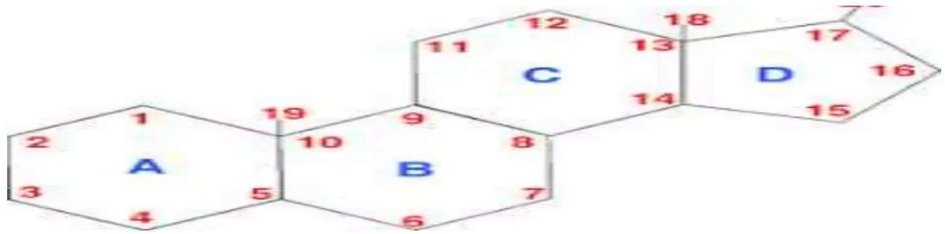
Microbial Biotransformation

Introduction

- Microbial biotransformation is a biological process in which organic compounds are modified from one form to another form to reduce toxicity
- These biotransformation reactions are catalyzed by purified enzymes present in microbial cells or pure cultures of microorganism.
- Microbial enzymes are highly versatile in nature .
- Microbial bioconversions are routinely used in commercial production of steroids , antibiotics , vitamins , citric acids etc .
- Steroids can be produced by chemical synthesis or by microbiological transformation.
- This process used as a manufacturing of pharmaceutical industries.

- Steroids are physiologically active compound which include progesterone , testosterone , corticosterone etc.
- Progesterone and estrogens are used as a oral contraceptives.
- Scientist Mamoli and Vercellone in 1937 made the first successful Microbial biotransformation of steroid .
- Peterson and Murray in 1952 reported the 11-hydroxylation of progesterone using a fungi Rhizopus arrhizus.
- Steroidal biotransformation are mainly affected by bacteria , actinomycetes and fungi.



Advantages

Selectivity

- One of the main advantages of biotransformation is its high selectivity.
- Biological catalysts can often produce specific products with high purity, whereas traditional chemical methods may produce unwanted by-products.
- Many biotransformation reactions use renewable starting materials and produce less waste than traditional methods.
- **Cost-Effective**
- In some cases, biotransformation can be a more cost-effective alternative to traditional chemical methods.
- Enzymes and microorganisms can often be produced at a relatively low cost, and the use of renewable starting materials can reduce production costs.
- **Mild Reaction Conditions**
- Biotransformation reactions can often be carried out at mild conditions such as low temperatures and atmospheric pressure, which reduces energy consumption and production costs.
- **Lower Environmental Impact**
- Biotransformation reactions often produce less toxic waste than traditional chemical methods, making it a more environmentally friendly approach.

Disadvantages

- **Limited Substrate Range**
- The substrate ranges of enzymes and microorganisms used in biotransformation can be limited, which can restrict the types of compounds that can be produced.
- **Sensitivity to Reaction Conditions**
- Enzymes and microorganisms used in biotransformation can be sensitive to reaction conditions such as pH, temperature, and substrate concentration, which can limit their effectiveness.
- **Product Inhibition**
- In some cases, the product of a biotransformation reaction can inhibit the enzyme or microorganism used, which can limit the yield of the reaction.
- **High Cost of Purification**
- Purification of enzymes and microorganisms used in biotransformation can be expensive and time-consuming, which can limit the feasibility of large-scale production.
- **Stability Issues**
- Enzymes and microorganisms used in biotransformation can be unstable, which can limit their shelf life and the overall feasibility of large-scale production.

Methods of Biotransformation

- Transformation of organic compounds may be accomplished by use of microorganism, isolated enzyme, immobilization techniques and solvent selection.
- Microbial cells serves as major tools for biotransformation .
- The main process used for biotransformation is fermentation .
- **The fermentation is carried out in two phases:**
- 1. Growth phase
- 2. Product formation phase

- Fermentation is carried out in the following phases
- **1.phase I – Growth phase –**
- In the growth phase a culture is grown in a nutritionally rich medium.
- The medium for the growth is simple or complex type.
- Aeration and agitation(stirring) are provided during growth and optimum temperature is maintained
- Time of incubation period is depends on type of culture and environmental condition.

Phase II – Transformation Phase

- Transformation Phase begins with addition of steroid at the end of the growth phase .
- Steroids may be added simultaneously with the inoculation
- Amount of steroid to be added depends upon transforming capacity of culture, toxicity of substrate or type of product.
- The biotransformation occurs under controlled conditions of temperature , p H , aeration and time .
- The submerged fermentation is carried out in a stainless steel tank with minimal nutritional quantities to allow maximum transformation and use of easy extraction and purification of transformation product.
- The microorganisms are grown in a suitable medium for 12-72 hrs depending on bacterium and fungus at optimum temperature, pH, aeration and agitation.

- After the transformation , microbial growth is separated from the fermentation liquor and extracted with a suitable solvent.
- Methylene chloride, chloroform and ethyl acetate solvents are commonly used for extraction of steroids .
- Product obtained from cell should be extracted separately.
- The extracted samples are analyzed.

Application of Microbial Biotransformation

- Transformation of steroids and sterols.
- Transformation of Pollutants.
- Transformation of Non-Steroid Compounds.
- Transformation of Antibiotics.
- Transformation of Pesticides.
- Petroleum Biotransformation.

Application of Biotransformation

- **Pharmaceutical Industry**
- Biotransformation is widely used in the pharmaceutical industry to produce a wide range of drugs.
- Enzymatic biotransformation has been used to produce drugs like antibiotics, statins (reduce cholesterol), and anti-inflammatory drugs.
- One example is the production of semi-synthetic penicillin, which involves the use of enzymes to modify natural penicillin to improve its properties.
- **Food Industry**
- Biotransformation is also used in the food industry to produce flavors, fragrances, and other additives.
- Microbial biotransformation is commonly used to produce compounds like vanillin, which is used as a flavoring agent in foods and beverages.

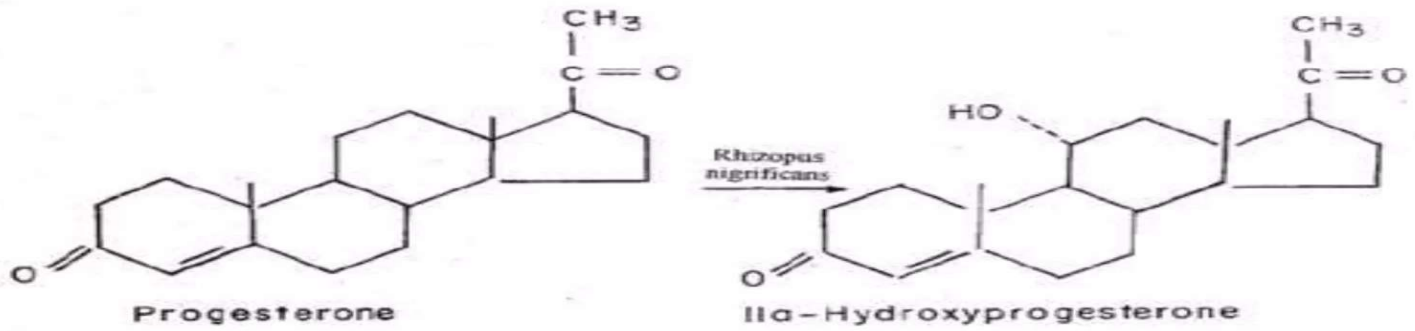
- **Chemical Industry**
- Biotransformation is also used in the chemical industry to produce a wide range of chemicals, such as detergents, solvents, and biofuels.
- Enzymatic biotransformation can be used to produce chemicals like esters or amides, which are widely used in the chemical industry.
- **Environmental Applications**
- Biotransformation can also be used in environmental applications, such as the treatment of wastewater or the remediation (used to remove toxins or contaminants)of contaminated soil.
- Microorganisms can be used to degrade pollutants, such as pesticides or industrial chemicals, into less harmful compounds.

Microbial Bioconversion

- The bioconversion reactions are classified as follows:
- A) Oxidation
- 1.Hydroxylation 2.Dehydrogenation
- 3.Epoxidation 4.Aromatization
- B) Reduction
- 1.Reduction of double bond
- 2.Reduction of ketone , aldehyde and acids
- C)Hydrolysis D) Esterification
- E) Isomerization F) Amide Formation
- G) Halogenation H)Decarboxylation
- I) Condensation

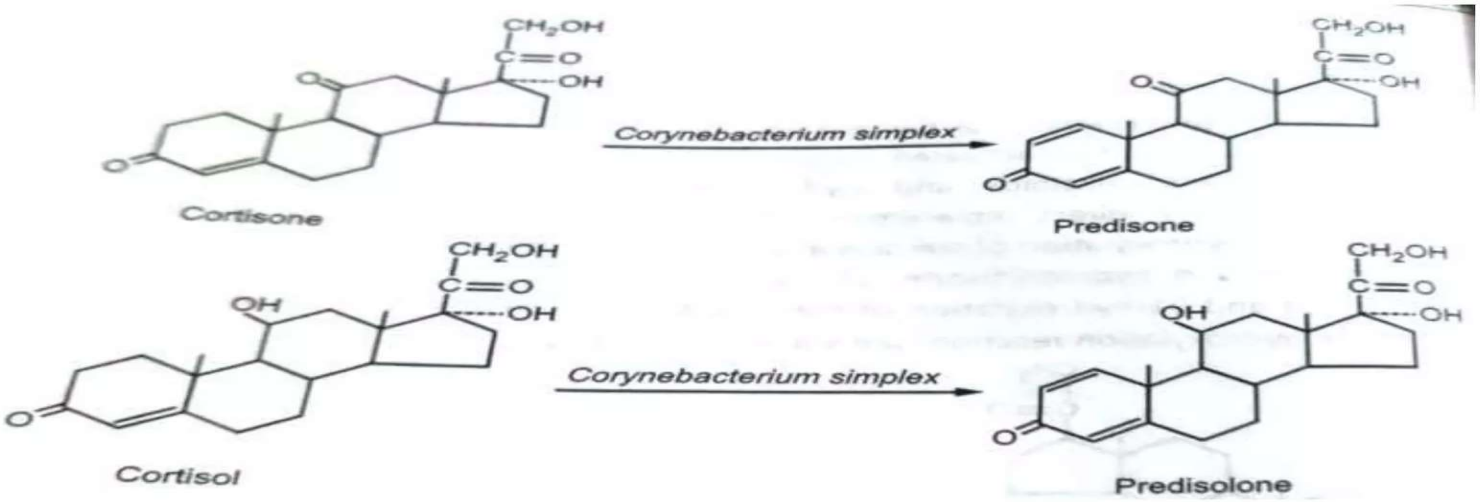
1.Oxidation

- I .Hydroxylation –
- Bacteria and fungi are used for hydroxylation at non activated carbons in substrate like steroids, prostaglandins , alkaloids and hydrocarbon.
- Microbial hydroxylation is done by direct replacement of hydrogen atom on given carbon .
- Microbial hydroxylation of steroids at C-11 increase demand of cortisone and hydrocortisone .



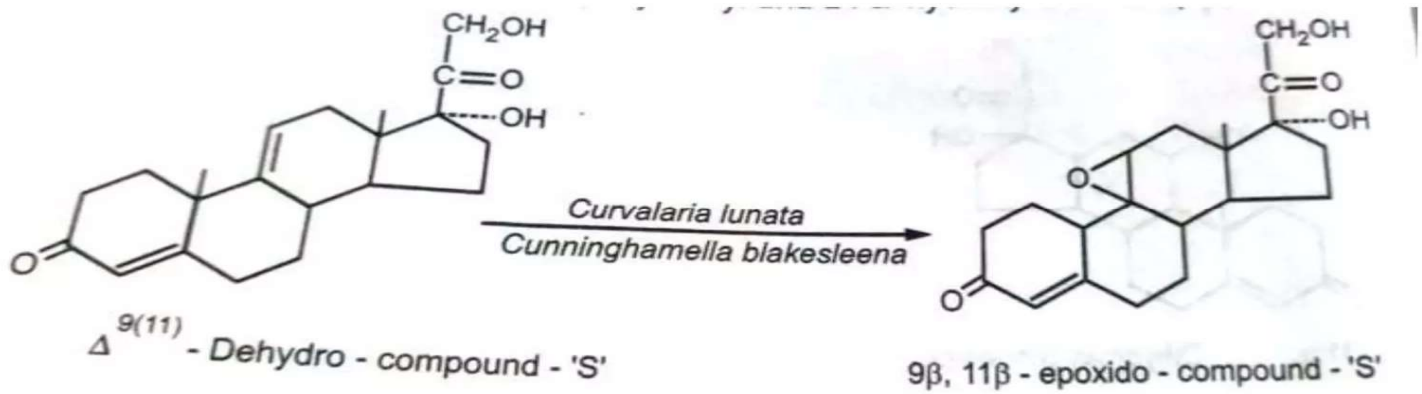
II .Dehydrogenation

- Bacterial and fungal species are capable of dehydrogenation of steroids .
- Addition of double bond on all 4 rings of steroid nucleus but microbes attacks the ring A



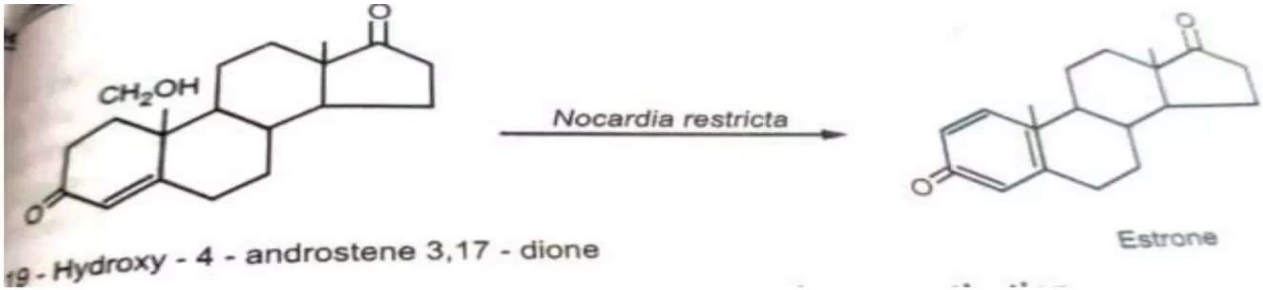
III .Epoxidation

- Epoxidation is very rare transformation.
- The microorganism which normally hydroxylate (addition of hydroxyl compound)saturated steroid will epoxidize (convert c=c bond into epoxide)



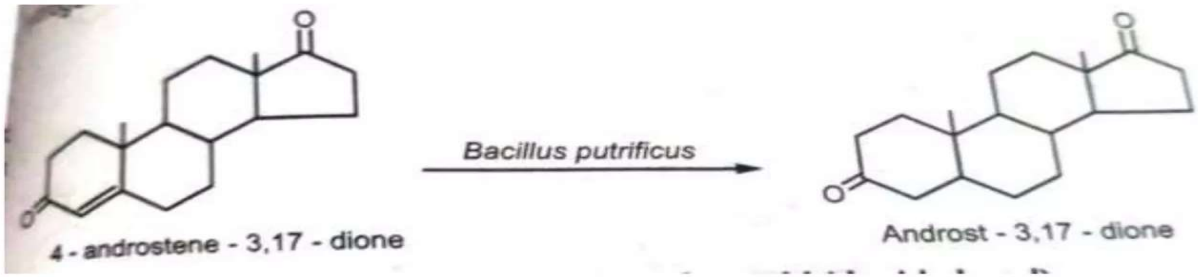
IV .AROMATIZATION

- 19 – hydroxyl cholesterol and 19 – hydroxyl beta sistosterol are converted to estrone with nacardia restricta by aromatization.
- C – 1 – dehydrogenation with substrate lacking methyl group at carbon 10 or suitably at carbon 19 result into aromatization .
- Aromatization is conversion of non aromatic ring to aromatic ring by using enzyme aromatase.



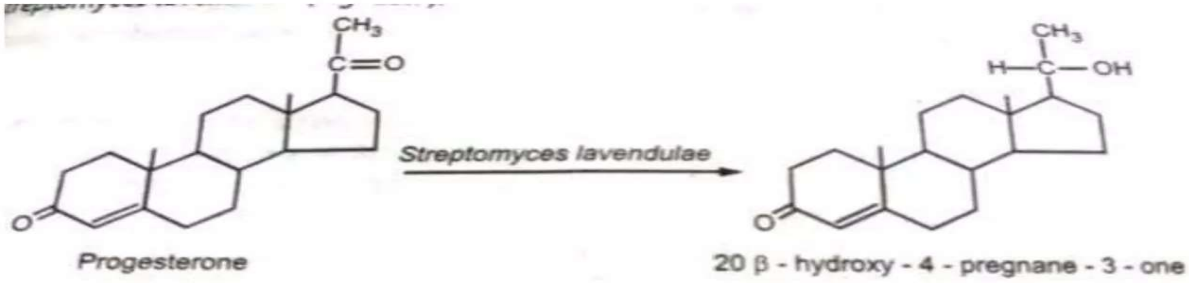
2.REDUCTION

- I .Reduction of double bond (-c=c-)–
- The enzyme oxido- reductases catalyze both dehydrogenation and reduction reaction .
- Ring hydrogenation or reduction occurs at ▲1, ▲ 4 , and ▲16 , ▲1.
- The conversion of 4 – androstane – 3 , 17 – dione to androsane – 3 , 17 dione with bacillus putrificus is reported for 4 hydrogenation ▲.



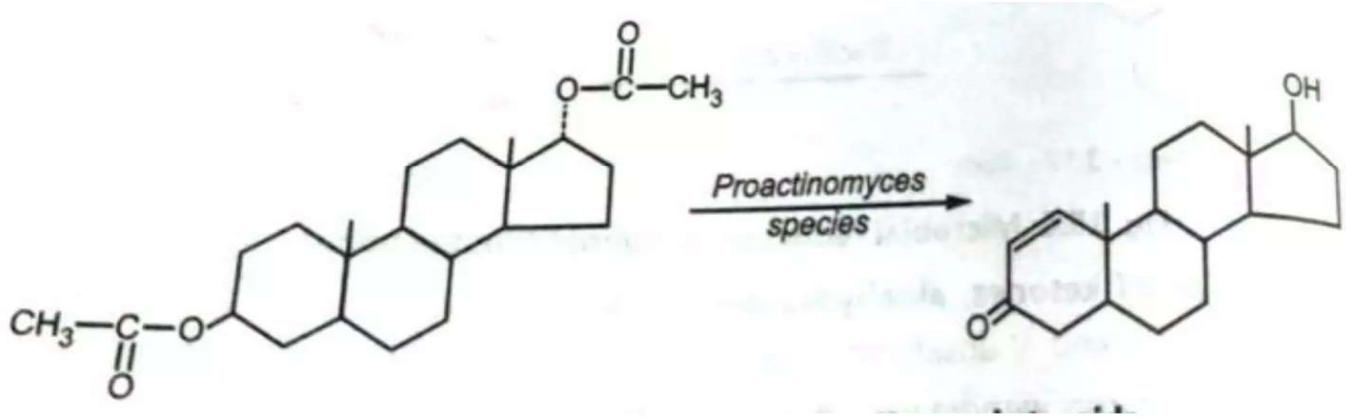
II .Reduction of ketones , aldehydes and acids

- Hydroxyl groups at C6, C11 and C17 , alpha – methyl group at C16 and Δ unsaturation decreases reduction in steroids .
- The presence of electron withdrawing groups at C-6 in 3 keto 4 Δ subtrate , shift direction from oxidation to reduction .
- Reduction of C-20 ketone occurs in presence of streptomyces lavendulae.



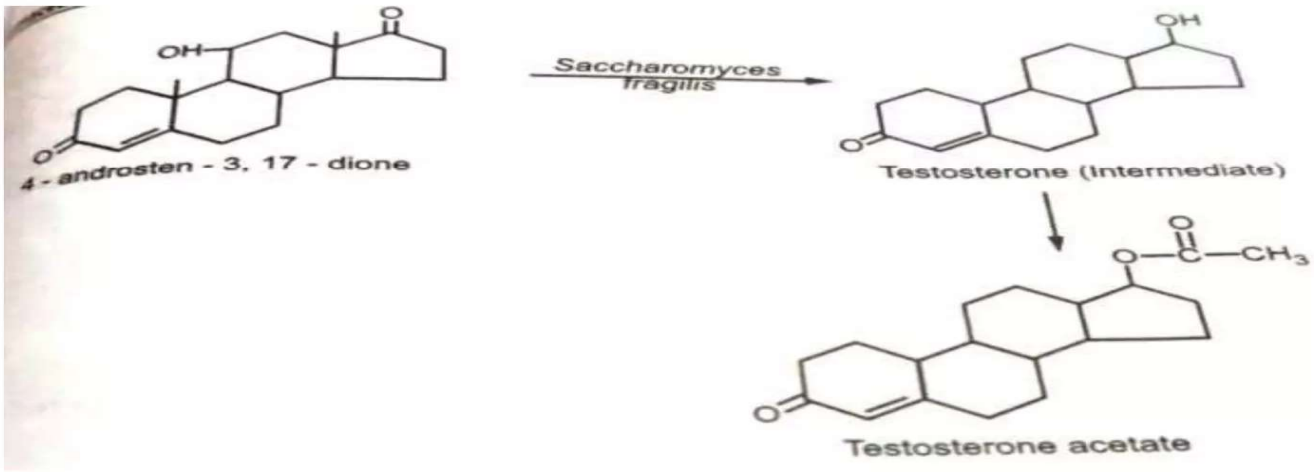
3.Hydrolysis

- A large number of esters , lactones , beta- lactum , glycosides, epoxides and amides can be hydrolyzed by using microorganism .
- In steroids hydrolysis 3 and 21 – acetates are generally hydrolyzed to hydroxylation or dehydrogenation .



4.Esterification

- Microbial transformation by esterification is prepared by esterification using sacchromyces fragilis .
- Esterification means combining organic acid RCCOH with alcohol ROH to form ester RCOOR.



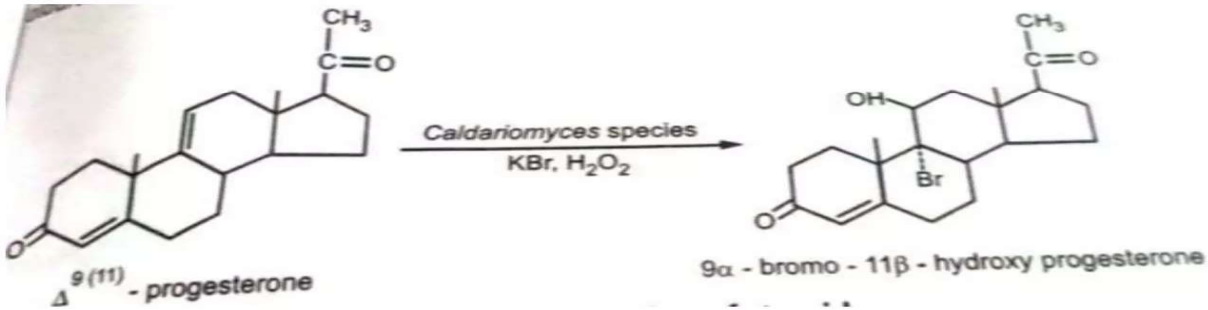
5.Isomerisation

- Deoxycorticosterone is prepared by isomerisation of the double bond from Isomerization of 5 to 4 with corynebacterium mediolanum .

- 6.Amide Formation –
- Amide formation is rare microbial biotransformation .
- Smith and his coworkers reported the transformation of steroids with streptomyces roseochromogenus .

7.HALOGENATION

- Halogenation reaction means replacement of one or more hydrogen atom .
- Halogenation carried out at p H 3 .
- Haloperoxidase enzymes from caldariomyces fumago catalase the halogenation reaction of steroids .



8. DECARBOXYLATION

- Decarboxylation is elimination of carboxyl group .
- L –lysine can be synthesized by stereospecific decarboxylation of meso α, α `- diaminopimelic acid (DAP) to L-lysine .
- The reaction is catalyzed by Bacillus sphaericus .

9. CONDENSATION -

- Microbial condensation was utilized in 1934 in the synthesis of natural ephedrine .
- Acetaldehyde reacts with benzaldehyde in presence of fermenting yeast and gives (R)- 1- phenyl – 1 – hydroxy – 2 – propanone .
- Propanone undergoes reductive condensation with methylamine to yield (1R , 2S) – ephedrine .