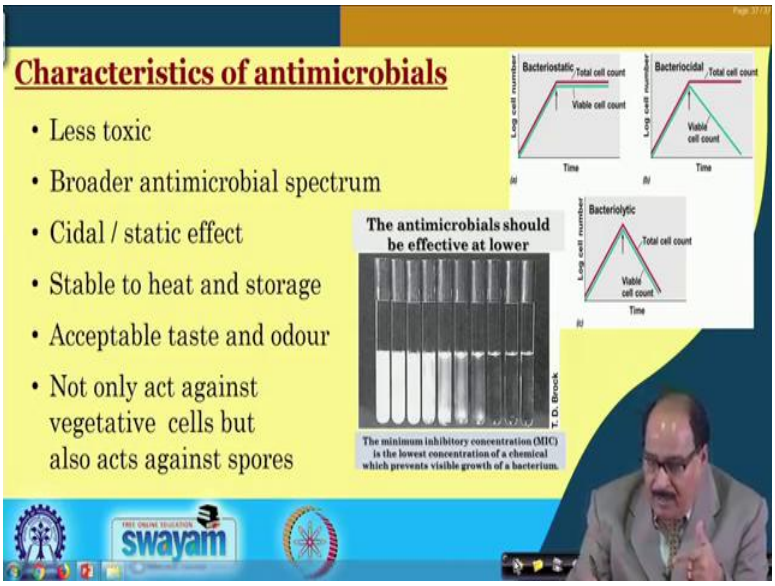
Novel Technologies for Food Processing and Shelf Life Extension
Prof. Hari Niwas Mishra
Department of Agricultural and Food Engineering
Indian Institute of Technology, Kharagpur

Lecture – 28
Antimicrobials & Bacteriocins

This lecture is about antimicrobials and bacteriocins.

Food antimicrobials are chemicals, which are added to or are present in food that retard microbial growth or kill microorganisms. Functions of food antimicrobials are to inhibit or inactivate spoilage microorganisms or pathogens. A number of compounds have been approved by international regulatory agencies.

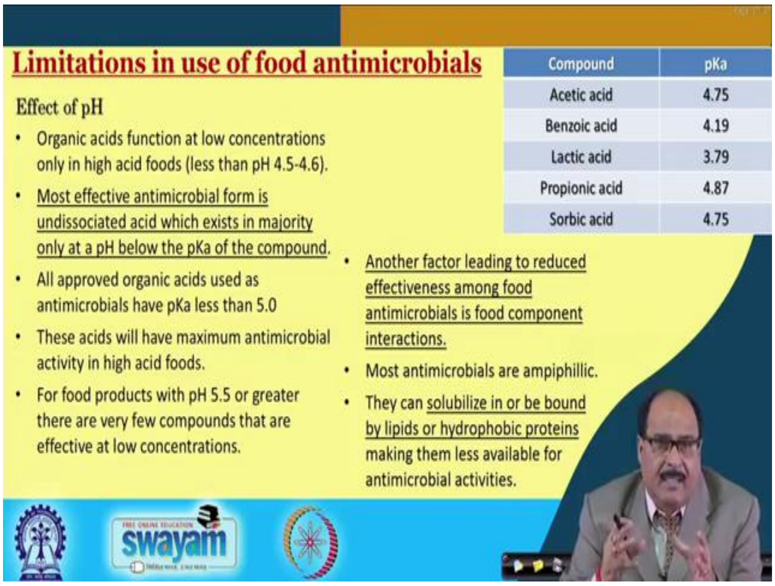


The chemical to be used in a food as an antimicrobial should have certain characteristics. It should be less toxic or non-toxic. It should have its antimicrobial activities over broad spectrum ranges. Particularly the bactericidal as well as bacteriostatic effect of the chemical should be well established. It should be stable to heat and storage, it should have acceptable taste and odour, it should not only act against vegetative cells, but also against spores of trouble creating microorganisms in food.

There are 3 effects of the chemical (See Fig.): bacteriostatic, bactericidal and bacteriolytic. Bacteriostatic means the bacteria comes under the stationary phase. Whereas the bactericidal means the bacteria is killed, the viable count decreases but, the bacteriolytic means total cell count and viable counts both decrease, they are completely killed.

And this of course, very important that these antimicrobials should be effective in lower concentrations, and the concentrations at which they are required to be added into the food to get the desired effect should not result into any undesirable taste, flavor and other characteristics of the food material.

So, these are some of the important characteristics, which should be there in the chemicals, in order to qualify it, for being used as a food antimicrobial.



There are; however, certain limitations in the use of food antimicrobials.

i) Effect of pH

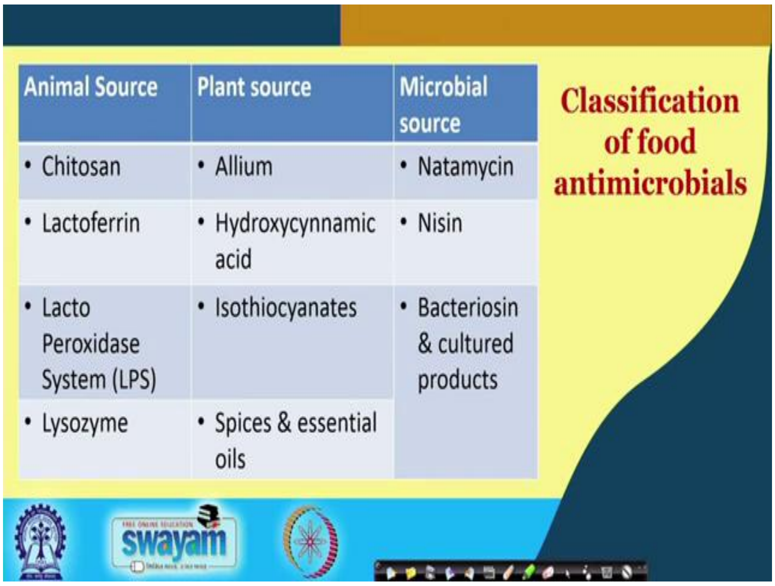
- Organic acids function at low concentrations only in high acid foods (less than pH 4.5-4.6).
- Most effective antimicrobial form is undissociated acid which exists in majority only at a pH below the pKa of the compound.
- All approved organic acids used as antimicrobials have pKa less than 5.0.

  All organic acids which are approved as antimicrobial, they have pKa (dissociation constant) less than 5 pKa, i.e. acetic acid has pKa of 4.75, propionic acid 4.87, sorbic acid has pKa of 4.75 (See Table).
- These acids will have maximum antimicrobial activity in high acid foods.
- For food products with pH 5.5 or greater there are very few compounds that are effective at low concentrations.

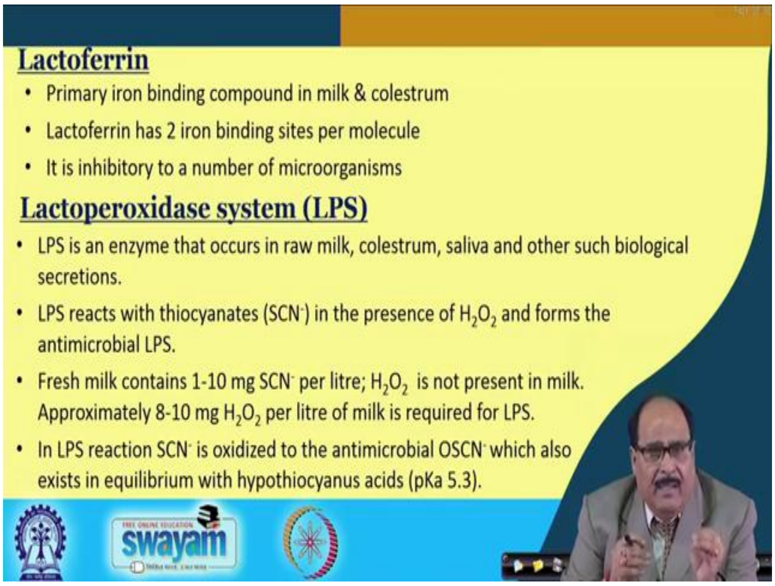
  Because, in these foods even very high concentration of these acids might be required which ultimately may influence the sensory characteristics or taste of the product.

ii) Another limitation to the use of these food antimicrobials is the interactions among the food constituents, i.e. the effectiveness of these antimicrobials get reduced because of the food component interaction.

Most of the antimicrobials are ampiphillic. So, they can solubilize in or be bound by lipids or hydrophobic proteins and because of this, they are binding with the hydrophobic proteins or lipids they become less available for their antimicrobial activities.



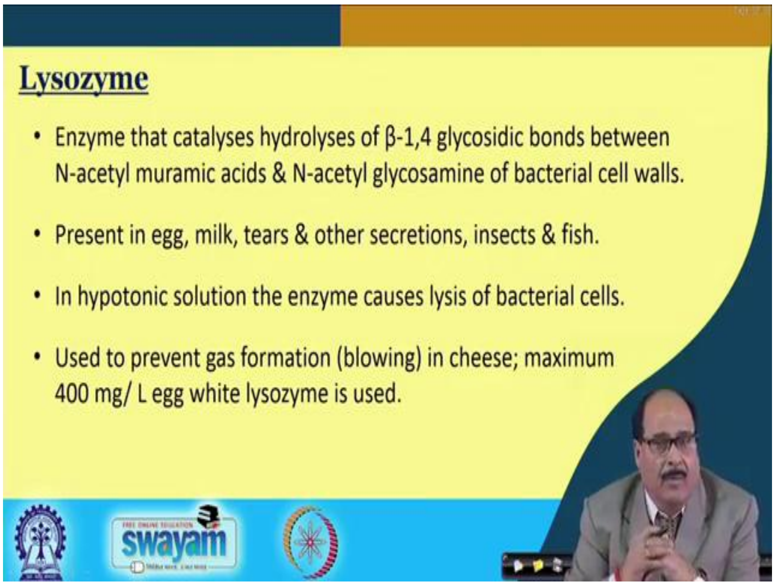
So, these food antimicrobials many a times are classified depending on the basis of the source from which they are obtained like, the antimicrobials obtained from the animal sources, includes chitosan, lactoferrin, lactoperoxidase system, and lysozyme. Those obtained from the plant sources include allium, hydroxycynnamic acid, isothiocyanates, or spices and essential oils, the antimicrobials of microorganism sources are the natural antimicrobials produced by microorganisms, include natamycin, nisin, bacteriocin and cultured products.



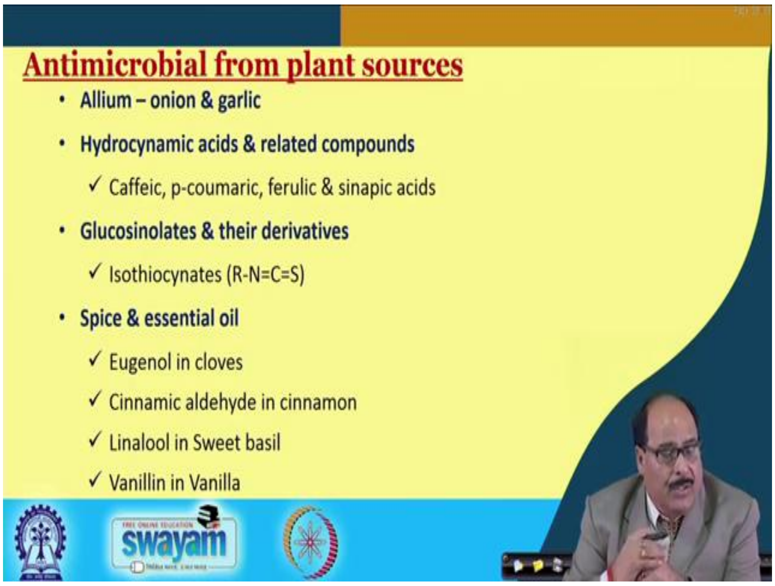
Lactoferrin: It is a primary iron binding compound in milk and colestrum, lactoferrin has 2 iron binding sites per molecule. And because of this iron binding activity, it becomes inhibitory to a number of microorganisms. And therefore, it can be used to prevent the growth or even to kill or inactivate the microorganisms in several food products.

Similarly the lactoperoxidase (LPS) system, it is an enzyme that occurs in raw milk, colestrum, saliva and other such biological secretions. This LPS reacts with thiocyanates in the presence of hydrogen peroxides. And, this forms the antimicrobial lactoperoxidase system. The fresh milk contains about 1-10 mg of the thiocyanate/L. Hydrogen peroxide, in fact, is not present in the milk. So, approximately 8-10 mg hydrogen peroxide/L of the milk might be required to be added for the generation of the LPS system.

So, in LPS reaction, SCN is oxidised to the antimicrobial O-SCN (hypothiocyanate) which also exists in equilibrium with hypothiocyanus acid, and its disassociation constant is 5.3. So, because of this equilibrium it results into the inhibitory activity.



Lysozyme is the enzyme that catalyzes hydrolysis of beta 1,4 glycosidic bonds between the N-acetyl muramic acid and N-acetyl glucosamine of the bacterial cell wall. This lysozyme is present in egg, milk, tears, and other secretions. It is also present in insects and fishes; in hypotonic solution of the enzyme, the lysis of the bacterial cell occurs. Lysozyme is used to prevent gas formation in cheese (blowing) and the maximum limit, which is recommended for use is 400 mg/L of egg white.

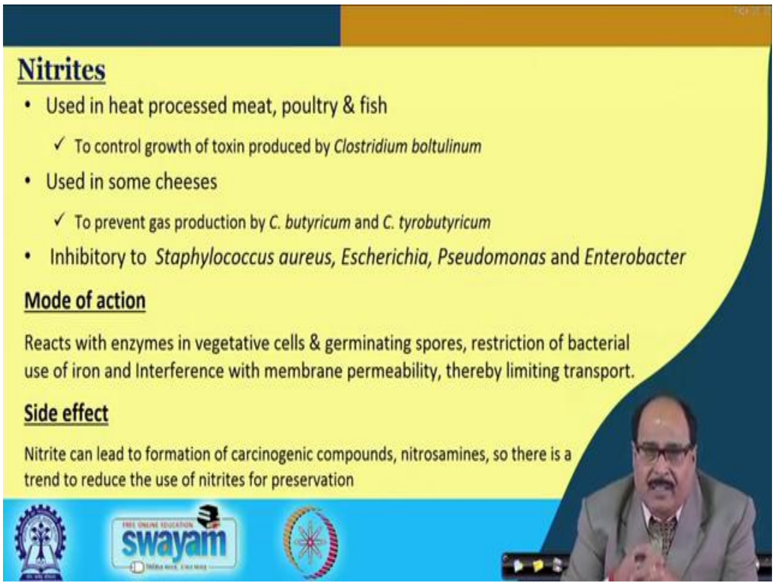


As far as the antimicrobials from plant sources are concerned, there are large number of compounds which have been identified and isolated. And in fact, their effectiveness against the microorganism has been proved by lot of reports in the literature.

Even in very traditional or ancient literature, the use of spices and spice oil extracts as antimicrobial system or antibacterial system, or for various medicinal purposes, etc. has been reported. For example, allium, onion and garlic, hydrocyanic acids, and related compounds in caffeic acid, p-coumaric acid, ferulic acid and sinapic acids etc.

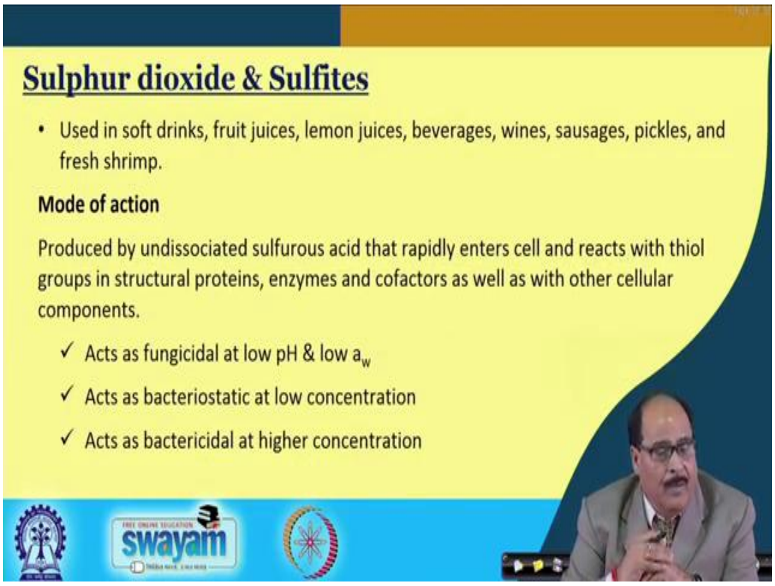
The gulcosinolates and their derivatives like isothiocyanates, spices and essential oils, like eugenol in cloves, cinnamic aldehyde in cinnamon, linalool in sweet basil, vanillin in vanilla, all these are reported to have antimicrobial or antibacterial activity, and they are used as food antimicrobial.

This slide shows a list of the food antimicrobials, which have been approved by regulatory agencies in different countries. Alkyl esters of para hydroxy benzoic acids, like parabens etc., acetic acid and acetate salts, benzoic acids and benzoate salts, dimethyl or diethyl dicarbonates, lactic acid and lactate salts, lysozymes, natamycin, nisin, nitrates and nitrites, phosphates, propionic acid and propionate salts, sorbic acid and sorbate salts, sulfur dioxide and sulfite derivatives, medium chain fatty acids and esters.

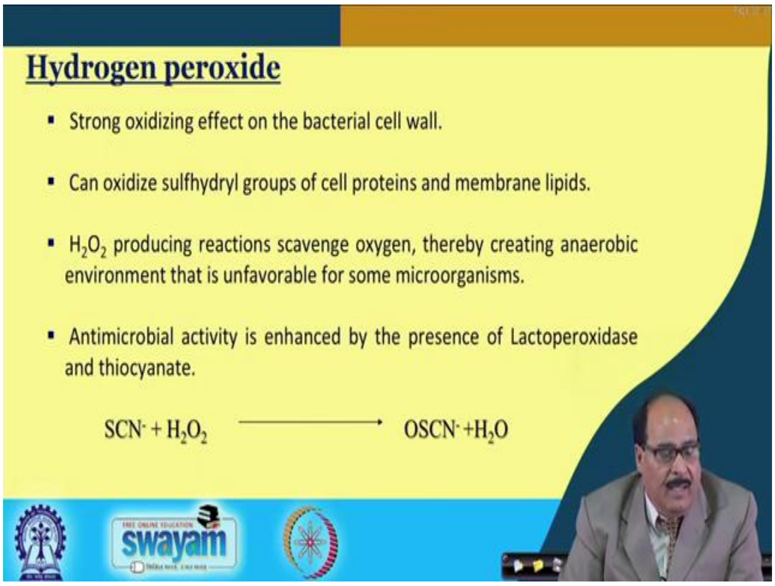


Nitrites are generally used in heat processed meat, poultry and fish, to control growth of toxin produced by clostridium botulinum. These are also used in some cheeses to prevent gas production by *Clostridium butyricum* and *Clostridium tyrobutyricum*. They are inhibitory to *Staphylococcus aureus, Escherichia, Pseudomonas, Enterobacter,* etc.

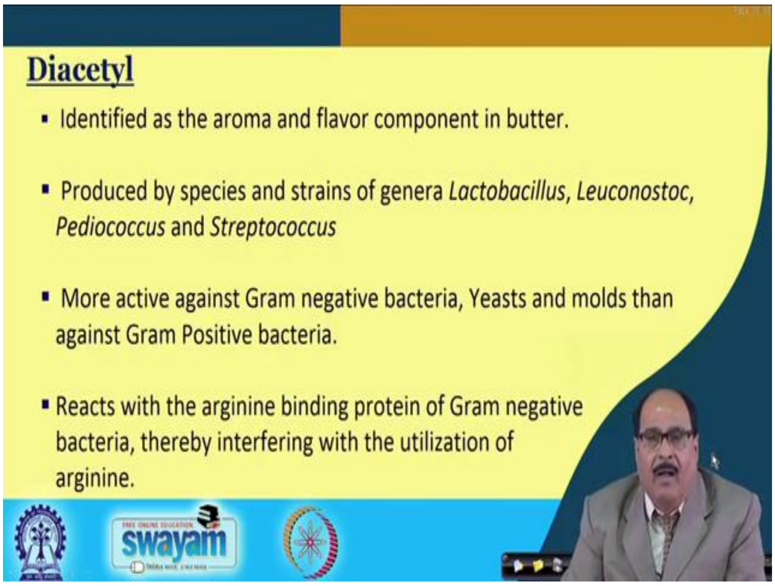
These nitrites react with the enzymes in vegetative cell as well as with the germinating spores, and they result into the restriction of bacterial use of iron, and interference with the membrane permeability, and thereby limiting the transport processes in the bacterial cell. And, the nitrites when they are used, they again disturb the homeostasis of the cell. There are certain side effects of the nitrites reported in literature that it can lead to formation of carcinogenic compounds like nitrosamines. So, there is a trend to reduce the use of nitrites for the preservation.



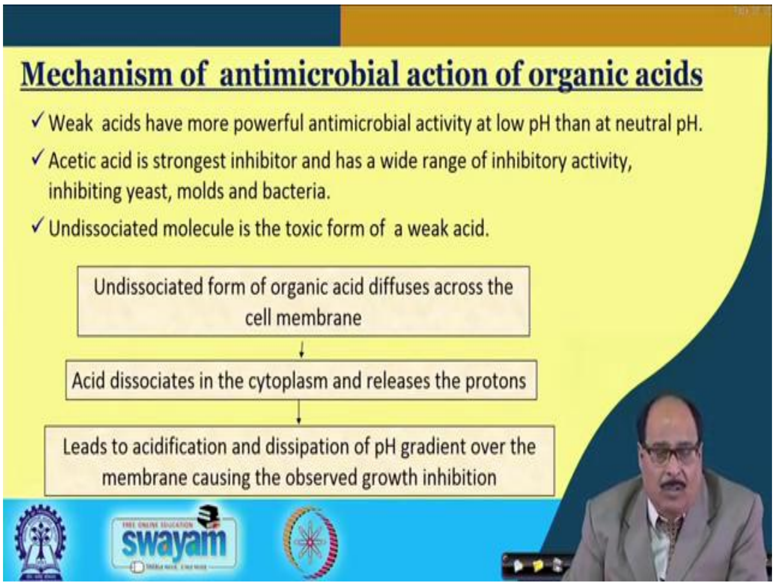
Sulphur dioxide and sulfites are used in soft drinks, fruit juices, lemon juice, beverages, wine, sausage, pickles, fresh shrimp, etc. These are produced by undissociated sulfurous acid that rapidly enters the cell wall and reacts with thiol group in the structural proteins, enzymes, and cofactors as well as other cellular components. And, this disturbs the normal physiological activity of the cell. It acts as a fungicidal at low pH and low water activity. SO_2 act as a bacteriostatic at low concentration and as a bactericidal at a comparatively higher concentration.



Hydrogen peroxide has a strong oxidizing effect on the bacterial cell wall. It can oxidize sulfhydryl groups of cell proteins and membrane lipids. Hydrogen peroxide producing reactions, scavenge oxygen, thereby creating anaerobic environment that is unfavourable for the growth of certain microorganisms. Antimicrobial activity of hydrogen peroxide is enhanced by the presence of lactoperoxidase and thiocyanates.

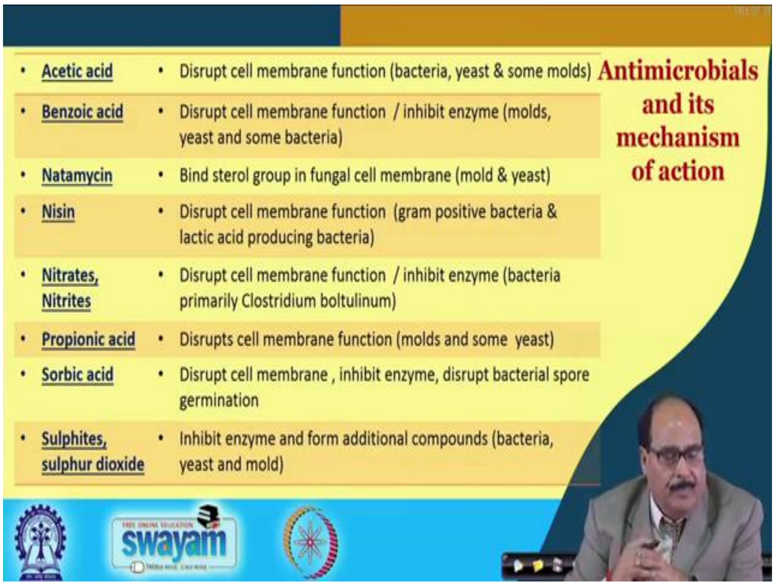


Diacetyl is identified as the aroma and flavor component in butter, it is produced by species and strains of genera lactobacillus, leuconostoc, pediococcus, streptococcus etcetera. It is more active against gram negative bacteria, yeasts and molds, and later reacts with the arginine binding protein of Gram negative bacteria. And thereby it interferes with the utilization of arginine as the bacterial cell wall and which influences its growth.



Mechanism of antimicrobial action of organic acids: The weak acids have more powerful antimicrobial activity at low pH than at neutral pH. Acetic acid is the strongest inhibitor, and has a wide range of inhibitory activity, inhibiting yeast mold and bacteria. And the undissociated molecule is a toxic form of a weak acid.

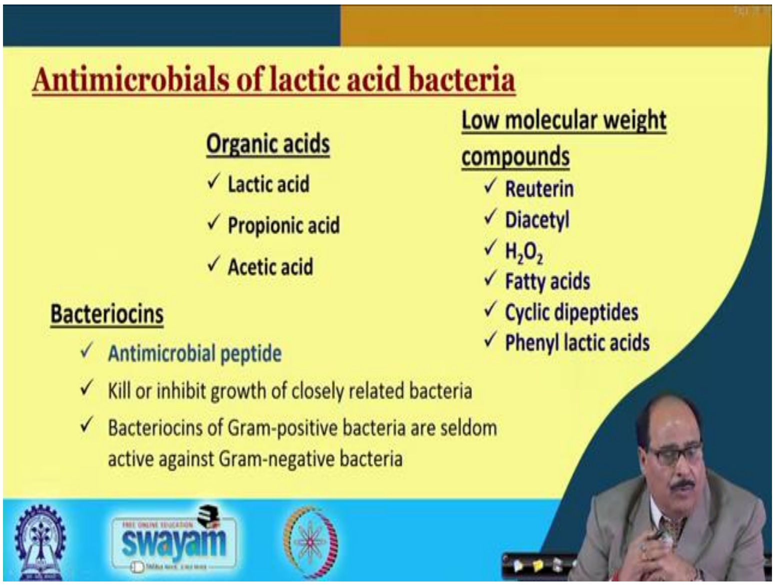
So, the undissociated form of organic acid diffuses across the cell membrane, the acid dissociates in the cytoplasm, and releases the proton, and ultimately leads to the acidification and dissipation of pH gradient over the membrane causing the observed growth inhibition. So, this is the way how these organic acids perform their antimicrobial action.



There are different antimicrobials like acetic acid, benzoic acid, natamycin, nisin, nitrates, propionic acid, sorbic acid, sulphites etc. and, their mode of action. Majority of them function by disrupting the cell membrane permeability or disturbing the cell homeostasis.

Bio preservation is a methodology to extend storage life and to enhance safety of foods using natural micro flora and/or their antibacterial products.

So, in the food either the microorganism in life forms or the bioactives, which are produced by the microorganism can be extracted and purified that can be used as antimicrobial in the bio preservation methods. In dairy products like milk, whey etc. where the microorganisms can be allowed to grow, in the fermented products like liquor or concentrate containing the bioactives, which are produced by the microorganism they can be used.



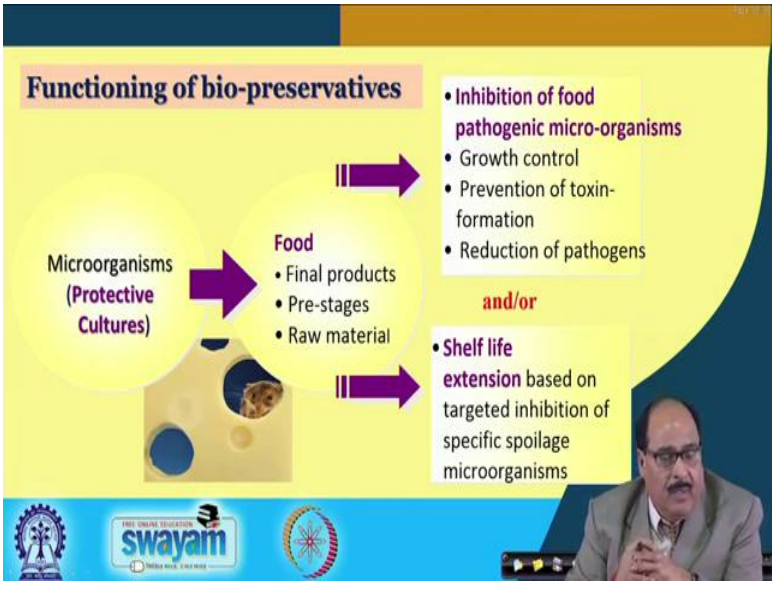
So, this has a wide ranging application in food processing. So, there are certain antimicrobials of lactic acid bacteria which are being used for bio preservation.

And, these include organic acid like lactic acid, propionic acid, acetic acid, or other low molecular weight compounds produced by the lactic acid bacteria, when they grow in the food etc. like reuterin, diacetyl, hydrogen peroxide, fatty acid, cyclic dipeptide, and phenyl lactic acids.

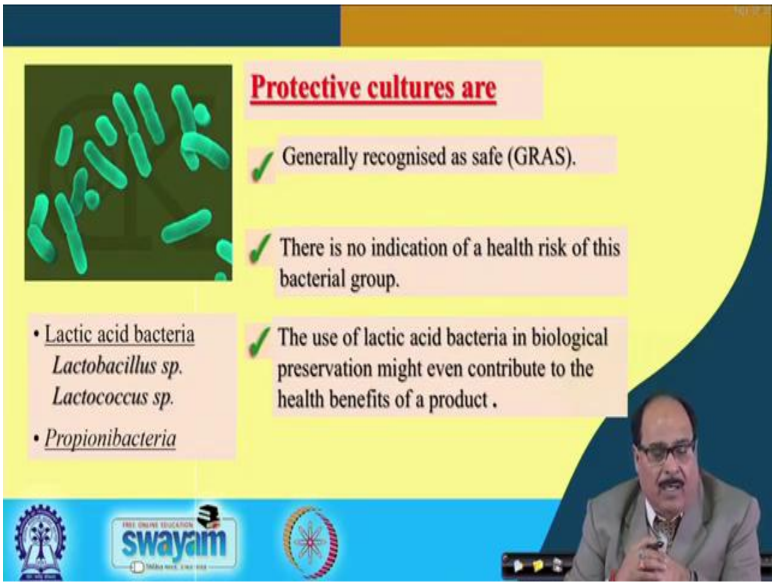
Another important component is the bacteriocin. These bacteriocins are actually antimicrobial peptides and they kill or inhibit growth of closely related bacteria. The bacteriocins of gram positive bacteria are seldom active against gram negative bacteria.

For the selection of microbial culture to be used as bio preservatives, certain points must be considered. It should be able to produce antimicrobials in the food; it should not affect the sensory characteristics of the food, it should result in limited number of acid or other bio active productions.

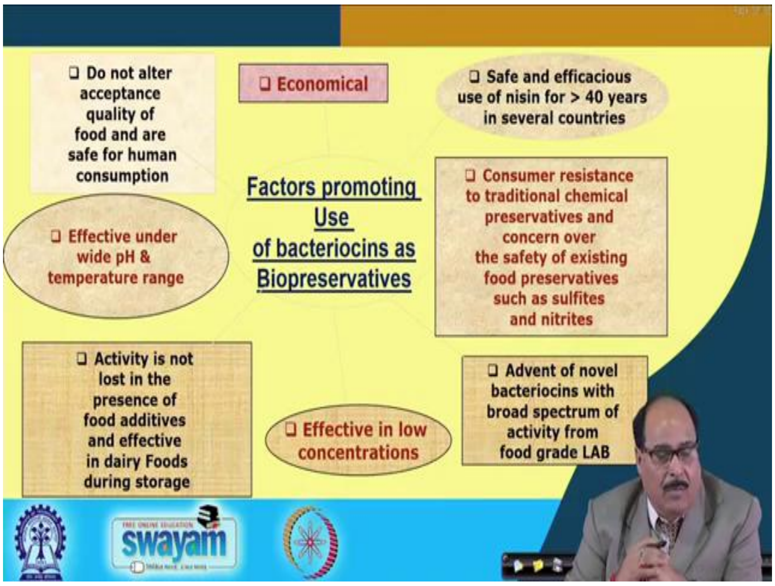
So, it should have weak protease activity, i.e. it should not adversely affect the protein and other components of the food, limited gas production, and absence of slime production.



Functioning of bio-preservatives: The microorganisms which are the protective cultures can be used in the food either in the final product, or even in the pre stages during the processing, or even they can be mixed with the raw materials. And, they result into the inhibition of growth of the pathogenic or spoilage microorganisms, or they can result into the extension of shelf life based on targeted inhibition of the specific spoilage microorganisms.

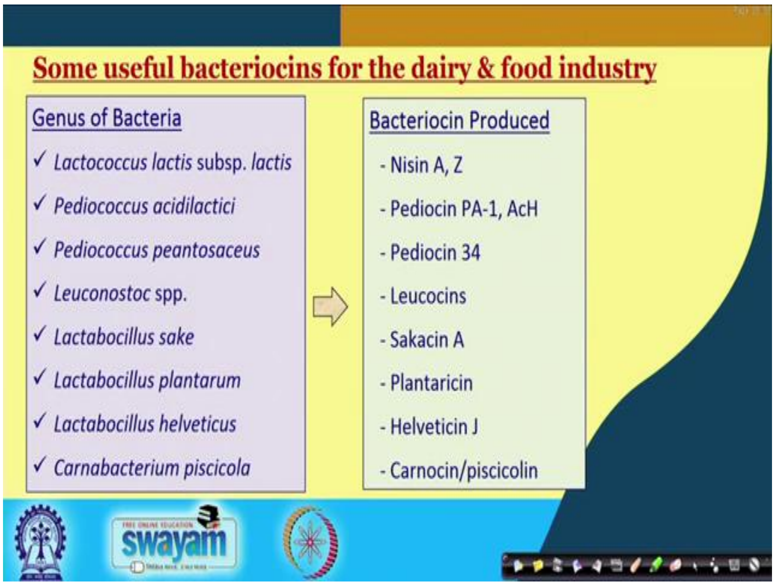


The protective cultures which are used in certain food operations or food process as bio preservatives, they should be listed in the GRAS. There is no indication of health risk of this bacterial group and which is used as bio preservatives. And, the use of lactic acid bacteria in biological preservation is recommended like, lactobacillus species, lactococcus species or propionibacteria etc. They might even contribute to the health benefits of the products, as they provide probiotic effects etc. into the food material; they result into the shelf life extension.



Factors promoting the use of bacteriocins as bio preservatives are listed: They do not alter acceptance quality of the food and are safe for human consumption; they provide effective barrier under wide pH and temperature ranges. Their activity is not lost in the presence of food additives and effective in dairy foods during storage.

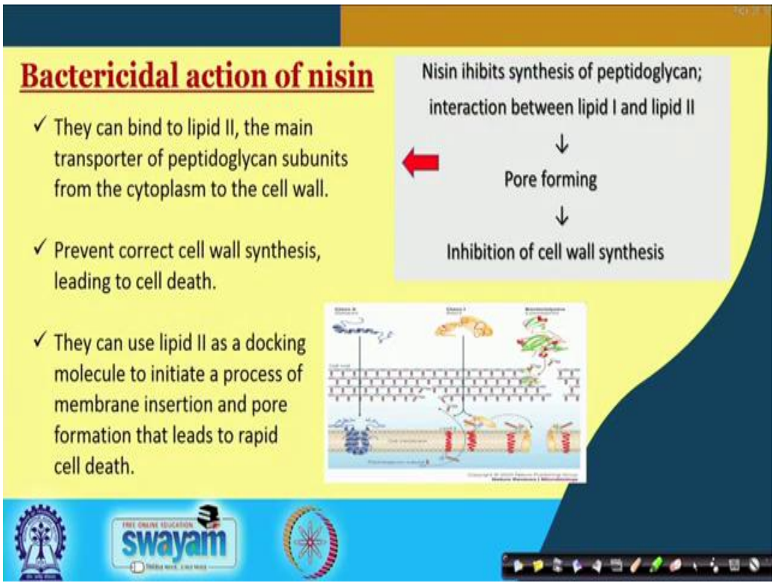
They are effective in low concentration; advent of novel bacteriocins with the broad spectrum of activity from food grade lactic acid bacteria has made the use of these bacteriocins and the microbials into the food possible. Even, the consumer resistance to traditional chemical preservatives and concerns over the safety of the existing food preservatives like sulfites and nitrites etc. has also led to the more use of these bacteriocins. Safe and efficacious use of nisin for more than 40 years in several countries is reported. So, the bacteriocins are economical and are safe to use in the food products.



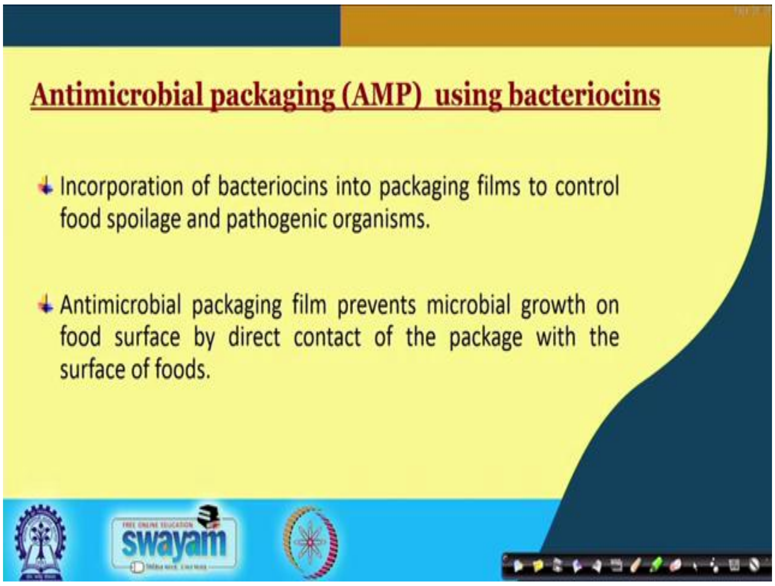
Some of the bacteriocins are produced by lactic acid bacteria. Nisin A and Z is produced by lactococcus lactis subsp lactis, pediocin PA-1, AcH is produced by pediococcus, then leucocins is produced by leuconostoc spp. and so on. So, there are many lactic acid bacteria, which can produce bacteriocins.

So, that is one way by which the microorganism itself can be used in the food where they produce the various low molecular weight compound acids and which provide the beneficial effect in extending its shelf life.

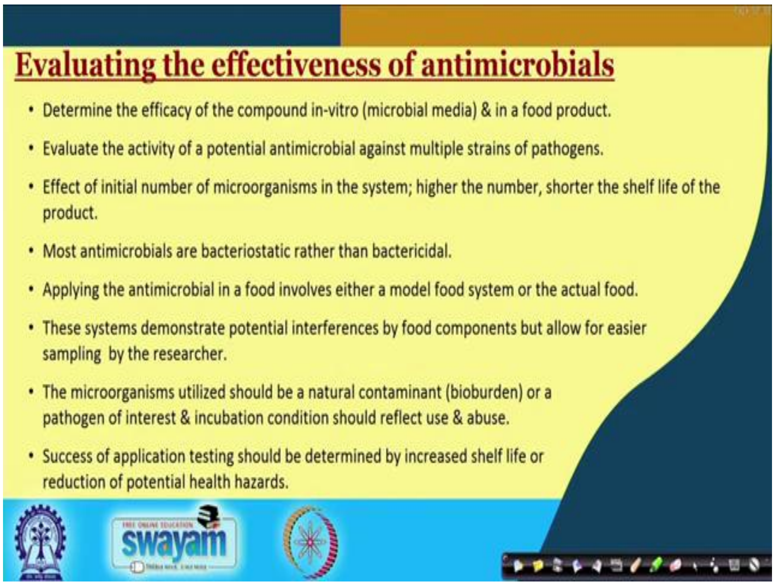
They can be grown in certain foods, extracted, purified and then use. However, the only commercially produced bacteriocin, which is used at present is nisin. It is produced by lactococcus lactis sub species lactis, and pediocin PA-1, which is produced by pediococcus acidilactici. So, the use of purified bacteriocin; however, have to be labeled as additive and they require regulatory approval. Nisin is approved for use as an antimicrobial in food by the joint FAO WHO Expert Committee on Food Additives.



Bactericidal action of nisin: Nisin inhibits synthesis of peptidoglycans. And, also it interacts between the lipid I and lipid II. And this may result into the pore formation and which finally, results into the inhibition of cell wall synthesis. So, they can bind the lipid II which is the main transporter of the peptidoglycan subunits from the cytoplasm to the cell wall. It prevents correct cell wall synthesis, leading to cell death.

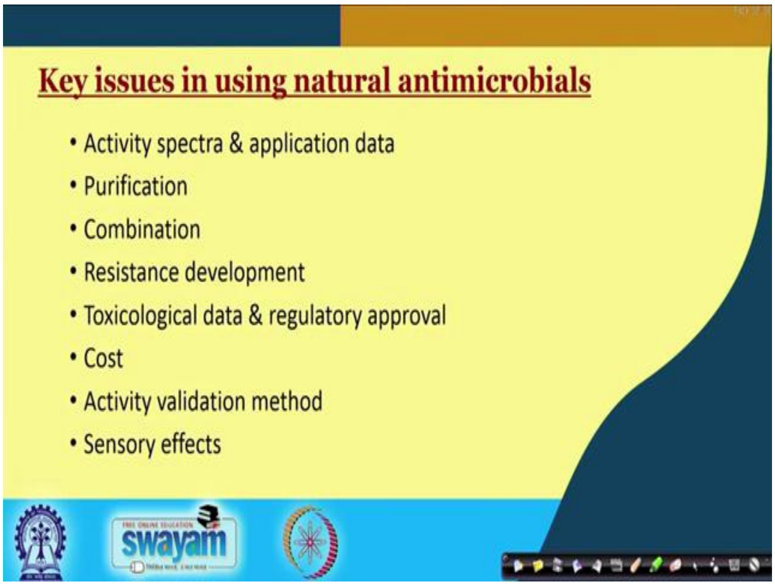


Antimicrobial packaging is another common use now a days, i.e. active packaging where these bacteriocins are used. Incorporation of bacteriocins into packaging films is used to control food spoilage and pathogenic microorganisms. Antimicrobial packaging film prevent microbial growth on food surface by direct contact of the package with the surface of food.



However, in order to make this use more widen and commercially more viable, one should have a system for evaluating the effectiveness of these antimicrobials. Once the efficacy of the compound in vitro is determined in the microbial media as well as in the food products, this would evaluate the activity or potential of an antimicrobial against multiple strains of pathogens. Because, many a times in the food, multiple micro flora are present. The antimicrobial are bacteriostatic rather than bactericidal. So, once the effect of the antimicrobial is removed, the bacteria will again revive.

So, applying the antimicrobial in a food involves either a model food system, or the actual food. These systems demonstrate potential interferences by food components, but allow for easier sampling by the researcher. The microorganisms utilized should be a natural contaminant or a pathogen of interest and incubation condition should reflect use and abuse. The success of application testing should be determined by increased shelf life or reduction of potential health hazards.



There are certain antimicrobials or bacteriocins at present that are being used in some of the food products in some countries. However, there are still certain issues, which should be resolved or which need an elaborated study, for making the natural antimicrobial more useful and its application more common.

These are activity spectra and application data; there are not much data available in the literature. So, generally this type of data obviously will make this process more popular.

Many a times it invites legal or regulatory problems, because these purified bio actives or antimicrobial might be considered by the regulatory agencies as chemical.

The data about the combined effect of different antimicrobials and resistance development will be of great immense. The microorganisms develop resistance to the chemicals after repeated or frequent use or after their exposure to these chemicals for a little more time. Toxicological data and regulatory approval require that it has to be added into the food for providing the desired effect in the food at that level in which it does not produce any toxic effect. There are many naturally occuring herbs or plant extracts etc., which have the potential antimicrobial effect. But many of them might be toxic otherwise. Another factor is the purity of the compound which is to be added that incurs cost. The bio-preservation of food has a great potential in producing safe and good quality food materials. So, the use of these natural antimicrobials or bio-preservatives is a promising way in improving the shelf life as well as quality and health value of the food products.