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Biotransformation of naphthalene by *Aspergillus ochraceus*

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ABSTRACT - In biotransformation there is addition, deletion, substitution and hydroxylation reaction takes place by phase I enzymatic reaction while conjugation reaction take place in phase II enzymatic reaction. Most enzymatic reaction carried in liver tissue of animal and also in lung, kidney, skin and intestine to less extent. Microorganisms have ability to modify chemically a wide verity of organic compounds. These change are called biological or microbial transformation or more generally bioconversion. Naphthalene is a simplest aromatic hydrocarbon in nature. Polycyclic compounds are environmental pollutants due to their toxic mutagenic and carcinogenic properties. Bitransformation of Naphpthalene by *Aspergillus ochraceus occurs.*

Key words - Ribbon fish, Drying methods, Biochemical composition

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Biotechnology employees microorganism as well as higher cell and their active principle with in the aim of achieving desirable conversion of various substrates. The desirable product of biotransformation are formed by enzymatic biotransformation of normal product or chemically synthesized substrate. Biotransformation reaction are also carried out in human body so as to convert xenobiotic toxic substance in to less toxic, easily extractable form. In biotransformation there is addition, deletion, substitution and hydroxylation reaction takes place by phase I enzymatic reaction while conjugation reaction take place in phase II enzymatic reaction. Most enzymatic reaction is carried out in liver tissue of animal and also in lungs, kidney, skin and intestine to less extent. Chemically microbial transformation reaction can be grouped under following categories : Oxidation, reduction, hydrolysis, condensation, and formation of new C-C bonds, introduction of hetrofunction. Oxidation reaction are particularly usful in industrial production (H.J.Rehm and G. Reed). Plant cell culture are being utilized for producing valuable product including secondary metabolites through biotransformation, a technique also utilized with the help of microbes. In this technique low cost precursors are used as a substrate and are transformed into value added high cost product (Gupta, 2002).

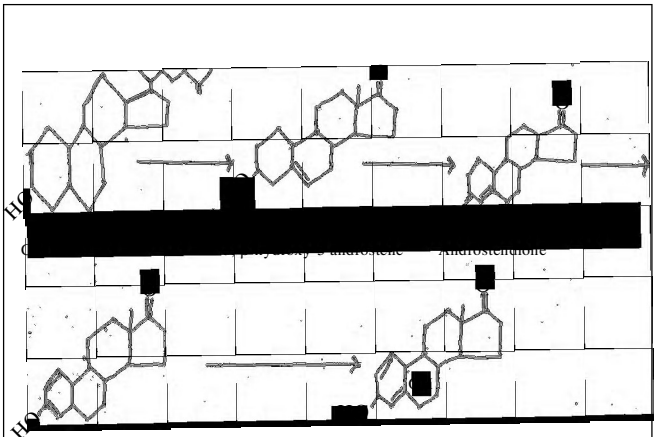

Fig. A : Biotransformation of chlolesterol to androstadlendione

Biotransformation of chlolesterol to androstadlendione:

Micro transformation or biotransformation have a selective advantage over non-biological chemical reaction

– Many non-biological chemical reaction call for a considerable input of energy to heat or cool the reaction vessels.

– Several solvent and inorganic catalysts are required in chemical reaction

– In non-biological chemical reaction, a few unwanted by-products which require addition purification are also formed.

– Bioconversions, unilike non biological reaction produced at ordinary cell temperature with water as the solvent.

– Bioconversion are high yielding and can reach 100 per cent (Trehan, 1997).

The use of fungi in effective transformation stems from the work of (Weaver *et al.,* 1960) showed that a strain of Rhizopus arrhizus would convert progesterone in to 11 alpha –progesteron. Hydroxylation is often the first step in the breakdown of an organic compound by a microorganism , but it is a reaction that is often difficult , lengthy and expensive to do chemically. The hydroxylation of steroids by fungal or bacterial biocatalysts have been known for many years (Weaver *et al.*, 1960)

For many years, fungi have been frequently used for hydroxylation of steroids, because specific microbial transformation can be carried out in much higher yield and at a lower cost than chemical procedure. Thus, *Curvularia lunata. Cochlibolus lunatus* and *Rhizopus nigricans* etc.are chosen by several groups as attractive model system to study steroids 11 beta 11 alpha –hydroxylase, respectively. Hydroxylation in the 11 beta position is an essenitial steps in the manufacture of the anti-rheumatic corticosteroids and this can be done on a manufacture scale directly with *Curvularia lunata*. *Curvularia lunata* CECT 2130 is able to introduce short alkyl chain and reduce the carbonyl group of benzolacetonitril. It also carries out alkylation –reduction reaction of beta ketonitriles. Another e.g. of a hydroxylation that has been carried out on a manufacturing scale is the conversion of the Schistosomicide lucanthone to the more active compound hycanthone, which is effected by *Aspergillus sclerotiorum.*

Naphthalene is a simplest aromatic hydrocarbon in nature . polycyclic compounds are environmental pollutants due to their toxic mutagenic and carcinogenic properties. Polycyclic aromatic hydrocarbon in nature are environmental pollutants due to their toxic, mitagenic and carcinogenic properties. Naphthalene being a simplest homologue in the polycyclic series has received considerable attention (Carle and Gibson, 1968). *Aspergillus ochraceus* is easily available as it is world widely distributed and is a common fungi. It can use aromatic hydrocarbon compounds for energy purpose. They can produce different secondary matabolites easily using aromatic ring compound. They are non –pathgenic in nature. They multiply readily to form large mass for application on large scale. Nutrtional requirements are simple.The fungi is used for biotransformation of various compounds example progesterone, hydroprogesteron production (Sharma).

Materials:

Micro-organism:

Fungus species: *Aspergillus ochraceus* (96 old culture)

Growth medium:

For mycelium-potato dextrose broth:

Preparation-Take 200 gm of potato , cut into small pieces and boil it for half an hour and collect the filtrate by using muslin cloth and make the volume of filtrate 1 liter by distill water . Add 20 gm glucose , 0.1 gm of yeast extract and sterilize it at 15 Ibs for 20 minute.

For spore:

0 per cent malt extract medium:

Preparation -Take 10 g of malt extract and dissolve in 100 ml distill water and sterilize at 127 degree Celsius for 20 minute.

Incubation time:

For mycelium—Inoculate a loopful culture of Aspergillius ochraceus in potato dextrose medium aseptically and incubate it for 96 hours at 30^0C.

Buffer:

Phosphate buffer (pH 7.4 , 5.0 mM):

Prepararion— For Stock solution- 1 M phosphate buffer

Take 11.84 g of K_2HPO_4 and 4.35 g of KH_2PO_4 and make volume 100 ml by distill water. For working s buffer-Take 5 ml stock from 1M phosphate buffer and dilute to 100 ml by distilled water and add 0.4 g of NaCl and adjust the pH to 7.4)

Substrate:

150mgs of naphthalene dissolve in minimum quantity of ethyl acetate.

Gel for Thin layer chromatography:

Silica gel in chloroform

Solvent system for TLC used:

Benzene: Acetone (3: 1)

EXPERIMENTAL METHODOLOGY

Take 96 hrs old culture *of Aspergillus ochraceus* grown on PDA media (potato dextrose media) Cut in to small pieces and add 50 ml (50 mM) phosphate saline buffer (For Stock solution- 1 M phosphate buffer.

Take 11.84 g of K_2HPO_4 and 4.35 g of KH_2PO_4 and make volume 100 ml by distill water. For working s buffer-Take 5 ml stock from 1M phosphate buffer and dilute to 100 ml by distilled water and add 0.4 g of NaCl and adjust the pH to 7.4) and add 50mgs naphthalene dissolved in 10 ml ethyl acetate.

Take 5 ml sample from thie reaction, add 5 ml ethyl acetate, immediately take zero reading. Then continuous shaking with rotator shaker (250 rev/min) and incubate at 30^0C. Again take 5 ml sample from the reaction mixture and add 5 ml ethyl acetate at 24 hrs , similar procedure carried out for 48, 72, 96 hrs.Take out the upper layer of ethyl acetate containing product from reaction mixture by using syringe. Sample is analyzed by Thin Layer Chromatography. Thin Layer Chromatography technique is based on the principle of adsorption as well partition chromatography , which depends upon the materials chosen for the preparation of thin layer. Here we have used silica gel for the chromatography which acts as absorbing material. Generally used for the separation of the acidic and neutral components. In thin layer chromatography the solid acts as adsorbing material and is called as stationary phase while the mobile phase is solvent system, which separates components depending on their partition coefficients.

The solubility of component depend upon the polarity of the component and that of solvent. Polar substance get dissolved in polar solvent while non-polar substance get dissolved in non-polar or less polar solvent adsorption as polarity increases.

Order of polarity of solvent:

Petroleum ether < benzene < acetone < methanol < ethyl acetate < acetic acid.

$$\text{RF value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

Visualization of spot and done using iodine chamber. Note the number of spot and measure the RF value.sample for product separation are taken at regular intervals.

Observation table
Solvent system = Benzene-Acetone (3:1)

	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs
	Observation Time in hrs.				
Number of spots	1	2	3	3	3
RF value	0.8	0.87	0.87	0.87	0.86
		0.85	0.84	0.85	0.85
			0.73	0.71	0.71
Standard Naphthalene (RF)	0.82	0.82	0.82	0.82	0.82

EXPERIMENTAL FINDINGS AND ANALYSIS

Biotransformation of naphthalene by Aspergillus ochraceus occurs.

Naphthalene give to products with the solvent system Benzene : Acetone (3: 1)

The product is non-polar due to solvent system is less polar

Outlook for future:

Determination of chemical nature and structure of product formed by biotransformation by using appropriate method such as HPLC, NMR, FTIR spectroscope.

– Determination of per cent concentration of product formed by biotransformation of naphthalene.

– To determine the types of enzymes involved in the biotransformation of naphthalene.

– To check the uses of products in therapeutic and pharmaceutical application.

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