Food Microbiology_PG Diploma (Dietetics)_Sem II

UNIT:IV_Chapter: 8_Microorganisms used in Food Biotech, Probiotics, Prebiotics

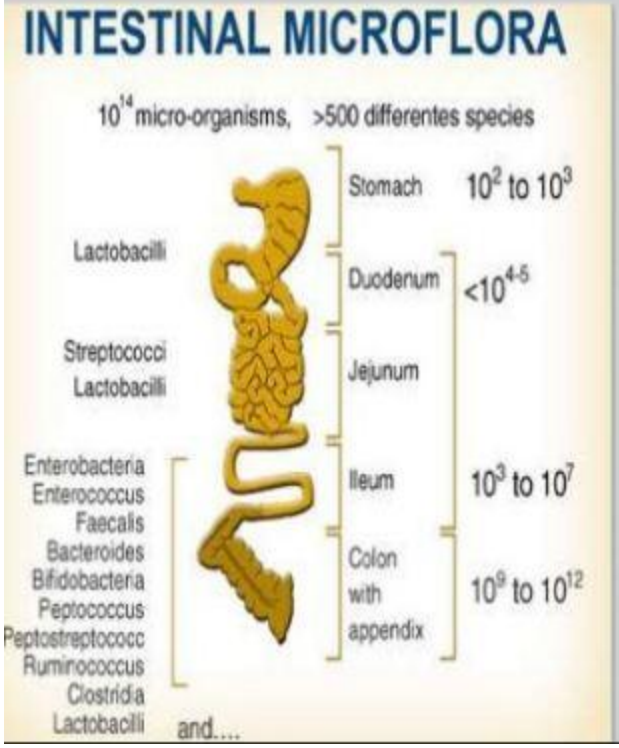
- World Health Organization:

"live microorganism which, when administrated adequate amounts, confer health benefits on hosts"

- A bacterial strain that
  - Survive stomach acid and bile
  - Adhere in intestinal lining
  - Grows and establishes temporary residence in intestine
  - Impart health benefits

- At the start of the 20th century, Russian noble prize winner and father of modern immunology, Elie Metchnikoff, a scientist at the Pasteur institute, was the first conceptualize "probiotics".
- In 1907 Metchnikoff proposed that the acid producing bacteria in fermented milk products could prevent "fouling" in the large intestine and if consumed regularly, lead to a longer, healthier life
- In early 1930's, in Japan, Minoru shirota developed a fermented milk product called Yakult (probiotic yogurt like product made by fermenting a mixture of skimmed milk with a special strain of Lactobacillus casei shirota).
- Probiotic term coined in 1965 by Lilly and Stillwell.

Facts about Intestine



- Trillions living bacteria exist in the human intestine
- We have more bacteria in our bodies (10 times greater) than the total number of our somatic and germ cells
- **We carry about 2 kg of bacteria !!!!!!!!**
- Over **500 species** of bacteria present in human colon.
- *Lactobacillus,Bifidobacterium* and *Acidophilus* comprise the majority of healthy bacteria in the colon along with other disease producing bacteria.

WHO NEEDS PROBIOTICS?

- People who suffer from yeast infections of any kind, including athlete's foot, jock itch, vaginal yeast infections and nail fungus.
- People with weak immune systems, frequent respiratory infections and congestion.
- People with food or respiratory allergies.
- People suffering from inflammatory bowel disorders, constipation or intestinal infections.

- **Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". The health benefit of the hosts targets primarily on the modulation of gut**

microbiota. Human gut microbiota includes the indigenous intestinal microflora that participates in diversified functions that improve host health

Characteristics of Effective Probiotics

- Able to survive the passage through the digestive system.
- Able to attach to the intestinal epithelia and colonize.
- Able to maintain good viability.
- Able to utilize the nutrients and substrates in a normal diet.
- non pathogenic and non toxic.
- Capable of exerting a beneficial effect on the host.
- Stability of desired characteristics during processing, storage and transportation.
- Anti-inflammatory,antimutagenic,immunostimulatory.

Advantages

- **Produce lactic acid**- lowers the pH of intestines and inhibiting bacterial villains such as *Clostridium, Salmonella,Shigella*, *E. coli,* etc.
- Decreases the production of a variety of toxic or carcinogenic metabolites.
- Aid absorption of minerals, especially calcium, due to increased intestinal acidity.
- Production of β-D galactosidase enzymes that break down lactose.

- Produce a wide range of antimicrobial substances acidophilin and bacitracin etc. help to control pathogenic bacteria .
- Produce vitamins (especially Vitamin B and vitamin k
- Act as barriers to prevent harmful bacteria from colonizing the intestines

Mechanism of Action of Probiotics

Inhibit Potentially Pathogenic Microorganisms (PPMs)

- Reduction in Intestinal pH
- Production of bacteriocins
- Competitive blocking of adhesion sites
- Competition for nutrients

Mechanism of Action of Probiotic

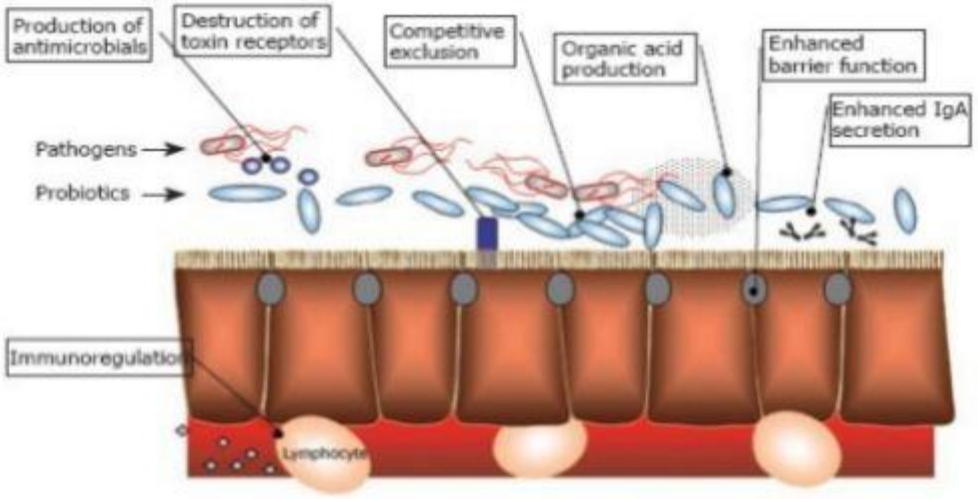
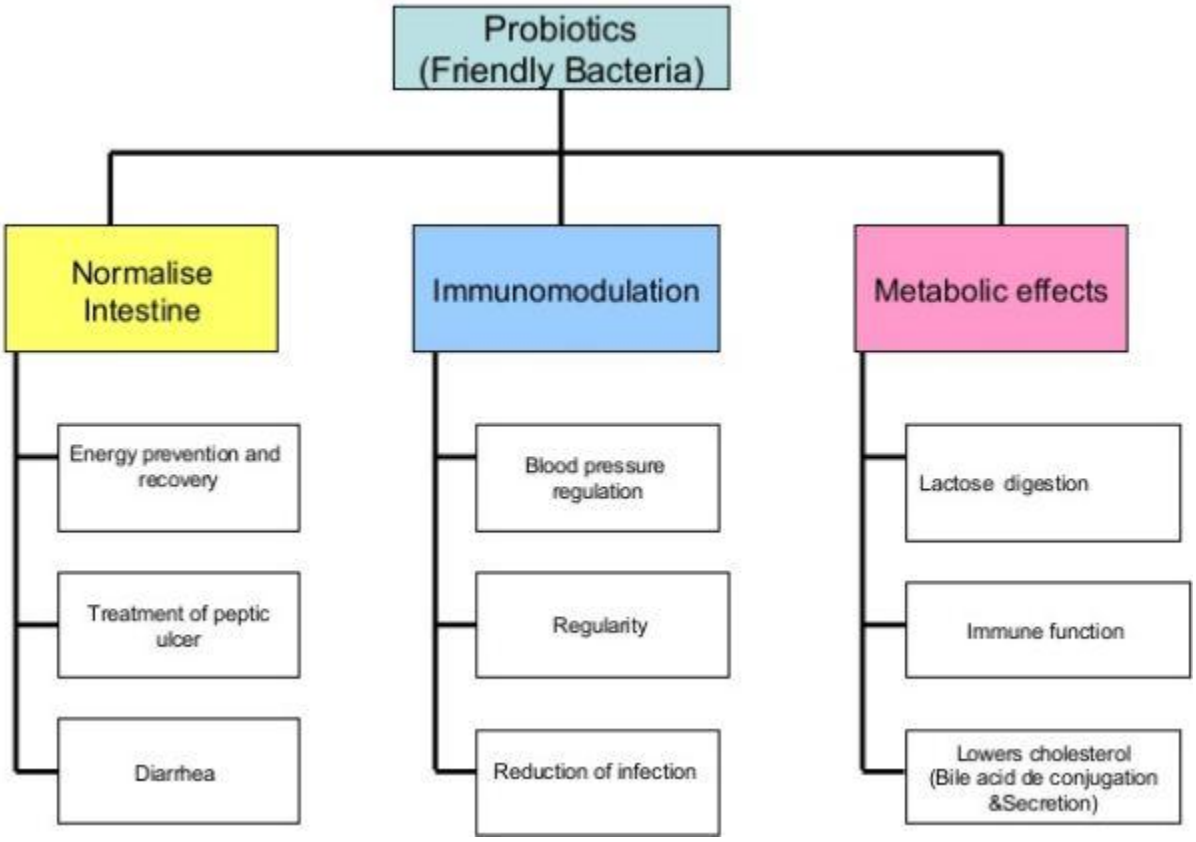


Figure 3 Mechanisms of probiotic activity.



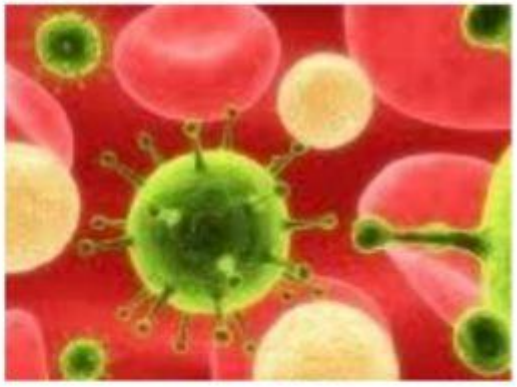
Probiotic strains

Lactobacillus species:

- *L. acidophilus*
- *L. plantarum*
- *L. casei subspecies rhamnosus*
- *L. brevis*
- *L. delbreuckii subspecies bulgaricus*

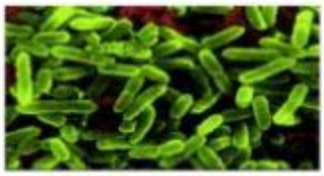
Bifidobacterium species

- ***B. adolescentis***
- ***B. bifidum***
- ***B. longum***
- ***B. infantis***
- ***B. breve***



Other Species of Probiotic

- ***Streptococcus salivarius* ssp. *thermophilus***
- ***Lactococcus lactis* ssp. *lactis***
- ***Lactococcus lactis s* ssp. *cremoris***
- ***Enterococcus faecium***
- ***Leuconostoc mesenteroides* ssp. *dextranicum***
- ***Propionibacterium freudenreichii***
- ***Pediococcus acidilactici***
- ***Saccharomyces boulardii***



Example of Probiotic Foods

Yogurt

- Usually made from milk (rarely, from cream) inoculated with *Streptococcus thermophilus* and either *Lactobacillus acidophilus* or *Lactobacillus bulgaricus*.
- Turkish in origin
- Available in innumerable forms and flavors
  1. Low fat chocolate yogurt
  2. Drinkable fruit-flavored goat yogurt
  3. Neon-colored yogurt in squeeze tubes

Frozen Yogurt

Laloo's Goat's Milk Frozen Yogurt, naturally chock-full of *S. Bulgaricus, L. Acidophilus* and *Bifidus.*

Juice

- *First probiotic juice launched in the fall of 2007 by Next Foods.*
- *Goodbelly, organic fruit juice-based probiotic beverage , contains L.Plantarum 299v, has effects on irritable bowel syndrome*
- *Three initial flavors include Brilliant Blueberry, Peach Mango and Strawberry Rosehip*

Attune Foods : Chocolate & Granola bars

Attune makes Wellness Bars in three chocolate varieties and three yogurt and granola varieties.

All products contain "more than 5 times the live active cultures in yogurt, with less sugar.

Kashi Vive Probiotic Digestive Wellness Cereal

Vive contains one billion CFUs of *Lactobacillus acidophilus* per serving.

Yakult Dairy Drink

- Probiotic, cultured dairy beverage
- Has citrus flavor
- Sold in single-shot containers that contain around 8 billion live and active *Lactobacillus casei shirota* cells per bottle

Ricera Rice Yogurt

An organic, non-dairy, rice "yogurt" with whole grains and live, active cultures, including *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus* and *Bifidobacterium bifidum.*

Multi-Probiotics

Research emerging on potential health benefits of multiple probiotic strains as a health supplement as opposed to a single strain.

1. Multibionta

Lactobacillus gasseri PA16/8, Bifidobacterium bifidum MF20/5, Bifidobacterium longum SP07/3

2. Acidophilus Pearls

Lactobacillus acidophilus, Bifidobacterium longum

3. Kyo-Dophilus

Lactobacillus acidophilus, Bifidobacterium bifidum, Bifidobacterium longum

4. Symprove live activated probiotic

Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus Casei, var. Rhamnosus, Enterococcus faecium, all in active state not freeze-dried

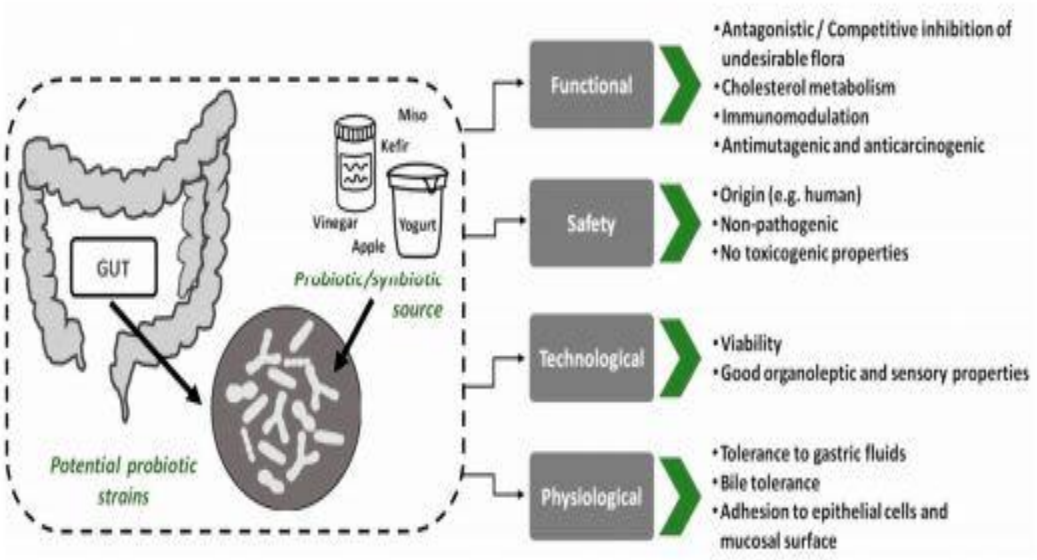


Figure 1. Criteria for the selection of probiotic strains.

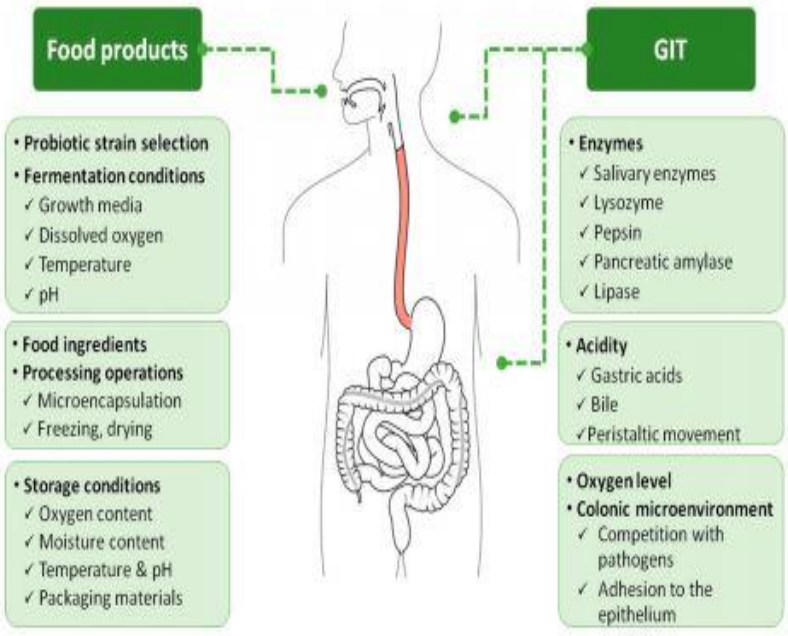


Figure 2. Factors affecting viability of probiotics in food products (during processing and storage), as well as in the gastrointestinal tract (GIT).

Side Effects of Probiotics

- Rare cases cause bloating, diarrhea, abdominal pain.
- If in excess cause infection that require medical attentions.
- People having on underlying disease or compromised immune system cause potential health problems like skin rash, fever, bloody stools etc.
- Sometimes interact with immunosuppressive drugs leading to life threating conditions. So people taking such drugs should avoid it.

Prebiotics

- A prebiotic is a non-digestible component which beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of colonic bacteria, thereby improving the health of the host .
- Examples : inulin, garlic, onions, chicory root, Asparagus, whole wheat, rye, barley

Characteristics of Prebiotics

- Should not be hydrolyzed or absorbed in the upper part of G.I tract.
- Should be a selective substrate for one or a limited number of potentially bacterial commercial to the colon culture protagonist.
- Should be able to alter the colonic micro flora towards a healthier composition or selectively stimulates the growth and or activity of intestinal bacteria associated with health and well being.
- Should help increase the absorption of certain minerals such as calcium and magnesium.
- Favorable effect on the immune system and provide improved resistance against infection.

Synbiotics

- PROBIOTICS + PREBIOTICS

- Foods containing the combination of probiotics and prebiotics are referred to as symbiotic.

- Improved survival in upper GIT and more efficient implantation.

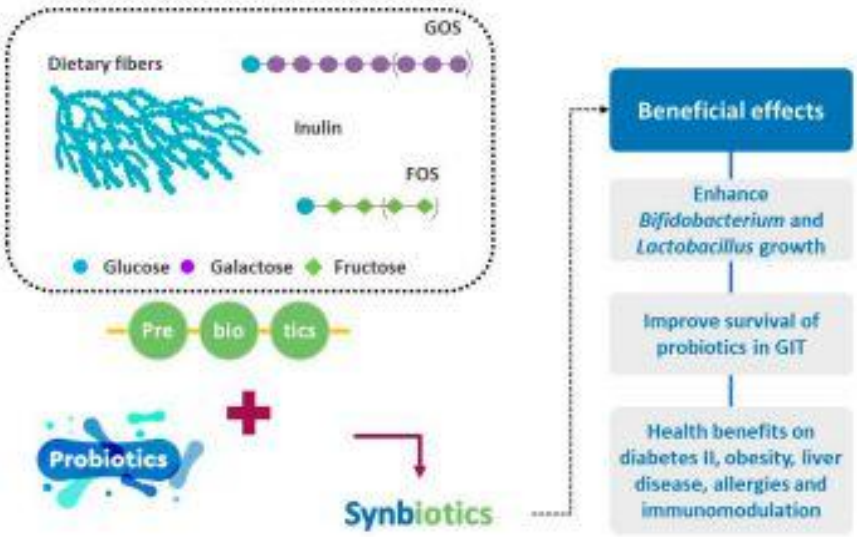


Figure 4. Beneficial effects of synbiotics.

Food applications of probiotic bacteria

Food	Microorganism	Coating Materials	Method
Apple juice	*Lactobacillus rhamnosus* GG	WPI alone and in combination with a modified resistant starch (RS)	Spray drying
Carrot Juice	*Lactobacillus casei*	Chitosan-Ca-alginate	Extrusion
Carrot juice	*Lactobacillus acidophilus*	Alginate-inulin-xanthan gum	Extrusion
Cheddar cheese	*Bifidobacterium longum*	Na-alginate and palmitoylated alginate	(i) droplet extrusion method (ADE) and (ii) emulsion method
Dry fermented sausages	*Lactobacillus reuteri*	Alginate	Extrusion
Fermented milk	*Lactobacillus casei* ATCC393	Chios mastic gum	Freeze drying
Fruit juice	*Lactobacillus paracasei* L26	Alginate	Extrusion
Fruit juice	*Lactobacillus rhamnosus* GG	Whey/alginate	Droplet extrusion with coating via electrostatic deposition
Fruit juices	*Bifidobacterium longum*, *Bifidobacterium breve*	poly-γ-glutamic acid	Freeze drying
Fruit juices	*Lactobacillus plantarum* and *Bifidobacterium longum*	Alginate or pectin coated with chitosan, gelatin or glucomannan	Extrusion
Ice cream	*Lactobacillus casei* Lc-01 and *Bifidobacterium lactis* Bb-12	Alginate and Hi-maize resistant starch	Emulsion
Iranian yogurt drink (Doogh)	*Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* Bb-12	Alginate	Extrusion

Kasar cheese	*Lactobacillus acidophilus* LA-5 *and Bifidobacterium bifidum* BB-12	Alginate	Emulsion or extrusion
kefir	*Bifidobacterium animals*	Sodium alginate	Extrusion
Mango juice	*Lactobacillus plantarum*	Calcium-Alginate-Soy Protein Isolate	Gelation
Mozzarella cheese	*Lactobacillus paracasei* ssp. *paracasei* LBC-1	Alginate	Extrusion
Oaxaca cheese	*Lactobacillus plantarum*	Aguamiel, Ag, or sweet whey, SW, as inner aqueous phase	Double emulsion
Pecorino cheese	*L. acidophilus*, *B. longum* and *B. lactis*	Na-alginate	Extrusion
Pecorino cheese	*Lactobacillus acidophilus* and a mix of *Bifidobacterium longum* and *Bifidobacterium lactis*	Alginate	Extrusion
Pomegranate juice	*Lactobacillusplantarum*	Alginate beads coating with double layer Chitosan	Extrusion
White-brined cheese	*Bifidobacterium bifidum* BB-12 and *Lactobacillus acidophilus* LA-5	Alginate	Emulsion or extrusion
Yogurt	*Lactobacillus acidophilus* LA-5	Pectin – Whey protein	Ionic gelation and complexation
Yogurt	*Bifidobacterium bifidum* F-35	Whey/alginate	Extrusion
Yogurt	*Lactobacillus acidophilus* ATCC 4356	Alginates	Extrusion
Yogurt	*Bifidobacterium animalis* subsp. *lactis* Bb12 and *Lactobacillus rhamnosus*	Alginate	Extrusion
Yogurt	*Lactobacillus plantarum*	Sodium alginate or pectin, coated with sodium alginate or chitosan	Extrusion
Yogurt	*Lactobacillus casei*	Sodium alginate (A), amidated low-methoxyl pectin (P), and blends	Extrusion
Yogurt	*Lactobacillus acidophilus*	alginate and chitosan	Extrusion
Yogurt—Ice cream	*Lactobacillus acidophilus* La-5	Na-alginate	Extrusion

Conclusions

- Probiotic bacteria confer health benefits by bolstering protective, structural and metabolic functions in the human body.
- Not all probiotics are equal.
- Disconnect between scientific evidence and allowable claims.
- Claims should be substantiated with well-controlled clinical studies.
- Products should be characterized for content and stability.

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Microorganisms used in food biotechtechnology

Introduction

Microorganisms have been used for preparing food products like bread, yoghurt or curd, alcoholic beverages, cheese, etc, for a long time without even knowing their involvement in fermentation. Louis Pasteur showed the role of microorganisms in spoilage and subsequent elucidation that fermentation also involves microorganisms. Once this fact was established, the scientists tried to isolate microorganisms, which were more efficient in producing better products or improvement of processes. Some species are useful for development of flavour unique to certain wines. Thus traditionally certain microorganisms were used in such fermented foods.

Organic Acids by Microorganisms

Citric acid is the most important organic acid produced by fermentation with an estimated annual production of about half a million tonnes with the value more than half a billion dollars. It is primarily used in foods. Some of the other acids produced in large quantities by fermentation are gluconic acid, lactic acid and ascorbic acid, each with production over 50,000 tonnes per annum.

Citric acid had been prepared from citrus fruits like lemon but now it is mostly produced by fermentation using *Aspergillus niger*, in large corrosion resistant fermenters having stirrers. Some yeasts like *Candida* have also been used to a smaller extent. A smaller amount is also made by older technique with surface fermenters. In submerged culture, when environmental conditions are controlled, organisms grow into small pellets. Sugar from cane molasses is commonly used in the medium, which needs to be controlled for trace metals like iron, copper etc. Maintenance of very low *p*H avoids by-products formation. High aeration rate is needed for higher yields. Conversion of glucose to product is high (70-90%) depending on the strain, purity of carbohydrate raw material, and environmental conditions. By-product formation of oxalic or gluconic acid can be reduced by strict control of growth conditions (Roehr *et al*, 1996).

A. niger strains have been developed by mutagenesis and screening, for higher productivity and adaptability to industrial fermenters. Some studies have been undertaken on parasexual recombination, diploidization, and heterokaryon formation, etc. (Visser, 1991). Although recombinant DNA technology has been reported for *Aspergillus* species, no reports are available on using this technique for commercial citric acid production. Genes cloned from *A. niger* for pyruvate kinase and phosphofructokinase will tremendously improve the commercial strains producing citric acid.

Lactic acid is another important acid produced by fermentation, although an equal amount is also chemically synthesised. The acid is mostly used for the manufacture of emulsifiers and as additives in food industry. It has two enantiomers, L(+)- and D(-)-lactic acid. The L-lactic acid is involved in normal human metabolism which can selectively be produced by fermentation and this is used in food applications, whereas the chemical synthesis produces DL-lactic acid.

Strains of *Lactobacillus delbruckii, L. casei, L. helveticus* and *L. acidophilus*, employed in commercial fermentation, can ferment a medium containing 12-15% sugar in 2 to 4 days with more than 90% yield (Kascak *et al*, 1996). Most lactobacilli cannot use starch. *L. amylophilus* and *L. amylovorus* are able to ferment starch to lactic acid (Zhang & Cheryan, 1991). The production of lactic acid or products like ethanol, acetic acid, etc. depends on the strains as well as the substrate and environmental conditions (Cheng

Biotechnology of Dairy Products

Lactic acid bacteria (*Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Bifidobacterium*, and *Lactococcus*) have been used to improve the flavour, texture, preservation and nutritive value of dairy as well as vegetable, cereal and legume fermentation products including yoghurt, buttermilk, cheese, pickled vegetables, idli, etc. (Luchansky *et al*, 1988; Wood, 1992). In addition, some are even used as probiotics, which contribute to the overall health of the user. In milk, the lactic acid bacteria ferment lactose and other sugars. Some proteases play role in the process along with the sugar metabolising enzymes. Formation of these products as well as compounds affecting flavour and texture gives the typical pleasant aroma, taste and body to the product. The metabolic activity also forms some useful vitamins. Many lactic acid bacteria like *L. acidophilus* and *L. sake* produce antimicrobial bacteriocins, which help in controlling unwanted microorganisms. Molecular strategies are being studied. Genetically engineered lactics with better fermentation efficiency, better shelf-life, nutritional and sensory properties for the product, etc. will be the target of these studies (Lin & Savage, 1986).

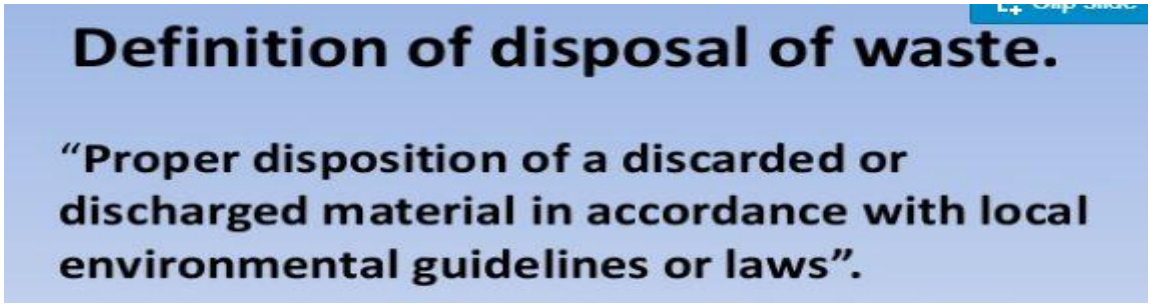
When cheese, yoghurt, etc. are made, undesirable contaminants can lead to poor flavour, low yield and food poisoning. Lactic acid bacteria can be genetically engineered to grow faster than the contaminants, as well as inhibit and destroy the growth of the contaminants including pathogens by producing antimicrobial agents. The starter cultures have been modified to produce an antimicrobial agent, which destroys cell walls of *Listeria monocytogenes*. Similar modification can also be carried out to protect against organisms like Salmonella.

Miscellaneous Microbial Products

Candida utilis has been used industrially in the production of SCP for food and fodder, waste treatment and the production of fine chemicals used as flavour enhancers (Boze *et al*, 1992). Among the products useful in foods, besides SCP, are 5'-GMP & 5'-IMP, ethanol, ethylacetate, acetylaldehyde, amino acids like serine, histidine, glutamic acid and lysine, xylitol, etc. *C. utilis* does not possess enzymes to hydrolyse starch, cellulose or pectic substrates. Two-step dual fermentation can be carried out using *C. utilis* with organisms like *S. fibuliger*, which produces amylases and can be used in starch wastes, and *T. reesei*, which has cellulases and can be used in cellulosic waste. Molecular genetics of *C. utilis* is not adequately studied. Although some transformations have been successfully carried out, no commercial strain has been developed by GMO including protoplast fusion. Since some of the enzymes are lacking in this organisms, incorporation of genes encoding these enzymes would produce a desirable modified organism with application in food industry.

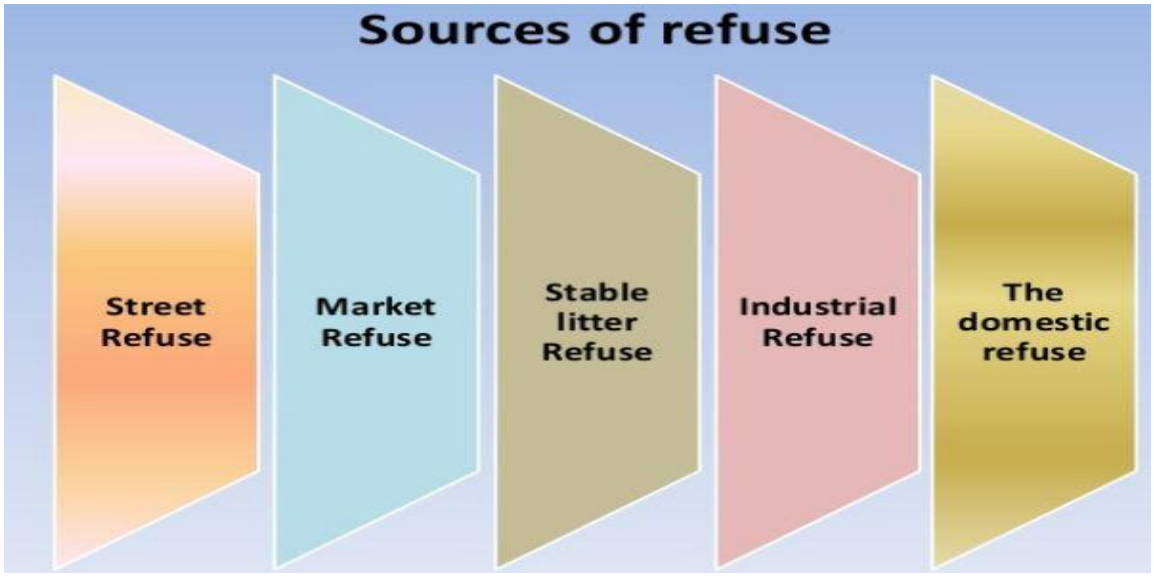
Bacillus species have provided traditional biotech products such as extracellular enzymes and insect toxins. *B. thuringiensis* strains with toxicities against a variety of pests have been exploited to the extent of getting the genes inserted into food crops for successful development of resistance against these pests. Protein engineering and molecular technologies will slowly replace screening programmes.

Chapter 7: Refuse disposal: Methods of collection, storage, and disposal



HOW IT AFFECTS HEALTH?

- It decomposes and favors fly breeding
- It attracts rodents
- The pathogens may be conveyed back to man's food through flies and dust.
- Water and soil pollution,
- An unsightly apperance, bad odors.
- Incidence of vector- borne diseases.



- Refuse that is collected by the street cleansing service or scavenging is called **street refuse**.
- Refuse that is collected from markets is called **market refuse**.
- Refuse that is collected from stables is called **stable litter**.
- **Industrial refuse** comprises a wide variety of wastes ranges from completely inert materials such as calcium carbonate to highly toxic and explosive compounds.
- The **domestic refuse** consists of ash, rubbish and garbage.

Storage

1.) The "**galvanized steel dustbin"** with close fitting cover is a suitable receptacle for storage of refuse.

- The capacity of a bin will depend upon the number of users and frequency of collection.
- The output of refuse per capita per day in India is estimated to vary from 1/10 to l/20 c.ft
- For a family of 5 members, a bin having a capacity of 5/10 or 1/2 c.ft would be needed. If collection is done once in 3 days, a bin having a capacity of 1½, or 2 c.ft would be adequate.

2.) A recent innovation in the western countries is the **"paper sack"**

3.) Public Bins: Public bins cater for a larger number of people.

- They are usually without cover in India and are kept on a concrete platform raised 2 to 3 inches above ground level to prevent flood water entering bins.
- In bigger municipalities, the bins are handled and emptied mechanically by lorries fitted with cranes.

Collection

- The method of collection depends upon the funds available.
- House-to-house collection is by far the best method of collecting refuse.
- People are expected to dump the refuse in the nearest public bin, which is usually not done.
- The collection methods normally practised in this country need drastic revision and improvement in the interest of better hygiene.
- The Environmental Hygiene Committee (1949) recommended that municipalities and other local bodies should arrange for collection of refuse.

- The open refuse cart should be abandoned and replaced by enclosed vans.
- Mechanical transport should be used wherever possible as it is more practical and economical than the 19th century methods.
- There is a wide variety of refuse collection vehicles of all shapes and sizes.
- The latest arrival in the western countries is the "Dustless Refused Collector" which has a totally enclosed body.

Methods of Disposal-:

- The principal methods of refuse disposal are :-
  (a) Dumping
  (b) Controlled tipping or sanitary land-fill
  (c) Incineration
  (d) Composting
  (e) Manure pits
  (f) Burial.

- The choice of a particular method is governed by local factors such as cost and availability of land and labour.

Dumping

- Refuse is dumped in low lying areas partly as a method of reclamation of land but mainly as an easy method of disposal of dry refuse.
- As a result of bacterial action, refuse decreases considerably in volume and is converted gradually into humus.
- A WHO Expert Committee (7967) condemned dumping as "a most insanitary method that creates public health hazards, a nuisance, and severe pollution of the environment".

- The drawbacks of open dumping are:

(1) the refuse is exposed to flies and rodents

(2) it is a source of nuisance from the smell and unsightly appearance.

(3) the loose refuse is dispersed by the action of the wind
and
(4) drainage from dumps contributes to the pollution of surface and ground water.

Controlled Tipping

- Controlled tipping or sanitary landfill is the most satisfactory method of refuse disposal where suitable land is available.
- It differs from ordinary dumping in that the material are placed in a trench or other prepared area, adequately compacted, and covered with earth at the end of the working day.
- The term "modified sanitary landfill" has been applied to those operations where compaction and covering are accomplished once or twice a week.

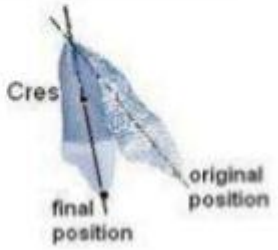
- Three methods are used in this operation :

1) **The trench method:** Where level ground is available.

- A long trench is dug out - 2 to 3 m (6-10 ft.) deep and 4 to 12 m. (12-36 ft.) wide, depending upon local conditions. The refuse is compacted and covered with excavated earth.

2) The ramp method : This method is well suited where the terrain is moderately sloping

Controlled Tipping:
If we want the following movement:



(3) The area method: This method is used for filling land depressions, disused quarries and clay pits.

- The refuse is deposited, packed and consolidated in uniform layers up to 2 to 2.5 m (6-8 ft.) deep.
- Each layer is sealed on its exposed surface with a mud cover at least 30 cm (12 inches) thick.
- Such sealing prevents infestation by flies and rodents and suppresses the nuisance of smell and dust.
- This method often has the disadvantage of requiring supplemental earth from outside sources.

- Chemical, bacteriological and physical changes occur in buried refuse.
- The temperature rises to over 60 deg. C within 7 days and kills all the pathogens and hastens the decomposition process.
- Then it takes 2 to 3 weeks to cool down.
- Normally it takes 4 to 6 months for complete decomposition of organic matter into an innocuous mass.
- The bulldozer achieves the tasks of spreading trimming and spreading top soil.

Incineration

- disposing hygienically by burning or incineration.
- It is the method of choice where suitable land is not available.
- Hospital refuse which is particularly dangerous is best disposed of by incineration.
- A preliminary separation of dust or ash is needed.
- All this involves heavy outlay and expenditure, besides manipulative difficulties in the incinerator.
- loss to the community in terms of the much

Incineration

Composting

- Composting is a method of combined disposal of refuse and night-soil or sludge.
- It is a process of nature whereby organic matter breaks down under bacterial action resulting in the formation of relatively stable humus-like material, called the compost which has considerable manurial value for the soil
- The principal by-products are carbon dioxide, water and heat.

COMPOSTING

- Composting is a method of combined disposal of refuse and night soil or sludge.

Organic matter

Bacterial action

Relatively stable humus-like material

Manurial value for the soil.

- The heat produced during composting -60 deg C or higher, over a period of several days- destroys eggs and larvae of flies, weed seeds and pathogenic agents.
- The end-product is a good soil builder containing small amounts of the major plant nutrients such as nitrates and phosphates.

- The following methods of composting are now used :
  - Bangalore method (Anaerobic method)
  - Mechanical composting (Aerobic method)

1)Bangalore Method-:

- Trenches are dug 90 cm (3 ft.) deep, 1.5 to 2.5 m (5-8 ft.) broad and 4.5 to 10 m (15-30 ft.) long, depending upon the amount of refuse and night soil to be disposed of.
- The pits should be located not less than 800 m (712 mile) from city limits.

- **The composting procedure is as follows :**

- First a layer of refuse about 15 cm (6 in) thick is spread at the bottom of the trench.
- Over this ,night-soil is added corresponding to a thickness of 5 cm (2 in).
- Then alternate layers of refuse and night-soil are added in the proportion of 15 cm (6 in) and 5 cm (2 in) respectively, till the heap rises to 30 cm (1 ft.) above the ground level.
- The top layer should be of refuse, at least 25 cm (9 in) thickness.
- Then the heap is covered with excavated earth.

"If properly laid, a man's legs will not sink when walking over the compost mass"

2)Mechanical Composting-:

- The refuse is first cleared of salvageable materials such as rags, bones, metal, glass and items which are likely to interfere with the grinding operation.
- It is then pulverised in a pulverising equipment in order to reduce the size of particles to less than 2 inches.
- The entire process of composting is complete in 4 to 6 weeks.

Manure Pits

- The problem of refuse disposal in rural areas can be solved by digging 'manure pits' by the individual householders.
- The garbage, cattle dung, straw, and leaves should be dumped into the manure pits and covered with earth after each day's dumping.
- Two such pits will be needed, when one is closed, the other will be in use.
- In 5 to 6 month's time, the refuse is converted into manure which can be returned to the field.

Burial

- This method is suitable for small camps.
- A trench 1.5 m wide and 2 m deep is excavated, and at the end of each day the refuse is covered with 20 to 30 cm of earth.
- When the level in the trench is 40 cm from ground level, the trench is filled with earth and compacted, and a new trench is dug out.

- The oxidation pond is an open, shallow pool 1 to 1.5 m (3-5 ft.) deep with an inlet and outlet.
- To qualify as an oxidation pond there must be the presence of (1) algae (2) certain types of bacteria which feed on decaying organic matter, and (3) sun-light.
- has been referred to by many different names – waste stabilization pond, redox pond, sewage lagoons, etc. The term "waste stabilization pond" is more appropriate.
- Over 50 ponds are working at present in India.

- The oxidation ponds are predominantly aerobic during sunshine hours as well as some hours of the night. In the remaining hours of the night, the bottom layers are generally anaerobic.
- Thus the sewage purification in oxidation ponds is brought about by a combination of aerobic and anaerobic types of bacteria.
- Mosquito nuisance is avoided by keeping weed growth in the neighbourhood of oxidation ponds to a minimum and the water line free from marginal vegetation.
- There is no odour nuisance associated with these ponds when they are properly maintained.