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Microbiology and Disease Management

Unit-1

3. Culture media for isolating plant pathogen-

Industrial Application of Microorganisms

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3. INDUSTRIAL APPLICATIONS OF MICROORGANISMS

Activities of microorganisms are very important to almost every sector of concern to mankind. Microorganisms are useful in agriculture, forestry, food, industry, medicines and environment. The scope of microbiology has enlarged manifold. Application of microorganisms in the industries are discussed below.

1) Organic acids: several organic acid including acetic, glucanic, citric, itaconic, gibberellic and lactic acids are produced commercially through microbial transformation.

Gluconic acid is produced by various bacteria including *Acetobacter* spp and several fungi such as *Aspergillus* and *Penicillium* spp. *A. niger* converts glucose to gluconic acid in a single enzymatic reaction (glucose oxidase). Fermentation is carried out at 30^0C with aeration and agitation. Gluconic acid is recovered by adding calcium hydroxide to form crystalline calcium gluconate. Free gluconic acid can be recovered by adding the acid.

Citric acid is also produced by *A. niger*. Citric acid is used as food additive, especially in soft drinks, as a metal chelating and sequestering agent and as a plasticizer. It is essential to limit the growth of the fungus so that high levels of citric acid can accumulate. The medium contains molasses, ammonium nitrate, $MgSO_4$ and potassium sulphate. Acid is added to lower pH. Metals added to the medium are removed from solution by cation exchange resins.

Itaconic acid is used as a resin in detergents. The transformation of citric acid by *Aspergillus terreus* can be used for commercial production of itaconic acid. Fermentation process involves a well aerated mineral salts medium at a pH below 2.2. At higher pH this microbe degrades in itaconic acid. Low levels of trace metals must be used to achieve acceptable product yield.

Gibberrellic acid and related gibberellins are important growth regulators of plants. Commercial production helps in boosting agriculture. The source of this acid is fungus *Gibberella fujikuroi*. This can be produced commercially using aerated submerged cultures. A glucose mineral salt medium incubation at 25^0C and slightly acidic pH are used for fermentation.

Lactic acid is used as preservative in foods, in leather production and textile industry. Various other forms of lactic acid are also used for other purpose. Fermentation is carried out by *Lactobacillus*, *Streptococcus* and *Leuconostoc* spp. The typical medium contains 10-15% glucose other sugar, 10% $CaCO_3$ to neutrlise the lactic acid formed and ammonium phosphate and nitrogen sources in trace. Carbohydrate sources used for fermentation; temperature of $45\text{-}50^0$C and pH 5.5-6.5. Agitation is needed without aeration. Process is complete within 5-7 days with approximately 90% of the sugar conversion to lactic acid.

2. Alcohol:

Alcoholic fermentation have been well known since ancient time, through little was known of the nature of the process. Generally *Saccharomyces* are used to produce various types of alcoholic beverages. The process relies on alcoholic fermentation conversion of sugar to alcohol by microbial enzymes.

i) Beer: The fermentation of beer is usually a batch process. In some countries the production of beer is carried out in a continuous flow through process. Beer is a product of the fermentation of barley grains by yeast. Barley seeds are allowed to germinate. During germination, amylase covert starch to sugar most of which is maltase. The process is called malting and the digested grain as malt. The next step is mash the grain with water and removes the fluid portion called the wort. Dried petals of vine, *Humulus lupulus* called hops are then added to the wort to give it flavor, colour and stability. Hops also prevent contamination of the wort, due to presence of two antimicrobial substances in petals. At this stage the fluid is filtered out and yeast is added in large quantities.

The yeast commonly used in fermentation of wort is one of the many strains of *Saccharomyces cerevisiae* developed by brewers. Some yeasts give a uniform cloudiness to beer and are carried to the top of the fermentation vat by foaming CO_2. Other yeast ferments the beer more slowly and produces a light beer with less alcohol. These yeasts are known as bottom yeasts and their product as lager beer.

Generally one week time is needed for normal fermentation. After a week the young beer is transformed to vats for primary and secondary aging. It may takes about six months more. For canning and bottling the beer is to be pasteurized at 140^0F for 13 min. to kill the yeast or may be filtered to remove the yeasts. Some yeast is used to seed new wort, the rest for animal feed or pressed to tablets for human consumption. Alcoholic content of beer is roughly 4 pre cent.

ii) Wine:

Wine fermentations are carried by controlled cultures of *Saccharomyces ellipsoidseus*, a variety of *S. cerevisiae* or with the strains of yeast naturally occurring on grapes. The former is common in U.S.A. and the latter in Europe.

Wine is made from ripe fruits, fruit juice or plant extract such as dandelions. Fermentation usually begins with crushing of the fruit to produce must. Sulpher dioxide may be added to control the process. In natural fermentation SO_2 is not used and the yeast begin to digest suger. Oxygen may be supplied to promote aerobic growth of the yeast. However, anaerobic conditions are established later. Alcohol production occurs within few days, though aging may take month or years. During this period secondary fermentation develops flavor, aroma and bouquet of the wine. Red wine becomes red as alcohol extract the colour of grape skin. For red wine fermentation is carried out at 24-27^{0}C for 3-5 days and white wine takes 1-2 weeks at 10-21^{0}C.

Additional CO_2production yields champagne and other sparkling wines which are naturally carbonated. Sherry wines result from inoculation with special yeast to have unique flaours. In dry wines most or all of sugar is metabolized, where as in sweet wines, fermentation is stopped before entire sugar is consumed. The strongest natural wines have about 16 per cent alcohol. Most table wines average about 10-12 per cent alcohol, with fortified wines reaching 22 per cent alcohol. In fortified wines brandy or other spirits are added to produce port, sherry and cocktail wines. For mass production, the wine is pasteurized, filtered and bottled.

iii) Distilled spirits:

They contain more alcohol than beer or wine. Alcoholic content is shown by a proof number which is the twice the actual percentage of alcohol. The process of distilled spirit begins with same type as for wine and beer, except that after the fermentation process the alcohol is collected by distillation to allow higher concentration of alcohol. The raw product is first fermented by *Saccharomyces*, then aged and finally matured in casks. At this time the process differs. The alcohol is concentrated by a distillation apparatus using heat and vacuum. During maturing, flaours from the chemicals as aldehydes, ethers and volatile acids are added. The alcohol content is then standardised by diluting it with water before bottling. There are four basic types of spirits: brandy is made from fruit juice; rum from molasses; whisky frommalted cereal grains; scotch from barley and bourbon from corn. The neutral spirit as vodka is made from potato starch and left unflaoured and jin flavoured with juniper oil.

3. Milk product:

Many products are made through microbial fermentation of milk, including buttermilk, yogert and many cheeses. Fermentation is primarily carried out by lactic acid bacteria. The differences in the flavour and aroma of the various dairy products are due to additional fermentation products that may be present in very low concentration.

i) Buttermilk, sour cream, kefir and koumis: Different products are produced by using different strains of lactic acid bacteria as shorter cultures and different fractions of whole milk as the starting substrate.

Sour cream uses *Streptococcus cremoris* and *S. lactis* for producing lactic acid and *Leuconostoc cremoris* for characteristic flavour. Butter is normally made by churning cream that has been soured by lactic acid bacteria. *Streptococcus cremoris* or *S. lactis* is used to produce lactic acid rapidly and *Leuconostoc citrovorum* produces necessary flavours. Kefir and koumis popular in Europe are fermentation products of *S. lactis, S. cremoris* other *Lactobacillus* spp and yeast.

ii)Yogert: It is made by fermenting milk with a mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* at 40^0C. Flavour is due to accumulation of lactic acid and acetaldehyde.

iii) Cheese: Cheese consist of milk curd that have been separated from the liquid portion of the milk (whey). The curdling of milk is done by enzyme rennin and lactic acid bacterial starter cultures. Cheeses are classified as soft (high water 50-80% content), semihard (about 45% water) and hard (less than 40% water). They are also classified as ripened and unripened. Cottage and cream are soft unripened cheese; Brie, camembert and limburger are soft, 1-5 months ripened cheeses; blue, brick, gorgonzola, Monterey, muenster and Roquefort are semisoft, 1-12 months ripened cheeses, whereas cheddar and Colby are hard, 3-12 months ripened cheeses.

Natural production of cheese involves lactic acid fermentation with various mixtures of *Streptococcus* and *Lactobacillus* spp used as a starter cultures. The flavour results from use of different microbial starter cultures, varying incubation times and conditions and the inclusion or omission of secondary microbial species late in the process. Ripening involves additional enzymatic transformation after the formation of cheese curd. Various fungi are also used in the ripening of different cheeses. The unripened cheese is inoculated with fungal spores. Blue cheese is produced by *Penicillium* spp. Roquefort cheese is produced by using *P. roquefortii* and camembert and brie by using *P. camembertii* and *P. candidum*.

4. Antobiotics:

There are thousands of antibiotics produced by microbes in nature. Alexander Fleming (1928) observed some substance from molds inhibiting growth of bacteria and concluded that active principle from *Penicillium* possesses antibacterial properties. He called this substance as penicillin. Rene Dubos in 1939 indicated that soil bacteria could produce antibacterial chemicals. Howard Florey and the German biochemist Ernst Boris Chan reisolated penicillin and carried out careful trials with highly purified samples. In 1940 their successful attempts were published in 1945. Fleming, Florey and Chan received Nobel prize for discovery and development of penicillin. Then a term antibiotic was introduced in medicine.

i) Penicillin: Among large group of penicillin darivatives, Penicillin G or benzyl penicillin is the most popular. Other types are penicillin F or penicillin V. all have the same basic structure with a beta-lactin nucleus and several attached groups.

Penicillins are active against a variety of Gram positive bacteria including *Streptococci* and *Staphylococci*. Penicillin functions during the synthesis of bacterial cell wall. There are two major drawbacks. One the anaphylactic reaction in allergenic cases causes swelling about the eyes or wrist, itchy skin etc. Second the evolution of some penicillin resistant bacteria that produce an enzyme, penicillase. This converts penicillin in to harmless penicilloic acid. Modern penicillins are produced from *Penicillium notatum* and *P. chrysogenum*.

ii) Semisynthetic Penicillin: In 1950 the betelactum nucleus of the penicillin molecule was identified and synthesised. Then various groups could be attached to this nucleus, creating a number of new penicillins now thousands of penicillins are prepared by semi synthetic process. Ampicillin is less effective against Gram positive cocci, but valuable against Gram negative rods. It can be taken orally and absorbed from the intestine. Amoxicillin and penicillin are useful to treat urinary tract infections. Carbeicillin is used for *Pseudomonas* and *Proteus* infections of urinary tract. Others are methicillin, nafcillin and oxacillin which are resistant to penicillase.

iii) Cephalosporins: They were developed in 1960. Cephalosporin C was isolated from the blue mold, *Cephalosporium*. A number related semi synthetic drugs developed from it are cephalexin, cephalothin, cephazolin and cephaloridine. These are alternatives to penicillin and are effective for Stephylococcal boils or wounds, Streptococci and bacterial pneumonia and urinary tract infection by Gram negative bacteria.

iv) Streptomycin (an aminoglycoside): This was discovered by Selman A. Waksman. It was produced from *Streptomyces griseus*. This drug was made available in 1947 and Waksman received Nobel prize in 1952 in medicine. Streptomycin in combination with isoniazid is important for treatment of tuberculosis; Gram negative infections as plague, brucellosis are also treated.

v) Other aminoglycoside antibiotics: Gentamycin is the first drug to be given for Gram negative bacteria. It is combined with carbencillin for *Pseudomonas* infections, with ampicillin for *Staphylococcus* infections of intestine and with cephalosporin for staphylococcal disorders. Neomycin is now used as eye bacterial conjunctivitis or other Gram negative infections. Neomycin (Neosporin) combined with polymyxin are useful for variety of skin infection by bacteria. All aminoglycoside are derived from the species of *Streptomyces*.

vi) Chloremphenicol: this is the first broad spectrum antibiotic discovered. It was isolated in 1947 by Ehrlich, Burkholder and Gotlieb. It inhibits a wide variety of Gram positive and Gram negative bacteria as well as several rekettsiae and fungi. It was originally isolated from the metabolites of *Streptomyces venezualae*. Its advantage is that it prevents haemoglobin incorporation into the R.B.C.-aplastic anemia. Die to its accumulation in blood of new borne child; it causes a toxic reaction and sudden breakdown of cardio vascular system- grey syndrome.

vii) Tetracyclines: They are also broad spectrum antibiotics. They include naturally occurring Chlorotetracyclin and oxytetracyclin isolated from species of *Streptomyces*. They may be taken orally, though they have side effect problem. They are used in Gram negative infections as brucellosis, plague, cholera, for primary atypical pneumonia, as substitutes for penicillin in syphilis, anthrax, gonorrhea and pneumonia and therapy of some protozoan infections as amoebiasis.

viii) Other antibiotics: Erythromycin obtained from *Streptomyces* is useful for primary atypical pneumonia, staphylococcal and streptococcal infections and syphilis. Vancomycin also a product of *Streptomyces* is given intravenously against Gram positive infections. Other antibiotics are rifampin for leprosy and tuberculosis; clindamycin and lincomycin are active against Streptococci, Staphylococci and other Gram positive organisms.

Bacitracin and polymyxin are obtained from *Bacillus* spp. Bacitracin is used as ointment for staphylococci and the polymyxin for Gram negative bacilli. Streptomycin a product of *Streptomyces* became popular in 1970 as substitute for penicillin in case of gonorrhea.

ix**) Antifungal antibiotics:** Nystatin a product of the *Streptomyces* is used as cream or ointment for infection of oral cavity, vagina or intestine due to *Candida albicans*. Grisofulvin is used for fungal infections of skin, hair and nails. It is effective against ringworm and aczema. This is a product of *Penicillium*. For serious systemic fungal infections, amphotericin B is used. It is effective for organisms of histoplasmosis, blastomycosis, cryptococcosis etc.

5. Biopesticides:

Several microbes are being developed as suitable biopesticides for management of insect and nematodal pests. Some fungi have good potential of their use as bionematicides to control nematodal pests of vegetables, fruits and cereal crops.

In order to minimise the use of chemicals in agriculture, biological control methods are being developed. Many of the microorganisms are used as pesticides to protect plants from the pests. Microbial populations can be used directly for controlling pests. Preparations of antagonistic microbial populations are called microbial pesticides. A microbial pesticides should be harmless to man and other valued plants and animal populations.

Microbial Insecticides:

The greatest commercial impacts of biocontrol agents have been made in the insecticide market. The most successful biocontrol agent so far been the insecticidal bacterium, *Bacillus thuringiensis*, whosae sales in forestry, agriculture and public health were much higher. Viruses, bacteria and fungi have been used as microbial insecticides.

a) Viral Insecticides:

Pathogenic viruses possess the potential for use as pesticidal agents. They attack insects and other arthropods the most commonly used viruses are, i) nuclear polyhedrosis viruses (NPV), ii) cytoplasmic polyhedrosis viruses (CPV), iii) granulosis viruses (GV). Pathogenic baculoviruses have been found principally for Lepidoptera, Hymenoptera and Diptera.viruses have been used in attempts to control outbreak of several insect pests including gypsy moth, Douglas fir tussock moths, pine caterpillers, red banded leaf rollers (pest of apple), spuce budworms, codling moths, alfalfa caterpillers, cabbage white butterflies, cabbage loopers, cotton bollworms, corn earworms, tobacco budworms, tomato worms etc. An interesting example of the use of viral pesticides is the attempt to control rabbit populations in Australia with myxoma virus.

b) Bacterial Insecticides:

There are several bacterial pathogens of insects that are being used at present as insecticides. They includes endospore forming *Bacillus* and *Clostridium* spas well as non endospore forming species of *Pseudomonas*, *Enterobacter*, *Proteus*, *Serratia* and *Xenorhabdus*. Of these *Bacillus thuringiensis* has been most extensively used. Commercial preperations of *B. thuringiensis* are registered by more than 12 manufacturers for use in several agricultural crops, forest trees and ornamentals for control of various insect pests. This bacterium has been testedsuccessfully against more than 150 insect species. Four separate toxins are produced by *B. thuringiensis*. Several vegetable insect pests have been managed by this bacterium. About 16 formulations based on exo- and endotoxins are used in U.S.A., France, Germany, USSR and Czechoslovakia. Some registered products are Thuricide, Sporcine, Condor, Cutlass, Foil and Invade, mostly in USA. In India also trials have been made for thuricide against insect pests of lac, cruciferous crops and white grubs of sugarcane.*Bacillus thuringiensis* has potential to control mosquito vectors of malaria. In India trials have been made for *B. thuringiensis*¸ *B. papillae* and *Serratia mariscens* to control insect pests of sugarcane.

c) Fungal Insecticides:

Fungal insecticides could became most common and effective means of control of pests in some countries, chiefly in USSR. Products of entomogenous fungi have been used for insects of field crops, forest trees as well as horticultural and vegetable crops. Several preparations have been produced, formulated and used commercially in USSR, Brazil, Cuba and Israel. Different kinds of formulations have been developed and applied in different ways. The most studies on entomogenous fungi have been concerned with species of the genera *Aschersonia*,*Beauveria*, *Metarrhizium*, *Verticillium*, *Hirsutella*, *Coelomomyces* and *Entornophthora*.

Microbail Nematicides:

Most studies have been made with fungal nematicides. The fungi of genera *Arthreobotrys*, *Dactylaria*, *Dactylella* and *Monachrosporium* have been studied in trials to control nematode genera *Meldogyne*, *Heterodera* and *Rotylenchulus* attacking mostly vegetables crops. These nematodes cause cyst and root knot diseases. There have been some limitations in the use of nematophagous fungi for the control of nematodes. It is difficult to manage fungi so that periods of nematode migration and trap formation coinside. Another group of fungi are found ideal nematicides. These are opportunistic fungi such as *Verticillium*, *Chlamydosporium*, *Dactylella oviparasitica* and *Paecilomyces lilacinus* that also attacks eggs and young females of cyst and root knot nematodes.

Microbial Herbicides:

Fungi could be suitable for herbaceous weeds. In classical strategy, weed parasitic fungi (mostly rust fungi) are introduced. Several products of fungi have been used on commercial scale in different parts of the world.

Thank You