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Production of secondary metabolites

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Abstract
Microorganisms are important for many reasons. Because, they produce things that are of value to us. These can be very large materials (e.g. proteins, nucleic acids, carbohydrate polymers, even cells) or smaller molecules and divided into metabolites that are essential for vegetative growth (primary) and those that are non-essential (secondary). Microbial technology has made significant advances in recent years with it's impact on the society. For thousands of years microorganisms have been used to supply products such as bread, beer and wine. A second phase of traditional microbial biotechnology began during World War I in the development of the acetone-butanol and glycerol followed by citric acid, vitamins and antibiotics.

Keywords: Secondary, metabolites, microbial technology

Introduction

Microbiology is a major participant in global industry

Microbial technology explores and exploits the microbial wealth for human requirements like production of microbial metabolites such as enzymes, organic acids, antibiotics, drugs and pharmaceuticals, through processes like recombinant protein expression, fermentation and downstream processing, bioleaching, soil and waste management etc. It is especially important in pharmaceutical, food and chemical industries (Barrlos *et al.*, 1998) [1].

Metabolites are the intermediate products of metabolism in biological systems. The term metabolite is usually restricted to small molecules. Metabolites have various functions: (1) biofuel production, (2) structure, signaling, catalytic activity and inhibitory effects on enzymes (usually as a cofactor to an enzyme), (3) defense and (4) interactions with other organisms (e.g. pigments, odorants etc).

There are two types of metabolites. They are primary metabolite and secondary metabolites. Primary metabolites are directly involved in normal growth, development and reproduction. A secondary metabolite is not directly involved in those processes, but usually has an important ecological function and it gives advantage for survival. Examples: antibiotics, pigments, resins and terrenes etc. the above mentioned metabolite types are dealt in detail from next paragraph.

Primary metabolites

Primary metabolism occurs in Trophophase, it is characterized by balanced growth of microorganisms and the metabolites produced during this stage are primary metabolites. There are two types of primary metabolites: (1) Primary essential metabolites, 2. Primary metabolic end products.

Primary essential metabolites

These are the compounds produced in adequate quantities to sustain cell growth. These compounds can be manipulated for industrial production Ex. vitamins, amino acids, nucleosides *etc.*

Primary metabolic end products

These are the normal and traditional end products of fermentation process of primary metabolism. They may or may not have any significant function to the organism, but they have many industrial applications. Ex. Ethanol, acetone, lactic acid *etc.*

Over production of primary metabolites

Usually the system is provided with regulation for the metabolite production. But for industrial purposes it can be biochemically manipulated and we can expect over production of primary

metabolites by eliminating the feedback inhibition. This can be accomplished by using auxotrophic mutants with a block in one of the steps in the biosynthetic pathway concerned with the formation of primary metabolites (this should be intermediate and not the final end product). Some of the examples of metabolic intermediates used in human day to day life are citric Acid (soft drinks, food etc.), lysine (essential amino acid, calcium absorption, building blocks for protein), glutamic acid (monosodium glutamate precursor), phenylalanine (aspartame precursor). Many microorganisms are used like yeasts, fungi and bacteria Ex. *Corynebacterium glutamicum* is used for the production of lysine. (Gonalez, 2003) [2].

Secondary metabolites

These are organic compounds that are not directly involved in the normal growth, development or reproduction of an organism. Secondary Metabolites are not part of the "central" metabolic pathways. But, they have wide applications in human life like allopathic medicines, flavoring substances/compounds and recreational drugs Ex. *Streptomyces* spp. (Actinomycetes) and *Penicillium* sp. (fungus) produce clinically important antibiotics, they produce antibiotics when the growth slows/stops in batch cultures. Biosynthetic pathway for most secondary metabolites are not clearly established. he regulation of the formation of secondary metabolites is more complex and differs from the primary metabolites Ex. About 300 genes are involved in the biosynthesis of chlorotetracycline. With such complex systems, the metabolic regulation is equally complex to achieve over production of secondary metabolites. (Robinson., 2001) [3].

The production environment for secondary metabolites has differernt stages of growth in the batch medium. They are:

1. **Trophophase:** The microorganisms have sufficient amounts of nutrients.
2. **Exponential phase:** Growth occurs at increasing rate. But, there is no product formation.
3. **Idiophase:** As the exponential growth ends, it enter idiophase the cells will have used up the supplied nutrients. So there will be nutrient limitation. Therefore growth slows down eventually ceases and product formation occurs. Idiophase is characterized by secondary metabolism where in the formation of certain metabolites referred to as secondary metabolites (idiolites) are formed. The product is available for harvest during this period.
4. **Senescence:** Product formation ceases. Degeneration/lysis of cells happen.

Over production strategies

Induction

The addition of certain compounds will induce the production of secondary metabolites. Ex. addition of methionine induces certain enzymes and enhances the production of cephalosporin antibiotic and presence of tryptophan regulates ergot alkaloid biosynthesis.

End product regulation

Some of the secondary metabolites inhibit their own biosynthesis. But, this can be resolved by using mutant isolates that are less sensitive to end product inhibition Ex. Penicillin production.

Catabolic regulation

A key enzyme involved in a catabolic pathway is inactivated by adding a commonly used substrate. Carbon source like glucose can inhibit the production of several antibiotics. Nitrogen source like ammonia and inorganic phosphate (up to 1mM) acts as regulators.

Autoregulation

In some microorganisms self-regulation of secondary metabolites occurs Ex. A compound designated as factor A involved in auto regulation for the production of streptomycin by *Streptomyces griseus*.

Production and Scale

A fermenter is the place where microbiological processes take place. Fermenters vary in size from 5 to 500,000 liters. The reaction can be aerobic or anaerobic. But, most are aerobic in nature. Large-scale fermenters are made up of stainless steel. Impellors and spargers supply oxygen to the growth of microorganisms.

Antibiotics

Antibiotics are chemical substances that can kill microorganisms or inhibit their growth. Therefore, they are being used to fight infectious diseases. In 1928, Alexander Fleming made an accidental discovery that the fungal *Penicillium notatum* produced a compound called Penicillin that killed wide range of bacteria without affecting host. There are around 10,000 different antibiotics known and these are secondary metabolites. Antibiotics are grouped under two categories. (Slavica, 2001) [4].

1. **Broad spectrum antibiotics:** They can control the growth of several unrelated organisms Ex. tetracyclin.
2. **Narrow spectrum antibiotics:** They are effective against selected species of bacteria Ex. penicillin, streptomycin.

Application of antibiotics

Antibiotics are particularly important as antimicrobial agents for chemotherapy e.g. Pneumonia, cholera, tuberculosis, leprosy etc. Some of the applications are as under:

1. The antifungal antibiotics: Grisofulvin has controlled the debilitating fungal skin diseases such as ring worm.
2. Antitumor (cancer) antibiotics: Ex. Actinomycin-D & Mitomycin C
3. Food preservative antibiotics: Ex. chlorotetracyclin & nicin (bacteriocin),
4. Used in animal feed &veterinary medicine Ex. Enduracidin, Tylosin
5. Antibiotics as tools in molecular biology: Certain antibiotics have been used to obtain some important information on DNA replication, transcription and translation.

Production of Antibiotics

Antibiotics may be produced by microbial fermentation or chemical synthesis or a combination of both.

Antibiotics: Isolation, Yield, and Purification

A simple plate assay for antagonism by antibiotic is Cross-streak method

- Used to test new microbial isolates for antibiotic production
- Most antibiotics fail toxicity and therapeutic tests in animals

- Time and cost of developing a new antibiotic is approximately 15 years and $1 billion
- Involves clinical trials and U.S. FDA approval
- Antibiotic purification and extraction often involves elaborate methods

Penicillin
- Penicillin (sometimes abbreviated PCN or Pen) is a group of antibiotics derived from *Penicillium* sp fungi.
- Penicillin antibiotics are historically significant because they are the first drugs that were effective against many previously serious diseases, such as Syphilis
- Penicillin are still widely used today, though many types of bacteria are now resistant.
- All penicillin are β-lactam antibiotics and are used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms.

Action of penicillin
Natural penicillin (penicillin V & G) are effective against several gram positive bacteria. They inhibit bacterial cell wall (peptidoglycon) synthesis and cause cell death.
Some person (approx. 0.5-02% of population) are allergic to penicillin.

Organisms for penicillin production
a. *Penicilliun notatum*
b. *Penicilliun crysogenum*

Commercial production of penicillin is done by *Penicillin crysogenum* strain – wis Q 176

Production process
Penicillin production is an aerobic process. Therefore, continuous oxygen supply is required. The required aeration rate is 0.5-1.0 vvm. pH should be maintained around 6.5 Temperature range is between 25-27°c. It is done with submerged process.
Precursor for penicillin-G production is phenyl acetic acid, because it is a secondary metabolite

Streptomycin
- Streptomycin is an antibiotic drug, it was the first antibiotic remedy for tuberculosis.
- It is derived from the actinobacterium *Streptomyces griseus*. Streptomycin is a bactericidal antibiotic.

Production
Similar to penicillin fermentation. The fermentation condition for optional production are temperature 27-30 °C, pH 6.5-7.5 aeration rate 0.5-1.0 vvm. The duration of fermentation process depends on strain used in between 6-8 days.

Tetracycline
- Tetracycline are broad spectrum antibiotics. They are effective against gram positive & gram negative bacteria
- Tetracycline is a prescription medication approved for treating common bacterial infections, such as pneumonia and urinary tract infections and treatment of '*Helicobacter pylori'* infection.
- They inhibit protein biosynthesis by blocking the binding of aminoacyl t-RNA to ribosome.
- Tetracycline production:
- The tetracycline can be produced by one or more of the following ways:

1. By chemical treatment of chlorotetracyclin.
2. By carrying out fermentation in a chloride free culture medium.
3. By employing mutants in which chlorination reaction by the addition of inhibitors.

E.g. Thiourea, 2-thiouracil.

Aromatic antibiotics
The antibiotics with aromatic rings in their structure are regarded as aromatic antibiotics.
Eg. Chloramphenicol, griesofulvin and novobiocin.

Griseofulvin
It is an anti fungal antibiotic used in the treatment of various fungal skin infections. Further griesofulvin is employed in the treatment of plant diseases caused by *Biotrytis* and '*Alternaria solani'*. Mechanism of action is not known.

Downstream Processing
- Products in a fermentor are impure and dilute, so need to be purified by downstream processing.
- This usually involves filtration to separate the microbial cells from the liquid medium, followed by chemical purification and concentration of the product
- Downstream processing can account for 50% of the cost of a process.
- Downstream processing is relatively easy since penicillin is secreted into the medium (to kill other cells), so there is no need to break open the fungal cells.
- However, the product needs to be very pure. since it being used as a therapeutic medical drug, so it is dissolved and then precipitated as a potassium salt to separate it from other substances in the medium.

Conclusion
Secondary metabolites are compounds produced are not very essential for microbial growth. But they confer ecological strength to the test organisms. Many developments in the field of biochemistry and biotechnology have helped man to explore numerous metabolic products. But the additional research and insight into physiological aspects of test organisms can reveal more products and further give way for scale up and use for human welfare.

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