
UNIT 4 BENEFICIAL ROLE OF MICRO-ORGANISMS

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4.0 OBJECTIVES

After reading this Unit, we shall be able to:

- describe beneficial role of microorganisms;
- explain fermentation – its science and technology;
- comprehend the needs and types of food fermentations;
- state common examples of different types of food fermentations; and
- attribute fermented foods as functional foods.

4.1 INTRODUCTION

Food microbiology can be divided into three focus areas; beneficial microorganisms, spoilage microorganisms, and disease causing microorganisms. Beneficial microorganisms are those used in food fermentation to produce products such as cheese, fermented meat (pepperoni), fermented vegetables (pickles), fermented dairy products (yoghurt), and ethnic fermented products such as sauerkraut, idli and kimchi. In fermented products (produced by natural or controlled fermentation), microorganisms metabolize complex substrates to produce enzymes, flavor compounds, acids, and antimicrobial agents to improve product shelf-life and to prevent growth of pathogens and to provide product attributes. Micro organisms with their enzymes, also breakdown indigestible compounds to make the product more palatable and easy to digest. In addition, the beneficial microorganisms also serve as probiotics to impart direct health benefit by modulating the immune system to provide protection against chronic metabolic diseases, bacterial infection, atherosclerosis, and allergic responses. Examples of beneficial microorganisms are *Lactobacillus acidophilus*, *Lactobacillus arabinosus*, *Lactobacillus lactis*, and *Pediococcus cerevisiae*. Food spoilage microorganisms are those which upon growth in a food, produce undesirable flavour (odour), texture and appearance, and make food unsuitable for human consumption. Sometimes uncontrolled growth of many of the beneficial microorganisms can also cause spoilage. Food spoilage is a serious issue in developing countries because of inadequate processing and refrigeration facilities. Examples of food spoilage microorganisms are *Brochotrix*, *Lactobacillus*, *Bacillus*, *Pseudomonas* spp. and some molds. The micro-environment created in a spoilt food generally discourages the growth of the pathogenic microorganisms, which are considered poor competitors.

4.2 FERMENTATION

The uniqueness of several microorganisms and their often unpredictable nature and biosynthetic capabilities, in a specific set of environmental conditions, have made them ideal candidates, in attempts to solve difficult problems in life sciences and other fields. Microorganisms have been used in various ways over the past many decades, to advance medical technology, human and animal health, food processing, food safety and quality, genetic engineering, environmental protection and agricultural biotechnology. The use of beneficial microorganisms in the food sector has been a long tradition, namely lactic acid bacteria and yeasts in fermentation processes; the former are widely used in the manufacture of fermented food and are among the best studied microorganisms. The fermentations may be by yeasts, bacteria, molds or combination of these organisms. Detailed knowledge of a number of physiological traits has opened novel potential applications for these organisms in the food industry, while other traits might be specifically, beneficial for human health.

The food and beverage industry exploits non-pathogenic microorganisms for the production of fermented foods. These foods are prepared from raw materials and acquire their characteristic properties by a process that involves microorganisms. In certain cases the endogenous enzymes of the wild micro flora nature to the raw material may play a decisive role. It is believed that fermented foods originated

from the Orient and date back to the prehistoric times. Originally, these were fermented “*spontaneously*” by autochthonous strains found in the raw materials or the environment; This was the start of traditional biotechnology. The most important were cheese, yoghurt, wine, vinegar, beer, bread and the traditional fungal fermentations used in Asia and Africa, for the production of food.

In the biochemical sense, the term fermentation refers to a metabolic process in which organic compounds (particularly carbohydrates) are broken down to release energy without the involvement of a terminal electron acceptor such as oxygen. Partial oxidation of the substrate occurs so that only a relatively small amount of ATP energy is released compared to the energy generated if a terminal electron acceptor is involved. Partial oxidation of a carbohydrate can give rise to a variety of organic compounds such as alcohols organic acids and acetone. The compounds produced by micro-organisms vary from organism to organism and are produced through different metabolic pathways.

The term fermentation can also be applied to any industrial process that produces a material that is useful to humans and if the process depends on the activity of one or more micro-organisms (consortia). These processes, known as industrial fermentations, are usually carried out on a large scale and in vessels in which the organisms are normally grown in liquid media. Some industrial fermentations are fermentation in the biochemical sense but the majority of microorganisms involved are aerobic and use oxygen (as terminal electron acceptor) and thereby metabolize carbohydrates completely.

A vast range of materials are produced by industrial fermentations. These include

- 1) Organic chemicals used as fuels, food additives, antibiotics and enzymes for use in the food and other industries. Vinegar is an example of a food additive produced by an industrial fermentation.
- 2) Organisms are produced on a large scale for the extraction of protein (single cell protein) that can be used as a part of the human diet. Quorn is an example of a single cell protein, It is produced from the fungus *Fusarium graminearum*. This mycoprotein, purified from the fungus, is currently available for use as a food and is incorporated into a range of dishes that appear on supermarket shelves. Meatless dishes with a high protein content made from Quorn are particularly appealing to vegetarians.
- 3) Yeast cells produced for use in industries such as the baking industry, which relies on the mass production of large amounts of baker’s yeast.
- 4) Foods produced on a large scale as a result of the activities of micro-organism, e.g. cheese, yogurt and bread.
- 5) Production of alcoholic beverages, e.g. beer and wines.
- 6) Cellular extracts used as food additives, e.g., yeast extracts from yeast cells produced as a by product of the brewing industry.
- 7) Industrial fermentations are now often considered under the heading of biotechnology, i.e. technology that uses living organisms and their products in the manufacturing and service industries.

4.3 FERMENTED FOODS AND THEIR IMPORTANCE

Fermented foods are those foods produced by the modification of a raw material of either animal or vegetable origin by the activities of micro-organisms. Bacteria, yeast and moulds can be used to produce a diverse range of products, that differ in flavour, texture and stability from the original raw material. The production of many fermented foods involves organisms that are biochemically fermentative. Lactic acid bacteria that ferment carbohydrates to produce lactic acid are particularly important, but yeasts also play a major role in some food fermentations, fermenting carbohydrates to produce ethanol and other organic chemicals. Moulds that do not ferment carbohydrates, also play an essential role in some food fermentations, for example, the production of cheeses (blue cheeses) and oriental foods (soy sauce). Fermented foods are an extremely valuable addition to the human diet for a whole variety of reasons:

- 1) **Increase in variety:** Fermented foods increase the variety of foods that are available, adding to our diet a group of highly nutritious products with unique characteristics. There are, for example, about 1000 different types of cheeses.
- 2) **Use of ingredients:** Fermented foods form an important ingredient for a wide variety of dishes and are often used to impart special flavours, e.g. pepperoni in pizzas, yoghurt in curries, cheeses in a whole range of dishes, including soups, and soy sauce in stir-fry dishes.
- 3) **Improvement in nutritional quality:** The fermentation process may improve the nutritional quality of a raw material. Here are some examples:
 - Tempeh fermentation raises the vitamin B₁₂ content of the original soybean .
 - Tapioca fermentation doubles the protein content of cassava and increases the level of essential amino acids.
 - The presence of yeasts in a fermented food will increase the vitamin B content.
 - Antinutritional factors such as phytates, glucosinolates and lectins may be removed by the fermentation process.
 - Fermentation may lead to an increase in the bio-availability of minerals.

These improvements in the nutritional value of raw material will have little effect in the balanced diets of Western populations. However, for populations that subsist on diets consisting largely of polished rice, maize or other starches, such as in Africa and Asia, the contribution that fermented foods make to the intake of B group vitamins and proteins is highly significant.

- 4) **Preservation:** Fermentation often preserves a raw material, improving safety with regard to food-borne pathogens and increasing shelf-life; compare the shelf-life of raw milk (only a few days) with the shelf-life of yoghurt (several weeks).
- 5) **Health benefits:** Some fermented foods are said to have definite health benefits, although the scientific evidence for this is limited. Reports suggest

that fermented milk products such as yoghurt can reduce serum cholesterol levels and help avoid cancers, particularly, those associated with the colon. 'Bio' yoghurts (AB and ABT yoghurts) are said to have a restorative effect on a normal micro flora, assisting recovery of a normal balanced flora after oral antibiotic therapy.

- 6) **Improved digestibility:** Some fermented foods are more easily digested than the original raw material. People who cannot digest lactose properly (show lactose intolerance) can often consume some types of fermented dairy products (particularly yoghurts) without harmful effects. Lactose intolerance is due to the absence of the enzyme galactosidase in digestive juices, which converts lactose to glucose and galactose. Ingestion of dairy products leaves unabsorbed lactose in the gut, which is fermented by the normal gut flora giving flatulence, abdominal pain and diarrhoea. The fermentation of milk converts the difficult to digest lactose to the more easily digested lactate, and the galactosidase in live starter culture organisms appears to assist in the digestion of any residual lactose. Legumes, e.g. soybean, contain oligosaccharides such as stachyose which are fermented in the gut to yield gas and the associated socially embarrassing flatus. The oligosaccharides are broken down to readily digestible monosaccharides and disaccharides during fermentation of legumes by moulds, thus removing the problem.

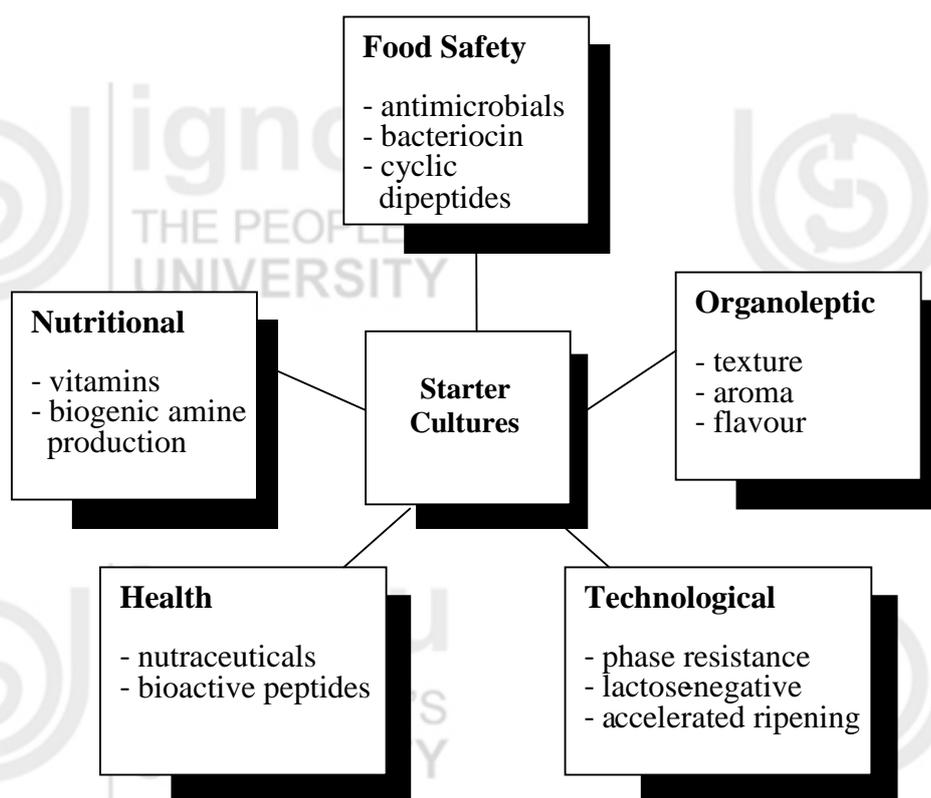


Fig. 4.1: Functional properties of starter cultures for food fermentation

- 7) **Detoxification of raw materials :** The fermentation process may remove toxic chemicals present in the raw material. Cassava fermentation, for example, removes a cyanogenic (cyanide producing) glycoside; cassava is toxic if eaten raw.

4.4 FOOD FERMENTATION - SCIENCE AND TECHNOLOGY

The science of fermentation is known as zymology. Food fermentation involves all those fermentation processes where either the ultimate product is used directly as a food, as an additive to food or is a basic ingredient to the food or the by-product formed during fermentation, food waste utilization, their disposal or proper management. As a science the food fermentation, has an element of biological sciences especially the microbiology, genetics and biochemistry, as a technology the food technology, chemical engineering along with integral component of sciences involved in food toxicity, acceptability and food nutrition. For any process to make fermented food, essential components are raw materials, micro-organism(starter cultures), fermentation vessel and associated controls, processing, recovery and packing systems, but from fermentation technology point of view, the fermenter and micro-organism involved assumes prime importance. Since the end product would serve as a food, it is essential to evaluate it from nutritional toxicology and sensory quality point of view and thus, the knowledge of these aspects would form an integral part of food fermentation technology, The technological dimensions of food fermentation towards the application of engineering sciences in designing the fermenters and associated controls for optimum fermentation and product recovery are immense. As the product on the commercial scale would be marketed, which can occur only if it is economical and therefore the economics of such products and the associated marketing aspects cannot be ignored. Similarly, transfer of the knowledge to the scale of technology operation required is of paramount importance. The scale up operations, harvesting, biocatalysis of micro-organisms, product recovery, effluent treatment etc. are of concern to fermentation technologists. Food fermentation technology could be organized based either on the product or the system used to make the fermented foods or products derived from it similar to food biotechnology (refer Fig.4.2)

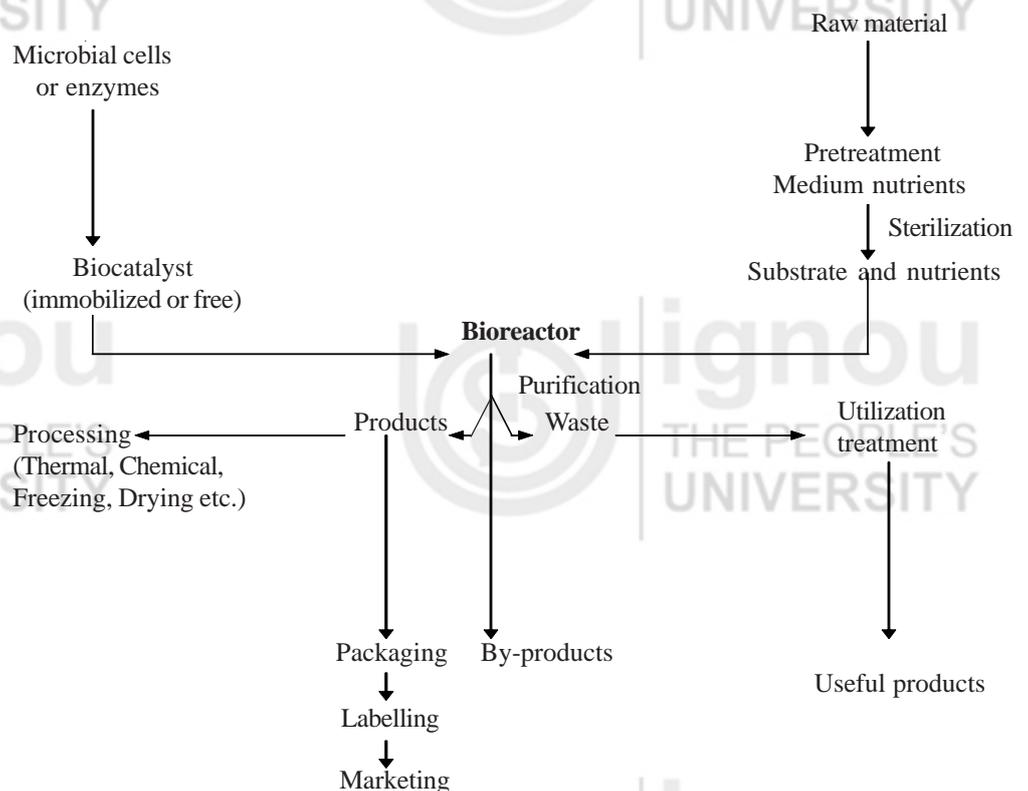


Fig 4.2: A generalized scheme of various operations employed in food fermentation technology

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1) What is fermentation?

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2) What is the purpose of fermentation?

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3) Define zymology.

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4.5 TYPES OF FOOD FERMENTATIONS

A number of different types of food fermentation can be recognized.

4.5.1 Acid Food Fermentation

These include acid fermented dairy products, e.g. cheese, butter, yoghurt and kefir; acid fermented vegetable products, e.g. sauerkraut, olives and various pickles; acid fermented meat products, e.g. the semi-dry fermented meats such as cerevelat and the dry fermented meats such as salami and pepperoni; sour dough breads.

The common feature of all these products is the use of lactic acid bacteria to carry out the basic fermentation process. Modern production systems usually involves the use of starter cultures. An exception is the fermentation of sauerkraut for which the process depends on lactic acid bacteria, that are natural inhabitants of the surface of cabbage leaves. Sometimes sugar is added to raw material to allow the lactic acid bacteria to produce sufficient acid for a successful fermentation. This is the case with fermented meats in which the sugar content of the raw material is very low. Salt may be added to suppress the growth of the normal spoilage micro flora and allow the lactic acid bacteria to dominate, e.g. sauerkraut, pickles and fermented meats. The raw materials may be pasteurized to eliminate pathogens and suppress natural contaminants that compete with the lactic acid bacteria, used in the starter culture.

4.5.2 Yeast Fermentation

Yeasts are important in food fermentation because of their ability to produce carbon dioxide and ethanol. Carbon dioxide is the important metabolic product in the manufacture of leavened bread whereas ethanol is metabolized in the production of beer, wine and spirits.

4.5.3 Solid State Fermentation

Solid state fermentation involves the use of a solid substrate into which the fermenting organism is inoculated. The organisms used are often molds. Examples are the 'koji' process and the second stage of tempeh fermentation.

4.6 COMMON EXAMPLES OF FOOD FERMENTATIONS

A very wide range of innumerable products of the food industry, such as sour cream, yoghurt, cheese, fermented meat, bread and other bakery products, alcoholic beverages, vinegar, fermented vegetables and pickles, etc., are produced through microbial fermentation processes. The efficiency of the strains of the organisms used, and the processes are being continuously improved to market quality products at more reasonable costs.

4.6.1 Oriental and Indigenous Fermented Foods

A large number of fermented foods can be grouped under the heading of oriental and indigenous fermented foods. Fermented foods of this type are produced in Asia and Africa and are often associated with specific countries or areas. Most of the products are unknown in the West but frequently have major nutritional role in the diets of the local population. Lactic acid bacteria are involved in some of the fermentation but yeast and moulds are often the main organisms responsible. Many are solid state fermentations or involve fermentations of more than one type. Some of the products are manufactured on large scale but many are carried out on a cottage industry or household basis. The major groups of these products with example/s are given below:

Table 4.1: Oriental and Indigenous Fermented Foods

S. No.	Product	Country/Region	Substrate	Micro-Organism(s) Involved	Nature and Use
1.	KANIMA	Nepal, Sikkim, & Darjeeling	Soyabeans	—	Solid snack food
2.	KHAMAN	India	Bengal gram	—	Solid, cake like breakfast food
3.	NATTO	Northern Japan	Soyabeans	<i>Bacillus natto</i>	Solid cake, as a meat substitute
4.	PAPADAM	India	Black gram	<i>Saccharomyces</i> sp.	Solid, crisp, condiment
5.	SOY SAUCE	Japan, China, Philippines & Oriental countries	Soybeans & Wheat	Lactic Acid Bacteria, <i>A. oryzae</i> , <i>A. sojae</i> , <i>Lactobacillus</i> sp., <i>Saccharomyces rouxii</i>	Liquid drink, seasoning agent for meat, fish and cereals.

S. No.	Product	Country/Region	Substrate	Micro-Organism(s) Involved	Nature And Use
6.	TEMPEH	Indonesia and nearby regions	Soyabeans	<i>Rhizopus sp.</i>	Fried in oil, roasted, as a meat substitute
7.	WARIES	India	Black gram flour	<i>Candida sp.</i> <i>Saccharomyces sp.</i>	Spongy, spicy condiment
8.	JALEBIES	India, Nepal & Pakistan	Wheat flour	<i>Saccharomyces bayanus</i>	Syrup filled confectionary
9.	KANJI	India	Rice & Carrots	<i>Hansenula anomala</i>	Sour liquid added to vegetables
10.	MISO	Japan, China	Rice & soyabeans	<i>Aspergillus oryzae</i> <i>Torulopsis etchellsii</i> <i>Lactobacillus sp.</i> <i>Saccharomyces rouxii</i>	Paste, soup base
11.	NAN	India Pakistan Afghanistan Iran	Unbleached wheat flour	—	Solid snack food/ bread
12.	DHOKLA	India	Bengal gram & wheat	—	Spongy condiment
13.	DOSAI	India	Black gram & rice	Yeasts	Spongy breakfast food
14.	IDLI	India	Black gram & rice	<i>L. mesenteroides</i> <i>T. candida</i> <i>Trichosporon pullulans</i>	Spongy, moist breakfast food
15.	TORANI	India	Rice	<i>H. anomala</i> <i>C. guilliermondii</i> <i>C. tropicalis</i> <i>G. candidum</i>	Seasoning for vegetables

4.6.2 Fermented Vegetable Foods

a) Sauerkraut

Sauerkraut is fermented fresh cabbage product. It is popular in USSR and Europe. The main organism involved in the fermentation of this pickle is lactic acid bacteria, *Leuconostoc mesenteroides* followed by *Lactobacillus plantarum*.

b) Cucumber pickle

Cucumber pickle is a fermentation product of fresh cucumbers. Several lactic acid bacteria are involved in preparation of this pickle. *Lactobacillus plantarum* is the most important organism required for fermentation of cucumber pickle.

4.6.3 Fermented Soyabean Products

a) Tempeh

Tempeh is a highly popular soyabean preparation in Indonesia. The chief organism in this preparation is the mold *Rhizopus oligosporus*. The boiled soybean seeds

are mashed and wrapped in banana leaves or kept in boxes or hollow tubes. It is inoculated with spores of tempeh fungus by addition of a portion of previous batch and allowed to ferment for about 20 hours at a temperature 32°C until there is a good growing mycelium but little sporulation. It is then sliced and prepared as per the taste such as roasting or frying. The taste of the tempeh is considered to be bland but it is highly nutritious.

b) Soya sauce

Soya sauce is a very popular preparation of Japan, which has received wide acceptance world over. This is prepared by inoculating *Aspergillus oryzae* (A. Soyae) in a mixture of soaked and steamed soy bean with roasted wheat in the ratio of 2:1. The mixture is incubated at 25-30°C for a period of 3 to 5 days. Subsequently, it is subjected to various processing steps using bacterium *Lactobacillus delbrueckii* and the yeast *Saccharomyces rouxii*. After 3 months, the final product is filtered, pasteurized and bottled for use.

c) Miso

It is made from fermented soyabeans and is a thick paste-like substance. Miso is brownish in color and tastes extremely salty and tangy on its own. *Saccharomyces rouxii* and *Torulopsis* are the yeasts and *Pediococcus halophilus* and *Streptococcus faecalis* are the bacteria, principally involved in Miso fermentation. While the most common use of miso is in Japanese-style miso soup recipes, miso also adds a unique burst of flavour to salad dressings, sauces and marinades, baked tofu, or vegetable dishes.

4.6.4 Fermented Dairy Products

The fermented dairy products assume greater importance in the human diets as invariably most of the diet consist of milk products especially the cheese, butter, yoghurt, curd etc. There are a number of fermented dairy products as shown below:

Table 4.2: Types of Fermented Dairy Products

Name	Country of origin	Milk types, conditions	Micro flora
Dahi (Dadhi)	India Persia	Milk (Cow/ Buffalo)	<i>L. lactis</i> subsp. <i>lactis</i> , <i>S. salivarius</i> subsp. <i>thermophilus</i> , <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>plantarum</i> , lactose fermenting yeast, Mixed culture
Srikhand (chakka)	India	Milk (Cow/ Buffalo)	<i>S. salivarius</i> subsp. <i>thermophilus</i> , <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> .
Lassi	India	Milk (Cow/ Buffalo)	<i>S. salivarius</i> subsp. <i>thermophilus</i> , <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> .
Cultured butter milk	Scandinavian and European countries	Milk (Cow/ Buffalo)	<i>L. lactis</i> subsp. <i>lactis</i> , <i>L. lactis</i> subsp. <i>diacetyllactis</i> , <i>Leuconostoc dextranicum</i> subsp. <i>citrovorum</i>
Acidophilus milk	Australia	Cow's milk	<i>L. acidophilus</i>
Yoghurt (bio-ghurt)	Middle Asia, Balkans	Cow's milk Goat's or mixed milk	<i>S. salivarius</i> subsp. <i>thermophilus</i> , <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>Micrococcus</i> and other lactic acid, bacteria, cocci, yeasts, molds

Name	Country of origin	Milk types, conditions	Micro flora
Kefir	Caucasus	Sheep's, Cow's, goat's or mixed milk, fermentation in skin bag or in wooden barrels	<i>L. lactis subsp. lactis</i> , <i>Leuconostoc spp.</i> , <i>L. delbrueckii subsp. caucassiuca</i> , <i>Saccharomyces kefir</i> , <i>Torula kefir</i> , micrococci, spore forming bacilli
Kumiss	Asiatic Steppes	Mare's, camel's or ass's milk fermentation in skin bag	<i>L. delbrueckii subsp. bulgaricus</i> , <i>Torula kumiss</i> , <i>Saccharomyces lactis</i> , micrococci, spore forming bacilli lactis, micrococci, spore forming bacilli
Leben, Labneh	Lebanon Arab countries	Goat's or sheep's milk, Fermentation in skin bag/ earthenware	<i>L. lactis subsp. lactis</i> , <i>S. salivarius subsp. thermophilus</i> , <i>L. delbrueckii subsp. bulgaricus</i> , lactose fermenting yeasts

4.6.5 Economically Important Fermentation Products

The growth of micro-organisms or other cells results in a wide range of products. Each culture operation has one or few set objectives. The process has to be monitored carefully and continuously, to maintain the precise conditions needed and recover optimum levels of products. Accordingly, fermentation processes aim at one or more of the following:

- production of cells (biomass) such as yeasts;
- extraction of metabolic products such amino acids, proteins (including enzymes), vitamins, alcohol, etc., for human and/or animal consumption or industrial use such as fertilizer production;
- modification of compounds (through the mediation of elicitors or through bio transformation); and
- production of recombinant products.

A. Microbial Biomass

Microbial biomass is produced commercially as single cell protein (SCP) using such unicellular algae as species of *Chlorella* or *Spirulina* for human or animal consumption, or viable yeast cells needed for the baking industry, which was also used as human feed at one time. Bacterial biomass is used as animal feed. The biomass of *Fusarium graminearum* is also produced for a similar use.

B. Microbial Metabolites

i) Primary metabolites

During the log or exponential phase organisms produce a variety of substances that are essential for their growth, such as nucleotides, nucleic acids, amino acids, proteins, carbohydrates, lipids, etc., or by-products of energy yielding metabolism such as ethanol, acetone, butanol, etc. This phase is described as the trophic phase, and the products are usually called primary metabolites. Commercial examples of such products are given in Table 4.3.

Table: 4.3: Examples of Commercially Produced Primary Metabolites

Primary Metabolite	Organism	Significance
Ethanol	<i>Saccharomyces cerevisiae</i>	alcoholic beverages
	<i>Kluyveromyces fragilis</i>	
Citric acid	<i>Aspergillus niger</i>	food industry
Acetone and butanol	<i>Clostridium acetobutyricum</i>	solvents
Lysine	<i>Corynebacterium</i>	nutritional additive
Glutamic acid	<i>glutamacium</i>	flavour enhancer
Riboflavin	<i>Ashbya gossipii</i>	nutritional
	<i>Eremothecium ashbyi</i>	
Vitamin B ₁₂	<i>Pseudomonas denitrificans</i>	nutritional
	<i>Propionibacterium shermanii</i>	
Dextran	<i>Leuconostoc mesenteroides</i>	industrial
Xanthan gum	<i>Xanthomonas campestris</i>	industrial

ii) Secondary metabolites

Organisms produce a number of products, in addition to the primary metabolites. The microbial growth phase, during which products that have no obvious role in metabolism of the synthesizing culture organisms are produced, is called the idiophase, and these products are called secondary metabolites. In reality, the distinction between the primary and secondary metabolites is not a straight jacket situation. Many secondary metabolites are produced from intermediates and end products of secondary metabolism. Some like the *Enterobacteriaceae* do not undergo secondary metabolism. Examples of secondary metabolites are given in Table 4.4.

Table 4.4: Examples of Commercially Produced Secondary Metabolites

Metabolite	Species	Significance
Penicillin	<i>Penicillium chrysogenum</i>	antibiotic
Erythromycin	<i>Streptomyces erythreus</i>	antibiotic
Streptomycin	<i>Streptomyces griseus</i>	antibiotic
Cephalosporin	<i>Cephalosporium acrimonium</i>	antibiotic
Griseofulvin	<i>Penicillium griseofulvin</i>	antifungal antibiotic
Cyclosporin A	<i>Tolypocladium inflatum</i>	immunosuppressant

iii) Production of Enzymes

Industrial production of enzymes is needed for the commercial production of food and beverages. Enzymes are also used in clinical or industrial analysis and now they are even added to washing powders (cellulase, protease, lipase). Enzymes may be produced by microbial, plant or animal cultures. Even plant and animal enzymes can be produced by microbial fermentation through techniques of genetic manipulations. While most enzymes are produced in the tropophase, some like the amylases (by *Bacillus stearothermophilus*) are produced in the idiophase, and hence are secondary metabolites. Examples of enzymes produced through fermentation processes are given in Table 4.5.

Table 4.5: Examples of Commercially Produced Enzymes

Organism	Enzyme
<i>Aspergillus oryzae</i>	Amylases
<i>Aspergillus niger</i>	Glucoamylase
<i>Trichoderma reesii</i>	Cellulase
<i>Saccharomyces cerevisiae</i>	Invertase
<i>Kluyzveromyces fragilis</i>	Lactase
<i>Saccharomycopsis lipolytica</i>	Lipase
<i>Aspergillus</i> species	Pectinases and proteases
<i>Bacillus</i> species	Proteases
<i>Mucor pusillus</i>	Microbial rennet
<i>Mucor meihei</i>	Microbial rennet

Check Your Progress Exercise 2

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1) What are the various types of fermentations?

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2) Give one example of each fermented food type-
Oriental, Indian, Caucasus and Lebanon.

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3) What are the major groups of commercially important fermentations?

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4.7 FERMENTED FOODS AS FUNCTIONAL FOODS

Dr. Elias Metchnikoff, a Nobel Laureate, around 100 years ago proposed that “*Lactic acid bacteria can render a great service in the fight against intestinal putrefaction*” and might “*postpone and ameliorate old age*”. This concept was developed further through the decades, and today, the emerging probiotics, prebiotics and synbiotics era is a subject of scientific debate and intense research. The use of probiotics, prebiotics, and synbiotics is a promising area for the development of functional foods.

Functional foods are generally characterized as foods similar in appearance to conventional foods, consumed as part of a usual diet, and providing health-related benefits beyond meeting basic nutritional needs. **A food can be considered naturally ‘functional’ if it contains a food component that affects one or more targeted functions in a beneficial way.** Dairy foods can be included in the functional food category because of their content of calcium, specific health-enhancing proteins, conjugated linoleic acid, sphingolipids, butyric acid, and probiotic cultures.

Dairy foods appear to be the preferred medium for introducing probiotic bacteria such as human-derived species of lactic acid bacteria (e.g., *L. acidophilus*, *L. casei*, *L. gasseri*, *L. rhamnosus*, *L. reuteri*, *Bifidobacterium bifidum*, *B. breve*, *B. infantis* and *B. longum*). *Lactobacillus spp.* (naturally found in the human small intestine) and various *Bifidobacterium spp.* (a major organism in the human large intestine) are the most commonly used probiotic cultures.

4.7.1 Probiotics

The term Probiotic means “for life”, is derived from the Greek language. It is now defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”. The most common probiotic organisms clinically useful include members of the bacteria such as *Lactobacillus* and *Bifidobacteria* species and some selected strains of *Streptococcus*, *Lactococcus* and members of yeast and molds such as *Saccharomyces*, *Aspergillus*, *Acanthosis* and *Candida* species.

4.7.2 Prebiotics

The term Prebiotic is defined as “short chained carbohydrates that are indigestible by human enzymes in the GIT (Gastro-intestinal tract) and selectively stimulate the growth and activity of specific species of bacteria in the gut, usually bifidobacteria and lactobacilli, with benefits to health”. The most commonly used prebiotics in

supplements are Fructo-oligosaccharides (FOS). Bifidobacteria, due to the presence of beta-fructofuranosidase enzyme are liable to break down and utilize FOS. This helps in stimulation of bifidobacterium growth in the GIT. FOS exhibits nutritional properties on colonic pH and stool bulking. It also increases bioavailability of essential minerals and decreases serum triglycerides.

The other types of prebiotic substrates include-

- Xylitol, Sorbitol, Mannitol
- Disaccharides-Lactulose, Lactilol
- Oligosaccharides-Raffinose, Soybean, Palatinose, Isomaltose, Lactosucrose
- Polysaccharides-In-ulin, resistant starch

4.7.3 Synbiotics

The term synbiotic is used when a product contains both probiotics and prebiotics. Since the word alludes to synergism, this term should be reserved for products in which the prebiotic compound selectively favors the probiotic compound, e.g., FOS in combination with strains such as Bifidobacterium *B. infantis*, *B. longum* etc. Combining probiotics with prebiotics could improve the survival of the bacteria crossing the upper part of the GIT, thus enhancing their effects in the large bowel. Moreover, the local and the systemic beneficial effects of probiotics and prebiotics might be additive or even synergistic.

4.7.4 Use of Probiotics

As per definition of probiotics the live microorganisms that are administered have to be in adequate amounts to confer a health benefit to the host. Clinical bodies have used probiotic levels of 1 billion to 10 billion or above.

Dosage

According to Earl Mindell, an internationally recognized expert on nutrition, healthy person should take 2-5 billion CFUs (colony stimulating units) of probiotics a day and those with GI conditions can take up to 10 billion CFUs per day. In acute infectious diarrhoea, lactobacillus is most effective at a dose of 10 billion CFUs during the first 48 hours, which translates to 5 billion CFUs per day. For prescription probiotics, the current daily intake recommended is 5-10 billion CFUs per day. Capsules and Sachets of Probiotic plus Prebiotic combination (Pro-wel) and Probiotic alone (Darolac) are commercially available. Benefits offered by Probiotic and Prebiotic combination formula are :-

- Maximum Colony Forming Units (CFUs): ensure complete action.
- Fructo-oligosaccharide (FOS): offers nutrition to the probiotics and normal intestinal flora.
- Acid-resistant cells: reach intestine in full force.
- Freezed-dried and nitrogen-flushed cells: offers excellent stability.
- Vegetable capsules: ensure universal appeal.

4.7.5 Health Benefits of Probiotics

Few well designed, well conducted human clinical trials of probiotics have been conducted over the past 30 years. Only in recent years, has the importance of choosing probiotic strains of demonstrated efficacy been recognized. With respect to health benefits of probiotics, research studies indicate the following:

- 1) Improved intestinal health.
- 2) Modulation of the immune response and immune function .
- 3) Reduced risk of cancer.
- 4) Reduced risk of heart disease.
- 5) Improved tolerance to milk.
- 6) Reduce food allergies.
- 7) Reduce ulcers by decreasing the growth of ulcer-inducing bacteria (i.e., *Helicobacter pylori*).

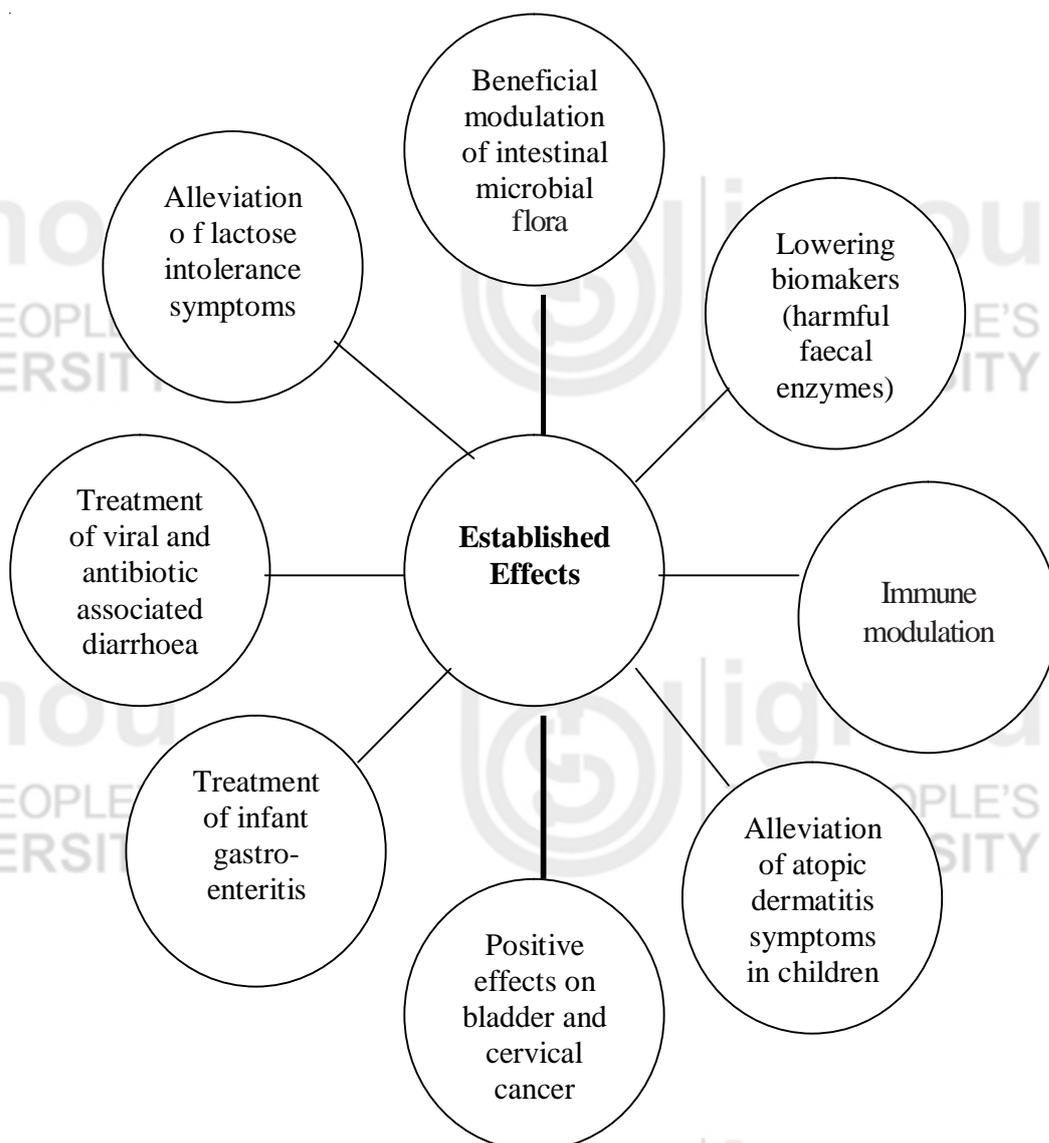


Fig. 4.3: Beneficial health effects claimed for probiotics

Check Your Progress Exercise 3

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1) What is a probiotic?
.....
.....
.....

2) What are the ideal properties of a probiotic?
.....
.....
.....

3) Define a prebiotic.
.....
.....
.....

4.8 LET US SUM UP

Fermentation is a relatively efficient, low energy preservation process which increases the shelf life and decreases the need for refrigeration or other forms of food preservation technologies. It is therefore a highly appropriate technique for use in developing countries and remote areas where access to sophisticated equipment is limited. Fermented foods are popular throughout the world and in some regions, make a significant contribution to the diet of millions of individuals.

In Asia the preparation of fermented foods is a widespread tradition. The fermented products supply protein, minerals and other nutrients that add variety and nutritional fortification to otherwise starchy and bland diets. For instance Soy sauce is consumed throughout the world and is a fundamental ingredient in diets from Indonesia to Japan. Over one billion litres are produced each year in Japan alone. Although fermentation of foods has been in use for thousands of year, it is likely that the underlying microbial and enzymatic processes responsible for the transformations were largely unknown. It is only recently that there has been a development in the understanding of these processes and their adaptation for commercialization. There is tremendous scope and potential for the use of micro-organisms towards meeting the growing world demand for food, through efficient utilization of available natural food and feed stocks and the transformation of waste materials. Genetic improvement of the organism is fundamental to the success of fermentation technology. Mutation and recombination are the two ways to meet this end.

The alarming increase in inappropriate use of antibiotics and enhanced bacterial resistance, along with renewed interest in ecological methods to prevent infections makes probiotics, prebiotics and synbiotics a very interesting field for research.

Evidence is beginning to accumulate, describing their beneficial effects in a variety of GI (Gastro Intestinal) and non-GI disorders. They offer dietary means to support the balance of the intestinal flora. They may be used to counteract local immunological dysfunction, to stabilize the intestinal barrier function, to prevent infectious succession of pathogenic microorganisms and to influence intestinal metabolism. Looking ahead, this field holds immense promise for the future in delivering novel therapies in different fields.

4.9 KEY WORDS

- Carbohydrates** : Organic compounds that consist of carbon, hydrogen and oxygen. They vary from simple sugars containing from three to seven carbon atoms to very complex polymers.
- CFUs** : Colony forming units.
- Enzyme** : A protein that acts as a catalyst. Every chemical reaction in living organisms is facilitated by an enzyme.
- Fermentation** : Enzymatic breakdown (catabolism) of carbohydrates generally in the absence of oxygen or changes in food caused by intentional growth of bacteria, yeast or mold.
- Fructo-oligosaccharides (FOS)** : A type of prebiotic/probiotic found in Jerusalem artichokes, shallots and onion powder which may improve gastrointestinal health.
- Food** : Those substances that are eaten or otherwise taken in the body to: sustain psychological and physiological life support growth and repair and replacement of tissues; and provide energy and nutrition. In essence, the sum of all the processes concerned with the growth, maintenance and repair of the body and/or its organs and systems.
- Food processing** : Using food as a raw material and changing it in some way to make a food product.
- Gastro intestinal** : Pertaining to the stomach, small and large intestines, colon, rectum, liver, pancreas, and gallbladder.
- Yeast** : Single cell organism, which as it grows converts carbohydrates by fermentation into alcohol and carbon dioxide.

4.10 ANSWERS TO CHECK YOU PROGRESS EXERCISES

Check Your Progress Exercise 1

Your answer should include the following points:

- 1) In the biochemical sense, the term fermentation refers to a metabolic process in which organic compounds (particularly carbohydrates) are broken down to release energy without the involvement of a terminal electron acceptor such as oxygen.

- 2) Food fermentation serves five main purposes:
 - Enrichment of the diet through development of a diversity of flavors, aromas, and textures in food substrates.
 - Preservation of substantial amounts of food through lactic acid, alcohol, acetic acid and alkaline fermentations.
 - Biological enrichment of food substrates with protein, essential amino acids, essential fatty acids, and vitamins.
 - Detoxification during food-fermentation processing.
 - A decrease in cooking times and fuel requirements.
- 3) The science of fermentation is known as zymology.

Check Your Progress Exercise 2

Your answer should include the following points:

- 1) The various types of food fermentations are acid, yeast and solid state fermentation.
- 2) An example of each fermented food type-
 - Oriental- Tempeh
 - Indian- Idli
 - Caucasus-Kefir
 - Lebanon- Leben
- 3) The major groups of commercially important fermentations are:
 - Microbial cells or biomass as the product, e.g. bakers yeast, lactobacillus etc.
 - Microbial enzymes: catalase, amylase, protease, pectinase, glucose isomerase, cellulase, hemicellulase, lipase, lactase, streptokinase, etc.
 - Microbial metabolites : Primary metabolites – ethanol, citric acid, glutamic acid, lysine, vitamins, polysaccharides etc. Secondary metabolites: all antibiotic fermentation

Check Your Progress Exercise 3

Your answer should include the following points:

- 1) Probiotics are dietary supplements containing potentially beneficial bacteria or yeasts. According to the currently adopted definition by FAO/WHO, probiotics are: 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host'.
- 2) Probiotics supplements are considered ideal, if they fulfill the following important characteristics: normal gut inhabitant, nonpathogenic, genetically stable, resistant to acid, capable of survival, proliferation and metabolic activity at the target site, high stability, viability at high populations and able to exert clinically documented health benefits

- 3) Prebiotics are a category of functional food, defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/ or activity of one or a limited number of bacteria in the colon, and thus improve host health.

4.11 SUGGESTED READING

Banwart, G.J. (1979). Basic Food Microbiology, AVI Publishing Co. Inc., Westport, Connecticut.

Frazier, W.C. and Westoff, D.C. (1996). Food Microbiology, Tata McGraw Hill Publishing Co. Ltd., New Delhi.

Pelczar, M. Jr., Chan, E.C.S. and Kreig, N.R. (1993). Microbiology, Tata McGraw Hill Inc., New York.

Garbutt, J. (1998). Essentials of Food Microbiology, Arnold International Student's Edition, London.