Biyani's Think Tank

Concept based notes

Industrial Biotechnology

[B.Sc. (Biotechnology) Part-II]

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Preface

I am glad to present this book, especially designed to serve the needs of the students. The book has been written keeping in mind the general weakness in understanding the fundamental concepts of the topics. The book is self-explanatory and adopts the "Teach Yourself" style. It is based on question-answer pattern. The language of book is quite easy and understandable based on scientific approach.

Any further improvement in the contents of the book by making corrections, omission and inclusion is keen to be achieved based on suggestions from the readers for which the author shall be obliged.

I acknowledge special thanks to Mr. Rajeev Biyani, *Chairman* & Dr. Sanjay Biyani, *Director* (*Acad.*) Biyani Group of Colleges, who are the backbones and main concept provider and also have been constant source of motivation throughout this Endeavour. They played an active role in coordinating the various stages of this Endeavour and spearheaded the publishing work.

I look forward to receiving valuable suggestions from professors of various educational institutions, other faculty members and students for improvement of the quality of the book. The reader may feel free to send in their comments and suggestions to the under mentioned address.

Note: **A feedback form is enclosed along with think tank. Kindly fill the feedback form and submit it at the time of submitting to books of library, else NOC from Library will not be given.**

Ritu Dhingra

Syllabus

Industrial Biotechnology

(B.Sc. Biotech : Part-II)

Note : Question No. 1 shall consist of questions requiring short answers and shall cover entire paper. The paper is divided into four sections. Students are required to attempt five questions in all, selection not more than one question from each section. All questions carry equal marks.

SECTION-A

1. Introduction, Classification of Microbial products. Microbial processes for production of organic acids, solvents antibiotics enzymes, polysaccharides, Tipids, pigments and aroma.
2. Equipments and Accessories for industrial processes. Stability of Enzymes. Enzymes stabilization by selection and genetic engineering, protein engineering.
3. Reaction Environment rebuilding, Chemical modification, in ramolecular cross linking, immobilization.
4. Application of enzymes in industry, analytical purpose and medical therapeutic.

SECTION-B

5. Microbial production of therapeutic agents: Pharmaceuticals Isolation of interferon, cDNAs; Engineering human interferon and human growth hormone; optimizing gene expression. Enzymes DNAase I and Alginate lyase against cystic fibrosis. Monoclonal antibody as therapeutic agents - Production of antibodies in *E. coli,* HIV therapeutic agents.
6. Vaccines: Subunit vaccines - Herpes simplex virus Foot and mouth disease, Tuberculosis, Peptide vaccines, Genetic immunization, Attenuated vaccines - Vector vaccines.

SECTION-C

7. Synthesis of the commercial products by recombinant in croorganisms: Restriction endonucleases, Small biomolecules - L-Ascorbic acid, indigo, amino acids, Antibiotics, Biopolymers.
8. Biorecombination and biomass utilization Microbial degradation of xenobiotics; Commercial production of fructose and alcohol; Silage fermentation, Utilization of cellulose.
9. Economically important primary and secondary metabolites. Production of single cell protein from carbohydrates, n-alkanes, methane and methanol for use in food and feed.
10. Production of beer wine, vinegar and distilled beverges, Microbial food products.

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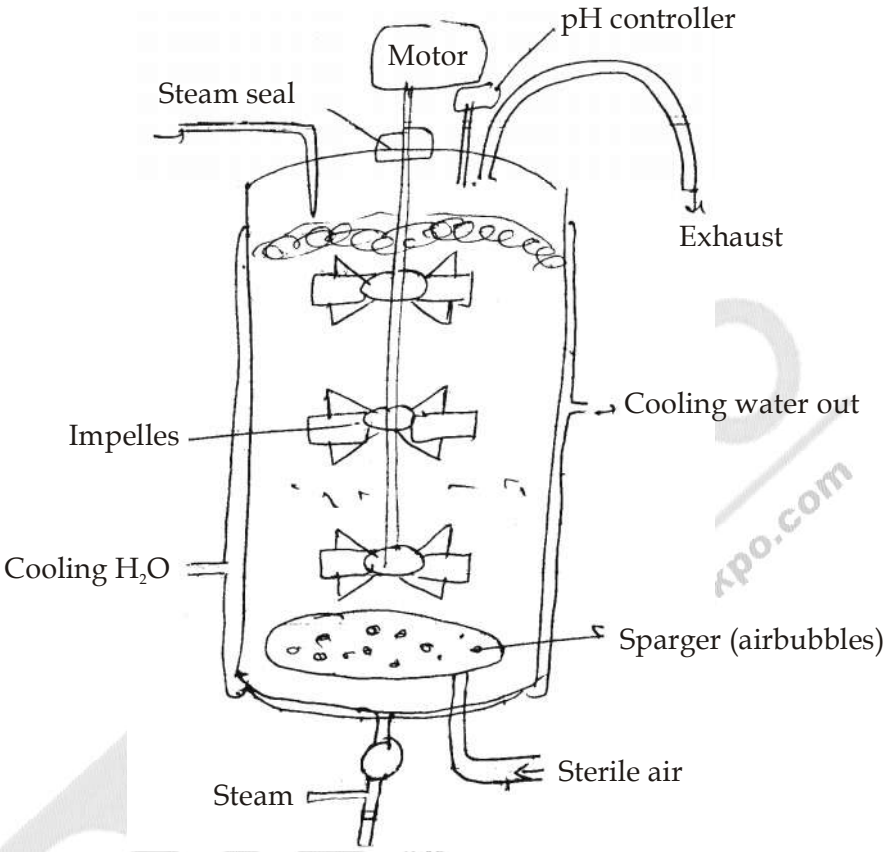
CONTENT

Section A
Introduction, Classification of Microbial products

Q.1 Write a detailed account on equipments and accessories for industrial processes?

Ans. Industrial uses of microorganism requires that they be grown in large vessel containing considerable amount of nutrient can be media, vessels are commonly called fermentor complicated in design since they must provide the '**control**' and '**observation**' of various stages of microbial growth and biosynthesis.

- Must be '**strong**' enough to with stand the '**pressure**' of large volume of aqueous medium.
- Some form of '**stirring**' should be available, if not accomplished by gas evolution, to mix both organism and medium so that nutrients and O_2 be more available to individual microbes.
- It should provide '**antifoam agents**' as demanded by foaming status of medium.
- It should provide '**aseptic means**' for withdrawl of culture as well as for introduction of inoculum at initiation of fermentation.
- For many fermentation '**air filters**' or some means of sterilization must be present.
- There must be a '**drain**' in the bottom of the fermenter for removing the fermentation broth from the tank.



Harvest fermentor

- Fermenters are available in varying sizes. These are stated based on total capacity of fermentor actual volume in a fermenter is always less than total volume, b/o a **head space** must be left at the tops of the fermentor.

  (Pilot plant – 25-100 gallon) Fermentor.

- There is a difference between capacity and working volume of a fermentor.

Fermenters, large or small are same what similar in mechanical design.

- An "**impeller**" is mounted to a shaft extending through a bearing in the lid of fermentor and driven by an external source such as a motor with adjustable belts, or by direct drive.

Driven by

- (Magnetic coupling) (Bakes's Yeast)
- In some fermentation process contamination is not a series problem because of the low pH of medum and inclusion of lactic acid prevent growth of microbes.
- Most aerobic fermentation requires that sterile air under pressure be introduced into the fermentation vessel.

  (Cheapest mean is to pass air through sterile filler composed of glass wool, C partical).
- The air passes into a sparger mainly consist of a pipe with minute holes that allow the air under pressure to escape as tiny air bubbles into the medium.

  (air bubble from sporges are picked up and dispersed through medium by act of impeller blade mounted above sparger).
- If imbeller is rotating rapidly enough the air bubble may escape directly through the shallow layer of medium above the impeller.

  (Thus the rotation rats of impeller should be great enough to allow thorough mixing of air into medium but not so great the air escapes without dissolves in the medium for use by microbes. (Size of air bubble α aeratn)
- In some fermentation tanks an impeller is not utilized. The medium is stirred by the direct rush of air bubble from the sparger at the bottom of tank.
- Fermentation tanks are never tightly sealed there must be some place for escape of fermentation gases evolved during growth of anaerobic microbes.
- For any given fermentor size of shorger is fixed and the air flow rate cannot be changed by altering the size of sparger holes.
- Impeller acts in a fermentation tank tend to spin the liq in a circular motion and create a vortex above impeller.
- Vertical baffles are attached to the inside wall of fermenter that disrupt the flow pattern of liq so that much greater mixing and turbulence of the will occur.

- Aeration and agitation of a liq medium can cause the production of foam.
- Proteolytic bacteria can cause serias problem b/o the foam due to peptides can be quite stable.
- If the foam is not controlled it will rise in the lead space of tank this condition cause of contamination of fermentation.
- The presence of excessive foam in a tank impedes aeration by limiting gas exchange between the medium and the atmosphere of head space.
- The usual procedure for controlling foam is to add an antifaming agents although a supplementary impeller blade may be effective.
- Antifoam agents lowers surface tension to the stability of foam bubbles so that they burst.

Temperature of fermentor must be maintained and this temp. must be below at or above ambient tank.

- Metabolically active microbes evolve heat which can accumulate to a considerable degree in the large volume of medium, because of this consideration the fermentor must be cooled to maintained the optimum fermentation temperature this is accomplished by spraying cold water on the outside of tank or by passing water through coils along inside wall of tank.
- Pure culture fermentations usually require that the medium be sterilized by auto claved.
- Various fermented products like lactic acid are corrosive and may attack inside lining of fermeutation tank so tanks must be constructed of materials resistant to corrosions.
- Certain fermentation are carried out 14 tanks constructed of wood, wooden tanks can be used for fermentation like lactic acid.
- Other materials used for constructs of fermentation tanks include Cu, Fe, glass, steel etc. e.g. Cu^{k} lined tanks useful in certain steps in brewing of beer, Fe^{+2} used for penicillin production.

STOCK CULTURES

- Microbes can be stored for future use in such a fashion that their growth and productive capacities remain unaltered. This maintenance of stock cultures plays an important role in industrial fermentation.
- 2 types of stock culture are present (1) Working stocks (ii) Primary stocks. Working stocks are used frequently and are maintained as agur slants stabs or broth, cultures and are held under refrigeration.
- Primary stocks are not maintained in a state of high physiological activity, transfers from these cultured are made when new working culture is required and to avoid death of cells is stock cultures stored at RT are maintained in agar absorbs primary cultures are also maintained at low temp.
- Although cultures may survive six month under refrigeration, usually they are transferred more quickly.
- Certain microbes cannot be stored at refrigeration temperature b/ o they die out at this temp. such culture can be held as agar slants at R.T.
- Sterile soil has widely use for the maintenance of microbes.
- Agar slant and stab cultures will survive of several years at RT if the growth is sub merged by under sterile mineral oil.
- Most widely used culture preservation method is lyophilization.
- If properly prepared and stored, most lyophilized cultures will remain viable for long without occurance of genetic changes.
- When needed cultures are recovered from ampoules by suspending cells in minimal amount of growth medium.

FERMENTATION MEDIA

- The choice of good medium is important for the success of industrial fermentation.
- Medium supplies nutrient for growth and energy and building of cell and biosynthesis of fermentation.
- A medium contains inorganic salts, H_2O vitamins and other GF, DO buffers and antifoam.

- Simple and complex composition. (Auto trophs require only a simple inorganics).
- These nutrient medium is further subdivided into "synthetic" and "crude media".
- In Synthetic media all the constituent are specifically defined and known compounds (amount chemical structure are known).
- Individual components are easily deleted (added).
- Foaming is not a series problem with synthetic media b/o they do not contain any protein or high mol. wt.
- With, these media recovery and purification of products are simple b/o mainly organic compounds are not include in the med and most of the compounds that interfere with recovery are known.
- These media can be expensive b/o the cost of relatively pure ingredients and the yield desived from this media is low.
- The alternative is the non synthetic/crude medium and allows much higher yields of fermentation products than does a synthetic medium.
- An example of crude medium is one containing blackstrap no molasses, corn steep liquor $CaCO_3$.
- It can allow the use of crude anti foaming agents without changing the nutritive balance.
- (Provides excess of both GF and nutritive agents). These nutrients be inexpensive. In this regard by product of agriculture provided the cheapest source of components particularly 'C' source.
- Media usually contain more than 70% or even 90% of water 'C' source of media can be simple/complex (can molasses, sugarcane, beet).

Molasses: Beet and cane molasses are the by products of sugar industry.

In the commercial products of sugar, juice from crushed sugarcane is concentrated to allow crystallization and then this sugar is separated from its mother liquor, this is further concentrated to allow recovery of additional sugar. So 52% of total sugar calculated as sucrose.

Cornsteep liquor: It is the water extract by product resulting from the steeping of earn during the commercial product of starch.

Sulfite Waste Liquor:

It is the spent sulfite liquor from paper-pulping industry. It is the fluid, remaining after wood for paper manufacture is digested to cellulose pulp sulphide under heat and pressure.

Growth factor: Growth factor is usually provided by the crude constituent of medium.

Q.2 Give a brief account on production of vinegar?

Or

Write Short note on: (i) Organic and production (ii) antibiotics (iii) enzymes (iv) solvents?

Ans. Production of organic acid

(i) Vinegar production – Vinegar word is derrived from 'vinaigre' means 'sour wine'.

Mainly two steps in vinegar production. In the 'first stage' yeast converts sugar into C_2H_5OH anaerobically while in the 'second step' ethanol is oxidized into acetic acid aerobically by acetobacter. This process is called acetification.

Substrate – Vinegar is produced by using fruit juice, malted cereals such as barley, rice, wheat, can etc.

Method – Mainly there are two methods (a) Slow or "let home" process (b) Quick or "French arleans" process.

(a) Slow process: In slow process, substrate is not allowed to move during acetification and fermented juice is used for acetic acid production.

Apple juice is specially used for alcoholic production by batch fermentation.

Barrel, is partially filled with the fermented juice and is allowed to undergo the acetification.

A film of vinegar bacteria called "mother of vinegar" should grow on the surface of liquid which indicates that ethanol is oxidized into acetic acid.

Demerit – Poor yield of vinegar, inferior quality.

(b) Quick process: In this process about ¼ barrel is filled with raw vinegar, and active vinegar bacteria. This is to be acetified. Now the barrel is filled, by alcoholic hydrolysate up to half filling and examine the bacterial film on the top.

This process takes weeks to month at 21-29" C and continuous process.

Major difficulty is dropping of gelatinous film of vinegar bacteria which results in retardation of the acetification.

(c) Generator process – It involves movement of ethanol liquid during acetification. A simple generator or tank made up of wood is used.

It gives high yield of acetic acid and leaves little residue e.g. alcohol.

(d) Makin's process: A fine mixture of vinegar bacteria and nutrient alcoholic solution is sprayed through jet nozzle into a chamber contining vinegar bacteria. This mixture is kept in circulation by filtered air.

(d) Submerged process: Medium containing 12% of ethanol is inoculated with acetobacter and is held at 24-29^{o}C.

(e) Finishing: Slow process, vinegar produced is less harsh the unit of vinegar is in given.

LACTIC ACID

Lactic Acid production by using chemical process was not economical and recovery purification were also not upto the mark, hence continuous effort were made to improve process.

(i) Fermentation:- Lactic acid is produced by several microbes which differ in their ability to produce either D (-) lactic acid, L (+) Lactic acid.

The racemic mixture is formed due to production of an enzyme called racemase.

Rhizopus oryzae produces only L (+) Lactic acid. There are mainly two important processes based an end product formation.

(a) Homs fermentative process -

Racto bacillus bacteria utilize the EMP, pathway to produce, pyruvic acid which is then reduced by lactate dehydrogenase to lactic acid.

(b) Hetero fermentative process – Process involves the action of Leuconostoc which produces lactic acid, CO_2, C_2H_5OH CH_3COOH and Water.

(ii) Medium and manufacture – Culture medium contains semi refined sugar, molasses maltose, sucrose with amonium hydrogen phosphate.

The colonies of Leuconostoc are transferred into large culture vessel kept at 45-55^{O}C. The inoculum volume is 5% and fermentation is carried ant for 5-10 days. Aeration and agitation are required.

Recovery: To the fermentation medium calcium carbonate is added, pH is adjusted to 10, broth is heated and filtered. Lactic acid is converted to calcium lactate. It decomposes sugar. The H_2SO_4 is added to remove Ca as $CaSO_4$. Lactic acid is crystallized as calcium lactate. The activated charcoal is added, to remove impurities and lactic acid is recovered.

Uses: It is a weak acid with good solvent properties and served as a preservative in food stuff.

Citric acid

(i) Fermentation - *Aspergillus niger*, has been the choice for the production of citric acid.

Large no. of fungi and yeast have also been used for citric acid production.

The advantages of using yeast rather than A. niger are the possibility of using very high sugar concentration.

In all the process, a variety of carbohydrates such as beet, molasses, cane molasses etc used in fermentation medium.

The fermentation is carried out by any of the process.

(i) Koji process (Solid state fermentation)

(ii) Liquid surface culture process.

(iii) Submerged fermentation process.

(a) Koji process: Mold is used in preparation called Koji in which wheat bran is substrate. The pH of bran is adjusted betn 4-5 moisture is picked up during steaming. After cooling the bran to 30-60^{0}C mass is inoculated with koji which was made by 'A. niger'. Since bran contains starch which by '*saccharification*' by amylase enzyme of A. niger induces citric acid production.

(b) Liquid surface culture process – In this case stainless steel shallow

pans or trays are used. Sterilized medium contain molasses and salts. Fermentation is carried out by blowing the spares of A. niger over the surface of solution for 5-6 days spare germination occurs within 24 hrs and a white mycelium grows over the surface of solution. Eight or ten days after inoculation the initial sugar concentration (20-25%) reduced to 1-3%. Small quantity of citric acid is produced during growth phase called primary metabolite.

Initially the pH remains 5-6 but on spare germination pH approaches the range of 1.5-2 as NH_4 ions are removed from solution.

(c) Submerged culture process:-Economical process, In this process Aspergillus is slowly bubbled in a stem of air through culture solution of 15 Cm depth.

Since the organism shows sub surface growth and produces citric acid in the solution. The yield is inferior as compare to other.

Recovery: After filteration the filterate is transferred and treated with H_2SO_4 to precipitate Ca as $CaSO_4$. This is subjected to the treatment with activated carbon. The purified solutions is evapourated in a circulating crystallizers.

Uses – Citric acid is an anhydrous crystalline chemical it is used in the soft drinks. Jaw wine and frozen truth and also used as artificial flavours.

Antibiotics: Antiobiotics are chemical substances secreted by some microbes which inhibit the growth and development of other microbes.

Penicillin: Penicillins differ from one another in the siede chain attached to its amino group. Most of these are 6-aminapenicillanic acid derivatives and all have β-lactum ring which is responsible for antibiotic activity. Penicillin acts against gram positive bacteria and inhibit cell wall synthesis.

Fermentation: Penicillin is produced by *P. Chrysogenum*, a fungus that can be grown in stirred fermenters. The inoculum under aerobic condition can be produced when there is glucose in sufficient amount in the media. If a particular penicillin is produced. Specific precursor is added in the media. The antifoam agents such as vegetable oil is added into the medium-before sterilization.

The spare suspension is inoculated in flask each containing 15 g barley seeds.

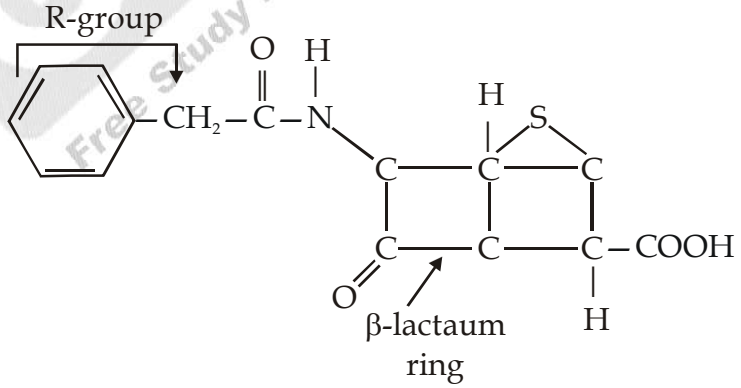
The preparation of inoculum takes place on barley seeds. These flask incubated at $25^{O}C$ for 7 days spores developed on barley seeds are suspended in distilled water to make spare suspension. After testing the antibiotic activity flask containing seeds are ready for seeding in fermentation. 3 phases of growth can be differentiating during cultivation of P. Chrgsogenum.

(a) First phases: In this phase growth of mycelium occurs yield of antiobiotic is quite low. NH_3 is liberated into the medium resulting into rise in pH.

(b) Second phase: There was intense synthesis of penicillin in this phase, due to rapid consumption of lactose. The mycelial mass increases the pH remain unchanged.

(c) Third phase: The concentration of antibiotic decreases in the medium.

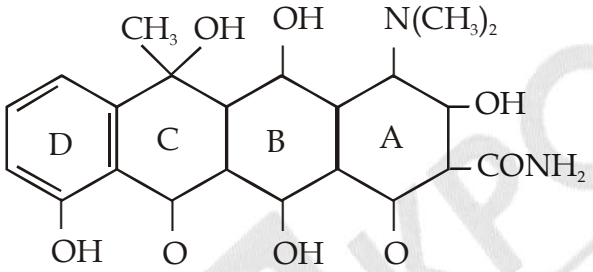
(ii) Recovery: When they fermentation cycle is completed the whole batch is harvested for recovery broth. Once the fermentation is completed the growth is separated from fungal mycelium and processed by absorption, precipitation and crystallization to yield final product. This basic product can then be modified by chemical procedure to yield a variety of semi synthetic penicillin such as ampicillin amoxyicillin etc.



Pencillia-G

(c) Tetracyclin – Due to broad antibiotic activity of the tetracycline these compounds are widely used in medicine. The tetracyclin are also valuable for relatively low toxicity.

There are 3 important microorganism namely S. aureafaciens S. ramosus and nlocardia which form tetracyclin.



Media composition: Sugar corn steep liquor $CaCo_3$ NH_4Cl is normally used for product of hetracyclin. The pH is kept at 6-7 yield depends upon pH, and composition of medium.

Chlorotetracyclin is isolated from the culture fluid by extraction, precipitation or absorption. This poorly soluble in common organic solvent but is soluble in water and insoluble in ether. Chlortetracyclin is more valuable than streptomycin or penicillin.

In 24-28 hrs of cultivation submerged spores of streptomycetes develop and then secondary mycelium grows. This process requires aeration. The ammonium salt and nitrates favour the biosynthesis of oxylebacyclin starch, glucose, maltose, glycerol are also used as carbon source. The consumption of carbohydrates increases the pH of medium decreases and biosynthesis of antibiotic slows down.

Chlorotetracyclin and oxytetracyclin inhibit phosphorylation process by preventing the inclusion of phosphorus into nucleic acid. The synthesis of protein is stopped.

Enzymes:

Pectinases: The wild disease in crops induced by fusarium species produced pectinases.

Two groups of pectotylic enzymes are produced by plant pathogens. One is pectinesterase or pectin methyl esterase which breaks the ester bond.

The other enzyme is transeliminas as which breaks 1, 4 glycosidic bond, thereby unsaturating the ring between 4^{th} and 5^{th} C atoms.

(i) Production:

A number of commercial firms produces fungal pectinases using A.niger and penicillin sps. The mycelium is developed on a medium containing pectin a nitrogen source, such as yeast extract, peptone etc fermentation with A. niger runs for 60-80 h. in fled batch cultures at pH 3- 4 at $37^{O}C$ using 2% sucrose and 2% pectin.

Purification is simple. Since the pectinase is present both in the cells as well as excreted to the medium, the enzyme is recovered from both saurces.

Harvest and Recovery: At the time of harvest, the mycelium is dried and its pectin as is extracted with water. It is then precipitated from its aqueous solution and from the culture broth.

Uses: Pectinases are used to clarify fruit juice and grapes must for the maceration of vegetables and fruits besides extraction of olive oil.

Invertase: This enzymes splits sucrose into glucose and fructose. Saccharoincyles Sp. are the richest source of enzyme invertase. It is produced in industries from baker's or brewers yeast.

Production: Industrially, enzyme invertase is produced by special strains of yeast which grow on bottom. The medium contains sucrose phosphate buffer and other minerals. The pH is adjusted to 4.5 and fermentation is carried out for 8h at 28-30°C.

Recovery: The yeast cells are filtered off compressed plasmolysed and autolyzed. The extracted invertase may be dried or held in sucrose syrup. It can also be purified by dialysis.

Uses: It is used in confectionary to make invert sugar for preparation of ice creams.

Proteases:-

The group of enzymes catalyze the hydrolysis of protein molecules. The enzyme is actually a mixture of proteinases and peptidases.

(i) Microorganisms involved: Various bacteria such as Bacillus

Pseudomonas, Clostridium and fungi are the sources of proteolytic enzymes.

(ii) Production: A high yielding strain is selected and inoculated in special culture media contains 2-6% carbohydrate protein and mineral salt. It is incubated for 3-5 days at abt 37°C with adequate aeration. The filteration is concentrated and the enzymes are used in this form from the culture is purified and absorbed auto. Some invert material. Many different media such as those containing wheat bran alfalfa meal are proved to be better for protease production.

(iii) Uses: Bacterial proteases help in digestion of fish liver to release fish oil to the tenderization of meat.

Steroid

These are complex organic compounds. Microbial preparation of many steroids by their enzyme action at the specific site led to the synthesis of novel varieties of steroids. Such processes are more viable and specific.

Steroid transformation is different with that of microbiological process due to the fact that in cater that organic acid solvent are synthesized from the ingredients in the medium which also serve as substrate for growth and reproduction of microorganism.

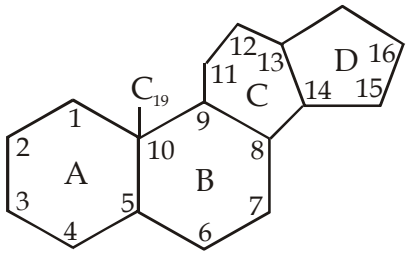
(i) Microorganism: Several fungi such as Rhizopus, Aspergillus penicillium and yeast are some of the important organism of steroid biotransformation.

(ii) Production: The production of steroid can be carried out by following steps:

(a) Cultivation of microbes: The appropriate microbe is grown in short period of time. generally glucose or sucrose are recommended as carbon source.

(b) Incorporation of inhibition into medium: A suitable amount of inhibition are added to inhibit the undesired enzyme activities.

(iii) Separation and purification: For this regular sample is to be analyzed chromatographically using TLC. The Spots are eluted and their quantity can be measured spectro photometrically. For this the product is extracted with a suitable solvent and then purified by using column chromategraphy or crystallization.



Structure of Steroid

Q.3 Write Short note on "Protein engineering"?

Ans. Many of the traditional fermentation processes for the manufacture of antibiotics enzymes and amino acids do not utilize wild type microbes because the yield of product is low. Rather then producing organism is subjective to successive rounds of mutagenesis until high-yielding strains are isolated.

The ability to alter amino acid sequence of a protein to meet a specific requirement is comes in Protein engineering.

Using identical techniques variants of clinically useful proteins have been isolated which have improved therapeutic properties.

Increased protein stability

The goal of many protein engineering programmes is to achieve increased protein stability. This may be either increase half life or thermostability of an enzyme used in an enzyme reactor increased shelf life of a protein for therapeutic use or resistance to inactivation resulting from oxidation of important amino acid residues. Increased thermostability is particularly important since there are benefits from operating enzymes reactors at elevated temperature. For example, reaction times are shorter and there is less risk of microbial spoilage of enzyme.

Examples – Enchanced specific activity of recombinant interferon.

Altering the kinetic properties of enzyme

Effective use of these enzymes requires alternation of enzyme kinetics.

For most applications the enzymes are used under conditions where there

is a vast excess a substrate and maximum reaction rate is the only parameter of importance. High concentration of product can inhibit the enzyme, leading to a decrease in the reaction velocity.

Example: Altering the pH dependence of enzyme catalyses.

One prospect for protein engineering is the tailoring of the pH dependence of enzyme catalysis to optimize activity in industrial process.

Chemical studies, shown that the pH dependence of catalyses by serine proteases alter with changes in surface charge. So a possible way of modifying pH dependence would be to alter the **electrostatic environment** of the active site by protein engineering and so change the pka values of ionic catalytic genes.

Increasing the Bioactivity of Proteins

Many human proteins are being tested as potential therapeutic agents.

Protein engineering is now being used to generate second generation variants with improve pharmaco-kinetics structure, stability and bioavailbility one example is oxidation resistant variant of antitrypsin.

Examples: Increasing the potency of hirudin

Hirduin, a 65 amino acid, polypeptide secreted from the leech salivary gland is a potent throw big inhibitor. Recombinant hirudin is an anti-coagulant when aspargine was converted to lysine or arginine the potency in a test tube clotting assay increased favour folds. When tested in an animal model the anti throm botic effects of the variants 20-fold higher than the native protein and five times higher than heparin.

Macromodification to proteins:-

In site-directed mutagenesis the aim is to replace one or more, amino acid in a protein sequence which will in same way improve the protein.

Example:- Novel interferons. Human interferons - α and β- elicit a number of biological response such as conferring resistance to viral infections macrophage and killer cell activation, inhibitory of cell proliferation and modulation of the immune response.

By constructing hybrids between different interferons, if may be possible to produce novel interferons with superior clinical properties e.g. increased potency or decreased toxicity.

Hybrids have also been constructed in which portion of the interferon-β molecule have been replaced with corresponding segment from interferon – α. Genetically engineered antibodies have been produced in similar way.

Q.4 Give an illustrated accounts on methods and effects of enzyme/Cell immobilization?

Ans. The term Immobilization means unable to more or stationary. An enzyme or tell physically attached to a solid support over which a substrate is passed and converted to product.

It is just like blocking the movement of an individual. During the lost 20-25 yrs the cell immobilization techniques with its origins in enzyme immobilization has attracted the attention of several research group.

Purpose of Immobilization

In cell immobilization

- We can rescue the cells or enzymes
- Recovery of product is easier.
- Purified enzyme/cells can be stored for long period without losing their effectiveness.
- Many purpose of cell immobilization is high productivity of desired substances.

Characteristics of immobilized Cell–

In immobilized cells enzymes are active and stable for a long period. In immobilized cells growth phase and product formation phase are uncoupled. The uncoupling means that productive cells cannot compete with the non productive cells.

Metabolism in immobilized cell:-

3 forms of metabolism can exist are:- Aerobic, anaerobic and anoxic.

"Aerobic" systems are limited in their degradation rate. Heavy biomass build up high oxygen demand.

Anoxic metabolism consist of placing microbes into a growth substrate

and placing the combination into an aquifier containing nitrate and sulphate.

Anaerobic metabolism occurs when microbes used toxic compounds as its main energy saurce and forms CO_2 and H_2O.

Methods of Immobilization:-

Immobilization can occurs either as a natural or through, artificial phenomenon.

The carrier matrices used for immobilization is usually inert polymer or inorganic material.

Ideal carrier matrics has the following property:-

- Low cost, inertness physical strength stability reduction in product inhibition reduction in microbial contamination and non specific adsorption.
- Choice for cell supporting material for application is related to following points.
- Bioreactor configuration.
- High carrier activity
- Low cost immobilization
- Physiological and environmental safety of the material used.
- Low, affinity of contamination

Various methods used for immobilization may be grouped into following types:-

(1) Adsorption

(2) Covalent bonding

(3) Cross linking

(4) Entrapment

All of these method have same purpose to retain high cell concentration with in a "certain defined region of space" such as bioreactor giving high productivity of a system.

(i) Adsorption: Least expensive and mildest immobilization method, uses

weak interaction forces such as H-bond, hydrophobic bond, van dewal usual forces to immobilized cells. The sensitivity of this interaction to pH makes the leakage of cell immobilized by this technique.

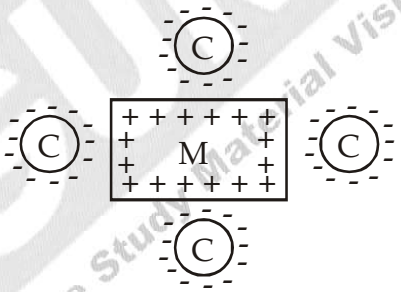
This was the first example of cell immobilization "Hattori "reported the binding of *E. coli* cells by an ion exchange resin so a variety of microbial cells were immobilized by adsorption on different supports like Kieselagur, wood, glass ceramic, plastic etc.

Klein have review the immobilized cells by adsorption.

Since the adsorption phenomenon is based on electrostatic interaction (Van del wal) between the charge support and microbial cell.

Unfortunately the actual charge on support surface is still unknown and this limit the proper choice for microbial attachment along with charge on cell surface.

All ceramic supports are comprised of varying proportion of oxides of Al_2O_3, SiO_3 Mg Zr etc result in bond formation between the cell and support. Several procedures of cell adsorption based on pH dependence are separated in the literature.



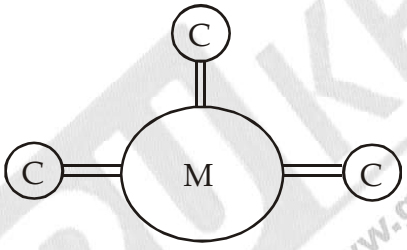
M = Matric
C = Cell

(2) Covalent bonding: The mechanism involved in this method is based on covalent bond formation between activated inorganic support and cell in the presence of a binding agent for covalent linking.

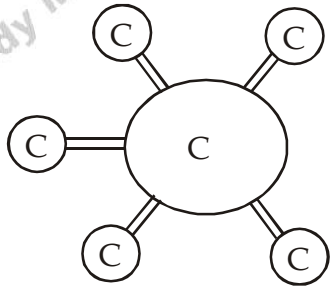
Cells of *S. Cerevisiae* were immobilized by coupling silinzied SiO_3 beads. The reaction requires introduction of reactive organic group an inorganic SiO_3 surface for the reaction between the activated support material and yeast cells. This organic group condense with alcohol grp., on SiO_3 surface. As a result the organic group is available for covalent bond

formation on the surface of SiO_3. It is also achieved by reacting the SiO_3 surface with glutaraldehyae and hydrogen cynide.

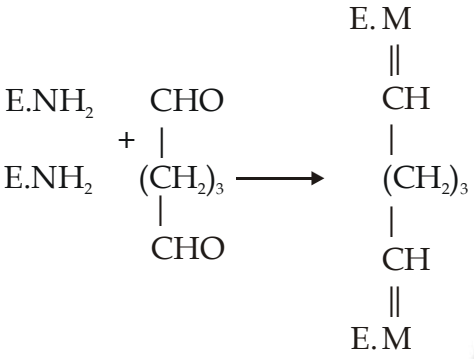
"Cabral" using inorganic carrier system have developed a system of enarg general interest. The addition of Ti^{+1} or Zr^{+}. Chloride salts to water results in pH dependent formation of gelatinous polymeric metal hydroxide precipitates where in R-OH or Oxides group bridge the metal by conducting such a precipitate in a suspension of microbial cells, the cells have been entapped, in the gel like precipitate formed. In continuous operation. Ti $(OH)_3$ immobilized cells of acetobacter were employed to convert R-OH to acetic acid.



Cross Linking: Microbial cells can be immobilized by cross linking each other with bi or multi functional reagent such as glutarpldehyde. A part from chemical cross linking, procedure employing physical process such as flocculation and pellelization also bemifits the immobilization technique because strong adherence forces of same cell culture.



The Fern Cross: Cross linking word is mostly used in the synthetis polymer science field it usually refers to the use of cross-links to promote a different in the polymers physical properties.



Cross linking of an enzyme is very expensive and insufficient since many molecules simply act as support for others so it generally performed, in conjunction with other method of immobilization. It not only link molecules to each other, but also link them to an inert carrier.

Glutaraldehyde has often been used to attach. Substrate auto carriers such as cellulose.

Bis diazobenzidine – 2, 2, - disulphuric acid. Glutaraldehyde Hexamethyl diisocyanate, Diazocbenzi dine are some cross linking reagents.

In Cross linking bond link one polymer chain to another. They can be covalent bonds or ionic bonds.

A novel method has been developed in which enzymes are immobilized as an enzyme polymer membrane, formed an the inner wall of the microchannel by cross linking method, resulting anicro reactor shows an excellent reaction performance and stability against denaturing agents.

Chemical methods of cross linking involves covalent bond formation between the cells by multifunctional reagents such as glutaraldehyde, toluene diisocyanate. However, toxicity of such type of reagents is a inviting factor for living cells and enzymes. Albumin and gelatin have been used to provide additional as spacers to minimize the close, proximity problems that can be caused by cross linking a single enzyme.

Physical cross linking of cells is well known method and lead to high cell density. Flocculating agents, such as polyamines polyethylene amine, polystyrene sulphonates and various phosphates have been used for immobilization because the absence of mechanical properties and poor stability are some initation.

Different types of cross linkers used in cross linking are:-

(1) Homobifunctional (2) Heterobifunctional

In Homo functional linkers have two identical reactive groups and often are used in one-step reaction to cross link protein to each other or to stabilize quaternary structure. It often results in self conjugation, inta-molecular cross linking.

Heterofunctional linkers possess two different reactive groups that allow sequential conjugations helping to minimize undesirable polymerization or self conjugation.

An ideal cross linkers have following properties:

(1) Chemical specifity.

(2) Spacer arm length: Required because steric effects represent the distance between potential reaction site for cross linking.

(3) Reagent water solubility and membrane permeability.

(4) Thermoreactive or photoreactive groups.

(5) Same or different reactive groups.

The use of cross linkers have made the study of receptor much easier. By derivatizing a receptor with a cross linker before or after contact with the ligand, it is possible to isolate the receptor-ligand complex.

The use of radio is divisable cross linker make if possible to identify a particular receptor by autoradiographic detection.

Cross linking is most often used to enhance other methods of immobilization normally by reducing cell leakage in other system.

Entrapment: Most studied method of immobilization is the entrapment of cells in polymer matrices. Matrices used are agar cellulose and its derivatives (resins, gelatin, PAM, polyester, polystyrene).

Advantages: Prevent wash out of biomass.

- Easier to operate in immobilized reactor.
- More biomass can be produced per volume of reactor.
- Cells live much greater period of time.

- More resistant to toxic.
- Many useful microbes will grow when immobilized but not in suspended reactor.
- Higher rate of degradation.
- Allow for longer microbial diversity.
- Could provide a more stable gene pool and enhance rates of genetic transfer.
- Shorter overall reaction time.
- Constant product quality.
- Improve substrate utilization.

Disadvantages:-

- Immobilization means additional cost.
- Immobilization often adversely affects the stabilization activity of the cells in such cases, suitable immobilization protocols should be develop.
- This approach cannot be used when one of substrate is insoluble.

Q.5 Write down different methods of enzyme stabilization including chemical modification?

Ans. Chemical modification is available for altering protein/enzyme/cell properties.

By cross linking with '**glutaraldehyde**' can be enhance the biocatalyst stability. By treatment of enzyme with 'PEG' (Polyethylene glycol) increase the solubility in organic solvents.

Chemical modification has been exploited for the incorporation of co-factors onto, protein, templates and for atom replacement in order to generate new functionality such as the conversion of a hydrolase into a peroxidase.

Chemical modification of protein surfaces improve their immobilization, on ionic exchangers.

The strongest **fibrinolytic proteases** in the six enzyme protein purified from earth worm "*Lumbricus rubellus*" has been modified chemically with human serving albumia. The modified enzymes cost the antigenecity of the native enzyme and reacted with antiserum against human serves albumia, fragmented and can't ligate with native enzyme to form precipitation lines fused with each other.

The Cnjugate was more resistant to inactivation by proteases inhibitor in rat plasma.

Enzyme labeled chemically which make them specific usually this procedure is used to identify a residue at the active site of the enzyme or other specific site on an protein such as an effector site or site where binding to another protein or nucleic acid occurs.

Example: In serine proteases and esterases which generally reacts with reagent like Di-isopropyl Flurophosphate for short and sulphony fluoride.

- Reagents should be selectively absorbed i.e. form a complex with the enzyme by non covalent binding and then react covalently.

For example at acidic pH iodo acetate reacts with N 'with his' or N^3 with his^{12} of ribonuclease. Reaction will be eight times with his ^{119} and considerably faster than free histidine.

Site directed reagents typically and chemically much less reactive than general modification reagent one uses an α-bromo and α-chloro carbonyl compound rather than iodo acetate/10 doacetamide.

Activity and stability of '**Chloroperoxide**' can be improve by chemical modification by using githoconic anhydride malic anhyaride or pthalic anhyaride.

By treatment with these chemicals thermo-stability can be improve by one to two fold and solvent tolerance. These modification can change the secondary structure of chloroperoxide.

Fluorescence spectroscopy proved that these modifications changed the environment of the reaction.

Chloroperoxidase is a heavily glycocylated monomeric heme protein secreted from filamentous fungus *aureomyces fumago*.

Such this technique very stabilized enzyme derivative may be achieved.

Stability of enzymes:

Purified enzymes generally cannot be stored for long periods without losing their effectiveness. Native enzymes are subjected to inactivation by chemical physical and biological factors, inactivation can occur either in storage or during use.

So there is need to stabilized enzymes because of high cost of enzyme production. Moreover, it should be possible to stabilize enzymes in commercial process in such a way that they can be used over and over again.

Methods of achieving enzyme Stability

There are many methods of achieving enzyme stability:

(1) Stabilization of Soluble enzymes: This is accomplished by a additives or by chemical modification. This improves stability of enzymes against physical and chemical agents without decreasing their solubility.

Such types of enzymes can be successfully stored but since they are still soluble they cannot be recycled after use.

In some cases enzymes cannot be successfully immobilized but must be used in soluble four.

Example:- Enzymes used in liquid detergent diagnostic reagents and as food additives. In these cases stabilization of enzymes is necessary in order to prolong shelf life of soluble enzymes. Substrate stabilization, stabilization by salt, solvent stabilization by polymers addition. By chemical modification are some methods of stabilization.

(2) Stabilization by immobilization:-

Bonding of an enzyme to another enzyme or to carrier must takes place without changing its so structure and active site of molecule substrate specificity and reaction specificity are not loss during the immobilization process.

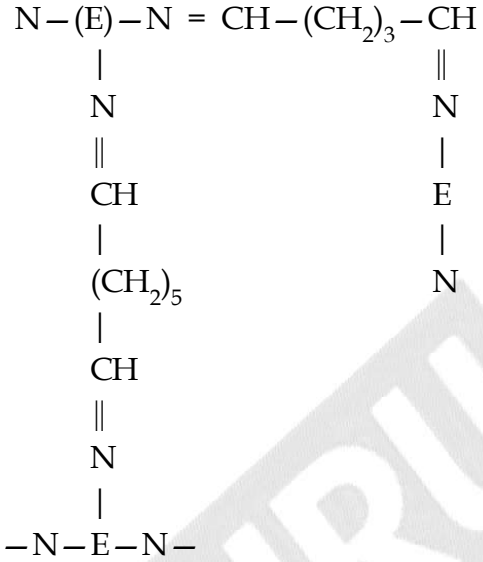
Different approaches of enzyme immobilization are:-

(a) Cross linked enzymes:-

"Gluturaldehyde" is commonly used as a polymerising agent.

This compound react with amino group of enzyme. (e.g. alcohol dehydrogenase) in formation of Schiff bases.

Cell immobilization can be accomplished with hexamethyl di-socyanate which reacts with enzyme by bond formation (peptide bond).



Cross linking of Enzyme with glutaraldehyde

(b) Carrier bound enzyme:-

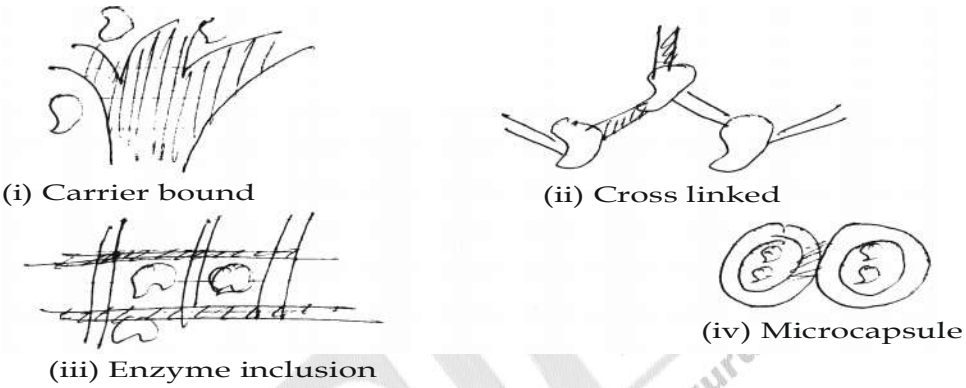
In this process bonding of enzyme can be done by adsorption onto carrier molecule.

It is most frequently used method in commercially.

- Structure of carrier, type of bonding site, particle size and surface volume ratio must be suitable for individual enzyme.
- Bonding by adsorption carrier least change or conformational alteration of the protein enzymes and is applicable of wide variety of enzymes.
- "Disadvantage" of this procedure is weak bonding and any temperature change in the bioreactor causes elution of enzymes from carrier and so activity loss.

'**Ionic exchange**' can be used in stabiliztion of enzymes in which enzyme can be held to carrier by ionic bond

It is easily accomplished method with low activity loss although changes in pH or ionic strength can also results in enzymes loss from carrier.



(3) Encapsulation of enzymes: When enzymes are physically enclosed at in gel/fibrous polymer there must be pores which are so small that the enzyme molecule cannot be washed out which are still large enough to permit the capsulation.

Diffusion of Low molecular substrate and products across the barrier.

Water insoluble polymers such as PAM, PUA and starch can be used to surround enzymes.

There gel can be cut up and can be used in a batch process.

(4) Microcapsules: Free enzymes or free cells can be surrounded by semi permeable polymer membrane.

Production takes place by polymerization reaction at the surface of aqueous enzyme droplets which are suspended in a non water soluble organic phase.

Inclusion in fibrous polymers is similar to microcapsulation. An emulsion of enzyme solution and organic water immisible both mix and forms fibrous polymers.

Section B
Microbial production of therapeutic

Q1 How enzymes are useful in industries? Write different applications of enzymes?

Ans. Application of enzymes in medical

Enzymes present in blood plasma or serum are carried ant routinely in most clinical chemistry laboratories and these play an important role in diagnosis.

For each plasma enzyme, there is a normal concentration range and hence a "normal range of activity", which can be determined.

If the cells of a particular tissue are effected by disease in such a way that many of them no longer have intact membranes then their content will leak out into the bloodstream at an increased rate and the enzymes associated with those cells will be found in the plasma in elevated amount since many enzymes or isoenzymes are characteristically associated with the cells of certain tissue plasma enzymes assay can help to identify the location of damaged cells.

"**Alanine transaminase**" (ALT) formerly known as glutamate pyruvate transaminase (GPT) catalyse the reaction

Alanine + 2- oxoglutarte pyruvate + glutamate.

This enzyme may be assayed by coupling via the product, pyruvate to an H-catalysed indicator reaction. ALT is found in high concentration in liver cells and in much smaller concentration elsewhere. Hence, a markedly raised plasma activity indicates a severe liver disease, usually viral hepatitis or toxic liver necrosis.

"**Alkaline phosphate**" catalyse the hydrolysis of organic phosphates at

alkaling pH is found principally in bone, liver kidney intestinal wall, lactating mammary gland and placenta.

Alkaline phosphatase in the liver is found associated with the cell membrane and so high plasma concentration of the liver isoenzymes indicates cholestosis (i.e. blockage of bile duct with a resulting back flow through the liver into the bloodstream) rather than simply damage to the liver cells.

"**Creatine Kinase** (CK)" also known with a degree of tantology as creatine phosphokinase (CPK) catalyses the reaction

Creation + ATP Phosphocreatine + ADP.

The enzyme is found mainly in the heart and skeletal muscle, and in brain, it is a dimer, made up from two types of polypeptide chain (B or M) thus B forms may be found.

'BB' the main form of brain, but also found to same extent in nerve tissue, thyroid, kidney and intestine. 'MB' found in heart muscle and the diaphragm, but not normally present in significant amount in skeletal muscle, and MM found in both heart and skeletal muscle. On electrophoresis, BB isoenzymes travels nearest to anode, the form is not normally present in plasma. In addition to electrophoresis affinity chromatography is proving useful in the analysis of CK isoenzymes.

MM form alone is found in high concentration in plasma when skeletal muscle cells are damaged (e.g. in the early stages of muscular dystrophy).

The digestive enzymes, trypsin, triacylglycerol lipase and α – amylase are produced in the panceas and these are found in increased amount in plasma in certain disease of the pancreas, particularly acute pancreatitis.

Cholinesterose is another enzyme which is usually assayed in plasma.

The enzyme catalyses the hydrolysis of choline esters such as acetyl choline.

Acetyl Choline + H_2O acetic acid + Choline

Enzymes as reagent in clinical chemistry:-

D-glucose in blood and other physiological fluids is analysed by means of procedure involving glucose oxidase. These methods are specific for

β-D-glucose but, except when solutions are freshly prepared.

Enzymes as analytical reagent:

Every enzyme catalyses a reaction which is both substrate-specific and product specific, because of this enzyme are extremely valuable as analytical reagent.

Another characteristics of enzyme catalysed reaction which makes them suitable for analytical application is that they proceed under relatively mild conditions (e.g. at neutral, pH and at around roam temperature).

Disadvantage in the use of enzyme is that they are often expensive and sometimes difficult to obtain. Hence if is important that they are not used in a wasteful fashion.

Principles of enzymatic Analysis

(i) End-point Methods: End-point methods of enzymatic analysis, also called total range or equilibrium methods, allow the reaction to go to equilibrium, whereupon the amount of product formed is determined.

Analysis should be performed under conditions where the concentration of the added enzymes is high to ensure rapid progress towards equilibrium, while the concentration of the substrate should be low enough.

(2) Kinetic methods: Most procedures for enzymatic analysis involve steady state kinetic methods, the initial velocity of the reaction is determined at fixed temperature and pH, and used to calculate the concentration of the substances being investigated.

(3) Immunoassay methods: Enzymes may also be involved in other immunoassay procedure, although it must be stressed that the role of enzymes in these is a secondary one they are used to replace radioisotopes as markers since they are not such a hazard to health and can be detected by techniques which are more generally available.

Any enzyme which has a sensitive and convenient assay procedure can be employed. There are currently two main types of these enzyme immunoassay (EIA) procedure:- ELISA and EMIT.

ELISA: resembles RIA in that it involves a heterogenous sys which must

undergo separation as part of the analysis procedure e.g. A pure specimen of antigen may be labeled by attaching it to an enzyme in such a way that the activity of the enzyme is not affected.

EMIT: is a hang gaseous procedure since no separation of component is required. As with same types of ELISA, an enzyme is attached to a specimen of antigen to act as a label.

In most types of EMIT procedure, enzyme activity is lost completely an binding to the antibody either as a result of steric hindrance or conformational changes.

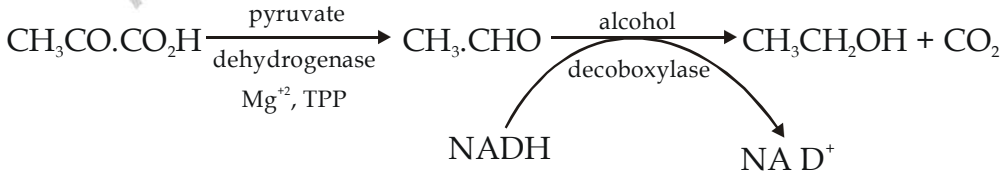
Enzymes may also be used as an alternative to radioisotopes as marker in immunoassays. Such procedures (enzyme-immunoassay) have been used for the determination of a variety of proteins and hormones.

Application of enzymes in Industry

Enzymes may be used in industry as component of living cells or after isolation, in free or immobilized forms. All of these may be referred to as "biocatalysts." Industrial processes have generally utilized enzymes in aqueous solution. However, much attention is now being given to possible applications involving enzymes which function in non-aqueous media.

Food and drink industry:

The traditional use of yeast in the baking and brewing industries arose because they contain the enzymes for alcoholic fermentation in consumer with other organisms. They metabolize hexose sugars to produce pyruvate but whereas animals convert this to lacitate under anaerobic conditions the anaerobic and product in yeast is ethanol, with CO_2 being involved.



In the "**baking of bread**" the preliminary process involves the mixing of wheat flour with yeast and water. Starch consist of D-glucose units inked by α – 14 glycosidic bonds with α 1, 6-bonds at branching points;

the enzymes α- amylase and β-amylase present in the flour cleaver same of α-1,4 bonds the eventual product being glucose, maltose which cannot be broken dawn further because of the presence of α-1,6 bonds. Glucose can then be metabolize by yeast. However, wheat flour often has a low α-amylose content. So it may be supplemented with malt flour or by fungal α-amylase. Wheat α-amylase is very heat stable, so it continues to act for a time during the baking process, this may lead to starch breakdown and cause the bread soggy.

For this reason, α-amylase is inactivated by brief treatment with superheated stem. "Proteases" from A. aryzae may be introduced to cause breakdown of wheat protein (gluten) thus shortening mixing time.

In "**brewing**" imalt produced by barley seeds. Reserve starch is broken down by amylase, to give glucose + maltose. Bacterial α-amylase, which is more heat stable than wheat α-amylase, is of increasing importance in the brewing industry. Fungal exo-1,4 α-glucosidase (amyloglucosidase) may also be added since this cleave α-1,6 and 1, 4 glycosidic bonds and so increase the yield of glucose + maltose.

In Industrial production of glucose from starch is first solubilized any partly degraded by bacterial and α-amylase and their with fungal amyloglucosidase can cleave both types of glycosidic band found is starch so gives good yield of glucose. Glucose may also be obtained from cellulose containing waste products by treatment with cellulose.

Invert sugar, a mixture of glucose + fructose is produced from sucrose by the action of yeast "β Fructofuranosidase" better known as "invertase." In confectionary crystalline sucrose is coated with chocolate and invertase causing the conversion of sucrose to invert sugar, Glucose invert sugar may also be produced from by the action of yeast Gluctose- isomerase.

Certain amino acids essential for animal growth are found in low amounts in the proteins of same food-stuffs. However, animals can only utilize L-amino acid whereas d and l-amino acids are produced by chemical processes.

The procedure introduced for the production of L-amino acid from racemic mixture is of great significance –acety H and L –amino acids are passed through a sys containing **"aminocyclase"** immobilized on a carrier, causing the deacylylation of L-form, which can then be separated,

the unchanged D-form is than subjected to racemization. The clarification of cider, wines and fruit juice is achieved by treatment with fungal pectinases. The pectins of fruit and vegetables play an important role in jam making and other processes by bringing about gel formation. However, they cause fruit drinks to be cloudy by preventing the flocculation of suspended particles. Pectinases are group of enzymes including "polygalacturonase" which breaks the main chain of pectinesterases, which hydrolyse inethyl esters.

Cheese production involves the conversion of the milk protein casein to paracasein by a defined hydrolysis catalysed by chymosin (rennin). In the presence of Ca^{+2}, paracaseiu clots and may be separated from the why, after which the clot is allowed to mature still in the presence of chymotrypsin, to form cheese. "Proteases" from animal, plants and microbes have been tried on their own.

"Papain" is used as a meat tenderizer. Papain may also be used in brewing industry to prevent chill hazes, caused by precipitation of complexes of protein and tannin at low temperature.

Application in other industries

Enzymes may also be of great value in pharmaceutical, industry e.g. for the conversion of naturally occuring penicillin G to 6- amino penicillanic acid. This reaction cleaves the side chain of the substrats at mildly alkaline pH values. Many commercial processes have been developed for the synthesis of 6-APA including the use of *E. coli* immobilized in polyacrylamids gel and the enzyme entrapped in fibres of cellulose acetate. This importance of 6-APA is that new side chain can be attached to give a variety of semi-synthetic penicillin e.g. amphicillin, a broad spectrum antibiotic. This may be done by chemical processes or by the use of enzyme under slightly acidic conditions, to favour the back reaction enzymes from different bacteria may be applied to catalyze the forward and backward reaction to make use of their slightly different characters and specificities.

"Washing powders" incorporating bacterial proteases have been available for several years. The enzymes are stable to alkali, high temperature detergents and bleaches. They will attack blood and other protein stains.

Bacterial proteases are also used in the "leather and textile, industry to losses wool and enable it to be separated from hide.

Q.2 What are interferons? How they are isolated? What is the mechanism of protection by interferons ?

Ans. Today, the "genes" (mostly complementary DNAs [cDNAs] for over 1,000 different proteins that are potential human therapeutic agents have been cloned. Most of these sequences have been expressed in host cells, and currently around 750 are undergoing clinical trials for the treatment of various human diseases.

Pharmaceuticals

Isolation of Interferon cDNAs: A number of different strategies have been used to isolate either the genes or cDNAs for human proteins, the target protein is isolated and a portion of the amino acid sequence is determined. From this information, a DNA coding sequence is deduced. The appropriate oligonucleotide is synthesized and used as a DNA hybridization probe to isolate the gene or cDNA from either a genomic or cDNA library. Alternatively, antibodies are raised against the purified protein and used to screen a gene expression library. For human proteins that are synthesized primarily in a single tissue, a cDNA library from the messenger RNA of this tissue is enriched for the target DNA sequence. For example, the major protein synthesized by the islets of Langerhans of the pancreas is insulin; 70% of the mRNA fraction isolated from these cells encodes insulin.

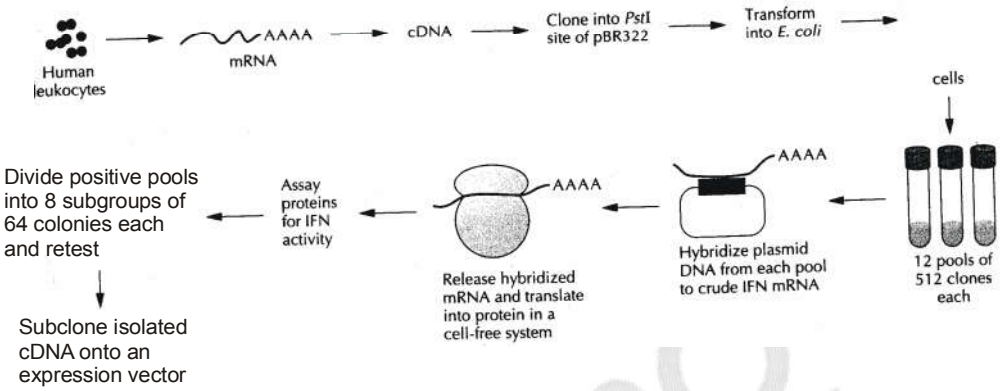
It was often necessary to devise innovative approaches to isolate human genes or cDNAs, especially when the protein encoded were found in very low concentrations or when the site of synthesis was not known. The human interferon (IFN) proteins, which include the α-, β-, and γ-interferons. (IFN-α, IFN-β, and IFN-γ), are naturally occurring proteins, each one with some what different biological activity. When the IFN cDNAs were initially isolated in the early 1980s, very little was known about the encoded proteins (interferon was originally thought to be a single protein), so a novel scheme had to be devised to overcome the scarcity of both the mRNAs and the proteins. The isolation of IFN cDNAs

included the following steps.

1. Size-fractionated mRNA was isolated from human leukocytes, reverse transcribed, and inserted into the *Pst*I site of plasmid pBR322.
2. The approximately 6,000 clones that were produced following transformation of *Escherichia coli* were divided into 12 pools of 512 clones each. Pools of clones rather than individual clones were tested to speed up the identification process.
3. Each pool was hybridized to a crude IFN mRNA preparation.
4. The hybridized input mRNA was separated from the cloned DNA-mRNA hybrids and translated in a cell-free protein synthesis system.
5. Each translation mixture was then assayed for IFN antiviral activity. The pools that showed IFN activity contained a clone with a cDNA that hybridized to IFN mRNA.
6. Positive pools were divided into eight subgroups of 64 clones each and retested. This subgrouping process was reported until a clone with the complete cDNA for a human IFN was identified.

Subsequently, whenever large quantities of the IFN are required, the IFN cDNAs can be sub cloned into an *E. coli* expression vector and expressed at high levels.

Human Interferons: After the isolation of the first IFN gene, researchers found that there are a number of different IFNs. On the basis of chemical and biological properties, the IFNs can be classified, into three different groups. IFN-α, IFN-β, and IFN-γ. The proteins IFN-α and IFN-β are synthesized in cells that have been exposed to viruses or viral RNA; IFN-γ is synthesized in response to cell growth-stimulating agents. IFN-α is encoded by a family of 13 different genes, IFN-β is encoded by two genes, and IFN-γ is encoded by a single gene. The IFN-α subtypes have different specificities. For example, the antiviral activities of IFN-α2 and IFN-α1 are approximately the same when assessed with a virus-challenged bovine cell line, but IFN- α2 is seven times more effective than IFN- α1 when human cells are treated with virus. IFN- α2 is 30 times less effective than IFN- α1 when mouse cells are used in this assay.



Isolation of IFN cDNA

Different members of the IFN- α gene family vary in the extent and specificity of their antiviral activity. Theoretically, this can be achieved by splicing a portion of one IFN- α gene with a DNA sequence from a different IFN- α gene to create a hybrid protein that exhibits novel properties, i.e., properties different from those of either of the contributing genes. In one study, hybrid genes from IFN- α2 and IFN- α3 were constructed in an effort to create proteins with novel IFN activities.

Comparison of the sequences of the two IFN- α cDNAs indicated that they have common restriction sites at positions 60, 92 and 150. Digestion of both cDNAs at these sites and ligation of the DNA fragments yielded a number of hybrid derivatives of the original genes. These hybrids were expressed in *E. coli*, and the resultant proteins were purified and examined for various biological functions. When tested for the extent of protection of mammalian cells in culture, some of the hybrid IFNs were found to have greater activity than the parental molecules. In addition, many of the hybrid IFNs induced test cells to synthesize (2′-5′) oligoisoadenylate synthetase. This enzyme generates (2′-5-)-linked oligonucleotides, which, in turn, activate a latent cellular endoribonuclease that cleaves viral mRNA. Other hybrid IFNs had an antiproliferative activity against various human cancers that was greater than that of either of the parental molecules. The creation of these hybrid IFNs demonstrates that potential therapeutic molecules can be constructed by combining functional domains from related genes.

Human Growth Hormone

Human growth hormone was one of the first therapeutic proteins to be approved for human use. The recombinant form of this protein is produced in *E. coli* and is identical to native pitutary-derrived human growth hormone. Infants and children who lack sufficient endogenous levels of growth hormone, patients with chronic renal insufficiency (defective kidneys), and individuals with Turner's syndrome respond to treatment with growth hormone, which stimulates tissue growth (including bone growth), increases protein synthesis and mineral retention, and decreases body fat storage.

The strategy of designing proteins by either functional domain shuffling or directed mutagenesis can be used to augment or constrain a protein's mode of action. For example, native human growth hormone (hGH) binds to both growth hormone and prolactin receptors on a number of different cell types. To avoid unwanted side effects during therapy, it is desirable that hGH bind only to growth hormone receptors. Because the segment of the growth hormone molecule that binds to the growth hormone receptor overlaps but is not identical to the portion of the molecule that binds to the prolactin receptor, it should be possible to selectively decreases the binding to the prolactin receptor.

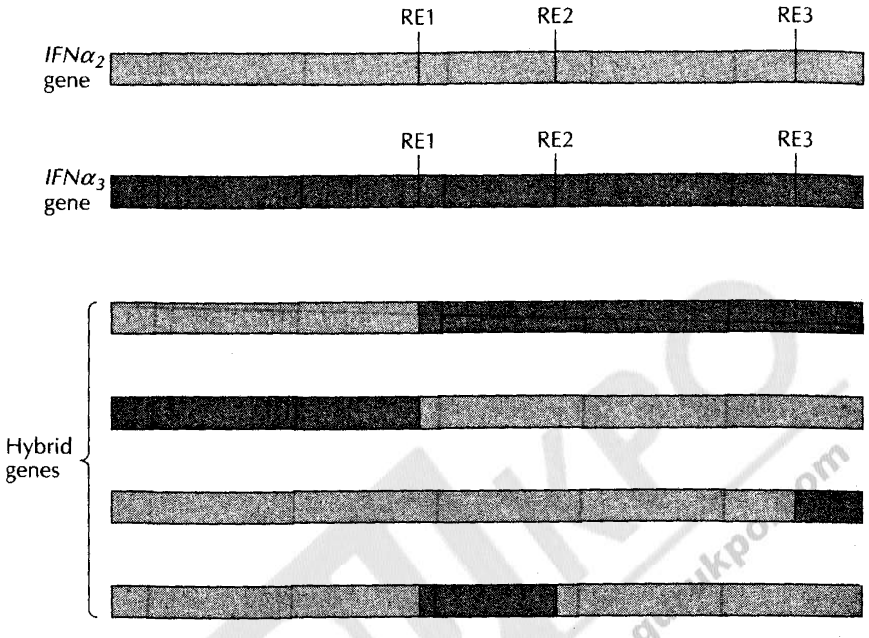


Fig. Structure of the IFN- α2 and IFN- α3 genes and four hybrid genes

Site-specific mutagenesis of the cloned hGH cDNA was used to change some of the amino acid side chains that act as ligands for Zn^{2+}, because this ion is required for the high-affinity binding of hGH to the prolactin receptor. As hoped, these modifications yielded hGH derivatives that bind to the growth hormone receptor but not to the prolactin receptor. These derivatives are being tested for safety and efficacy in humans.

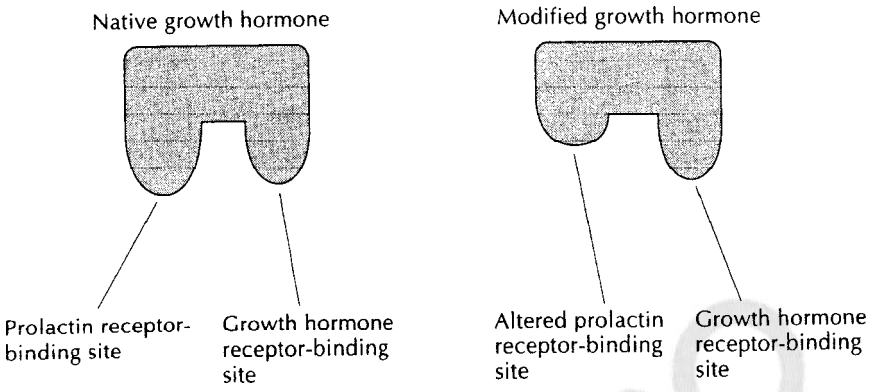


Fig. Schematic representation of native and modified hGH

Q3 Write brief note on: (i) Optimizing gene expression (ii) DNase 1 (iii) alginate 1 yase?

Ans. Optimizing Gene Expression

After designing a protein, it is important that the expression of the gene be optimizied. Initially, researchers determine whether an authentic product can be synthesized in sufficient quantities in either a prokaryotic or a eukaryotic expression system. Unfortunately, not all organisms are equally effective in synthesizing a functional form of a heterologous protein; therefore, comparative assessments must be conducted.

In a study of the expression of the gene for human interleukin-3 in different host cells, the "best" organism was *Bacillus licheniformis*. Although somewhat higher levels of expression were achieved with one of the *E. coli* constructs, the product was a 20-kilodalton (kDa) fusion protein of interleukin-3 and a portion of *E. coli* β-galactosidase and not the 15-kDa mature authentic protein. Such a fusion protein is not usually acceptable as a therapeutic agent. The yeasts Kluyveromyces lactis and Saccharomyces cerevisiae and the human cells were able to glycoslyate interleukin-3, although the level of expression in these cell types was relatively low. Glycosylation, which is not essential for the activity of interleukin-3, resulted in an apparent size heterogeneity.

Therapeutics Produced and Delivered by Intestinal Bacteria

Ulcerative colitis and Crohn disease, both diseases of the intestinal tract, affect approximately 1 in every 500 to 1,000 people in the developed

countries of the world are associated with excess type–2T-helper-cell cytokines, including interleukin-4 and interleukin-5, and type 1 T-helper-cell cytokines, including TNF- α, IFN- α, and interleukin-2, are overproduced. The treatment for Crohn disease often includes trying to lower the levels of cytokines, especially TNF- α One approach has been targeted interleukin-10 as a means of controlling Crohn disease because this cytokine modulates the regulatory T cells that control inflammatory responses to intestinal antigens. However interleukin-10 is not clinically acceptable because it needs to be administered by either frequent injections or rectal enemas. To overcome the problem of the delivery of interleukin-10, one group of researchers genetically engineered the bacterium *Lactococcus lactis* to synthesize and secrete interleukin-10. *L. lactis* is a non pathogenic, non invasive, non colonizing gram-positive bacterium that is often used in the production of fermented foods. Moreover, lactobacilli, including *L. lactis*, have for many years been used as probiotics. A probiotic is a live microorganism that confers a health benefit by altering the indigenous microflora of the intestinal tract.

To test whether interleukin-10-secreting *L. lactis* could be used to treat inflammatory bowel disease, experiments with mice were performed. First, interleukin-10-secreting *L. lactis* was fed to mice with ulcerative colitis that had been induced by 5% dextran sulfate in their drinking water. Second, strains of mice that are genetically incapable of synthesizing interleukin-10 and provide an animal model for ulcerative colitis, were tested. In both of these cases, the engineered *L. lactis* significantly alleviated the symptoms of the disease, establishing that this approach works in principle. However, these mouse models for inflammatory bowel disease are not identical to the disease in humans.

Interleukin-3 synthesis in different host systems

Host System	Promoter	Expression level (units)	Protein form
B. licheniformis	Amylase	300	15 kDa (mature)
E. coli	*lacZ*	500	20 kDa (mature)
E. coli	*lacZ*	20	15 kDa (mature)
Human cells	Metallothionein	2	20-40 kDa
K. lactis	Lactose	20	20-100 kDa
S. cerevisiae	Mating factor α	20	20-100 kDa

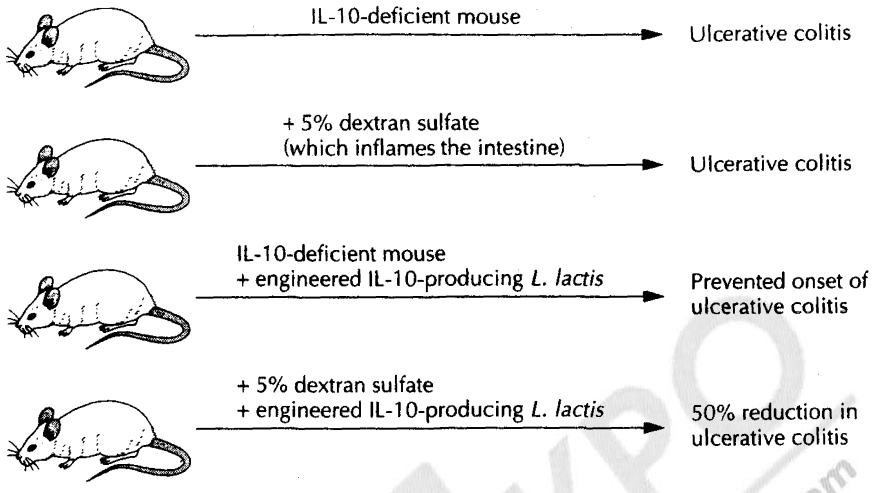


Fig. (IL-10)- secreting bacteria on inflammatory bowel disease in mice.

Enzymes

DNase 1: Cystic fibrosis is one of the most common fatal hereditary diseases in Europeans. Individuals with cystic fibrosis are highly susceptible to bacterial infections in their lungs. Antibiotic treatment of patients who have these recurring infections eventually leads to the selection of antibiotic-resistant bacteria. The presence of bacteria, some alive and some lysed, causes the accumulation of a thick mucus in the lungs of these patients, making breathing very difficult and acting as a source for further infection. The thick mucus in the lungs is the result of the combination of the alginate that is secreted by the living bacteria and the DNA that is released when bacterial cells and degenerating leukocytes that accumulate in response to infection are lysed to overcome this problem, we isolated the gene for the human enzyme deoxynuclease I (DNase I) and subsequently expressed this gene in Chinese hamster ovary (CHO) cells in culture. DNase I can hydrolyze long polymeric DNA chains into much shorter oligonucleotides. The purified enzyme was delivered in to the lungs of patients with cystic fibrosis. The DNase I decreased the viscosity of the mucus in the lungs and made it easier for these patients to breath. While this treatment is not a cure for cystic fibrosis, it nevertheless relieves the most severe symptom of the disease in most patients.

In response to the infection of the lungs of cystic fibrosis patients by pathogenic bacteria, leukocytes infiltrate the airways, and eventually both the bacteria and leukocytes are lysed leukocytes release both DNA and the protein action. The monomeric form of actin binds very tightly to DNase I and inhibits its DNA hydrolytic activity. This interaction limits the effectiveness of DNase I as a therapeutic agent. On the basis of the X-ray crystallographic data of the structure of bowine DNase I complexed with either monomeric actin or DNA, which is 78% identical to human DNase I, a model of the ternary complex between human DNase I, monomeric actin, and DNA was constructed. The model indicated that the binding of actin to DNase I interferes with the binding of DNA to the enzyme, and it was possible to predict which amino acid residues interacted with actin and were therefore targets for change. Changing amino acid 144 from alanine to arginine or changing residue 65 from tyrosine to arginine decreased the binding of DNase 1 to actin up to 10,000 fold. In addition, the actin-resistant mutants had 10 – to 50 – fold more activity than the wild-type enzyme. It is not known whether any additional benefit might be realized by combining the amino acid changes from several actin-resistant mutants.

Alginate Lyase

Aglinate is a polysaccharide polymer that is produced by a wide range of sea weeds and both soil and marine bacteria. Alginate is composed of chains of the sugars β-D-mannuronate and α - L- guluronate. The properties of a particular alginate depend on the relative amounts and distribution of these two saccharides. For example, stretches of α-L-guluronate residues form both interchain and intrachain cross-links by binding calcium ions, and the β-D-mannuronate residues bind other metal ions. The cross-linked alginate polymer forms an elastic gel the structure of an alginate polymer is related to its viscosity, which is in turn directly proportional to its molecular size.

The excretion of alginate by mucoid strains of *Pseudomonas aeruginosa* that infect the lungs of patients with cystic fibrosis is significantly contributes to the viscosity of the mucus found there so depolymerization of the alginate would help clear blocked airways. Since the enzyme alginate lyase can liquefy viscous bacterial alginate, it is a good candidate as a therapeutic agent for cystic fibrosis patients.

An alginate lyase gene has been isolated from a *Flavobacterium* sp., a gram-negative soil bacterium that is a strong producer of this enzyme activity. *A flavobacterium* clone bank was constructed in *E. coli* and screened for alginate lyase-producing clones by plating the entire clone bank onto solid medium containing alginate. Following growth, colonies that produced alginate lyase formed a halo around the colony when calcium was added to the plate. In the presence of calcium, all of the alginate in the medium, except in the immediate vicinity of an alginate lyase-positive clone, becomes cross-linked and opaque. Since hydrolyzed alginate chains do not form cross-links, the medium surrounding an alginate lyase-positive clone is transparent. Analysis of a cloned DNA fragment from one of the positive colonies revealed an open reading frame encoding a popypeptide with a molecular mass of approximately 69,000 Da. Detailed biochemical and genetic studies indicated that this polypeptide is a precursor of the three alginate lyases produced by the *Flavobacterium* sp. After the 69,000- Da precursor is produced, a proteolytic enzyme cleaves off an N-terminal peptide of about 6,000 Da. The 63,000 –Da protein can lyse both bacterial and seaweed alginates. Cleavage of the 63,000-Da protein yields a 23,000-Da enzyme that depolymerizes seawood alginate and a 40,000-Da enzyme that is effective against bacterial alginate. To produce large amounts of the 40,000 –Da enzyme, the DNA corresponding to this enzyme was amplified by the polymerase chain reaction (PCR) and then inserted into a *Bacillus subtilis* plasmid vector fused to a *B. subtilis* α-amylase leader peptide to direct the secretion of this protein, and placed under the transcriptional control of a penicillinase gene promoter. Transformation of *B. subtilis* cells with this construct yielded colonies with large halos on solid medium containing alginate after calcium was added. When these transformants were grown in liquid medium, the recombinant alginate lyase was secreted into the culture broth. Further tests showed that this enzyme efficiently liquefied alginates that were produced by mucoid strains of *P.aeruginosa* that had been isolated from the lungs of patients with cystic fibrosis. Additional studies are necessary to determine whether recombinant alginate lyase is an effective therapeutic agent.

Q4 What is monoclonal antibody? Describe the production of monoclonal antibodies and discuss their applications?

Ans. Today with the discovery of hybridoma technology a no. of antibodies have been approved for treating human disease. This technique can be used to maintain a continuous supply of pure monospecific antibody. To avoid the problem of cross-reactivity human monoclonal antibodies with both specific immunotherapeutic properties and lowered potential for immunogenicity have been produced.

Structure and Function of Antibodies: An antibody molecule (immunoglobulin) consists of two identical light (L) protein chains and two identical heavy (H) protein chains, held together by both hydrogen bonding and precisely localized disulfide linkages. The N-terminal regions of the L and H chains together form the antigen recognition site of each antibody. The sites that recognize and bind antigens consist of three complementary – determining regions (CDRs) that lie within the variable (V_H and V_L) regions at the N-terminal ends of the two H and two L chains. The CDRs are the part of an antibody molecule with the greatest variability in amino acid sequence. In addition to the variable regions, each L chain contains one constant region, (C_L), and each H chain has three constant regions, or domains (C_{H1}, C_{H2}, and C_{H3}). When antibodies are digested with the proteolytic enzyme papain, three fragments are release two identical (Fab) fragments, each of which contains an intact L chain linked by a disulfide bond to the V_L and C_{H1} regions of the H chain, and on Fc fragment, which consists of two H chain fragments, each containing the C_{H2} and C_{H3} domains and joined by a disulfide bond. The Fab fragment retrains the antigen-binding activity. In fact, the N-terminal half of the Fab fragment, which is called the Fv fragment, contains all of the antigen-binding activity of the intact antibody molecule. The amino acid sequence of this portion of the antibody varies considerably from one molecule to another.

In an intact antibody molecule, the Fc portion elicits several immunological responses after antigen-antibody binding occurs:-

- The complement cascade is activated. The components of this system break down cell membranes, activate phagocytes, and generate signals to mobilize other components of the immunological response system.

- After the Fab region binds to a soluble antigen, the Fc portion of an antibody can be bound to Fc receptors of phagocytic cells, which engulf and destroy the antibody-antigen complex.

Preventing Rejection of Transplanted Organs: Passive immunization was reconsidered as a way of preventing immunological rejection of a transplanted organ.

The mouse monoclonal antibody OKT3 was the first to be approved by the FDA for use as an immunosuppressive agent after organ transplantation in humans. Lymphocytes that differentiate in the thymus are called T cells. Various members of the T-cell population act as immunological helper and effector cells and are responsible for organ rejection. The OKT3 monoclonal antibody binds to a cell surface receptor called CD3, which is present on all T cells. This action prevents a full immunological response and spares the transplanted organ from rejection.

Treating Brain Tumors: Monoclonal antibodies that are potentially useful as therapeutic agents are initially examined in mice. The epidermal growth factor receptor gene is often amplified and mutated in human cancers. One common mutation of the human epidermal growth factor receptor gene is an in-frame deletion of 801 base pairs (bp), which results in the expression of a novel protein on the surface of the tumor cell. This tumor-specific protein is an ideal target for immunotherapeutic treatment.

Production of Antibodies in *E. coli:* Hybridoma cells, like grow, slowly, do not attain high cell densities, and require complex and expensive growth medium. Because of the cost of their production attempts have been made to genetically engineer bacteria, plants, and anti-mals to act as "bioreactors" for the production of monoclonal antibodies.

An elaborate series of manipulations makes it possible to select, as well as produce, functional antibodies in *E. coli*. Different steps in prepartion of antibodies are :

1. cDNA is synthesized from mRNA isolated from human antibody-producing cells (B lymphocytes).
2. The H and L chain sequences in the cDNA preparation are amplified separately by PCR.
3. Each amplified cDNA preparation is treated with a specific set of

restriction endonucleases and cloned into a bacteriophage ë vector. The cDNA sequences of the H and L chains each have distinctive restriction endonuclease recognition sites, an arrangement that facilities the directional cloning of each sequence into a separate bacteriophage ë vector. At this stage of the process, many different H and L chain sequences are cloned.

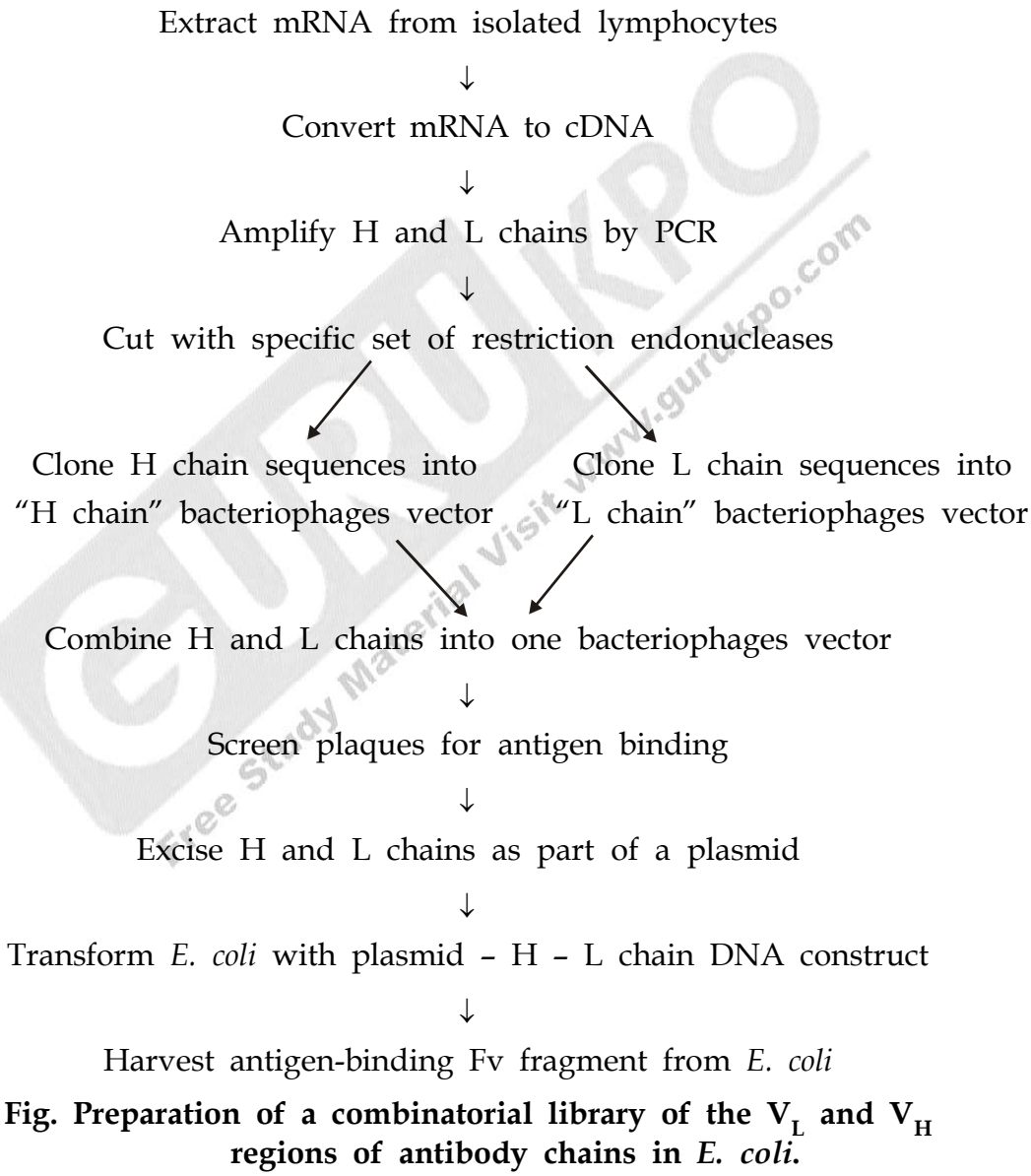


Fig. Preparation of a combinatorial library of the V_L and V_H regions of antibody chains in *E. coli*.

4. The cDNAs of one H and one L chain are cloned into a single "combinatorial" vector, thereby enabling the bacteriophage to coexpress both chains, thus forming an assembled antibody Fv fragment.
5. The H and L chains are expressed during the lytic cycle of bacteriophage ë, so that the library of combinatorial bacteriophages clones can be screened for the presence of antigen-binding activity.

Q.5 Define Vaccine. Explain different types of vaccines including subunit vaccines and enumerate their advantages and limitations. How is alternation of vaccines achieved?

Ans. Vaccination protects a recipient from pathogenic agents by causing an immunological resistance to infection. Injected oral vaccine induces the host to generate antibodies against the pathogen. So during exposure the pathogen is inactivated, its proliferation is prevented. Modern vaccines typically consist of either a killed (inactivated) or a lie, non virulent (altenuated) form of an infectious agent.

There are many limitations of vaccine production:-

- All pathogens can not be grown in culture and so no vaccine have been developed.
- Production of animal and human viruses requires expensive animal cell culture.
- Not all disease (e.g. AIDS) are preventable through the use of traditional vaccine.
- Production rate of animal and human viruses are quite low so making vaccine production costly.

At this time "recombinant DNA technology" has provided a means of creating new generation of vaccine that overcome the drawbacks of traditional vaccine.

Subunit Vaccines: Vaccines that use components of a pathogenic organism rather than the whole organism are called "subunit" vaccines;

recombinant DNA technology is widely used for developing new subunit vaccines.

There are advantages and disadvantages to the use of subunit vaccines that can initiate undesirable side effects in the host organism.

Herpes Simplex Virus: Herpes simplex virus (HSV) is a cancer-causing agent and also causing sexually transmitted disease, severe eye infections, and encephalitis protection against HSV would be best achieved by a subunit vaccine, which would not be oncogenic.

The primary requirement for creating any subunit vaccine is identification of the component(s) of the infectious agent that elicits antibodies. HSV-1 envelope glycoprotein D (gD) is such a component, because, after injection into mice, it elicits antibodies that neutralize intact HSV. The complete sequence of the gD gene encodes a protein that becomes bound to the mammalian host cell membrane. A membrane bound protein is much more difficult to purify than a soluble one. So the gD gene was modified by removing the nucleotides encoding the C-terminal transmembrane-binding domain. The modified gene was then transformed into CHO cells, where the product was glycosylated and secreted into the external medium. In laboratory trials, the modified form of gD was effective against both HSV-1 and HSV-2.

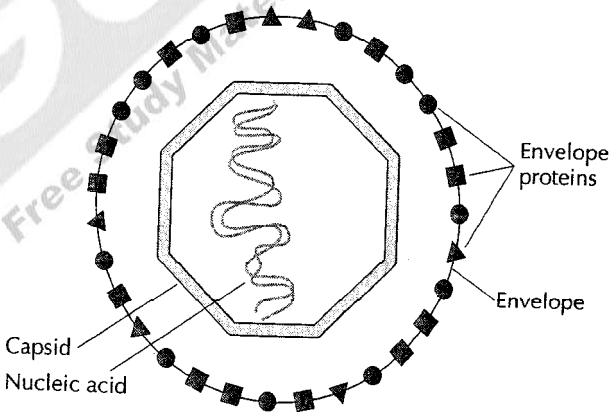


Fig. Schematic representation of an animal virus

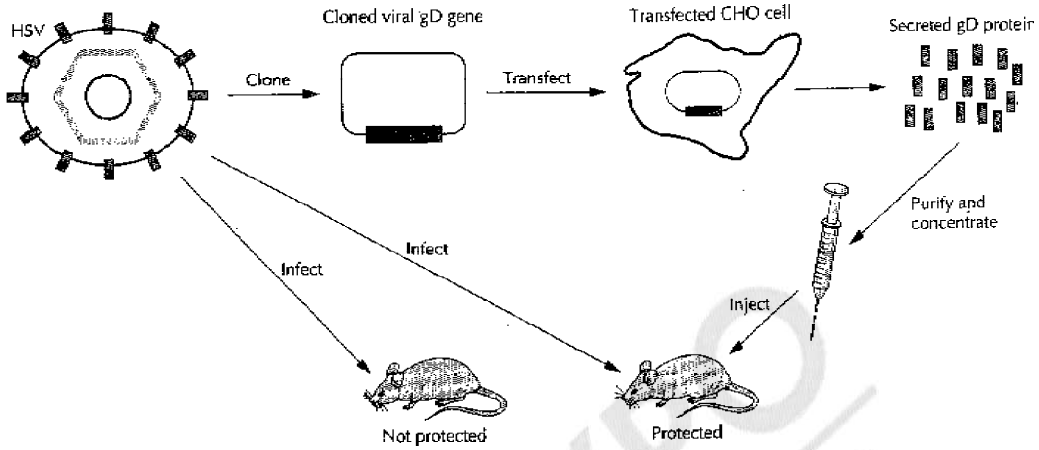


Fig. Schematic representation of the development of a subunit vaccine against HSV. The isolated HSV gD protein gene is used to transfect CHO cells. Then the transfected cells are grown in culture and produce gD protein. Mice inoculated with the purified gD protein are protected against infection by HSV.

Foot-and-Mouth Disease: Food-and-mouth disease virus (FMDV) has a destroying impact on cattle and swine and is extremely virulent, but, for the most part, it has been possible to keep the negative effects of this virus to a minimum by using formalin-killed FMDV preparations as a vaccine.

Research on FMDV found that the major antigenic determinant that induces neutralizing antibody is capsid viral protein 1 (VP1). Although purified VP1 is a much less potent antigen than intact viral particles, it can still elicit neutralizing antibodies by itself and therefore can protect animals from infection by FMDV. Thus, the gene for VP1 became a target for cloning.

The genome of FMDV is composed of single-stranded RNA (approximately 8,000 nucleotides long). Therefore, for recombinant DNA manipulations, it was necessary first to synthesize a double-stranded complementary DNA (cDNA) of the entire genome. This cDNA was then digested with restriction enzymes, and the fragments were cloned in an peptide 141 to 160 elicited sufficient antibody to protect animals against subsequent challenges with FMDV. By contrast, inoculation with complete VP1 or

peptide 9 to 24, 17 to 32, or 25 or 41 yielded lower levels of neutralizing antibodies.

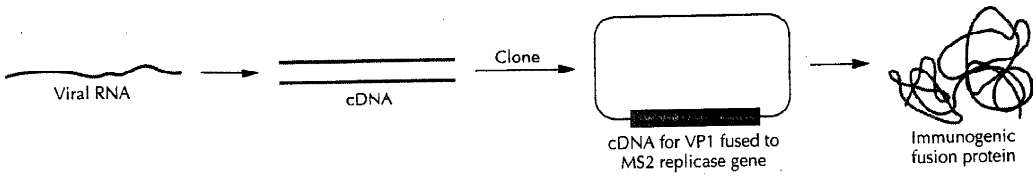


Fig. Schematic representation of the development of a subunit vaccine against foot-and-mouth disease.

In an additional experiment, a longer peptide consisting of amino acids 141 to 158 joined to amino acids 200 to 213 by two praline residues elicited high levels of neutralizing antibodies in guinea pigs, even when it was injected without any carrier protein. This "two-peptide" molecule was more effective than either of the single peptides alone and prevented FMDV proliferation in cattle as well as in guinea pigs.

The amount (dose) of peptide material that had to be used to elicit an immunological response was approximately 1,000 times the amount of inactivated FMDV needed to elicit the same response. To overcome this problem, DNA encoding FMDV VP1 peptide 142 to 160 was linked to the gene encoding a highly immunogenic carrier molecule, hepatitis B core protein (HBcAg). When the gene for this fusion protein was expressed in either *E. coli* or animal cells in culture, the protein molecules self-assembled into stable "27-nm particles", with the FMDV VPI peptide located on the outer surface of the particle. These particles are highly immunogenic in laboratory animals. Therefore, HBcAg may be an effective carrier molecules for such short synthetic peptides. A comparison of the immunogenicitiy, in guinea pigs, of a variety of FMDV peptide vaccines, all of which contained the VPI peptide 142 to 160 sequence, revealed that a fusion protein containing HBcAg and FMDV VP1 amino acids 142 to 160 was approximately one-tenth as immunogenic as inactivated FMDV particles, 35 times more immunogenic than a fusion protein containing *E. coli* β-galactosidase and FMDV VPI amino acids 137 to 162, and 500 times more immunogenic than the free synthetic peptide composed of amino acids 142 to 160. Because synthetic peptides fused to HBcAg do not interfere with the assembly of the 27-nm hepatitis

B virus-like particles, and because these particles are nearly as immunogenic as the intact virus from which the synthetic peptide was derived, this approach may become a general method for the delivery of peptide vaccines.

Nevertheless, there are certain limitations to using short peptides as vaccines:

- To be effective, an epitope must consist of a short stretch of contiguous amino acids, which does not always occur naturally.
- The peptide must be able to assume the same conformation as the epitope in the intact viral particle.
- A single epitope may not be sufficiently immunogenic.

Genetic Immunization: DNA Vaccines: A novel strategy that elicits an antibody response without the introduction of an antigen has been developed. In this case, the gene encoding an antigenic protein is incorporated into cells of a target animal, where the antigen is synthesized. In the initial experiments, gold microprojectiles were coated with *E. coli* plasmid DNA carrying an antigen gene under the transcriptional control of an animal viral promoter. A biolistic system was used to deliver the microprojectiles into cells in the ears of mice.

In one experiment, in more than 75% of the trials, the input gene was taken up into the mouse cells and directed the synthesis of the protein antigen, which in turn activated in the mouse an immune response that led to the production of antibodies against the target protein. One distinctive feature of "genetic immunization" is that the costly and time-consuming procedure of either purifying an antigen.

The feasibility of genetic immunization has been examined in detail. In one series of experiments, mice were injected in the quadriceps of both legs with an *E. coli* plasmid carrying the cDNA for influenza A virus nucleoprotein under the transcriptional control of either a Rous sarcoma virus or a cytomegalovirus promoter. Although the expression of the nucleoprotein was too low to detect, nucleoprotein-specific antibodies were observed in the blood of the test mice 2 weeks after the initial injection. This cross-protection is in sharp contrast to traditional influenza virus vaccines, which are directed against surface antigens of the virus, so that each vaccine is specific to a single strain of influenza virus.

An advantage of genetic immunization, besides by passing the need for purified protein antigens, is that it can trigger a response only against the protein encoded on the plasmid and not against the plasmid itself.

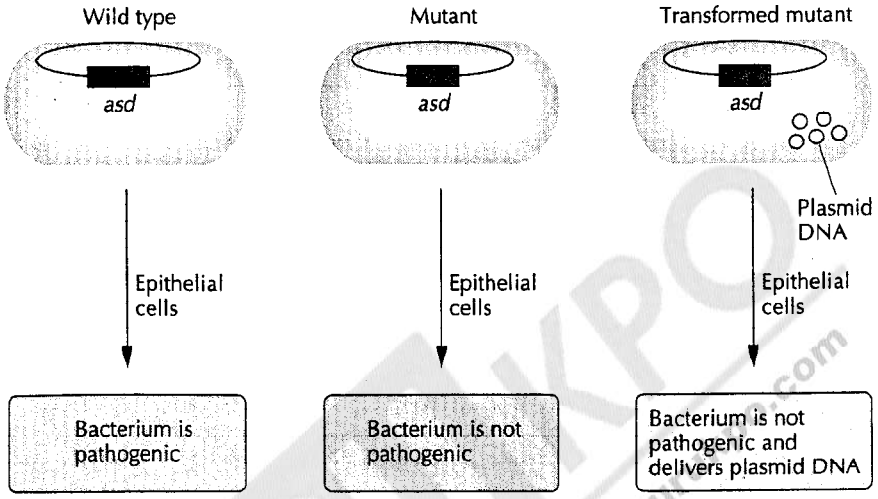


Fig. Use of non pathogenic *S. flexneri* to deliver foreign DNA to mammalian epithelial cells.

The greatest potential advantage of this approach is that with the *Shigella* system, DNA for vaccination may be delivered orally, greatly simplifying the delivery of a variety of vaccines.

Attenuated Vaccines: These vaccines are either non pathogenic organisms that have been engineered to carry and express antigenic determinants from a target pathogenic agent or engineered strains of pathogenic organisms in which the virulence genes have been modified or deleted.

Cholera: It is usually advantageous to develop a live vaccine, because they are generally much more effective than killed or subunit vaccines. The major requirement for a live vaccine is that no virulent forms be present in the inoculation material. with this objective in mind, a live cholera vaccine has been developed. Cholera is a fast-acting intestinal disease characterized by fever, dehydration, abdominal pain, and diarrhea. It is transmitted by drinking water contaminated with fecal matter.

The bacterium *V Cholerae*, the causative agent of cholera secretes large amounts of a hexameric enterotoxin, which is the actual pathogenic agent.

This protein is a hexamer that consist of one subunit, the A subunit, that has ADP-ribosylation activity and stimulates adenylate cyclase, and five identical B sub units that bind specifically to an intestinal mucosal cell receptor. The A subunit has two functional domains: the A_1 peptide, which contains the toxic activity, and the A_2 peptide, which joins the A subunit to the B subunits. This vaccine generates only moderate protection, which normally lasts from about 3 to 6 months.

A strain of *V. cholerae* was created with part of the coding sequence for the A_1 peptide deleted. This strain cannot produce active enterotoxin; therefore, it is non-pathogenic and is a good candidate for a live vaccine.

It was necessary to engineer a strain carrying a defective A_1 peptide sequence that could not revert.

(1) A plasmid containing the cloned DNA segment for the A_1 peptide was digested with the restriction enzymes *Cla*II and *Xba*I, each of which cut only within the A_1 peptide-coding sequence of the insert.

(2) To recircularize the plasmid, an *Xba*I linker was added to the *Cla*I site and then cut with *Xba*I.

(3) T4 DNA ligase was used to join the plasmid at the *Xba*I sites, thereby deleting a 550-base-pair (bp) segment from the middle of the A_1 peptide-coding region. This deletion removed 183 of the 194 amino acids of the A_1 peptide.

(4) Then, by conjugation, the plasmid containing the deleted A_1 peptide-coding sequence was transferred into the *V. cholerae* strain carrying the tetracycline resistance gene within its A_1 peptide DNA sequence.

(5) Recombination (a double crossover) between the remaining A_1 coding sequence on the plasmid and the Tet^r gene-disrupted A_1 peptide gene on the chromosome replaces the chromosomal A_1 peptide-coding sequence with the homologous segment on the plasmid carrying the deletion.

(6) After growth for a number of generations, the extrachromosomal plasmid, which is unstable in *V. cholerae*, is spontaneously lost.

(7) Cells with an integrated defective A_1 peptide were selected on the basis of their tetracycline sensitivity. The desired cells no longer had

the tetracycline resistance gene but carried the deleted A_1 peptide sequence.

Vector Vaccines

Vaccines Directed against Viruses: Vaccinia virus is a member of the poxvirus family. This completely sequenced virus has a double-stranded DNA genome that contains 187 kilobase pairs (kb) and encodes approximately 200 different proteins. Vaccinia virus DNA replicates within the cytoplasm of infected cells. Cytoplasmic, rather than nuclear,

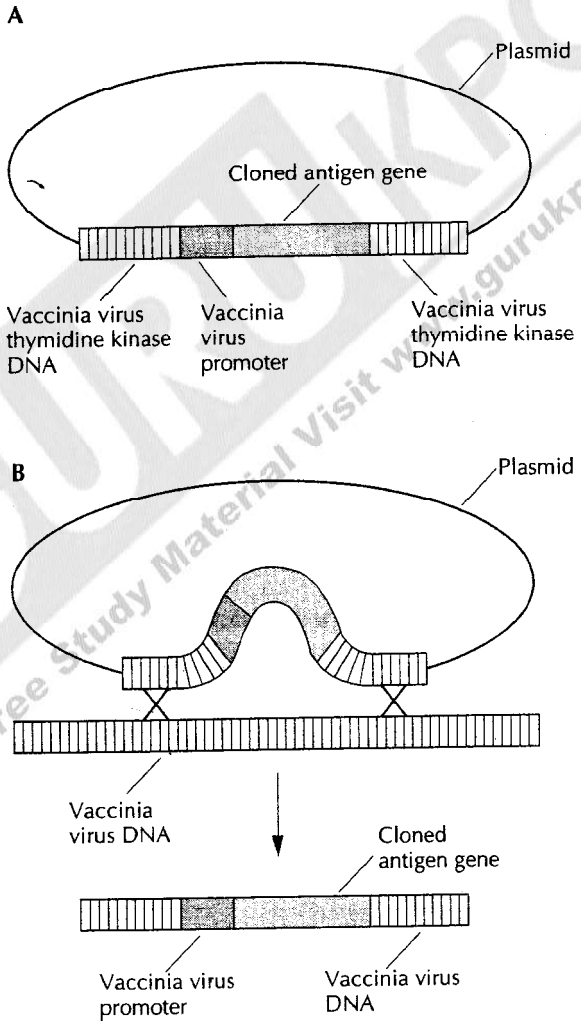


Fig. Method for the integration of a gene into vaccinia virus

replication is possible because vaccine virus DNA contains genes for DNA polymerase, RNA polymerases, and the enzymes to cap, methylate, and polyadenylate messenger RNA (mRNA). Thus, if a foreign gene is inserted into the vaccinia virus genome under the control of a vaccinia virus promoter, it will be expressed independently of host regulatory and enzymatic functions.

Vaccinia virus has a broad host range, is well characterized at the molecular level, is stable for years after lyophilization (freeze-drying), and is a usually benign virus. For these reasons, it is a strong candidate as a vector vaccine. The important features of a vector vaccine are the delivery and expression of cloned genes encoding antigens that elicit neutralizing anti-bodies against pathogenic agents. Unfortunately, the vaccinia virus genome is very large and lacks unique restriction sites. Therefore, it is not possible to insert additional DNA directly into the viral genome. The genes for specific antigens must be introduced into the viral genome by in vivo homologous recombination.

(1) The DNA sequence coding for a specific antigen, such as HBcAg, is inserted into a plasmid vector immediately downstream of a cloned vaccinia virus promoter and in the middle of a non essential vaccinia virus gene, such as the gene for the enzyme thymidine kinase.

(2) This plasmid is used to transform thymidine kinase-negative animal cells in culture, usually chicken embryo fibroblasts, that have previously been infected with wild-type vaccinia virus, which produces a functional thymidine kinase.

(3) Recombination between DNA sequences that flank the promoter and the neutralizing antigen gene on the plasmid and the homologous sequences on the viral genome results in the incorporation of the cloned gene into the viral DNA. Although the recombination event is rare, the absence of thymidine kinase activity in the host cells and the disruption of the thymidine kinase gene in the recombined virus render the host cells resistant to the otherwise toxic effects of bromodeoxyuridine. This selection scheme enriches for cell lines that carry a recombinant vaccinia virus.

(4) The definitive selection of cells with a recombinant vaccine virus is made by DNA hybridization with a probe for the antigen gene.

Tuberculosis: Tuberculosis, one of the most important infectious diseases worldwide, is caused by the bacterium *M. tuberculosis*. This bacterium can form lesions in any tissue or organ, which leads to cell death. The lungs are most commonly affected. Patients suffer fever and loss of body weight, and without treatment, tuberculosis is often fatal approximately 2 to 3 million deaths a year result from these infections. Antibiotics have been used to treat patients infected with *M. tuberculosis*. However, numerous multidrug-resistant strains of *M. tuberculosis* are now prevalent.

Currently, bacillus Calmette-Guerin (BCG), an attenuated strain of *Mycobacterium bovis* that was developed between 1906 and 1919, is used as a vaccine against tuberculosis. However, the use of this vaccine has some drawbacks. First, live BCG cells can cause tuberculosis in immunocompromised individuals, such as AIDS patients. Second, individuals treated with BCG respond positively to a common tuberculosis diagnostic test, which makes it impossible to distinguish between individuals infected with *M. tuberculosis* and those inoculated with BCG cells.

The extent of the immunoprotection elicited by purified *M. tuberculosis* extracellular proteins was examined. Following growth of the bacterium in liquid culture, six of the most abundant of the approximately 100 secreted proteins were purified. Each of these proteins was used separately and then in combination to immunize guinea pigs. The immunized animals were then challenged with an aerosol containing approximately 200 cells of live *M. tuberculosis* – a large dose for these animals. The animals were observed for 9 to 10 weeks before their lungs and spleen were examined for the presence of disease-causing organisms. In these experiments, some of the purified protein combinations provided a slightly lower level of protection against weight loss, death, and infection of lungs and spleen than did the live BCG vaccine. Prominent among the proteins that provided protection was the *M. tuberculosis* major secretory protein, α-antigen. While this and possibly other *M. tuberculosis*-secreted proteins might eventually be part of a safe and efficacious vaccine for the prevention of tuberculosis in humans, it is necessary to

develop a suitable delivery system for these proteins. In theory, the optimal delivery system for an antigen that provided protection against tuberculosis should be (1) able to multiply in the mammalian host, (2) non-pathogenic, and (3) able to express and secrete the protective antigen. All of these requirements are satisfied by the available BCG strain. Therefore, an *E. coli*-mycobacteria shuttle vector that contained the gene for the 30-kDa protein (α-antigen) under the control of its own promoter was introduced into two different BCG strains. Transformed cells continued to express a high level of 30-kDa protein after vaccination of a test animal.

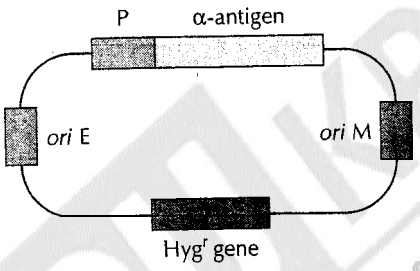


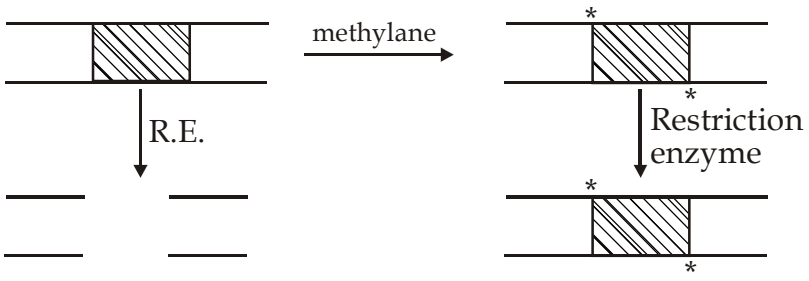
Fig. The plasmid construct used to transform BCG to make it a more effective vaccine.

Section C
Synthesis of the commercial products by recombinant in croorganisms

Q1 Give a brief account on production of respiration endonuclease by Recombinant DNA technology?

Ans. Recombinant DNA technology would not be possible without use of "restriction endonuclease." More than 300 different restriction endonucleases are commercially available. These enzymes occur naturally in micro-organism including species that are aerobic anaerobic, mesophilic thermophilic. For each of these organism a fermentation protocol has to be developed to achieve the maximum yield of restriction endonuclease. For this *E. coli* cells grow rapidly to high cell densities and can be engineered to averex press each target restriction enzyme. Although isolation and expression of foreign genes in *E. coli* is well established but the host organism is a living entity that can be affected by the production of a heterologus protein. Example, overexpression of a heterologous protein may drain the host organism, so adversely affect its growth.

In addition the presence of a heterologous protein, may be lethal to the host example restriction endonucleases digest DNA at sites that are present on all DNA molecules. So an organism that express a cloned restriction endonucleases gene is likely to have its own DNA degraded unless a protection mechanism is present. Microbes that make restriction endonuclease have evolved self-protection system methylation of one or more of the bases of the DNA within the recognition sequence prevents the homologous restriction endonuclease from cutting the DNA at this site.



One way to circumvent the problem of host DNA degradation by heterdogous restriction endonucleases is to clone and express the genes for both the restriction enzyme and specific modification enzyme in the host organism. Cloning both of these genes into the same organism is technically complex unless both the restriction endonuclease and methylation genes are close to each other on the chromosome.

The strategy was used to isolate the gene for the restriction enzyme *PsE*1 from *Providencia stuartii* shows how one restriction endonuclease gene was cloned into *E. coli* :

(1) The chromosomal DNA from P. stuartii was digested with Hind III and ligated into the Hind III site an plasmid pBR322.

(2) Following the introduction of P. stuartii clone library into E. coli HB101 cells, transformants were grown in liquid medium before being infected with bacteriophage ë

(3) Transformants that were resistant to lyses by ë were grown and samples were osinotically shocked fore lease the periplasmic protein which were assayed for PstI restriction enzyme activity.

(4) Positive clones were assayed for Pst1 methylase activity.

A strategy also has been used to isolate the genes for restriction and modification (methylation) enzyme system. It consist of following steps:-

(1) A clone bank was made from the DNA of Donor organism that has previously identified restriction endonuclease. Plasmid vector should have one recognition site for R.E.

(2) The long bank was in produced into *E. coli* by transformation. This step increase the amount of recombinant plasmid DNA and allows

the expression of modification enzyme.

(3) Plasmid DNA was isolated from transformed cells that had been grows in liquid media.

(4) The plasmid DNA preparation is treated with the target R.E.

(5) *E. coli* cells are transformed with the restriction endonuclease treated plasmid DNA preparation.

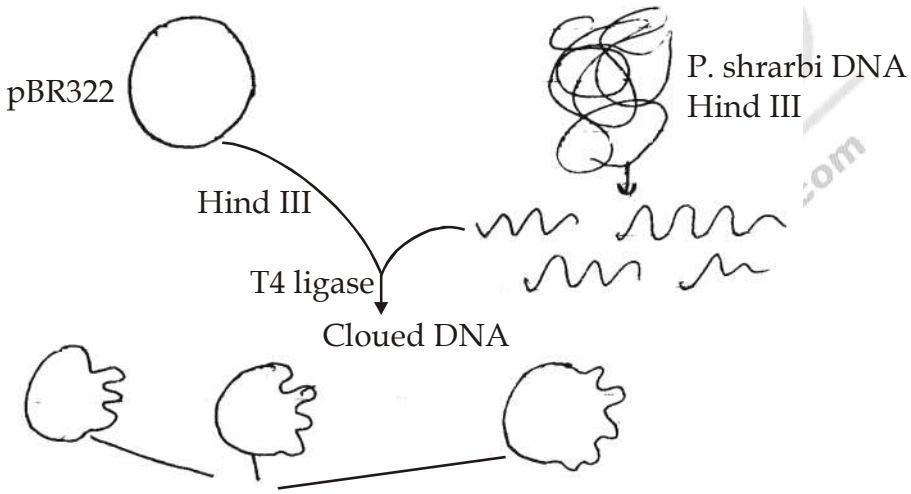


Fig. Transformed into *E. coli* grow in liquid culture intect with bacteriophage ë

The rationale for this procedure is that the clones that carry and express the modification enzyme will produce plasmid DNA that is resistant to digestion by the target R.E. because their DNA will be methylated at the recognition sites example transformation of *E. coli* by PBR322. Hind III clone bank of desulfovibrio desulfuricans DNA plasmid DNA was isolated and digested with the restriction enzyme Ddel. Plasmid that encode the Ddel modification enzyme are not digested by Ddel because the 8 Ddel recognition site of pBR 322 are methylated. After Ddel treatment remaining plasmid mixture is used to transform *E. coli*. Only intact plasmid yield transformants and these carry gene for a functional D del modification enzyme. All other plasmids are degraded by R.E. Resulting transformants must be assayed for D del restriction enzyme activity to determining which clones have the genes for both the

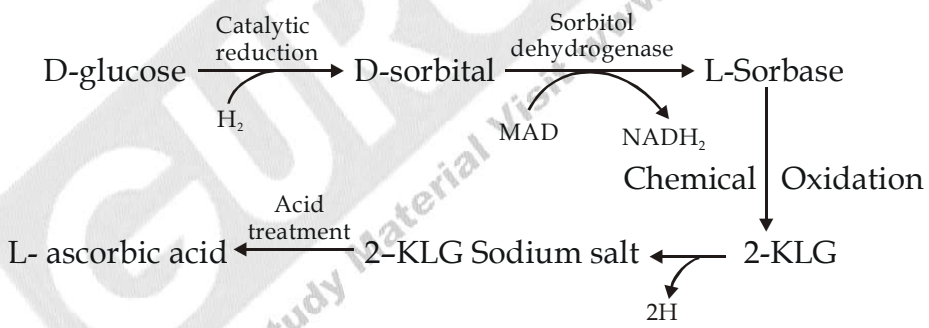
modification enzyme and R.E.

This strategy is effective for any, R.E. gene most restriction enzymes are encoded on the same operon as their cognate modification enzyme and its cloned into a plasmid vector that has at least one recognition site for target enzyme.

Q7 Give a detailed account of biotechnological production of (i) ascorbic acid (ii) Indigo (iii) amino acid?

Ans. With recombinant DNA technology our goal is to create an organism with a novel enzymatic activity that can convert an existing substrate into a commercial compound, that can be produced only by a combination of chemical treatments and fermentation.

Synthesis of L-Ascorbic acid: L-ascorbic acid is currently synthesized commercially by starting with glucose that includes one microbial and a number of chemical step (microbial conversion).



The host step in the process is the acid-catalyzed conversion of 2-keto-L-gluconic acid (2-KLG) to L-ascorbic acid. It is possible to synthesized 2-KLG by different pathway. Example – Some bacteria can convert glucose to 2,5 DKG (Diketo Gluconic acid) and others convert 2,5 DKG to 2-KLG.

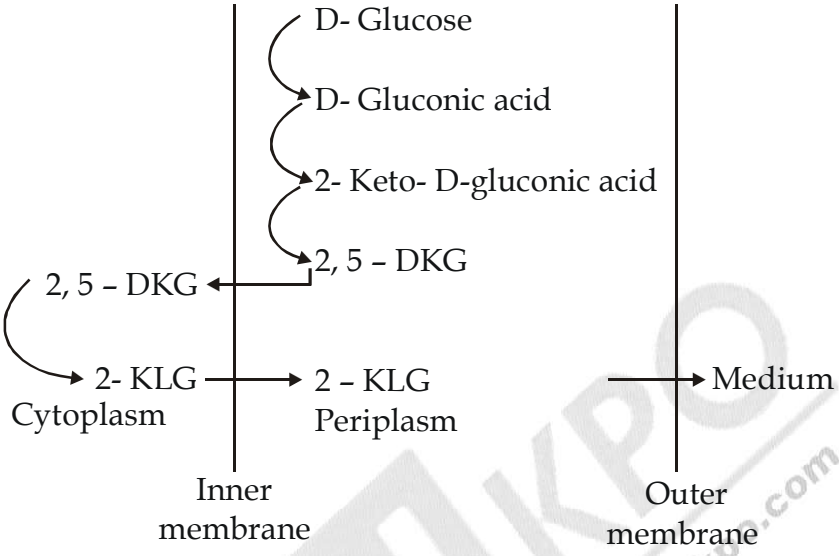
Synthesis of ascorbic acid could be improve by producing 2-KLG from glucose by cofermentation with suitable organism but, If has problems of its own example the two fermentive organism might have different temperature and pH the medium requirements and growth rates also might differ in such a way that the fermentation conditions are optimal

for one organism and suboptimal for other. This leads to the washout of one organism. This compatibilities may be overcome by utilizing a tandem fermentation process in which two organisms are cultivated in succession that requires two fermentation process so the best way to convert glucose into 2,5-DKG would be to engineer a single microbe that carries all the required enzymes.

The simplest strategy for constructing a single organism i.e. able to convert D-Glucose to 2-KLG is to isolate the 2,5 – DKG reductase gene from the corybacterium sp and express is in '*E. herbicole*' "First-Step", in cloning 2, 5 – DKG reductase gene from corynebacterium sp involved purifying enzyme and determining the sequence of first 40 amino acid. On the basis of these amino acid sequence, two by 3- nucleotide DNA hybridization probes were synthesized. This approach was taken because at the time the time that this work was done, mixed probes were not available.

A corynebacterium DNA clone bank was screened with these probes. A clone that hybridized with both probes was isolated and sequenced, it contained 2,5- DKG reductase gene. DNA sequence that were upstream of ATC start signal were deleted and replaced with transcriptional signal that function in E. coli because the regulatory sequences are not efficiently utilized by *E. coli*. This construct was sub cloned and a broad host range vector, which was used to transform E. herbicola.

The transform Erwinia cells were able to convert D-glucose directly to 2-KLG. The endogenous Erwinia enzymes, localized in inner membrane of bacterium converted glucose to 2, 5 – DKG and the cloned 2,5 –DKG reductase localized in cytoplasm catalyzed the conversion of 2,5 – DKG to 2-KLG. Thus, the genetic manimulation which was able to produce the end product of the engineered metabolic pathway.



Conversion of D-glucose to 2-KLG by recombinant *E. leubicola*

The commercial utility of the cloned 2,5-DKG reductase gene might be improved by replacing amino acid of enzyme to create mutants with increased catalytic activity and enhance thermal stability.

The predicted structure of 2, 5- DKG consist of eight parallel β-strands arranged close together surrounded by 8- α helices that are joined to the β-strand through loops of various lengths. By comparison this structure with other proteins three of the loops might be involved in substrate binding.

Future work on 2,5-DKG reductase is likely to focus on combining these amino acid changes into a single version of enzyme.

Synthesis of Indigo

A number of bacteria (Pseudomonas) have the ability to use a variety of organic compounds as their carbon source. The genes encoding the enzymes for the degradation of organic compounds are located on large plasmid. For synthesis of indigo for example:- Plasmid MAH7, has two separate operons that contain this plasmid to grow an naphthalene as the sole carbon source.

In first step "MAH7" plasmid DNA was digested with Hind III and the

fragments were ligated with linear Hind III-digested plasmid pBR322. This clone bank was introduced into *E. coli* cells and transformants were selected on the basis of their resistance to ampicillin and sensitivity to tetracycline. All transformants were then tested for production of non volatile metabolites that might results from the hydrolysis, of radiolabeled naphthalene.

During characterization of transformants if was obtained that when minimal growth medium contain trptophan, it turned blue analysis of blue colour revealed that the transformed *E. coli* cells were synthesizing the dye indigo. Synthesis was achieved in four steps:-

(1) Conversion of tryptophan in the growth medium to indole by tryptophanase, produced by *E. coli*.

(2) Oxidation of indole to cis-indole-2,3-dihydrodiol by naphthalene dioxygenase, which is encoded by the DNA that was cloned from the MAH 7 plasmid.

(3) Spontaneous elimination of water and

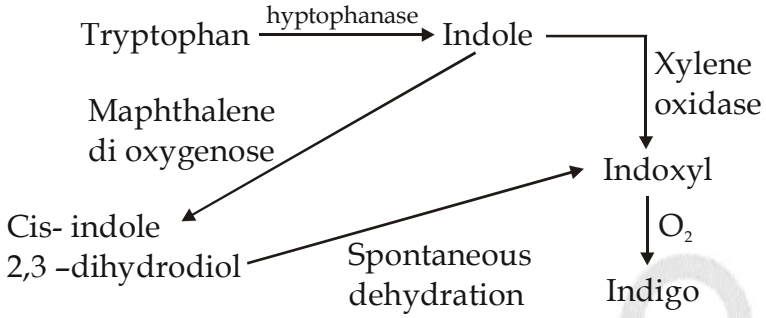
(4) Air oxidation to form indigo.

So the combination of enzymes from two different pathways and two different organisms results in the synthesis of dye indigo. Introduction of gene for enzyme, xylene oxidase, which is encoded in the TOL plasmid, can convert tryptophan to indoxyl, which then oxidizes to indigo.

Indigo, a commercially important blue pigment that is used to dye both cotton and wood was isolated from plants but currently synthesized chemically. Indigo the colouring agents in blue jeans is the largest selling dye in the world. The ability to produce indigo from bacteria opens the possibility of developing and efficient and economical process for its production. This process would avoid the use of hazardous compounds such as aniling, formaldehyde and cyanide, which are needed in the chemical synthesis of indigo.

Although this recombinant system has not yet been commercialized, a microbial process for synthesis of indigo might include a bioreactor, in which the recombinant. *E. coli* is chemically immobilized to a solid matrix. This could between continuously by adding tryptophan to one end and

removing indigo from the other hand.



Synthesis of amino acid:

Amino acid are used in food industry as flavor enhancers, antioxidants used in medicine in infusion solutions far post operative treatments and in chemical industry used as starting materials for manufacture of polymers and cosmetics.

Amino acids are commercially produced either by extraction from protein hydrolysates or as fermentation products of either corynobacterium or Brevi-bacterium spp. both are gram-positive non sporulating bacteria. The productivity of these organism has been improved by mutangenesis and subsequent screening for strains that over produce certain amino acid. But this way of developing new strains is slow and inefficient. By using biochemical information about enzymes, it is more expeditious to isolate and manipulate the specific genes example the pathway leading to the biosynthesis of amino acid contains a number of different enzymes, each of which may be either activated or inhibited by a number of metabolite present in cell this makes it difficult to know which enzyme to manipulate to enhance the yield of end product.

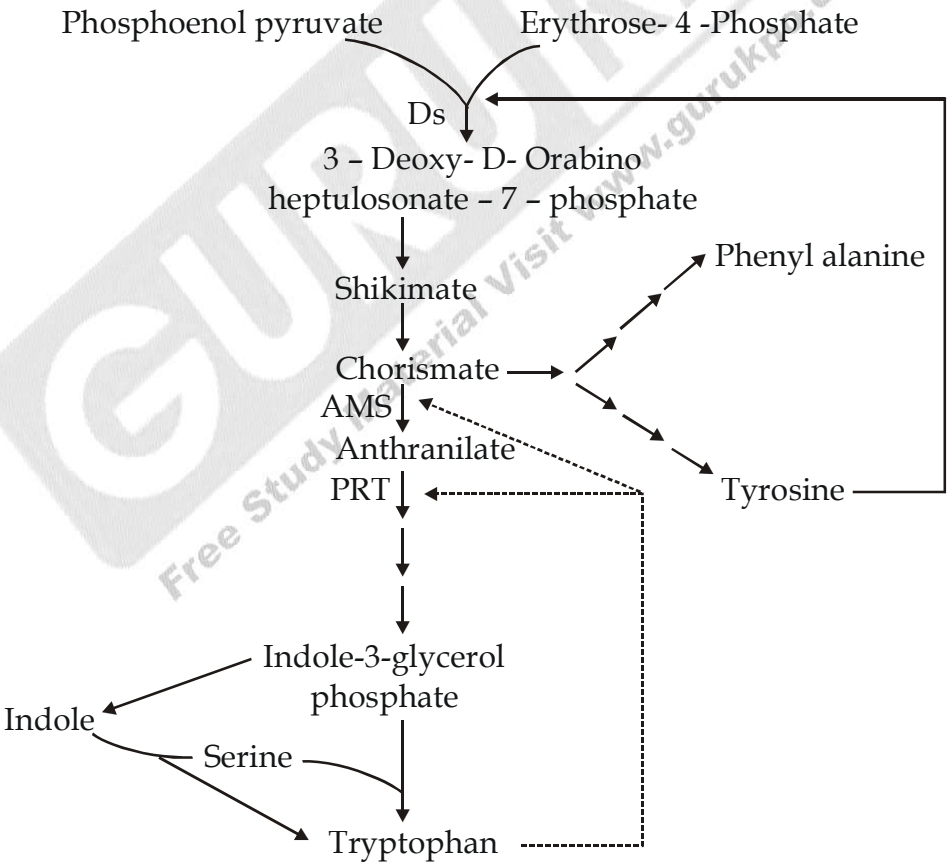
It is necessary to construct expression reactors that are specifically for corynebacterium because most broad host range plasmid vector replicate only in gram negative organism. Such cloning vehicles high take the form of *E. coli* coynebacterium shuttle vectors *E. coli* portion of plasmid could encode resistance to antibiotics. Because both *E. coli* and corynebacteria spp. are susceptible to these antibiotics, they could be used as selectable markers. Many *C. glutamium* genes are not efficiently expressed in *E. coli*. So far selection schemes that depend on gene expression the entire clone bank should be transformed into *C. glutamicum*. The transformation

frequency is very low when DNA is introduced into *C. glutamicum* by either direct transformation or electroporation. However, effective transformation of *C. glutamicum* is achieved when foreign DNA is introduced by conjugation or after formation of protoplast. Transformation is possible by adding PEG to facilitate the uptake of exogenous plasmid DNA.

For increasing the amino acid output, the synthesis of essential amino acid tryptopham was enhanced by introducing into wild type *C. glutamium* cells, a second copy of the gene encoding anthranilate synthetase:

Isolation of anthranilate synthetase gene can be done by following steps:-

(1) A library of Brevibacterium flavour chromosomal DNA was cloned into *C. glutamium E. coli* shuttle vector and introduced into a mutant strain of *C. glutamium* that produce no anthranilic acid.



Pathway and regulation of tryptophan biosynthesis in C. glutamium

(2) The mutant strain was unable to grow on minimal medium unless anthranilic acid was added. So, transformants were selected by their ability to grow in the absence of anthranilic acid.

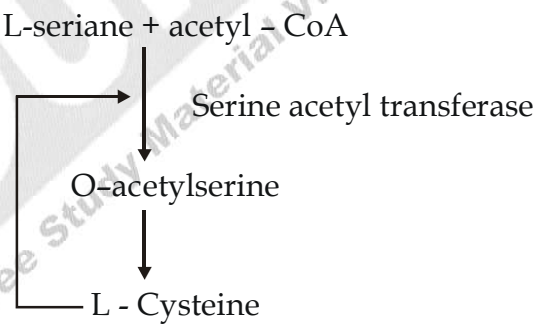
(3) The vector carrying anthranilic acid synthetase gene was transferred to a wild type strain of C. glutanium.

The amount of tryptophan produced in the mutant and wild-type C. glutamium strains – one without and one with the vector carrying the cloned anthranilic acid synthetase gene.

By addition of this gene to C. glutamium, the level of over production reflects more efficient utilization of precursor material. So by cloning, it was possible to generate much more of the end product. Higher level of trypophan production was achieved when modified genes introduced into C. glutamium cells.

An alternative to producing amino acids in corynebacterium and Breui bacterium spp. is to produce them in *E. coli*, where both metabolic pathways and genetic manipulation are much better characterized.

'L – cysteine', has been obtained by extracting it from acid hydrolysate of human hair and animal feathers.



Biosynthesis of L - Cysteine from L - serine from acetyl CoA

Q.8 Write short note on:

(i) Antibiotic production by biotechnology.

(ii) Biopolymers

Ans. Antibiotics: The universal use of antibiotics to treat bacterial disease has

resulted in an enormous improvement in human health. Most important antibiotics have been isolated from the gram positive soil bacterium strephomyces, although fungi and other gram positive and gram negative bacteria are also source of antibiotics.

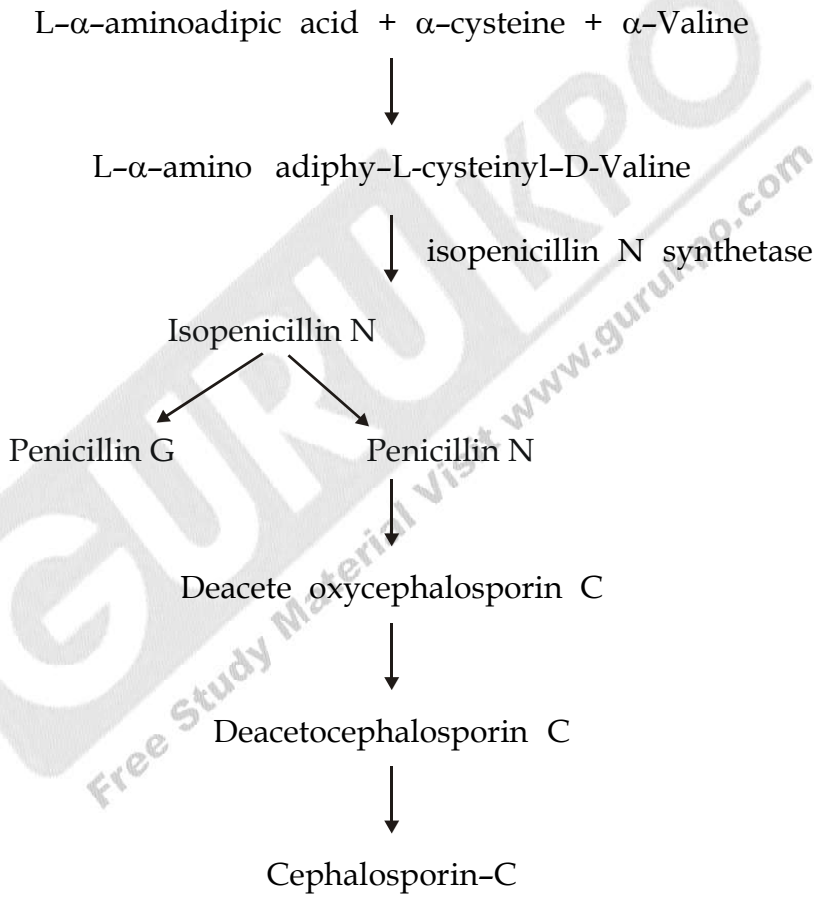
(i) Recombinant DNA technology can be used to develop new, structurally unique antibiotics with increased activities against selected targets and decreased side effects.

(ii) Genetic manipulation can be used to relatively rapidly and inexpensively enhance yields and hence lower the cost of production of existing antibiotics.

For genetic manipulation of streptomyces it must be transformed. Unlike *E. coli*, Streptomyces strain do not exist as individual cells but as aggregates called mycelial filaments. So the cell wall must be removed to release individual cells prior to DNA transformation. Colonies that grew in the presence of selective antibiotics would contain a mixture of transformed and non transformed cells. However as a consequence of protoplast formation prior to transformation. All colonies that grow in the presence of antibiotics contained only transformed cells, the protoplast are first plated ant a solid medium to enable the cell walls to regenerate and then over laid with a selective medium that contains either neanycin or thiostreptor, act as selection agents for transformed cells.

Cloning antibiotic biosynthesis gene: This strategy (isolating the complete set of antibiotic resistance gene) consist of transforming one or more mutant strain that are unable to synthesize the antibiotic with DNA from a lane bank constructed from wild type chromosomal DNA. following the introduction of clone bank DNA into mutant cells, transformants are screened for their ability to produce the antibiotic. Then the plasmid DNA is used as a DNA hybridization probe to screen another clone bank of wild type chromosomal DNA to isolate clones with regions that overlap the probe sequence. In this way, DNA segments that are usually bigger than initial complementing DNA can be identified and cloned. Antibiotic biosynthesis genes are clustered at a single site on chromosomal DNA. Genes that are adjacent to complementing gene are also involved in the biosynthesis of larger antibiotic.

Through genetic and biochemical studies one or more enzymes can be identified and purified N-terminal sequence of enzyme can then be determined thus oligodeoxybo-nucleotide probes can be prepared. This approach has been used to isolate the gene for "isopenicillin N-synthetase" from "P. chrysogenum". This enzymes catalyzes the oxidative can consation of the compound S-α- cysteinyl–D-valine to isopenicillin N, an intermediate in the biosynthesis of penicillin, cephalosporin and cephamycine.

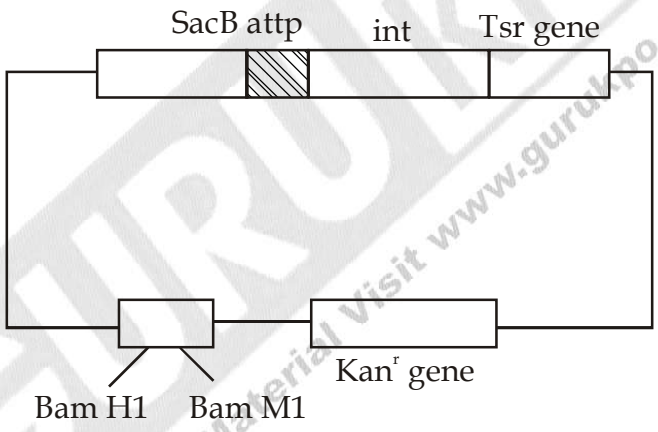


Biosynthetic pathway for penicillin and cephalosporin in P. Chrysogenum

There are a number of examples of the cloning and transfer of large DNA fragments of antibiotic bio-synthetic genes. In this if is necessary to use a vector that can accept and maintain pieces of large DNA.

Researchers have employed BACs that have been engineered to replicate autonomously in *E. coli* and when introduce into streptomyces, to integrated into chromosome. A gene cassette for integration into host chromosome was incorporated into BAC.

This vector has two Bam H1 site so that clan banks of streptomyces DNA can be prepared by partially digesting the chromosomal DNA and ligating into Bam HI digested vector. Kan^R kanamyc in resistance gene allows the presence of the vector to be selected in *E. coli*. Tsr^r thiostrepton resistance gene, which allows the selection of transformed streptomyces strain att P, and int facilities DNA integration into streptomyces chromosome.



Synthesis of Novel antibiotics: New antibiotics with unique properties may be produced by genetic manipulation of genes, involved in biosynthesis of existing antibiotics.

The new antibiotics represent, minor structural variants of the preexisting antibiotics examples are: Actinar hodine by wild type S. colicolor, C. Granaticin and dihydro granatine in produce by S. violacearaber and Lybrid antibiotics produced are mederrhodine A and dihydrogranatirhodine.

Engineering of Poly ketide antibiotics: "Poly ketide" means a class of antibiotics that are synthesized through the enzymatic condensation of small carboxylic acids such as acetate propionate and butyrate. Various polyketides produced by plant and fungus majority produced by actinomycetes.

Poly ketide antibiotics are synthesized by a complex enzymatic mechanism analogous to that used for the synthesis of long chain fatty acid. Synthatases that catalyze the biosynthesis two classes of polyketides synthatese are present of aromatic polyketides make up one class and consists of one polypeptide with an active site for each reaction.

Second class includes synthesis that are assemblies of several polypeptides that have separate. These enzymes have a number of different domains that has a separate enzymatic activity.

The complete synthesis of a polyketide requires the participation of several multifunctional enzymes.

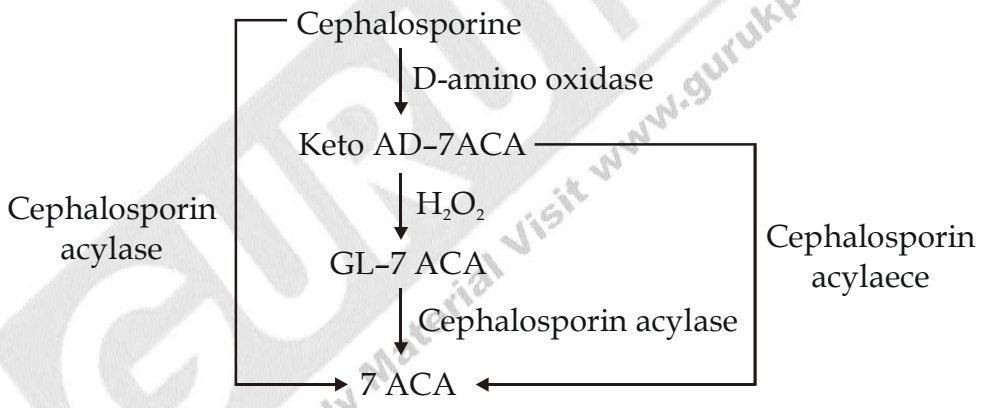
Improving antibiotic production: Genetic engineering can also be used to enhance the yield and rate of production of known antibiotics. Large-scale production of antibiotics by streptomyces is limited by the amount of oxygen available to the cells. Low solubility of oxygen in aqueous media, results in oxygen depleted culture medium that causes poor cell growth and reduced antibiotic yield. To overcome this problem. To improve the design of bioreactors. By genetic manipulation of streptomyces strain it is possible to utilize the available oxygen.

Some aerobic microbes used oxygen poor environment and synthesize a hemoglobin like molecule that can sequester oxygen from medium and then deliver into the cells example vitreoscilla produces a hamodimeric hence protein that is functionally similar to eukaryotic hemoglobin. Gene for ultreoscilla hemoglobin was isolated and sub cloned into a reactor expression of the vitreoscilla hemoglobin gene in S. codicolor the vitreoscilla hemoglobin represented approx 1% of total cellular protein. When both transformed and non-transformed S. Coelicolor cultures were grown in the presence of low level of DO, the transformed cells with a functional vitreoscilla hemoglobin produced 10 times more actinohodine per gram of cells and had greater cell densities than non transformed cells.

A compound 7 aminocephalosporanic acid is synthesized chemically from the antibiotic cephalosporine and is used as starting material for chemical synthesis of a number of cephalosporin. But there is no organism that can synthesize 7 ACA. However, a novel 7 ACA biosynthesis

has been constructed in fungus. "Acremonium chrysogenum, which normally synthesizes only cephalosporine. The genes involved in this novel engineered pathway encodes α-amino oxidases and comes from the fusarium solani and cephalosporin acylase comes from Pseudomonas diminute. Both of these genes were sub cloned anto an A. Chrysogenun rector under control of a promoter.

In first step cephalosporin C is converted into the compound 7-B-Cephalosporanic acid by amino oxidase. Some of this products reacts with the H_2O_2 to form 7-B-Cepholosporanic acid (GL - 7ACA), cephalosporin C-keto, AD-7ACA are each hydrolyzed by cephalosporin acylase to form 7ACA. However, in the absence of the D-amino acid oxidase, only 5% of cephalosporin C is converted to 7 ACA to both enzymes are essential for 7 ACA.



Genetically engineered biosynthesis of 7ACA from Celphalosproin C.

Biopolymers: These are large multiunit macromolecules synthesized by microorganism plants and animals.

Same of these polymers have physical and chemical properties that are useful in food-processing manufacturing and pharmaceutical industries.

By new genetic engineering we can modify existing biopolymers to enhance their physical and structural characters.

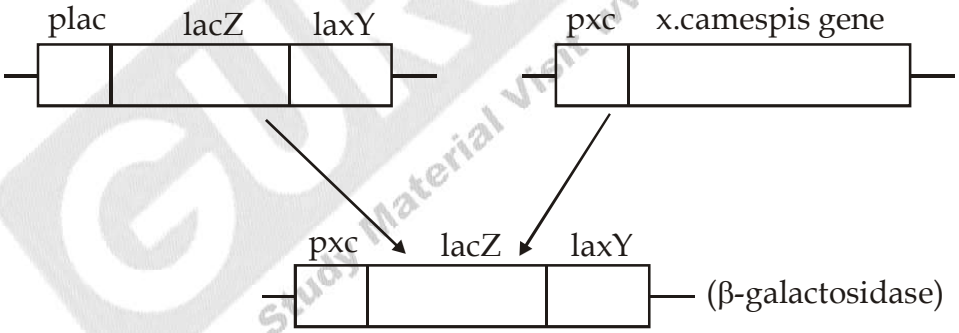
Xanthangum production by engineered Xanthomonas: "*X. Camestris*" is a gram negative aerobic soil bacterium that produces commercially important biopolymer xanthan gum, as a by-product of its metabolism.

This polymer has a cellulosic backbone made up of a straight chain polymer of glucose unit its trisaccharide chain includes one glucouronic acid and two mannose residue which are attached to every second glucose residue of the backbone.

Xanthangumgum was a high viscosity is stable in extreme physical and chemical environment and exhibit physical and chemical properties similar to plastic. It's physical properties makes it useful as stabilizing or suspending agent. For production of Xanthan wild type X. Campestris can efficiently utilize glucose, Sucrose and starch but not lactose as disposal of whey (consist of water lactose and protein) is a major problem of diary industry but this by-product can be used in creative ways.

Whey could be used as a carbon source for growing microorganism. With this in mind, X. Camestris was genetically engineered to grow an whey.

The *E. coli* lac-Z Y genes, encodes the enzymes β - galactosidase and lactose permease, were cloned ante a broad-host-range plasmid under transcription control of an X-Camestris phage promoter.



Engineering *E. coli* lacZ and lac Y (lactose permease) genes for constitutive expression in X. camestris

This construct was introduced into *E. coli* and then transferred from *E. coli* to X. Camestris by tripartite mating. Transformants expressed the enzyme β- galactosidase and lactase permease at high level utilize lactose as 'C' source. By contrast wild type X. Camestris produce high level of xnthanum gum only when grown on glucose.

Synthesis of an animal adhesive biopolymer in microbial cells

An adhesive protein biopolymer originally isolated from the blue mussel *Mytilus edulis* in microbial cells. This biopolymer is strong water proof adhesive protein that enable the mussel to attain very tightly to a variety of surfaces.

The cDNA for adhesive protein was isolated from a cDNA library that was constructed with messenger RNA (mRNA) isolated from the gland that actively secrets byssal adhesive. Both cDNA and adhesive protein have unusual feature that might make cloning expression and production in a heterologous host of a functional adhesive protein difficult. First, Highly repetitive nature of adhesive protein cDNA could make if unstable as a result of recombination and subsequent loss of cloned sequence.

Second, proline lysine and tyrosine represent about 70% of the amino acid of protein. So very high level of synthesizes may not be achievable because intracellular aminacyl-tRNA pools might be limiting when cDNAs for the adhesive protein were cloned ante yeast expression vector and introduced into yeast cell. Novel forms of adhesive protein were synthesized and represented a significant fruition of total cell protein. Thus there were no problems concerning the production of moderate amount of the adhesive protein. Considerably higher expression level were attained when a chemically synthesized adhesive protein gene sequence was used.

Microbial synthesis of Rubber

Natural rubber, is an extensively used biopolymer that is obtained from a large number of different plant, biosynthesis of rubber starts from simple sugar and requires 17-enzyme catalyzed steps with the final step being polymerization of isopenty pyrophosphate onto an allylic pyrophosphate. Last step is catalyzed by enzyme rubber polymerse. In synthesis of rubber by genetic engineered microbes initial step, is: a cDNA library was constructed by using mRNA from rubber producing plant Hevea brasiliensis. This library they, screened with a short synthetic DNA hybrillization probe whose sequence was based on the amino acid sequence of a portion, of rubber polymerase enzyme.

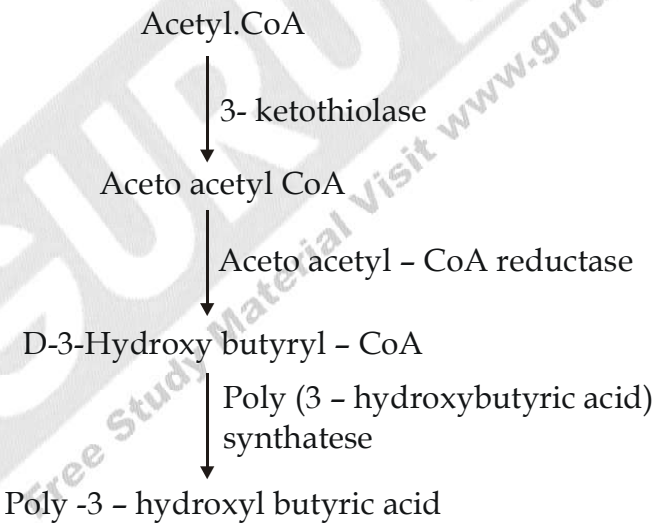
Antibodies directed against purified enzymes for proving that cloned

cDNA expressed rubber polymerase. This cDNA clone can be used in concert with other genes in the rubber synthesis pathway.

Production of Polyhydroxyalkanates

PHA, are a class of biodegradable polymers that are produced by a number of different microbes. These compounds have thermoplastic / elastic properties depending an polymer composition.

There compounds and its derivatives are produced by fermentation of *A. eutrophus*. This organism grows very slowly and utilize only a limited number of 'C' sources for growth making production cost very expensive besides these when genes for the biosynthesis of this polymers were transferred to *E. coli*, the resultant transformants grew rapidly to a high cell density and accumulated very large amount of Poly-3-hydroxy butyric acid. It is synthesized from acetyl CoA in 3 steps catalyzed by enzyme.



Synthesis of poly (3-hydroxybutyric acid) from acetyl CoA

Q.9 What is SCP? How many types of microorganisms are used for production of SCP?

Ans. The term "Single-cell protein" (SCP) was coined in 1966 and is used today for microbial biomass used as food and feed additives.

Either the isolated cell protein or total cell material may be called SCP.

Microbes are eaten in a lot of food products such as cheese, vinegar, mushroom koji.

Because of insufficient food supply and high protein content of microbial cells, the use of biomass produced in fermenter would be an ideal supplement to conventional food supply. SCP is of great value because of its high protein vitamins, lipid and all essential amino acids.

However the following points must be considered with regards to safety.

(1) There is possibility that microbes may produce highly toxic substances.

(2) Slow digestion of microbial cells may cause indigestion and allergic reaction.

(3) High nucleic acid content can be hazardous to health.

Following substrates are being studied for SCP production like:- methanol, carbohydrates, alkanes cellulose and waste materials.

Production of Single-Cell protein from alkanes:

Alkanes can be catabolized by many yeast and some fungi and by source bacteria. Candida oleophila and Saccharomycopsis lipolytica have been most studied yeast for SCP production.

Disadvantages of using alkanes is that they are not easily soluble. During growth in bioreactors with impellers large alkane dress are formed which remain suspended considering the low water solubility of alkanes.

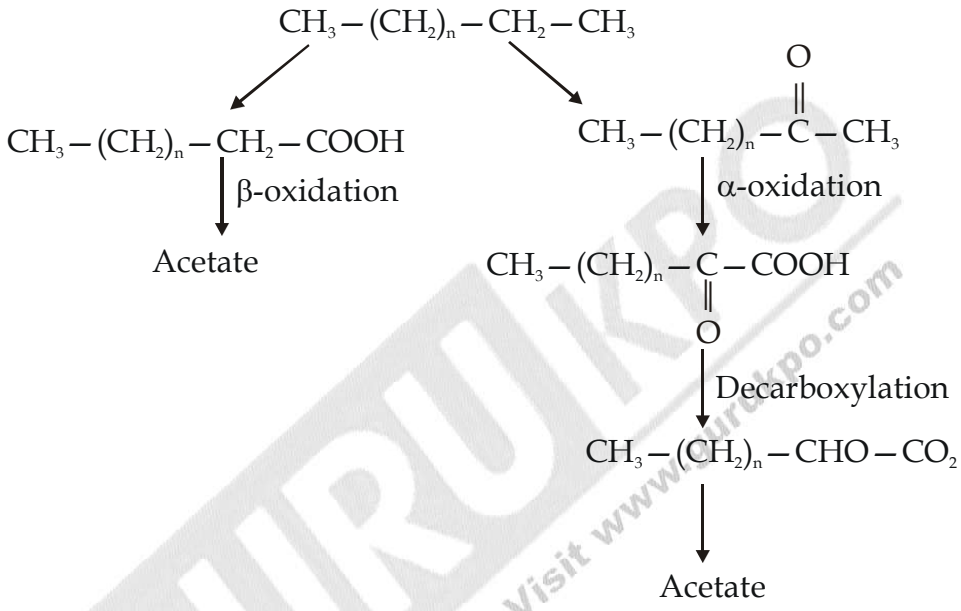
Cells form emulsifying substances which converts the insoluble alkanes into droplets, they can reach the cytoplasmic membrane through the cell wall via passive diffusion, cells growing on alkanes are enriched in lipids and these play an important role in transfer of alkanes.

Catabolism of Longer Chain alkanes

The first step in the utilization of alkanes is the introduction of molecular oxygen into the molecule. Two pathways for oxygen introduction are terminal and subterminal oxidation. In terminal oxidation monocarboxylic acid is produced via intermediate stages of primary alcohol. After

terminal oxidation acetyl-cost by β-oxidation.

In subterminal oxidation ketone is produced via a secondary alcohol α-oxidation with decarboxylation and β-oxidation has been found in Candida.



In comparision to glucose Candida toopicalis grows much more poorly an alkanes, but yield is better and the oxygen uptake rate is similar.

"Methane" is the chief component of natural gas, is a desirable energy source for SCP production. However in contrast-to higher hydrocarbons methane cannot easily be liquefied and high risk of explosion.

Methane utilizing bacteria are methylomanas metanica Methylococcus capsulatus. Enzyme methane oxygenase oxidizes methane to methanol, which then inhibit the growth of bacteria, and channeled into 1^o metabolism methanol is preferred starting material for system using C substrate.

$$CH_4 + XH_2 \rightarrow CH_3OH + H_2O + X$$

Methane can be obtained as a very pure gas. Methane utilizing bacteria are obligate methylotrophs.

Methanol fermentation:

Extensive research on methanol utilizing organisms was, carried at. Methanol can by obtained from natural gas methane, oil or coal. Wood could also be used as a starting material for methanol production. Bacteria yeast and fungus may considered for the production of SCP from methanol.

Besides the obligate methylotrophic bacteria that grow only an C_1 compound, facultative bacteria metabolize longer chain hydrocarbon.

In contrast to bacterial yeast are unable to use any C compound other than methanol. In methanol fermentation bacteria are employed for these reasons Rapid growth, higher protein content and better yield.

Methanol is oxidized via bacteria into CO_2

CH_3OH ? HCHO HCOOH ? CO_2

In first step to formaldehyde requires enzyme methanol dehydrogenase.

Methanol can also be oxidized by H_2O_2 with peroxidase activity. The H_2O_2 being produced by methanol oxidase. After oxidation of methanol into formaldehyde:-

Conversion of formaldehyde to formate with reduced glutathione (GSH) by "formaldehyde dehydrogenase", reaction also occur in yeast.

$$HCHO + NAD + H_2O \xrightarrow{GSH} HCOOH + NADH_2$$

By unspecific methanol dehydrogenase,

Two/Three ATP molecule per mole of substrate are obtained. In last step oxidation of formate occurs is common to all methanol utilizing bacteria. It involves NAD dependent formate dehydrogenase and yield 3ATP per mole of substrate.

HCOOH + NAD CO_2 + $NADH_2$

This CO_2 may be taken up in Photosynthetic organism via ribulose diphosphate cycle (Colvin cycle).

Fermaldehyde other products can be metabolized by different metabolic pathway like:- Serine – pathway, dihydroxyacetone pathway, ribulose mono-phosphate cycle etc.

Production process:

Imperial chemical industries (ICI) was the first company to develop continuous methanol fermentation for production of SCP.

ICL studied the effect of CO_2, O_2 and methanol concentration and effect of pressure differential between bottom and surface of bioreactor, on productivity.

They develop a "pressure cycle fermenten", a combination of air lift and loop bioreactor.

It consist of 3 units:-

Air lift column, down flow tube with heat removal and gas release space. The organism used for industrial SCP production is Pseudomonas methylotropus, a methanol oxidizer.

In the product recovery process, partial cell lysis is first achieved, via heat and acid treatment and nutrient solution is then clarified by decanting. Water is then recycled back into the fermentor.

Single Cell protein from wood ? At this time

Cellulose from natural sources and waste wood gives an attractive starting material for single-cell protein production.

Cellulose sources must by pretreated physically and chemically to break down cellulose into sugars. It can be enzymatically with cellulose or chemically with acid hydrolysis.

In enzymatic treatment extracellular cellulase can be used that is excreted by both bacteria and fungi.

Cellulose consist of 3 different enzymes that are:- Endo β-1,4 glucose, Exo – β-1,4 gluconase β-1,4 glucosidase.

Bacteria and fungi used in SCP production are: Cellulomonas, actinomycetes, Trichoderma, pennicillium, etc.

SCP from Carbohydrates:-

Large scale cultivation of yeast is widely used is used in manufacture of baker's yeast. In addition several large scale yeast processes employ whey as a starting material (Whey = principle sugar is lactose).

A promising product is a enycoprotein. The fungus Fusarium gramunearum is continuously an glucose supplemented with biotin and ammonium ion serving as the nitrogen source. The pH is regulated at 6.0 and incubation temperature is $30^{O}C$ from 1 kg of glucose 1 kg wet wt. of fungal mycelium is obtained, with a protein content of 136 gm. The culture taken directly from the fermenter is heated to $64^{O}C$ to inactivate proteases and to activate endogenous RNAases. After so min. heating the rRNA is reduced and its breakdown products diffuse from the cells. The dried protein is formed into structures that resembles park chicken. The final product is produced in the fermenter.

Q.10 Write brief notes on Production of (i) Beer (ii) distilled beverages and other microbial food products?

Ans. Production of Beer:-

Types of Beer:- There are 2 major types of beer which are distinguish on the basis of fermentation differences "Bottom fermented beers" named because as the fermentation subsides bottom yeast tends to flocculate and settle. "Top fermented beers" are named analogously. During fermentation top yeast rises to the surface where it recovered by skimming.

Lager beers: Bottom fermented beers are generally called Lagers. Two kinds of lager beers are there light and dark.

This beer is light bodied with a dry crysp bitterness.

Ales: Top fermented beers are called "ales". They are distinguish on the basis of color and body. The darker, sweeter mild ales are occasionally called brown ales.

Elements of the brewing process:-

Malt: Malt is prepared from barley which is sprouted under control condition and their dried. It is kilned at higher temperature for a longer time and have a reduced enzymatic activity. Regular malt are kilned at enough to arrest growth.

Malt provides carbohydrates and proteins and also the carbohydrates and proteases which break them down. The simple sugar and amino acid produced serves as nutrients for yeast growth and metabolism.

Adjuncte: Brewing adjuncte are prepared from unmalted cereals-mainly cares (maize) or rice and are pure carbohydrates without significant amounts of lipids, enzymes or other protein. Brewers use adjuncts to impart desirable qualities of products.

Water: For brewing beer good quality of water is a primary requirement. Pure water is free of turbidity color odor and taste. It is free of organic matter, iron, heavy metals and sulfides.

Hops: The hop plant "*Humulus lupulus*" is a dioecious and herbaceous plant in which only female flowers are used in brencing and i.e. commonly referred to as hops. Hops are rich in essential oils in which various compounds like hydrocarbons (mainly terpens) and oxygenated compounds.

Resins (15% of total hops wt) are responsible for the bitterness which hops impart to beer.

Most of the flavor of hops in beer is due to α-acids.

Culture Yeast: Culture yeast is an essential ingredient in spontaneous fermentation process.

Additives: These are usually used during conditioning or finishing. Some brewers use yeast food for better fermentation. These additives provide nutrients for yeast as trace metals.

Process:

Malting: When barley is first received for malting it is cleaned of dust and foreign material and then stored. Following storage broken barley grains and residual foreign materials are removed is Barley is then sieved into 3 sizes. Large and medium size corn are malted separately whereas small size once are sold for animal feed.

These steps are essential for obtaining unifous germination essential for preparation of good quality.

In beginning of malting steeping of barley is done in water at 12^{o}-$15^{o}C$ for 2-3 days.

After steeping germination begins, embryo is activated and hydrolytic action on the endosperm moves outward from the embryo, process called

as "modification." Properly modified malt is readily friable. The final step in malting is screening a process which removes the rootlets, that are undesirable in brewing.

Brewing and mashing: The malt is ground to a meal or coarse flour by a dry milling process. In mashing ground malt and brewing water are mixed. This mash is then heatch. Infusion and decoction are 2 different methods of heating:- "**Infusion mashing**" consist of step wise heating to several successively higher temperature below the boiling point. "**Decoction mashing**" is accomplished by with drawing a portion of the mash, heating it to boiling and pumping it bark thereby raising the temperature, of entire mash. This sequence is repeated several times. In united states, where adjuncts are used extensively a double mash system with some of the features of both the infusion and decoction methods is common. In this system at optimum temperature (40^{o}-60^{o}C) alt malt mash is heated.

Fermentation:- Fermentation is central to the beer-making process, its principal ingredients are "Wart" and yeast. Wart is the rang material from which beer is made by the metabolic action of brewer's yeast.

The clarified wart is inoculated with yeast is suitable strain which has been grown in a pure culture propagator or recovered from an earlier fermentation.

Primary fermentation: - Twenty four to forty eight hours after inoculation clumps of foam appears on the surface due to evolution of carbon dioxide. At this time yeast gradually increase in size and it corresponds to the logarithmic growth phase of yeast. Yeast growth during larger fermentation accumulate to 4-5 times the inoculation. As the fermentation proceeds, specific gravity, decreases due to utilization of carbohydrates, amino acids by yeast and also due to production of ethanol with densities less than that of water. At the end of primary fermentation, the fermenters is cooled to about 0^{o}C. During this period most of the yeast settles ant and 'young beer" as if is often referred to, is removed from the settled yeast and pumped to a storage tank at 0-2^{o}C a process called "Fassing". Brewing may also be a continuous process.

Continuous process:-

The manpower requirement is less because the process is automatically controlled. Equipments required for the process is relatively complex and high level of technical skill is required to operate it. Although the fermentation time is greatly reduced by fermentation process.

Aging and Finishing: - Aging may or may not involve a secondary fermentation. Finishing process includes clarification carbonation and use of various additives (like caramel antioxidants, proteolytic enzymes).

The purpose of clarification is not only to remove residual yeast but also to remove larger aggregates which render the beer colloidally, unstable at low temperature (5°C) Beer is aged near 0°C to foster the precipitation of colloidal complexes. Although suspended material may be removed by centrifugation and filteration and solubilized by protolytic enzymes.

Turbidity during aging arises from microbial contaminants that can be removed by filteration.

Secondary fermentation is carried out in closed tanks so that the beer becomes fully carbonated during process.

After final filteration, the beer is packaged 14 cans/bottles.

Distilled Beverages

(i) Whiskey:- Whiskey is an alcoholic distillate from a fermented mash of grain is bottled at a minimum of 80^0 proof. Bourbanrye "wheat or malt whiskey" is being distilled at less than 160^0 proof and matured at not more than 125^O proof.

"Corn Whiskey" is distilled at less than 160^0C proof made from a mash of 80% car.

For production of Beverage alcohol the corny grains are sampled graded and checked for possible presence of undesirable contaminants such as high bacterial counts in the malted-barley.

Mashing and Conversion: Dry milting may be accomplished with attrition, hawner or roller wills.

Attrition and hamner mills works satisfactorily for mashing. Roller mills may be preferred for pressure cooking due to low incidence of flowing when operated properly.

Meal should be held for only short periods of time due to susceptibility to hygroscopic action and to infection. Ideally meal should be slurried almost immediately after grinding.

Wet milling may also be used in the manufacturing of distilled beverages.

After steeping of corn in water the hulls and germ are then separated by milling from starch and the gluten. Starch and gluten are surried once again and separated. In order to fractionate the starch an acid treatment is introduced.

Volume of liquid corn slurry then introduced for mashing or cooking may vary from 76-114 ltrs/vessel. Temperature will range from 66° to 177°C and holding times at selected temperatures from 30 sec units pH should be 5.2-5.5 and good starch exposure. Conversion of starch to fermentable sugar begins, with the α-amylase attacking the long chain sugar molecule.

Fermentation:- Yeast propagation for whiskey making accomplished from pure culture methods exclusively through combination of pure culture.

A typical procedure will maintain a pure culture or slants which will provide the inoculation for plant scale liquid culture in regular short term cycle multi-plant companies provide starter cultures from a centrallocation where the yeast will be transferred under sterile conditions from the slants to a small liquid medium. After 18-20 hrs internals the volume of culture media will be increased. Temperature during culture should not exceed 28-29°C.

The barley malt in the yeast mash serves 2 purposes conversion of starch into fermentable sugar and contribution of flavor to the whiskey. In distillers mash pH control is critical when fungal enzymes are used. AtpH-5.5 best activity is achieved optimum conversion takes place when the enzyme are added to the fermentor at 21°-24°C.

Fermenters may be constructed from concrete wood steel or stainless steel. As soon as the first converted mash is received in the fermentar, a 2.5-3% by volume yeast inoculum should be introduced.

Operating schedule should be so designed that when laboratory indicates fermentar is " worked out" the beer should be put into a beer well for immediate distillation.

Distillation: In distillation a typical product from a beer could be drawn off at 110^{o} to 115^{o} proof. A further step "doubling" could produce a whiskey from 120^{o} - 145^{o} proof.

The future might bring an expansion in multicolumn whiskey distillation units which are capable of making uniform bourbons which mature more rapidly than the heavier flavored whiskeys.

Bourbon Whiskey: It is the most popular category of American whiskey Bourbon must contain at least 51% corn 30% rye, and 10% barley malt distillation methods for several years did not utilize fractionation.

In the Whiskey the only component removed was a portion of water. Current bourbon production methods may specify a mash of 80-85% corn, 8-10% rye, and 8-12% malt.

Rye Whiskey:- Rye Whiskey must be made from a mash containing at least 51% rye grain.

Flavours of rye whiskey may range from a mild pleasant pleasing sensation to very hash hardly palatable characteristics. Previous distillation methods included the use of charge stills as one of the types of equipments to produce rye. A charge still is a series of chambers in a vertical stack arrangement. The vapors rise through the still but the liquids are manually transferred from top to bottom. The mash is exposed to high temperature for extended periods of time and so protein decomposition is induced.

Corn Whiskey:- Corn Whiskey must be distilled from a mash containing

at least 80% corn. A good corn whiskey will have a pleasingly mild, slightly sweet, clean taste.

Light Whiskey: It's a new category of whiskey. The distillation proof specified ranges from 160^{O} to less than 190^{O}.

Canadian Whiskey: Canadian Whiskeys are distilled only in Canada from corm mash, rye and barley malt. Both Canadian and scotch whiskey at this time enjoy a label statement advantage, when sold outside the united States, as 3 years in wood enables the bottling without age declaration.

By Canadian whiskeys are lighter or less flavourful than many other distilled spirit.

Scotch Whiskey: It is made only in Scotland. There are 2 basic types:- malt and grains.

Malt is a key component of scotch whiskey infant many malt distillers in recent years made their own malt. Process of malting the barley begins by cleaning the grain and is followed by sleeping the grains for 2-3 days in warm water. Water is drained and the soaked grains are spread over the floor to an even depth for germination.

After germination the malt is heated to stop further development. The object of germination is the production of α and β amylase that convert the starch grains into sugar. Fermentation temperature ranges from 22°C-27°C. The inoculation of yeast may be supplied in dry form.

The completed whiskey is chilled to same temperature and tight filtered to produce a bright sparkling liquid.

Rum: Rum is defined as an alcoholic distillate from the fermented juice of sugar cane syrup. Sugar cane molasses or other by products. It is one of the oldest distilled beverages in the world.

Some distillers use the pure cane juice, but more typical is the use of

black strap molasses. There is same evidence that fungal amylase might enhance the yield.

Many distillers use pure culture yeast but some west Indies producers continue to use spontaneous fermentation.

Most large rum distilleries use continuous multicoloumn stills.

Processing and bottling methods are similar to other beverage alcohol products.

M.Sc./B.Sc. (Part II) Examination, 2011

(FACULTY OF SCIENCE)

(Common to Three and Five Year Integrated Course)

BIOTECHNOLOGY

Paper BT-603

INDUSTRIAL BIOTECHNOLOGY

Year-2011

Time.: 3 Hours **Max. Marks :: 50**

Attempt FIVE questions in all, selecting ONE question form each section. Questions No. 1 is compulsory. Each question carries equal 10 marks.

1. Define/Explain the following:
   (i) Alternative splicing library
   (ii) Antigen capture immunoassay
   (iii) Baculoviruses
   (iv) Bioaugmentation
   (v) Checkerboard hybridization
   (vi) Complementary DNA
   (vii) Core enzyme
   (viii) Cosmid
   (ix) DNA vaccine
   (x) Metagenomic library.

Section-A

2. What do you understand by 'closing vectors" ? Explain their general properties giving suitable examples.
3. Write short notes on the following: -

(i) Biosynthetic Plastics

(ii) Biosynthesis of β Lactam antibiotics.

4. What are molecular probes? How can these be prepared and labeled ? Discuss briefly their uses in biotechnology research.

Section-B

5. What do you understand by 'Humanization of Monoclonal Antibodies" Discuss the clinical application of humanized antibodies.
6. Write short notes on any two of the following

(a) Immune memory and vaccination

(b) DNA vaccines

(c) Edible vaccines

7. Describe the uses of pharmacogenomics in biomedicine involving diagnosis and treatment of disease. Discuss using suitable examples.

Section-C

8. Write an essay on " Natural Attenuation of Pollutants".
9. Describe the process of manufacture of Beer and Wine in industries.
10. How can you use recombinant microorganisms for the commercial production of antibiotics? Discuss the technology and process in details.