia *et al.*, 2021).

Biomass basically refers to the organic matter & materials such as grass, wood structures, and various types of algae, commercial & agricultural crops, microbiological cells and their residues or wastes, including some animal waste. The ecological advantages of biomass or agricultural wastes related to the production of solid, liquid and gaseous fuels is attracting wide global attention (Carrier *et al.*, 2019). Petrochemical based non-biodegradable plastics are causing threatful environmental effects therefore these biodegradable, non-conventional polymer based bioplastics are best replacement over traditional plastics.

Methodology

In our current research for the production of bioplastic substrates such as agricultural remains including Wheat Straw & Rice Straw were collected locally from the agri-fields of Bichpuri, Agra, Uttar Pradesh.

Pre-treatment: Pre-treatment of agricultural waste involves several washing to remove dirt & dust of the straws collected from agriculture fields. These straws were dried, and grinded to form straw powder, further washed with distilled water and dried in vaccum oven at 60°C for 24 hrs. Now powdered straw is chemically treated by immersing it in 30% ethanol for 6 hrs chemically treated powder was washed thoroughly with distilled water and dried for 24 hrs at 60°C (Rohmawati *et al.*, 2018).

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Further processed powder was immersed in 4% HNO_3 for 2hrs by using water bath followed by several times washing and then placed in vaccum oven for proper drying at 60°C for 30 hrs. (Mostafa *et al.*, 2015).

Cellulose Extraction: Extraction of cellulose from agricultural waste was done by using NaOH (Rohmawati *et al.*, 2018). In the current experiment for optimising the concentration of alkali for maximum yield of cellulose, five different concentrations of NaOH i.e. (0.5M, 1M, 1.5M, 2M, 2.5M) were used (Mostafa *et al.*, 2015). 4gms of processed straw powder was added to each of these five solutions of NaOH and placed on heating mantle at 90°C for around 3 hrs to extract cellulose.

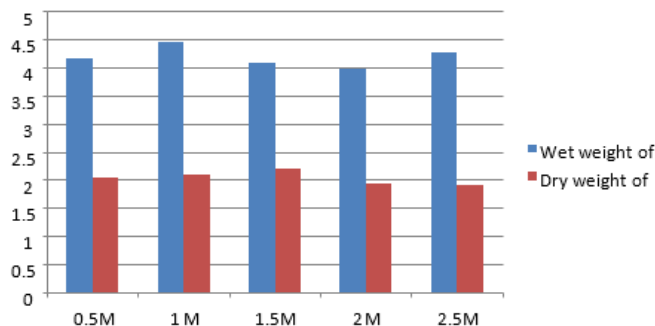


Fig 1: Cellulose yield at different concentrations of NaOH

Bleaching of Extracted Cellulose: Three different types of bleaching agents were used i.e. NaOCl (Sodium Hypochlorite), H_2O_2 (hydrogen peroxide) and Na_2SO_3 (sodium sulfite) at different concentrations to optimize the bleaching agent. 5gms of Extracted Cellulose was immersed in each bleaching agent of given concentration for 20hrs at room temperature.



Fig 2: Extracted Cellulose fibres treated with different bleaching agents at varied concentrations showing comparative variation in their appearance

Conversion of extracted cellulose to carboxy-methyl cellulose: 4gms extracted cellulose was weighed and isopropanol was added to it while stirring continuously, then strong alkali like NaOH solution (25% w/v) was mixed to the cellulose mixture and stirred for almost one hour at 35°C . Furthermore to this mixture, 4.8 g of monochloroacetic acid was added & stirred continuously for more than 3hrs at 52°C (Sunardi *et al.*, 2017). Finally, the formed slurry was neutralized with glacial acetic acid followed by washing with ethanol. The whole product was dried in oven at 65°C for 24 hrs and then stored in airtight container at for further use.

Synthesis of Egg-Shell Nanoparticles:

Formation of nanoparticles was done by adding CaCO_3 (egg shell powder) in diluted HCl at 120 for 2 hrs, which will give CaCl_2 as product. Then hydrolysis is initiated, by dissolving NaOH in a drop-wise manner to the mixture of CaCl_2 at room temperature which results in precipitation of $\text{Ca}(\text{OH})_2$ to form a crystalline structure. This crystalline structure is aged for 18 hrs at room temp. Finally calcium hydroxide nanoparticles are filtered & dried in vaccum oven for at 60°C for 20 hrs, further grinded to form fine powder. The final product was calcium nanoparticles (Habte *et al.*, 2019).

Synthesis of bioplastic with different combinations of substrate (carbon source):

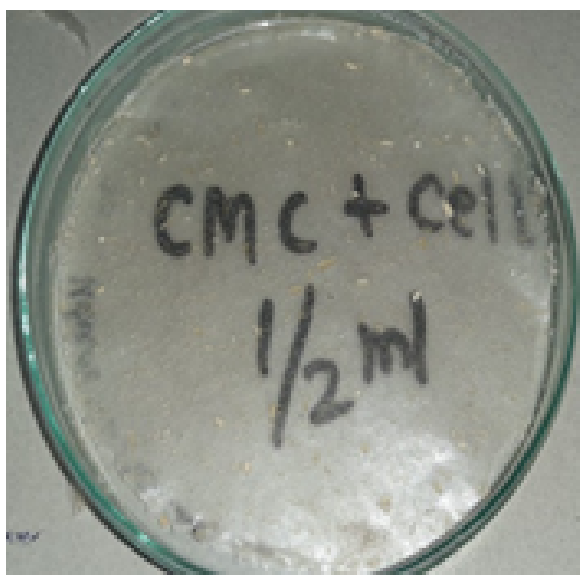
Five different combinations of carbon substrate viz. Cellulose + CMC, CMC + Potato Starch, CMC + Arrowroot Starch, Cellulose + Arrowroot Starch, Cellulose + Potato Starch; were used to make samples of biodegradable plastic. For bioplastic production, 0.75gm of different carbon substrate combinations was added to 40ml distilled water, the mixture was then stirred using homogeniser and varied amounts of plasticizer (2ml, 1ml, 0.5ml) was added to it. This mix was then heated for a while to make viscous slurry and then poured on petri dishes to make plastics (Azmin *et al.*, 2020).

Table 2: Combinations of bio-plastic samples using various combinations of substrate and varying concentrations of plasticizer

S.No.	Combinations of bio-plastic (0.75 g + 0.75g)	Plasticize (amount in g)	Nanoparticle (g)	Properties
1.	CMC + Cellulose	Glycerol (2 ml)	-	Much rigid, less thin, non-sticky, less transparent, less easier to peel, enough elastic, weak plastic
2.	CMC + Cellulose	Glycerol (1 ml)	-	Moderately rigid, appropriate thickness, less sticky & transparent, easier to peel, moderate elastic & strength
3.	CMC + Cellulose	Glycerol (0.5 ml)	-	Less rigid, less thin, less sticky, less transparent, very tough to peel, less elastic, weak plastic
4.	CMC + Cellulose	Glycerol (1 ml)	0.2 g	Moderately rigid, appropriate thickness, less sticky, less transparent with white spots, easier to peel, moderately elastic, moderate strength, decreased solubility in hot water
5.	CMC + Cellulose	Glycerol (0.5 ml)	0.2 g	Much rigid, more thin, less sticky, less transparent with white spots, easier to peel, moderately elastic, moderate strength, decreased solubility in hot water
6.	CMC + Potato Starch	Glycerol (2 ml)	-	Less rigid, thin, sticky, transparent, easy to peel, highly elastic, weak plastic
7.	CMC + Potato Starch	Glycerol (1 ml)	-	Moderately rigid, thin, sticky, transparent, easier to peel, moderately elastic, moderately weak plastic
8.	CMC + Potato Starch	Glycerol (0.5 ml)	-	Less rigid, very thin, sticky, transparent, difficult to peel, less elastic, very weak plastic
9.	CMC + Potato Starch	Glycerol (1 ml)	0.2 g	Less Brittle, tough to peel, thin, less sticky, less transparent with white spots, very less elastic, moderate strength, breakable, not easily soluble in water
10.	CMC + Potato Starch	Glycerol (0.5 ml)	0.2 g	Brittle, tough to peel, thin, less sticky, less transparent with white spots, less elastic, easily breakable, moderate tensile strength, not easily soluble in water
11.	CMC + Arrowroot Starch	Glycerol (2 ml)	-	Highly elastic, less rigid, thin, sticky, transparent, easy to peel, weak plastic
12.	CMC + Arrowroot Starch	Glycerol (1 ml)	-	Moderate elastic, moderately rigid, lesser thin, sticky, transparent, easy to peel, weak plastic
13.	CMC + Arrowroot Starch	Glycerol (0.5 ml)	-	Less elastic, more rigid, moderately thin, less sticky, transparent, easy to peel, less weak plastic
14.	CMC + Arrowroot Starch	Glycerol (1 ml)	0.2 g	Moderately elastic, thin, less sticky, less translucent with white spots, easier to peel, less strength, weak plastic
15.	CMC + Arrowroot Starch	Glycerol (0.5 ml)	0.2 g	Moderately elastic, moderately thin, less sticky, less transparent with white spots, easier to peel, less strength, weak plastic
16.	Cellulose + Arrowroot Starch	Glycerol (2 ml)	-	Less rigid, thin, less sticky, translucent, easy to peel, highly elastic, weak plastic

17.	Cellulose + Arrowroot Starch	Glycerol (1 ml)	-	Moderately rigid, moderate thickness, less sticky, translucent, moderately easier to peel, less elastic, normal plastic
18.	Cellulose + Arrowroot Starch	Glycerol (0.5 ml)	-	More rigid, thin, less sticky, translucent, less easy to peel, less elastic, very weak plastic
19.	Cellulose + Arrowroot Starch	Glycerol (1 ml)	0.2 g	Brittle & rigid, less thin, less sticky, yellowish & translucent with white spots, uneasy to peel, increase strength
20.	Cellulose + Arrowroot Starch	Glycerol (0.5 ml)	0.2 g	Less Brittle & rigid, more thick, less sticky, less translucent with white spots, uneasy to peel, moderate strength
21.	Cellulose + Potato starch	Glycerol (2 ml)	-	Much rigid, thick, less sticky, less transparent, difficult to peel, less elastic, less strong plastic
22.	Cellulose + Potato starch	Glycerol (1 ml)	-	Very less rigid easily breakable, thick, sticky, translucent, difficult to peel, less elastic, weak plastic
23.	Cellulose + Potato starch	Glycerol (0.5 ml)	-	Not rigid, very thin, less sticky, less transparent, difficult to peel, less elastic, very low strength

The physical and mechanical properties of bio-plastics made with the above combinations of CMC + Cellulose quite impressive and acceptable to replace conventional kind of chemical based plastics, hence addition of



Ca-nanoparticles was done to all the combinations to find out the conclusion that whether those nanoparticles will/will not improve the properties of required

biodegradable plastic. Physical form of these plastic samples was not appropriate as their properties were not suitable to utilize them as a substitute for conventional plastic hence addition of nanoparticles was not done with this combination.

Results

Effect of pre-treatment: Agricultural waste collected from agri-fields of Bichpuri, Agra was pre-treated to loosen the binding between cellulose, hemi cellulose and lignin. Cellulose and Hemicellulose was treated with acids to solubilize easily, for this diluted nitric acid was used which would not affect cellulose but can solubilize the hemi cellulose, along with that ethanol is used to hydrolyze whole substrate and provides additional cleaning of dirt and dioxins.

Effect of concentration of NaOH: This study shows that 1.5M concentration of sodium hydroxide has been recorded as the optimum concentration to get best outcome with highest yield of cellulose.

Effect of temperature on cellulose extraction: Three different temperature ranges (70°, 80° and 90°) were used at 1.5M conc. of NaOH to check the effect of temperature on extraction process and the time taken to complete the extraction was approximately 4hrs, 3hrs 20mins and 3hrs respectively.

Effect of pH on extraction: As suggested with the observation of optimum concentration of sodium hydroxide to be used it was concluded that the extraction process gives much yield when pH increases to a particular point. Afterwards, the yield decreases as pH increases.

Effect of bleaching agent: This study clarifies that the best bleaching agent that overall gives better texture, fine and modified fibres with good bleaching was Sodium

Hypochlorite.

Effect of concentration of bleaching agent: Desired value of bleaching agent was noticed as 5% or much higher concentration of sodium hypochlorite the other bleaching agents at any conc. does not provide proper bleaching to the extracted material.

Effect of quantity of plasticizer: This experiment finds out that 1ml glycerol in 1.5gm dry ingredients (diluted in 40ml water) is best among other concentrations that gives best product with any of the five varied combinations used to make bioplastic.

Effect of different combinations of substrates: Table 2. given below briefly describes the difference in physical properties of bioplastic made from different combinations of material.

Table 2: Different combinations of bio-plastic samples showing different physical properties

	Cellulose + CMC	CMC + Potato Starch	CMC + Arrowroot Starch	Cellulose + Arrowroot Starch	Cellulose + Potato Starch
Stickiness	Normally Sticky	Much Sticky	Less Sticky	Less Sticky	Much Sticky
Transparency	Less transparent	Transparent	Transparent	Translucent	Translucent
Solubility or degradability in Water	132 days	70 days	92 days	116 days	102 days
Degradation in Soil (of pH 5-6)	112 days	65 days	104 days	108 days	95 days

Effect of nanoparticles: The conclusion made by the addition of egg shell nanoparticles to the bioplastic is somehow different from the expected results as it clearly disturbed the transparency of bioplastic and do not helped in enhancing the strength or other required physical parameters. Although, addition of egg shell nanoparticles to the material increases brittleness reduces elasticity, increases rigidity and increase degradation time of the biodegradable plastics.

Discussions

Sodium hydroxide is preferably used in the experiment as it has high pH value and is considered best among other alkalis to extract

cellulose. This study involves the use of egg-shell waste to create carbon nanoparticles that are considered to be a promising and hopeful source of strength in bioplastic. In the earliest trials of bioplastic making only cellulose has been used to create such plastic using plasticizer and those plastic samples were very light in weight, do not possess any tensile strength (i.e. do not even hold proper shape) and no elasticity. The use of starch and CMC has been done to increase tensile strength, flexibility & elasticity and this also increases the weight of bioplastic. These bioplastics were heavier than that of plastic samples made with cellulose only. Bioplastic samples without starch were dry and not peeled easily. The bioplastic films made with

CMC & Starch were transparent and easily peel able with good and improved physical properties. Bioplastic films made with the combination of starch and cellulose are sticky as compared to other films, & this could be due to starch that has been used, i.e. potato starch. Stickiness in potato results in sticky bioplastic samples, but this stickiness reduces by switching to Arrowroot Starch.

Conclusion

Various parameters and materials used in this experiment to make bioplastic have been optimized. It was concluded that Pre-treatment of agricultural waste is quite essential for cellulose extraction. Best concentration of alkali used to extract cellulose has been found with the proper bleaching agent and its optimum concentration to properly bleach the extracted cellulose fibres has also been done. Calcium nanoparticles have not created a hopeful difference in the physical & mechanical properties of biodegradable plastic sample made using agricultural waste.

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Medicinal marvels, significance of phytochemical and versatile applications of *Adhatoda vasica*

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Abstract

Medicinal plants have been used from old times and are extensively used for the cure of different afflictions and are gaining acceptance among the people of both urban and rural areas of the world. The importance of their genuine usage, therapeutic dosage and role of their agile ingredients in current treatment system are in focus in today's world. The present study was aimed to investigate the phytochemical evaluation and antimicrobial estimation of *Adhatoda vasica* commonly known as adulsa in Indian origin. The accomplishment of the development of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased the ability of pharmacists and scientists to respond to the challenges that have come out with the spreading of professional services in abetment of people's life.

Key words: Medicinal plants, herbs, phytochemical, anti-microbial, *Adhatoda vasica*, *Adulsa*.

Introduction

On the report of World Health Organization, medicinal plants are the foremost source to obtain a wide variety of newer herbal drugs and newer medicines in present scenario. The stature of herbal medicine and herbal drugs has been rapidly emerging all over the world during the last few years. About 80-85% of the

individuals of prosperous countries use orthodox medicine, which has compounds derived from medicinal plants (Patel *et al.*, 1984). During the last century, during investigation of the innate environment, scientist has made great findings that have enabled him to use a considerable number of biological resources such as plants.

The ancient man discovered the health-giving values of some herbs by hit and trial methods. Plants are rich in extensive varieties of secondary metabolites production such as antibiotics, tannins, toxins, alkaloids, flavonoids, phenols, steroids, terpenoids, glycosides and volatile oils. Bacterial strains which are impenetrable to antibiotics are increasingly becoming a problem to human well-being. Currently used antibiotic factors are failing to bring an end to many bacterial strain infections due to super resistant strains. Plants have a great potential and strength for producing newer drugs of great benefit to human health. Medicinal plants could be used as a rich source from which anti-microbial factors may be acquired (Patel *et al.*, 1984).

Adhatoda vasica, commonly known as *Adulsa* is an important Indian medicinal plant which has been used in rasayanas and homoeopathy system of medicine. *Adulsa* plant has been involved in miscellaneous numbers of pharmaceutical activities which incorporates respiratory tract infection, cough syrups formulation, medicament, anti-spasmodic and bleeding pills (Vinothapooshan *et al.* 2010). Just now various researchers showed greater interest in anti-microbial activity of *Adulsa* plant against several bacterial species in

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different researches. Different parts of *Adhatoda vasica* plant such as leaf is beneficial in the treatment of bronchitis, tuberculosis and other asthametic and bronchiole disorders (Srivastava *et al.*, 2015). A decoction of the leaves of *Adulsa* may be used to get relief in situation of excessive cough and other indications of cold (Vinothapooshan *et al.* 2010). The soothing activity of *Adulsa* plant helps during irritation in the throat and the expectorant will help in loosening of phlegm deposits in the airway.

In this review paper, the major focus is on traditional uses of different parts of *Adulsa* plant as well as, ethnopharmacological and toxicological studies, published and unpublished, are reviewed and commented upon. The data have been assessed from the point of view of correctness, dependability, applicability and acceptability for the overall evaluation of the safety of *Adathoda vasica*.

Ethnopharmacology and bibliography of *Adhatoda vasica*: *Adathoda vasica*, latin name *Adhatodae vasicae folium*, botanical name *Nees* - a small (1.0-2.5 m height), thick, evergreen shrub or bush with 10-15 cm prolonged and 3-10 cm wider leaves having a herbaceous woody stem, substantial and dense flowers, small and clubbed fruit is widely administered all round the tropical regions of South-east (Singapore, Malaya, Sri Lanka, Upper and Lower Myanmar, southern China, Laos, and the Malay Peninsula and Indonesian Archipelago) (Patel *et al.*, 1984, Vinothapooshan *et al.* 2010, Chakrabarty *et al.*, 2001). In Indian origin the *Adulsa* plant is distributed throughout the plains, in underneath Himalayan ranges, also found in Chandigarh and Punjab, West-Bengal and Assam (Rahman *et al.*, 2004).

Researches performed during the last three to four decades over *Adulsa* plant has revealed that the alkaloids (plant substance used in

drugs) vasicine, vasicinone and deoxyvasicinone (quinazoline ring derivatives) are specifically present in the leaves possess respiratory restorative action whereas its thick roots contain alkaloids, vasicinolone, vasicol and peganine (Gupta *et al.*, 1977; Chihara *et al.*, 1997). Vasicine $C_{11}H_{12}N_2O$ (1,2,3,9-tetrahydropyrrolo [2, 1-b] quinazolin-3-ol,) also called Peganine (Huq *et al.*, 1967). Deoxyvasicinone (2, 3-Trimethylene-4-quinazolinone) possesses antimicrobial, anti-inflammatory and antidepressant activities (Al-Shamma *et al.*, 1981). Vasicinone ($C_{11}H_{10}N_2O_2$) shows pronounced bronchodilatory activities *in vivo* and *in vitro*.

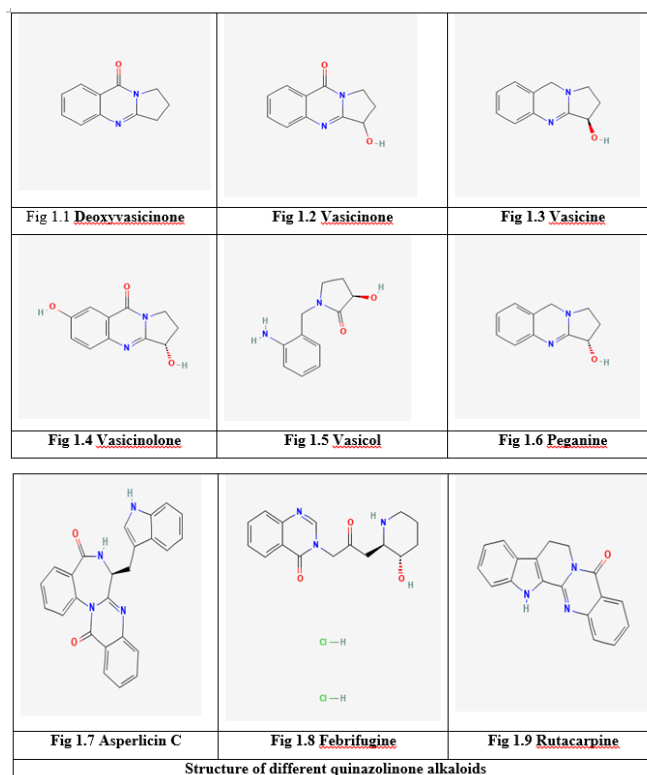


Fig 1: Various alkaloids present in leaves (vasicine, vasicinone and deoxyvasicinone), and roots (vasicinolone, vasicol and peganine) of *Adathoda vasica*

The botanical description of the plant is as follows: (Nadkarni, 1976)



Fig 2: Adulsa, Malabar nut, *Adhatoda vasica* Plant

Kingdom : Plantae
Division : Angiosperms
Class : Eudicots
Order : Lamiales
Family : Acanthaceae
Genus : Justicia
Species : *Adhatoda (Adhatoda vasica)*

Plant Parts Used of *Adhatoda Vasica*: The leaves, roots, flowers, stem and bark of this plant are used in therapeutic applications.

Conventional uses of *Adhatoda vasica*: *Adhatoda vasica* plant is mainly used as a bronchodilator stimulant. Mixture of the leaves of *A. vasica* is a magnificent agent for the abolition of wide variety of ants especially white ants, flies, and mosquitoes (Mahbubur *et al.*, 2008). The extricate obtained by boiling the leaves of Adulsa plant in combination with fruit of *Phyllanthus emblica*, used with honey is very good for asthmatic patients. The fresh extricate of Adulsa leaves mixed in honey and ginger extract is recommended for symptoms of persistent cough, chronic bronchitis and asthma (Mahbubur *et al.*, 2008). Extricate of *A. vasica* leaves is also recommended in malarial fever. Leaves of Adulsa plant are squashed and the extricate is drunk orally for dry cough and phlegm congestion in the course of cold (Singh *et al.*, 2011). Paste of the fresh macerated roots is applied on the abdomen region and vaginal region minutes before child birth for easy child birth. Leaves along with

flowers of Adulsa plant possess expectorant and anti-asthmatic properties. Juice made from the Adulsa bark and leaves are used for vomiting and as an anti-helminthic. The fruits of same are used for cold, antispasmodic and bronchitis activity (Khan *et al.*, 2010).

Medicinal Applications of *Adhatoda vasica*

Mitigating Power and remedial Properties: The leaves, roots and the flowers of Adulsa plant are substantially used in aboriginal medicine as a remedy for cold, cough, bronchitis and asthma (Khan *et al.*, 2010).

Bronchitis and Asthma: In case of acute bronchitis stages it gives unfailing relief especially where the sputum is thick and sticky. It liquidifies tough sputum so that it is raised up more effortlessly. For relaxation in asthma and bronchitis, smoking dried leaves should be done (Waheed *et al.*, 2015).

Tuberculosis: In Rasayana and homoeopathy, a preparation made from *Vasaka* flowers, known as gulkand is used to treat tuberculosis. Fresh petals of *Vasaka* flowers should be discolored and placed in a pot of chill clay. Finally sugar crystals are added and the jar is kept in the sun rays for heating. It should be stirred after intermittent time intervals in the morning and evening sessions. Conserved is ready for use in about a month. Even the juice from its leaves is useful in treating tuberculosis (Srivastava *et al.*, 2013). 30 ml. of Adulsa leaf juice thrice a day relieves the irritable cough by its soothing action on the nerve and by liquefying the sputum, which makes expectoration easier (Kumar *et al.*, 2010).

Coughs: For coughs, 7 leaves of the plant are boiled in water, strained and mixed with 24 grams of honey. This decoction provides relief. Similarly a confection of *Vasaka* flowers eaten in doses of 12 grams twice daily relieves cough. About 60 grams of flowers and 180 grams of jiggery should be mixed for preparing this confection (Kumar *et al.*, 2010).

Intestinal Worms: Its leaves, bark, the root-bark, the fruit and flowers are useful in the removal of intestinal parasites. The decoction of its root and bark in doses of 30 grams twice or thrice a day for 3 days can be given for this purpose. The juice of its fresh leaves can also be used in doses of a teaspoon thrice a day for 3 days (Jain *et al.*, 1980).

Diarrhea and Dysentery: The juice from its leaves should be given in doses of 2 to 4 grams in treating diarrhea and dysentery.

Skin Remedy: A poultice of its leaves can be applied with beneficial results over fresh wounds, rheumatic joints and inflammatory swellings. A warm decoction of its leaves is useful in treating scabies and other skin diseases (Srivastava *et al.*, 2013).

Evaluation of Phytochemicals: The chemical mixture present in *Adhatoda vasica* plant incorporate essential oils, fats, resins, sugar, gum, amino acids, proteins and vitamins 'C' etc (Waheed *et al.*, 2015).

The phytochemical estimation of *Adulsa* leaves shows that it contains phenols, tannins, alkaloids, anthraquinone, saponins, flavonoids and reducing sugars (Waheed *et al.*, 2015). But pharmacologically the most studied chemical component in *Adulsa* plant is vasicine, a bitter quinazoline alkaloid, the novel alkaloid recovered from leaves and distinguished as 1, 2, 3, 9-tetrahydro-5-methoxypyrrol [2, 1-b] quinazolin-3-ol (Waheed *et al.*, 2015) mainly present in the leaves, roots and flowers. Besides vasicine, the leaves contain several alkaloids (Vasicinone, Vasicinol, Adhatodine, Adhatonine, Adhvasinone, Anisotine and Hydroxypeganine), betaine, steroids and alkanes (Waheed *et al.*, 2015). The pharmacological activities of vasicine and vasicinone are well known. Recent investigations on vasicine showed broncho-dilatory activity equivalent to theophylline, both in natural and in artificial conditions (Srivastava *et al.*, 2015). Both the alkaloids in combination showed pronounced

bronchodilatory activity. Vasicine also exhibits strong respiratory stimulant activity. There has also been a report of thrombopoetic (platelet increasing) activity with vasicine. Uterine restorative activity and midlling hypotensive activity of the alkaloids have also been observed in *Adulsa* plants (Singh *et al.*, 2000).

Vasicine is metabolized to vasicinone and analysis of plant leaf extract showed that it contained 0.85% vasicine and 0.027% vasicinone. Sitosterol, α -glucoside-galactose and deoxyvasicine have been isolated from the roots of the plant (Singh *et al.*, 2000). 2'-4-dihydroxychalcone-4-glucoside has also been recognized in the flowers (Singh *et al.*, 2000). Flowers of *Adhatoda vasica* mainly contain kaempferol and quercetin (www.agefotostock.com). A new triterpenoid, 3-hydroxy-D-friedoolean-5-ene, along with the known compounds, epitaraxerol and peganidine have been isolated from the aerial parts of *Adhatoda vasica* Nees (www.agefotostock.com). The leaves also contain a very small amount of an essential oil and a crystalline acid. An analysis published in India in 1956 showed the seeds as containing 25.8% of deep yellow oil composed of glycerides of arachidic 3.1%, behenic 11.2%, lignoceric 10.7%, cerotic 5%, oleic 49.9% and linoleic acids 12.3% and α -sitosterol (2.6%). Elemental analysis using atomic absorption spectrophotometry revealed the presence of major (K, Na, Ca and Mg) and trace (Zn, Cu, Cr, Ni, Co, Cd, Pb, Mn and Fe) elements in *Adhatoda vasica* (Singh *et al.*, 2000).

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Methods of gene cloning and expression vectors

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Abstract

The techniques of DNA cloning and expression vectors play a vital role in the emerging field of biotechnology. The most significant application of expression vectors is the production of heterologous proteins. Cloning vectors are typically plasmids used to propagate DNA, which replicate in *E. coli* to high numbers and contain a multiple cloning site (polylinker) with restriction sites suitable for inserting a DNA fragment. In contrast, expression vectors are designed to allow transcription of the cloned gene and its translation into protein. While they share some features with general cloning vectors, such as the multiple cloning sites and a selectable marker, they typically have a lower copy number within cells and lack a screenable marker. Expression vectors also have additional features that enable them to express genes and produce proteins, including a promoter, ribosome binding site, ATG starts codon, a multiple cloning site (polylinker) that ensures inserts are ligated in a predictable reading frame, and often (not always) a fusion tag to aid in purification steps. Therefore, cloning and expression vectors are important

tools in recombinant DNA technology. This article reports on the basics of cloning methods and different expression systems in the current context.

Keywords: DNA, recombinant DNA technology, cloning, vectors, genes, promoters.

Introduction

Genes are the building blocks of heredity and are composed of DNA. Some genes are responsible for producing functional proteins, while others are not. Clones are genetic copies of a cell, DNA sequence, or entire organism. For example, identical twins are clones produced through cell division. The word “gene” was coined by Danish botanist Wilhelm Johannsen to describe Mendelian units of heredity and distinguish between an individual’s outward appearance (phenotype) and genetic traits (genotype). Johannsen suggested “genes” as a term for Mendelian factors of inheritance, derived from the Greek word “genos,” meaning “birth,” which also spawned the word “genome.” Clones can occur naturally through asexual reproduction in plants or binary fission in bacterial cells. Animals capable of parthenogenesis also produce clonal offspring. Biotechnological methods are used to produce clones artificially, such as molecular cloning to copy specific gene fragments or reproductive cloning to create a multicellular clone or whole organism through somatic cell nuclear transfer (Brown, 1990).

Recombinant DNA technology (RDT) encompasses all techniques involved in

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constructing, studying, and using recombinant DNA molecules. Genetic engineering is a term coined in the 1970s to describe the field of RDT, which involves taking pieces of DNA and combining them with other pieces of DNA in labs. This transformed genetic material containing DNA molecules from two or three different origins is called a recombinant (or Chimera). Recombination occurs naturally or through technology by exchanging DNA sequences between different molecules, resulting in a recombinant DNA molecule made up of DNA fragments from more than one organism. Genetic engineering directly manipulates and alters one or more genes, usually by adding a gene from another species to an organism's genome to confer a desired phenotype. Humans have indirectly influenced genomes through controlled breeding and selecting offspring with desired traits (Lodge *et al.*, 2007).

Paul Berg, Herbert Boyer, and Stanley Cohen are pioneers in the field of genetic engineering. Boyer, a co-founder of Genentech, won the Biotechnology Heritage Award in 2000 for his distinguished academic career and development of RDT, which has brought new hope to millions worldwide. Cohen, who created rDNA plasmids, received the Biotechnology Heritage Award in 2016. Boyer also received the Perkin Medal in 2007 and the Winthrop-Sears Medal in 2005 from the Society of Chemical Industry.

Historical background

During the mid-19th century, Gregor Mendel established a set of rules that explained the inheritance of biological characteristics. He referred to each heritable property of an organism as being controlled by a factor, which is now known as a gene. In 1900, the rediscovery of Mendel's laws marked the birth of genetics, a science aimed at understanding genes and how they work. W. Sutton proposed in 1903 that genes reside on chromosomes, and by 1910, T.H. Morgan and his co-workers

developed techniques for gene mapping. By 1922, they had produced a comprehensive analysis of the relative positions of over 2000 genes on the four chromosomes of the fruit fly, *Drosophila melanogaster*. Before experimental proofs of Avery, MacLeod, and McCarty in 1944 and Hershey and Chase in 1952, hardly anyone believed that deoxyribonucleic acid (DNA) was the genetic material. Delbrück, Chargaff, Crick, and Monod were among the most influential contributors to the second great age of genetics. Between 1952 and 1966, the structure of DNA was elucidated, the genetic code was cracked, and the processes of transcription and translation were described. According to DNA folklore, Kary Mullis invented the polymerase chain reaction (PCR) during a drive along the coast of California one evening in 1985.

Cloning methods

The process of creating identical copies of a specific gene or DNA sequence through genetic engineering techniques is known as gene cloning. This process can also be referred to as DNA cloning, molecular cloning, or recombinant DNA technology. When extracting DNA from an organism, all of its genes are obtained. Gene (DNA) cloning involves copying a specific gene to form "clones." This technique is used to isolate and amplify genes of interest. There are two methods for DNA cloning: cell-based DNA cloning and cell-free DNA cloning (PCR).

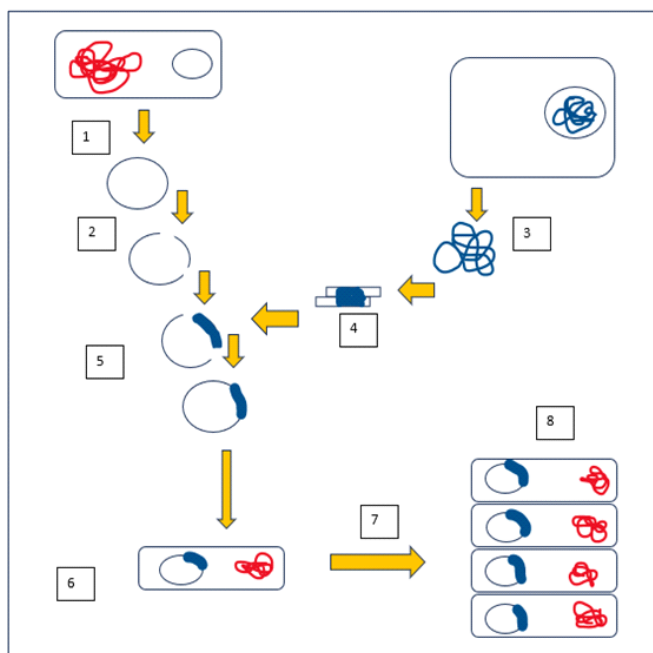
Principle of gene cloning

To clone a desired gene, a fragment of DNA is inserted into a vector. This vector acts as a vehicle and carries the gene into a host cell, usually a bacterium. The vector then multiplies, creating multiple identical copies of itself and the gene. As the host cells divide, the recombinant DNA molecule is passed down to the progeny, resulting in a large number of identical cell clones. Each cell in the colony contains one or more copies of the recombinant DNA molecule, and the gene is

now considered to be cloned. Other types of living cells can also be used for this process (Schamhart *et al.*, 1999).

Requirements for Gene Cloning (Cell-based)

1. DNA fragment containing the desired genes to be cloned.
2. Restriction enzymes and ligase enzymes.
3. **Vectors** – to carry, maintain and replicate cloned gene in host cell.
4. **Host cell**– in which recombinant DNA can replicate.



1- Isolation of plasmid, 2- Cut plasmid DNA, 3- Isolate gene of interest, 4- Prepare gene of construct, 5- Insert the gene of interest in the created nick, 6- Ligate the nick, 7- Place the recombinant plasmid in the transformation vector, 8- Allow bacterium to reproduce

Fig. 1 Steps in gene cloning

A. Isolation of the DNA fragment or gene

- The first step in cloning a gene is to identify and isolate the specific DNA or gene fragment of interest.
- A restriction endonuclease (RE) enzyme can be used to cut DNA at specific recognition nucleotide sequences, known as restriction sites.
- These restriction sites can result in either

blunt or sticky ends.

- Another method of isolating the desired gene is to synthesize a complementary DNA strand using its mRNA.
- This is accomplished using a reverse transcriptase enzyme.

B. Selection of suitable cloning vector

- A vector transports a gene of interest (GoI) into a host and replicates itself along with the GI to create multiple copies.
- Cloning vectors have size limitations for the insert they can carry.
- Depending on the size of the insert and its intended use, a suitable vector is chosen.
- Plasmids and bacteriophages (phage ϕ) are the most commonly used cloning vectors among all available options.
- Other types of vectors available for gene cloning include bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs), and mammalian artificial chromosomes (MACs).

C. Essential Characteristics of Cloning Vectors

The cloning vectors are carriers of DNA molecules known as the Gene of Interest (GoI). These vectors have certain common features that include:

- They should be able to self-replicate inside the host cell.
- They must have a unique restriction site for restriction enzymes.
- The introduction of GoI should not affect the vector's self-replication.
- They must possess a selectable marker gene that can be used to identify the transformed cell later on. Usually, this gene is an antibiotic resistance gene that is absent in the host cell.
- They should be easily isolated from the host cell.

D. Formation of Recombinant DNA

- Cut open the plasmid vector using the same restriction enzyme that was used to isolate the Gene of Interest (GoI) from the DNA fragment
- Mix the GoI DNA fragment and plasmid vector together
- Allow DNA ligase to facilitate the base pairing of the GoI DNA fragment and plasmid vector
- The resulting DNA molecule is a hybrid of the two DNA molecules, which is referred to as recombination in genetics
- This new hybrid DNA molecule is also known as a recombinant DNA molecule.

E. Transformation of the recombinant vector into a suitable host

- Recombinant DNA is typically transformed into a suitable host cell
- This is done to replicate the recombinant DNA molecule and obtain multiple copies of the gene of interest
- It also enables the expression of the gene of interest, allowing it to produce the necessary protein product
- Some bacteria are natural transformers and will automatically uptake the recombinant vector, such as *Bacillus*, *Haemophilus*, and *Helicobacter pylori*
- Other bacteria require artificial methods such as Ca^{++} ion treatment, electroporation, or PEG incorporation.

F. Isolation of Recombinant Cells

- Transformation process generates mixed population of cells in a colony
- Colony contains both transformed and non-transformed host cells
- Only useful transformed cells are filtered out
- Plasmid vector contains selectable marker gene
- Marker gene used to isolate or select recombinant cells from non-recombinant cells
- pBR322 plasmid vector contains

Ampicillin-resistant and Tetracycline-resistant genes

- *pst1* RE utilized to knock out Ampicillin-resistant gene from plasmid
- Transformed cell made susceptible to Ampicillin after Ampicillin-resistant gene is knocked out

G. Multiplication of Selected Host Cells

1. The screening process must be completed first.
2. The host cells need optimal conditions to grow and multiply in petri-plates and form transformed colonies.
3. The transformed cells are placed into fresh culture media to replicate the recombinant DNA, resulting in multiple copies of the desired gene.
4. If the aim is to obtain many copies of the gene of interest (GoI), the host cells are allowed to replicate.
5. If the goal is to produce a specific product, such as protein, hormones, or secondary metabolites, the vector containing the GoI must be provided with the necessary conditions to express the desired product.

H. Isolation and Purification of the Product

The final step of the process is to separate the multiplied gene of interest (GoI) that is attached to the vector and isolate the desired product encoded by the GoI. This is achieved through purification processes of the isolated gene copy or protein.

Applications of gene cloning

1. Isolate the chosen gene (GoI) and determine its nucleotide sequence.
2. Identify and analyze the control sequences of DNA.
3. Investigate and study the function of the protein, enzyme, or RNA.
4. Identify any mutants related to specific diseases.
5. Engineer defected cells in organisms for specific purposes, such as insulin production, insect resistance, hormone production, and TAP.

Expression vectors

Vectors called expression vectors are used to carry and express DNA inserts. They can be either plasmids or viruses, and are sometimes referred to as expression constructs. These vectors are engineered to introduce genes into specific cells, and contain regulatory elements such as enhancers and promoters to ensure efficient transcription of the gene of interest. Simple expression constructs are called transcription vectors, as they only allow for transcription of the cloned foreign gene, not its translation. Protein expression vectors facilitate both transcription and translation, leading to the production of recombinant protein.

Promoters and termination sequences are necessary for transcription and translation. Promoters in expression vectors must have on/off switches to regulate the production of the gene product, as excessive amounts of the protein can be toxic for the cell. An example of a commonly used promoter is the mutant version of the lac promoter, lacUV. This promoter initiates high levels of transcription under induced conditions. Some expression vectors also have a ribosomal binding site upstream of the start codon, which facilitates efficient translation of the cloned foreign gene. Expression vectors are widely used in molecular biology, particularly in techniques like site-directed mutagenesis.

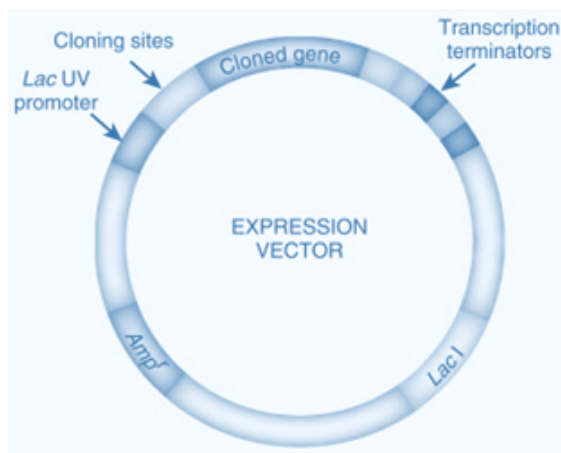


Fig. 2 Basic elements of Expression vectors

How expression vectors work?

After the expression construct is introduced into the host cell, the gene of interest is transcribed, resulting in the production of the encoded protein. To ensure efficient transcription, regulatory elements such as enhancers and promoters are often engineered into the plasmid. Expression vectors are frequently used in biotechnology and molecular biology to create stable proteins, including insulin, the main treatment for diabetes. However, purifying the protein of interest from the host organism presents a challenge, which is why the gene of interest should always have a “tag” to simplify the purification process. Expression vectors are also used in techniques like site-directed mutagenesis, where cloning vectors insert the gene of interest into a plasmid that replicates in bacteria. To ensure successful protein expression, expression vectors must have strong promoter and termination signals, proper distance between the promoter and cloned gene, inserted transcription termination sequences, and portable translation initiation sequences (Dieckman *et al.*, 2002).

The following are some detailed explanations of the important components of expression vectors:

A. Promoters - Promoters ensure a reliable transcription of the gene of interest. For efficient mRNA synthesis with RNA polymerase, strong promoters are also necessary. When constructing an expression vector, it's critical to keep in mind the regulation of the promoter (Ayoubi and Van, 1996). The strongest promoters are found in bacteriophages T5 and T7. In *E. coli*, the promoter is regulated in two ways:

- a) **Induction** - Chemical switches are added to turn on the transcription of the gene.
- b) **Repression** - Chemical switches are added to turn off the transcription of the gene.

The most commonly used promoters in *E. coli* expression system are:

- i. **lac promoter:** The transcription of the lac Z gene is regulated, as it is responsible for producing β -galactosidase. This gene can be induced by IPTG, also known as isopropyl-thio-galactosidase. By fusing the lac promoter sequences to the target gene, it can express lactose-dependent expression. However, the lac promoter has some limitations. It is not strong enough to produce high levels of the desired protein and still carries out basal level transcription even without an inducer molecule present.
- ii. **trp promoters-** A group of genes related to tryptophan production is regulated by this entity. Tryptophan serves as its inhibitor, while 3- indoleacrylic acid triggers its activation.
- iii. **tac promoters-** This is a promoter that combines the lac and trp promoters through hybridization. It is more potent than either of them. When isopropylthiogalactosidase (IPTG) is present, the tac promoter becomes active.
- iv. **PL promoters:** The PL promoter is a synthetic translation initiation region (TIR) that includes convenient cloning sites. These cloning sites allow for the precise fusion of target DNA sequences to vector transcriptional and translational signals by providing all or part of an AUG translation initiation codon. This strong promoter is responsible for the transcription of DNA in *E. coli*, and its repressor, referred to as the repressor, is produced by the λ cl gene. The PL promoter is utilized in combination with the fg mutant host to produce a temperature-sensitive form of the repressor. At low temperatures, the repressor protein represses transcription, while at high temperatures, the cloned gene's transcription occurs because the repressor is inactivated. To efficiently transcribe cDNA in mammalian cells, the promoter must be located upstream of the cloned cDNA. In most cases, viral promoters, such as the CMV promoter (derived from cytomegalovirus) and the SV40 promoter (derived from simian virus

40), are employed for their reliable strong constitutive expression. The yeast expression vectors available commercially may contain active constitutive or inducible promoters.

- a) **Constitutive Promoter-** A constitutive promoter is a specific type of promoter that remains unregulated and permits the continuous transcription of its corresponding gene. An example of a constitutive promoter is the GAP promoter of the gene that encodes glyceraldehyde-3-phosphate dehydrogenase.
- b) **Inducible promoter-** Inducible promoters regulate gene expression and can be turned on or off during specific stages of development or at certain times. Examples of inducible promoters include AOX1, GAL1, GAL10, nmt1, nmt42, and nmt81.

The AOX1 promoter is responsible for activating the gene that produces alcohol oxidase. This promoter is triggered by methanol and is ideal for producing the protein in *Pichia pastoris*.

- A. Two examples of promoters are GAL1 and GAL10, which can be induced by galactose and are ideal for protein expression in *Saccharomyces cerevisiae*. Additionally, the nmt1, nmt42, and nmt81 promoters can be induced by thiamine and are useful for protein expression in *Schizosaccharomyces pombe*.
- B. **Reporter genes-** The reporter gene is responsible for producing a protein that can be easily detected and quantified through a simple assay. It is a valuable tool for measuring gene expression efficiency and identifying the protein's location within a cell. The rate of the structural gene's expression is influenced by regulatory sequences located upstream of it. The gene's expression rate can be measured by replacing its protein-encoding portion or fusing it with another gene that produces a different protein. This additional protein's presence can be easily detected. Reporter genes are helpful for identifying promoters, enhancers, and other proteins or regulatory elements that bind to them (**Brown, 2020**).

There are four commonly used genes that act as reporters in genetic research. These genes are:

- a) The lac Z gene of *E. coli* which produces a blue color in the presence of X-gal. The intensity of the blue color indicates the level of the gene and can be easily quantified.
- b) The CAT gene of *E. coli* which encodes for the enzyme chloramphenicol acetyltransferase. This enzyme transfers acetyl groups from acetyl CoA to the antibiotic chloramphenicol.
- c) The luciferase gene of the firefly *Photinus pyralis* which is responsible for the oxidation of luciferin resulting in the emission of yellow-green light. This light emission can easily be detected even at low levels.
- d) The green fluorescent protein (GFP) gene of the jellyfish *Aequorea victoria* which was discovered by Shimomura. This protein fluoresces green when irradiated with UV or blue light and is used as a tag to monitor protein localization in genetic engineering.

Ribosome binding sites and translation initiation sites After the promoter, you'll find the ribosomal binding site (RBS), which helps with the smooth translation of the cloned gene. In prokaryotes, the Shine Dalgarno sequence is the translation initiation site that's part of the RBS. The consensus sequence of the translation initiation site includes a set of 8 base pairs located upstream of the AUG start codon. For eukaryotes, the Kozak sequence is the specific sequence where ribosomal machinery for the translation of mRNA is assembled (Lewis *et al.*, 1998).

- C. **Poly-linkers:** The vector contains specific recognition sites for restriction enzymes which are used to excise the vector at the restriction site for cloning the desired foreign gene. These sites are referred to as poly-linkers or multiple cloning sites (MCS) because they are often found close together. Typically, these regions are between 50 to 100 base pairs in length and can have up to 25 restriction sites clustered within them.

- D. **Poly A (Adenylation) tail:** The poly-A tail found at the end of mRNA plays an important role in protecting it from degradation by exonucleases or endonucleases. This tail is essential for stabilizing the mRNA and terminating transcription and translation. The addition of the poly-A tail requires a nucleolytic enzyme complex and a poly-A-polymerase.

- E. **Expression systems:** To produce a protein, an expression system is necessary. There are two types of expression systems: prokaryotic and eukaryotic. Each has its own benefits and limitations that can be considered when creating an expression system. However, no single expression system can be universally used for the production of heterologous proteins (Miller, 1988).

- a) **Prokaryotic expression systems-** In prokaryotes, the specificity of the RNA polymerase promoter is controlled by the sigma factor. *E. coli* is a popular prokaryotic expression system that produces proteins at high levels. To ensure safety during large-scale experiments and fermentation, *E. coli* strains are genetically manipulated for the production of recombinant proteins. The purification of proteins has become simpler with the use of recombinant-fusion proteins that can be purified through affinity chromatography, such as glutathione-S-transferase and maltose-binding fusion proteins.

- b) **Eukaryotic expression systems-** When it comes to cloning genes, using a eukaryotic expression system can solve many of the challenges faced with a prokaryotic expression system. Eukaryotic expression systems, such as yeast, mammalian cells, and insect baculovirus cells, are viable options to consider.

The advantages of using a eukaryotic expression system are: The cloned gene produces a protein that is expressed at high levels. The proteins can be purified easily using specific tags included in the vector, such as His or Myc (Miller, 1989).

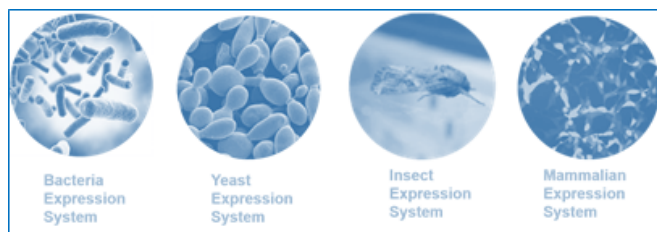


Fig. 3. Different expression systems

The disadvantage of the eukaryotic expression system includes: The eukaryotic cells grow at a slow pace compared to the prokaryotic cells.

- F. Fusion protein-** The proteins produced by expression vectors can either be native polypeptides or fusion proteins. Fusion proteins are advantageous in purifying and analyzing the protein. They are also known as chimeric or hybrid proteins, which result from the coding sequence of two different genes that are fused together and form a single polypeptide sequence after translation. Fusion proteins protect the gene of interest from the proteases that exist in the host cell, preventing it from being degraded. When expressed separately, the proteins are prone to degradation and undergo proteolysis. A fusion vector system involves inserting the target gene into the coding sequence of the host gene. To construct fusion proteins, the coding sequences of two different genes are ligated at the DNA level. Prior knowledge of the nucleotide sequence of the coding genes or segments is essential to guarantee that the ligation produces the correct reading frame (Clark and Pazdernik, 2015).

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Nanomedicine Breakthroughs: Advancing Therapeutic Potential through Nanotechnology-Based Drug Delivery Systems in Medical Science

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Abstract

The use of nanotechnologies in medicine, particularly in the creation of novel drug delivery systems, has gained growing interest. Drugs can target specific sick locations with the assistance of nanoscale carriers, extending treatment efficacy and reducing unwanted side effects. Despite significant drawbacks, a lot of pharmaceutical and medical device businesses have already used medical nanotechnology. In some situations, medications with a high potential for toxicity, like cancer chemotherapy drugs, can be delivered with a higher level of safety. Living cells are microscopic virtual machines that participate in all biological processes, such as cell signaling, metabolism, energy production, and nutrient transport. Considering this, it can be said that this technique is a strong contender for use in therapeutic biology and medicine. We talk about the value of nanoscience in this review as various nanotechnology platforms are applied in other areas of medicine. Additionally, we are talking about the potential applications of nanotechnology in human health in the future.

Keywords: Nanomedicine, Nanocarriers, Therapeutics, Dendrimers.

Introduction

Nanotechnology is the only discipline that can collaborate with traditional disciplines like applied health, molecular chemistry, molecular science, pharmaceutical science, the field of optics, etc. to find novel features of matter. By developing a more efficient healthcare system, nano-medical equipment, and therapeutic approaches in the last several decades, science and technology have frequently been well-designed to address the challenges in the field of medicine and health sciences. Historically, Professor N. Taniguchi first used the word “nanotechnology” in 1974. In 1986, Drexler created the initial conception (Feynman’s thoughts) of nanotechnology and published it in the book “Vehicles of Creation: The Arrival of the Nanotechnology Era” (Bayda *et al.*, 2019). The influence of nanotechnology on humans and animals is currently opening new research opportunities and transforming the field of health science, making it a crucial topic for consideration as a therapeutic tool. Nanotechnology is a highly speculative multidisciplinary field that was developed to engineer biological elements such as atoms, molecules, and supramolecular systems at nanoscales of approximately 1-100 nm to overcome current challenges by developing novel devices and identifying material structures with distinctive properties to study and comprehend the deadly biological problems followed by disease diagnosis and treatment (Secretariat, 2006; Sichert *et al.*, 2015). The most dominant and

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economically successful technology of these decades has emerged as nanotechnology, which is incredibly important for human life. It should be noted that the machinery found in live cells is both important and exceedingly small (nanoscale). They play a significant role in practically every biological process, including nutrient transport, energy synthesis, metabolism, and cell signaling. As a result, it can be said that nanotechnology is a strong candidate to provide new technologies at the level of the individual particles of matter to deal with biology and medicine (Zandonella, 2003). Numerous clinically advantageous nanoscale substances are developed; nanomedicine is quickly becoming the gold standard in the field of health sciences (Moghimi and Farhangrazi, 2013; Zarrintaj *et al.*, 2017). Numerous benefits for generating and distributing various nanomedicine products can be obtained by modulating diseased/abnormal tissues/cells in a way that is clinically correlated and pathophysiologically sound. To overcome the disease prognosis, tissue-specific therapeutic targeting techniques may thus have a variety of tactics (Stirland *et al.*, 2013). Researchers and physicians worldwide now find it relatively easier to not only identify diseases like cancer so early but also to successfully treat those diseases, thanks to a breakthrough in nanotechnology research. Nanotechnology and nanomedicine, which have made early and accurate diagnosis and rapid treatment possible, have been a blessing to scientists (Champion *et al.*, 2007). Nanomaterials, particularly metal nanoparticles, have drawn increased interest recently in the broad field of medical science. Nowadays, there is a lot of research being done on how to best synthesize nanomaterials such as polymers, emulsions, micelles, dendrimers, liposomes, nano capsules, and nanoparticles.

One of the most important uses of nanotechnology today is nanomedicine, which is dedicated to creating medical devices at the

nanoscale to create a high-quality healthcare system. With the help of this method, we can better comprehend human physiology and combat various devastating diseases like cancer and cardiovascular conditions. The importance of nanomedicine is primarily applied for imaging, disease diagnosis, tissue engineering, and designing more effective, safe, and cost-effective drug delivery systems to precisely deliver drugs to target sites that can speed up treatment outcomes by reducing toxicities and off-target effects (Dobrovolskaia, 2015). Bebo *et al.*, state that the US-FDA has so far approved about 51 nanomedicines. After that, 77 products are in the pre-clinical stage, with about 40% of them currently in the clinical trial stage. Most of these authorized nanomaterials are nanocrystal and polymeric liposomal compositions. However, the researcher hopes to create more effective materials for clinical trials, such as micelles, protein-based nanoparticles (NPs), and a variety of inorganic and metallic particles (Sahoo *et al.*, 2017).

Nanotechnology has the potential to foster ground-breaking developments in technology that will benefit human health in the areas of medicine, communications, genomics, and robotics. To debate and investigate every use of nanotechnology is quite broad and varied (El-Sayed *et al.*, 2020).

The importance of nanotechnology to human health is highlighted in this review, with a focus on its use in nanomedicine for imaging, evaluation, diagnostics, selective drug delivery systems, and efficient treatment approaches for human diseases. Here, we also illustrated the risk connected to it and its potential for future development in the medical sciences.

Kinds of Nanocarriers

In recent years, nanocarrier-assisted drug delivery methods have become more popular for biological uses. varied designs of nanocarriers are being created to suit specific

criteria since varied therapeutic needs call for distinct types of drug delivery systems. For the transport of drugs, several nanocarriers have been developed. These include carbon nanotubes, inorganic nanoparticles, carbon dots, polymeric micelles, liposomes, nanogels, and dendrimers (Shen *et al.*, 2016; Li *et al.*, 2020).

Carbon Nanotubes

Carbon nanotubes are distinctive tubular structures with a significant length-to-diameter ratio and a nanometer diameter. The concentric carbon shells that make up the nanotubes can range in number from one to hundreds, with a distance between each shell of about 0.34 nm. The honeycomb configuration of the carbon atoms in the graphite sheets and the carbon network of the shells are closely connected. The fascinating mechanical and electrical characteristics of nanotubes are due to their almost one-dimensional (1D) structure and the arrangement of the carbon atoms in the shells, which is like graphite (Popov, 2004). The CNTs must first be functionalized to be water-soluble and biocompatible to be suitable for biomedical usage (Liu *et al.*, 2009). The CNTs must first be infused to be water-soluble and safe to be suitable for biomedical usage (Liu *et al.*, 2007). Based on the function of the nanotubes, which may also be responsive to various stimuli, operational CNTs can act as carriers for a variety of therapies (from small molecules to peptides to nucleic acids) and are able to carry the active agents to a variety of organs (Hasnain *et al.*, 2019; Bianco *et al.*, 2005). CNTs have the ability to breach the blood-brain barrier (BBB) and treat neurological illnesses because they can enter cells with ease (Carter *et al.*, 2019). Regarding the safety character of the CNTs, research indicated that endocytosis of the CNTs causes oxidative stress to the cells, exposing a tight relationship between CNTs and fibrosis, cancer, and inflammation, hindering the translational potential of this nanocarrier

(Mohanta *et al.*, 2019; Dong and Ma, 2019).

Inorganic Nanoparticles

When compared to organic materials, inorganic nanoparticles are significantly more durable, hydrophilic, and safe. It has distinctive physical and chemical features, including a high surface area per unit volume, special optical, and magnetic capabilities, and it may be functionalized with a variety of ligands to increase their affinity for the target molecules or cells. Inorganic nanoparticles can manage medication release profiles, preserve pharmaceuticals from degradation, and lower dosages and delivery rates, which significantly reduces the toxicity of drugs in general and cancer therapies in particular (Paul and Sharma, 2020). These inorganic nanoparticles as medication carriers have several benefits. First, they have a predetermined size and are simple to prepare. More intriguingly, whereas organic nanoparticles like liposomes and microspheres solely act as drug reservoirs, they frequently display multiple medically beneficial roles, such as acting as exothermic reactors and contrast agents (Murakami and Tsuchida, 2008). The processes of drug release in most organic drug carriers, including liposomes and lipid microspheres, are passively controlled and include drug diffusion, particle erosion, particle degradation, polymer swelling, and drug diffusion through a membrane or wall. It is acceptable to state that gold nanoparticles may display active regulated release that is triggered by extraneous stimuli in this manner. Since the internal regions of ox-NHs allow deposition metal crystals (Miyawaki *et al.*, 2006). Ox-NHs might be given the characteristics of both iron oxide and gold nanoparticles. Additionally, by adding “nano caps” for their nanometer-sized pores to regulate drug release through the pores, ox-NHs could create a novel sort of drug release (Ajima *et al.*, 2006).

Carbon Dots

A promising new development in nanomedicine is fluorescent carbon dots (CDs) with a size lower than 10 nm, great biocompatibility, and little to no cytotoxicity. The use of green-emitting CDs with a carboxyl-rich surface as a trackable drug delivery agent for localized cancer treatment in a mouse model is demonstrated for the first time in this report. The natural carboxyl groups on the CDs and the amine moiety on the cancer medicine Doxorubicin (DOX) form a non-covalent link that allows the CDs and DOX to be conjugated (Zeng *et al.*, 2016). CDs offer hope for overcoming the difficulty of transporting medications over the BBB for the treatment of neurological illnesses because of their unusually small size. To treat glioblastoma brain tumors, Leblanc's team created carbon dots coupled with therapeutic medicines and a targeted ligand (Hettiarachchi *et al.*, 2019). To establish that the carbon nitride dots could penetrate the BBB, they tested the medication in the zebrafish model (Liyanage *et al.*, 2020). However, additional testing is still required to confirm the safety profile of these carbon dots (Havrdova *et al.*, 2016).

Polymeric Micelles

Polymeric micelles, are aggregation colloids created in solution by the self-assembly of amphiphilic polymers, display a novel way to address a few problems with drug administration, such as the drug's poor water solubility and poor permeability through biological barriers. Polymeric micelles often have a smaller size, simpler preparation and sterilization procedures, and good solubilization qualities compared to other nanocarriers. Unfortunately, these characteristics are also linked to a reduced stability in biological fluids and a more challenging characterization (Ghezzi *et al.*, 2021). Several methods, including the addition of albumin, have been researched to

increase the drug loading capacity of biocompatible polymeric nanocarriers. After some modification, the FDA approved the creation of the albumin-based polymeric nanocarrier Abraxane, which transports the anti-cancer medication Paclitaxel for the treatment of breast cancer (Chen *et al.*, 2018). Additionally, the surface-functionalization of the micelles can aid in preventing the gastrointestinal system from degrading payloads like protein medicines, enabling the oral administration of protein pharmaceuticals like insulin (Anselmo *et al.*, 2019; Veisheh *et al.*, 2015). The drug-releasing characteristics of polymeric micelles have also been improved. A programmable multistage drug delivery system with lymph node targeting was recently developed by Schudel *et al.* They created thiol-reactive oxanorbornadiene (OND) linkers and attached them to nanoparticles of polymeric micelles that are readily absorbed by lymph nodes. The half-life of the nanocarrier can be programmed to range from hours to days depending on different OND substituents. Compared to free drug administration, such multi-staged intra-lymphatic drug release can aid in enhancing the immunotherapeutic effects (Schudel *et al.*, 2020). But there are still several obstacles to be overcome. For instance, polymers can decrease the bioactivity of protein therapies when they are conjugated with proteins (Ekladios *et al.*, 2019). PMs are therefore the subject of substantial research and may show to be an efficient means for resolving several outstanding problems in medication delivery (Kotta *et al.*, 2022).

Liposomes

The spherical vesicles known as liposomes have an aqueous core inside one or more concentric phospholipid bilayers. Liposomes are an effective medication delivery mechanism for many pharmaceuticals since they are harmless and biodegradable. By stabilizing chemicals, removing barriers to cellular and tissue uptake, enhancing

medication biodistribution to target regions in vivo, and lowering systemic toxicity, they have increased the therapeutic efficacy of pharmaceuticals (Guimarães *et al.*, 2021). The basic self-assembled lipid bilayers that make up traditional liposomal nanocarriers deliver medicines in their watery cores. By coating the surface with polyethylene glycol (PEG), or “PEGylation,” the lipid bilayers can be further stabilized (Suk *et al.*, 2016). Their absorption by the RES can be considerably reduced by PEGylation, but the clearance cannot be totally avoided. Additionally, liposome targeting effectiveness and therapeutic efficacy have been continuously improved while liposome toxicity has been reduced. Tang *et al.* presented a new alteration to the liposome’s surface using a “do not-eat-me” technique to get around the problem of being swiftly cleaned by RES (Tang *et al.*, 2019). Liposomal carriers are frequently utilized as nanocarriers for the delivery of drugs because they significantly increase therapeutic efficacy by stabilizing the payload and facilitating uptake by the targeted tissue (Sercombe *et al.*, 2015; D’Mello *et al.*, 2017). The first approval of Doxil, a liposomal medication containing the anti-cancer medicine doxorubicin hydrochloride, by the Food and medicine Administration (FDA), has paved the way for the clinical application of nanocarriers (Li *et al.*, 2020). Among all the nanocarrier-assisted medicine submissions to the FDA throughout the following years, liposomes have remained the predominate nanocarrier, indicating the efficacy and safety of this kind of nanocarrier. Most liposomal nanocarriers available thus far are designed to be injected (D’Mello *et al.*, 2017). Research is ongoing into the potential of liposomal nanocarriers for the transport of nucleic acids in addition to drugs (Xue *et al.*, 2015). The fact that systemic delivery of these nanocarriers results mostly in hepatic uptake is a significant barrier to targeted therapy using liposomal nanocarriers to carry nucleic acids. Tissue-specific antibodies can be added to the

liposomal carriers to increase their tissue specificity. For instance, Parhiz *et al.* recently coupled the mRNA-carrying liposomal nanocarriers with antibodies specific to the vascular cell adhesion molecule, PECAM-1, to accomplish the transport of transgenes to the pulmonary vasculature. This innovative method significantly decreased hepatic uptake and assisted in directing the nanocarriers to the desired organs (Parhiz *et al.*, 2018). A liposomal nanocarrier expressing the mRNA of thrombomodulin, a naturally occurring endothelial inhibitor of thrombosis, inflammation, and vascular leakage, was created by Marcos-Contreras *et al.* using a similar methodology. These liposomal mRNA nanocarriers were effective at reducing TNF-induced acute brain inflammation by concentrating on the inflamed cerebral vasculature (Marcos-Contreras *et al.*, 2020).

Nanogels

Nanogels (NGs) are cross-linked, 3D networked structures that may swell in water and range in size from 1 to 1000 nm. Because of their capacity to react to both exogenous stimuli-triggers like light and temperature as well as endogenous biological triggers like pH and biomolecule recognition, biocompatible and “smart” NGs made from stimuli-responsive polymers are particularly appealing. Importantly, the sensitive NGs can be used for therapeutic administration and diagnostic applications after being exposed to these physical or biochemical signs (Preman *et al.*, 2020). Drugs can be released from nanogels passively through diffusional release or chemically or biologically destroying the structures’ functionalized components (Madan *et al.*, 2014). However, there are other challenges in the way nanogels’ clinical application. For instance, when deformity happens, formerly beneficial characteristics like their swellability, drug loading capacity, and affinity target adhesion might be significantly impacted (Myerson *et al.*, 2019). Additionally, ongoing work is being done to

improve their biodistribution, prevent their rapid clearance, reduce the toxicity brought on by the surface charge, and improve control over targeted release of drugs and nanogel disintegration (Soni *et al.*, 2016). In order to achieve these objectives, Myerson *et al.* recently created a cross-linker modulated nanogel with antibodies specific to endothelium markers for enhanced targeted drug delivery (Myerson *et al.*, 2019).

Dendrimers

Emerging polymeric frameworks called dendrimers are renowned for their well-defined structures, adaptability in drug delivery, and high functioning, many of whose characteristics are like those of biomolecules (Madan *et al.*, 2014). Using non-covalent interactions, the pharmaceuticals can either be physically trapped in a dendrimer or covalently attached to dendrimers (Chauhan, 2018). The ability of dendrimers to entrap hydrophilic or hydrophobic molecules with high molecular weights and their high surface to volume ratio, which makes them possible carriers for gene therapy, make them extremely promising for usage in biomedical applications (Madan *et al.*, 2014). In order to combat multidrug resistance, which is frequently encountered in cancer treatments, Pan *et al.* recently created a polyamidoamine dendrimers-based nanomedicine that incorporates a small interfering RNA (siRNA) that can lower a multidrug resistance (MDR)-related protein onto the dendrimer (Pan *et al.*, 2019). To lessen the toxicity of dendrimers, attempts have been undertaken to carboxylate, PEGylate, or acetylate the terminal amine groups of dendritic polymers. Fast removal from the body and comparatively quick circulation time are some additional dendrimer drawbacks, albeit all of them are being improved by recent technological developments (Zhu *et al.*, 2019).

Nanotechnology in disease treatment

The simple fact that nanotechnology has

special functional qualities and features to work in harmony with our bodies' natural systems makes it a potential area in the medical industry and healthcare system. As is commonly known, live cells exist in nanoscale ranges. Thus, it was proposed that the domain of nanotechnology is a fascinating one for biology and medicine. Due to its focused delivery and nature, nanotechnology is now being used in medicine more and more frequently each day. Various nanoscale materials have been developed with a variety of clinical applications, such as illness detection, medication delivery, and molecular medical imaging, in the past. These materials have been followed by certain products that have undergone clinical trials (Pelaz *et al.*, 2017).

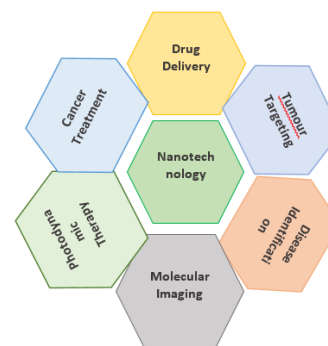


Fig.1. Application of nanotechnology in different field of medicine and healthcare

Application in drug delivery

There is currently a great deal of interest in drug delivery research to successfully deploy drug molecules and therapeutic agents to their intended locations for the treatment of various ailments (Obeid *et al.*, 2017). The formulation of the drug-targeting system is based on an appropriate concentration, therapeutic efficacy, and a prolonged circulation period. This effective drug targeting strategy was developed by using the pathophysiological modulation that occurs with the progression of illnesses. The binding and distribution of medications to specific locations may be facilitated by this tailored delivery. The main use of nanotechnology in medication delivery

has been concentrated in NPs, which are mostly advised for the treatment of malignancies (Devulapally and Paulmurugan, 2014). The effectiveness of these NPs as drug delivery systems, however, differs based on their size, shape, and other innate biophysical/chemical characteristics. The term “NP” is also known as nano structures, nanospheres, nanovehicles, and nanocarriers. Gregory Gregoriadis developed the liposome, the first NP for a drug delivery system (Gregoriadis, 2016). Later, several NPs-based medication carriers emerged, and several are now being studied for a variety of disorders. There are several NP-based therapeutic drug delivery systems in use today that have been specifically formulated as polymer micelles, emulsions, and solid particles. Some nano-based medications, including Caelyx®, Doxil® and Abraxane®, are marketed for the treatment of cancer. The optimal method of medicine delivery is typically oral administration because it is noninvasive. Oral administration of peptide or protein drugs has not yet been accomplished (Bajracharya *et al.*, 2019). To get over these obstacles in medication distribution, however, nanotechnology will be used to encapsulate such pharmaceuticals in nanoparticles, changing the drug carrier’s characteristics. Additionally, it improves medication site-specific targeting, preventing the delivered drug’s accumulation to lessen the off-target effect. NPs-based medications are currently most frequently combined with natural goods to lessen toxicological problems.

Application in tumour targeting

Using quantum dots and in-vivo targeting a therapeutic human cancer cell, this method was first shown in nude mice. It’s interesting that a subdermal tumour just needs a tiny depth of penetration for imaging. Polyethylene glycol coupled with quantum dots and functionalized with an anti-membrane antigen (prostate-specific) antibody was

injected into naked mice. Antigen-antibody binding, the retention effect, and the permeability of vascular tumours were the initial causes of conjugation. The inherent vascular permeability of cancer tissue lacking lymphatic drainage protects against this impact. Quantum dots in polyethelynglycol were used without antibody conjugation to study the influence and permeability on a mouse model of cancer induction. However, it has been discovered that this approach is less effective than active probe targeting (Suk *et al.*, 2016).

Application in disease identification

Nanotechnology has shown promise in locating SNPs using a variety of methods, such as partial aggregation upon detecting target DNA. Additionally, signal amplification can only be accomplished through optical nanocarriers, which can then be enhanced utilizing hybrid techniques, such as combining nanotechnology and sensing techniques. Graphene, Au, Si, Ag and quantum-based nanostructures have all been extensively researched in the past for use in diagnostic applications (Mukhtar *et al.*, 2021).

Application in molecular imaging

Rapid developments in nanotechnology promise fundamental advancements in the identification and treatment of neuroinflammatory diseases. For in vivo molecular magnetic resonance imaging (mMRI) of cerebrovascular endothelial targets like vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and the glycoprotein receptor GP IIb/IIIa expressed on activated platelets, a variety of iron oxide nano- and microparticle agents have been developed. Nanoparticle imaging probes have also been successful in targeting molecular markers of glioma cells, such as matrix metalloproteinase-2 (MMP-2), and markers for brain tumour angiogenesis, such as alpha (v) beta (3) integrin (v-3) (Baeten *et al.*, 2008).

Application in photodynamic therapy

Human cell anti-carcinoembryonic antibodies were used to attach LoVo to a TiO₂ nanoparticle. When the conduction band of TiO₂ is exposed to UV light, one electron in the valance bond becomes excited, which leads to the creation of two photoinduced electrons and holes (Arruebo *et al.*, 2009). When exposed to UV light, the production of reactive oxygen species causes the presence of holes and photoinduced electrons to have redox (reduction, oxidation) reaction characteristics, which results in the demise of malignant cells (Fujita *et al.*, 2007). These antibodies are used to label with (FITC) Fluorescein isothiocyanate and then analyzed in confocal laser microscopy to increase optical detection. To hasten the incorporation of conjugated nanoparticles in cancer cells, the electroporation approach is used. The in-vitro investigation demonstrates that most malignant cells were photo-killed in about 90 minutes. Using gold-coated silica nanoparticles, photodynamic treatment is applied to external tumours existing in the body. This causes irreversible heat destruction to malignant cells present in the human breast when exposed to NMR rays in an in vitro setting (Aigner, 2007).

Application in cancer treatment

The greatest cause of death worldwide is cancer. The most enigmatic and complex disease, cancer is thought to be a multi-stage carcinogenesis process involving many physiological cell processes, including cell signaling and death. Numerous advancements have been achieved in conventional cancer therapies like radiotherapy and chemotherapy, which don't seem to be working very well. Since it is hampered by various drawbacks including chemoresistance (Russi *et al.*, 2019). Two crucial aspects of the development of nanomaterials for cancer therapy are the ability to identify tumours and reach the target tumour site without harming healthy cells. Numerous nanoparticles show

promise in their capacity to recognize very sensitive and specific cancer cells via various ways. These nanoparticles stand out from other cancer treatments thanks to their distinctive qualities (Misra *et al.*, 2010). The three most often utilized therapeutic nanoformulations for cancer chemotherapy currently offered by the FDA are Doxil, Daunorubicin (Daunoxome), and Abraxane. Paclitaxel is delivered by the albumin-bound nanoparticle Abraxane (Abraxis), and doxorubicin is delivered by the liposome-mediated drug delivery system Doxil. These two nanoformulations are frequently utilized to treat metastatic breast cancer. Targeted liposomes with folate receptors have been proven to successfully deliver doxorubicin in vivo and to overcome multidrug resistance in cancer cell lines (LaRocque *et al.*, 2009). A crucial aspect of cancer therapy is the interaction between chemotherapy and antiangiogenic drugs. According to Sengupta *et al.*, tumour malfunction in the blood arteries may reduce the delivery of chemotherapeutic medications and boost the production of drug resistance genes. Using nanoparticles with two layers, a drug delivery system was created. The first layer is a core made of poly lactic-co-glycolic acid (PLGA), which is coupled with doxorubicin and bound to a PEG and combretastatin conjugated liposome. Combretastatin is an anti-angiogenic medication, whereas doxorubicin is a cancer treatment agent. This nanoparticle was easily absorbed by the tumour when it was intravenously injected into mice with tumor-mediated (carcinoma or cell-derived melanoma) disease. They significantly slowed the growth of tumours. In mice models of melanoma and lung carcinoma, this nanoparticle formulation has demonstrated effectiveness, and similar efficacy in human models is anticipated (Sengupta *et al.*, 2005). Theranostics is a biomedical technique that integrates diagnosis and treatment in a single step. The primary components of medical care,

such as diagnosis and therapy, will be combined with the aid of theranostics to speed up, simplify, and improve the effectiveness of treatment. To enable non-invasive and efficient cancer treatment, biocompatible nanoparticles are being created as cancer screening agents. Shim et al. have developed a collective cancer diagnosis and therapy (theranostics). They investigated the possibility of combined stimulation using tiny gold nanoparticles encapsulating Si RNA stimulation-enhanced gene silencing and stimulus-responsive optical imaging (Singh *et al.*, 2011).

Risks connected to nanotechnology

An expanding area of study, nanotechnology has a variety of uses in fields such as medicine, health care, and human welfare. Along with these uses, worries about the potentially harmful effects of these nanoparticles caught the attention of many researchers, and various studies were conducted to determine their toxicity. These studies' findings show that the following characteristics of nanoparticles may cause those particles' hazardous effects.

Smaller size of the nanoparticle

These nanoparticles are reduced to their most attractive forms to maximize efficiency, but at this small diameter, they are practically impossible to process further, and if they ingest undesirable cells or tissues, their concentration can have hazardous effects.

Different form could have hazardous effects

According to nanoparticle characterization, different shapes of the same nanoparticles can have dangerous effects on the same target. For example, rod-shaped ZnO nanoparticles are more toxic to the lungs' epithelium than spherical ZnO nanoparticles.

Lung injury

Inhaling nanoparticles in any form causes humans to accumulate in the mucosal layers of the epidermis, pass through the lungs,

brain, liver, and spleen, and cause toxic effects on physiology. For instance, TiO₂ nanoparticles exposed to a rat model system suggested that the nanoforms of these compounds are relatively more toxic than the bulk material and cause pulmonary inflammations (Smijs and Pavel 2011).

The drawbacks of nanomedicine

There is virtually little research on the entirely new field of nanomedicine. To be on the safe side, we still want to see the practical application of nanomedicine; we should note that it is always in the experimental stage. We are unable to state with certainty what the health dangers of nanotechnology are due to a lack of knowledge regarding the drawbacks of nanomedicine. Nevertheless, we can accept the truth that nanoparticles can enter our bodies in a variety of ways, which may cause considerable worry. Although nanomedicine has numerous uses and many benefits, it cannot be said to be flawless. The fact that the size range is drastically reduced as they move from micro- to nanoparticles provides a reasonably consistent justification for this evaluation.

The mononuclear phagocytic system (MPS) in the organs captures nanoparticles. For nanostructures intended for pharmacological activity in another portion of the body, the same characteristic might have proven problematic. The chemical reactivity of nanoparticles is amplified by their increased surface area, which creates essential instability in their behavior under various conditions and their capacity to pass the plasma membrane and enter cells. Reactive oxygen species (ROS) are created when the chemical reactivity of nanoparticles is increased. ROS can directly result in undesirable consequences by causing oxidative stress, inflammation, and damage to DNA, proteins, and membranes. The fundamental disadvantage of nanomedicine is that apart from their size, nanoparticles do not share any characteristics. Therefore, each

particle needs to be evaluated separately.

When nanoparticles are injected into the body, they pass through cell membranes, enter capillaries, travel from the injection site to other areas of the body, and then strangely enter the BBB. Effects that have not been seen with traditional treatment could be brought on by nanoparticles. such as harm to different cellular components, like the mitochondria or nucleus. Additionally, they might trigger blood clotting processes and improve platelet aggregation. Despite being designed to decrease systemic adverse drug effects, carrier systems can be hazardous in and of themselves.

Outlook for the future

At a compound annual growth rate (CAGR) of 13.5%, the size of the worldwide nanomedicine market is expected to rise from an estimated \$53 billion (about \$160 per person in the US) in 2009 to more than \$100 billion (about \$310 per person in the US) in 2014. The potential for developing a system that is more effective for the creation of nanoproducts is constrained. It is essential to comprehend how nanomaterials are distributed within the body. This is related to a second limitation that calls for imaging techniques to track the biodistribution of nanomedicine over time. The safety of nanomedicine has not yet been fully established, despite rigorous standards and ongoing identification of potential benefits. Nanotechnology is anticipated to be used in diagnostics, molecular research methods, and instruments as it advances concurrently in other domains.

Although instead than spending money on novel and cutting-edge nanotechnology applications, attention should be directed towards resolving current problems, such as ensuring that affordable and accessible diagnostic options are available for the underprivileged. The majority who require primary healthcare facilities will be served by it. To develop nanotechnology-based therapy options, additional research is required with a

stronger emphasis on transport processes, endocytosis, biological barriers, degradation pathways, and control over its potential harmful effects.

Conclusions

In this review, we covered various nanocarrier types used in drug delivery systems. The platform for drug delivery has been significantly enlarged using nanocarriers, whether with single-featured nanomaterials and/or structures or in a hybrid approach. Nanoparticle applications are simple and effective. Though, nothing is known about the toxicity of nanomaterials. One of the biggest obstacles in the realm of health and medicine is the variety of nanoparticles and the number of contraindications. Despite this, numerous crucial studies were conducted in medicine to address the fundamentally distinct features of nanoparticles. In this article, we've discussed the current state of several nanoparticle kinds and their diagnostic and therapeutic applications in treating a range of medical issues. Nanotechnology is an interdisciplinary field that spans a wide range of scientific disciplines, including biology, chemistry, electrical engineering, and materials science. Additionally, it is challenging for one researcher to become an expert in all subjects. Therefore, to ensure that developments can result in useful goods, nanotechnology developers should cooperate.

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Plant Genome Construction of Molecular Maps, Functional Genomics and Comparative Genomics

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Abstract

Mapping genome of an organism is an important tool to provide a guide for the sequencing experiments by showing the exact positions of genes and other distinctive features in the chromosomal DNA. Whole genome sequencing of eukaryotic genomes is greatly facilitated by high-density genome maps. The high-density maps serve as foundation during organizing and assembling the nucleotide sequences of genomes. Distinctive features on the genome map are used as landmarks to aid assembly of master sequence from huge number of short sequences obtained from genomic library. First step for manipulation of genes for various applications is cloning that relies on mapping close to a convenient marker. A complete physical genetic map of genome is necessary for comparative genomics studies with other genomes. High resolution comparative physical maps will reveal regions of collinearity and rearrangement and will have important implications for phylogenetic studies and genome evolution.

Keywords: Whole Genome Sequencing, High-Density Genome Maps, Repetitive DNA, Physical Maps, Marker

Introduction

Since the early 1950s, the development of genetics has been exponential with several landmarks, including determination of DNA as the genetic material in 1944, discovery of the double-helix structure of DNA in 1953, the development of electrophoretic assays of isozymes and a wide range of molecular markers that reveals differences at the DNA level. A genome map, like any other map, defines the relative positions of features that are of interest, or which can serve as reference points for navigation. The features that are located on a genome map are collectively referred to as markers and can include both genes and noncoding sequences.

Many methods exist for making genome maps, differing in the types of markers that can be mapped and in their accuracy. Only one technique, fluorescence in situ hybridization, allows the position of markers in the genome to be observed directly. In all other methods, a less direct approach is necessary. A common theme in these indirect methods is that some means is first used to isolate a portion of the genome from the remainder and to identify the markers present in that portion. The markers found can then be assumed to be physically linked in the genome.

Good maps are invaluable in many aspects of genome analysis. Genetic linkage maps although often of low-resolution, are unique in that they allow the position of a gene to be determined even when nothing about the gene is known except for its phenotypic effect

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Comparison of genome maps of different species can reveal conservation of the order of genes over large regions of chromosomes. Such comparative mapping can give insight into genome evolution, and can provide clues as to the location of a gene in one species if its position in the genome of another is known. Higher resolution maps can direct the early stages of genome sequencing or, if accurate enough, can help to guide the assembly of the complete sequence and ensure its long-range continuity and freedom from gaps.

The convention is to divide genome mapping methods into two categories

1. **Genetic mapping-** Genetic mapping is based on the use of genetic techniques to construct maps showing the positions of genes and other sequence features on a genome. Genetic techniques include cross-breeding experiments or, in the case of humans, the examination of family histories.
2. **Physical mapping-** uses molecular biology techniques to examine DNA molecules directly in order to construct maps showing the positions of sequence features, including genes.

Genetic Mapping

The early geneticists understood that recombination between genes take place by an exchange of segments between homologous chromosomes in the process now called crossing over. Each crossing-over is manifested physically as a chiasma, or cross-shaped configuration, between homologous chromosomes; chiasmata are observed in prophase I of meiosis. Each chiasma results from the breaking and rejoining of chromatids during synapsis, with the result that there is an exchange of corresponding segments between them. A genetic map is also called a linkage map or a chromosome map. The linkage of the genes in a chromosome can be represented in the form of a genetic map, which shows the linear order of the genes along the chromosome with the distances between adjacent genes

proportional to the frequency of recombination between them. Recombination frequency between two loci is defined as the ratio of the number of recombined gametes to the total number of gametes produced. Recombination fraction, can be denoted by r , however, has a domain of $0 \leq r \leq 0.5$, with $r = 0$ indicating perfect linkage and $r = 0.5$ meaning complete independence of the two loci.

Principles of genetic mapping

Genetic mapping is based on the principle that genes (markers or loci) segregate via chromosome recombination during meiosis (i.e., sexual reproduction), thus allowing their analysis in the progeny. During meiosis, chromosomes assort randomly into gametes, such that the segregation of alleles of one gene is independent of alleles of another gene. This is stated in Mendel's second law and is known as the law of independent assortment. Genes that are closer together or tightly-linked will be transmitted together from parent to progeny more frequently than those genes located far apart.

Requirements for genetic mapping

Mapping population- The first step in producing a mapping population is selecting two genetically divergent parents, which show clear genetic differences for one or more traits of interest. They should be genetically divergent enough to exhibit sufficient polymorphism and at the same time they should not be too genetically distant so as to cause sterility of the progenies

Show very high levels of segregation distortion during linkage analysis.

In self-pollinating species, mapping populations originate from parents that are both highly homozygous (inbred). In cross pollinating (outcrossing) species, the situation is more complicated since most of these species do not tolerate inbreeding. Selection of populations is critical to successful linkage mapping. F₂ populations are developed by selfing F₁ hybrids derived by crossing the two

parents while BC population is produced by crossing F1 back into one of the parents. Recombinant Inbred Lines (RILs) are developed by single-seed selections from individual plants of an F2 population; such selections continue for six to eight generations.

A Double Haploid (DH) population is produced by doubling the gametes of F1 or F2 population. Plants will be regenerated using tissue culture techniques after induction of chromosome doubling from pollen grains or haploid embryos resulting from species crosses. RILs, NILs and DHs are permanent populations because they are homozygous or 'true-breeding' lines that can be multiplied and reproduced without genetic change occurring.

Selection of molecular markers for mapping

In traditional plant breeding, genetic diversity was usually diagnosed through observational selection. But now, this work is determined at molecular level based on DNA changes and their effects on the phenotype. Once DNA was extracted from plant, changes in the samples are determined using PCR or hybridization and subsequent agarose or acrylamide gel electrophoresis to recognize different molecules based on their size, chemical composition or charges. Genetic markers are biological compounds which can be determined by allelic variations and can be used as experimental probes or labels to track an individual, tissue, cell, nucleus, chromosomes or genes. In classical genetics, genetic polymorphism represents allele diversity. While in modern genetics, genetic polymorphism is the relative difference in genetic locus of the genome. DNA markers involve several sets of markers and divided into two main categories: PCR-based molecular markers and hybridization-based molecular markers. Restriction Fragment Length Polymorphism (RFLP) is a hybridization-based molecular marker while

the other markers like microsatellites or Simple Sequence Repeats (SSRs), Expressed Sequence Tags (ESTs), Cleaved Amplified Polymorphic Sequence (CAPS), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphisms (AFLPs), Inter Simple Sequence Repeat (ISSR), diversity arrays technology (DArT), and Single Nucleotide Polymorphism (SNP) are all PCR-based [4] and have been used for map construction in several plants. The first large-scale efforts to produce genetic maps were performed mainly using RFLP markers, the best-known genetic markers at the time [21]. The major strength of RFLP markers includes codominant inheritance, good transferability between laboratories, locus-specificity that allows synteny (conserved order of genes between related organisms) studies, and high reproducibility.

Polymorphism screening and genotyping of the mapping population

The third step in the construction of a linkage map is to identify sufficient number of markers that reveal differences between parents. In general, cross-pollinating species possess higher levels of polymorphism compared to inbreeding species. Once sufficient numbers of polymorphic markers have been identified between parents, they must be used to genotype the entire mapping population. A genetic map is as good as the data that were used to construct it. Therefore, the data must be critically checked for all possible errors, such as typographical error, missing data, genotype coding error, order of genotypes along all loci, etc. It is absolutely essential that the order of the individuals is identical over all loci in the data file. The most likely effect of a typing error is to introduce a double recombination, so that an individual's genotype at three neighboring loci might change from a true genotype of ABA to ABB.

Linkage analyses and map construction

Linkage analyses and mapping are computerized. Several computer packages are

presently available for genetic linkage mapping but the most widely used are Join Map, Map marker/exp, Linkage and Map Manager QTX. Join Map is a commercial program while all others are freely available from the internet. The basic principles in map construction are basically the same for the different statistical programs, and the major steps in linkage analyses are described using Join Map as an example.

Genes were the first markers to be used

The first genetic maps, constructed in the early decades of the twentieth century for organisms such as the fruit fly, used genes as markers. To be useful in genetic analysis, a gene must exist in at least two forms, or alleles, each specifying a different phenotype, an example being tall or short stems in the pea plants originally studied by Gregor Mendel. In starting, the only genes that could be studied were those specifying phenotypes that were distinguishable by visual examination. So, for example, the first fruit-fly maps showed the positions of genes for body color, eye color, wing shape, and suchlike, all of these phenotypes being visible simply by looking at the flies with a low-power microscope or the naked eye. This approach was fine in the early days but geneticists soon realized that there were only a limited number of visual phenotypes whose inheritance could be studied, and in many cases their analysis was complicated because a single phenotype could be affected by more than one gene.

For humans it is possible to use visual characteristics but, since the 1920s, studies of human genetic variation have been based largely on biochemical phenotypes that can be scored by blood typing. These phenotypes include not only the standard blood groups, such as the ABO series, but also variants of blood serum proteins and of immunological proteins such as the human leukocyte antigens (the HLA system). A big advantage of these markers is that many of the relevant

genes have multiple alleles. For example, the gene called HLA-DRB1 has at least 290 alleles and HLA B has over 400. This is relevant because of the way in which gene mapping is carried out with humans.

DNA markers for genetic mapping

Genes are very useful markers but they are by no means ideal. One problem, especially with larger genomes such as those of vertebrates and flowering plants, is that a map based entirely on genes is not very detailed. This would be true even if every gene could be mapped because in most eukaryotic genomes the genes are widely spaced out with large gaps between them.

Mapped features that are not genes are called DNA markers. As with gene markers, a DNA marker must have at least two alleles to be useful. There are three types of DNA sequence feature that satisfy this requirement: restriction fragment length polymorphisms (RFLPs), simple sequence length polymorphisms (SSLPs), and single nucleotide polymorphisms (SNPs).

Cloned DNA fragments can be mapped, and therefore act as markers, even when little or nothing is known of their sequence. Techniques for mapping such cloned fragments include physical mapping and fluorescence in situ hybridization.

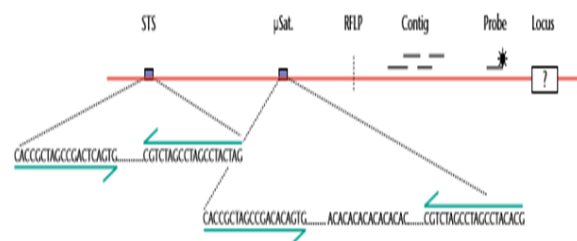


Fig-1 cDNA Strand

Techniques for mapping such cloned fragments include physical mapping and fluorescence in situ hybridization. If the anonymous cloned fragment contains a restriction site whose location differs from one allele to the next then genetic linkage mapping can be used.

Genetic linkage mapping

In organisms that reproduce sexually, meiosis breaks the parental chromosomes at random, recombines the fragments and segregates the shuffled chromosomes into gametes, and thence to offspring. If two markers, A and B, lie close together on a chromosome, then it is unlikely that a meiotic break will occur between them. Hence, A and B will seldom recombine, and will usually segregate together to the same gametes and hence to the same offspring. In contrast, if markers B and Z lie at opposite ends of a chromosome, it is much more likely that a meiotic break will fall between them, whereupon they may segregate independently. Markers lying on different chromosomes will also segregate independently. There is one complicating factor, however. Meiotic recombination occurs by an exchange of homologous segments between chromosomes. Hence, some way must be found to distinguish between the copies of the markers on each parental chromosome, so that the pattern of segregation can be followed.

The analysis of linkage data to produce maps is far from simple and several mathematical methods exist. The first step is normally to estimate the distances between all possible pairs of markers in the study. In the ideal case, this can be done by finding the proportion of meiotic events in which recombination occurs between the two markers.

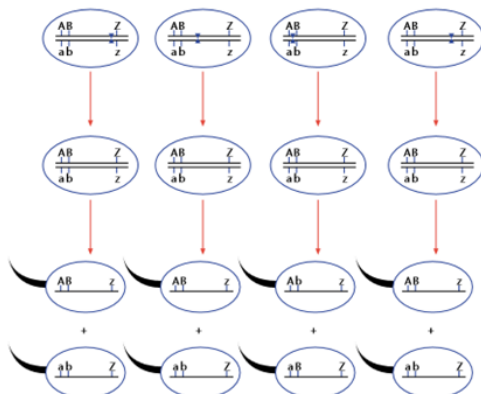


Fig-2 Meiotic division of Recombinant Strands

Genetic linkage mapping is a powerful method, but suffers from some limitations. In particular, only polymorphic markers can be mapped, and the resolution that can be achieved is limited. In humans, recombination occurs only every 100Mb or so on average, limiting the resolution to around 1Mb if a few hundred individuals are genotyped. Markers situated on either side of such 'recombination hotspots' will recombine more often than would otherwise be the case, leading to an overestimation of the distance between them.

Radiation hybrid mapping

In the mid-1970s, Stephen Goss and Henry Harris discovered Radiation hybrid mapping. If cultured human cells were subjected to high dose of radiation and then fused to unirradiated hamster cells, hybrids would be produced. Such 'radiation hybrids' would initially contain many fragments of human chromosomes and hence would express many human proteins. DNA is prepared from each cell line in the panel, and is screened to determine which donor-derived genome markers it contains. The markers are usually STSs (including ESTs), which can easily be detected using PCR. Radiation hybrid (or RH) mapping is closely analogous to genetic linkage mapping. Instead of recombination, it is the radiation that randomly breaks the chromosomes; hybrid cell formation replaces meiotic segregation as a way to shuffle and segregate these fragments.

HAPPY mapping

RH mapping overcomes many of the limitations of genetic linkage mapping. In HAPPY mapping, DNA is first prepared from the species whose genome is to be mapped. To prevent unwanted mechanical breakage to the naked, fragile DNA molecules, this is normally done by embedding the living cells in agarose gel, and then treating them with a solution of detergent and proteases. These diffuse in to the agarose, lysing the cells and stripping away proteins and other cellular debris, which

diffuse out of the agarose; the long molecules of chromosomal DNA remain trapped and protected within the agarose. The DNA is now broken randomly, either by melting the agarose and mechanically shearing the DNA solution, or by treating the embedded DNA with gamma radiation. The result is a pool of random DNA fragments, whose average size depends upon the violence of the mechanical shearing or on the dose of radiation used. This broken DNA is then diluted to a very low concentration, and about one hundred samples are dispensed into separate tubes. The complete set of samples is referred to as a 'mapping panel'. Most importantly, each sample is exceedingly small, containing less than one genome's worth of DNA fragments.

The *in vitro* nature of HAPPY mapping eliminates some of the potential limitations of RH maps. This aggregation of DNA fragments is done by simple dilution, and is therefore not affected by centromeres, telomeres or other biologically active sequences. Since there is no 'host genome', the samples can be screened for markers without interference from homologous host sequences. As a result, HAPPY maps are relatively immune to artefacts.

Physical mapping

Physical mapping involves finding a contiguous series (or 'contig') of cloned DNA fragments which contain overlapping portions of the genome. The overlaps define the positions of the clones relative to one another. The starting point for physical mapping is a library of cloned genomic fragments, normally prepared by either random mechanical breakage or partial restriction digestion of genomic DNA. The fragments are usually cloned in bacterial hosts (normally *Escherichia coli*), using bacteriophage, cosmid, plasmid or other vector systems. For physical mapping of large genomes, it is desirable to use clones containing large inserts, such as P1 artificial chromosomes (PACs) or bacterial artificial chromosomes (BACs) which can carry inserts in excess of 100kb. Even larger

fragments (over 1Mb) can be cloned in yeast (*Saccharomyces cerevisiae*) using yeast artificial chromosome (YAC) vectors; however, such clones are often unstable, undergoing deletions or internal rearrangements.

Once the library is established, overlapping clones can be identified using several different approaches. In STS content mapping, the library is screened to identify all clones that contain a specific STS marker. Screening can be done either by PCR using the appropriate primers (in which case only clones containing the STS will give a PCR product); or by arraying the clones on a nylon membrane, lysing them to expose their DNA, and probing the membrane with labelled DNA corresponding to the STS in question. In either case, all clones containing a given STS marker are identified. If two clones are found to contain the same STS, then they must represent overlapping parts of the genome. If one clone contains two different STSs, then those two STSs must be consecutive in the genome. If enough clones are characterized for enough STSs, then a contiguous overlapping path of clones is defined. A second common approach to physical mapping is by the use of restriction fingerprinting. In this case, each member of the clone library is digested in turn with one or more restriction enzymes, and the sizes of the resulting fragments are measured by gel electrophoresis. If two clones are found that have several fragment sizes in common, then they must represent overlapping parts of the genome, with the shared fragments coming from the region of overlap. Again, if enough clones are fingerprinted then the hope is to find a contiguous series of overlapping clones. If one clone contains two different STSs, then those two STSs must be consecutive in the genome. If enough clones are characterized for enough STSs, then a contiguous overlapping path of clones is defined. A second common approach to physical mapping is by the use of restriction fingerprinting. In this case, each member of the clone library is digested in turn with one or

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Fluorescence in situ hybridization

In contrast, fluorescence in situ hybridization (FISH) is an elegantly direct way of observing the physical arrangement of markers along the chromosomes. Fluorescently labelled probes are hybridized to metaphase chromosomes on a glass slide, and their position observed using a fluorescence microscope. The first requirement for conventional FISH is a population of cells in which a high proportion are in metaphase, the stage in the cell cycle where the chromosomes are condensed as distinct bodies. This is typically achieved by culturing lymphocytes or fibroblasts in the presence of a mitogen such as phytohemagglutinin, then treating them with calcemic to block the cell cycle at metaphase. Treatment with bromodeoxyuridine (which is incorporated into the replicating chromosomes) and ethidium bromide improves the morphology of the chromosomes for FISH. The cells are swollen in a hypotonic salt solution and stabilized with a fixative. Drops of the cell suspension are placed on glass slides, to which the chromosomes adhere. Their DNA is denatured, and is then ready to be hybridized to the probe. by the incorporation of a hapten such as biotin or digoxigenin. They are denatured, allowed to hybridize to the corresponding sequences of the meta phase

chromosomes, and the surplus washed away. Nonspecific hybridization is normally suppressed by using an unlabeled competitor such as total genomic DNA. To detect the probes, the slide is incubated with fluorescently labelled proteins which bind to the hapten in the probe and render it visible in the fluorescence microscope. At the same time, the chromosomes themselves can be visualized by a fluorescent counterstain, such as DAPI of a different colour to the fluorophore used to detect the probe.

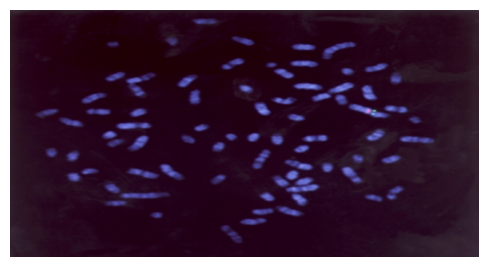


Fig-3 Color fluorescence in-situ hybridization

The resulting images clearly reveal the location of the bound probe, typically as two adjacent spots corresponding to the two chromatids. The characteristic banding pattern of the chromosomes gives the absolute location of the probe in the genome. By using different combinations of haptens and fluorophores, two or more probes may be hybridized and detected simultaneously. Such multi colour FISH allows the order and spacing of the probes to be determined more accurately than is possible with independent, single-probe experiments.

Comparative genomics

Comparative genomics uses evolutionary theory to glean insights into the function of genomic DNA sequences. By comparing DNA and protein sequences between species or among populations within a species, we can estimate the rates at which various sequences have evolved and infer chromosomal rearrangements, duplications and deletions. This evolutionary reconstruction can then be used to predict functional properties of the DNA. Sequences that are needed for functions common to the species being compared are

expected to change little over evolutionary time, whereas sequences that confer an adaptive advantage when altered are expected to have greater divergence between species. Furthermore, sequence comparisons can help in predicting what role is played by a particular functional region, e.g., coding for a protein or regulating the level of expression of a gene.

There are two drivers for comparative genetics. One is a desire to have a much more detailed understanding of the process of evolution at the gross level (the origin of the major classes of organism) and at a local level. The second driver is the need to translate DNA sequence data into proteins of known function. The rationale here is that DNA sequences encoding important cellular functions are more likely to be conserved between species than sequences encoding dispensable functions or non-coding sequences. More recently, Bofelli *et al.* (2004) have shown that by comparing genomes that are very distantly related, e.g., mammals and fish, it is possible to identify conserved sequences that, presumably, have a significant function.

Orthologs and paralogs are key steps in gene evolution

In order to compare genome organization in different organisms it is necessary to distinguish between orthologs and paralogs.

Orthologs are homologous genes in different organisms that encode proteins with the same function and which have evolved by direct vertical descent.

Paralogs are homologous genes within an organism encoding proteins with related but non-identical functions.

The orthologs evolve simply by the gradual accumulation of mutations, whereas paralogs arise by gene duplication followed by mutation accumulation. Good examples of paralogs are the protein superfamilies. There are many biochemical activities that are common to most or all living organisms, e.g., the citric acid cycle, the generation of ATP, the synthesis of nucleotides, DNA replication, etc.

It might be thought that in each case the key proteins would be orthologs. Indeed, “universal protein families” shared by all archaea, eubacteria, and eukaryotes have been described (Kyrpides *et al.* 2023).

Protein evolution occurs by exon shuffling

Analysis of protein sequences and three-dimensional structures has revealed that many proteins are composed of discrete domains. These so-called mosaic proteins are particularly abundant in the metazoan. The majority of mosaic proteins are extracellular or constitute the extracellular parts of membrane bound proteins and thus they may have played an important part in the evolution of multicellularity. The individual domains of a mosaic protein are often involved in specific functions which contribute to its overall activity. These domains are evolutionarily mobile which means that they have spread during evolution and now occur in otherwise unrelated proteins.

Study of the genes that encode mosaic proteins reveals a strong correlation between domain organization and intron–exon structure (Kolkman & Stemmer 2001); i.e., each domain tends to be encoded by one or a combination of exons and new combinations of exons are created by recombination within the intervening sequences. This process yields rearranged genes with altered function and is known as exon shuffling. Because the average intron is much longer than the average exon and the recombination frequency is proportional to DNA length, the vast majority of crossovers occur in non-coding sequences. The large number of transposable elements and repetitive sequences in introns will facilitate exon shuffling by promoting mismatching and recombination of non-homologous genes.

Comparative genomics of bacteria

By mid-2004 the website of the National Center for Biotechnology Information listed 173 bacteria (19 Archaea and 154 Eubacteria) whose

genomes had been sequenced. Simple analysis of the sequence data reveals two features of note. First, the genome sizes vary from 0.49 Mb (*Nanoarchaeum equitans*) to 9.1 Mb.

When the different genomes are arranged in size order (Fig. 18.1) some interesting features emerge. First, the archaebacteria exhibit a very much smaller range of genome sizes. This could be an artifact of the small number of genomes examined but more probably reflects the fact that most of them occupy a specialized environment and have little need for metabolic diversity. The exception is *Methanosarcina acetivorans*. This bacterium is known to thrive in a broad range of environments and at 5.8 Mb has the largest archaeal genome.

Larger microbial genomes have more paralogs than smaller genomes

Comparison of the *P. aeruginosa* (6.3 Mb) and *E. coli* (4.5 Mb) genomes indicates that the large genome of *P. aeruginosa* is the result of greater genetic complexity rather than differences in genome organization. Distributions of open-reading frame (ORF) sizes and inter-ORF spacings are nearly identical in the two genomes. If the larger genome of *P. aeruginosa* arose by recent gene duplication one would expect it to have a similar number of paralogous groups compared to the other large bacterial genomes and a larger number of ORFs in each group. In fact, the number of ORFs in the paralogous groups in *Pseudomonas* is similar to the other genomes.

Comparative genomics of eukaryotes

The minimal eukaryotic genome is smaller than many bacterial genomes

The smallest eukaryotic genome that has been sequenced is that of the obligate intracellular parasite *Encephalitozoon cuniculi*. This has a genome size of only 2.9 Mb although its close relative *E. intestinalis* may have a genome that is even smaller 2.3 mb. Genome compaction in these organisms is achieved by a reduction in the length of intergenic spacers

and a shortness of most putative proteins relative to their orthologs in other eukaryotes. Even so, *E. cuniculi* has approximately 2000 ORFs, which is 7–8 times the number in the minimal bacterial genome.

Comparative genomics can be used to identify genes and regulatory elements

A complete genome sequence can be very difficult and identifying regulatory elements can be even harder still. A powerful method for finding functional elements such as genes and regulatory regions is to align orthologous genomic sequences from different species and search out regions of sequence conservation. The rationale for this approach is that mutations in functional DNA will be deleterious and thus counter-selected thereby resulting in a reduced rate of evolution of functional elements. An example of a comparative analysis with a narrow scope is the genomic comparison of *S. cerevisiae* with three other species of *Saccharomyces*.

The gene analysis resulted in a major revision of the *S. cerevisiae* gene catalog that affected 15% of all genes, reduced the total count by about 500 genes, and identified 43 new small ORFs (50–99 amino acids). A comparative analysis with a more divergent scope is that between the pufferfish (*Fugu rubripes*) and human genomes (Aparicio *et al.* 2002).

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A Review on Recombinant DNA, Gene Cloning, Molecular Probes, Chimeric DNA and Gene Libraries

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Abstract

The recombinant DNA technology was only an idea that desirable qualities in live bodies may be improved by manipulating the expression of target genes. However, in recent years, this field has made significant contributions to human progress. This technology allows for the safe, affordable, and sufficient production of critical proteins for health problems and dietary purposes. This technique has broad applications and the ability to improve vital areas of life, such as health, food resources, and resistance to diverse unfavorable environmental effects. Genetically engineered plants, particularly in agriculture, have boosted resistance to hazardous agents, increased product yield, and demonstrated increased adaptation for better survival. Furthermore, recombinant medications are increasingly being used with confidence, with commercial approvals coming quickly. Techniques such as recombinant DNA technology, gene therapy, and genetic changes are also commonly employed in bioremediation and the treatment of serious diseases. Because of the enormous advancement and wide range of applications in the field of recombinant DNA technology,

this review article focuses primarily on its significance and potential applications in daily life. Gene cloning, molecular probes, and chimeric DNA are examples of tools. Genomic libraries will be employed in the future to create recombinant DNA for crop enhancement.

Keywords: Recombinant, cloning, probe, gene libraries

Introduction

Recombinant DNA technology entails changing genetic material outside of an organism to produce living things or their products with improved and desired traits. Using the right vector, this approach entails inserting DNA fragments from a range of sources that have the desired gene sequence. The manipulation of an organism's genome can take place either by adding one or more new genes and regulatory elements, or by recombining existing genes and regulatory elements to reduce or prevent the expression of endogenous genes. Recombinant DNA techniques to promote agricultural and pharmaceutical innovations took longer than predicted due to unforeseen challenges and hurdles to attain the desired outcomes. Researchers frequently utilise gene cloning in molecular biology labs to make duplicates of a certain gene for later uses, such as sequencing, mutagenesis, genotyping, or heterologous protein expression. A DNA fragment of interest is transferred from one creature to a self-replicating genetic component, like a

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bacterial plasmid, in the classic method of gene cloning. Today, this method is frequently employed to isolate lengthy or understudied genes and protein expression. Polymerase chain reaction (PCR) is a relatively contemporary method for amplifying a gene of interest. The time saved in producing a pure sample of the target gene is the benefit of using PCR over conventional gene cloning, as mentioned above. However, PCR-based gene isolation can only amplify specific genes with specific sequences. Probes are segments of DNA or RNA to which a label has been applied. The label enables us to see where the DNA bonds, whether it is in an isolated piece of pure DNA, a chromosome, or both. To track probes, we mark them with various chemicals. It can be chemically attached to a probe using radioactive or fluorescent material. A gene library is a collection of gene clones that serve as an organism's genetic blueprint.

Recombinant DNA

Recombinant DNA molecules are DNA molecules produced using genetic recombination techniques used in laboratories (such as molecular cloning), which combine genetic material from several sources to produce sequences not normally seen in the genome. A DNA strand that has been formed by joining two or more fragments from various sources is known as recombinant DNA. The fact that all DNA molecules have the same chemical structure and only differ in nucleotide sequence makes recombinant DNA conceivable. Because they can be created from DNA from two distinct species, much like the mythological chimera, recombinant DNA molecules are frequently referred to as chimeric DNA. Palindromic sequences are used in rDNA technology to create sticky and blunt ends. Chimeric DNA, also known as recombinant DNA or hybrid DNA, is a molecule made by the combination of the DNA of two distinct animals.

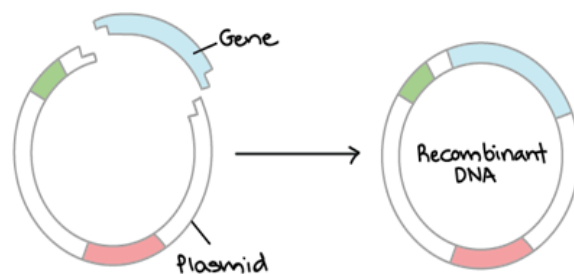


Fig. 1: construction of recombinant DNA

How is Recombinant DNA made?

Recombinant DNA can be produced in three main ways. They are Non-Bacterial Transformation, Phage Introduction, and Transformation. Each is discussed in further detail below.

(A) Transformation

The selection of a DNA fragment to be inserted into a vector is the first stage in the transformation process. The second step entails using a restriction enzyme to cut that segment of DNA, followed by DNA Ligase to ligate the DNA insert into the vector. The insert has a selectable marker that makes it possible to recognise recombinant molecules. It is common practise to utilise an antibiotic marker so that when a host cell is exposed to a specific antibiotic, the host with the vector will survive because it is resistant.

Transformation is the process of inserting the vector into a host cell. *E. coli* is one illustration of a potential host cell. To accept the foreign DNA, the host cells need to be appropriately prepared.

Antibiotic resistance, colour changes, or any other trait that can distinguish changed hosts from untransformed hosts are all examples of selectable markers. Different vectors have various characteristics that make them appropriate for various applications. Size, a high copy number, and symmetrical cloning sites are a few possible characteristics.

(B) Non-Bacterial Transformation

This process is extremely comparable to Transformation, which was previously discussed. The main distinction between the two is that the non-bacterial does not use *E. coli* as the host. The DNA is microinjected right into the cell's nucleus, causing the transformation to take place. In biolistic, DNA-coated gold or tungsten particles or other high-velocity microprojectiles are fired at the host cells.

(C) Phage Introduction

Transfection, which is the same as transformation, is what happens during phage introduction, however phages are utilised in place of bacteria. The packing of a vector is done *in vitro*. This creates phage plaques containing recombinants using lambda or M13 phages. By utilising a variety of selection techniques, the recombinants that are produced can be recognised by distinctions between recombinants and non-recombinants.

How does rDNA work?

When the host cell produces protein from the recombinant genes, recombinant DNA functions. Without the addition of expression factors, the host will not manufacture a sizable amount of recombinant protein. The presence of signals that give the cell instructions for the transcription and translation of the gene is necessary for protein expression to occur. The promoter, the ribosome binding site, and the terminator are some of these signals. These signals are present in the expression vectors, where the foreign DNA is introduced. Signals are unique to each species. Since *E. coli* is unlikely to comprehend signals from human promoters and terminators, these signals in this scenario must be generated by *E. coli*.

Why is rDNA important?

Recombinant DNA has been more significant in recent years, and as hereditary illnesses become more common and agricultural land is

depleted, their significance will only increase in the twenty-first century. The areas where recombinant DNA will have an impact are listed below.

- Drought-resistant and heat tolerance
- Developed vaccine
- Prevent sickle cell anemia
- Production of clotting factors
- Insulin production
- Germline and somatic gene therapy
- Pharmaceuticals production

Gene Cloning

A gene of interest is found and copied (cloned) using all the DNA that has been taken out of the organism. This procedure is known as gene cloning. using gene cloning to duplicate and isolate a particular gene of interest. It is discussed how to transform bacteria with plasmids containing genes for antibiotic resistance in order to create gene libraries and how to choose bacteria colonies that have the desired gene. The newest area of study in biology is CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats). Endonuclease Cas9 performs the roles of genomic molecular scissors, and it is utilized to target a particular spot of the gene using an engineered single-stranded guide RNA (gRNA) with a specified sequence (Jameel *et al.*, 2023)

Steps: The basic steps in gene cloning are:

- Restriction enzymes are used to break down DNA taken from an organism known to carry the desired gene into fragments the size of genes.
- The same restriction enzyme is used to cleave bacterial plasmids.
- One test tube contains both the chopped plasmids and the gene-sized DNA. A gene-sized fragment of DNA and a plasmid frequently anneal to generate a recombinant plasmid (recombinant DNA).
- Electroporation or thermal shock is used to introduce the recombinant plasmids into the bacteria.

- The germs are dispersed across plates and given time to organize into colonies. All the colonies on all the plates are referred to as a gene library together.
- The colony harboring the gene of interest is found in the gene library by screening for one of three things: The protein encoded by the gene of interest; A DNA marker whose location has been identified close to the gene of interest; The DNA sequence of the gene of interest or a very comparable gene; Antibiotic resistance genes are naturally present in the bacterial plasmids used in gene cloning.

Library Construction

In addition to the extracted DNA, a plasmid, restriction enzymes, and a gene library must be created. Using restriction enzymes, the extracted DNA is split into fragments that are the size of genes to create a gene library. The DNA sequence is cut by these enzymes by severing the links between the nucleotides in a DNA strand after they have read the nucleotide sequence of the DNA and recognised particular sequences. The tiny DNA fragments are combined with bacteria plasmids that have also undergone the same restriction enzyme cutting in a test tube.

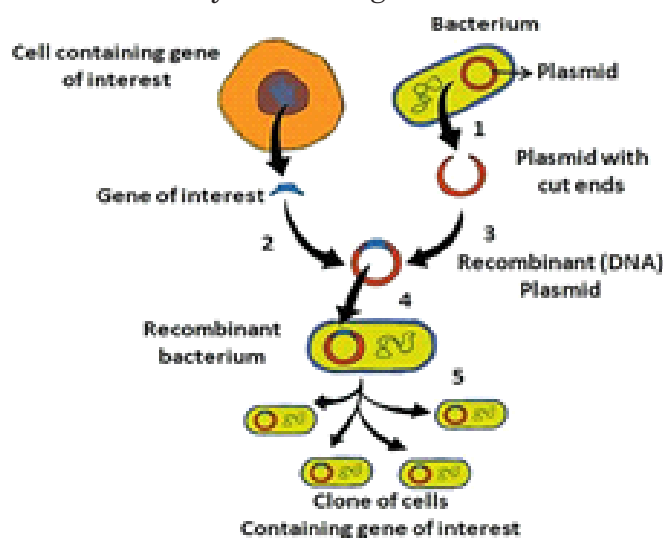


Fig.2: Gene cloning

(A) Plasmids

Small DNA circles called plasmids are present in bacterial cells naturally in addition to the bacteria's other DNA. They can be replicated numerous times in a single bacterial cell yet only comprise a small fraction of the hundreds of genes that make up a bacterium's genome. Recombinant DNA, also known as DNA in a "new combination," is created during the library creation when a portion of the enzyme-cut DNA combines with the cut plasmids.

(B) Cloning Vectors

A cloning vector is a DNA molecule that, when a foreign DNA molecule is integrated, has the ability to duplicate that foreign DNA molecule within itself to produce many clones of recombinant DNA. Plasmids and phages are two types of vectors that are utilised for cloning.

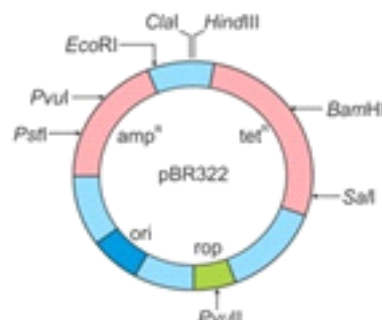


Fig. 3: *E. coli* vector pBR 322

CRISPR-Cas12a-mediated direct cloning

It is easier to find drugs based on natural products when biosynthetic gene clusters (BGCs) from microbial genomes are directly cloned. Here, we created CAT-FISHING (CRISPR/Cas12a mediated rapid direct biosynthetic gene cluster cloning), a quick yet effective in vitro platform for directly capturing large BGCs. This platform combines Cas12a and the sophisticated features of bacterial artificial chromosome library creation (Liang *et al.*, 2022)

Types of cloning vectors

1. **Plasmid:** A plasmid's size is 4361 base pairs, and its cloning range is 0.1 to 10 kb. While remaining isolated from *E. Coli*, the marker gene is ampicillin and tetracycline resistant. Example: PBR322
2. **Bacterial Artificial Chromosome (BAC):** The cloning limit for BAC is between 35 and 300 kb. Chloramphenicol and the lactose metabolising gene are the marker genes. It is created artificially and is a modification of f-plasmid. Example: pUvBBAC
3. **Yeast Artificial Chromosome (YAC):** The cloning vector is 100–1000 kb in size, and the YAC is 11400 bp in size. The marker resembles that found in yeast. It is artificial and contains an isolated *Saccharomyces cerevisiae* yeast centromere.
4. **Bacteriophage:** Its size is 48502 bp, of which 1/3 is not necessary. Only roughly 4-5 kbp of the donor DNA can be recombinant. Lambda genome is one instance.
5. **Cosmid:** The cloning limit for cosmids is between 30 and 50 kb, and their size is 7900 bp. Super COS1 is an illustration of it and has characteristics that are similar to both phase and plasmid.
6. **Human Artificial Chromosome:** This artificial chromosome is utilized to transfer human genes, and since it can contain a significant amount of DNA, cloning is not constrained by it.

Gene library: A gene library is a collection of gene clones that serves as an organism's genetic blueprint. A library in molecular biology is a collection of DNA fragments that have been molecularly cloned, stored, and spread throughout a population of microorganisms. There are two main gene libraries, which also include genomic and cDNA collections.

Application of gene library:

The origin of the original DNA pieces affects how gene libraries are used. Each DNA fragment in a gene library is typically individually inserted into a cloning vector before being introduced into a population of bacteria or yeast. This ensures that each

organism carries, on average, one construct. The DNA molecules found inside of organisms are copied and spread when the population of those organisms increases in culture.

Genomic libraries:

A genomic library is a collection of clones that includes every piece of an organism's genome. The size of the genome and the insert size both affect how many clones make up a genomic library.

Since every cell in the body has the same DNA, the genomic library is typically the same for tissues from various sources of an organism.

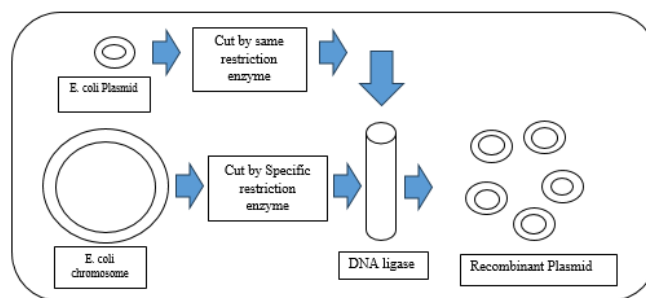


Fig. 4: Synthesis of genomic libraries

Applications of genomic libraries include: There are major applications of genomic library

- (1) determining the entire genome sequence of a specific organism;
- (2) acting as a source of genomic sequence for the production of transgenic animals via genetic engineering
- (3) In vitro investigation of the function of regulatory sequences
- (4) Investigation of genetic alterations in tissues

cDNA libraries:

A cDNA library is a collection of cDNA that has been converted by the enzyme reverse transcriptase from mRNA from a specific source. It contains the genes that are actively transcribed in that source under the physiological, developmental, or environmental conditions that occurred at the time the mRNA was isolated. cDNA libraries are useful in reverse genetics; however, they only represent a very small (less than 1% of an organism's whole genome).

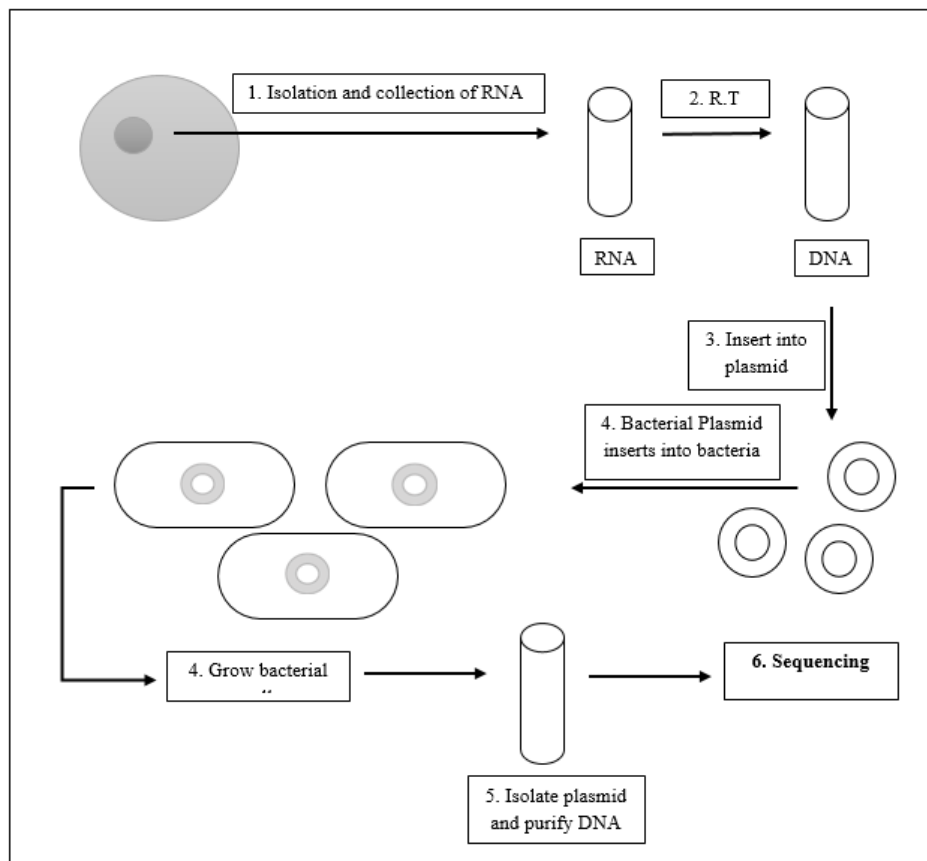


Fig. 5: Synthesis of cDNA library from mRNA

Applications of cDNA libraries include

There are major applications of cDNA libraries

- (1) Identification of novel genes;
- (2) Cloning of full-length cDNA molecules for in vitro gene function analysis
- (3) Investigation of the activities of mRNAs expressed in various cells or tissues
- (4) Investigation of alternative splicing in various cells or tissues

Molecular Probes

A nucleic acid molecule (single-stranded DNA or RNA) with a high affinity for a specific target (DNA or RNA sequence) is known as a probe. The probe and target base sequences must be complementary to one other, although they may not have to be perfectly complimentary depending on the circumstances. When proper labelling and detecting techniques are utilised, the hybrid (probe-target combination) can be exposed. Gene probes are used to detect nucleic

acid sequences in a variety of blotting and in situ procedures. They can aid in the identification of microorganisms and the diagnosis of infectious, hereditary, and other disorders in medicine.

1. Probe Design

The probe design depends on whether a gene probe or an oligonucleotide probe is desired.

- (A) **Gene Probes:** Gene probes are typically more than 500 nucleotides long and contain all or most of a target gene. They can be produced in two ways. Cloned probes are typically employed when a specific clone is available or when the DNA sequence is unknown and must first be cloned before being mapped and sequenced. It is common practise to use restriction enzymes to cut the gene and excise it from an agarose gel, however if the vector has no homology, this may not be necessary.

(B) Oligonucleotide Probes: Oligonucleotide probes are typically designed to target specific sequences within genes. The most frequent oligonucleotide probes are 18-30 bases, however modern synthesizers can efficiently produce probes with at least 100 bases. The following guidelines can be used to manually pick oligonucleotide probe sequences from a known gene sequence: The length of the probe should be between 18 and 50 bases. Longer probes have longer hybridization periods and lower synthesis yields, while shorter probes lack specificity.

The GC base composition should range between 40 and 60%.

Detection by Radioactive Labels: Some DNA probes can be marked with radioactive isotopes (for example, ^{32}P , ^{35}S , ^{125}I , ^3H). Autoradiography or Geiger-Muller counters are used for detection.

Detection by Nonradioactive labels: There are some nonradioactive labels that are used detect to the current position. Biotin, Chemiluminescent enzyme labels, fluorescence chemicals, Fluorescence chemicals, Antibodies and Digoxigenin System.

Hybridization method The difficulty in creating different ISH techniques is raising the sensitivity of the probes that are used to see the target sequence. The following methods are used to see the probe: (1) radioisotope labeling, which is thought to be very sensitive but has a short half-life, high exposure, and health risks. Pardue *et al.*, 1969; Huber *et al.*, 2018. (2) using non-radiolabeled probes and poly(rA)/poly(dT)-antibodies (emitting a signal via rhodamine-conjugated secondary antibody; the alternative is the rRNA biotin technique, which uses indirect detection by using avidin-based detection); In contrast to smaller micro-deletions found in the genome, fluorescent probes employed in interphase FISH can identify genetic aberrations in the

150–200 kb range.

Therefore, scientists are focused on the creation of methods to improve detection limits Huber *et al.*, 2018; Gozzetti *et al.*, 2000; Looi *et al.*, 1992.

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Single cell proteins: An additional nutrition to improve malnutrition

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Abstract

In today's fast-paced world, people often require an additional source of energy. Single proteins are an excellent option for obtaining nutritional energy, as they can help combat hunger and malnutrition. A study conducted by Transparency Market Research (TMR) predicts that the market for single-cell protein (SCP) will reach a value of US\$24.5 million by 2030. SCP is a term used to describe unrefined or refined protein derived from microbes, fungi, yeast, or algae that humans or animals consume. The high protein content of SCP has made it a promising alternative to protein derived from agriculture. Additionally, SCP contains other nutrients such as vitamins and lipids. The production of SCPs could potentially be used to feed the world's growing population. Therefore, this article will discuss the various methods and applications of single-cell proteins (SCP).

Keywords: SCP, hunger, protein, biomass, animal, human, algal, bacterial, mold.

Introduction

Dead, dried cells of microorganisms including yeast, fungus, bacteria, and algae are known as single-cell proteins (SCP). For their protein

composition, these microorganisms grow on various carbon sources. Carol Wilson originally introduced the phrase "single cell protein" in 1967 to replace the less appealing terms "petro protein" and "microbial protein." Most of the microorganisms utilized are unicellular, and the protein they produce is referred to as "single-cell protein." Yeast is typically used for SCP due to its excellent nutritional qualities in terms of protein content and an excellent supplement for animal feed. SCP does include nutrients including vitamins, lipids, and minerals, among others. A lack of protein in third-world countries led to the large-scale production of SCP, which led to the creation of SCP technology for use in both cattle and humans. A third of a square mile's worth of SCP fermenters are thought to be able to supply 10% of the world's protein needs (Lähteenmäki-Uutela *et al.*, 2021).

Need for microbial production of SCP

- SCP technology can alleviate global food scarcity
- It can meet 10% of the world's protein requirements using just one-third of a square mile for production
- SCP technology is not affected by climate conditions
- SCP technology offers high protein content, all essential amino acids, and microorganisms rich in vitamins
- SCP technology has a high ratio of surface area to volume, small doubling time, high growth rate, flexibility in substrate use,

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independence of cultivable land and climate, works continuously, is eco-friendly, cost-effective, energy-efficient, and can also be genetically controlled (Nalage *et al.*, 2016).

Sources of microorganisms and the selection criteria

- Various microorganisms are used for SCP production, including algae, fungi, protozoa, and bacteria.
- Microorganisms are selected based on their ability to use carbon and nitrogen sources, their tolerance to growth conditions, their resistance to viral infection, non-toxicity, non-pathogenicity, and nutritive value of cell mass.
- Spirulina is the most extensively used algae for SCP production, while Chlorella, *Senedesmus*, and *Dunalliella* are also widely used for their biomass.
- Fungi species such as *Aspergillus*, *Fusarium*, *Candida*, *Chaetomium*, *Trichoderma*, and *Penicillium* are ideal candidates for SCP production due to their ability to utilize various substrates and withstand abiotic conditions.
- Bacteria like *Bacillus*, *Lactobacillus*, *Pseudomonas*, and *Aesonomas* are also used for SCP production, but their success rate is not as promising.
- Mixed cultures have shown better results in terms of stability and resistance to contamination.
- The main challenges for SCP production from algae are their unpleasant odor and tastelessness.
- Algae primarily grows using carbon dioxide (CO₂) and sunlight.
- Fungal species can be grown on a variety of substrates, including sulfite waste liquor, prawn shell wastes, dairy waste (such as whey), and molasses.
- Lignocellulosic waste can also be used for specific fungi intended for SCP production.
- For bacteria, C1 - C4 compounds,

agricultural, animal, and food processing wastes, methane, and methanol are among the substrates that have been utilized.

Nutritional aspects of SCP- Microbial protein, also known as SCP, meets the essential amino acid requirements and scoring patterns recommended for adults by the FAO/WHO. In addition to its high protein content, ranging from 60% to 82% of its dry cell weight, SCP also contains fats, carbohydrates, nucleic acids, vitamins, minerals, and essential amino acids, such as lysine and methionine. These amino acids are typically lacking in many plant and animal proteins. There are numerous potential feedstocks for SCP production, including industrial wastewater, agricultural wastes, petroleum residues, methane, heptane, methanol, bio-gas, CO₂, ethanol, molasses, brewery residues, cellulosic biomass, and other industrial and agricultural residues. Microbial protein grown on agricultural wastes is becoming an important source of protein supplements due to its high protein content and the short growth cycle of microorganisms, which allows for rapid biomass production. Moreover, microbes can grow on inexpensive nutrient sources, resulting in economically advantageous protein supplements for balanced nutrition (Sharif *et al.*, 2021).

Methods for production of single-cell proteins

The process of producing single-cell proteins involves fermentation, where specific strains of microorganisms are multiplied on appropriate raw materials through a technical cultivation process. This process aims to promote the growth of the culture and cell mass, which is followed by separation processes. To begin the process, microbial screening is conducted to obtain suitable production strains from samples of soil, water, air, or inorganic/biological materials. These strains are then optimized through selection, mutation, or other genetic methods. Technical

conditions for cultivation are established, and metabolic pathways and cell structures are determined. Process engineering and apparatus technology are also utilized to ensure that the production is suitable for large-scale use, taking into account economic factors such as energy and cost, as well as

safety and environmental concerns. Additionally, legal regulations are considered to protect innovation and ensure safe operation, including operating licenses and product authorizations for specific applications (Spalvins *et al.*, 2018).

Submerged fermentation

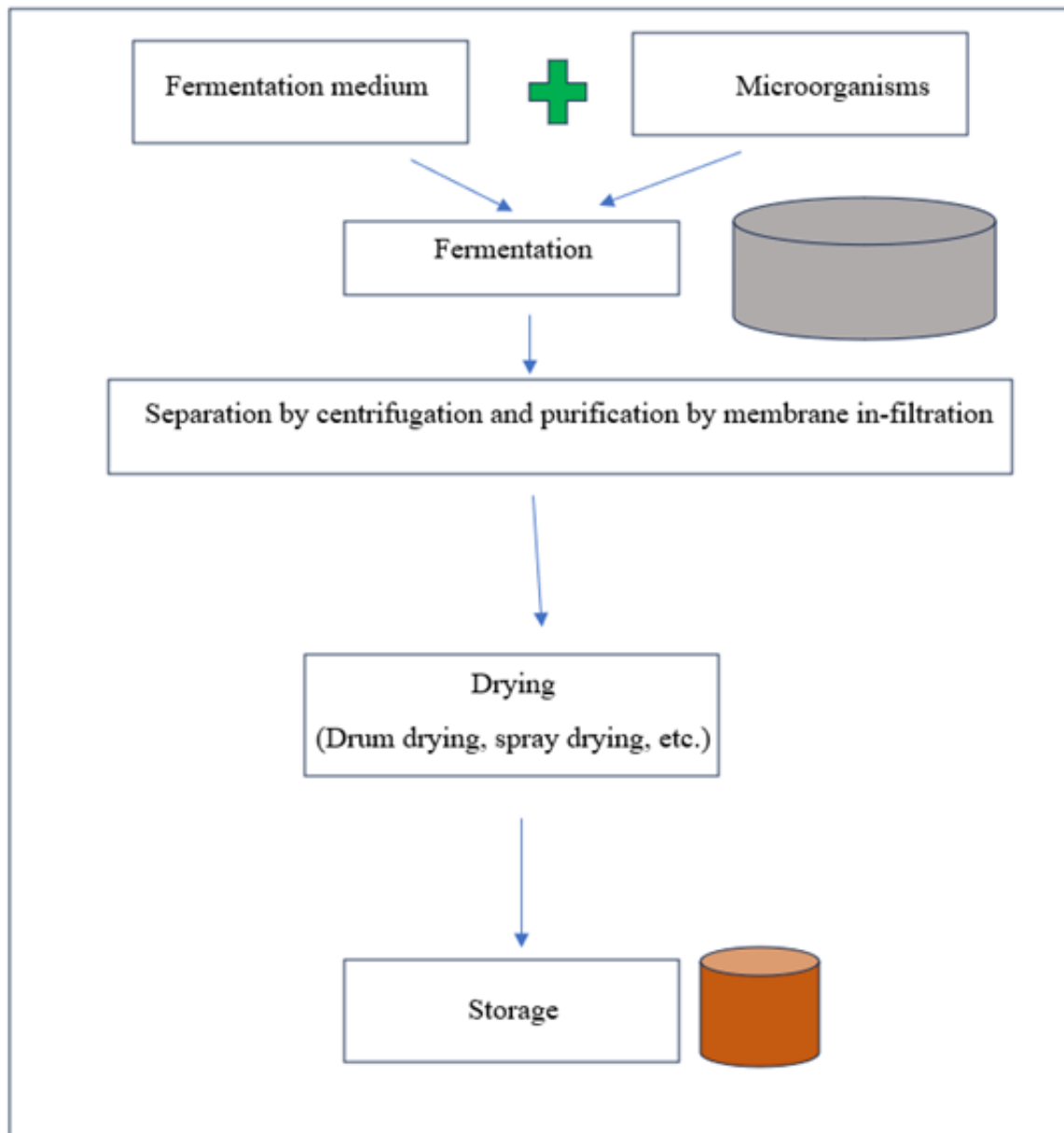


Fig. Steps involved in the production of single-cell proteins

For the submerged process of fermentation, the substrate is kept in a liquid state and contains all the necessary nutrients for growth. The fermenter is operated continuously, and the product biomass is harvested using various techniques. After harvesting, the product undergoes filtration or centrifugation before being dried. Aeration is a crucial step in the process, as heat is generated during cultivation and removed using a cooling device. Different methods are used to harvest microbial biomass, with centrifugation used for single-cell organisms like yeast and bacteria, and filtration used for filamentous fungi. It's important to recover as much water as possible before the final drying stage, which should be done under clean and hygienic conditions.

Semisolid fermentation

In the process of semisolid fermentation, the substrate isn't completely cleared and is often used in solid states like cassava waste. Submerged culture fermentations require a significant capital investment and have high operating costs due to several operations involved, including stirring and mixing of a multiphase system, transport of oxygen from gas bubbles through the liquid phase to microorganisms, and heat transfers from the liquid phase to the surroundings. To identify mass and energy transportation phenomena, a U-loop fermenter is designed. Producing single-cell protein involves preparing a suitable medium with a proper carbon source, preventing contamination of the medium and the fermenter, producing microorganisms with desired properties, and separating the synthesized biomass for processing. The carbon source used can be n-alkenes, gaseous hydrocarbons, methanol and ethanol, renewable sources like carbon oxide molasses, polysaccharides, effluents of breweries, and other solid substances (Malav and Dube, 2017).

Solid state fermentation

There has been extensive research on Solid State Fermentation (SSF), with numerous publications describing different types of bioreactor designs, process conditions, and microorganisms used for creating valuable products such as SCP, feeds, enzymes, ethanol, organic acids, B complex vitamins, pigments, and flavors. The process involves depositing a solid culture substrate, like rice or wheat bran, on flatbeds and seeding it with microorganisms. This mixture is then left in a temperature-controlled room for several days. Liquid State Fermentation, on the other hand, is used for unicellular organisms like bacteria or yeasts and is performed in tanks that can be as large as 1,001 to 2,500 square meters at an industrial scale. To achieve liquid aerobic fermentation, it is necessary to constantly supply the microorganism with oxygen by stirring the fermentation media. Precisely managing the synthesis of the desired metabolites requires regulating temperature, soluble oxygen, ionic strength, pH, and nutrients. The main steps in the process include medium preparation, fermentation, separation, and downstream processing (Suman *et al.*, 2015).

Technologies (processes) used Worldwide

There are various processes involved in producing feed protein like the Bel process, Pekilo process, Bioprotein process, Pruteen process, Quorn production, Symba process, Waterloo process, and high-rate algal ponds. This article will focus on the Pruteen process which involves the use of methanol-based processes. The process was developed in Europe, the former society union, Japan, and the USA, using bacterial species such as *Hyphomicrobium*, *Methylococcus*, *Methylophilus*, and *Methylotrophus*, yeasts including *Candida boidinii*, *Pichia angusta*,

and *P. pastoris*, and even filamentous fungi like *Gliocladium deliquescens*, *Paecilomyces variotii*, and I. The ICI in the UK developed the most adventurous process in 1980, using the methylotrophic bacterium, *M. methylotrophus*, to produce pruteen, a feed protein for chicken and pigs. The process is known to be the world's largest continuous aerobic bioprocess system, involving a 3000m pressure cycle airlift fermenter with an inner loop and working fluid volume of 1.5×10^4 L. The Pruteen process is capable of producing up to 50,000 tonnes of pruteen protein.

Factors affecting single protein production

SCP production is a complex and costly process. The production is greatly affected by the factors such as:

Selection of kind of microorganisms

- Consider growth rate, productivity, and yields when choosing a microbial strain for SCP production.
- Take into account temperature and pH tolerance, oxygen requirements, heat generation during fermentation, and growth morphology.
- Evaluate genetic stability in the fermentation process.
- Consider ease of recovery of SCP and requirements for downstream processing.
- Evaluate the final product's structure and composition in terms of protein content, amino acid profile, RNA level, flavor, aroma, color, and texture.

Other major factors are safety and acceptability

It's important to note that most SCP products are intended for animal feed and not for human consumption. Therefore, they must meet specific safety requirements. However, for proteins that are meant for human

consumption, obtaining regulatory approval is a complicated and costly process that influences the choice of production organism. When producing SCP products, it's crucial to consider the nucleic acid content, as many microorganisms have high levels of nucleic acid due to the fermentation conditions that promote rapid growth rates and elevated RNA levels. This can be problematic as the digestion of nucleic acids generates purine compounds that can cause gout-like symptoms or form kidney stones. Additionally, the digestion of some microbial cells within the gut, sensitivity or allergic reactions to microbial protein, and the absorption of toxic or carcinogenic substances from certain growth substrates are additional concerns that must be thoroughly examined (Matassa *et al.*, 2016).

Parameters for optimum SCP production

The parameters include the microorganisms used, culture conditions, pre-treatment of substrates, nutrient supplementation, types of fermentation processes, strain improvement, and genetic engineering techniques.

Applications of SCP

- Provides immediate energy
- Beneficial for maintaining healthy eyes and skin
- Excellent source of protein for undernourished children
Contains essential nutrients such as vitamins, amino acids, and minerals

Used in therapeutic and natural medicines for

- Lower blood sugar levels in diabetic patients
- Reduce body weight
- Lower cholesterol levels
- Reduce stress
- Prevent the accumulation of cholesterol in the body

Used in Poultry and dairy farms

- Protein and nutrient-rich food source
- Commonly used for animals such as cattle, birds, and fish

Technological advantages of single-cell protein

- Single-cell protein is composed of proteins, fats, carbohydrates, ash ingredients, water, phosphorus, and potassium.
- Single-cell protein technology offers year-round production, and waste management through the use of waste materials as substrate, a small land requirement, and less time required for production.
- It can be a solution during food crises by providing an alternative substrate for cultivation and aiding in the disposal of waste created during the process.
- Additionally, it is useful for recycling waste.

Side effects/drawbacks of SCP technology

Despite the very striking features of single-cell protein as a nutrient for humans and animals, various problems deter its adoption globally (Reihani and Khosravi-Darani, 2019). Such problems include:

- i. the concentration of nucleic acid is higher than other conventional protein sources and it is the characteristic of all rapidly growing organisms
- ii. the problem which occurs with the consumption of high nucleic acid-containing protein (18-25 g/100g protein dry weight) is the production of a high concentration of uric acid level in the blood causing health disorders such as gout and kidney stone
- iii. single cell protein from bacteria has also been found to be associated with these pitfalls which include: high ribonucleic acid content, high risk of contamination during

the production process, and recovering the cells is a bit problematic

- iv. about 70 to 80% of the total nitrogen is represented by amino acids while the rest occurs in nucleic acids
- v. it is also the point to be noted that the microbial cell wall may be indigestible
- vi. there may be intolerable colour and flavors (especially in algae) and in yeast there may also be possible skin reactions from the consumption of foreign protein and gastrointestinal reactions may occur resulting in nausea and vomiting
- vii. single cell proteins from algae may not be suitable for human consumption because they are rich in chlorophyll (except *Spirulina*)
- viii. it has low density i.e., 1-2 gm dry weight/liter of the substrate and there is a lot of risk of contamination during growth.
- ix. the filamentous fungi show slow growth rate than yeasts and bacteria there is high contamination risk and some strains produce mycotoxins hence they should be well screened before consumption.

Conclusion

The amount of single-cell protein (SCP) produced relies on the type of substrate and composition of the culture medium used, which greatly impacts cell growth rate. The ideal substrate for SCP production should be non-toxic, abundant, renewable, affordable, contain carbohydrates and nutrients, and support fast growth. Microorganisms use various carbon sources to produce SCP, including conventional materials like starch, molasses, fruit and vegetable waste, and brewery residues, as well as unconventional substrates such as petroleum by-products, methane, and ethanol. Agricultural and agro-industrial waste products like sugarcane bagasse, rice husk, and wheat straw residue have also been utilized for SCP production due to their low cost and natural abundance (Jones *et al.*, 2020).

The availability of energy and carbon sources, as well as the ability to utilize these sources, are critical factors that affect microbial growth. Bacteria, fungi, yeast, and algae can use cheap raw materials like lignocelluloses, starch, and organic waste as carbon and energy sources for cell growth. Some raw materials may require pre-treatment or hydrolysis before use. SCP is becoming increasingly popular as it requires minimal land area for growth and aids in waste recycling. Using agro-industrial residues in bioprocesses like SCP cultivation provides alternative substrates and helps solve pollution problems that may arise from their disposal (Abdel-Azeem and Sheir, 2020).

SCP has a high protein content of 60-70%, making it a suitable protein source for both human and animal consumption. They are also rich in vitamins, particularly B complex, and low in fat. However, SCP also contains a high concentration of nucleic acids, which can be reduced through chemical treatment, activation of endogenous nucleases, thermal shock, or treatment with sodium hydroxide or sodium chloride. Huei-Hsiung Yang developed a simple method for reducing nucleic acid in *Brevibacterium* JM98A by incubating non-proliferating cells at pH 10.3 and 55°C for three hours (Ritala *et al.*, 2017).

SCP research has been driven by the concern over potential food shortages if the world's population is not controlled. However, to ensure the future success of SCP, food technology problems must be addressed to make SCP more familiar as a food source, and its production must be compared favorably with other protein sources. Scientists in universities and industries, particularly in oil, are increasingly interested in SCP as a potential solution for future food shortages in less developed countries.

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Development of Transgenic Plants against Biotic Stress through Agrobacterium Mediated Gene Transfer Method

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Abstract

Crops can now be modified genetically in unique ways, which has the potential to solve significant issues with contemporary agriculture. Using *Agrobacterium* as a biological vector has made it possible to introduce genes into plants. While simpler and more effective, *agrobacterium*-based techniques have the drawback of not being suitable for all plant species. Recent research suggests that by creating unique plant cell culture protocols and specifying inoculation and co-cultivation conditions, these host-range constraints may be circumvented. The most popular technique for introducing new genes into plants relies on *Agrobacterium tumefaciens* inherent ability to transfer DNA. In the natural world, a broad variety of dicotyledonous plant species develop crown galls, which are tumors caused by this soil bacterium. T-DNA, a component of the Ti-plasmid of the *Agrobacterium*, is transported and incorporated into the plant genome during this infection. Because of this inherent ability, we used this bacterium to introduce foreign genes (incorporated into the Ti-plasmid) into plant chromosomes naturally. In order to create a transgenic Bt

cotton crop that is resistant to the cotton bollworm bug and reduces losses by 50 to 60%, the desired Bt gene isolate from *Bacillus thuringiensis* was inserted into cotton. Future transgenic crops will aid with biotic and abiotic stress as well as improve crop management practices for productivity and quality.

Key words: Transgene, *Agrobacterium tumefaciens*, Ti plasmid, T-DNA, Bt gene, cotton

Introduction

Transformation technology is an effective method for examining the function of genes in increasing crop tolerance to abiotic stress. Plant genetic transformation is the intentional insertion of specific DNA segments into the genome of the receiving plant, excluding implantation by fertilisation or hybridization. Other non-sexual gene transfer methods for plants include somatic hybridization, which combines the nuclear and cytoplasmic genomes of two separate protoplasts, and cybridization, which occurs through cytoplasmic fusion (Lindsey, 1992). Genetic transformation, on the other hand, is the only approach that allows for both the insertion and integration of specific DNA sequences. Extensive research in fundamental molecular and cellular biology is required to find and isolate the gene responsible for the trait of interest in the donor organism in order to implant a desired gene into the host (Brasileiro and Carneiro, 1998).

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Cotton is an important industrial crop that contributes significantly to the world economy, which has numerous setbacks as a result of biotic and abiotic challenges. Despite these issues, genetic transformation and regeneration limits in cotton prevent further biotechnological advancements

A simple and improved *Agrobacterium*-mediated transformation protocol of tomato (*Solanum lycopersicum*) cultivar Rio Grande was developed to inspect the potential of producing transgenic tomatoes. In this study, regeneration and transformation efficiency as assessed in response to the seedling age, explant type, co-infection, co-cultivation duration, selection pressure (kanamycin), and the optimal concentration of plant growth regulators (PGR) 6-benzylaminopurine (BAP), gibberellic acid (GA3), and indolebutyric acid (IBA) in a Murashige and Skoog (MS) basal medium. To accomplish this goal, 2- to 4-wk-old tomato explant cotyledons, hypocotyl, and cotyledonary nodes were excised and transformed with the EHA105 *Agrobacterium tumefaciens* strain harboring pBIN19 binary vector containing *uidA* reporter gene and *nptII* as a selectable marker (Hashmi *et al.*, 2022). For this, meristematic shoot tips were employed as explant sources, GFP and NPTII genes were used as reporter and marker genes, respectively, and one of the cotton strains (Cukurova 1518) that is extensively grown in Turkey was chosen. Utilising the in vitro selection process allowed for the provision of the efficient post-co-cultivation conditions. (Ozyigit *et al.*, 2022). Only the discovery of plant tissue culture techniques made genetic transformation in plants possible. For the transformation procedure to be effective, foreign DNA must first be introduced into a plant cell or plant tissue; a transgenic plant is then regenerated from the changed cell. This process is enabled by the totipotency phenomenon, which is the ability of individual plant cells to develop into entire new plants under favorable conditions and in the presence of growth regulators and nutrients. Plants in which one or more genes

from another species have been introduced into the genome through genetic engineering techniques are used in this technique.

- Add a new characteristic to the plant that does not exist naturally in the species.
- Increase supply of food with reduced cost and longer self-life. Like flavor sever tomato (first transgenic food crop 1994)
- Improve the trait quality, increase crop production, increase insect resistance power Increase herbicide tolerance power, Increase nutrition value

Requirements of transgenic plants:

Nucleic acid manipulation

The process of generating transgenic plants comprises several processes, including gene discovery, isolation, and sequencing; another crucial element is clone engineering, which involves the building of the insert incorporating the known coding region as well as its promoter and terminator regions. A genomic library must be established and probed to isolate the genes of interest, and if the identified gene is expressed in a sexually compatible species, traditional breeding can be used. All of these methods are referred to as recombinant DNA technology, and they rely on a variety of novel technologies. Restriction enzymes, DNA ligase, Cloning vectors, and genomic and cDNA libraries are among the technologies addressed.

a. Restriction enzymes

The construction of cloned DNA molecules depends on restriction enzymes, also known as restriction endonucleases, which recognise a specific sequence of nucleotides in double-stranded DNA and cut the DNA at that sequence's location. Most restriction enzymes recognise sequences of four to eight base pairs and hydrolyze a single phosphodiester bond on each strand. Many of these cleavage or restriction sites have double-stranded DNA as a characteristic.

b. DNA ligases

DNA ligases are used to connect the fragments of DNA created by restriction enzymes, allowing the transfer of specific DNA sequences from one molecule to another.

c. Cloning Vectors

Once identified and separated, the DNA fragment carrying the desired genetic information is placed into a molecular cloning vector, a DNA molecule capable of copying and amplifying genetic information. Plant transformation uses primary cloning vectors obtained from bacterial plasmids. Cloning vectors such as bacteriophage DNA, eukaryotic viral DNA, and bacterial or yeast artificial chromosomes (BAC or YAC) can all be employed.

d. Genomic and cDNA Libraries

Genomic libraries are created by cloning an organism's whole genome. First, using restriction enzymes or mechanical shearing, a collection of all of a species' DNA fragments is created. These fragments are connected to a vector capable of receiving large, lengthy inserts (such as YACs and BACs), requiring fewer clones to obtain a representative sample of the whole genome. Large inserts also enable for the cloning of an entire gene, including its 5' and 3' flanking regions, in a single clone or a small number of distinct clones.

Methods for the Development of Transgenic Plants

There are two methods of transgenic plants

1. Direct Method

a. Physical Methods

- Microinjection
- Particle bombardment Methods
- Electroporation
- Silicon Carbide Fibers

b. Chemical Methods

- PEG
- Liposome

2. Indirect methods

- Agrobacterium-mediated gene transfer like callus, planta method
- Plant virus vector like CaMV, TMV

Agrobacterium Mediated Gene Transfer

Agrobacterium tumefaciens is an aerobic, Gram-negative bacterial species that is found in soil and belongs to the Rhizobiaceae family. It is a pathogen that infects a wide range of host plants (over 600 species), and it has the ability to transfer part of its genetic material into higher plants. The bacteria will recognise and come in contact with the plant cell during the initial phase of the *A. tumefaciens* infection process. Signalling molecules such as low molecular weight phenolic compounds, amino acids, and sugars generated by the wounded root are responsible for mediating this process of recognition and interaction. The signalling molecules encourage the activation of the tumor-inducing (Ti) plasmid's Vir region's virulence genes (vir genes) in *A. tumefaciens*. The vir region contains six groups of genes, virA, virB, virC, and virD, which are either necessary or help the host cell change more effectively (virE and virG).

- The gene VirA encodes for a receptor that responds to phenolic chemicals like acetosyringone that leak from injured plant tissues.
- There are 11 genes in the Vir B operon, which encodes proteins that form a structure resembling a pore or pilus.
- The helicase protein known as Vir C binds the overdrive sequence.
- Vir E1 protein is a sort of chaperon that aids in the folding of VirE2
- Vir D4 is the coupling protein that interacts with T-pilus
- Vir D1 encode for a topoisomerase relaxing the double helix
- Vir D2 create endonucleases which target the direct repeat boundaries of the T-DNA segment

- Starting at the right border, VirE2 binds to the T-strand to protect it from nuclease damage and intercalates with lipids to produce channels in the plant membranes through which the T-complex travels.
- After binding to a consensus sequence and being phosphorylated by virA, virG initiates vir-gene expression.
- Vir H—Responsible for host specificity; nothing is known about this gene and it is not described in all Ti plasmids. (Hwang *et al.*, 2017)

Vectors

(A) Ti Plasmid: the natural Ti plasmid of *A. tumefaciens*. The transferred DNA (T-DNA) region contains the oncogenes for cytokinin (PC) and auxin (PA) synthesis as well as the gene for opine synthesis (SO). The Right Border (RB) and Left Border (LB) areas, which are both crucial for the T-DNA transfer process, border the T-DNA region. The Ti plasmid also contains an OR for *A. tumefaciens* replication, a virulence region (Vir), a conjugative transfer region (TC), an expression region for genes involved in opine catabolism (CO), and an expression region for genes involved in conjugative transfer.

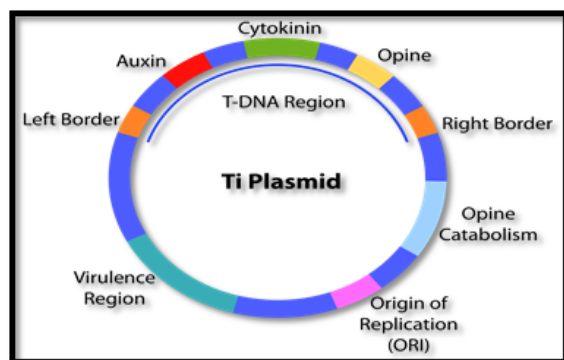


Fig 2: *Ti plasmid of Agrobacterium tumefaciens*

(B) Co-integrate Plasmid: The PC, PA, SO, TC, and CO portions of the T-DNA are taken out, and an exogenous T-DNA section is added, to disarm the Ti plasmid. The selection marker gene (SMG), the promoter (P), and terminator (T) regions are all introduced by the foreign T-DNA. A gene (R) for antibiotic resistance is introduced as well to enable the selection of the altered bacteria.

- (C) Helper T-DNA:** The T-DNA binary vector system uses the Ti plasmid with excised T-DNA as a helper.
- (D) Binary Vector:** A binary vector contains the selectable marker gene (SMGs) for both the plant selectable marker gene 1 (SMG1) and the *A. tumefaciens* selectable marker gene 2 (SMG2) in addition to the gene of interest (GI). For *E. coli*, it also possesses a source of replication. Plant transformation can be carried out using *A. tumefaciens* bacteria that have a helper Ti plasmid carrying the binary vector. growth of altered cells, which causes tumour development. Additionally, the T-DNA contains genes that code for the enzymes needed to create opianes (modified amino acids or carbohydrates). Large amounts of protein are produced as a result of an *A. tumefaciens* infection and are catabolized by the bacteria. *A. tumefaciens* infections allow sections of a prokaryotic genome (the T-DNA region of Ti plasmid) to be incorporated into the DNA of a eukaryotic organism, a natural characteristic unique to this bacteria. For transgenic plants to be produced, it was crucial to comprehend this natural process of genetic element transfer between other organisms.

Laboratory Procedures for Transgenic Plant Development

There have been several distinct techniques for plant transformation identified. The standard laboratory procedures required to produce transgenic plants are:

- (1) Isolation, creation of a gene construct, and cloning of the desired gene
- (2) The process of preparing plant material, including leaf pieces, embryos, protoplasts, and others
- (3) Gene transformation (DNA transfer)
- (4) Transgene integration screening
- (5) Plant regrowth from a modified cell

(1) Isolation, creation of a gene construct, and cloning of the desired gene

The gene of interest is extracted from the donor organism's total DNA and cloned onto a vector to transform *E. coli* competent cells in this stage. The gene of interest can be

extracted by creating a genomic library and recognizing the gene with appropriate probes, as well as amplifying the gene with specific primers targeting the coding region. The extracted gene of interest is used to build the vector. The vector must also contain a selection marker gene in order to track the successfully converted plant cells. In the case of *A. tumefaciens* genetic transformation, the desired gene should be either transferred to a binary vector or co-integrated into the bacteria's Ti plasmid.

A. Gene Construct: A construct is a man-made fragment of DNA carried on a vector that can be used to insert genetic material into a target tissue or cell. A functional gene construct is made up of three parts: a promoter area, a gene coding region, and a terminator/stop region. Furthermore, some contain unique sequences such as an enhancer, silencer, antibiotic resistance gene, or reporter sequences.

Methods in joining of gene of interest to vector (forms gene construct)

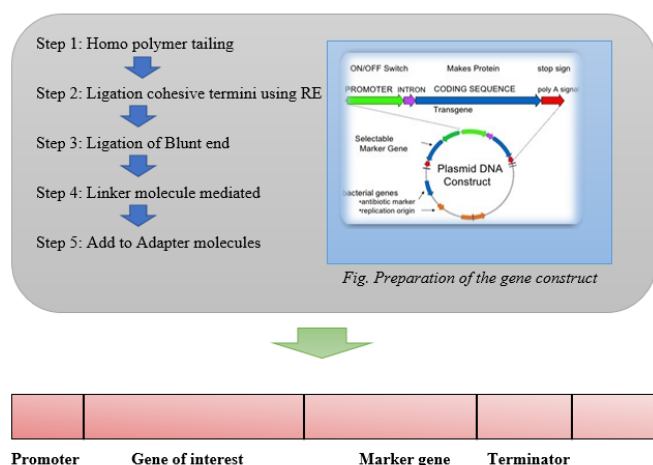


Fig. 2 Different steps of gene construct

(2) Preparing of plant material

To avoid contaminating the plant material that will be converted, this process must be carried out under aseptic circumstances. The plant tissue should be sterilised with ethanol and sodium hypochlorite before use. Leaf fragments must be kept in an enzyme solution with the cut surface in contact with the

solution to form protoplasts. The protoplasts could be used for electroporation or PEG therapy. In order to facilitate the process of infection and the transfer of exogenous DNA when utilising *A. tumefaciens* for genetic transformation, incisions must be created in the explants.

These cuts are done during the preparation process, slicing the explants into small pieces. The explants are stored in a regeneration medium containing full strength Murashige and Skoog (MS) salts (Murashige and Skoog, 1962), vitamins, zeatin, sucrose, and acetosyringone before co culture with *A. tumefaciens*.

(3) Gene transformation (DNA transfer)

The method employed to deliver DNA to the target plant cell will determine the procedure. Specific conditions will vary depending on the plant species and tissue type used. Co-cultivation with explants occurs after the pre culture step in the transformation procedure mediated by *A. tumefaciens*.

First, the *A. tumefaciens* culture with the plasmid encoding the gene of interest is cultured at 28°C in a liquid medium containing appropriate antibiotics, and the cultures are centrifuged. The bacterial cells are resuspended in liquid MS media supplemented with myo-inositol and sucrose (Murashige and Skoog, 1962). To allow the DNA to transfer, the explants are immersed in this solution. The explants are then dried with sterile filter paper before being returned to the pre culture medium. The explants are incubated in this media for around 2 days before starting the regeneration process.

(4) Transgene integration screening

There are some screening methods that can be used for the identification of transgene.

- Blue/White Screening
- Replica Plating Technique
- Selectable Markers
- Genome Sequencing
- Molecular Techniques: there are some techniques like Agarose Gel

Electrophoresis, PAGE, ELISA, DNA Probes, Isotopic and non-isotopic labeling

- Nucleic Acid Hybridization based technique
 - Southern Blotting: The process of transferring DNA from agarose gels to nitrocellulose membranes.
 - Northern Blotting: RNA transfer to nitrocellulose membrane.
 - Western Blotting: Protein electrophoretic transfer to nitrocellulose membrane.

(5) Plant regrowth from a modified cell

Undifferentiated cells have unlimited potential and can develop into any kind of plant tissue. Calli (undifferentiated cells) arise when explants or protoplasts are exposed to a selected regeneration media. The calli are then moved to an elongation medium for around 30 days following this regeneration stage. Sections of the differentiated and lengthened shoots are then moved to the rooting media. The plants are then moved to a substrate in the greenhouse once they have formed shoots and are rooted.

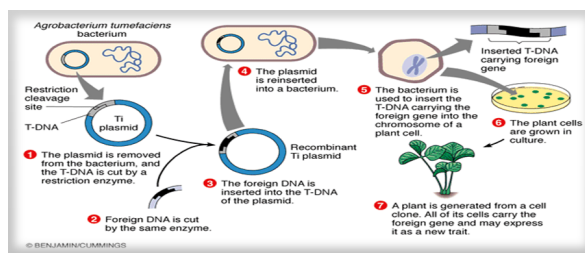


Fig. 3 Agrobacterium-mediated gene transfer (Source here)

Identification of Transgenic Plants

The selection genes result in the production of proteins with enzymatic activity that provide the transformed cells some easily observable trait, such as fluorescence emission or the capacity to proliferate in the presence of a typically toxic chemical. Depending on how the new trait is discovered, these genes are referred to as marker genes or reporter genes.

a. Marker Genes

Plant cells with marker genes are resistant to harmful chemicals such as herbicides and antibiotics. The feature is detected by adding the harmful substance to the culture medium, allowing only plant cells expressing the marker gene to grow normally. The *nptII* gene obtained from *E. coli* is the most often utilised selection agent in plant transformation. When expressed in plants, this gene encodes neomycin phosphotransferase, which provides resistance to antibiotics such as kanamycin, gentamicin A, neomycin, and others. The *hpt* gene, which was also identified from *E. coli*, codes for hygromycin transferase and confers resistance to hygromycin. Herbicide resistance genes have also been employed as selection markers on numerous occasions.

The *bar* gene, which was identified from the bacterium *Streptomyces hygroscopicus* and encodes phosphinothricin acetyltransferase, an enzyme that inactivates the herbicide phosphinothricin, is a popular alternative. Another extensively used marker that imparts resistance to the herbicide glyphosate is the *aroA* gene derived from the bacteria *Salmonella typhimurium*.

b. Reporter Genes

Reporter genes enable the identification of changed cells without the use of selective media because they encode proteins that are more visible. The *gus* gene, which encodes the enzyme -glucuronidase, is widely employed as a plant transformation marker. A histochemical examination can reveal the presence of this gene, which creates a blue precipitate in the presence of the suitable substrate. The *luc* gene, which encodes the luciferase enzyme from the firefly *Photinus pyralis*, and the *gfp* gene, which produces a fluorescent protein derived from the jellyfish *Aequorea victoria*, are two more extensively used reporter genes.

A luminometer detects yellow-green light produced by luciferase through a redox reaction. The GFP protein is fluorescent by nature and may be identified immediately under UV light. The marker gene, together with the gene of interest, must be introduced into the plant genome. Recombination events, on the other hand, can result in the insertion of only the marker gene. Plants selected by markers or reporter genes should be cultivated until there is enough plant tissue for DNA extraction to ensure that both genes were inserted. To validate the existence of the gene of interest, this DNA should be examined using PCR or DNA hybridization (Southern blot or dot blot). Alternative techniques for determining whether the inserted transgene is expressed include Northern blot hybridization of transcripts and protein detection through ELISA or Western blot.

Use of Transgenic Plants

Plants that have been genetically modified (GM) can be divided into three “generations.” The first generation was intended to enhance the manufacturing process and typically possessed agronomic features such as disease and insect resistance as well as herbicide tolerance. The second generation was created with the consumer in mind, and it comprises GM plants with improved nutritional features, both qualitatively and quantitatively, as well as plants with improved post-harvest quality. The third generation comprises GM plants that are designed to behave as biofactories, synthesizing substances for use in the pharmaceutical (vaccines, hormones, and antibodies), biosanitation, and manufacturing industries.

Effect

The usage of transgenic cultivars can have direct effects on agroecosystems due to the presence of functional exogenous genes in the plant, or indirect effects due to changes in the production management system. These effects

can occur in both soil and air. Changes in pesticide usage patterns, conservation practices, agricultural production, the ecology of invasive plants, and even the trophic chain (by altering nontarget organisms such as predators, natural enemies, and phytophagous species) can all occur.

Applications

Transgenic plants have various applications:

1. Resistance to Biotic Stress

- Insect Resistance like Corn, Cotton and Potato for Bt insect resistance
- Virus Resistance like Potato, Papaya, Squash, Melon etc.
- Disease Resistance like fungal and bacterial

2. Resistance to Abiotic Stress

- Herbicide Resistance like
- Glyphosate Resistance like Soyabean (80%) Maize, Soyabean, Cotton etc.

3. Improvement of crop yield and quality

- Extended self-life of fruit like Fleavr Savr Tomato
- Improved Nutrition like Golden Rice
- Improved coloration

4. Production of low-cost pharmaceutical

- Edible Vaccine like Banana, potato, wheat, Rice, Maize etc
- Essential Nutrition

Conclusion

In the future, researchers want to be able to provide transgenic plants that are resistant to stress. Transgenic plant improvements have had and will continue to people's lives. Transgenic plants provide a novel method for manufacturing and administering human antibodies known as edible vaccination. Future generations of GM plants are anticipated to be suited for improving the nutrient quantity and extending the shelf life of fruits such as Flavr Savr Tomato.

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Cell-free DNA: an opportunity to revolutionize advance disease detection

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Abstract

The vast majority of the DNA in a genome is neatly enclosed inside cells in the human body with the help from particular proteins, safeguarding it from degradation. However, under a number of conditions, certain DNA fragments are 'released' from their containers and found outside the cell, in body fluids. These tiny nucleic acid fragments are commonly referred to as cell-free DNA (cfDNA). Mandel and Metais made the initial discovery of cfDNA in 1948, then the scientists got aware of such degraded nucleic acid fragments in bodily fluids¹. However, only in the last two decades or so, since genome sequencing tools became more widely available, have they truly found out what to do with the information they have gathered.

Keywords:

A valuable asset

cfDNA consists of double-stranded deoxynucleic acid fragments that are roughly 200 base pair or less in length, have a low molecular weight, and are present in low concentrations². cfDNA can be created and released from a cell in a variety of circumstances, including when the nucleic acids in the cell decay. Because a variety of factors affected the deterioration, the amount, size, and source of the cfDNA can also vary. Furthermore, the release of cfDNA could occur

in conjunction with a number of activities, including those essential for normal development, those linked to the development of certain malignancies, and those linked to a number of other disorders (Stroun *et al.* 2000). One of the first findings of cfDNA levels in disorders came from studies looking into an autoimmune condition called systemic lupus erythematosus, in which the body's own immune system targets specific cells. The goal of cell-free DNA testing is to identify genomic variations linked to hereditary or genetic disorders by analyzing free (i.e., non-cellular) DNA present in biological samples in a lab. It is not surprising, then, that researchers throughout the world are increasingly discovering cfDNA to be a powerful tool for understanding human diseases and applying that knowledge to enhance diagnosis, monitoring, and prognosis⁴.

Examining a new life

By far the most common use of cfDNA has been in screening fetuses for specific chromosomal disorders, a procedure known as non-invasive prenatal diagnostics. The application derives from one of the Lancet publications on cfDNA in pregnancies, which was published in August 1997⁵. With the emergence of low-cost genome-sequencing methods, doctors will be able to sequence cfDNA fragments that correspond to foetal DNA. They can then utilize it to understand specific chromosomal disorders involving chromosomal copy number changes. Such mutations can result in disorders such as Down syndrome, which is caused by a

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chromosome 21 change (there are three copies of chromosome 21 instead of two, therefore it is also known as Trisomy 21) Swarup *et al.* (2007)

As a result, clinicians may now test moms from a few millilitres of blood drawn after nine or ten weeks of pregnancy to ensure the growing foetus is free of such chromosomal abnormalities using a cfDNA-based approach. The test is nearly 99% accurate for trisomy 21 or Down syndrome and slightly less accurate for other prevalent trisomies (of chromosomes 13 – Patau syndrome and chromosome 18 - Edwards's syndrome)⁷.

Prior to the genome-sequencing era, screening for such defects would have required putting a small needle into the body to remove the amniotic fluid and cells surrounding the developing baby (called as chorionic villus sampling or amniocentesis) and analyzing them in the lab. This method carries risks to both the foetus and the mother, many a times leading to miscarriage. It is not surprising, then, that the cfDNA-based technique has now become the gold standard for screening high-risk pregnancies. However, the test has limits, which implies that a positive cfDNA test result should always be followed up by a confirmation test. Lo *et al.* (1998)

Early Cancer Detection

Another promising application of cfDNA is in cancer diagnostics, prognostics, and therapeutics. The majority of cancer-related deaths in humans result from detection in advanced stages, when treatments are less effective. Multiple cancer types have shown clinical benefits of early identification of cancer, however it is still difficult to deploy screening methods (Sung *et al.* 2020 and Guide to Cancer Early Diagnosis (World Health Organization, 2017). Recent studies have demonstrated the potential for non-invasive early cancer detection using genome-wide fragmentation and methylation analysis (Mathios *et al.* 2021 and Siejka-Zielińska *et al.* 2021),¹².

Early cancer screening gives doctors the chance to find tumours when treatments are less gruesome, cures are more likely, and outcomes are better. Using cfDNA, there aren't many research examining the simultaneous detection and localisation of various cancer types. Circulating Cell-free Genome Atlas (CCGA) (NCT02889978 and NCT03085888) study was started by Liu and his team, with the goal to ascertain whether genome-wide cfDNA sequencing in conjunction with machine learning could detect and localise a wide range of cancer types with enough precision to be taken into consideration for a general population-based cancer screening programme. A total of 6689 participants were examined, which included 2482 cancer patients and 4207 non-cancer ones. In the CCGA sub-study, whole-genome bisulfite sequencing (WGBS), which probes genome-wide methylation patterns, performed better during discovery work than whole-genome sequencing (WGS), which probes copy-number variants (CNVs), and targeted sequencing approaches, which probe single-nucleotide variants (SNVs)/small insertions and deletions (SIDs) (Liu *et al.* 2020).

A wide variety of cancer types were found using cfDNA sequencing of relevant methylation patterns at metastatic and non-metastatic phases, with specificity and sensitivity performance approaching the target for population-level screening. These findings confirm the viability of applying this tailored cfDNA methylation analysis to ongoing clinical trials in the target group for early cancer identification¹³. cfDNA will prove to be an emerging tool or medium for treating the cancer patients.

A less invasive, more affordable, and potentially effective alternative to tumor biopsy is cfDNA analysis in liquid biopsy. The molecular characterization of cfDNA, which is of great significance for the prevention and treatment of tumor recurrence, enables liquid biopsy based on cfDNA to not only detect cancer recurrence more quickly than the

available radiological imaging technology, but also to provide insights on the molecular evolution of minimal residual disease (MRD) in tumor progression (Siejka-Zielińska *et al.* 2021).

Practically Endless Applications

Due to its accessibility, noninvasiveness, and possibility for tumor specificity through quantitative detection or targeted sequencing, cfDNA analysis has gained growing attention. The use of cfDNA is expanding, and one of these uses is in figuring out why a body rejects a donated organ. Here, donor-derived cfDNA, also known as dd-cfDNA, can be used to obtain an early yet precise estimation of how well the organ is being assimilated. This is a compelling hypothesis because alterations in cfDNA levels in the blood would occur before any biochemical or molecular indicators that scientists now use as proxies for organ acceptance¹⁴. That is, if something is going to go awry, the cfDNA may convey a signal before other markers do. Prenatal medicine has already been greatly impacted by cfDNA, and other sectors including as oncology, transplant medicine, and cardiovascular illnesses may follow in the near future (Yan *et al.* 2021).

In fact, cfDNA appears to have an almost endless number of uses, particularly as nucleic-acid sequencing becomes quickly more accessible to the general public and develops more uses of its own in therapeutic contexts. According to some sources, cfDNA may one day serve as a biomarker for conditions ranging from type 2 diabetes and non-alcoholic fatty liver disease to metabolic illnesses like Alzheimer's disease, neuronal tumors, stroke, and traumatic brain injury^{16,17}. In a real sense, cfDNA genomics aspires to put us on the right track for a healthy world by enabling more accurate disease screening and early diagnosis.

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RECENT NEWS

Paraguay Grants Approval to HB4® Wheat

HB4® wheat has received approval in Paraguay, according to the Instituto de Biotecnología Agrícola (INBIO). This approval allows the introduction of the HB4® event to wheat improvement programs so that in four to five years, wheat varieties will be ready to be produced and commercialized for their seeds.



As a genetically modified crop, HB4® wheat went through a thorough risk assessment process for commercial planting in Paraguay, including environmental, human and animal nutrition, and safety aspects. The approval process is comparative with its non-genetically modified counterpart.

The results of these studies were submitted to various regulatory agencies. During the evaluation process, the required additional studies were carried out, and all of them confirmed the safety of HB4® wheat. In addition to drought tolerance, HB4 wheat is also tolerant to the herbicide glufosinate ammonium, a technology that has been used safely for 20 years.

New Zealand Broadens Research on Genetically Modified Grasses

AgResearch, a scientific research center in New Zealand, is expanding research and

development initiatives on genetically modified and gene-edited grasses. The center has included clover and endophytes to its research efforts, which initially focused on High Metabolizable Energy (HME) ryegrass. HME ryegrass has completed field trials in the US, but was temporarily withdrawn in Australia because of the complex requirements of the regulators.



HME ryegrass is a genetically modified grass that has been engineered to have higher levels of lipids in its leaves. This breakthrough has been achieved by increasing the expression of two genes that are involved in lipid production and photosynthesis. Aside from HME ryegrass, the researchers are now working on high-condensed tannin white clover, which is in the early stages but is showing promising results, such as reduction of methane emissions by 15%. They are also developing gene-edited endophytes to improve pest defense and reduce potential toxicity to animals simultaneously.

Harnessing Genetic Information Unlocks the Gene Editing Potential of Grass Pea

New genome sequence information of grass peas revealed key biochemical steps on how the plant produces toxins. The data can aid researchers in potentially applying genome

editing tools on grass pea to make it less or non-toxic and become an alternative crop with natural resilience to flood and drought for farmers.

Grass pea has been used for centuries as an insurance crop because it can survive drought and flood when other crops fail. It is nutritious and high in protein. However, its pea contains the neurotoxin B-L-ODAP (ODAP), which can cause neurolathyrism that can lead to irreversible paralysis when eaten by malnourished people.

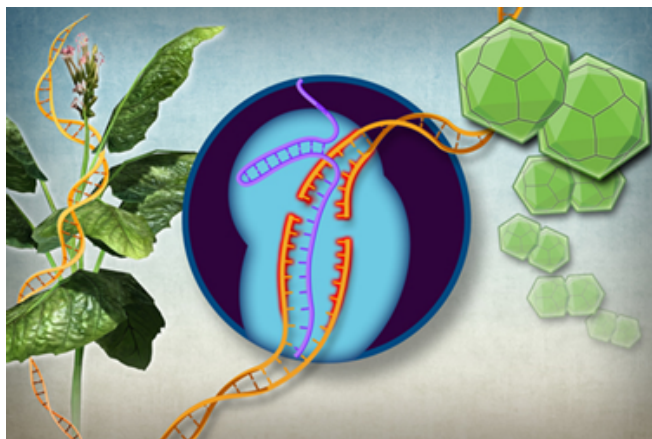


The newly available genome sequence of the plant makes it possible for breeders and researchers to develop varieties of grass peas with low or zero ODAP. By understanding the plant's mechanism that enables it to produce the neurotoxin, scientists can use gene editing and other modern breeding methods to alter or eliminate genome pathways and prevent the plant's neurotoxin production. This can render the crop safe for consumption. Along with its natural resilience to drought and flood, non-toxic grass pea can be considered a climate-smart crop for future generations to plant.

Revolutionary Multiplex Gene Editing Alters Plant Growth Patterns

Technological and scientific advancements have improved in the past two decades. Today, the combination of genomics, gene editing, and artificial intelligence is propelling the next wave of breeding advancements.

With tools such as CRISPR, plant breeders can cut a specific DNA sequence, then rely on the cell's natural DNA repair mechanisms to introduce changes at that site. The outcomes are just like those of traditional breeding but conducted in less time, with much greater precision and far fewer resources. Single edits are not, however, able to alleviate some of the world's biggest challenges which call for increasing yield with reduced use of natural resources. Multiplex gene editing, an advanced form of gene editing in which multiple edits are made to multiple genes at the same time, can help in these challenges, with the potential to meaningfully change how plants grow and use resources.



Multiplex gene editing is significant. At Inari in Massachusetts, the technology can increase yields by 10-20% in corn, soybeans, and wheat, up to 20 times the current industry average. Scientists at Inari are also using multiplex gene editing to reduce corn's need for nitrogen fertilizer and water by 40% without sacrificing productivity.

JRC Policy Report Examines the Effects of Gene-Edited Wheat with Reduced Gluten for Celiac Disease Safety

The Spanish National Research Council (CSIC) and Wageningen University & Research (WUR) used NGT-CRISPR-Cas targeted mutation to produce low-gluten, celiac-safe wheat that will help individuals with gluten intolerance. The policy report shared the advantages of this product when it becomes available in the market.



The GM gluten-free wheat products will be cheaper for people that follow a celiac-safe diet. The products can also eliminate the undesirable consequences of a gluten-free diet. Other advantages include the increase in income for the EU farmers and the boost in export volumes that will benefit the European Union (EU) agri-food system.

CRISPR Utilized to Reduce the Population of Mosquitoes that Spread Malaria

Scientists at the University of California Berkeley (UC Berkeley) and the California Institute of Technology (Caltech) created a system that uses CRISPR technology to

terminate female mosquitoes that can spread diseases. This has the potential to stop the spreading of malaria across the globe.



Malaria is one of the world's deadliest illnesses, which can cause hundreds of thousands of deaths each year. To address this issue, scientists at UC Berkeley and Caltech produced a system called Ifegenia, or "inherited female elimination by genetically encoded nucleases to interrupt alleles." The procedure employs CRISPR technology to disable the *femaleless (fle)* gene, which governs sexual development in *Anopheles gambiae* mosquitoes. Ifegenia functions by genetically encoding the two primary CRISPR elements within African mosquitoes. The elements consist of a Cas9 nuclease, which creates the cuts, and a guide RNA that leads the system to the target site.

"We crossed them together, and in the offspring, it killed all the female mosquitoes," said Andrea Smidler, first author of the study. On the other hand, the male mosquitoes inherit Ifegenia, but it doesn't affect their reproduction. Ifegenia can also be used on other mosquito species that spread diseases, like the ones that transmit Chikungunya, Dengue (break-bone fever), and Yellow Fever viruses.

Allergen-free Chicken Eggs via Gene editing

Researchers in Japan have created a gene-edited allergen-free chicken egg called the OVM-knockout that may be safe for people who are allergic to egg whites. The researchers used genome editing to produce the egg without the protein ovomucoid, which accounts for approximately 11% of all the protein in egg whites.

Chicken egg allergies are most common in children. Though most children outgrow this allergy by age 16, some of them carry the allergy into adulthood and some people with egg white allergies are unable to receive certain flu vaccines. To produce the OVM-knockout eggs, the research team first needed to detect and eliminate the ovomucoid protein in the egg whites. They engineered TALENs to target a piece of RNA called exon 1, which codes for specific proteins. The eggs produced from this technique were then tested to ensure there was no ovomucoid protein, mutant ovomucoid protein, or other off-target effects.



According to Ryo Ezaki, assistant professor at the Graduate School of Integrated Sciences for Life at Hiroshima University in Hiroshima, Japan, the eggs laid by homozygous OVM-knockout hens showed no evident abnormalities, and the albumen contained neither the mature OVM nor the OVM-truncated variant. Ezaki added that the

potential TALEN-induced off-target effects in OVM-knockout chickens were localized in the intergenic and intron regions. Also, the plasmid vectors from genome editing of the chickens were only momentarily present. These results show the importance of safety evaluations and reveal that the eggs laid by this OVM knockout chicken solve the allergy problem in food and vaccines.

Possible Applications of Gene Drive Technology in Agriculture

Gene drive technology is a genetic phenomenon occurring in nature and causing an identified trait to spread through a species through sexual reproduction over several generations, with an inheritance rate higher than the Mendelian rate of 50%. While gene drives have been used for public health and conservation, the approach could also be used in other species that reproduce sexually in short generation times, including agricultural pests.

The negative impact of weeds and pests on important crops has prompted increasing interest in using genetic techniques, including gene drive, to control these organisms. Controlling insect pests in agricultural production has been done using pesticides, and these tools have contributed to improvements in reducing food loss and waste. Ongoing research using new tools to help protect crops and minimize agriculture's environmental footprint looks at a wide range of tools and practices, including those that would leverage gene editing techniques.



Researchers are now in the early stages of exploring gene-drive-based strategies to help reduce agricultural pests. These strategies are focused on controlling pests that damage or compete with crops or carry disease. Gene drive could also be used to spread desired crop and livestock traits faster than traditional breeding. However, this option is limited because gene drives cannot alter asexually reproducing organisms, such as plants grown for food, and are unsuitable for species with long generation times. Determining the best tool to solve the issue at hand is the first step. Gene drive approaches may or may not meet these needs and requirements, and a case-by-case assessment would help determine if it is a helpful tool to investigate.

Scientists Develop Breakthrough Gene Therapy for Inherited Blindness

Researchers from a leading biotechnology company have announced a groundbreaking gene therapy that shows promising results in treating inherited blindness. The team successfully used a modified virus to deliver a functional copy of the faulty gene responsible for the condition, effectively restoring vision in a group of patients.

The inherited form of blindness, known as retinitis pigmentosa, affects millions of people worldwide. It is caused by mutations in specific genes that play a crucial role in the functioning of photoreceptor cells in the retina. Until now, there has been no definitive cure for this debilitating condition.

In this new study, scientists designed a viral vector capable of safely carrying the corrective gene into the target cells within the retina. The virus was engineered to specifically target the defective gene and replace it with a functional version. After administering the gene therapy to a small group of patients, researchers observed a significant improvement in their vision.

The initial results indicate that the treatment not only halted the progression of blindness but also resulted in noticeable vision

restoration. Patients reported enhanced light perception, improved visual acuity, and expanded visual fields. Follow-up examinations and tests revealed no adverse side effects or complications associated with the therapy.

While the study is still in its early stages, these findings offer hope for individuals affected by inherited blindness. Researchers are optimistic about the potential of this gene therapy approach and are planning to conduct larger clinical trials to further evaluate its safety and effectiveness.

If future trials yield similar results, this breakthrough gene therapy could revolutionize the field of ophthalmology and provide a viable treatment option for inherited retinal diseases. The implications of this research extend beyond blindness, as the gene therapy platform could be adapted to target other genetic disorders and offer potential solutions for various diseases.

This remarkable achievement highlights the tremendous potential of biotechnology and gene therapy in advancing medical treatments. As further research and development progress, it brings us one step closer to a future where inherited blindness and other genetic conditions are no longer untreatable.



Scientists Develop Climate-Resilient Crops

In a significant breakthrough, scientists at a prominent agricultural institute have made

substantial progress in developing climate-resilient crops through the application of advanced biotechnology techniques. By employing genetic modification, these researchers have successfully engineered plants that demonstrate enhanced tolerance to adverse environmental conditions, including drought, heat, and pests. This achievement holds tremendous potential in addressing the pressing global issue of food security, which has become even more critical due to the challenges posed by climate change.

By introducing specific genetic modifications into the crops, the scientists have bolstered their ability to withstand prolonged periods of water scarcity and extreme temperatures. These climate-resilient plants exhibit traits such as improved water-use efficiency, deeper root systems for better access to water, and mechanisms to cope with high temperatures. Additionally, they possess enhanced natural defense mechanisms against common pests and diseases, reducing the reliance on chemical pesticides.

The development of climate-resilient crops is a significant stride towards ensuring a stable and sustainable food supply in the face of changing climatic conditions. As climate change continues to impact agricultural productivity and threaten food security worldwide, these genetically modified plants offer hope for increased resilience and yield stability in the agricultural sector.

By reducing crop losses caused by extreme weather events and pest outbreaks, these climate-resilient crops have the potential to mitigate the adverse impacts of climate change on food production. Furthermore, they hold the promise of enabling farmers to cultivate crops in regions that were previously unsuitable due to harsh environmental conditions.



However, it is important to conduct rigorous safety assessments, environmental impact studies, and engage in transparent dialogue to address potential concerns associated with genetically modified organisms (GMOs). Ethical considerations and regulatory frameworks play a crucial role in ensuring responsible and sustainable deployment of climate-resilient crops.

Overall, the successful development of climate-resilient crops through advanced biotechnology techniques represents a significant stride in the quest for sustainable and resilient agriculture. Continued research, collaboration, and informed decision-making are essential to harness the full potential of these innovations, ultimately contributing to global food security in the face of a changing climate.

Gene-editing technique CRISPR used to successfully treat genetic disorder in human patients

In a groundbreaking development, the revolutionary gene-editing technique known as CRISPR has been successfully utilized to treat a genetic disorder in human patients. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a powerful and precise tool that enables scientists to edit genes with unprecedented accuracy.

The successful application of CRISPR to treat a genetic disorder marks a significant milestone in the field of genetic medicine. Genetic disorders are caused by mutations or abnormalities in an individual's DNA, leading to various health conditions. CRISPR offers the potential to correct these genetic defects by targeting and modifying specific genes responsible for the disorder.

The specific details of the case may vary, as there have been numerous advancements in CRISPR technology since my knowledge cutoff in September 2021. However, the general process involves using CRISPR to introduce specific modifications into the patient's genome, aiming to rectify the genetic defect causing the disorder.



The success of using CRISPR in human patients demonstrates the immense potential for targeted gene therapies and opens doors for treating a wide range of genetic disorders. By directly editing the DNA sequence, scientists can potentially eliminate or mitigate the effects of harmful mutations, offering hope for those affected by genetic conditions.

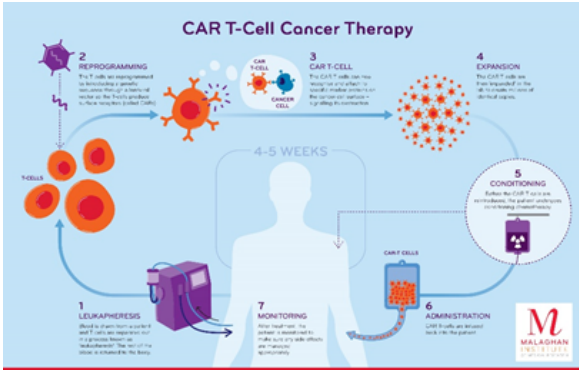
It's important to note that while the successful treatment of a genetic disorder using CRISPR is a remarkable achievement, there are still challenges and ethical considerations associated with this technology. Rigorous testing, careful evaluation, and thorough regulatory oversight are essential to ensure the safety and effectiveness of CRISPR-based therapies before they can be widely implemented.

In conclusion, the use of CRISPR as a gene-editing technique to treat genetic disorders in human patients represents a significant breakthrough in the field of genetic medicine. This development brings hope for the future of personalized and targeted therapies, paving the way for potential treatments for various genetic conditions. However, further research and ethical considerations are necessary to refine and ensure the responsible use of CRISPR technology in clinical applications.

Breakthrough in cancer immunotherapy: CAR-T cell therapy achieves unprecedented remission rates

In a groundbreaking development in the field of cancer treatment, CAR-T cell therapy has achieved unprecedented remission rates, signaling a major breakthrough in cancer immunotherapy. CAR-T (Chimeric Antigen Receptor T-cell) therapy is a form of personalized medicine that harnesses the power of a patient's own immune system to fight cancer.

The therapy involves extracting a patient's T-cells, a type of white blood cell crucial for immune response, and genetically modifying them in the laboratory to express a chimeric antigen receptor (CAR) on their surface. This receptor allows the modified T-cells to recognize and target cancer cells specifically. Once the CAR-T cells are reinfused into the patient's body, they seek out and destroy cancer cells, leading to potential remission or even cure.



The remarkable aspect of this recent breakthrough is the unprecedented remission rates achieved with CAR-T cell therapy. Clinical trials and studies have shown impressive responses in patients with various types of cancer, including hematological malignancies such as certain forms of leukemia and lymphoma.

In some cases, patients who have exhausted all conventional treatment options, including chemotherapy and stem cell transplantation, have experienced complete remissions. These remissions are characterized by the absence of detectable cancer cells in the body, leading to improved survival rates and enhanced quality of life for patients.

The success of CAR-T cell therapy can be attributed to its specificity and potency. By genetically modifying the patient's T-cells to express CARs targeting cancer-specific antigens, the therapy precisely targets cancer cells while sparing healthy cells, minimizing adverse side effects. Additionally, CAR-T cells have demonstrated potent cytotoxic activity, effectively eliminating cancer cells that may have been resistant to other forms of treatment.

Although the remission rates achieved with CAR-T cell therapy are highly encouraging, there are challenges associated with its implementation. The therapy can lead to significant side effects, including cytokine release syndrome (CRS) and neurotoxicity, which require careful management. Additionally, CAR-T cell therapy is currently limited to certain types of cancer and may not be suitable for all patients.

Despite these challenges, the breakthrough in achieving unprecedented remission rates with CAR-T cell therapy represents a major advancement in cancer treatment. It offers renewed hope for patients with limited treatment options and demonstrates the potential of harnessing the immune system to combat cancer effectively. Further research and clinical trials are ongoing to expand the

use of CAR-T cell therapy to other cancer types and refine its safety and effectiveness, bringing us closer to a new era in cancer immunotherapy.

Scientists create lab-grown human organs for transplantation, reducing waiting lists and improving outcomes

In a groundbreaking achievement, scientists have successfully created lab-grown human organs that could potentially be used for transplantation. This remarkable development has the potential to revolutionize the field of organ transplantation by reducing waiting lists and improving outcomes for patients in need of organ transplants.

The process of creating lab-grown organs, also known as organoids or bioengineered organs, involves taking a small sample of the patient's own cells or using stem cells and culturing them in a laboratory environment. By providing the necessary growth factors and conditions, these cells are coaxed into self-organizing and forming three-dimensional structures that closely resemble real organs.



The successful creation of lab-grown organs offers several advantages over traditional organ transplantation. One significant benefit is the potential to overcome the shortage of donor organs. Currently, there is a severe shortage of suitable organs available for transplantation, leading to long waiting lists and a high mortality rate among patients awaiting transplants. Lab-grown organs could alleviate this shortage by providing an alternative source of organs for transplantation.

Another advantage is that lab-grown organs can be tailored to match the recipient’s specific needs. Since the organs are created from the patient’s own cells or from stem cells that can be derived from a donor, the issue of organ rejection is minimized. This could potentially eliminate the need for immunosuppressive drugs, which are typically required to prevent the recipient’s immune system from rejecting a transplanted organ.

Additionally, the use of lab-grown organs allows for the study of diseases and the testing of potential therapies in a controlled laboratory environment. These organoids can provide valuable insights into disease mechanisms, drug efficacy, and personalized medicine approaches.

While the creation of lab-grown organs is a remarkable achievement, there are still significant challenges to overcome before they can be widely used in clinical transplantation. One major hurdle is scaling up the production process to create organs that are suitable for transplantation in larger animals or humans. Additionally, ensuring the functionality and long-term viability of lab-grown organs is critical for their success in transplantation.

Nevertheless, ongoing research and advancements in tissue engineering and regenerative medicine are making significant strides towards making lab-grown organs a reality.

Researchers

Engineer

Drought-Resistant Crops Using

Gene-Editing Technology, Addressing

Food Security Challenges

In a significant breakthrough to address global food security challenges, researchers have successfully engineered drought-resistant crops using gene-editing technology. Drought poses a significant threat to agriculture, leading to reduced crop yields and food scarcity in many regions. By utilizing gene-editing techniques, scientists have developed crops that are more resilient to drought conditions, offering the potential to

improve agricultural productivity and ensure food availability in the face of climate change. The gene-editing technology employed in this research is often based on CRISPR-Cas9, a revolutionary tool that enables precise modifications to the DNA of organisms. Scientists have identified specific genes involved in drought response and water-use efficiency in plants. By selectively editing these genes, they aim to enhance the plant’s ability to withstand drought stress and maintain productivity even under limited water availability.

Through targeted genetic modifications, researchers have been able to introduce traits that enable plants to conserve water, enhance their ability to absorb and utilize water efficiently, and optimize their response to drought-induced stress. These genetic changes can range from modifying the expression of certain genes involved in water transport and storage to enhancing the production of stress-responsive proteins that protect plants from damage.



The development of drought-resistant crops holds immense potential in mitigating the impact of water scarcity on agricultural systems. By improving crop resilience to drought, farmers can reduce yield losses and maintain agricultural productivity in regions prone to water limitations. This technology has the potential to benefit a wide range of staple crops, such as maize, rice, wheat, and soybeans, which are vital for global food security.

Additionally, the use of gene-editing technology offers advantages over traditional breeding methods in developing drought-resistant crops. Traditional breeding often involves crossing plants with desirable traits, which can be time-consuming and limited by the availability of suitable germplasm. Gene editing allows for more precise modifications, enabling scientists to target specific genes and accelerate the development of drought-resistant varieties.

While the successful engineering of drought-resistant crops using gene-editing technology is an exciting advancement, it is essential to consider the potential implications and address any concerns associated with genetically modified organisms (GMOs). Regulations, transparency, and ethical considerations surrounding the release and cultivation of genetically modified crops need to be carefully managed to ensure safety and public acceptance.

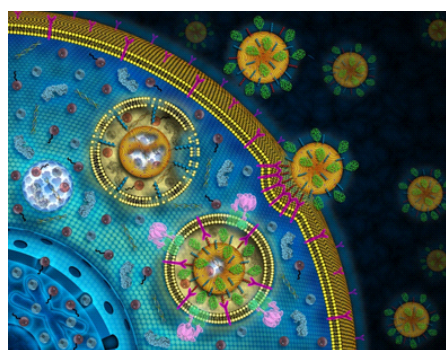
In conclusion, the use of gene-editing technology to engineer drought-resistant crops represents a significant step forward in addressing food security challenges. By enhancing plants' ability to withstand drought stress and maintain productivity under limited water availability, these advancements have the potential to bolster agricultural resilience and ensure food availability in a changing climate. Continued research, responsible regulation, and stakeholder engagement are essential to fully harness the benefits of this technology and promote sustainable agriculture for a more food-secure future.

Scientists Develop Novel Nanotechnology-Based Delivery System for Enhanced Vaccine Efficacy and Stability

In a remarkable scientific achievement, researchers have developed a novel nanotechnology-based vaccine delivery system that offers enhanced efficacy and stability.

Vaccines are crucial in preventing infectious diseases, and advancements in vaccine delivery technologies can greatly improve their effectiveness and accessibility.

The new vaccine delivery system utilizes nanotechnology, a field focused on manipulating materials at the nanoscale level (one billionth of a meter). By engineering nanoparticles or nanocarriers specifically designed for vaccine delivery, scientists can overcome various challenges associated with traditional vaccine formulations.



One key advantage of this nanotechnology-based delivery system is its ability to enhance the immune response generated by the vaccine. The nanocarriers can be designed to mimic the size, shape, and surface properties of pathogens, thereby triggering a stronger and more targeted immune response. Additionally, the nanocarriers can protect the vaccine antigens from degradation, ensuring their stability and longevity, which is particularly important for vaccines requiring cold storage or in resource-limited settings.

Furthermore, the precise control over the composition and structure of nanocarriers allows for the incorporation of various adjuvants or immune-enhancing substances. Adjuvants are substances that can enhance the immune response to vaccines, leading to improved efficacy. By combining antigens with adjuvants within the nanocarriers, the vaccine delivery system can stimulate a robust and long-lasting immune response, potentially requiring fewer doses for optimal protection.

The nanotechnology-based vaccine delivery system also offers advantages in terms of targeted vaccine delivery. Nanocarriers can be designed to selectively target specific cells or tissues, such as immune cells or specific organs, improving the vaccine's efficacy by directly delivering the antigens to the desired sites of action. This targeted delivery can enhance vaccine uptake, reduce side effects, and enable a more tailored immune response. Additionally, the nanocarriers can be engineered to have controlled release properties, gradually releasing the vaccine antigens over time. This controlled release profile can extend the duration of immune stimulation, leading to a sustained and durable immune response. While the development of this nanotechnology-based vaccine delivery system

is an exciting advancement, further research and clinical trials are necessary to evaluate its safety and efficacy in humans. Additionally, manufacturing scalability and cost-effectiveness need to be addressed for widespread adoption.

In conclusion, the development of a novel nanotechnology-based vaccine delivery system represents a significant step forward in vaccine technology. The ability to enhance vaccine efficacy, stability, targeted delivery, and controlled release can revolutionize immunization strategies and contribute to improved global health. Continued research and development in this field hold the potential to overcome existing challenges in vaccination, leading to more effective and accessible vaccines for a wide range of infectious diseases.

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NATIONAL AND INTERNATIONAL CONFERENCES

- 1) ICCBB—Ei, Scopus 2022 : 2022 6th International Conference on Computational Biology and Bioinformatics (ICCBB 2022)—Ei Compendex, Scopus Bali, Indonesia When: Dec 26, 2022 - Dec 28, 2022 Submission Deadline: Aug 5, 2022
- Venue : Kuala Lumpur, Malaysia
- 2) ICBB 2022 : 2022 International Conference on Biotechnology and Bioengineering (12th ICBB) Pozzuoli, Italy (Webinar) When: Sep 27, 2022 - Sep 30, 2022 Submission Deadline: Aug 31, 2022
- 3) ACM IMIP 2023 : ACM—2023 5th International Conference on Intelligent Medicine and Image Processing (IMIP 2023) Tianjin, China When: Mar 17, 2023 - Mar 20, 2023 Submission Deadline: Oct 15, 2022
- 4) ICSTR 2023 : 5th International Conference on Stem Cells, Tissue Engineering & Regenerative Medicine Rome, Italy When: May 18, 2023 - May 19, 2023
- 5) Bio International Convention Boston, United States June 5 – June 8, 2023
- 6) Bio Asia-Taiwan Taipei, Taiwan July 27 – 31, 2023
- 7) Bio Spain Barcelona, Spain September 26-28, 2023
- 8) International Conference on Solar Power Technology (ICSPT) -18th Aug,2023 -Venue : Kyoto, Japan
- 9) International Conference on Artificial Intelligence, Machine Learning and Big Data Engineering (ICAIMLBDE) - 18th Aug,2023
- 10) International Conference on Mechanical, Manufacturing, Industrial and Civil Engineering (ICMMICE) - 18th Aug,2023 - Venue : Kuala Lumpur, Malaysia
- 11) International Conference on Recent Advances in Science, Engineering and Technology (ICRASET) - 18th Aug,2023 - Venue : Daegu, Korea (south)
- 12) BPA — BioPharm America 2023 13 Sep 2023 - 14 Sep 2023 Raleigh, United States
- 13) ECCE14 & ECAB7 — The joint 14th European Congress of Chemical Engineering (ECCE14) & 7th European Congress of Applied Biotechnology (ECAB7). 17 Sep 2023 - 21 Sep 2023 Berlin, Germany
- 14) EMBL Course — Genome engineering: CRISPR/Cas from cells to mice 17 Sep 2023 - 22 Sep 2023 EMBL Heidelberg, Germany
- 15) ISBB 2023 : INTERNATIONAL SUMMIT ON BIOTECHNOLOGY & BIOINFORMATICS San Francisco, USA When: Sep 14, 2023 - Sep 16, 2023 Submission Deadline: Jan 16, 2023
- 15) Food biotech Congress 2022 : 3rd World Congress on Food Science and Biotechnology Amsterdam, Netherlands When: Nov 2, 2022 - Nov 3, 2022 Submission Deadline: Jan 16, 2023
- 16) “4th Edition of Euro-Global Conference on Biotechnology and Bioengineering” (ECBB 2024) is scheduled as a Hybrid Event in Germany and Virtually from September 19-21, 2024



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INFORMATION FOR CONTRIBUTORS

GENERAL

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Declarations to be made regarding ethical issues

Manuscripts that deal with clinical findings should be enclosed with a statement on informed consent of the patients under study. If humans and animals are the subject of a clinical study, it is essential for the study to have been carried out in accordance with the ethical standards of the country/ countries where the research described in the article has been conducted. A declaration to that effect must accompany the manuscripts.

Author conflict of interest statement

Authors must acknowledge the organizations that have provided financial support for their work. Any other conflict of interest must be declared while submitting the manuscripts.

CATEGORIES OF MANUSCRIPTS

General articles not exceeding 5000 words, 8 display items (tables and figures)] discuss current trends in research in a field that would be of interest to readers outside the field. These include interdisciplinary topics, science policy and science administration, some aspects of the application of science and technology to human needs or the impact of science and

technology on society/ecosystem/life. The articles should include an abstract, introductory paragraph, brief subheads at appropriate places, illustrations that will help a general reader and references.

Review articles (not exceeding 7000-8000 words, cited references to be limited to about 100 in number) are expected to survey and discuss current developments in a field. They should be well focused and organized, and avoid a general 'textbook' style.

Research articles (not exceeding 5000 words) report research result of major significance. They should include an abstract, an introductory paragraph and brief subheads.

News (not exceeding 1000 words) can cover important scientific events or any other news of interest to scientists in general.

MANUSCRIPT PREPARATION

Manuscripts should be typed/printed double spaced on one side of white paper (21×28 cm). The pages should be numbered consecutively, starting with the title page and through the text, reference list, tables and figures legends. The title should be brief, specific and amenable to indexing. Not more than five keywords should be indicated separately; these should be chosen carefully and must not be phrases of several words. Abstract and summary should be limited to 100 words and convey the main points of the paper, outline the results and conclusions, and explain the significance of the results. The full length paper should have the following headings.

SHORT TITLE : A short title, not exceeding 35 letters, should be typed at the top of first page in first letters capitals and underlined.

TITLE : The title should be short and informative, typed in first letters capital, Latin names underlined

AUTHORS : Names of authors to be typed in first letters capital.

ADDRESS : Address of the institution where the work was carried out. Footnote should indicate the authors for correspondence.

MAIN HEADINGS : Each full-length research paper should be divided into the following main headings which are to be bold and typed in first letters capitals on the left hand side of the page: Abstract, Introduction : Materials and Methods, Results, Discussion, References.

SUB-HEADINGS : Typed flush left in first letter capital, bold and italicized.

Abstract : A brief abstract, not exceeding 200 words should be typed after address.

Key words : The Abstract should be followed by about five key words indicating the contents of the manuscript.

Introduction : Introduction should be brief and pertinent.

Materials and Methods : Materials and Methods should include the source and nature of material, experimental design and the techniques employed.

Result : Result should contain data which are essential for drawing main conclusion from the study. Wherever needed the data should be statistically analyzed. Same data should not be presented in tables and figure form.

Discussion : The discussion should deal with the interpretation of the results. Wherever possible, Results and Discussion can be combined.

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Rynold, M.P. (1994). The Archaean grey gneisses and the genesis of continental crust. In: Archean Crustal Evolution (ed. Candie, K.C.) Elsevier, Amsterdam, pp. 205–259.

Sengar, R.S. (2013). Estimation of population growth and extinction parameters from noisy data. Ecol. Appl., 13 : 806–813.

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Proceedings of ..5th World Congress of Cellular & Molecular Biology (WCCMB, 2012).., November 02- 06, School of Biotechnology, Devi Ahilya University Indore, India & World Society of Cellular & Molecular Biology, France. 142–143.

Abbreviations : Use standard abbreviations. Some of the common abbreviations are given below: A (absorbance); h (hour); min (minutes); sec (seconds); cpm (counts per minutes); Ci (Curie); molwt (molecular weight); kD (kilo Dalton); kb (kilo base); sp act (specific activity); wt (weight); SD (standard deviation); SE (standard err); DAF (days after flowering)

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