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ANTIBIOTICS PRODUCTION FROM LIGNOCELLULOSIC WASTE MATERIALS

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ABSTRACT
Antibiotics have become a necessity to fight infections. These antibiotics are produced by microorganisms and industrially they are produced in fermentation tanks by using desired microorganisms. The fermentation media contains carbon source, nitrogen source and trace elements along with anti-foaming agents. The carbon source best suited for efficient production of bioactive compounds by microorganisms includes glucose and lactose sugar. By changing the source of obtaining glucose for media preparation the antibiotics production can be made more cost efficient and also more sustainable. Lignocellulosic waste materials includes plant dry matter which is easily available and this can be converted into cellulose and further into glucose by various physical, chemical or biological methods. With increasing demands of antibiotics, lignocellulose can be considered as a reliable raw material for their production.

KEYWORDS: Antibiotics, Lignocellulose, Biomass, Fermentation

INTRODUCTION

Antibiotics are substances or molecules of low molecular weight, produced as secondary metabolite by microorganisms which are not necessary for them but can kill or stop the growth of many other microorganisms. Industrially they are produced by fermentation with the help of microorganisms. The production takes few days to obtain products and the production is done by batch process. The production process consists of 3 basic steps: isolating a desired microorganism; Fueling growth of culture by media preparation; refining and isolating final products.3) The media used for growth of microorganism can be liquid or solid and its main constituents include carbon, nitrogen, and Sulphur. Antibiotic production is done usually by solid state fermentation in which organisms grow on non-soluble material or solid substrate in absence of free water. Before starting fermentation suspensions of antibiotic producing organism is made in lab with sufficient growth factors provided in small amount of media. Then these suspensions are introduced into seed tanks for further growth where they are provided with ideal environment and essential growth factors. The seed tanks have mixers and are continuously pumped with sterilized air. After a day the material in these seed tanks are transferred to primary fermentation tanks. Fermentation tanks are large stainless steel vessels equipped with motor, aerator, agitator, pH and temperature control in which microorganisms are grown. After three to five days antibiotics can be isolated and purified using ion exchange or solvent extraction methods. The dissolved antibiotic is then recovered in powder form using various organic chemical agents. The powdered antibiotics obtained are refined further in desirable forms and at each stage of manufacture the quality of compounds are checked at regular basis.3) 10)

The nutrient media used for growth of microorganisms consists of a carbon source which has dual role of biosynthesis and energy generation. Nitrogen source can be inorganic such as ammonium salts or organic such as amino acids or proteins. Glucose is considered a good source of carbon as it provides faster growth than other sugars and is consumed first. The glucose content needs to be regulated, it is used up first to produce more and more cells and little or no antibiotics are formed, whereas the second best carbon source is used for idiolite formation. Hence glucose plays an important role in growth of microorganisms. Higher glucose concentration causes faster growth but acts as repressing carbon source for production of secondary

metabolite. Low concentration of these repressing carbon source is necessary for slow growth and antibiotic biosynthesis.10) 14)

Lignocellulose is plant dry matter and is most abundantly available raw material on earth. It is composed of carbohydrate polymers (cellulose and hemicellulose) and aromatic polymer (lignin). The carbohydrate polymers contain different sugar monomers which are tightly bound to lignin.7) To extract fermentable sugar from lignocellulosic waste, it is necessary to extract cellulose from the lignin first and then use acid or enzymatic methods to hydrolyze celluloses to break down into simple monosaccharides. Cellulose in lignocellulosic waste is most abundant and is composed of β(1-4) linked D-glucopyranosyl units. The degree of polymerization in these chains are up to 14000 glucose units. The next component is hemicellulose of which xylans such as 4-O-methylglucuronoxylans and arabino 4-O-methylglucuronoxylans; and β-mannans such as gluco and galacto-gluco-mannans are predominant. Lignin hinders the biodegradation of these polysaccharides. Cellulose is degraded enzymatically by three classes of enzymes endo-cellulases of different specificities, exo-cellulases and β glucosidases. These enzymes constitute a cellulosic complex and degrades cellulose to glucose. Enzymatic action on cellulose is initiated by endo-cellulase on less crystalline or amorphous region of cellulose and creates chain ends where exo-cellulase acts. Enzymes for degrading xylans are (1-4) β-xylanases, β-D-xylosidases, α-L-arabinosidases and α-D-glucuronidases. The enzyme complex responsible for degradation of mannans include (1-4) β-D-mannanases, β-D-mannosidases, β-D-glucoosidases and α-D-galactosidase. For lignin degradation basidiomycetes and especially white rot fungi are characterized best, the enzymes has not been isolated and purified yet for in vitro lignin oxidation.8) Before using enzymes for conversion, the lignocellulose has to be pretreated using physical (mechanical and irradiation), chemical (acid, alkali and organic solvents) and biological methods. Among these pretreatment methods chemical method is considered for effective, especially acidic pretreatment. In acidic pretreatment, acid acts as catalyst in hydrolysis of carbohydrate and thus it loosens lignin-hemicellulose barrier which protects cellulose. The acids used for pretreatment are mineral acids - sulfuric, hydrochloric, hydrofluoric, phosphoric, nitric and formic acids, in their concentrated or dilute forms and organic acids – maleic, acetic and oxalic acid. During pretreatment other than main constituents – lignin, hemicellulose and cellulose few by-products are also formed which interfere with the fermentation capacity of microorganisms. These by-products formed mainly includes aldehydes, ketones, weak acids and phenolic compounds that acts as inhibitors which has to be removed by a process called detoxification.12)

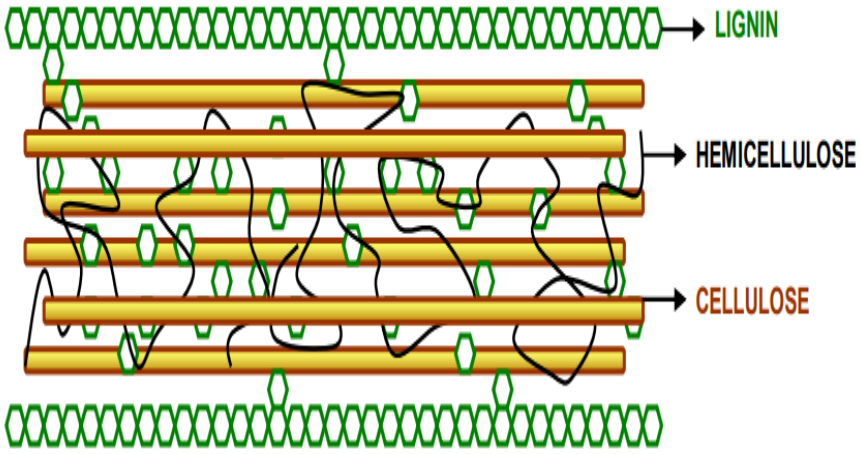

Figure 1- Lignocellulose structure showing cellulose, hemicellulose and lignin fraction.8)

The enzymes used for conversion of lignocellulose into glucose are obtained from microorganisms. The best known producer of cellulase is Trichoderma viride, but it is a poor secretor of cellobiases. This deficiency of cellobiases can be alleviated by adding exogenous source of cellobiases produced by mutant strains of Aspergillus niger and Aspergillus phoenics. Mutants of T.viride QM 6a namely T.reesei QM 9414 have been produced which has more efficacy of extracellular cellulase. Another T.viride mutant NG-14 produces highest amount of enzymes and the basidiomycetes strain produces highest amount of endo-cellulase. 4) 11)

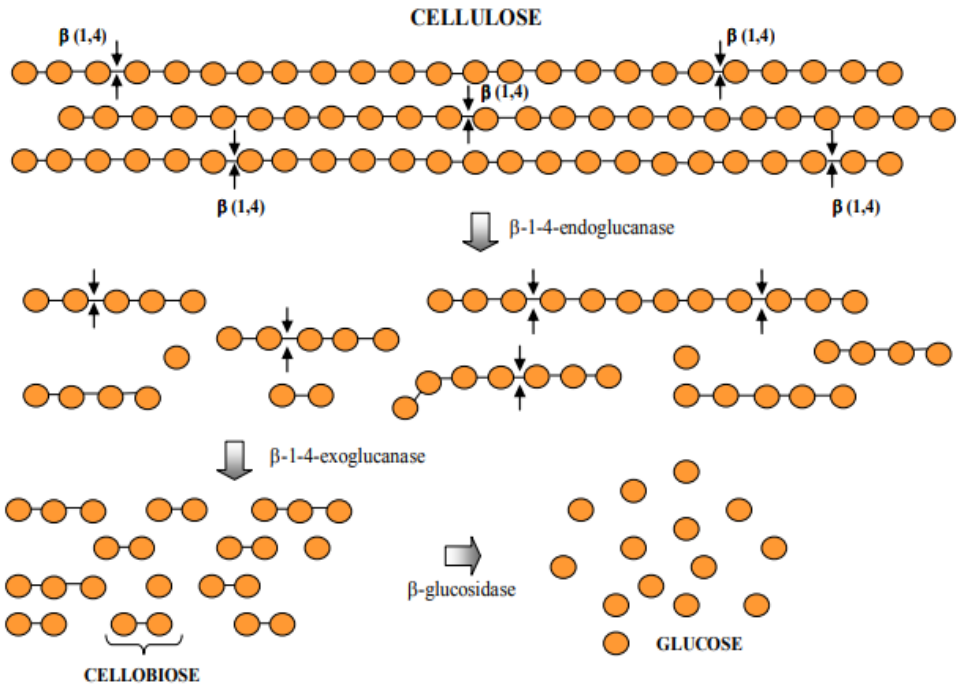

Figure 2- Representation of cellulase enzyme over cellulose structure.8)

MICROBIAL PRODUCTION OF ANTIBIOTICS AS SECONDARY METABOLITES

Microbial metabolism is the means by which a microbe obtains nutrients and energy it needs to live and reproduce. Primary metabolism is an interconnected series of enzyme mediated catabolic, amphibolic and anabolic pathways, which results in biosynthetic intermediates, energy production and conversion of biosynthetic precursors into essential macromolecules. Primary metabolism is essential for all living things. Certain microbes are capable of synthesizing special metabolites by general basic enzymes or by special synthetases produced by specialized cell or under specific nutritional conditions. These are called secondary metabolites or idiolites because they are produced in idiophase (stationary phase of growth curve). These special metabolites have unusual chemical structures and are not essential for the growth of producing organism, but they do have survival function in nature. Microorganisms produce antibiotics only when the specific growth rate decreases below a certain level. When the media provided has enough nutrients microbes do not require to produce antibiotics, but when nutrients are limiting microbes produce antibiotics for their survival. The biosynthesis of antibiotics starts when one or more nutritional growth limiting components are deficient. Depletion of such factors arrests the growth of microbes and initiates idiolite biosynthesis. A secondary metabolite is called secondary only because it is not needed for exponential growth of producing culture. The information for antibiotic biosynthesis is present in both chromosomal and extrachromosomal genes. There are hormones like signaling molecules responsible for initiation of antibiotic biosynthesis. These hormones like signaling molecules are a group of small diffusible signaling molecules that interacts with specific receptor proteins to initiate complex regulatory cascades of antibiotic biosynthesis. The intermediates of biosynthetic process also serves as auto regulators or cross regulators to regulate the production.2)

TYPES OF MICROORGANISMS USED AND THEIR ISOLATION

There are several antibiotics producing microorganisms which produces many structurally different antibiotics. These microbes can be isolated from water bodies or soil. Cephalosporin producing Acremonium chrysogenum, Geldanamycin producing Streptomyces hygroscopicus, Erythromycin producing Saccharopolyspora erythraea, Streptomycin producing Streptomyces griseus, Tetracyclin producing Streptomyces aureofaciens, Vancomycin producing Amycolatopsis orientalis, Rifamycin from Micromonospora. There are many Bacillus species found in soil which produces dozens of antibiotics. The main antibiotic producing species are: B. cereus (Zwittermycin, Cerexin); B.brevis (Tyrothricin, Gramicidin);

B.circulans (Circulin); B.licheniformis (Bacitracin); B.laterosporus (Laterosporin); B.polymyxa (Colistin, Polymyxin); B.subtilis (Bacitracin, Polymyxin, Subtilin, Difficidin, Mycobacillin); B.pumilus (Pumulin).2)9) These can be isolated from soil or water. Soil samples can be collected from different locations and made into suspensions with distilled water in test tubes. Serial dilution can be performed to minimize the number of colonies present. These serially diluted suspensions can be inoculated on nutrient agar and different bacteria can be identified using Bergey's manual. After identification different colonies can be grown in different petri plates containing nutrient agar media. These can be further sub cultured and kept for long. In a similar way bacteria can be isolated from water bodies by collecting different samples, diluting it and inoculating it on nutrient agar.9)15)

SELECTION AND SCREENING OF ANTIBIOTIC PRODUCING MICROBES

For selection of microbes various morphological and biochemical tests are performed. To obtain morphological characteristics, tests like Gram staining, endospore staining, capsular staining and motility tests are performed. Biochemical tests conducted involves IMViC, sugar fermentation, organic acid production, alkaline production, starch hydrolysis, and catalase test. The results obtained through these tests can help in identification and selection of bacteria of interest. Screening can be done by using test organisms. The test organisms used for primary screening are usually collected from a clinical laboratory. S.aureus, Pseudomonas aeruginosa, E.coli, Klebsiella pneumonia and Salmonella typhi are some of the test bacteria used for testing antimicrobial activity of antibiotic producing microbes using agar well diffusion method and zone of inhibition is measured. Secondary screening is done by disc diffusion method. For this Mueller Hinton agar is used and standard antibiotic discs are used as control. Minimum inhibitory concentration (MIC) and minimum bacterial concentration are determined (MBC). These MIC tests are performed when the culture enters stationary phase, observed through growth curve. The cultures are taken in centrifuge tubes and centrifuged at high speed. Only the supernatant from these tubes are taken to do MIC tests, as supernatant is considered to be containing active compounds.6)13)

RAW MATERIALS USED FOR PRODUCTION OF ANTIBIOTICS

The components used to make fermentation broth are considered primary raw materials. The broth is an aqueous solution of all the necessary compounds required for proliferation of microorganisms. Typically it consists of a carbon source which is often molasses, soy meal, acetic acid or hydrocarbons. It is majorly lactose or glucose sugar. The other ingredient is nitrogen source required for metabolic activities and it is ammonium salt mostly. Trace elements are also required for proper growth and these includes: phosphorus, Sulphur, magnesium and zinc. In addition anti foaming agents are added to prevent foam formation during fermentation. 3)10)

EXTRACTION AND PURIFICATION OF ANTIBIOTICS

Isolation can be initiated after 3-5 days of fermentation as by that time growth reaches stationary phase and production of secondary metabolites is achieved. The fermentation broth is processed by various purification method. For antibiotics which are water soluble, ion exchange method is used for purification.3) The compound is first separated from waste organic materials in the broth, and then sent through various equipment for further separation, which removes other water soluble components. For fats soluble antibiotics, solvent extraction method is used. For this the broth is treated with organic solvents, which can dissolve antibiotics. This dissolved antibiotic is then recovered using organic chemical means and at the end powdered form of antibiotics is obtained. This is further purified and converted into desirable forms.10)

WHY LIGNOCELLULOSIC WASTE CAN BE USED AS RAW MATERIAL

Lignocellulosic waste materials contains several high value substances such as sugars which is majorly utilized by bacteria if used for fermentation processes, minerals and proteins. Generally these lignocellulose wastes are thrown into landfills and it creates environmental problems. Since it is considered waste material, it will serve as a low cost material and only transportation charges will be there.5) Lignocellulosic wastes can be utilized in both submerged and solid state fermentation, thus can be easily utilized and its conversion to

cellulose, hemicellulose and then to glucose is also feasible using microorganisms. Apart from being cost effective it serves as environment friendly method of waste management.1)11)

CONCLUSION

Antibiotics are a major class of pharmaceuticals being produced. There are several antibiotics available for different class of microbes and there exists several methods for producing them, fermentation being the common way. Main component needed for growth of microbes is carbon source which is used as molasses traditionally. Lignocellulose can be used to produce glucose using Aspergillus niger, Aspergillus phoenics, T.viride QM 6a and T.reesei QM 9414 preferably in solid state fermentation. The glucose produced is used by antibiotic producing microbes for their growth and with limiting concentration of glucose secondary metabolites are produced by them. There are many microorganisms known for antibiotics production which are used. Lignocellulose can be a promising raw material for production of antibiotics due to its low cost and environment friendly nature of material. Lignocellulosic waste have been used as substrate for antibiotic production by Streptomyces sp. AS4. This would provide a great scope for better and cost effective production of many bioactive compounds.

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