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# Quest




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# Editorial

Though the progress of science is made possible by funding received from common man's tax; paid to government, gap between science and common man has been an issue of consideration in past. It is indeed becoming a major issue to consider even in present day.

Science progresses every single day, new discoveries and inventions are made even as you read this. However until and unless those inventions and discoveries are brought into the knowledge of common man, it doesn't serve the purpose.

As a method of knowledge dissemination various channels of distribution is need of the day. Quest is a small but appreciable effort of the student and by the student of ARIBAS. Beginning from the 1st issue Quest slowly but surely making its way in this direction.

In this Deepabali wish the light of knowledge ignite young mind of creativity for the benefit of Aam Admi (common man).

# Index



## Notice to Authors

Manuscripts submitted to Quest should adhere to below mentioned criteria.
Research News: About 400 words (1 page)
Research Article: About 2000 words (4 pages)

Common for all: -
Font: Calibri
Font Size: 14
Columns: 2
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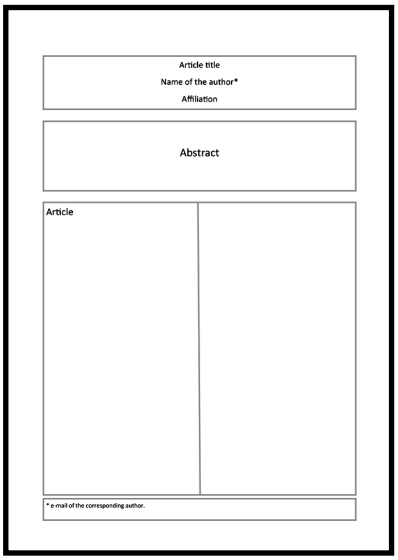
References: 1) In text citing, S No, Superscript.
2) Author's name (s), *Journal name*, **Volume No**, Page No**,** (year).



## Prebiotic effects on gut function and human health

Prebiotics which are short chain carbohydrate that alters the metabolism of gut microbiota in a benificial manner.

Prebiotic effect of GOS (galacto oligosaccharides) which is a resulting molecule of transgalactosylation of hydrolysed lactose by an enzyme beta galactosidase.

Galacto-oligosaccharides and bowel function, constipation is a common problem and its prevalence increases with age. Severe constipation requires treatment with laxatives, especially increased dietary fibre is recommended for the prevention of mild constipation. Alternative can be use of oligosaccharides which act as soluble fibre and have a bifidogenic effect GOS occuring naturally in human milk can be produced from lactose.

Several clinical trials have shown that the supplementation of infant formula with a mixture of GOS and FOS stimulate the growth of intestinal bifidobacteria and lactobacilli in infants. Supplementation of infant formulas with GOS & FOS has decreased stool consistency and increased defecation frequency to levels similar to those found in breastfed infants.

The laxative effect of GOS is believed to be caused by its action as a soluble fibre. Oligosaccharides pass undigested into the large intestine and stimulate bacterial fermentation in the colon. The bacterial fermentation of oligosaccharides increases bacterial mass, which in turn increases faecal bulk. The bacterial fermentation of oligosaccharides increases bacterial mass, which in turn increases faecal bulk. Undigested oligosaccharides and fermentation products may also produce an osmotic effect in the gut, which increases the water content of faeces

Clinical trials showed that use of GOS reduces mild constipation in constipated people, ingestion of prebiotic mixture GOS/FOS can modulate bowel function of infants feeded with formulas as breast feeded infants.

*Contributed By Deval Patel IG-IBT 9th Sem*

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## Excerpts on Significance of Helminth Infections in Human Gut

Helminths are among the most common infectious agents reported worldwide. They pose an important threat to public health especially in the developing countries. The communities severely affected by helminth infections belong to low income groups and dwell in unhygienic environments. The major helminths associated with human diseases are pinworm or seatworm (*E. vermicularis),* Hookworms *(Ancylostoma duodenale and Necator americanus),* Roundworm (*Ascariasis lumbricoidies),* Whipworm (*Trichuria trichura) and* Trematodes **(**Schistosoma)

Almost every person is infected by Parasites at some time in their life. About 85% of the world's populace is estimated to be infected with helminths of one or the other kind. The severity of infection may vary with person to person either leading to silent commensal residence to clinical diseases. Due to the global commotion by parasites in the body's normal functioning and maintenance, some scientists believe that parasitic infection is often responsible for many chronic diseases such as cancer, diabetes, liver dysfunction, HIV infection and others. Helminthes are difficult to diagnose using lab tests.

A parasitically diseased person may often present with symptoms of other diseases, such as influenza and colds, migraine headaches, polyps, neurological disorders, anemia, chronic fatigue, general tiredness, frequent constipation, chronic weight problems, iron deficiency, etc.

Universally, infectious diarrhea is a common cause of morbidity and mortality in children. The etiological agents of infectious diarrhea consist of bacteria, viruses, helminthic and parasites. The incidence of helminthic and parasitic infection among children is more in the lower socio-economic community where multiple factors like insanitary environmental condition, over-massing, and defective feeding programs of nurslings and children exist.

There are many drugs in the market that show killing of helminthes however, their killing is non specific and also eliminates the normal microflora of human gut. Most of the drugs used for mass administration show variety of side effects. Further more, several incidences of development of resistance against the routine anthelmintic drugs has been reported. Hence elimination of helminthes from human gut is posing problems day by day.

In 2001, emissaries at the World Health Assembly unanimously endorsed a resolution (WHA54.19), which urged endemic countries to start seriously tackling worms, specifically schistosomiasis and soil-transmitted helminths. The strategy for control of soil-transmitted helminth infections is to prevent and control morbidity through the sporadic treatment of at-risk population living in endemic areas. People at risk are preschool-aged children; school-aged children; women of motherhood age (including pregnant women in the second and third trimesters and breastfeeding women).

WHO recommends periodic treatment with anthelminthic (deworming) medicines, without previous individual diagnosis to all at-risk people living in endemic areas. Treatment should be given once a year when the occurrence of soil-transmitted helminth infections in the community is over 20%, and twice a year when the prevalence of soil-transmitted helminth infections in the community go beyond 50%. This intervention reduces indisposition by reducing the worm burden. In addition education on health and hygiene reduces transmission and reinfection by encouraging healthy behaviours and provision of inadequate sanitation is also important but not always possible in resource-constrained settings. Periodic deworming can be easily integrated with child health days or vitamin A supplementation programmes for preschool-aged children, or integrated with school-based health programmes would help to keep the disease under control. Schools provide an important entry point for deworming activities, as they provide easy access to health and hygiene education components, such as the promotion of hand washing and improved sanitation.

Recent research has highlighted the significance of helminth infections in the gut in controlling the inflammatory diseases and complete elimination of helminthes from the gut is posing serious consequences on health of inhabitants of developed countries in the form of different lifestyle diseases related to inflammation.

The global target is to eliminate morbidity due to soil-transmitted helminthiasis in children by 2020. This will be obtained by regularly treating at least 75% of the children in endemic areas (an estimated 873 million). Hence a lot of significance is attached to helminth infections in human gut.

*Contributed By Jeki Patel IG-MBT Sem XI*

# Heterogeneity in lignin deposition

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Approximately 95% of plant biomass is composed of lignocellulosic material. Lignocellulose typically contains 45% cellulose, 25-30% hemicellulose and up to 25% lignin. These three types of polymers are strongly intermeshed and chemically bonded by non-covalent forces cross-linkages.

Lignin is second most abundant biopolymer after cellulose contributing more than 25% of the global plant biomass (Lewis et al., 1999). It is an aromatic polymer derived mainly from the polymerization of three different hydroxycinamyl alcohols: p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol deposited in the cell walls of supporting and conducting tissues such as fibers and tracheary elements of higher plants (Hatfield and Vermerris, 2001). Biosynthesis of lignin formation proceeds via polymarisation of free radicals forms of precursor's, i.e. the monolignols para -coumaryl, coniferyl, and sinapyl alcohols. In the final polymer p-hydrophenyl (H), guaiacyl (G), and syringyl (S) type units, respectively. It seems plausible that lignin polymerization pattern and assembly is guided by orientation of cellulose and structure of hemicellulose. They are C6-C3 phenylepropanoids and differ from each other only by the degree of methoxylation. In lignin, the C6 -C3 units are interconnected by several types of ether and carbon–carbon linkages.

Interest in lignin gained momentum due to its hindrances in cell wall extensibility and the cell elongation (Musel *et al*., 1997). It also acts a strong barrier to the plant pathogens (Nicholson and Hammerschmidt, 1992) and participates in wound healing (Hawkins and Boudet, 1996). Lignin composition, quantity and distribution also affect the agro industrial uses of plant material. Digestibility and dietary conversion of herbaceous crops are affected by differences in lignin content and composition (Akin *et al.,* 1991). Lignin is an undesirable component in the conversion of wood into pulp and paper and remova1 of lignin is a major step in the paper making process. During the manufacture of high quality paper, lignin is chemically separated from the polysaccharide components of wood during pulping and bleaching reactions. Lignin extraction consumes large quantities of chemicals and energy leading to a poor environmental image for the industry (Higuchi, 1985; Odendahl, 1994; Biermann, 1996). For these reasons, new biological approaches to pulping are continuously being researched. This shows the importance of lignification which involves the deposition of lignin in the cell wall and cell wall domains of growing and differentiating plant cells. Cell wall peroxidase is widely believed to be involved in the lignification process (Wallace and Fry, 1994).

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Generally the lignin content is typically higher in coniferous wood (25- 40%) than in angiosperm wood (18-25%) (Adler, 1977). In coniferous lignin is mainly composed of guaiacyl (G), whereas in angiosperms lignin is a copolymer syringyl (S) and guaiacyl (G) units (Nimz *et al*., 1981). H units derived from *p*-Conefryl alcohol are present in both angiosperms and gymnosperms in small amounts, but are most abundant in grasses (Nimz *et al*., 1981). In addition, relatively high amounts of hydroxycinnamyl acids, most of all *p*-coumarate and ferulate are also found in grass lignins (Ralph *et al*., 1998). In dicotyledonou**s** plant**s**, the lignin polymer is made predominantly from the monolignol**s** coniferyl and **s**inapyl alcohol (Baucher *et al*., 1998), whereas the lignin of gymnosperms, lacks **s**inapyl alcohol. Grass lignins contain guiacyl-, syringyl-, and p-hydroxyphenyl-units.

Lignin content and composition are also known to vary among the individuals of population, tissues and individual cell types within a tissue and with the developmental stages and the environmental conditions (He and Terashima, 1991. In *Asparagus*, for example, the lignin content is approximately three fold higher at the base of the stem than that at the top (Hennion *et al*., 1992). The same type of variation has been reported for the stem internodes of *Alfalfa* and was correlated with a difference in both lignin and hemicellulose extractability (Baucher *et al*., 1998). Lignin in vessel cell walls of angiosperm trees contains more G-units than fibre walls and both in angiosperm and gymnosperm trees, H-type lignin is more abundant in the areas of cell corners and middle lamella compared to the other cell wall layers (Grünwald *et al*., 2002). Lignification is also affected by stress conditions within the plants; for example, compression wood formed on the lower side of bent stems of conifers is characterized by higher lignin content and higher amounts H-type lignin compared to normal wood (Önnerud and Gellerstedt, 2003).

.Lignin composition, quantity, and distribution also affect the agro industrial uses of plant material. Lignin limits the digestibility of forages. Hence, plant varieties with altered lignin contents may have improved performance as fodder crops or in the production of pulp and paper ( Pilate *et al*., 2002; Baucher *et al*., 2003; Boudet *et al*., 2003). Lignin is also suggested to be a limiting factor of the cell wall extensibility and the cell elongation (Musel *et al*., 1997). Simultaneously lignin is also responsible for the hardness of the wood so if we increase the content in  then naturally the hardness is also increase and it will be beneficial for the wood industries. The calorific value of the wood depends upon the lignin content of the plants. Calorific value increases as the lignin content in the plant increases.

Histo-chemical methods, such as ultraviolet micro-spectrophotometry and the Mäule's color reaction coupled with micro-spectrophotometry, allow visualization of the distribution of lignin in tissues without the destruction of cell walls (Fukazawa, 1992). Ultraviolet microscopy and micro spectrophotometry are useful for detection of the distribution of lignins, exploiting differences between the wavelengths of maximum absorption by guaiacyl and syringyl units. Ultraviolet photographs recorded at 280 nm and ultraviolet absorption spectra derived from thin sections reveal the localization of guaiacyl units in the cell walls of hardwoods. However, these methods are not as useful for detection

of syringyl units in the cell walls because syringyl units absorb ultraviolet light much more weakly than guaiacyl units (Fergus and Goring, 1970a, b). The Mäule's color reaction provides an effective method for the detection of syringyl units as a result of differences among the colors of the *o*-quinones that are formed during the reaction (Meshitsuka and Nakano, 1978). When thin sections are treated by the Mäule's color reaction, cell walls that contain syringyl units turn predominantly reddish purple, whereas cell walls that contain guaiacyl units remain yellowish in color (Watanabe *et al*., 1997). Yoshinaga *et al.* (1989) reported that the Mäule's color reaction coupled with spectroscopy allowed investigations of the localization of syringyl units and guaiacyl units in the cell walls of different cell types in hardwoods.

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# Mushroom Cultivation

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**Abstract:** Edible mushrooms are collected from the wild. They are currently threatened by deforestation. A survey was carried out and to gather information on their household consumption, income generation and to determine how their cultivation could improve rural livelihood. Mushrooms are sources of food, income and of medicinal value. The market for mushrooms continues to grow due to interest in their culinary, nutritional, and health benefits. They also show potential for use in waste management. However, as fungi, mushrooms have life cycles very different from those of green plants. The choice of species to raise depends both on the growth media available and on market considerations. Oyster mushrooms, which grow on many substrates, are easiest for a beginner. Shiitake mushrooms already have earned considerable consumer demand. Only two mycorrhizal mushrooms, morels and truffles, have been commercially cultivated. Mushroom cultivation offers benefits to market gardens when it is integrated into the existing production system. A careful analysis of potential markets must be the first step in deciding whether to raise mushrooms to sell. Mushrooms are cultivated only on small scale but efforts are underway to extend improved methods of its cultivation to the rural communities, thereby providing them with alternative livelihood and thus ease the pressure on the forests.

## Introduction:

The forest contributes to all aspects of rural life, providing food, fodder, fuel, building materials and household items. People have harvested mushrooms from the wild for thousands of years for food and medicines. Of the estimated 1.5 million species of fungi, about 10,000 produce the fruiting bodies we call mushrooms. While commercial harvesting of wild mushrooms continues today, most of the world's supply comes from commercial mushroom growers. The Chinese first cultivated shiitake (*Lentinula edodes*) mushrooms around 1100 AD, with domestication efforts beginning centuries earlier. White button mushrooms (*Agaricus* spp.), most familiar to American sand Europeans, were first domesticated in France in 1650. Commercial production began in the United States in the 1880s. *Agaricus* is the leading mushroom crop worldwide and accounted for 99 percent of the 1997 United States' mushroom production. Oyster mushrooms (*Pleurotus*spp.) were more recently domesticated, and now rank second in world production. Shiitake mushrooms, which are very popular in Asian cultures, rank third. Mushrooms (fungal sporocarps) represent one of the world's greatest untapped resources of nutritious and palatable food and they possess extensive enzyme complexes, which enable them to flourish successfully on a wide variety of inexpensive substrates, such as lignin, cellulose, hemicelluloses, pectin and other industrial wastes which are not suitable for animal feed. Mushrooms, which are used as food, are assuming greater importance in human diets worldwide than ever before. Edible mushrooms are considered as healthy food because their mineral content is higher than that of meat or fish and most vegetables (Chan, 1981).Furthermore, it is known that the protein content of fresh mushrooms is about twice that of vegetables and four times that of oranges (Chan, 1981). Mushrooms are prized for their exclusive flavor and deliciousness; they are rich in proteins, contains less fat, less carbohydrate and salt and rich in fi-

ber.

**What is Mushroom**:

Mushrooms are the fleshy fungi which constitute a major group of lower plant kingdom. The mushroom is a common fungal fruit body that produces basidiospores at the tip of club like structures, called basidia, which are arranged along the gills of the mushroom. Beneath the mushroom, in the soil, is the mold colony itself, consisting of a mat of interwined hyphae, sometimes several feet in diameter. The mushroom first appear as white tiny balls consisting of short stem (stipe) and a cap (pileus), which begin to open up like an umbrella. The delicate membrane or veil (velum) enveloping the cap tears off, if allowed to develop fully, and lamella (gills) radiating from the stalk in to the cap become visible. These gills become darkened as the basidiospores (seeds) develop into millions and fall to the ground for starting their lifecycle once again for second generation of mushroom. Since mushrooms grow independently of sunlight so they can be grow in complete darkness but darkness is not an essential prerequisite. They are relatively fast growing, do not require fertile soil, since grow on composted or uncomposted agro-wastes additional to floor, air space is also utilized resulting in higher production. It is a labour intensive indoor activity which can help the landless, small and marginal farmers to raise their income, diversity economic activity and can create gainful employment especially for unemployed/under-employed youths, weaker section of the society and women folk. It produces nutritious food from unused resources, available surplus in India (25 million tones of agriculture waste) and also can earn foreign exchange.

**Mushroom cultivation:**

Mushrooms may be grown successfully in a variety of places. Commercial and amateur mushroom cultivation is done indoors. The various installation and required for mushroom cultivation vary with the size of a mushroom house.

The protocol for cultivation of mushroom requires following steps:

**1) Preparation of compost:**

1. Wet the saw dust by spraying water or leaving it overnight after mixing all the constituents except wheat straw.
2. Spread the wheat straw over the cement floor on the following day and wet it thoroughly by sprinkling water.
3. Spread the pre-mixed constituents over the wheat straw surface and mix thoroughly.
4. Stack this mixture into a pile of 1.30metres wide and 1.30metres high, using indigenously fabricated wooden-mould.
5. Allow the compost to decompose for 28-30 days under aerobic conditions in the compost pile.
6. Dismantle the heap repeatedly and prepare pile again and again at periodic intervals by
7. Placing the outer compost inside and inner compost layers in the outer periphery, the
8. Process called turning of the compost pile, to obtain uniform fermentation of the entire Pile.

**2) Filling of Tray Beds:**

1. Spread the prepared compost on the platform.
2. Mix 3kg of calcium carbonate to it.
3. Fill the compost in all corners and edges of a tray.
4. Compress firmly the compost in the tray using a wooden board.
5. Leaving 1 cm clear space on the top of the tray.

**3) Spawning:** Spawning means planting mushroom mycelium, growing on a suitable substrate, in the compost. Government and non government agencies prepared and sold the mycelium. NCMRT-National center for mushroom research and training, chambaghat, solan (HP), India one such central government institute.

Mycelium of mushroom propagated vegetatively on sterilized cereal grain is known as "spawn". Commercial mushroom growers purchase spawn from any of about a dozen spawn companies. Farmers have a choice of growing different strains, ranging from smooth white, off-white, cream, to brown capped mushrooms. These strains vary in flavor, texture, and growing requirements. Spawn is introduced and thoroughly mixed into the compost with a special machine that mixes the compost and spawn with small tines or finger-like devices (figure below and to the right). After spawning, the compost is maintained at approx. 24$^{o}$C, and relative humidity and $CO_2$ levels are kept high to minimize drying of the compost. The spawn will begin to grow and produce a thread-like network of mycelium throughout the compost. Complete colonization of the compost usually requires 12-20 days, depending on the spawning rate and environmental conditions.

Perform the spawning by spreading the spawn on tray beds when half filled with compost and again after the tray is filled completely. During spawning, the spawn is gently mixed with fore-fingers and pressed uniformly each time.

Cover the trays with newspaper sheets.

Sprinkle water on newspaper sheets, to provide humidity.

Stack the inoculated trays vertically, one over the other, depending on the height of the room.

Continue water spraying twice a day or less depending upon available humidity in the atmosphere throughout the spawn running and cropping period.

**Note:** Maintain temperature of the room between 24 and 25°C for 12-15 days for running of the spawn, i.e. formation of mycelium strands all over the tray beds.

**Observation:** observe for the white cottony mycelium over the compost surface and colour of the compost that changes from dark to light brown which is indicative of successful completion of spawn running period.

**4) Casing :** Casing means covering the compost with a thin layer of soil or soil like material after the spawn has spread in the compost (spawn run).To promote mushroom formation, casing soil is added as a surface layer (1.5 - 2 inches deep) on the colonized compost. The important transition from the vegetative to the reproductive stage of mushroom takes place in the casing layer, which is usually a mixture of peat and lime-

stone. Mushrooms form only after the compost is covered with a layer of casing material. In addition to stimulating fruit body formation, the casing layer provides moisture essential for high yields and anchorage for the developing mushrooms. Casing materials do not provide any nutrients to the mushroom mycelium. Environmental conditions after casing are the same as during spawn growth. The compost temperature is kept around 24$^{o}$C for up to 5 days after casing to allow for the spawn to grow through the casing layer. Soil has been the universal casing material. But all soils as such cannot be used as 'casing soil' with advantage, so it is especially prepared soil that can be used for casing.

**Pining:** Primordia or "pins" are knots of mycelium that eventually develop into mushrooms (figure to the left).Once the mycelium has reached the surface of the casing layer, the mushroom is induced to pin by reducing both the air temperature (to 16-18$^{o}$C) and the $CO_2$ concentration (to 0.08%). Fruiting occurs in well-defined flushes or breaks with the first harvestable mushrooms appearing 18 to 21 days after casing.

**5) Watering the beds**: spray the beds over the casing soil with a fine nozzle of a sprayer to maintain relative humidity between 70 to 80 per cent.

**Observation**: observe the beds for mushroom crop which can be expected after 5 to 20 days. Mushrooms mostly appear in- "flushes" and at a temperature of 15°C, it generally takes 7 to 8 days to come to the button stage from the first appearance of the formation of a pin-head. There is an interval of 8 to 10 days between flushes.

**6) Harvesting of mushrooms:** Harvest the crop everyday (in the morning and evening depending upon the market demand) to get a good quality of mushrooms (the cap still being tight to its stalk is the right stage to harvest the mushrooms.) Harvesting is done by holding the cap with fore-fingers slightly pressed against the soil and twisted out. The mycelial strands and soil particles clinging to the base of the stalk are cut of with a knife. Specially desingned wooden box is used for collection of mushrooms from multistoried trays, each provided with the hooks for resting it against the side board of the mushroom tray.

**7) Storage:** Store the mushrooms at 4°C in a refrigerator for a few days to avoid the quality deterioration, because mushrooms are a highly perishable commodity. The white colour turns brown and then black in a couple of hours at high summer temperature. Soon after water oozes out and the mushrooms become unfit for cooking.

**Limitation for Mushroom:**

High cost of energy for year round production.

Unorganized production and sale particularly by seasonal farmers.

Lake of facilities to produce quality of compost, causing material, spawn and processed products.

Limited consumer demand in some part of the country.

Spore allergy to certain people.

**Importance of Mushroom cultivation in India**

Mushroom cultivation is labour intensive ac-

tivity.

Mushroom harvesting is not automatic process.

It helps in maintaining the cycle of nature by decomposing agro residues.

Good source of high quality of protein rich in vitamins and minerals. It is good for vegetarian population.

It provides excellent opportunity to educate rural youth and provide job.

Opportunity to use wastelands.

Rural women who are educated or uneducated easily handle.

**Salient features:**

100% risk free project: running cost is very low and not require heavy machinery.

Low working capital: raw material is waste use so capital cost is low.

Very high returns use waste and get income

Easy financing and long term business.

Pollution free environment friendly project

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# Microbial Transformation of Steroids: Current Trends in Cortical Side Chain Cleavage

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**Abstract:** Steroids have ample clinical applications ranging from anti-inflammatory, immunosuppressive, progestational, diuretic, and anabolic to contraceptive agents. Economical mode of production of pharmacologically active steroidal compounds involves microbial transformation of abundantly available precursor steroids to important drug intermediates and further conversion of these intermediates to active compounds by simple chemical or microbial processes. Alternative modes of bioconversion of steroids are primarily standardized using precursors with cortical side chains and the results extrapolated for complex steroids. Microbial transformation after entrapment of whole cells, extractive bioconversion in biphasic medium and aqueous two phase systems are the current trends in cortical side chain cleavage.

## Introduction

Steroid biotransformation is a multimillion dollar industry and pharmaceutical uses of steroids are numerous. Specific microbial transformation steps have been incorporated for synthesis of new steroids and their evaluation as drugs and hormones. Biotransformations have provided adequate tools for the large scale production of natural or modified steroid analogues[1]. Highly complex structure of steroids molecules renders the use of biocatalysts for the production pharmacologically important steroid drug intermediates. The production of steroid drug hormones is good example of the successful application of microbial technology in large scale industrial processes[2]. Alternative modes of bioconversion are the areas that address the problems of insolubility of steroids in the bioconversion medium and substrate inhibition observed during the catalysis by enzymes and microbial cells.

## Precursor steroids

Natural sources of pharmacologically active steroidal compounds are scarce, uneconomical to isolate and non-feasible as stereochemical considerations limit the use of compounds derived from one animal as drugs in another. A large number of naturally occurring, extractable steroidal compounds have complex side chains and cannot be used as drugs. Among the abundantly available steroid precursors are cholesterol, steroidal sapogenins, steroidal alkaloids and phytosterols. Cholesterol can be extracted from the waste blood collected from slaughter houses. Plant derived steroids are extracted from parts of plants cultivated for the purpose and from press mud generated from edible oil extraction units.

## General scheme for steroid drug production

Steroid drugs are synthesized by chemical or microbial routes, both of which involve conversion of steroid precursors to drug intermediates and final conversion of intermediates

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to active drugs.


Microbial transformations cleave the complex side chains of precursor steroids in one single step and incorporate desirable modifications in steroid nucleus.

## Chemical transformations v/s Microbial transformations

The Chemical synthesis and transformations of steroids requires multiple steps and makes the use of reagents that have health risks and cause serious environmental disposal problems. The conversion of precursor steroids through microbes as compared to chemical process is less expensive, non toxic and less time consuming. During bioconversion, microbes provide enzymes which act upon and convert organic compounds or modify it. Microbial transformations are regio-specific and stereo-specific, whereby organic compounds are modified into desirable isomers of products involving simple chemically defined reactions catalyzed by the enzymes in the microbial cells. One of the major biotechnological aspects in microbial transformation is the application of a wide range of the microorganisms including bacteria, fungi and microalgae in converting steroid substances into the pharmacologically active compounds or other useful intermediates[3].

## Steroids with cortical side chains

Steroids with cortical side chains are formed as intermediates, both during in-vitro bioconversions of complex precursor steroids like sterols and steroidal sapogenins involving microorganisms as well as during in-vivo steroid biosynthesis. Pregnenolone, progesterone and their derivatives are compounds that have pharmacological activity as well as potential for utilization as starting materials for synthesis of other intermediates during steroid drug production.

Pregnenolone is a natural hormone, produced primarily by adrenal glands, but also in the brain, skin, liver, testicles, and ovaries. In the body pregnenolone is used as pregnenolone itself, or is converted into androgens, estrogens and other steroids. Pregnenolone is also being considered as a potential treatment for schizophrenia[4].

## Potential microbial transformations of steroids with cortical side chain

The bioconversion of 3β-acetoxypregna-5, 16 -diene-20-one (commercially known as 16-DPA) by a mixed culture of *Pseudomonas diminuta* and *Comamonas acidovorans* has been reported[5]. Unlike the mixed culture-mediated steroid conversions reported in the literature, neither of the two isolated bacteria could modify 16-DPA individually in pure cultures, but successfully converted 16-DPA to androsta-1,4-diene-3,17-dione (ADD) in a mixed culture. This bioconversion is of particular interest as 16-DPA is available in large quantities.

Biotransformation of hydrocortisone, a corticosteroid by *Neurospora crassa* FGSC 4335 was investigated[6] and the microorganism was reported to produce two major metabolites. The studies with growing cells of *Pseudomonas putida* MTCC 1259 were carried for biotransformation of a steroid with cortical side chain, namely, 11β,17α-dihydroxy-4-pregnene-3,20-dione-21-O-succinate[7]. They

reported that side-chain degradation did not require addition of inhibitors of ring cleavage enzymes.

Wide varieties of fungi are capable of side-chain cleavage of progesterone[8]. During side-chain cleavage of progesterone, usually 17-acetate is formed by introduction of oxygen between C-17 and C-20 then an esterase cleaves the acetate leaving the 17-hydroxy steroid, testosterone. Then the 17-hydroxyl group is oxidized to 17-ketone, some of the organisms may form the 1-dehydrogenated derivatives. Adrost-4-ene-3,17-dione [AD], and Androsta-1,4-diene-3,17-dione [ADD] are among several important compounds obtained by degradation of steroid side-chain which may be useful for the production of androgens, estrogens and other compounds by further chemical modification. 1-dehydrotestolactone may also be produced which is approved for the treatment of mammary cancer. Androst-4-ene-3,17-dione can be chemically converted to spironolactone which is an important drug in the treatment of hypertension. Androst-4-ene-3,17-dione may also be chemically reduced to give testosterone and some derivatives that have important medicinal uses.

## Alternative Bioconversion Methods

Steroids are insoluble in water and uniform dispersal of steroids in bioconversion medium is the major problem associated with steroid bioconversions that limits the yield of such processes. Further, the yield of products is restricted by upper limit of substrate concentration as the enzymes involved in bioconversions are inhibited by high concentration of their substrates. Alternative methods like use of immobilized cells, emulsification of substrate, use of organic solvents in biphasic systems and aqueous biphasic systems for increasing the solubility and dispersal of steroids have been attempted by many researchers.

The use of immobilization of microbial cells provides the ability to minimize the deactivation of biocatalyst and controls reaction times, reuse of cells for many reaction cycles and lowers the total production cost of cell mediated reactions. Cells can be used for many reaction cycles and lowers the total production cost of cell mediated reactions.

The 11β-hydroxylation and 1-dehydrogenation on a cortical side chain containing precursor by using immobilized spores of *Cunninghamella elegans* was studied and maximum production of cortisol and prednisolone were obtained after 72 h transformation period using immobilized spores of *C. elegans* of concentration 2 x $10^7$ spores/ml for both entrapment and adsorption[9]. The highest transformation efficiency was recorded on using 1.6% w/v glass wool (92%) compared to that when the fungal spores were entrapped in 3% alginate (84%). Each immobilized microbial system was stable and could be used for the sequential reactions repeatedly (operational period, 18 days using entrapped *C. elegans* in alginate beads and 45 days using adsorbed spores on glass wool).

The most obvious benefit of the immobilization technique is the capability of continuous cycling which provides a means for using them in continuous processes maintaining high cell population to achieve fast reaction rates[10].

Attempts were made to immobilize *Aspergillus terreus* mycelia on a new hydrophobic matrix polytetrafluoroethylen for conversion of

progesterone to 11α-hydroxyprogesterone[11]. Utilization of the immobilized cells in repeated batch processes indicated that the cells retained about 84% of their activity after reuse for eight successive cycles.

Immobilized *Arthrobacter simplex* cells were used for transformation of microcrystalline hydrocortisone to predisolone. Immobilized bacterial cells showed higher yield than free form in aqueous system. In two phase system of butyl acetate to aqueous media with ratio 1:30, three bacterial strains namely, *Bacillus simplex, Bacillus sphaericus & Arthrobacter simplex* in immobilized form gave highest prednisolone yield[12].

A major problem in the biotransformation is the poor solubility of substrates in aqueous medium solution, which leads to extremely low productivity. Therefore the use of aqueous/organic solvent two- liquid-phase systems is a preferred way to improve substrate solubility, enabling operation at high substrate concentration and facilitating subsequent product recovery. In Biphasic system the cells are in the aqueous phase and steroid dissolved in the organic phase is considered as ideal set up. An idea based on that the enzymatic process carried out in a biphasic system water–*water-immiscible* organic solvent was attempted thereby the enzyme is localized in the aqueous phase which eliminates the traditional problem of stabilizing the enzyme against inactivation by a nonaqueous solvent[13].

The 15α-Hydroxylation of a steroid (13-ethylgon-4-en-3, 17-Dione) by *Penicillium raistrickii* in an ionic liquid/aqueous biphasic system has been reported[14]. Biphasic processes are used in whole-cell biotransformation to overcome the low water solubility of substrates and products as well as their inhibitory effects on the biocatalyst. Commercially available ionic liquids (ILs) were used in a biphasic system for the 15α-hydroxylation reaction. The yield was 70 % after 72 hr as compared to a 30 % yield in a monophonic aqueous system. This suggests the potential industrial application of ILs-based biphasic systems for steroid biotransformation.

A novel aqueous two phase system based on polyethylene glycol (PEG) and monosodium glutamate was attempted for the 1-dehydrogenation of hydrocortisone-based substrates[15]. This system led to higher substrate solubilties and biocatalyst-steroid separation level when compared with other alternative systems. Reports on success of aqueous two phase systems for complex bioconversions involving multiple enzymes are scarce in literature.

## Conclusion

The recent approaches in bioconversion of steroids with cortical side chains are directed towards improvement of solubility of substrates in bioconversion medium. Of the unexplored areas is the use of aqueous two phase systems for complex steroid bioconversions requiring cascades of enzymatic reactions for desired product accumulation.

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