APPLIED MICROBIOLOGY

Microbial production of Flavours and fragrances; Fats and oils; Dyes; Bioplastics (PHAs); Polysaccharides; Pharmacologically active substances from marine microbes; Anti-cancer agents and Microbial biotransformation

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Introduction

Microbes have been employed by man for the production of wine, bread, cheese etc since antiquity without knowing that the microorganism are involved in such product generation. These activities remained exclusively an art in the society until the discovery of microbes in seventeenth century by Leeuwenhoek and experiments of Pastuer in 1860's, which dispelled all myths and proved that the microbes bring about fermentation. This followed rapid development in basic techniques for isolation, preservation and handling of microbes and by the beginning of twentieth century the art of harnessing the potential of microbes became the science of microbiology. The role of microbes in industry, agriculture, medicine and environment became increasingly important in all these years and today the microbiology is more an applied than basic science.

The potential of microorganism was further transformed into production of a range of products at industrial scale with the inputs from chemistry, genetics and engineering sciences. Now the microbial cell is considered to be as mini factory and the substrates (carbon, nitrogen sources etc) in the medium as raw materials which are converted by a microbial cell into finished goods i.e. product. It has become feasible to convert inexpensive substrate into a high value product with the selection of a right microorganism and controlled growth or reaction environment. The metabolic diversity of microbes is being explored to produce novel products for industry. In this present chapter, some important products e.g. polysaccharides, anticancerous agents, flavours, single cell oil and others of microorganisms will be discussed to understand the potentials that microbes have for providing materials and molecules to man in order to fight disease, hunger and malnutrition.

Microbial Production of Flavours and Fragrances

Plants and animals are the natural sources of flavours and fragrances since ages. However, the man used microbial systems for the first time to impart new aromas to the fermentation products (beer, wine, cheese). Vanillin (1874) and coumarin (1868) were the first synthetic fragrance and flavour compounds made available for use in the food industry. Presently flavours and fragrances are widely used in food, beverage, cosmetic, detergent and pharmaceutical formulations, and their worldwide demand was estimated to be US$ 16 billion in 2003. Most of these flavouring and fragrance compounds are prepared by chemical technology and only a small fraction of the demand is met from plant or through microbial sources.

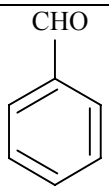
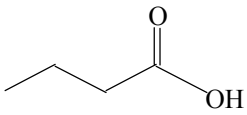
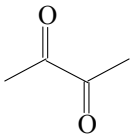
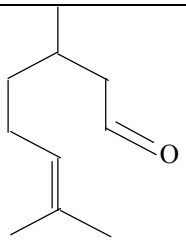
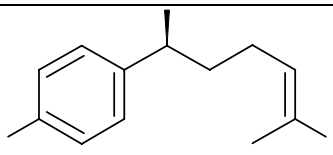
Flavours and fragrances are divided into two categories: a) natural and b) nature-identical. Natural flavours are prepared by extraction from plants or by enzymatic or microbial processes, and flavours and fragrances that are synthesized chemically or conversions of natural substrates are referred to 'nature-identical'. Vanillin is the most consumed flavour, which occur in tropical Vanilla orchids (Vanilla planifolia) meet only less than 1% of the world market demand. The cost of production of synthetic vanillin is much cheaper (i.e. >US$15/kg) as compared to natural plant product (US$1200-4000/kg).

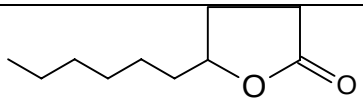
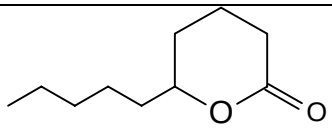
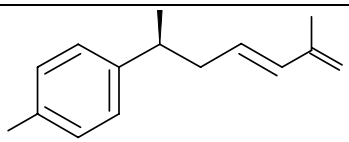
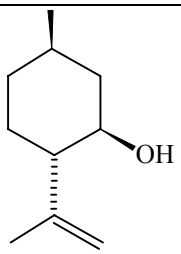
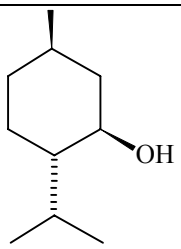
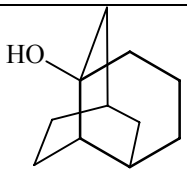
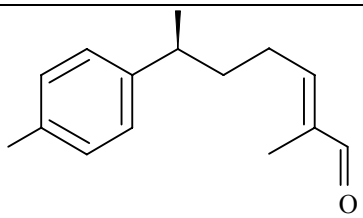
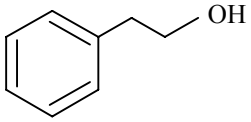
The 'natural' flavours are produced either by de novo synthesis in microbes or plants or through single-step biotransformation of natural substrates by enzymes or microbes or plant cells (e.g. synthesis of nootkatone using citrus cell cultures). In de novo synthesis, microbes transform carbon or nitrogen compounds into flavour molecules. Lipases, esterases, proteases, nucleases and some glycosidases are some of the enzymes extensively used in industry for the synthesis of flavouring compounds since they catalyse single-step

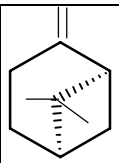
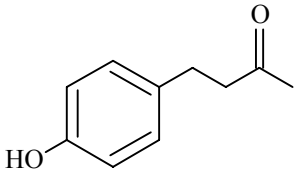
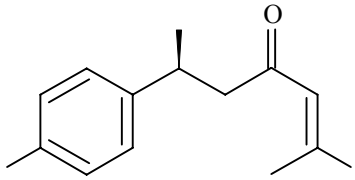
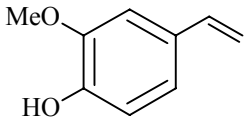
transformation of substrates into flavouring molecules. Microbiological or enzyme based processes have been developed, where lignin, phenylpropanoids and phenolic stilbenes are converted to the desired flavours and fragrances.

There are some aroma compounds e.g. esters (ethyl and butyl acetates, ethyl butyrate, caproate, isobutyrate, isovalerate, 2-methyl butyrate, menthyl acetate), aldehydes and ketones, (acetaldehyde, diacetyl), acids (acetic, butyric, caproic, caprylic, isobutyric, isovaleric, 2-methyl butyric) and lactones (gamma-decalactone) which can either be produced by microbial fermentation or by using enzymes (Table 1).

Table 1: Flavours and Fragrances Produced by some Microorganisms or using Enzymes

Product	**Chemical structure**	**Microorganism/ enzyme involved in production**	**Flavour**
Benzaldehyde		*Ischnoderma benzoinum* (a bracket fungus)	Almond
Butyric acid		*Clostridium butyricum* (bacterium)	Apple and pine apple
2,3-butanedione		*Lactic streptococci* (bacterium)	Flavour component of dairy products
Citronellal		*Rhodotorula minuta* (yeast)	Rose-like odor
(+)-Curcumene		*Saccharomyces cerevisiae* (baker's yeast)	Flavour component of many essential oils

γ-Decalactone		*Yarrowia lipolytica* (yeast)	Peach
δ-Decalactone		Enzymatic reduction of the α, β-unsaturated compound (massoia lactone)	Coconut-peach
(+)-Dehydro-curcumene		*Saccharomyces cerevisiae* (yeast)	Flavour component of many essential oils
(-) Isopulegol		Lipase (*Pseudomonas* sp.)	Citrus type fragrance
(-)-Menthol		Lipase (*Candida rugosa*)	Mint
Norpatchoulenol		*Pithomyces* sp. (mould)	Expensive fragrance compound
(+)-Nuciferal		*Saccharomyces cerevisiae* (yeast)	Flavour component of many essential oils
Phenolethanol		*Kluyveromyces* sp. (yeast)	Rosary

β-pinene		Lipase	Spearmint flavour
Raspberry Ketone		*Beauveria bassiana* (fungus)	Raspberry
Thaumatin and monellin	Proteins	*Kluyveromyces* sp. (yeast)	Chocolate flavours
(+)-Turmerone		*Saccharomyces cerevisiae* (yeast)	Flavour component of many essential oils
Vanillin		*Pycnoporus cinnabarinus* (fungus)	Vanilla

Microbial Production of Fats and Oil

Fats and oils obtained from microbial sources have a structural similarity to edible oils. The common sources of microbial fats and oils are oleaginous molds. Some of the eukaryotic microorganisms accumulate large percentage of triacylglycerols as cellular storage lipids and their lipids consist of high proportions of polysaturated fatty acids which have nutritional and nutraceutical significance. These microbial lipids are known as single cell oils (SCO).

In an oleaginous microorganism, oil and fats accumulation starts when nitrogen depletes but still there is an excess of carbon source (i.e. glucose) in the growth medium. Few yeasts such as Rhodotorula spp., Lipomyces starkeyi and Cryptococcus curvatus are capable of accumulating lipid up to 40 and 70% of their biomass. Very high fat contents have been reported in Aspergillus sydowii (97.4%) and Fusarium oxysporum (92.5%) and both these molds contain high level of unsaturated fatty acid (oleic acid). The lipid of A. sydowii comprise 35.4% saturated and 64.6% unsaturated fatty acids, while that of F. oxysporum 43.4% saturated and 56.6% unsaturated fatty acids.

Oil from moulds usually contains both short chains (C8-C12) to long chains fatty acids (C22-C24). Fungal lipids contain plenty of polyunsaturated fatty acids, linolenic acid and oleic acid. The constitution of oil depends on the composition of the medium e.g. F. oxysporum when grown in glucose containing medium, the fatty acids so formed resembles edible palm

oil, while the same cultured in sucrose containing medium produces lipid with long chain fatty acids. Some of the microbial lipids are listed in Table 2. The constituents of which are similar to edible plant oil.

Table 2: Fats and Oils from Microbial Sources

Organism	**Fats and oil**	**Resemblance**
Apiotrichum curvatum (yeast)	Oleic acid, palmitic acid, stearic acid and linolenic acid	Cocoa butter
Aspergillus niger (mold)	Oleic acid and unsat-urated fatty acids	Groundnut
Aspergillus sp. (mold)	Oleic acid and unsat-urated fatty acids	Seed fat of Meduca latifolia
A. sydowii (mold)	Oleic acid	Groundnut
Bacillus subtilis (Marine bacteria from *Aurora globostellata* marine sponge)	γ- Linolenic acid, eicosapentaenoic acid and branched chain fatty acids	-
*Crypthecodinium cohnii (*microalga)	Docosahexaenoic acid	-
Fusarium oxysporium (mold)	Saturated and unsaturated fatty acids	Groundnut
F. equisetti (mold)	Saturated fatty acid	Palm oil
Mucor circinelloides (mold)	γ- Linolenic acid	-
Mortierella alpine (mold)	Arachidonic acid	-
Pseudomonas sp. (Marine bacteria from *Heteronema erecta* marine-a sponge)	γ- Linolenic acid	-
Schizochytrium sp. (mold)	Docosahexaenoic acid	-

The oleaginous microorganisms are of commercial interest because they utilize wastes, transform low-value substrates into high value products such as SCO and reduce organic wastes to manageable size. *Apiotrichum curvatum* and *Candida bombicola* (yeasts) use whey as substrate and accumulates lipids as oil droplets. *A. curvatum* also accumulates 60% fat (on dry weight basis) in form of intracellular oil droplets and decrease 97% of the chemical oxygen demand of whey permeate. It grows very well on ripe banana vis-a-vis accumulates higher content of oil.

Microbial oils are cheap and efficient source of some polysaturated fatty acids (PUFA) of pharmaceutical and nutraceutical importance. Bacteria associated with higher marine organisms have emerged as better source of highly pure PUFA oils than fish and fungal oils. A few bacterial species belonging to the genera *Mycobacterium, Streptomyces, Nocardia* and *Rhodococcus* are also the potential bioresources for the production of triacylglycerols. The single cell oil can supplement conventional oils to some extent in meeting the demand of the ever-expanding human population for edible oils.

Microbial Production of Dyes

Production of dyes by microorganisms dates back to 1983 when Ensley *et al.* (1983) combined naphthalene dioxygenase system of the *Pseudomonas* in *E. coli*. In *E. coli* tryptophan is converted into indole by tryptophanase and then it is transformed into indigo dye (blue pigment widely used in textiles industry) which otherwise occur naturally in plants (e.g. *Indigofera suffruticosa*) by the naphthalene dioxygenase. The chemical production of indigo in the laboratory involves lengthy procedures and drastic chemical reactions. Therefore, indigo production using microorganisms has revolutionised the production of this dye (Fig. 1).

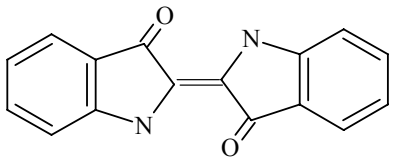


Fig. 1: Chemical Structure of Indigo Dye

The species of *Monascus, Rhodotorula, Bacillus, Achromobacter, Yarrowia* and *Phaffia* produce a number of pigments. Astaxanthin (carotenoid) is a red pigment obtained from *Phaffia rhodozyma*, *Haematococcus pluvialis* and other organisms and used in the pharmaceutical industry. Phycobiliproteins obtained from cyanobacteria and some algae have important application in food industries and as fluorescent marker in biochemical assays. Microbes as source of pigments have many advantages over plant sources (independence from weather conditions, faster growth on cheap substrates and easy manipulation of production conditions). However, most of the dyes from microbial sources are sensitive to heat, light and acidity, which make them commercially less viable.

Biodegradable plastics

The synthetic polymers especially plastics have revolutionized the industrial and domestic market because of their fabulous stability, durability and mechanical and thermal characteristics. Plastics are widely used for packaging, grocery carts, dent-resistant body panel of cars and others, and pose threat to the environment as these are resistant to degradation in landfills and emit toxic gases when incinerated. Therefore, the efforts are underway to develop biodegradable plastics possessing properties similar to their synthetic counterparts. As a result, a number of bioplastic materials have been synthesized which can be categorized into three major groups.

1. ***Photodegradable plastics***
   These plastics have photosensitive groups attached to the backbone of the polymer making them susceptible to the attack by light. Photosensitive components commonly used in such plastics are di-ketones, aminoalkyferrocene and carbonyl-containing species. The polymer degradation is triggered by UV light in the presence of UV sensitizers. Photodegradable plastics become weak and brittle on prolonged exposure to sunlight. The degradation follows in a two-stage process. UV light initially breaks some bonds releasing low molecular weight materials like waxes, and then converted to carbon dioxide and water by the bacterial action.

2. ***Biodegradable starch-based polymers***

In this type of biodegradable plastics, starch is used as a backbone to which short fragments of polyethylene are grafted. Biodegradation of such polymers is due to the enzymatic lysis of glycosidic linkages in starch leading to the formation of monosaccharides, disaccharides and oligosaccharides that are readily metabolized by microbes as a carbon source. This causes the polymer matrix to disintegrate into small polyethylene fragments, which serve as the substrate for other bacterial species. There is a range of starch-based plastics:

- **Thermoplastic starch products (TPS):** These contain more than 70% maize starch and are generally used as shopping bags, bread bags, bait bags, over wrap and 'flushable' sanitary products. Due to high starch content, these plastics are highly hydrophilic and get easily degraded in water. These natural starch polymers are commercialized by a Melbourne based company, Plantic Technologies Ltd.
- **Starch and synthetic aliphatic polyester blends:** These biodegradable plastics are prepared by blending 45% wheat starch with degradable polycaprolactone (PCL). The two commercially available such products are Mater-Bi ™ by Novamont (Italy) and Bioflex™ by Biotech (Germany). These plastics get completely biodegraded within eight weeks in soil.
- **Starch polybutylene succinate/ polybutylene succinate adipate polyester blends:** These are made by blending of polybutylene succinate (PBS) or polybutylene succinate adipate (PBSA) with cornstarch in the presence of 5% of compatibiliser (maleic anhydride functionalized polyester), which impart phase stability to starch based polymer blends. Plasticizers are added in such polymers in order to improve flexibility and decrease brittleness because of higher starch content (>60%). These are used as biscuit trays or film products and their half-life decline with increasing starch content.
- **Starch polyvinyl alcohol blends:** Such plastics are synthesized easily by blending polyvinyl alcohol (PVOH) with starch and are readily biodegraded in nature. The starch-PVOH blends are degraded mainly via hydrolysis and biodegradation of the sugar molecules. Some commercially available starch-PVOH blends are Novon™ (Chisso Corp, Japan), Novon™ (Warner Lambert, USA) and Mater-bi™ (Novamont, Italy).

3. ***Biodegradable polyesters***

The polyesters are the main constituents of biodegradable plastics because they contain hydrolysable ester bonds. These are divided into two groups: linear polyesters and aromatic polyesters. Except polyhydroxyalkanoates, all polyesters are synthesized chemically e.g. aliphatic polyesters are synthesized by condensation or polymerization reaction of diols and dicarboxylic acids. The linear polyesters are biodegradable in soil and water. The aromatic polyesters such as polyethylene terephthalate (PET) are more resistant to microbial attack. All polyesters usually get degraded by hydrolysis. Some of the important biodegradable polyesters are listed in Fig.2.

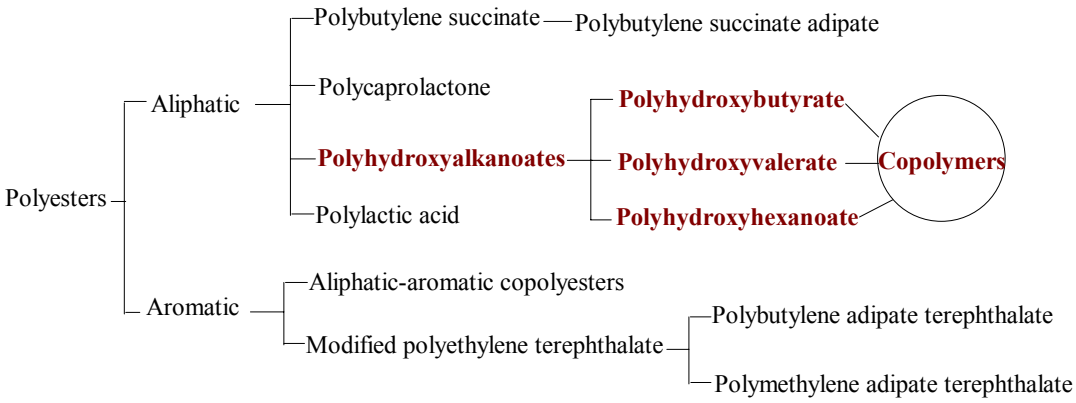


Fig. 2: Classification of Polyesters

Polyhydroxyalkanoates (PHA)

These are aliphatic polyesters naturally produced by about 75 genera of gram positive and gram-negative bacteria on sugar-based media. The efficient PHA synthesis is achieved when these bacteria are starved of nutrients. Under such stringent conditions, these microbes accumulate intracellularly about 90% of PHA of the total cell weight (Fig. 3). The polymers act as a carbon and energy source for the microorganism. The molecular mass of PHA varies between 50,000 and 1000000 Da (Fig. 4). PHAs are produced biologically by growing microorganisms on the renewable substrates (triglycerides, cellulose, sucrose and starch), wastes or byproducts of some industries (whey, molasses and glycerol) and chemically from 4-hydroxybutyric acid, propionic acid and CO_2. The most extensively studied PHAs are polyhydroxybutyrate (PHB) (Fig. 5) and polyhydroxyvalerate (PHV). The PHAs are used for manufacturing molded bottles and plastic films under the Biopol™ trademark.

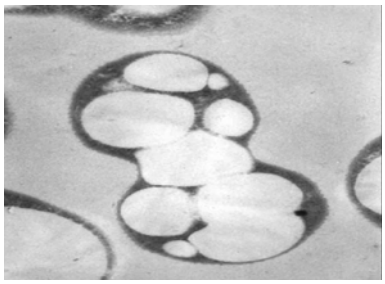
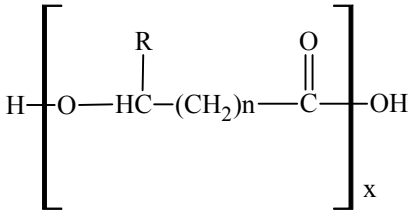


Fig. 3: PHA as white patches in *Pseudomonas putida*



R=upto C13

x= 1-3 or more

Fig. 4: PHA (Polyhydroxyalkanoates) -polyester produced by microbes

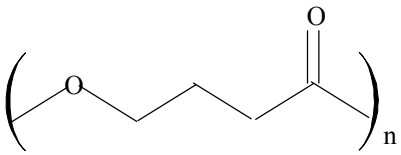


Fig. 5: Structure of PHB (Polyhydroxybutyrate)

The composting degrades PHAs and the commercially available Biopol™ (PHA)gets degraded completely in 10-week composting period at 60°C, 55% moisture, and C: N ratio of 18:1.

PHBH Polyesters

Poly-hydroxybutyrate-co-polyhydroxyhexanoates (PHBHs) are recently discovered natural biodegradable polyesters. The PHBH is produced by microbial systems using sucrose, fatty acids or molasses as carbon source. These are 'aliphatic-aliphatic' copolyesters. Procter & Gamble Co. developed such materials by blending PHB and PHA to obtain the desired stiffness or flexibility. These bioplastics based on PHBH have also been developed by Kaneka Corporation (a Japanese manufacturer) and marketed by Procter & Gamble Co. under the tradename Nodax™ (Fig. 6). PHBHs are easily decomposed by aerobic and anaerobic bacteria.

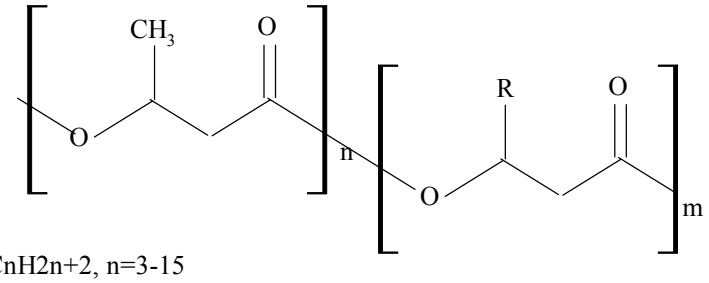


Fig. 6: Aliphatic-aliphatic copolyester [NodexTM P (3HB-co-3HHX)] produced by blending of PBA and PHA

Polylactic Acid (PLA) Polyesters

Polylactic acid is an aliphatic polyester produced by ring opening of the lactide group or by poly-condensation of naturally produced lactic acid. Lactic acid is formed as a by-product of corn wet milling during starch fermentation. PLA blended with starch make it more biodegradable and also reduce its cost of production. This copolymer as such does not qualify to be used as substitute for plastics due to its brittleness. However, using low molecular weight plasticizers such as glycerol, sorbitol and triethyl citrate, this drawback can be overcome. Since microbial enzymes attack its ester linkages, it gets quickly biodegraded in soil. Many PLA products are marketed under the trade name Lacea (Mitsui Toatsu, Japan), Lucty (Schimadzu, Japan) and Nature Works (Cargill Dow, USA). These are used as drink cups, take-away food trays, containers and planter boxes. The PLA polyesters have good rigidity characteristics and can replace polystyrene and polyethylene terephthalate for the selected applications.

Microbial Polysaccharides

The polysaccharides and gums are used as gelling agents, thickeners and stabilizers in food and pharmaceutical preparations. These are generally derived from plants and microbes. Gums obtained from plant and algal sources require skilled manpower for collection, and seasonal fluctuations immensely affect the quality and yield of the product. A number of microbes (bacteria, yeasts and fungi) produce polysaccharides and about 20 different types of microbial polysaccharides of commercial importance have been reported hitherto. Microbial polysaccharides constitute small fraction of the total polysaccharides used. In nature these gums are produced by microbes as exudates that provide them defense against environmental influences (e.g. desiccation), bacteriophage attack, attachment to surfaces, nutrient gathering and antigenicity.

Microbial polysaccharides are generally produced by the selected microbes under controlled fermentation conditions in stirred tank fermenters using complex media having high C: N ratios (10: 1). Glucose or sucrose is generally used as carbon and energy source. The high viscosity of the fermentation broths cause inefficient mixing and aeration leading to reduction of mass transfer and formation of heterogeneous polysaccharide. Fed batch fermentations are also used when high sugar concentration interferes with the growth and production of the polysaccharide. The polysaccharides are usually recovered from the fermentation broth by precipitation (with organic solvent or acid or salt) followed by drying and grinding. Some characteristics, general production conditions and applications of a few important microbial polysaccharides are given below:

Xanthan

Xanthan is a bacterial polysaccharide, which was discovered at NRRL (National Regional Research Laboratories, US Department of Agriculture) in 1950s. A plant pathogenic bacterium *Xanthomonas campestris* produces this gum. Xanthan is non -toxic and has been approved by the Food and Drug Administration for use as food emulsifier or stabilizing agent for oil or water emulsion such as salad dressing. About 20,000 tons of xanthan is produced annually for food and non-food applications (Table 3). It is a heteropolysaccharide and its basic structural unit consists of 2 glucose units, 2 mannose units and 1 glucuronic acid. The molecular weight of xanthan varies from 2×10^6 - 20×10^6 Da. It is basically a cellulosic backbone and to its every second glucose residue a trisaccharide side chain is attached (Fig. 7). Xanthan is stable at both acid and alkaline pH and forms pseudoplastic dispersion in water. Relatively low polysaccharide concentrations produce highly viscous solutions and the viscosity does not change much with the increase in temperature. Because of these characteristics and its inherent safety, xanthan has been placed under Generally Regarded as Safe (GRAS) category for use in food or pharmaceutical formulations in USA, EU and in a number of other countries. In India, Central Food Technological Research Institute (CFTRI), at Mysore has developed technology for production of xanthan gum and it is available for commercialization. Industrially used xanthan comprises of 37% glucose, 43.4% mannose, 19.5% glucuronic acid, 4.5% acetate and 4.4% pyruvate. The viscosity of the xanthan gum depends on the terminal pyruvate number and molecular mass.

Table 3: Industrial Applications of Xanthan Gum

S No.	Industry	Applications
1.	Petroleum	Flocculent and lubricant
2.	Textile	Suspending agent for dyes, pigments and controlling agent in printing
3.	Paints and inks	Stabilizer and emulsifier for thixotropic paints
4.	Ceramics	Suspending agent in ceramics glazes
5.	Paper and pulp	Rheology modifier for high size press and roll coating
6.	Adhesives, toothpastes and cosmetics	Used to control viscosity and modify flow
7.	Food	Used to improve texture of bread, mouth feel of bakery products and freeze-thaw stability of frozen foods. Used as thickening (in juice, drinks, chocolates, and pickles) and gelling agent (in dairy)

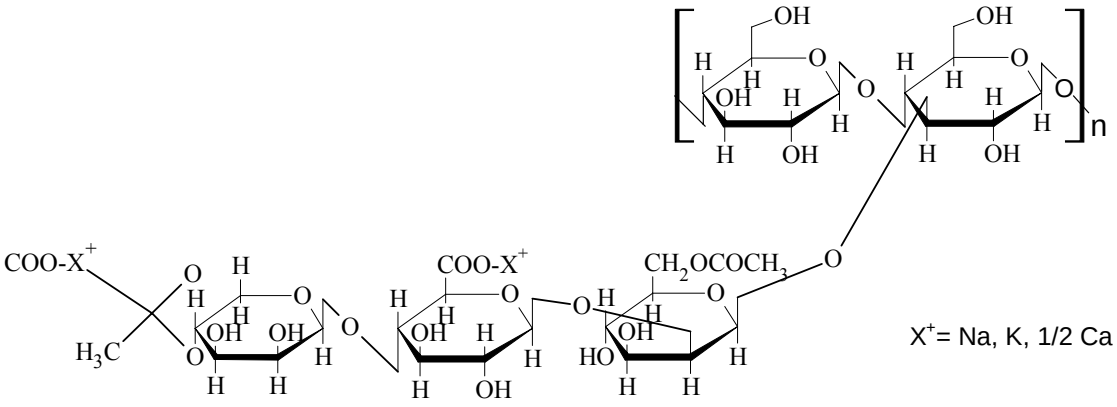


Fig. 7: Basic Repeating Unit of Xanthan Gum

Industrial production

The media used for the production of xanthan by *Xanthomonas* commonly constitute inexpensive carbon (sucrose/ sugarcane molasses/ whey, etc.) and nitrogen (ammonium or nitrate salts/yeast extract/soy meal/ peptone) and phosphorus (added in form of phosphate buffer) sources. The fermentation is carried out in a stirred tank reactors for about 2 days at 25-35 ˚C. The polysaccharide secretion starts in the log phase and continues in the stationary phase of growth. The pH of the medium decreases due to the production and accumulation of organic acid and it significantly reduces the xanthan production. Therefore for the maximum xanthan production, the pH of the broth is maintained around pH 7.0 during fermentation.

After the completion of fermentation, the culture broth is diluted to 100 cP (Centipoise, a unit of viscosity) with 33% ethanol. The broth is centrifuged to remove the cells and 1% KCl is added. The gum is precipitated by the addition of chilled 95% ethanol (3 volumes). The

major producers of xanthan are Merck and Pfizer (United States), Rhone Poulenc and Sanofi-Elf (France) and Jungbunzlauer (Austria).

Bacterial Alginates

The sources of alginates are *Azotobacter vinelandii*, *Pseudomonas aeruginosa*, *P. mendocina*, *P. fluoroscens* and *P. putida*. The alginates from different sources vary in their composition. The structural unit of *Azotobacter* alginate consists of D-mannuronic acid and L-guluronic acid. The polysaccharide contains the alternating blocks of polymannuronic acid (poly M), polyguluronic acid (poly G). The bacterial alginates differ from commercial algal alginates in having larger molecular mass. The alginate from *Pseudomonas* is a homopolymer of D-mannuronic acid. It may have single or double acetyl group substituted to D-mannuronosyl moiety. Due to the presence of acetyl group, these alginates don't bind to Mg^{2+} and Ca^{2+} and do not form gels with these divalent ions. Chemical deacetylation increases the ion binding capability of alginate. The number of acetyl group substitution on uronic acid cause substantial conformational changes. They are widely used in immobilization of microbes and as ion exchanger.

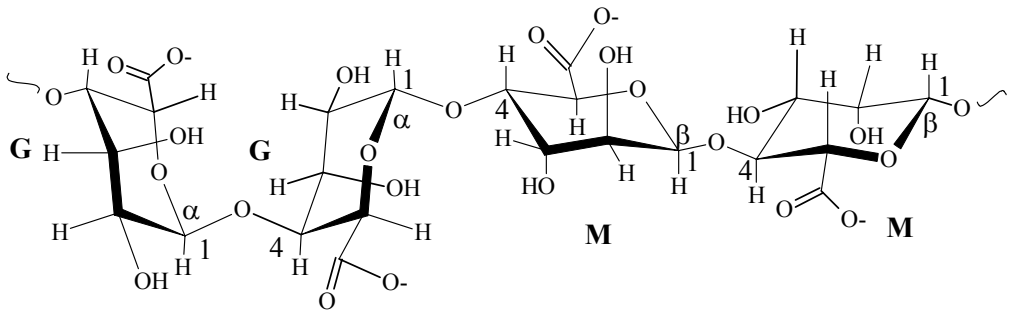


Fig. 8: Structure of Bacterial Alginate

Production

Bacterial alginate production has not reached a commercial stage as the alginate is unstable (mucoid Pseudomonas aeruginosa) and most of the bacterial strains also secrete alginate hydrolyzing enzyme, which ultimately decreases the molecular weight of the polysaccharide. However, mutant strains have been developed to overcome this problem. The mutant C-14 of Azotobacter vinelandii produced 6.22 g/liter of bacterial alginate and viscosity of the purified bacterial alginate was five times higher than algal alginate at a low concentration (0.6%). The pseudoplasticity of bacterial alginate was higher than that of algal alginate.

Curdlan

The curdlan is a simple unbranched glucose homopolymer having β 1-3 glucosidic linkage (Fig. 9) and is named so because it '**curdles**' on heating. Curdlan is produced by Alcaligenes faecalis var. myxogenes and organism also secretes a heteropolysaccharide succinoglucan, which is β-1-3 glucan containing 10% succinic acid. Molecular weight of curdlan varies from 5.3 x 10^4 to 2.0 x 10^6 Da. Alcaligenes faecalis var. myxogenes 10C3 is a mutant that only produces curdlan. The main carbon sources used in the media for the industrial production of curdlan by Alcaligenes are maltose and sucrose. The viscosity of broth does not increase during fermentation because of insoluble nature of curdlan that form gels at higher

temperature (above 54˚C). Curdlan has a unique feature to produce high set gels and is widely used in food industry. It also has immunostimulating effects.

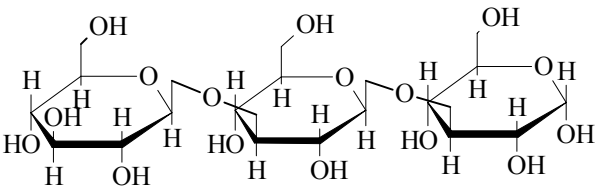


Fig. 9: Basic structural unit of curdlan having 1-3 glucosidic linkage

Gellan

Gellan gum (E418) is an exopolysaccharide produced by *Sphingomonas elodea* (previously called *Pseudomonas elodea*). It is a high molecular weight heteropolysaccharide consisting of 50,000 monomeric residues.

The repeating unit of gellan gum is a linear tetrasaccharide (-L-rhamnopyranosyl, D-glucopyranosyl, D-glucuronopyranosyl and D-glucopyranosyl with O (2) L-glyceryl and O (6) acetyl substituents on the 3-linked glucose) (Fig. 10). It is de-esterified by alkali treatment prior to its use in food. The type of gel produced depends on the degree of its acylation. The acylated gellan forms soft, elastic, transparent and flexible gels but de-acylated gellan forms hard, non-elastic brittle gels. The gellan gels are firm but brittle and mimic 'melting in the mouth' sensation.

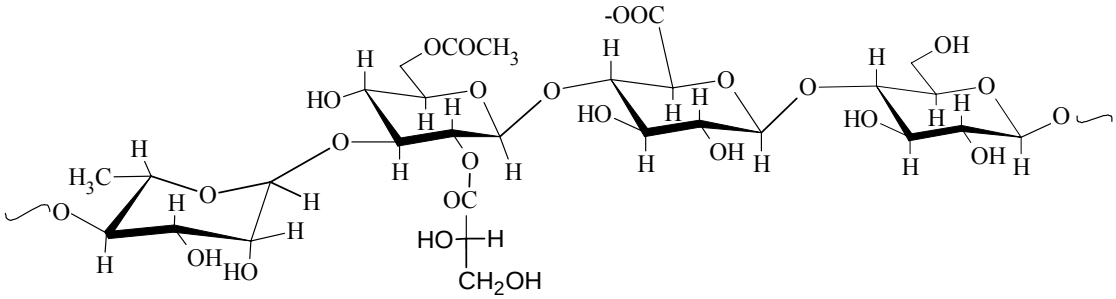


Fig. 10: Basic structural repeating unit of gellan (a tetrasaccharide)

Production

For gellan gum production, *Sphingomonas elodea* is grown in glucose (as a carbon source) in simplified media. The large amount of gellan gum is produced during batch fermentation when 4% inoculum of 8-h-old culture is added to the production medium. The maximum yield of gellan gum is 17.7g/liter, and the highest conversion efficiency is 57.12% in a 30-liter fermenter.

Hyaluronic Acid

Hyaluronic acid (HA) is a polysaccharide known as glucosaminoglucan (GAG) because it has one sugar modified with amino group (-NH_2). GAG polysaccharides are found in animals and provide structural integrity to their tissues. The basic structural unit of HA contains two sugars 1) glucuronic acid and 2) N-acetyl glucosamine. These two sugars are joined alternately by β 1-3 and β 1-4 glycosidic bond (Fig. 11). The molecular weight of hyaluronic acid varies from 10^4 to 10^7 Da depending on the source.

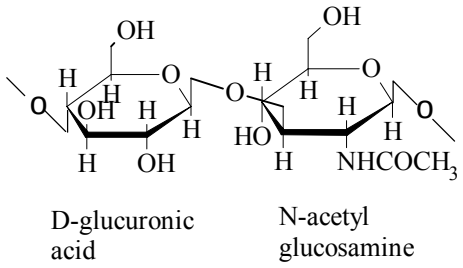


Fig. 11: Hyaluronic acid repeating unit having β 1-3 and β 1-4 glycosidic linkages

Hyaluronic acid (HA) plays some important biological (structural and functional) roles in bacteria and higher animals including humans. Naturally occurring HA may be found in the tissue of higher animals as intercellular space filler and in the vitreous humour of the eye and in the synovial fluid of articular joints. In gram-positive Streptococci, it appears as a mucoid capsule around the bacterium.

The use of animal-derived HA for human therapeutics raised many ethical problems because of the risk of viral infection.

Production

Industry has now turned to microbial fermentation processes for the production of HA. In bacterial fermentation, HA is released into the growth medium and the polymer characteristics can be easily manipulated. *Streptococcus epizooticus* strain is commercially used in HA synthesis. The molecular weight of HA is controlled by several fermentation parameters. The high molecular weight HA is produced at 28^{o}C, high initial glucose concentration (40 g/l) and culture aeration using *S. zooepidemicus*. The culture pH and agitation do not have much effect on the molecular weight of HA in bacterial fermentations.

Applications

HA is the longest GAG and form stiff gelatinous polysaccharide. The smaller HA serves as an excellent lubricant. These properties contribute largely to its biological function. HA finds its use as a hypoallergenic surgical material for reconstructing and building up of lost tissues and it has become popular biomaterial in plastic and reconstructing surgery especially involving joints and eyes. Many companies in this area are launching new HA based nutrient supplement which have curative properties against arthritis, cancer and other ailments. The commercial value of HA is much higher than that of other microbial polysaccharides. Its world market is estimated to be of $US 500 million and it is sold at a rate of $ US 100,000 per kg in the international market.

Pullulan

Pullulan is a water-soluble fungal exopolysaccharide secreted by *Aureobasidium pullulans* when starch is used as the substrate. It is a linear polysaccharide and the basic repeating unit is maltotriose (three glucose units linked via α-1, 4 and the maltotriose units are connected by α-1, 6 glycosidic bond) as depicted in Fig. 12.

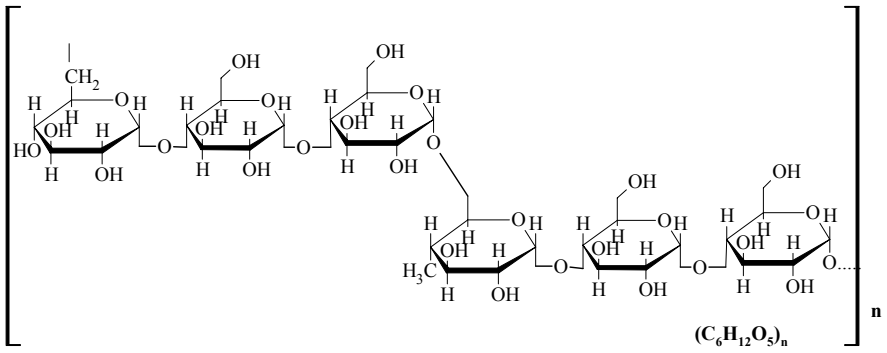


Fig. 12: Structural unit of pullulan consisting of repeating unit is maltotriose

Production

For pullan production, beet molasses is used as a substrate. The use of sulphuric acid pretreated molasses in the culture medium results in higher polysaccharide yield. The chemical structure of pullulan generally depends on the carbon source, strain of Aureobasidium pullulans and fermentation conditions.

Application

Pullulan has good filming properties and used as a food coating material. Pullulan films adhere firmly on the food materials thus avoid do not allow distortion of shapes of foodstuffs during printing. It results in production of high quality images and can be used to create novel confectionary and bakery products. This polysaccharide is also used as an ingredient of low calorie food because it is slowly digested. It is also used as adhesive and because of its smooth textures it finds application in skin care and facial cleansing products. A new nanoparticle known as cholesterol pullulan (CHP) has been developed (pullulan is mixed with protein or drug or enzyme within cholesterol moiety) for therapeutic use. CHP is finding an interesting application in encapsulation and delivery of cancer specific antigen and is being used for vaccine therapy of cancer.

Bacterial Cellulose

Common vinegar bacterium *Acetobacter xylinum* is the best-known bacterium used for the large-scale production of bacterial cellulose (Fig. 13). This bacterium uses variety of substrates and forms cellulose free of lignin and hemicelluloses with high degree of crystallinity. Bacterial cellulose possesses most of the desirable properties to be used as industrial material than their plant counterparts especially because of its exceptional purity, ultra fine network structure, high biodegradability and unique mechanical strength (Table 4).

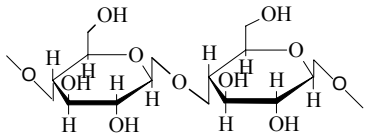


Fig. 13: Bacterial cellulose containing linear chain of glucose linked by β 1-4 linkage

Table 4: Applications of microbial celluloses in different industries

S. No.	Industry	Uses
1.	Food industry	• Consumed as deserts known as Nata de Coco and Manchurian tea in Japan and Philippines • Diet drinks, ice creams chips, snacks and candies. • Ice cream and salad dressing as thickner
2.	Others	As weight reduction base, base for artificial meat, sausage, meat casings and serum cholesterol reduction
3.	Pharmaceutical industry	As wound care bandage, artificial arteries, vessels, skin, temporary skin substitute and for dressing of chronic wounds like diabetic ulcers, pressure sores and venous ulcers
4.	Electronics	Synthesis of acoustic diaphragms for audio speakers
5.	Environment	Production of activated carbon fiber sheet for absorption of toxic gases and as oil spill cleanup sponge
6.	Cosmetic industry	Skin creams, astringents, base for artificial nails and thickener and strengthener for fingernail polish
7.	Others	Binder in papers, in water purification via ultrafilters and reverse osmosis membranes and in mineral and oil recovery

Production

The static culture method is the most successful method for microbial cellulose production, which is being practiced for many years by the Nata industry of Japan. The low shear forces in static culture promote higher productivity. The microbial cellulose is an extracellular polysaccharide and is excreted into the culture medium. The cellulose membrane so formed around the bacterial cells becomes a barrier for substrates and oxygen necessary for the cells to produce cellulose. Thus, novel fermentation approaches like agitated culture systems were developed to overcome the problems associated with the mass culture of *Acetobacter*. Ethanol, glycerol and sucrose are efficiently used as carbon sources and corn steep liquor (CSL) is used as suitable organic nitrogen sources for bacterial cellulose production. An efficient large-scale fermentation technology will be required to produce microbial cellulose at a reasonable price in the market.

Chitin and chitosan

Chitin is a biodegradable polysaccharide of high molecular weight found in some fungi and in an alga. Chitin and chitosan have the same chemical structure. The chemical structure of

chitin resemble that of cellulose but N-acetyl-D-glucosamine is linked by β-D- (1-4) linkage in a linear chain instead of glucose (Fig. 14).

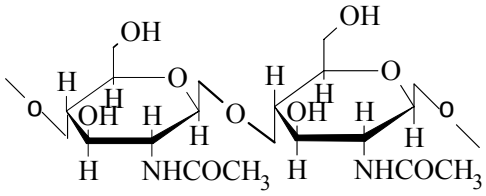


Fig. 14: Structural repeating unit of Chitin

In nature chitosan is found in the majority of Mucoralean fungi (*Zygomycetes*). Chitosan is the deacetylated form of chitin, which makes it soluble in most diluted acids allowing its use in a number of industrial applications (Table 5). It consists of repeating units of D-glucosamine (Fig. 15). Variation in viscosity and degree of deacetylation results in the production of a wide range of chitosan.

Table 5: Industrial applications of microbial chitin and chitosan

S No.	Industry	Uses
1.	Food	• As a fat trap • Thickener and stabilizer in sauces and dessert creams • Purifying agent for water and to clarifying drinks
2.	Medicine	As dressing in artificial skin (Betschitib W™), corneal bandages, suture thread in surgery and implants or gum cicatrization in bone repair or dental surgery.
3.	Agriculture	Applied as the natural fertilizer and the pesticides because of their plant protecting and antifungal properties
4.	Environment	As chelating agents for treating drinking water by separating organic compounds, traps heavy metals, precipitates certain anionic wastes and captures pollutants such as DDT and PCBs in treating sewage
5.	Cosmetic	Skin creams, shampoos, varnishes

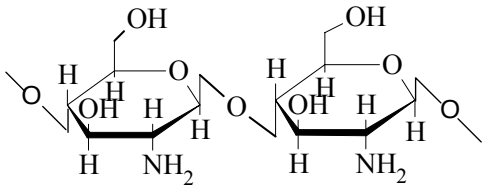


Fig. 15: Structural repeating unit of chitosan

Production

The production of chitosan through chemical methods involves many steps and the chitosan so synthesized has limited medical applications due to some undesirable physicochemical properties of the mycelia of Zygomycete fung*i* has been used as an alternative source of

chitosan. *Mucor racemosus* and *Cunninghamella elegans* have been standardized for faster chitosan production in submerged culture. Maximum chitosan yield is normally obtained in the early growth phase. *Mucor racemosus* produces about 35 mg of chitosan/gm dry mycelia, while *Cunninghamella elegans* yields around 21 mg chitosan/gm dry mycelia in 24h.

Scleroglucan

Scleroglucan is produced by the fermentation using many fungal strains like *Sclerotium rolfsii*, *Sclerotium glucanicum, S. delphinii, Helotium sp.* The chemical structure consists of β-1-3-D-glucopyranosyl unit backbone with every third unit linked by single β-1-6-D-glucopyranosyl unit (Fig. 16). The molecular weight of polysaccharide is 1.3 x 10^5 Da.

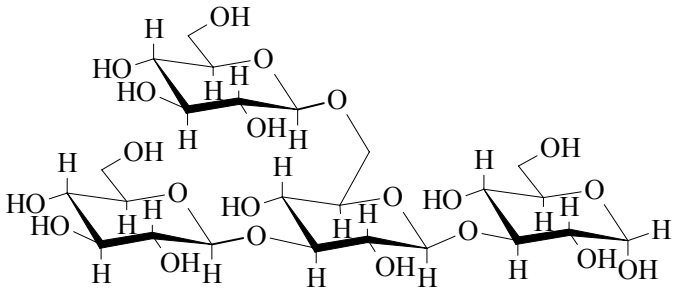


Fig. 16: Structural unit of scleroglucan

Sclerotium glucanicum grows in the medium containing glucose, nitrate and mineral salts. The polysaccharide formed is precipitated after filtration with alcohol.

Application

Scleroglucan is readily soluble in water and gives highly viscous solutions. Scleroglucan find application in the foodstuffs as thickening agent. It forms film when dried and used as edible coating. It is also used in petroleum recovery, agricultural sprays and in pharmaceutical formulations.

Dextran

Dextran is α-1-6- D-glucan produced by fermentation of Leuconostoc mesenteroides, L. dextranicum, Streptococcus mutans and Acetobacter sp. by the action of the enzyme dextransucrase on sucrose (Fig. 17). Dextran was the first microbial polysaccharide approved for its use in food. Dextran is used as a blood plasma extender in blood transfusions. A complex of iron with dextran known as Iron Dextran is used as a source of iron to baby piglets, which are often anemic at birth. It finds its extensive use as matrix (Sephadex) in liquid gel chromatography.

Polysaccharides of Alcaligenes

Wellen gum is an extracellular high viscosity polysaccharide produced by a strain of *Alcaligenes* and used in oil field drilling. It is a polysaccharide gum, which primarily comprises of principally a heteropolysaccharide containing the neutral sugars D-glucose, D-glucuronic acid, L-rhamnose and L-mannose and glucosidically linked acetyl ester groups. Rhamsan gum is another polysaccharide produced by *Alcaligenes* which has excellent

suspending properties and unusual compatibility with salts, making it useful in agricultural applications.

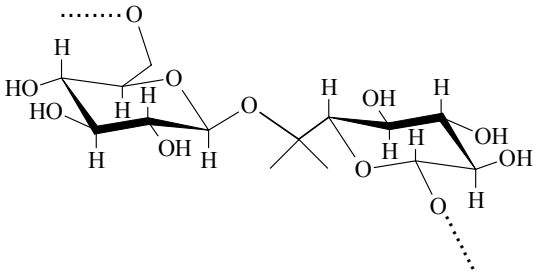


Fig. 17: Basic structural unit of α-1-6- D-glucan (Dextran)

Microbial Production of Pharmacologically Active Substances from Marine Microbes

Soil and water bodies are considered as the repositories of microorganisms. Man has explored soil extensively and the water reservoirs including lakes, rivers and oceans have been comparatively less searched for microbes and microbial products. The oceans present a huge diversity of marine microbes and pharmacologically active substances, which display unique structural and chemical properties. Marine bacteria, cyanobacteria and fungi have shown a very good potential as source for new molecules with pharmacological activity. In spite of large number of pharmacologically active compounds reported from marine organisms, only a few marine products (e.g. cephalosporin) could reach industrial level because of problems associated with the large-scale culture of marine microbes. The pharmacological activities exhibited by products of marine microorganism include antitumor, immunosuppressive, antibacterial, antifungal, antiproliferative and anti HIV activities.

The first marine microbial product reaching industrial level was a β-lactam antibiotic cephalosporin produced by a marine fungus *Cephalosporium* sp. in 1965. It is followed by the production of some other antimicrobial products like chalcomycin B, 5-hydroxyramulosin. Chalcomycin is a 16-membered macrolide antibiotic synthesized by the marine bacterium *Streptomyces* sp. (Fig. 18).

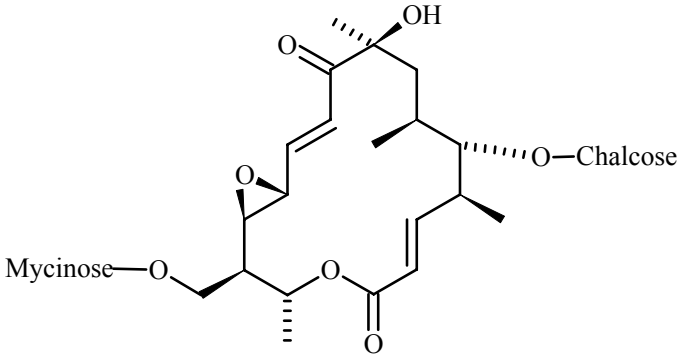


Fig. 18: Chalcomycin (Macrolide antibiotic)

Marine bacterium *Alteromonas* sp. (from the sponge *Halichondria okadai*) produces tetracyclic alkaloid alteramide. Alteramide is the new macrocyclic lactam, which form a

hexacyclic product by intramolecular photochemical cyclization (Fig. 19). It has cytotoxic activity against leukemia P-388, lymphoma L 1210 and epidermal carcinoma KB cells.

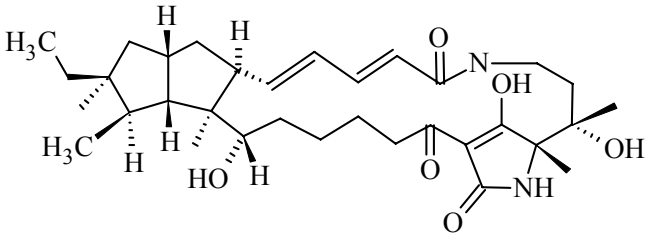


Fig.19: Alteramide A (Anti tumor agent)

Symbiotic microorganisms of the marine environment synthesize a diverse variety of bioactive secondary metabolites. A few of the pharmacologically active compounds reported from marine microbes are listed in Table 6.

Table 6: Some Pharmacologically Active Molecules from Marine Microorganisms

Product	**Microorganism**	**Use**
Thiocoraline	*Micromonospora marina* (marine actinomycetes)	As antitumor agent and antibiotic against gram-positive organism.
Microcolin A	*Lyngbya majuscule* (alga)	Immunosuppressive agent
Lyngbya toxin		
Curacin		An anti-proliferative agent.
Chalcomycin B	*Streptomyces*	Macrolide antibiotic
Dolastatins	Cyanobacteria (symbiont)	Potent cytotoxic activity
Scytonemin	Cyanobacteria from *Stigonema* spp.	Hyper proliferative and anti-inflammatory activity
Anatoxin A	*Anabaena ciecinalis*.	Alkaloid toxin having pharmacological potential
Sorbicillacton A	*Penicillium chrysogenum* from *Ircinia fasciculate* (sponge)	Antileukemia activity
Epothilone A	*Myxobacterium*	Anti-cancerous agent
Cephalosporin	*Cephalosporium* sp. (Marine fungi)	Antibiotic
Alteramide A	*Alteromonas* sp.	Anti-tumor agent
Asperazine	*Aspergillus niger*	Anti leukemic agent
Macrolectin A-F	*Alteromonas* spp	Protect against HSV I and HSV II and AIDS
	Lyngbya lagerhaimanii and *Phormidium tenice*	Anti HIV
Macrocyclic polyesters	*Hypoxylon oceanicum*	Antifungal agent

Ascosalipyrrolone A	*Ascochyta salicorniae* (marine fungus)	Antiplasmodial activity
Sesquiterpenoid secondary metabolites	*Drechslera dematioidea* (algicolous fungus)	Antiplasmodial activity
5-Hydroxyramulosin	*Phoma tropica* (marine fungus from the alga *Fucus spiralis*)	Antimicrobial activity.
Epicoccamide	*Epicoccum purpurascens* (marine fungus from jellyfish *Aurelia aurita*	Pharmacological properties
Proteosome inhibitor	*Salinospora* sp.	Anticancer agent
Herbamide B	Cyanobacteria	Activity against Chagas Disease (American Trypanosomiasis)
Diketopiperazines	*Micrococcus* sp.	Neuroprotective agent
Brominated biphenyl ethers	*Vibrio* sp.	Antibiotic
Polybrominated biphenyl ethers	*Oscillatoria spongeliae* (endosymbiotic cyanobacterium)	Antibiotic
Theonegramide	Filamentous heterotrophic bacteria	Antifungal cyclic peptide
Neuroactive compounds	*Antarcticum vesiculatum* and *Psychroserpens burtonensis*	Neuroactive agent
Antimicrobial metabolites	*Micrococcus luteus*	Antimicrobial activities
Quinolones and phosphatidyl glyceride	*Pseudomonas* sp.	Antimicrobial activities
C_{16} aromatic acids	*Pseudoalteromonas rubra*	Myorelaxant properties
Thiomarinol	*Alteromonas rava*	Effective against antibiotic-tolerant *Staphylococcus aureus* strains and Gram-negative bacteria
2,3-indolinedion (isatin)	*Alteromonas* sp. from shrimp (*Palaemon macrodactylus)*	Its derivatives are used as fungicides, herbicides and also for synthesis of indigo dyes.

The potential marine microorganisms have been studied only to a lesser extent, and therefore, there is a vast scope for exploration of marine microbes for new molecules with desired biological and pharmaceutical activities.

Microbial Production of Anticancer Agents

Cancer is one of the most devastating diseases and although surgery, radiotherapy and chemotherapy are some of the routine treatments prescribed to the patients suffering with this disease yet the use of anticancer agents of biological origin is becoming very effective remedy for some form of cancers. In the last more than four decades, lots of efforts have been

put to discover and develop anticancer agents and as a result a number of important drugs like taxol, camptothecin analogs, topotecan and irinotecan, vinblastine and vincristine, doxorubicin and bleomycins have come out in the market. There are different types of microbial anticancerous agents which include: a) antitumor antibiotics; b) anticancer enzymes and other bioactive compounds.

a) Antitumor antibiotics

These are used as cytostatic agents in cancer treatment and brief description of some important antitumor antibiotics is given below:

Aclacinomycin

It is an anticancerous oligosaccharide anthracycline antibiotic produced by bacterium *Streptomyces galilaeus*. It intercalates with DNA of the tumour cell and interferes with action of topoisomerases I and II causing inhibition of replication, repair of DNA and RNA and protein synthesis.

Adriamycin

Adriamycin or doxorubicin is an anthracycline antibiotic produced by *Streptomyces peucetius* and is commonly used in the treatment of uterus and ovarian cancers. It is widely used in chemotherapy and interferes with the replication of DNA by intercalation. The commercially available adriamycin is sold under the brand name of Doxil®, which is administered as liposome-encapsulated dosage manufactured by Johnson & Johnson. This encapsulation reduces its cardiotoxicity and is used in the treatment of Hodgkin's disease, breast cancer, lung cancer, soft tissue sarcoma and Kahlers disease. It is also used in combinatorial therapy experiments with rapamycin and shows promising results in the treatment of Akt positive lymphomas.

Chromomycin A_3

It is a C-glycoside (oligosaccharide with aromatic chromophore) produced by *Streptomyces griseus* and also known as toyamycin and aburamycin. It is used as antibiotics, anticancerous agent, fluorescent dyes and inhibitors of nucleic acid synthesis. It binds to G/C sequences or to dimer resulting in wider, shallower minor groove with major groove compression. However, due to immunosuppressive and cytotoxic properties, its use is limited.

Actinomycin D

It is a chromopeptide antibiotic produced by *S. antibioticus*. It was first reported antibiotic having anticancerous property and is marketed under the trade name Dactinomycin. It intercalates with DNA and is administered intravenously. Though actinomycin is quite effective in the treatment of a number of cancers, yet it has limited clinical use as it damages genetic material.

Mitomycin C

Mitomycin C is benzoquinone that inhibits DNA replication by producing cross linkages and is produced by *Streptomyces caespitosus*. It is used as drug against different types of cancers e.g. breast, stomach, oesophagus and bladder cancers.

Mithramycin

Mithramycin is also a C-glycoside (tricyclic pentaglycosidic antibiotic) isolated from *S. plicatus, S. argillaceus* and *S. atroolivaceus*. It binds to GC rich region of minor groove of DNA leading to inhibition of RNA synthesis, mRNA expression and protein synthesis. It finds its use against bone and testicular tumours.

Bleomycin
It is a glycosylated linear non ribosomal peptide antibiotic produced by *Streptomyces verticillus*. It was initially marketed by Bristol-Myers Squibb under brand name of Blenoxane. It chelates metal ion leading to the production of a pseudoenzyme which interact with oxygen and form superoxide and hydroxide free radicals that split DNA. It is used in the treatment of hodgkin's disease, squamous cell carcinomas, testicular cancer and pleurodesis.

Daunomycin
Daunomycin (daunomycin cerubidine) or daunorubicin belongs to anthracycline family produced by *Streptomyces peucetius*. It is used in combination with other drugs and is mostly prescribed in the treatment of specific type of leukemia (acute myeloid leukemia and acute lymphocytic leukemia) and neuroblastoma.

Neocarzinostatin
It is a chromoprotein produced by *Streptomyces carzinostaticus*. It intercalates with DNA, where cycloaromatisation results in a biradical intermediate that removes hydrogens from the sugar moiety of DNA and causes single- and double-strand breaks in the DNA. It also has proteolytic activity against histones and is used with copolymer of styrene- maleic anhydride in the treatment of liver cancer.

b) Anticancer bioactive compounds

Thiocoraline
It is an anticancerous bioactive compound isolated from *Micromonospora marina*-a marine actinomycetes that causes the arrest of G1 phase in human colon cancer cell lines since it interferes with DNA replication by inhibiting DNA polymerase activity.

Alteramide
It is a tetracyclic alkaloid produced by a marine bacterium *Alteromonas* sp. (associated with the sponge *Halichondria okadai*). Alteramide A possesses cytotoxicity against leukaemia, lymphoma and epidermal carcinoma.

Epothilone
It is a new type of anticancerous agents produced by marine bacterium *Sorangium cellulosum* (myxobacterium) that emerged as a potential drug against those cancers that resist chemotherapy. Its mode of action is more or less similar to taxol (Fig. 20).

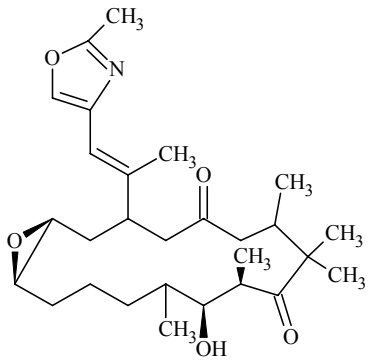


Fig. 20: Chemical structure of epothilone A

Sorbicillacton A
Sorbicillacton A is an anti-leukaemia substance produced by *Penicillium chrysogenum* (isolated from a sponge *Ircinia fasciculate*). Sorbicillacton A has entered into pre-clinical testing.

c) Anticancer enzymes

L-asparaginase
L-asparaginase is a microbial enzyme produced by many bacteria (e.g. *Bacillus* sp., *Pseudomonas ovalis*, *Mycobacterium phlei*, *Citrobacter* sp., *Erwinia carotovora*), yeast (*Candida utilis*, *Pichia polymorpha* and *Saccharomyces cerevisiae*), fungi (*Aspergillus nidulans, Mucor* sp. and *Cylindrocapron obtusisporum*), algae (*Chlamydomonas* sp.) and actinomycetes (*Streptomyces karnatakensis*, *Thermoactinomyces vulgaris*), which is used in the treatment of some cancers. There are some basic differences between normal and malignant cells e.g. tumour cells have lost the capability to synthesize L-asparagine, as a result they depend on its supply from the blood. L-asparaginase is an enzyme that hydrolyzes asparagine and if administered into the blood stream, it converts blood asparagines into aspartic acid. Normal cell circumvents this problem since they synthesize asparagines required for their growth and maintenance. The cancerous cells exhaust all external asparagines rapidly and ultimately die due to starvation. L-asparaginase is available in the market for pharmaceutical applications under different names e.g. elspar, oncaspar, and erwinase.

L-glutaminase
L-glutaminase is a widely distributed enzyme and present in plants, animal tissue and microorganisms (bacteria, yeast and fungi). Major glutaminase producers are *Escherichia coli, Bacillus subtilis, B. licheniformis, Saccharomuces cerevisiae, Pseudomonas aeruginosa, Aspergillus sojae, Clostridium welchii and Pseudomonas fluorescens.* The glutaminase enzyme possesses antitumor activity and hence causes the selective degradation of glutamine dependent tumor cells by depriving the cell of glutamine. The use of enzyme to deprive neoplastic cells of essential nutrients helps in the treatment of malignancies. *Archromobacter* is known to possess highest glutaminase activity and used as effective antitumor agent. The microorganism has both L-asparaginase and L-glutaminase activity and selectively kills human leukemic leukocytes. *Pseudomonas* 7^0A glutaminase-asparaginase enzyme system is effective against solid as well as ascites tumors and it has an unusual long biological half life, making it an effective agent against cancer metastasis.

Microbial Transformations

The metabolic and biocatalytic potential of the microorganisms has been exploited to transform the nonconventional substrates into desirable products. Microbial catalysis and transformations have been explored and perfected to the extent that a vision has emerged that why to synthesize a compound in an organic chemistry laboratory when there is microorganism to it. The microorganisms or their enzymes are now used to carry out the conversion of substrates to desired products and such reactions or processes are popularly known as microbial transformation or biotransformation. Microbial transformations have many advantages e.g. a) reaction-specific processes b) higher stereo-and region-specificity c)

milder reaction condition and are therefore preferred over chemical transformation in industries usually involved in preparative organic chemistry.

Microbial transformation reactions are mainly categorized into oxidation/ reduction, hydrolysis, condensation and isomerization reactions (Fig. 21).

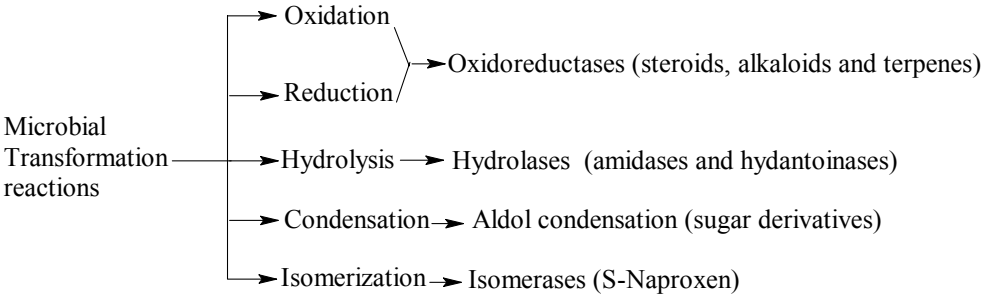


Fig. 21: Type of transformation reactions catalyse by enzymes

In biotransformation reactions, microbial cultures are grown in a suitable medium and then used as a source of enzymes. The poorly soluble substrates are either used in low concentration or mixed with emulsifiers. Lipophilic substrates are transformed in biphasic or polyphasic system, in which enzyme is present in aqueous phase and substrate is in the solvent phase. Some important microbial transformations are described in the following sections:

Steroids and sterol transformation

Steroids are natural hormones secreted by adrenal gland. Steroids share a basic structure called cyclopentanoperhydrophenanthrene (Fig. 22). Steroids and its derivatives are of commercial use because of their therapeutic (glucocorticoids) and contraceptive properties (e.g. progesterone and estrogen). These are also used as sedative and antitumor agents. Cortisone is of commercial importance because of its anti-inflammatory action against rheumatoid arthritis and can be transformed to predisolone (by the introduction of double bond in ring A), which is more effective steroid as compared to its precursor (Fig. 23).

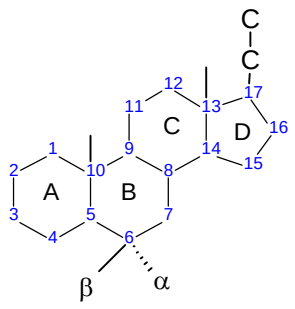


Fig. 22: Basic structural unit (cyclopentanoperhydrophenanthrene) of steroid

Chemical steroid synthesis involves 31 reaction steps to yield 1g cortisone from 615 g of deoxycholic acid (purified from beef bile). Transformation of simple natural precursor molecule to product using microbes (*Rhizopus arrhizus* and *Aspergillus niger*) reduced the cost of manufacturing cortisone from 200$ (1949) to 1$ (1979) and shortened 31 chemical

steps to 11 steps. The introduction of oxygen atom in C-11 is crucial for anti-inflammatory activity (Fig. 24).

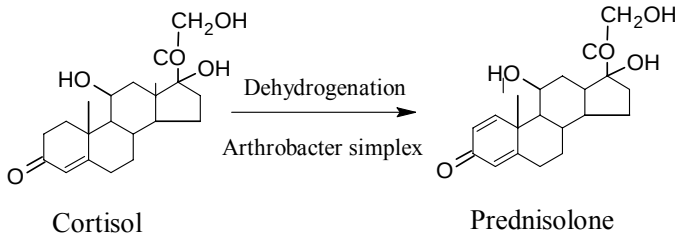


Fig. 23: Microbial transformation of cortisol to prednisolone

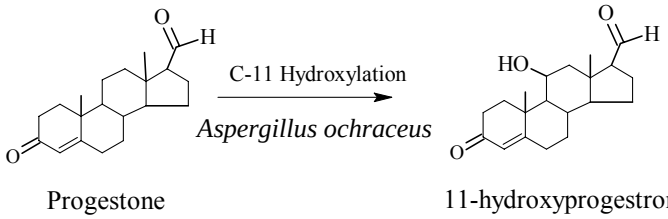


Fig. 24 Microbial hydroxylation of progestone to 11-hydroxyprogestone

The microbial steroid transformations were made cost effective by substituting expensive precursor by cheaper plant based sterol, which is modified by mycobacteria. Plant sterols like stigmasterol, sitosterol (byproducts of soyabean oil production), campesterol (byproduct of paper industry) and diosgenin (Maxican yam roots- *Dioscorea composite* or *Testudinaria sylvatica*) or animal sterol (cholesterol) are the precursors used in steroid transformation reactions catalyzed by microbes. Presently all steroids can be easily hydroxylated at specific position using microbial monooxygenases (e.g. 11α- or 11β- hydroxylase, 17α- hydroxylase and 21α- hydroxylase). These microbial transformations are accomplished at 37ºC in aqueous medium at atmospheric pressure. The market demand for four major steroids (cortisone, aldosterone, prednisone and prednisolone) is about 700,000 kg/year (Table 7).

Table 7: Production of some steroids using microorganisms

Steroid	**Microorganism**	**Producer**
Cortisol	*Curvularia lunata*	Pfizer Inc., Gist-Brocades
Prednisolone	*Arthrobacter simplex*	Schering Corp., Upjohn Co.
11α- Hydroxyprogesterone	*Rhizopus nigricans*	Upjohn Co.
Triendiol	*Septomyxa affinis*	Schering Corp., Upjohn Co
Androstadiendione	*Mycobactrium* sp.	G.D. Searle and Co., Upjohn Co
1-Dehydrotestololactone	*Cylindrocarpon radicicola*	E. R. Squibb and Sons

Microbial Transformation of Antibiotics

Penicillin and cephalosporin are the most widely used antibiotics but with the emergence of resistance to these natural antibiotics in pathogenic bacteria necessitated the development of new antibiotic derivatives. The derivatives of the natural antibiotics are made enzymatically

or chemically by modification of existing antibiotics and are called semisynthetic antibiotics (Table 8). Moreover natural penicillins such as Penicillin G or Penicillin V are not effective against gram-negative organisms thus there is a need either to develop new antibiotics or to modify the existing ones. Ampicillin was the first semisynthetic penicillin developed in 1959.

Table 8: Some of natural and semisynthetic antibiotics

β-lactam antibiotics	
Narrow spectrum penicillins	Benzathine penicillin Benzylpenicillin (penicillin G) Phenoxymethylpenicillin (penicillin V) Procaine penicillin
Narrow spectrum penicillinase-resistant penicillins	Methicillin Dicloxacillin Flucloxacillin
Moderate spectrum penicillins	Amoxicillin Ampicillin
Broad spectrum penicillins	Co-amoxiclav (amoxycillin+clavulanic acid)
Extended spectrum penicillins	Piperacillin Ticarcillin Azlocillin Carbenicillin
Cephalosporins	
First generation cephalosporins	
Moderate spectrum.	Cephalexin Cephalothin Cephazolin
Second generation cephalosporins	
Moderate spectrum with anti-*Haemophilus* activity.	Cefaclor Cefuroxime Cefamandole
Second generation cephamycins	
Moderate spectrum with anti-anaerobic activity	Cefotetan Cefoxitin
Third generation cephalosporins	
Broad spectrum.	Ceftriaxone Cefotaxime
Broad spectrum with anti-*Pseudomonas* activity.	Ceftazidime
Fourth generation cephalosporins	
Broad spectrum with enhanced activity against Gram positive bacteria and beta-lactamase stability	Cefepime Cefpirome

Carbapenems	
Broadest spectrum of beta-lactam antibiotics.	Imipenem with cilastatin Meropenem Ertapenem
Monobactams	Aztreonam (Azactam^{TM})
Beta-lactamase Inhibitors	Clavulanic acid Tazobactam Sulbactam

Semi-synthetic penicillins have many advantages like broad spectrum of activity, resistance to penicillinase and can be taken orally (acid stability). Amoxicillin and ampicillin are effective against gram-negative organisms. The precursors of semisynthetic antibiotics are produced enzymatically (penicillin G amidase, penicillin acylase, hydantoinase, etc), which are further modified by chemical reactions (Fig. 25).

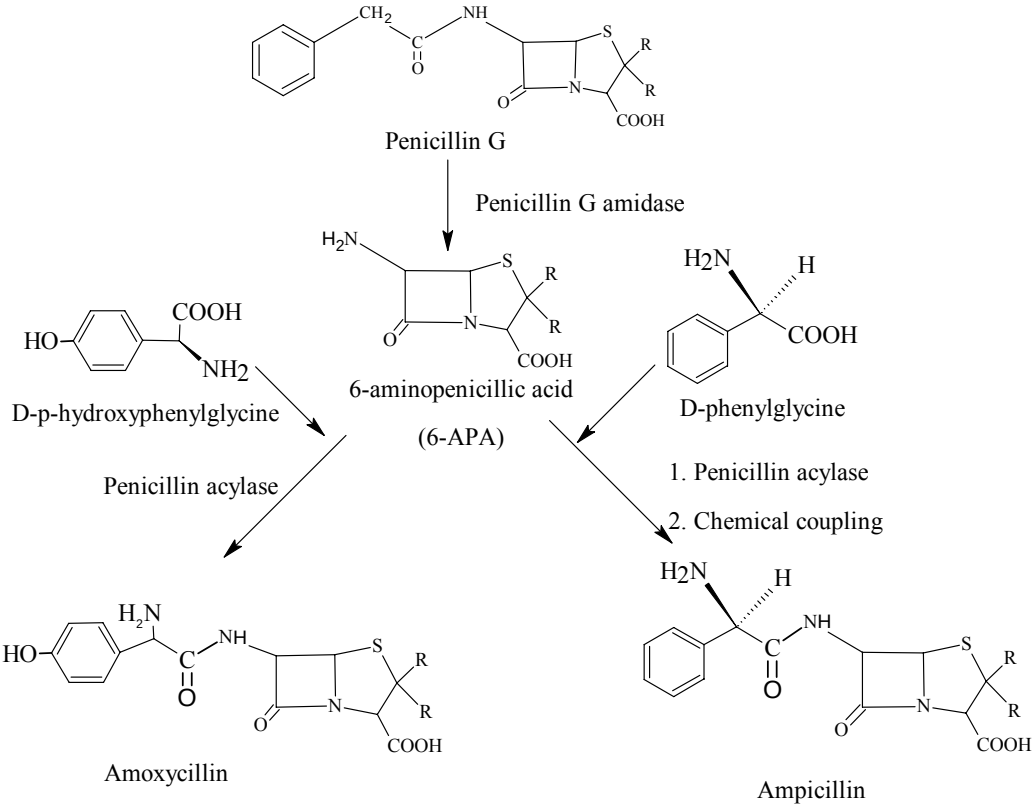


Fig. 25: Enzymatic synthesis of semi-synthetic antibiotics

Microbial Production of L-ascorbic Acid

L-ascorbic acid is an antioxidant used in food industry and vitamin formulations. The annual world production of L-ascorbic acid is about 80,000 tons and its worldwide market was estimated to be over $600 million in 2002. In Reichstein Grüssner synthesis of L-ascorbic acid, D-sorbitol is converted into L-sorbose by sorbitol dehydrogenase of *Acetobacter suboxydans* /*A. xylinum* in a single step reaction. L-sorbose is then further converted to L-ascorbic acid by chemical reactions (Fig. 26). The overall productivity of process is approximately 1 kg L-ascorbic acid/ 2 kg glucose.

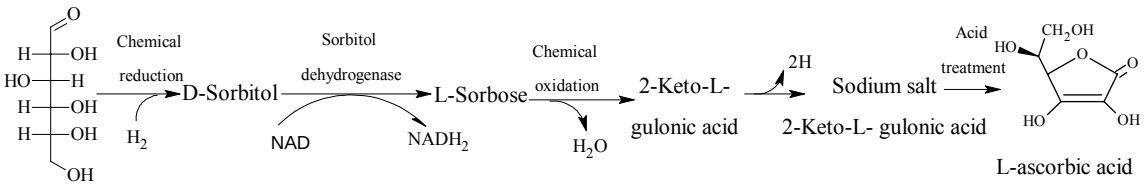


Fig. 26: Reichstein Grüssner synthesis of L-ascorbic acid

Besides Reichstein Grüssner synthesis, two-stage fermentation was developed for commercial production of L-ascorbic acid. In the first step, glucose is converted into 2, 5-diketo-D-gluconic acid (2, 5-DKG) by *Erwinia* sp. which is further reduced to 2-keto gulonic acid by *Corynebacterium* sp. The total productivity of this process is about 86%. 2-keto-D-gulonic acid can then be easily transformed to L-ascorbic acid (Fig. 27).

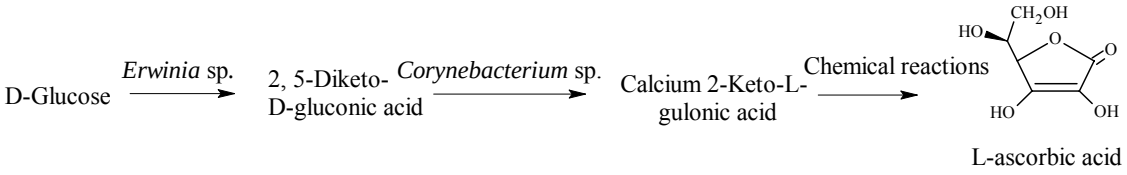
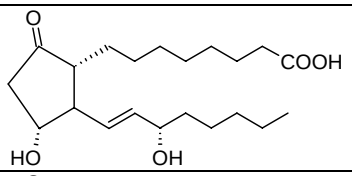
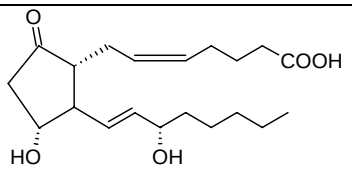
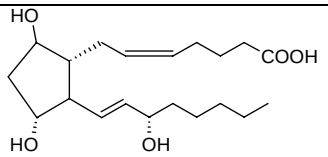


Fig. 27: Commercial production of L-ascorbic acid by two-stage fermentation

Prostaglandins

Prostaglandins are tissue hormones having vast medical applications. These are biologically active lipids synthesized by some fungi (e.g. *Mortierella* sp., *Cunninghamella* sp. and *Candida albicans*) using unsaturated fatty acids (e.g. arachidonic acid). Arachidonic acid on oxidization by the cyclooxygenase enzyme of the fungus gives rise to prostaglandins (PGE_2, $PGF_{2\alpha}$). Some of the medically relevant prostaglandins are listed in Table 9.

Table 9: Some medically relevant prostaglandins

Prostaglandin	**Chemical structure**	**Application**
Prostaglandin E_1		Used in the treatment of congenital heart failure and digestive disease.
Prostaglandin E_2		As contraceptive, alleviation of pain during childbirth
Prostaglandin $F_{2\alpha}$		As aparturifacients in obstetrics, gynaecology and promoter of intestinal movement after abdominal operations.

Production of Biologically Active Chiral Compounds and Commodity Chemicals

Biocatalysts find wide application in chiral drug synthesis since the active principles of the drugs are stereospecific and the undesired stereoisomer/enantiomer can in some cases cause serious side effects. Conventional organic synthesis generally end up in racemic mixtures making them unfit for use as drugs. Since the enzyme-mediated reactions are highly stereospecific, therefore enzymes are now preferred for the synthesis of optically pure drugs e.g. S- atenolol (a drug for hypertension). It interacts with β_1 adrenergic receptors (class of G protein-coupled receptors that are targets of the catecholamines) of heart lowering heart rate, cardiac output and blood pressure. In S-atenolol production, the racemic precursor is first resolved using *Nocardia*, *Rhodococcus*, *Corynebacterium* or *Mycobacterium.* These bacteria oxidize all the S-isomer of racemic mixture to (R)-1, 2-O-isopropylideneglyceric acid leaving the R-isomer (R-1, 2-O-isopropylideneglycerol) unchanged. These alcohol and acid compounds can be easily separated. Thereafter, the desired key precursor (R-1, 2-O-isopropylideneglycerol) can be transformed to optically pure S-atenolol (Fig. 28).

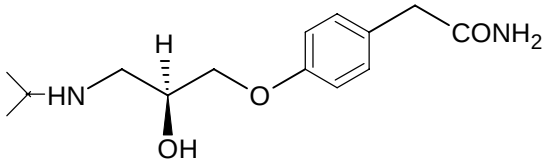


Fig. 28: S-atenolol- a drug for hypertension

Isomerases from fungus *Cordyceps militaris*, *Beauvaria bassiana* and *Exophiala wilhansii* are used to convert R-naproxen to S-naproxen because only S isomer has analgesic and antipyretic property which is a well known nonsteroidal anti-inflammatory drug (Fig. 29).

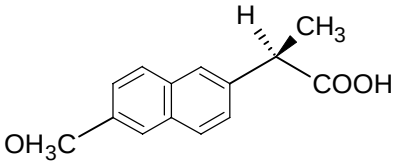


Fig. 29 Anti-inflammatory drug S-naproxen

The enzymes are also employed in pharmaceutical industries for the synthesis of fine chemicals. Nicotinamide and nicotinic acids are synthesized by the hydration of 3-cyanopyridine to nicotinamide using nitrile hydratase and hydrolysis of 3-cyanopyridine to nicotinic acid using nitrilase at industrial scale. Mandelic acid is another chiral intermediate which is produced by conversion of mandelonitrile by nitrilase by BASF (Germany) in tonnes. It is a precursor for semisynthetic antibiotics. Microorganisms are also used to produce a variety of commodity chemicals like ethanol, acetone, butanol, lactic acid and glycerol, etc. (Table 10). In 1980s, Japan started developing biotransformation process for the conversion of acrylonitrile to produce acrylamide using microbes.

Table 10: Fine and Commodity Chemicals Produced by Microorganisms

Fine Chemical	**Microorganism**	**Uses**
Atorvastatin	*Alcaligenes* sp.	Cholesterol - lowering drug
Vitamins (B_{12}, riboflavin, β-carotene)	*Rhodococcus rhodochrous* J1, *Eremothecium ashbyii, Propionibacterium freudenreichii* ATCC 6207	Dietary additives
Mandelic acid	*Alcaligenes faecalis*	Cosmetics
Amino acids (L-glutamic acid, L-lysine, tryptophan, praline, serine, leucine)	*Corynebacterium glutamicum*, *Escherichia coli*	Therapeutic application
Commodity chemicals		
Dihydroxyacetone	*Acetobacter* sp.	As a cosmetic material
Ethanol	*Zymomonas mobilis*	As a solvent in the manufacture of varnishes and perfumes, as preservative, in the preparation of essences and flavorings, as a disinfectant and in tinctures (e.g., tincture of iodine), and as a fuel and gasoline additive
Citric acid	*Aspergillus niger*	As food additive, plasticizer, detergent, and abrasive
Lactic acid	*Lactobacillus* sp.	As an acidity regulator and used in the cosmetics industry
Itaconic acid	*Aspergillus terreus*	In medicine, cosmetics
L-malic acid	*Saccharomyces bayanus*	In wine production
L-glutamic acid	*Corynebacterium glutamicum*	As a food additive
Acrylamide	*Pseudomonas chlororaphis* B23	Production of polymers and used as flocculates for petroleum recovery.
Acrylic acid	*Rhodococcus rhodochrous* J1	Starting materials for the synthesis of various kinds of polymers
Propene oxide	*Flavobacterium*	As a fumigant such as in the sterilization of packaged foods and in the preparation of surfactants and oil demulsifiers
Formaldehyde & formic acid	*Pseudomonas putida*	As disinfectant and as preservative and antibacterial agent in livestock feed
Hydroquinone	*Mycobacterium* sp. B-394	As photographic developer
(E, E)-Hexa-2, 4-dienedioic acid	*Arthrobacter* sp.	As raw material for new functional resins, pharmaceuticals and agrochemicals

Benzene-1, 2, 3-triol	*Citrobacter* sp.	As developer in photography, for staining fur, leather and hair, for manufacturing various dyes
Indigo	Recombinant *Escherichia coli*	As dye in textiles.

The communication, transportation, education and globalization are fast changing the socioeconomical scenario around us. New comforts have been added vis-s-vis new challenges in food, medicine and environment have crop up. To meet these challenges microbes, microbial processes and products will play a very significant role in coming years. Genetic, protein and metabolic engineering programmes related to microbes will generate more efficient microbial systems leading to availability of quality products in the reach of common man. The scope of applied microbiology will increase manifold in the near future.

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