Catalysts Connect

July-Aug-Sept-Oct 2017 Volume 27

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MESSAGE FROM THE MANAGING DIRECTOR

Dear Friends,

I am sure you all had a wonderful Deepawali celebration with your friends and family! I take this opportunity to wish all of you a very prosperous year ahead!

As you may be aware, our corporate and business development teams have moved to a new office and the R&D facility has been enhanced as well. I would like to congratulate all of you for enabling the same.

As we begin our next quarter of calendar year 2017, each one of us must gear up for a dash to meet our personal, team and company targets for the year 2017. Champions are those who are able to motivate themselves and lift their game at an appropriate time to ensure success.

For all of us at Catalysts Group success is not incidental but a way of life!

The challenges as always will be there for us to overcome. Rather than using them as an excuse, use your experience and acumen to work your way through these challenges. I am sure that your hard work, dedication and commitment will ensure a continuous growth of the company.

Do Well! Do Big! Go get your Destiny!

Munish Madaan

MESSAGE FROM THE DIRECTOR

Dear Friends,

I hope you all had a wonderful pollution free Diwali full of celebrations.

Like pollution chocks our lungs making it difficult to breathe and puts additional stress to filter the bad air, same way our negative energy chocks our feelings & thoughts making it not only uncomfortable to think right but also puts stress on mind to react & take bias / wrong decisions resulting in harmful actions.

This Diwali let's try to give positive energy to all our thoughts, people around us & even to materialistic things we own. Everything responds to us in the same manner. So, lets give energy to our body for a good health, energy to our soul for enlightenment, energy to our mind for a better focus & calmness, energy to nature for continued blessings to us with its natural resources and energy to this entire universe for peace in all our lives.

We also seek your blessings & wishes for our new office from where we further improve to give you newer technologies with enhanced services in the times to come.

Wishing you all a very Happy New Year in advance. May the even year 2018 removes all odds & brings our life to even's.

Best wishes

Aditya Malhotra

Unveiling of Catalysts New Corporate Office at Patparganj, New Delhi & New Wing of R&D Lab at Sahibabad

Microorganisms in Food Production

Joole Chauhan, Research & Development Department

Nature uses microorganisms to carry out fermentation processes and for thousands of years mankind has sed yeasts, molds and bacteria to make food products such as bread, beer, wine, vinegar, yoghurt and cheese, as well as fermented fish, meat and vegetables.

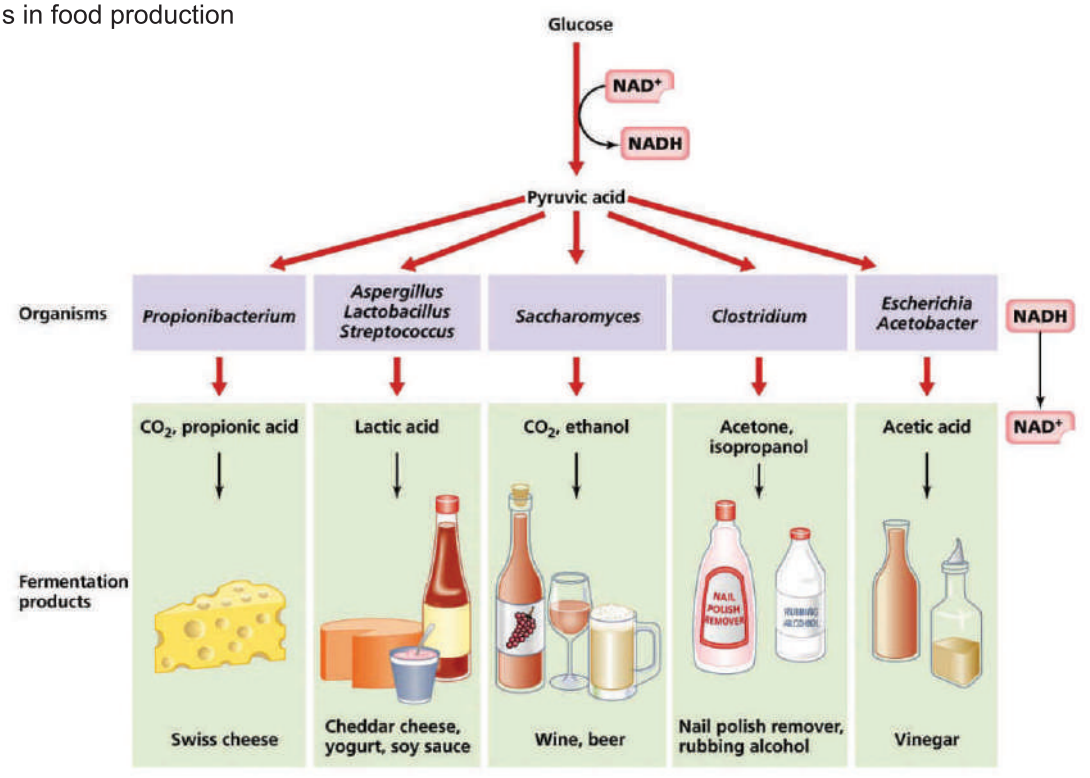
Fermentation is one of the oldest transformation and preservation techniques for food. This biological process allows not only the preservation of food but also improves its nutritional and organoleptic qualities (relating to the senses: taste, sight, smell, touch). A well conducted fermentation will favor useful flora, to the detriment of undesirable flora in order to prevent spoilage and promote taste and texture.

A BIT OF HISTORY

The first realization that microorganisms were involved in food production processes was in 1837, when scientists discovered the role of yeast in an alcoholic fermentation. Later, when the world renowned French chemist and biologist Louis Pasteur was trying to explain what happened during the production of beer and vinegar in the 1860, he found that microorganisms were responsible. However, it wasn't until after the Second World War that the food industry began to develop the biotechnological techniques we rely on today to produce a wide variety of better, safer foods under controlled conditions.

FEATURES

- Bacteria in food production
- Yeasts in food production
- Molds in food production



BACTERIA IN FOOD PRODUCTION

Bacteria are used to make a wide range of food products. The most important bacteria in food manufacturing are Lactobacillus species, also referred to as lactic bacteria.

a. Dairy Industry

It would be impossible to make cheese without a starter culture. As the culture grows in the milk, it converts the sugar lactose into lactic acid, which ensures the correct level of acidity and gives the cheese its moisture. As the cheese ripens, the culture gives it a balanced aroma, taste, texture. It is also responsible for the 'holes' in cheeses such as emmental. Choosing the right mixture of culture is essential for a high-quality cheese.

In yoghurt and other fermented milk products, the culture is responsible for the taste and texture of the final product. Depending on the acidity, the product will have either a mild or strong taste, and the viscosity depends on the quantity of polysaccharides – chains of sugar molecules – that are produced. In recent years, probiotic cultures have become popular in dairy products because of their health benefits. These cultures are all very carefully selected strains, and there is good evidence that they help improve digestion, safeguard the immune system, and keep the body's intestinal flora in balance.

b. Meat Industry

Meat starter cultures are used to make dried, fermented products such as salami, pepperoni, chorizo and dried ham. Lactic bacteria develop the flavor and colour of the products. In addition, a wide variety of molds are used to ripen the surface of sausages, preserving the natural quality of the product and controlling the development of flavor.

c. Wine Industry

Yeasts are responsible for the fermentation process which produces alcohol in wine. However, lactic bacteria also play an important role, as they convert the unstable malic acid that is naturally present in wine into the stable lactic acid. This conversion gives the stability that is characteristic of high-quality wines that improve on storage.

d. Health Food Industry

Lactic bacteria are used in many different tablets and capsules sold as supplements in the health food industry. Our hectic modern lifestyles often lead to an imbalance in the intestinal flora; travel and medical treatment are two of the major culprits. By taking supplements containing lactic bacteria, this balance can be restored, improving the quality of life.

DID YOU KNOW?

While yeast is responsible for causing bread to rise, lactic bacteria also play an important role as they form the 'sour dough' fermentation. This offers a number of benefits to bakers, such as a dough that is easy to handle and uniform, with a shorter resting time, a good aroma and a product that stays fresher for longer. Lactic bacteria are also playing an important role in the preparation of sauerkraut.

YEASTS IN FOOD PRODUCTION

Yeasts have two main uses in food production: baking and making alcoholic beverages. They have been used in this way since ancient times – there is evidence that ancient Egyptians used yeast in bread making, and we have been making fermented drinks like beer and wine for millennia.

a. Baking

Baked goods like bread rise because of the presence of yeast as a raising, or leavening, agent. The most common yeast used in bread making is Saccharomyces cerevisiae. It feeds on the sugars present in the bread dough, producing the gas carbon dioxide. This forms bubbles within the dough, causing it to expand. Other ingredients in the mixture have an effect on the speed of the fermentation – sugar and eggs speed it up; fats and salt slow it down.

b. Brewing

Several different yeasts are used in brewing beer, where they ferment the sugars present in malted barley to produce alcohol. One of the most common is Saccharomyces cerevisiae, the same strain used in bread making, this is used to make ale-type beers and is known as a top-fermenting yeast as it forms a foam on the top of the brew. Bottom-fermenting yeasts, such as Saccharomyces pastorianus, are more commonly used to make lagers. They ferment more of the sugars in the mixture than top-fermenting yeasts, giving a cleaner taste.

c. Winemaking

The alcohol in wine is formed by the fermentation of the sugars in grape juice, with carbon dioxide as a byproduct. Yeast is naturally present on grape skins, and this alone can be sufficient for the fermentation of sugars to alcohol to occur. A pure yeast culture, most often Saccharomyces cerevisiae, is usually added to ensure the fermentation is reliable. Sparkling wine is made by adding further yeast to the wine when it is bottled. The carbon dioxide formed in this second fermentation is trapped as bubbles.

DID YOU KNOW?

Yeasts are also common in kefir products, in semi-soft ripened cheeses and fermentation of soy drinks.

MOLDS IN FOOD PRODUCTION

Molds are essential components of several food products, such as some cheeses, sausages and soy sauce.

a. Cheese Making

Three main types of cheese rely on molds for their characteristic properties: blue cheese, soft ripened cheese and rind-washed cheese.

To make blue cheese, the cheese is treated with a mold, usually Penicillium roqueforti, while it is still in the loosely pressed curd form. As the cheese matures, the mold grows, creating blue veins within it which gives the cheese its characteristic flavor. Examples include Stilton, Roquefort and Gorgonzola.

Soft ripened cheese such as Brie and Camembert are made by allowing Penicillium camemberti to grow on the outside of the cheese, which causes them to age from the outside in. The mold forms a soft white crust, and the interior becomes runny with a strong flavor.

Rind washed cheeses like Limburger also ripen inwards, but here, as the name suggests, they are washed with brine and other ingredients like beer and wine which contain mold. This also makes them attractive to bacteria, which add to the flavor.

b. Meat Fermentation

A wide variety of molds (i.e. Penicillium chrysogenum and Penicillium nalgiovense) are used to ripen surfaces of sausages. The mold cultures plays a role in aroma formation and improve the texture of the sausages. They also contribute to shortening of the ripening period and preserving the natural quality and in that way expanding the shelf life of the meat product. Inoculations of sausages with molds were traditionally done with the indigenous flora of the slaughters, the so-called house flora.

c. Soy Sauce

Traditional soy sauce is made by mixing soybeans and other grains with a mold-either Aspergillus oryzae or Aspergillus sojae and yeast. Historically, this would have been left to ferment in the sun, but nowadays it is mostly made under industrial conditions. The key flavor ingredients formed in this process are salts of the amino acid glutamic acid, notably monosodium glutamate.

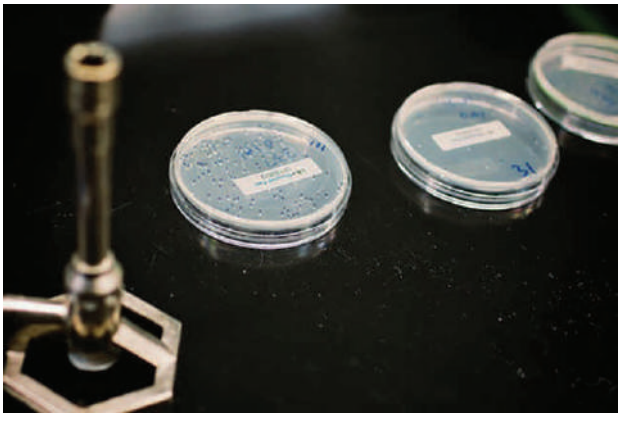
DID YOU KNOW?

Currently, more than 3500 traditionally fermented foods exist in the world. They are of animal or vegetable origin and are part of our daily life. Alcoholic drinks are not the only fermented drinks; cocoa beans, coffee grains and tea leaves are fermented after harvest in order to develop their typical flavor profiles. Moreover, fermented products have geographical specificities: in Europe, cheese and bread are widely consumed. In Africa, products manufactured from fermented manioc play a key role in the diet and, in Asia, products derived from soy or fish are consumed on a daily basis.

Reference: www.effca.org

Antimicrobial Enzymes as an Emerging Microbe Control Strategy

Dr Archana Prakash, Research & Development Department



Antimicrobial enzymes are widespread in nature, where they play a critical role in defending living organisms from bacterial attack. These enzymes are now increasingly being exploited against microbial systems. As a group, they possess abilities to directly attack the microorganism, interfere with biofilm formation, destroy the biofilm, and/or catalyze reactions which result in the production of antimicrobial compounds. In some cases, antibacterial enzymes are now an established technology. For example, antimicrobial and anti-biofilm enzyme liquid formulations are frequently exploited for cleaning surfaces. Enzymes may be incorporated or grafted into and onto polymer materials to prevent microbial colonization. The formulations may contain one or more enzymes, or enzymes combined with other antimicrobial or anti-biofilm agents.

Major antimicrobial enzymes successfully used to control microbes are given below:

A. SUBTILISINS

Subtilisins are amongst the most widely used proteolytic enzymes for the control of biofilm in industry. Subtilisins are produced by *Bacillus sp.* and are serine proteases that cleave proteins in which serine serves as the nucleophilic amino acid. Subtilisins hydrolyze adhesins (bacterial proteins essential for attachment onto solid supports and other bacteria), thus preventing co-aggregation of microorganisms a condition which allows bacteria to communicate with other and participate in multispecies biofilm formation.

B. LYSOSTAPHIN

Lysostaphin, first isolated from Staphylococcus simulans, is a metalloendopeptidase. It cleaves *Staphylococci* cell walls on the third and fourth glycine residues of the pentaglycine cross-bridge. This enzyme is attracting significant interest for the control of multidrug resistant MRSA. Lysostaphin exhibits three different catalytic activities: glycylglycine endopeptidase, endo-beta-Nacetyl glucosamidase and –acetyl muramyl-L-alanine amidase activity. This makes it able to effective hydrolyze of S. aureus cell walls.

C. BACTERIOPHAGE LYSINS

Bacteriophages occupy every niche in the biosphere where their bacterial hosts are present. Bacteriophage endolysins are re-emerging as a new class of antibacterial agents for application in

agriculture, health, food and environmental maintenance. These double- stranded DNA (dsDNA) phages express two types of essential proteins, endolysins and holins during their replication inside their bacterial host, which are critical for host cell lysis. Holins are small proteins that permeabilize the bacterial membrane, allowing endolysin access the peptidoglycan layer of the host bacteria. The term endolysins is used to describe bacteriophage muralytic enzymes collectively. These include enzymes such as – acetylmuramidases, endo-β-N-acetylglucosaminidases, lytic transglycosylases, endopeptidases,and – acetylmuramoyl- l-alanine amidases. The group C streptococci C1 phage endolysin was the first to be applied topically for treating bacterial infections and is reemerging as alternative drug to deal with increasing resistance to erythromycin and clindamycin in penicillin allergic women.

D. ALPHA-AMYLASE

Alpha-amylase inhibits the formation of, and hydrolyzes existing biofilms of S. aureus. It can also be used in conjunction with proteases to enhance removal of biofilms and prevent bacteria from adhering onto surfaces. A combination of proteases and amylases was effective in removing a Pseudomonas fluorescens biofilm.

E. DISPERSIN B

Dispersin B (DspB), is another important antimicrobial and anti-biofilm enzyme. It is produced by *Actinobacillus actinomycetemcomitans*. DspB hydrolyzes β-1,6-N-acetyl-d-glucosamine, an important polymer needed for biofilm attachment onto surfaces. Its combination with proteases, DNAses and glycolytic enzymes enhances the removal of biofilms although DspB alone is also able to attack biofilms of several different species of bacteria. An engineered DspB from T7 phage hydrolyzed *Staphylococcus* and E. coli exopolysaccharides and reduced bacterial cell counts by a factor of 100 000. Coating with triclosan1 DspB and chlorhexidine silver sulfadiazine was effective in combating contamination of catheters by S. aureus S. epidermidis and E. coli.

F. ALGINATE LYASE

Alginate lyase is another antimicrobial enzyme, which cleaves β-glycosidic bonds of bacteria alginate polymer. Alginate is a copolymer made up of alpha-L-guluronate and beta-D-mannuronate. Alginate lyases are widely distributed and have been found in algae, invertebrates and microorganisms.

G. LACTOPEROXIDASE

Lactoperoxidase is the second most abundant natural enzyme in milk and plays an important role in preserving milk and is found in tears and thyroid glands. Its antimicrobial activity is based on the oxidation of the thiocyanate anion SCN. Depending on the pH two antimicrobial agents namely; hypothiocyanite OSCN and hypothiocyanous acid that are capable of oxidizing the sulfhydryl groups of enzymes and proteins, thereby inhibiting microbial growth It is effective against both Gram-negative and Gram-positive

bacteria. The lactoperoxidase system is variously used for preserving food, boosting the airway host defense system of humans, and plant and crop protection. Recently the lactoperoxidase (LPO) system has been shown to play an important role in maintaining oral hygiene.

H. MYELOPEROXIDASE

Myeloperoxidase (MPO) is a common neutrophilic enzyme, plays an important role in the human defense system by oxidizing chloride anions using H_2O_2, yielding very potent antimicrobial agents, namely hypochlorite anion or undissociated hypochloric acid depending on the pH. The naturally occurring SCN− ions in human saliva are the preferred substrate of MPO. The generated radicals by MPO inhibit the growth of bacterial but are non-toxic to the surround cells.

I. ANTI-QUORUM SENSING OR QUORUM-QUENCHING ENZYMES

Other emerging groups of antimicrobial enzymes include the so called “anti-quorum sensing or quorum quenching” enzymes. Bacteria use quorum sensing to regulate various physiological activities, including virulence, competence, conjugation, antibiotic and bacteriocin production, motility, and spore and biofilm formation. Among the quorum-sensing molecules proposed as targets are the acyl homoserine lactones (AHLs), which are implicated in the regulation of bacterial virulence in greater than 50 bacterial species. These quorum-sensing molecules move in and out of the cell through passive diffusion or active transport. Lactonases quorum-quenching enzymes hydrolyzes the ester bond of the homoserine lactone ring of acylated homoserine lactones, thereby preventing AHLs from binding to their target transcriptional regulators. AHL acylase hydrolyses the amide linkage between the acyl chain and the homoserine moiety of AHL molecules. The first quorum-quenching enzyme encoded by the aiiA gene was identified from a soil bacterial isolate belonging to a Gram-positive Bacillus sp.

Several antimicrobial enzymes based products have already been commercialized for application in the healthcare, food and biomedical industries. Antimicrobial enzymes that targets different bacterial cellular components and biofilm formation are increasing investigated. But use of these enzymes in fermentation industries upon the targeted cellular component of undesired organism need to be studied. Increasing research in this area can help to solve contamination problem which affects the recovery. The challenge for the future research activities in this area is to harness all the advances in biotechnology (protein engineering, synthetic biology, domain swapping and gene shuffling, bioinformatics, metagenomics, large-scale DNA sequencing technologies) for the development of novel and more potent antimicrobial and antibiofilm enzymes.

Reference: Barbara T1 et al., Biotechnol. J., 2013, 8: 97-109

The difference Between Scotch and Whiskey

Ashwini Agrawal, Business Development Department

What's the difference between scotch and whiskey

READ TO BE AN EXPERT

While savoring the smooth taste of your favorite dark spirit, have you ever wondered the difference between scotch and whiskey (also written as whisky)? Why is one expensive than the other or why does the taste differ according to the 'age' of the alcohol? The blends and malts used to produce these distilled alcoholic beverages create all the difference. We have scooped out the answers into four simple differences so that next time you sound like an expert!

1. THE DIFFERENCE OF GRAINS AND MALTS

Whiskey is made by fermenting the grain mash. Malts of various types of grains, like corn, wheat, barley and rye are used and aged in charred white oak casks to prepare the distilled alcoholic beverage. Unlike wine, whisky does not mature further once it is bottled.

Scotch, a malt whiskey, is made in Scotland from only water and malted barley. Scotch whiskey must be aged for a minimum of three years but mostly it lies in the woods for 8 to 10 years or more which smoothens the taste of this fine dark beverage.

2. THE DIFFERENCE IN PRODUCTION

Whiskey: The still (apparatus used to mix liquids) used for making whiskey is generally made of copper because the metal removes sulphur-based compounds from the alcohol that would otherwise make the beverage unpleasant to drink.

Scotch: A scotch must be processed strictly according to the law and only in a single distillery. It must be processed at a distillery in Scotland and should be fermented only by adding yeast. It must mature in Scotland in oak casks containing no added grains or substances.

3. THE DIFFERENCE IN MATURATION PROCESS

Apart from the grains used, the difference in taste between whiskey and scotch is only because of the maturation process. As it matures, the alcohol becomes smoother because the flavor of the grains mature and the color of the beverage turns golden. Smallest changes in the maturation process can cost a difference in the taste of the alcohol. A whiskey matured in American white oak, which has a mellower, finer aroma would taste different from European oak that provides more intense aroma and tannins. And that is where the prices differ too.

4. BLENDS

Whiskey: Blended whiskey would mean blending types of whiskeys and spirits with different grains, colors and flavors. It is generally the mixing of high-quality spirit whiskeys with less expensive spirits.

Scotch: Single malt scotch means that it has been processed at one single distillery. There are three types of blends you would find in scotch whiskeys:

1. **Blended Malt Scotch Whiskeys:**
   This means that two or more single malt whiskeys from different distilleries are blended together.
2. **Blended Grain Scotch Whiskeys:**
   This means that two or more single grain scotch whiskeys from different distilleries are blended together.
3. **Blended Scotch Whiskey:**
   This is a blend of the above two. It means that two or more single malt scotch whiskeys are blended with one or more single grain scotch whiskeys.

CRYOPRESERVATION

Sutrishna Roy, Research & Development Department

Cryopreservation is the preservation of cells and tissue by freezing. Cryopreservation is based on the ability of certain small molecules to enter cells and prevent dehydration and formation of intracellular ice crystals, which can cause cell death and destruction of cell organelles during the freezing process. Two common cryoprotective agents are dimethyl sulfoxide(DMSO) and glycerol. Glycerol is used primarily for cryoprotection of red blood cells, and DMSO is used for protection of most other cells and tissues. A sugar called trehalose, which occurs in organisms capable of surviving extreme dehydration, is used for freeze-drying methods of cryopreservation. Trehalose stabilizes cell membranes, and it is particularly useful for the preservation of sperm, stem cells, and blood cells.

Most systems of cellular cryopreservation use a controlled-rate freezer. This freezing system delivers liquid nitrogen into a closed chamber into which the cell suspension is placed. Careful monitoring of the rate of freezing helps to prevent rapid cellular dehydration and ice-crystal formation. In general, the cells are taken from room temperature to approximately −90°C (−130°F) in a controlled-rate freezer. The frozen cell suspension is then transferred into a liquid-nitrogen freezer maintained at extremely cold temperatures with nitrogen in either the vapour or the liquid phase. Cryopreservation based on freeze-drying does not require use of liquid-nitrogen freezers.

An important application of cryopreservation is in the freezing and storage of hematopoietic stem cells, which are found in the bone marrow and peripheral blood. In autologous bone-marrow rescue, hematopoietic stem cells are collected from a patient's bone marrow prior to treatment with high-dose chemotherapy. Following treatment, the patient's cryopreserved cells are thawed and infused back into the body. This procedure is

necessary, since high-dose chemotherapy is extremely toxic to the bone marrow. The ability to cryopreserve hematopoietic stem cells has greatly enhanced the outcome for the treatment of certain lymphomas and solid tumour malignancies. In the case of patients with leukemia, their blood cells are cancerous and cannot be used for autologous bone-marrow rescue. As a result, these patients rely on cryopreserved blood collected from the umbilical cords of newborn infants or on cryopreserved hematopoietic stem cells obtained from donors. Since the late 1990s it has been recognized that hematopoietic stem cells and mesenchymal stem cells (derived from embryonic connective tissue) are capable of differentiating into skeletal and cardiac muscle tissues, nerve tissue, and bone. Today there is intense interest in the growth of these cells in tissue culture systems, as well as in the cryopreservation of these cells for future therapy for a wide variety of disorders, including disorders of the nervous and muscle systems and diseases of the liver and heart.
Cells can live more than a decade if properly frozen. In addition, certain tissues, such as parathyroid glands, veins, cardiac valves, and aortic tissue, can be successfully cryopreserved. Freezing is also used to store and maintain long-term viability of early human embryos, ova (eggs), and sperm. The freezing procedures used for these tissues are well established, and, in the presence of cryoprotective agents, the tissues can be stored over long periods of time at temperatures of −14°C (6.8°F).

Research has shown that whole animals frozen in the absence of cryoprotective agents can yield viable cells containing intact DNA upon thawing. For example, nuclei of brain cells from whole mice stored at −20 °C (−4°F) for more than 15 years have been used to generate lines of embryonic stem cells. These cells were subsequently used to produce mouse clones.

PRINCIPLE OF CRYOPRESERVATION

Cryopreservation is the use of very low temperatures to preserve structurally intact living cells and tissues. The biological effects of cooling are dominated by the freezing of water, which results in the concentration of the solutes that are dissolved in the remaining liquid phase. Rival theories of freezing injury have envisaged either that ice crystals pierce or tease apart the cells, destroying them by direct mechanical action, or that

damage is from secondary effects via changes in the composition of the liquid phase. Cryoprotectants, simply by increasing the total concentration of all solutes in the system, reduce the amount of ice formed at any given temperature; but to be biologically acceptable they must be able to penetrate into the cells and have low toxicity. Many compounds have such properties, including glycerol, dimethyl sulfoxide, ethanediol, and propanediol. In fact, both damaging mechanisms are important, their relative contributions depending on cell type, cooling rate and warming rate. A consensus has developed that intracellular freezing is dangerous, whereas extracellular ice is harmless. If the water permeability of the cell membrane is known it is possible to predict the effect of cooling rate on cell survival and the optimum rate will be a trade off between the risk of intracellular freezing and effects of the concentrated solutes. However, extracellular ice is not always innocuous: densely packed cells are more likely to be damaged by mechanical stresses within the channels where they are sequestered and with complex multicellular systems it is imperative not only to secure cell survival but also to avoid damage to the extracellular structure. Toxicity is the major problem in the use of vitrification methods. Whether freezing is permitted (conventional cryopreservation) or prevented (vitrification), the cryoprotectant has to gain access to all parts of the system. However, there are numerous barriers to the free diffusion of solutes (membranes), and these can result in transient, and sometimes equilibrium, changes in compartment volumes and these can be damaging. Hence, the processes of diffusion and osmosis have important effects during the introduction of cryoprotectants, the removal of cryoprotectants, the freezing process, and during thawing. These phenomena are amenable to experiment and analysis, and this has made it possible to develop effective methods for the preservation of a very wide range of cells and some tissues; these methods have found widespread applications in biology and medicine.

MECHANISM OF CRYOPRESERVATION

The cryopreservation technique followed by the regeneration of plants involves following steps:

1. **Selection of material:**
   Selection of proper plant material is important. Two important factors depend on it such as (a) nature and (b) density. Any tissue can be selected for this purpose. e.g. meristem, embryo, ovules, seeds etc.. The density should be high.

2. **Addition of cryoprotectant:**
   Generally two cryoprotectant should be used together instead of single one as they are more effective. These are sucrose, alcohols, glycols, some amino acid (proline), DMSO (dimethyl sulfoxide). They are chemical which prevent cryo destruction.

3. **Freezing:**
   The sensitivity of cells to low temperature depends on the plant species.
   There are different types of methods:
   - Slow freezing method:
     The tissue or plant material is slowly frozen at slow cooling rate. The advantage is the plant cells are partially dehydrated and survive better.
   - Rapid freezing method:
     It involves plunging the vials in liquid nitrogen. The temperature decreases from -300 to -1000 degree rapidly. This is combination of both slow and rapid freezing method. The process is carried out in step wise like manner.
   - Dry freezing method:
     In this method dehydrated cells and seeds are stored.

4. **Storage in liquid nitrogen:**
   The maintenance of the frozen cells or material at specific temperature is very important. In general the temperature is kept -70 to -196 degree. Prolong storage is done at temperature of -196 degree in liquid nitrogen. To prevent damage, continuous supply of nitrogen is done.

5. **Thawing:**
   It is usually carried out by plunging the vials into warm water bath with vigorous swirling. As thawing occurs the vials are transferred to another bath at 0 degree.

6. **Washing and reculturing:**
   The preserved material is washed few times to remove the cryoprotectant. This material is then recultured in a fresh medium

7. **Measurement of viability:**
   Measurement of viability There is possibility of death of cells due to storage stress. Thus viability can be found at any stage. It is calculated by formula: No of cells growing / no of cells thawed * 100

8. **Regeneration of plants:**
   The viable seeds are cultured on non specific growth medium. Suitable environmental conditions are maintained.

PRESERVATION OF MICROBIOLOGY CULTURES

Bacteria and fungi can be kept short-term (months to about a year, depending) refrigerated, however, cell division and metabolism is not completely arrested and thus is not an optimal option for long-term storage (years) or to preserve cultures genetically or phenotypically, as cell divisions can lead to mutations or sub-culturing can cause phenotypic changes. A preferred option, species-dependent, is cryopreservation.

Fungi

Crypreservation is a hallmark method for fungi that do not sporulate (otherwise other preservation methods for spores can be used at lower costs and ease), sporulate but have delicate spores (large or freeze-dry sensitive), are pathogenic (dangerous to keep metabolically active fungus) or are to be used for genetic stocks (ideally to have identical composition as the original deposit). As with many other organisms, cryoprotectants like DMSO or glycerol (e.g. filamentous fungi 10% glycerol or yeast 20% glycerol) are used. Differences between choosing cryoprotectants are species (or class) dependent, but generally for fungi penetrating cryoprotectants like DMSO, glycerol or polyethylene glycol are most effective (other non-penetrating ones include sugars mannitol, sorbitol, dextran, etc.). Freeze-thaw repetition is not recommended as it can decrease viability. Back-up deep-freezers or liquid nitrogen storage sites are recommended. Multiple protocols for freezing are summarized below (each uses screw-cap polypropylene cryotubes)

a. Non-sporulating fungi or embedded mycelia: 10% glycerol is added to the tube and agar plugs of fresh culture are added and immediately frozen in liquid-nitrogen vapour (−170°C). Cultures are thawed at 37°C and plated.

b. Spores or mycelia from agar plate: 10% glycerol or 5% DMSO spore or mycelia suspension are made and frozen.

c. Liquid mycelia: Mycelia are macerated (not for use with human pathogenic fungi) and mixed to make a final concentration of 10% glycerol or 5% DSMO.

Bacteria

Many common culturable laboratory strains are deep-frozen to preserve genetically and phenotypically stable, long-term stocks. Sub-culturing and prolonged refrigerated samples may lead to loss of plasmid(s) or mutations. Common final glycerol percentages are 15, 20 and 25. From a fresh culture plate, one single colony of interest is chosen and liquid culture is made. From the liquid culture, the medium is directly mixed with equal amount of glycerol; the colony should be checked for any defects like mutations. All antibiotics should be washed from the culture before long-term storage. Methods vary, but mixing can be done gently by inversion or rapidly by vortex and cooling can vary by either placing the cryotube directly at −50 to −95 °C, shock-freezing in liquid nitrogen or gradually cooling and then storing at −80 °C or cooler (liquid nitrogen or liquid nitrogen vapor). Recovery of bacteria can also vary, namely if beads are stored within the tube then the few beads can be used to plate or the frozen stock can be scraped with a loop and then plated, however, since only little stock is needed the entire tube should never be completely thawed and repeated freeze-thaw should be avoided.

12 Winter Health Tips for Children

Brace yourself for a long list of winter health tips and advice, protecting children from the harsh winter is not very hard provided that you follow some simple steps from basic hygiene to maintaining health of children.

1. **WASHING HANDS**

   Many of us use water very sparingly for our kids during winter since it is cold and untouchable. Try washing your little ones hands in warm water or hand santiser before they eat as it is the main route of infection for the communicable diseases like cold, flu, pneumonia.

2. **WASH YOUR WINTER WEAR REGULARLY**

   We seldom wash our sweater, jerkins as it takes a long time to dry. We think that in winter there is less sweating so it will not become soiled soon. But woolens have the tendency to attract dust and it may lead to dust allergy and asthma. Wash your wollens regularly.

3. **AERATE THE ROOMS**

   I can hear you thinking ,"Aerating the room in the winter!! Are you kidding? "
   Do you know that a closed warm room may attract more microbes than a room that has air flow in it? I am not asking you to open all the windows and doors. just leave the windows and doors open for a few minutes when the sun is shining bright so that clean air can flow through the room, also UV rays of the sunlight are a natural disinfectant.

4. **GENERAL THUMB RULE FOR WINTER WEAR FOR CHILDREN**

   There is a general thumb rule for dressing up the kids which is "Parents put one more layer on the child then they themselves need to keep warm"
   But this depends on the parent, kid and on the weather that particular day.

5. BATHE THE HAIR REGULARLY

Even I hesitate to wash the children's hair frequently in winter. It is advisable to wash the hair atleast once a week. Apply oil regularly to prevent a dry and frizzy hair.

6. DON'T FORGET THE SUNSCREEN

Even though it is winter, don't forget to apply sunscreen generously when the kids go out to play.

7. MOISTURIZE MOISTURIZE!!

Moisturizing is the key for maintaining perfect skin health of children in winter. If not adequately moisturized (atleast twice a day) it may lead to dry skin and eczema. After bath, dry the body properly and apply the moisturizer cream evenly throughout the skin and massage lightly.

8. COVER THE EARS

Protect the ears from the cold daft with a snug fitting hat.

9. CORRECT THE POSITION

Never give bottle of milk while the child is in lying position, as there is greater chance for the milk to flow to Eustachian tube. It may collect in the Eustachian tube and become a breeding zone for microbes and thereby resulting in middle ear infection.

10. CLEAN THE NOSE REGULARLY

The mucus may harden to thick crusts inside the nose more during winter. So it is necessary to clean the nose regularly. Wet a cotton swab in warm water and gently wipe the area.

11. MAINTAIN ADEQUATE HYDRATION

Even adults don't feel to drink water in the winter. Hydrate your child with adequate water intake. Warm water seasoned with jeera is a good choice for winter months.

12. WHAT FOOD TO GIVE TO KIDS DURING WINTER?

It is of utmost importance that you feed your child properly during the winter to maintain his health.

a. **Whole Grains:** Whole grains like Rice, wheat, jowar, bajra, oats as they have a warming up effect.
b. **Root Vegetables:** Root vegetables like carrot, beet, potato and radish build the immunity naturally.
d. **Fruits:** Fruits like apple, pomengranates and orange are most preferred fruits in winter.
e. **Dry Fruits:** Dry fruits can be made as powder, mixed in rotis and can be mixed in milk too.
f. **Green Leafy Vegetables:** Include more of greens in the meals, they are rich in Vitamin A , C and K.
g. **Garlic:** Try to include more garlic in the child's diet, as garlic contains a compound called allicin which has antiviral and antibacterial properties.
h. **Non-Vegetarian:** Although chicken and mutton are good for winter, they digest very slowly. In the winter, digestion is slower than usual and for children it may take even longer for digestion. So prevent frequent use of non veg in the child's diet.

PARENT SHOULD BE AWARE OF

- Fast breathing or working hard to breathe
- Not waking up or interacting
- Having no tears when crying
- Bluish skin color
- Being unable to eat or drink
- Enjoy the Winter with the Kids

Although there are so many reasons to worry over our children during winter…

"KEEP CALM AND ENJOY THE WINTER WITH THE KIDS"

LOOKING AT MIRROR

Ashish Kr. Sharma, Production Department

One day all the employees reached the office and they saw a big advice on the door on which it was written: "Yesterday the person who has been hindering your growth in this company passed away. We invite you to join the funeral in the room that has been prepared in the gym". In the beginning, they all got sad for the death of one of their colleagues, but after a while, they started getting curious to know who was that man who hindered the growth of his colleagues and the company itself.

The excitement in the gym was such that security agents were ordered to control the crowd within the room. The more people reached the coffin, the more the excitement heated up. Everyone thought: "Who is this guy who was hindering my progress? Well, at least he died!" One by one the thrilled employees got closer to the coffin, and when they looked inside they suddenly became speechless. They stood nearby the coffin, shocked and in silence, as if someone had touched the deepest part of their soul. There was a mirror inside the coffin: everyone who looked inside it could see himself.

There was also a sign next to the mirror that said: "There is only one person who is capable of setting limits to your growth: **it is YOU**." You are the only person who can revolutionize your life. You are the only person who can influence your happiness, your realization, and your success. You are the only person who can help yourself. Your life does not change when your boss changes, when your friends change, when your partner changes when your company changes. Your life changes when YOU change, when you go beyond your limiting beliefs when you realize that you are the only one responsible for your life. "The most important relationship you can have is the one you have with yourself".

MORAL:

The world is like a mirror: it gives back to anyone the reflection of the thoughts in which one has strongly believed.
The world and your reality are like mirrors lying in a coffin,
which show to any individual the death of his divine capability to imagine and create his happiness and his success.
It's the way you face Life that makes the difference.

YOUR FEELINGS ARE YOUR GOD

Renuka Malhotra, Management

Health, money and relationships are generally three types of problems which every person faces in his/her life. Whether you want to change your health, money, relationship or anything else, imagine what you want, Imagine and feel the love of having it. Imagine every scene and situation you can, with what you want and feel that you have it now. Try spending seven to ten minutes each day imagining and feeling having what you want. Do it each day until you feel or though you already have your desire fulfilled. Do it until you know that your desire belongs to you as you know your name belongs to you.

Some desires may just take few days to get fulfilled and others may take longer. Then, SIMPLY GET ON WITH YOUR LIFE, GIVING AS MUCH LOVE AND AS MANY GOOD FEELINGS AS YOU CAN BECAUSE THE MORE LOVE YOU GIVE, THE FASTER YOU WILL RECEIVE WHAT YOU DESIRE.

It will be more clear with an example-If a person is injured & is undergoing best medical treatment but his condition is not improving, it means he is imagining the injury more rather than the feeling of full recovery.

The way to tip the balance to recovery is to imagine and to feel having full recovery. The fact that you can imagine a full recovery means that it already exists. Impregnate your magnetic field with good feeling about anything and everything that makes you good.

After you have imagined and felt having what you want, you are literally in a new world, so don't contradict the new world by telling everyone about an injury that is not improving because then you are imagining the worst again, and you are back in the old world. If you can imagine the worst, that is what you will receive back. If you can imagine the best, that is what you will receive back.

If somebody asks how your injury is doing, you can say"I am feeling much better and my body is following" or you can say "This has been a blessing because it has made me appreciate my body and health more than I ever have in my life."

When you become more aware of how you are feeling and you care about more of your feelings, you will get to the point where the slightest dip in your good feelings will be unbearable for you and you will get yourself to feeling good right away. You are supposed to feel good and be happy most of the time, because you are meant to have an amazing life and there is no other way, you can receive it!

LET'S TOGETHER MAKE A VOW

"I am still determined to be cheerful and happy, in whatever situation I may be;
for I have also learned from experience that the greater part of our happiness
or misery depends upon our dispositions and not upon our circumstances."

Fun & Celebrations@Catalysts

welcome

NAME : Sumit Chauhan
DEPARTMENT : R&D
DATE of JOINING : 21 Sept, 2017

NAME : Florencia Joseph
DEPARTMENT : HR
DATE of JOINING : 22 Sept, 2017

WHO WE ARE

Catalysts was established in 2003. Having its corporate office in the largest state of Uttar Pradesh in India and Manufacturing units in the Hill state of Uttrakhand. It is a leading research and quality certified Biotech company. We are engaged in delivering enzyme based eco-friendly solutions to many industry verticals. We are a multilevel quality certified company having certification of ISO 9001:2008, FSSC 22000 and HALAL.

Our Process expertise based enzyme formulation are a key competitive advantage for Catalysts and thus for our customers. We have a modern fully-equipped technology centre, where application research is done extensively using substrates received from client side.

Our technical team provides real time process and troubleshooting support to various industries like Molasses Ethanol, Grain Ethanol, Carbohydrates processing, Malt extraction, Brewing process and sugarcane juice processing.

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(Let's Connect)

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