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Chapter: Application of Micro-organisms in food and Dairy Industry
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Table of Contents

Lesson: Application of Microorganisms in Food and Dairy Industry

Introduction

Food is the essence of life for all living organisms. We all eat food from plant and animal sources to get energy, to grow in size and to protect ourselves from diseases. Similarly microorganisms utilize food nutrients to carry out all their physiological functions and growth. Association of microorganisms with foods is well known to all of us in our daily life, *e.g.*, preparing dahi/curd by adding lactic acid bacteria to milk, souring and curdling of milk (spoilage of milk) if kept for long without refrigeration, development of green patches of fungi on bread during summers, gastroenteritis on consumption of contaminated food at a restaurant *etc*. These examples represent the advantages and disadvantages of the presence of microorganisms in food. Their growth in food may bring certain desirable changes as in case of fermentation of foods or undesirable changes as in case of spoilage of milk and bread. Some foods are eaten raw while others are processed to make them more palatable and exotic. Hence, it is important for us to understand the role of microorganisms in food production, spoilage, preservation and safety.

Sources of Microorganisms in Foods

Milk and eggs are generally sterile unless produced from infected animals. Interior tissues of meat are also free of microorganisms at the time of slaughter. Fruit juices/milk in tetra packs is stored at room temperature as long as the tetra pack is not opened. After opening, it requires refrigeration and consumption within 24 hours. Then why milk, meat, eggs, etc. cannot be stored for long and spoil if not placed in refrigerator? It is mainly because microorganisms find entry into these foods from various sources on coming in contact with the outer environment and grow there. This is called contamination of foods with microorganisms. Various sources of contamination of foods are given in Table 1 below.

Table 1: Various sources of contamination of food.

Source: Author

Sources of Contamination	Microorganisms
Soil	*Acinetobacter*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Pseudomonas*, *Psychrobacter*, fungi
Water	*Acinetobacter*, *Aeromonas*, *Alteromonas*, *Pseudomonas*, *Vibrio* and any pathogen entering from sewage contamination
Air and Dust	*Bacillus, Clostridium, Micrococcus, Paenibacillus,* Fungal spores
Food Utensils and Equipment	Depends on the type of food handled, *e.g.,* milk (lactic acid bacteria, *Escherichia, Bacillus* etc.), Bread (molds, *Bacillus* etc.)
Plants and Plant Products	*Citrobacter, Erwinia, Enterobacter, Burkholderia,* lactic acid bacteria*, Listeria, Pectobacterium*, yeast etc.
Food Handlers (cloths,	*Staphylococcus, Enterococcus, Clostridium, Bacillus,*

hairs, hands, nose, mouth, etc.)	*Corynebacterium, Escherichia* etc.
Animal Hides	Lactic acid bacteria, organisms found in soil, organisms associated with food handlers and gastrointestinal tract of animals
Animal Feeds	*Salmonella, Listeria, Bacillus, Clostridium, Enterococcus* etc.
Gastrointestinal Tract	*Escherichia, Enterococcus, Salmonella, Shigella, Hafnia, Campylobacter, Citrobacter* etc.

Interesting facts: Marry Mallon nicknamed 'Typhoid Marry' was an asymptomatic carrier of typhoid causing bacteria, *Salmonella typhi* in her gallbladder and continued to shed this bacteria in her faeces. She was responsible for infecting 51 people with typhoid, three of whom died, in New York City, USA, while working as a cook. She rarely cleaned her hands after using toilet and before cooking. It is an example of contamination of food from food handlers. That is why chefs and waiters at restaurants or workers in food processing units wear caps and gloves to cover their hairs and hands, respectively. It also emphasizes the need to maintain good personal hygiene with cleaning of hands before handling foods.

Food as Substrate for Growth of Microorganisms

Once microorganisms enter the food they will try to survive and grow by making use of nutrients present in that food. However, their survival and growth is dependent on the availability of favourable conditions both inside food (intrinsic) and its outside environment (extrinsic) (Table 2). Intrinsic environment of food constitutes its pH and oxidation–reduction (O/R) potential, amount of water, type of nutrients and antimicrobial properties. Extrinsic environment is the surroundings of the food in which it has been kept. It includes temperature of storage, relative humidity and gaseous atmosphere during storage. Knowledge of these factors associated with foods is important for any food microbiologist to assess:

a) What kind of microorganisms can grow and spoil a particular food?

b) What kind of preservation method can be applied to that food to prevent its spoilage?

Table 2: Effect of intrinsic and extrinsic food environments on the growth of microorganisms.

Source: Author

Food Environment	Effects on Microorganisms
Intrinsic	
pH	Foods like fruits and fruit juices, vegetables and dahi have low pH, which favour growth of yeast and fungi. Foods like milk, cream, meat and fish with near neutral pH favour growth of bacteria.
Water Content	Water content of food is measured as its water activity (a_W), *i.e.*, the ratio of vapour pressure of solution to vapour pressure of solvent. Bacteria require higher values of a_W for growth than fungi. The minimum a_W values for growth of spoilage bacteria and fungi are 0.91 and 0.80, respectively. Dried foods (milk powder, nuts, grains, dried fruits, etc.), frozen foods (Frozen vegetable,

	meat, etc.), jellies, candies remain unspoilt for long because of either their less water content or water is unavailable for the growth of microorganisms. On the other hand, on absorption of moisture from environment or rehydration, these products spoil much faster.
O/R Potential	The O/R potential of a food substance is positive if it is oxidised and negative if reduced. Food with +ve O/R potential e.g. fruit juices, ground meat, etc. will favour growth of aerobic microorganisms while food with –ve O/R potential e.g. cheese, solid meat, etc. will favour the growth of anaerobic microorganisms.
Nutrients	Simple nutrients are utilized first by microorganisms followed by breakdown of complex carbohydrates and proteins into simpler ones. Growth of bacteria and fungi will depend on their ability to utilize these complex macromolecules and on the availability of accessory growth factors.
Protective constituents	Natural fresh foods contain antimicrobial properties restricting the growth of microorganisms e.g. Eggs contain lysozyme (active against Gram +ve Bacteria), avidin (binds biotin), conalbumin (binds iron), etc. Fresh milk contains lactoperoxidase system and lactoferritins. Vegetables, fruits, nuts and eggs have an outer natural covering like skin/shell that prevents the entry of microorganisms inside such foods.
EXTRINSIC	
Temperature of storage	Temperature at which a food material is stored will govern the type of microorganisms growing in that food. Food stored i) under Refrigerated conditions (~7°C) - favours *psychrophiles* and *psychrotrophs* (optimum temp for their growth is between 20°C to 30°C) b) Between 30°C to 40°C – favours mesophiles c) Above 40°C – favours thermophiles
Relative humidity	Higher relative humidity of storage environment than a_W of food – food surface will absorb moisture and promote growth of microorganisms at the surface. Lower relative humidity of storage environment than a_W of food – food surface will lose moisture resulting into dried and wrinkled texture.
Gaseous atmosphere	Carbon dioxide and Ozone gases are used during storage of foods to restrict the growth of microorganisms.

Food Spoilage

Microorganisms on entering the foods make the food environment suitable for their growth. They start utilizing food nutrients by producing enzymes that act on proteins, carbohydrates and fats. During this process, certain biochemical, textural and organoleptic (aroma, taste, etc.) changes may take place that render the food unsuitable or unacceptable for consumption. This state of food is generally referred as food spoilage. Foods have been categorized into three groups on the basis of their ease of spoilage.

1) Perishable foods spoil readily within few days, *e.g.,* meat, fish, milk and milk products, eggs, fruits and vegetables.

2) Semi-perishable foods do not spoil for long (two-three weeks) if stored properly, *e.g.*, potatoes, apples.

3) Non-perishable foods do not spoil for very long (a few months) if stored properly, *e.g.,* grains, sugar, pulses, beans, flour.

How does anyone judge whether a certain food material is spoilt or not?

Whenever a cooked dish is stored either in a refrigerator or outside for some time/days, we first smell it for any changes in its aroma/flavor, taste a bit of it to find any change in its original taste or sometimes touch it to look for development of stickiness. Bad/sour smell or change in taste or sticky threads (ropiness) appearing upon touching are some of the parameters that all of us commonly use to define whether the food is edible or not. If not, it is called spoilt food!

Similarly, simple visualization for the presence of any green, black, blue or white patches on the food surface, as we sometimes notice on long stored bread (Figure 1.a) and fruits and vegetables, will define a spoilt food product. At times, upon noticing any soft or mushy or pulpy or discoloured spot on fruits and vegetables (Figure 1.b), we decide either to remove the spoiled part or discard that piece altogether. Even the puffiness of the food packet due to gas production (if not desired) in foods stored in sealed containers is unacceptable (Figure 1.c).

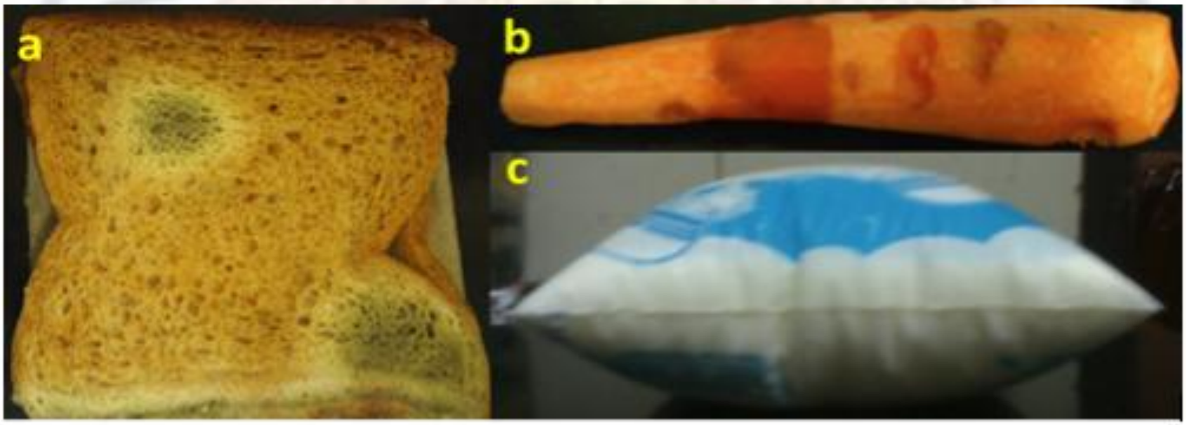

Figure 1: Microbial Food Spoilages. a) Moldiness of Bread. b) Bacterial soft rot of carrot. c) Gas accumulation in the poly pack containing buttermilk.

Source: Author

Some examples of common microbial spoilage of foods are given in Table 3 below.

Table 3: Most Common Microbial Spoilages of Foods and Causative Microorganisms.

Source: Author

FOOD PRODUCT	**SPOILAGE**	**CAUSATIVE MICROORGANISM**
Bread	Moldiness	*Rhizopus stolonifer*, *Aspergillus niger*, *Penicillium expansum*, *Neurospora sitophila,* etc.
Milk	Curdling	Lactic acid bacteria, coliform bacteria, enterococci, etc.
Meat	Sliminess	*Pseudomonas*, *Acinetobacter*, *Moraxella*, *Alcaligenes*, *Aeromonas*, etc.

Vegetables & Fruits	Bacterial Soft Rot	*Pectobacterium carotovorum* subsp *carotovorum*
	Gray Mold Rot	*Botrytis cinerea* (fungus)
	Watery Soft Rot	*Geotrichum candidum* (fungus)

Food Preservation

All the categories of foods particularly perishable ones will be spoiled or decomposed by microorganisms very fast unless certain special methods are adopted to enhance their life during storage. In Indian conditions of milking, milk can remain unspoilt for only a few hours. Similarly, meat has a very low shelf life if stored at room temperature. This small window period of safety hampers the transport of perishable foods for long distances. Therefore, immediately after their production certain measures are applied to extend their shelf life before these food items reach consumers. Even the consumer would like to have some hours or days of safety period before that food is actually consumed or further processed. This extension of time for which any food material remains or stored unspoiled compared to its natural state is called food preservation. All methods of preservation are based on any of the following principles and used alone or in combination thereof.

1) Preventing microorganisms from entering into the food product: Lesser the number of microbes in food, longer is the time taken for its spoilage. Preventing contamination by applying aseptic and hygienic conditions during food production will increase shelf life. One such method is packing foods in cans, bottles, poly packs, tetra packs, etc. which keeps away microorganisms (Figure 2).

2) Removing microorganisms from foods physically: Some liquid foods like fruit juices, bear, wine, etc. are passed through filters to remove microorganisms. Spoiled parts of vegetable and fruits are trimmed to stop further spread of spoilage. Vegetables and fruits are washed with clean water to get rid of soil and its associated organisms.

3) Killing of Microorganisms: Microorganisms present in foods are directly killed and reduced in number by applying the following methods.

a) High temperatures – Boiling, frying, baking, roasting, etc. are the methods of cooking food which apply high temperatures around 100°C killing most of the non- spore forming bacteria, yeast and molds. The commercial methods used are pasteurization and Ultra High Temperature (UHT) treatment. Pasteurization is the heat treatment given to liquid foods to kill all pathogenic and few spoilage organisms followed by fast cooling. The milk that we get in the market in poly packs from Mother dairy or Amul or any other brand is pasteurized at 62.8°C for 30 min (low-temperature long-time, LTLT method) or 71.7°C for 15 sec (high-temperature short-time, HTST method) to kill the most heat resistant non- spore forming pathogenic bacteria *Coxiella burnetii*, a causative agent of Q fever. In ultrahigh temperature treatment (UHT), milk is heated at 135-140°C for a few sec and packed under aseptic conditions in tetra packs. The shelf life of pasteurized milk under refrigeration is 24 hours while UHT milk can be stored for six months at room temperature.

Figure 2: Aseptically packaged fruit juice and milk in tetra packs with shelf life of 6 months at room temperature without any chemical preservative. In addition milk is treated at Ultra High temperature.

Source: Author

b) Irradiation – Foods are irradiated using Gamma rays generated from ^{60}Co or ^{137}Cs. These ionizing radiations are deep penetrating and kill microorganisms without raising the temperature of the food product. Fresh poultry, meat, seafood, fruits, vegetables and cereal grains can be preserved by irradiation.

c) Mechanical destruction – Some advanced and novel methods like high hydrostatic pressure and pulsed electric fields are being researched to kill microorganisms in foods.

4) Restricting the growth and activity of microorganisms – Shelf life of foods may also be enhanced by manipulating the growth conditions available in the food from optimum to unfavourable. These new conditions will not allow microorganisms to multiply in the food, thereby preventing food spoilage. The following methods fall into this category:-

a) Storage of foods at low temperature – A common practice followed at our homes involves storing our leftover cooked food, milk, meat, eggs, vegetables, fruits in a refrigerator which generally maintains a temperature in the range 4 - 10°C. At this temperature, the biological activity of microorganisms is severely reduced though not completely arrested, that is why it takes a longer duration for microorganisms to spoil the refrigerated food. The shelf life of foods can be further extended by freezing them and storing them under frozen state e.g. frozen peas, vegetables, meat, ready to eat foods, etc.

b) Drying foods – Availability of free water in food is an essential requirement for growth of microorganisms. During the drying process, water is evaporated beyond the minimum water requirement of spoilage organisms. These dried foods like milk powders, baby foods, grains, dried fruits (apples, figs, apricots etc.), nuts, etc. remain unspoiled for very long time if stored properly under dry conditions.

c) Maintenance of anaerobic conditions or a modified atmosphere – Evacuating air from food filled containers and replacing it with inert gases like nitrogen or carbon dioxide will create anaerobic conditions which will limit the growth of aerobic spore forming bacteria like *Bacillus* sps.

d) Use of chemicals for preservation – Many chemicals has been approved for use in foods as preservatives to contain the activity and growth of microorganisms. Fish and vegetables are stored in brine (salt) solutions. Sugar is added to jams, jellies, chocolates, condensed milk, and fruit syrups to prevent microbial growth. Organic acids like benzoic, sorbic and propionic are used in tomato ketchup, soft drinks, pickles, cheeses, bread, etc. to control yeast and molds. Even biopreservatives like nisin produced by lactococci are being used extensively in cheese and canned foods to prevent the germination of *Clostridium botulinum* spores.

The preservative methods mentioned above are not applicable to all types of foods and have one or the other limitation. Changes in nutritive value, texture, natural flavour or development of side effects are some concerning issues. Health conscious consumers are more concerned about the natural properties of food products and tend to reject foods containing chemical preservatives or lacking natural aroma and texture. Therefore, development of new preservation strategies to retain the natural value of foods is of utmost significance for future microbiologists.

Food Safety

We all have experienced diarrhoea, dysentery, vomiting along with abdominal cramps many times in our life. The questions that strike our mind immediately are (a) Is it food poisoning? (b) If yes, then what did we eat the previous day and from where? (c) What kind of water did we drink and from which source? It implies that such ailments are mostly attributed to either ingesting stale/contaminated food or drinking contaminated water. Food is rejected if spoiled mainly because of changes in taste, aroma, texture, etc. At the same time, it is also suspected of containing microbial toxins and the causative agents of gastrointestinal infections. This is the reason why even tasting of small amount of such stale food is prohibited. But what about those food items which do not show any sign of food spoilage yet are carrying large number of food pathogens. Therefore, it becomes necessary to study and investigate the presence of such pathogens and their toxic metabolites.

Food borne diseases are categorized into two groups 1) Food borne intoxications and 2) Food borne infections.

Food borne intoxications: It refers to the food poisoning caused by the presence of a microbial toxin produced by microorganisms in the food before its ingestion. There are three main types of food intoxications: 1) botulism caused by a neurotoxin produced by *Clostridium botulinum* in food, 2) staphylococcal intoxications caused by an enterotoxin produced in food by *Staphylococcus aureus* and 3) mycotoxicosis caused by a mycotoxin produced by fungi in food, *e.g.* aflatoxin produced by *Aspergillus flavus*. In all such cases favourable storage conditions of food promote toxin production by microbes. If it is not adequately heat treated before consumption, toxins are ingested along with food, thereby causing food poisoning.

Interesting Facts: The botulinum toxin is one of the most toxic substances known with an estimated 50% human lethal dose of 1 *n*g/Kg body weight. However, it is being widely used for cosmetic purposes as 'Botox' to treat the frown lines between eyebrows. It is also used as therapeutic agent to treat the conditions like excessive blinking, severe underarm sweating, squints, cervical dystonia, chronic migraines, etc. It is estimated that the global Botox market will touch $2.9 billion in 2018.

Food borne infections: It refers to the food borne illnesses caused by the ingestion of large number of viable bacterial cells present in food. When such a high number of live bacterial pathogens enter the intestinal tract, these either produce enterotoxins or invade the intestinal tissues and establish infection. In most of the cases, infection is not lethal and may be controlled in few days with proper rehydration and salt balance schedule. Some common food borne infections, their causative agents and foods involved are presented in Table 4 below.

Table 4: Food borne infections, their causative agents and foods involved.

Source: Author

FOOD BORNE INFECTION	MICROORGANISMS	FOODS INVOLVED
Salmonellosis	*Salmonella typhimurium*, *S. Enteritidis*, *S. Gallinarum*	Milk, ice-cream, eggs and poultry products, potato salad
Shigellosis	*Shigella dysenteriae*, *S. flexneri*, *S. Sonnei* and *S. Boydii*	Shellfish, fruits and vegetables, chicken, dips and salads
Listeriosis	*Listeria monocytogenes*, *L. Innocua*	Raw milk, soft cheeses, poultry, fresh and frozen meats, seafood, fruits and vegetables
Escherichia coli gastroenteritis	*Escherichia coli* (enteroaggregative EAggEC, enterohemorrhagic EHEC, enteroinvasive EIEC, enteropathogenic EPEC, enterotoxigenic ETEC)	Water, milk, meat, poultry, salads
Vibriosis	*Vibrio parahaemolyticus*	Raw seafood like oysters, clams
Yersiniosis	*Yersinia enterocolitica*	Cakes, seafood, meat, vegetables, milk
Campylobacteriosis	*Campylobacter jejuni*	Raw poultry, raw milk, water, meat, vegetables

Food Fermentation

Microbial fermentation of foods is another way of food preservation being practiced since time immemorial.

Benefits of Fermented Food

The fermented products like cheese, pickles, sausages, etc. are the best examples of food preservation through fermentation. However, these days new food products are being developed using this technique to enhance their nutritive value, biological value, flavours and aroma, and therapeutic value (Figure 3).

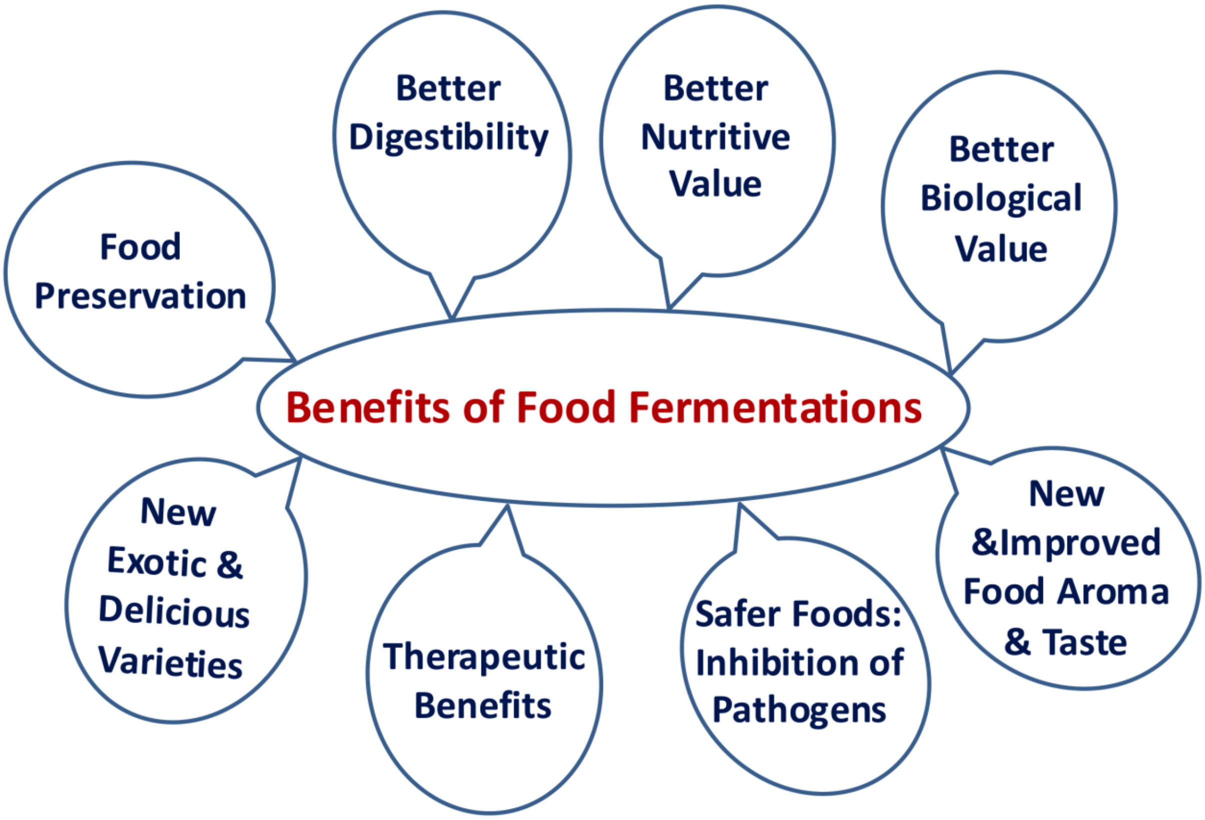

Figure 3: Benefits of fermentation process in food production and development.

Source: Author

Raw products or substrates like milk, vegetables, flour, beans, meat, fish, etc. are fermented with microorganisms and transformed into delicious products which have occupied an important place in our diet (Figure 4). These fermented products are used either as staple foods or as supplementary foods to enhance the palatability of meals.

Figure 4: Fermented Foods. a) Dahi, b) Cheese, c) Soya Sauce, d) Bread, e) Fruit Yogurt.

Source: Author

Microorganisms used in Fermentation

Fermentation is carried out by desirable microorganisms called starter culture which are added intentionally to raw foods (inoculation) and allowed to grow at a fixed temperature and time (incubation). Microorganisms used in food fermentations can be classified into three broad categories (Figure 5):-

1) Lactic acid bacteria: Following genera produce lactic acid from hexoses.

Lactococcus (*L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *diacetylactis*)

Streptococcus (*S. salivarius* subsp. *thermophilus*)

Lactobacillus (*L. delbrueckii* subsp. *bulgaricus*, *L. plantarum*, *L. acidophilus*, *L. casei*)

Pediococcus (*P. acidilactici*)

Leuconostoc (*L. mesenteroides* subsp. *cremoris*)

Some of them produce only lactic acid (homo-fermentative) while others produce lactic acid with carbon dioxide and other organic acids (hetero-fermentative).

2) Yeast: They produce alcohol and carbon dioxide from sugars and carbohydrates. Most common yeasts used in food fermentations are *Saccharomyces cerevisiae*, *Torula cremoris*, *Torulopsis kefyr*, etc.

3) Molds: They belong to genera *Aspergillus* (*A. oryzae, A. soyae, A. glaucus*), *Rhizopus* (*R. oligosporus*) and *Penicillium* (*P. roqueforti*) and used to produce Soya sauce, Tempeh and cheese, respectively.

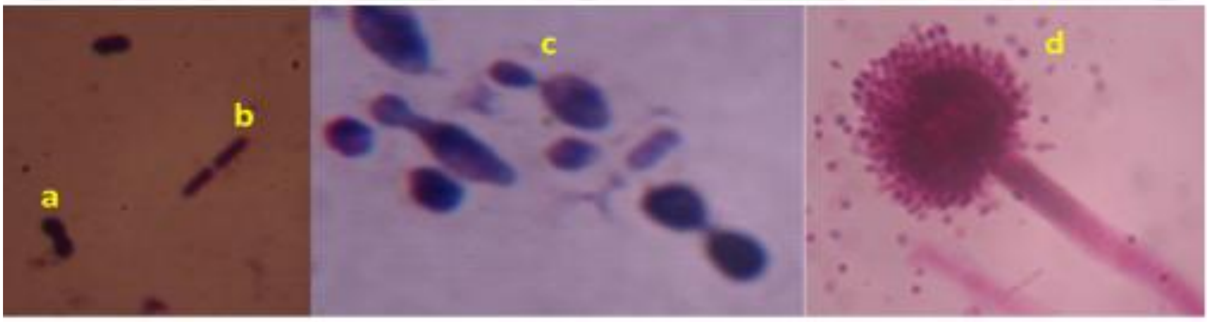

Figure 5: Fermentative Microorganisms. a) *Lactococcus lactis* (1000X), b) *Lactobacillus* (1000X), c) *Saccharomyces cerevisiae* (1000X), d) *Aspergillus* (45X).

Source: Author

Common Fermented Foods

Some of the fermented foods like dahi, cheese and bread are discussed in detail and summarized in Table 5.

Fermented Dairy Products

Dahi/Curd: Dahi or curd, a common fermented milk product associated with our daily diet, is relished in South Asian subcontinent. Almost every household prepares it at home using small part of previous batch of dahi called as '*khatta*', '*jamun*'. The market dahi available in Halwai/Indian sweet shops contains mixed flora constituting species of *Lactococcus*, *Lactobacillus*, *Streptococcus* and some times even yeast, while commercially produced dahi contains pure cultures of lactic acid bacteria. In industry, pasteurized or boiled milk after cooling to room temperature is inoculated with pure and known culture of lactic acid bacteria at the rate of 1-2% and incubated at 28°C for 6-8 hrs until a set product is formed with the production of 0.6-0.8% lactic acid. The set dahi is refrigerated to restrict over fermentation and access production of lactic acid.

Cheese**:** It is liked and produced all over the world for its better shelf-life and nutritive value. It is generally prepared from pasteurized milk which is coagulated by the addition of rennet (rennin from calf abomasums and rennin like enzymes from microbes that curdle milk) and fermented later by lactic acid bacteria and in some cases by molds. Once the moisture/water (whey) from the coagulated product is removed by heating and pressing, it is stored at 10°C for 1-12 months for ripening. During this ripening process, milk protein casein is converted into small peptides and free amino acids and milk fat is hydrolysed into glycerol and fatty acids. It enhances nutritive and biological value of milk and imparts a typical flavour and taste to cheese. Swiss cheese is specifically known for the presence of eyes or holes produced due to accumulation of carbon dioxide produced by *Propionibacterium shermanii* or *P. freudenreichii.*

There are about 3000 varieties of cheeses produced all over the world which are classified into the following categories on the basis of their moisture content and ripening period (Figure 6).

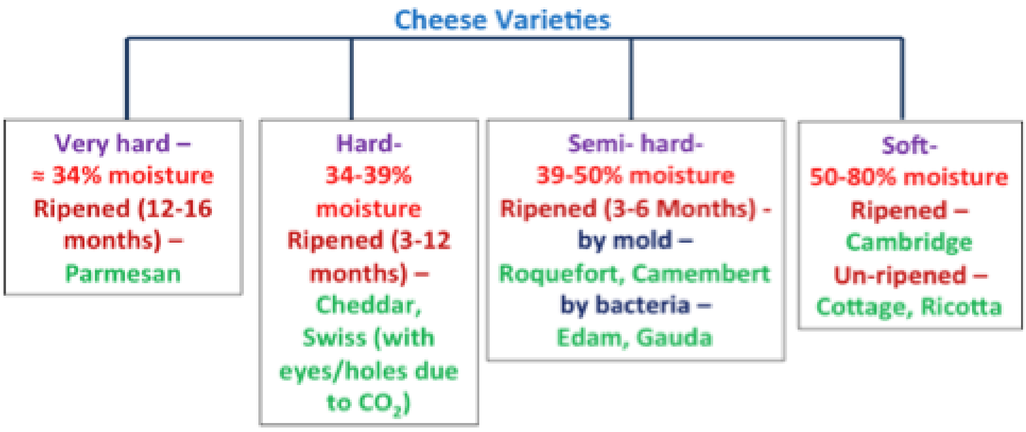

Figure 6: Classification of Cheeses.

Source: Author

Table 5: Fermented milk products and their associated starter culture bacteria and fungi.

Source: Author

Fermented Product	**Microorganisms**	
Curd/Dahi, lassi, chach (butter milk)	Lactic acid bacteria: *Lactococcus lactis* subsp *lactis* *Lactococcus lactis* subsp *diacetylactis* *Lactobacillus* sps *Streptococcus salivarius* subsp *thermophilus*	Dahi/Curd is a popular fermented milk product of South Asian countries, generally fermented at home using the curd from previous batch with 0.6-0.8% lactic acid.

Yogurt (Plain, Flavored, Fruit, Frozen)	Lactic acid bacteria: *Lactobacillus delbruekii subsp bulgaricus Streptococcus salivarius subsp thermophilus*	Yogurt is a popular product of Western countries. The two cultures are mixed in 1:1 ratio and fermentation is carried out at 43°C for 3.5 hrs.
Cheese (Cheddar, Gouda, Parmesan, Mozzarella, Swiss, Roquefort, Camembert, etc.)	Lactic acid bacteria: *Lactococcus lactis* subsp *lactis* *Lactococcus lactis* subsp *cremoris* *Lactobacillus* sps *Streptococcus salivarius subsp thermophilus* Others depending on the type of cheese: *Propionibacterium shermanii* or *P. freudenreichii* (Swiss cheese) *Penicillium roqueforti* or *P. camemberti* (Roquefort and Camembert cheese).	Milk is coagulated by rennin like enzymes and fermented by lactic acid bacteria and ripened by lactic acid or propionic bacteria or fungi during cheese production. Water expelled out from curd during heating and pressing is rich in lactose, water soluble proteins and vitamins and minerals, is called whey.
Kefir (an effervescent and alcoholic milk based drink)	Lactic acid bacteria: *Lactococcus lactis* subsp *lactis*, *Leuconostoc* sps, *Lactobacillus caucasicus*, Yeast: *Saccharomyces kefir*, *Torula kefir*	It is a popular Russian acidic and mildly alcoholic product fermented by cultures embedded in white yellowish kefir grains. It contains 0.9-1.1% lactic acid, 0.1-1.0% alcohol and carbon dioxide to cause effervescence.
Kumiss (an effervescent and alcoholic drink generally made from Mare's milk)	Lactic acid bacteria: *Lactobacillus delbruekii subsp bulgaricus, Lactobacillus acidophilus* Yeast: *Saccharomyces lactis*, *Torula kumiss*	It is popular in Southern Russia and Central Asia and prepared from Mare's milk. It contains 0.7-0.9% lactic acid, 1-2% alcohol and carbon dioxide for effervescence.
Other fermented milk products: Acidophilus milk, Bulgarian milk, Shrikhand, etc.		

Fermented Non Dairy Products

Bread: It is one of the most ancient human foods fermented by microorganisms. Bread is manufactured using wheat flour (whole or all purpose) either alone or mixed with other cereal flours. The flour is mixed with active baker's strain of *Saccharomyces cerevisiae (*for fermentation), α- and β- amylases (to convert starch into maltose and glucose) and supplemented ingredients like vitamin A and reconstituted into dough. Dough is allowed to ferment at a temperature of 22°C for 2-3 hrs during which carbon dioxide is produced and accumulated to raise the volume of dough. This accumulated carbon dioxide is responsible for stretching of wheat protein gluten resulting into the typical structure of bread. Fermented dough is made into a loaf and baked at 180°C for 1 hr. Heating during baking will inactivate all yeast cells and stop further fermentation. The baked loaf is cooled, sliced into pieces and wrapped under aseptic conditions.

Table 6: Fermented Plant and Meat Products and their Associated Starter Culture Microorganisms.

Source:

http://www.wiley-vch.de/books/biotech/pdf/v09indig.pdf http://www.fao.org/docrep/015/i2477e/i2477e00.pdf

Fermented Product	**Raw material**	**Microorganisms**
Plant Based Fermented Foods		
Bread	Wheat, Rye, Oats, etc.	Yeast: *Saccharomyces cerevisiae* (Baker's strain)
Soya sauce (two stage fermentation)	Soya beans	Ist stage - Fungi: *Aspergillus oryzae* IInd stage- *Lactic acid bacteria: Lactobacillus delbueckii subsp delbrueckii,* and Yeast: *Zygosaccharomyces rouxii*
Idli and Dosa	Rice and black gram (*urad* beans)	Lactic acid bacteria: *Leuconostoc mesenteroides*
Pickles	Vegetables (cabbage, olives, cucumber, etc.)	Lactic acid bacteria: *Leuconostoc mesenteroides, Lactobacillus plantarum, Pediococcus cerevisiae*
Animal based Fermented Foods		
Sausages; Salami, Bologona	Ground meat and beef	Lactic acid bacteria: *Pediococcus acidilactici, Pediococcus pentosaceus, Lactobacillus plantarum*

Probiotics

Increased consumption of fermented milk products like lassi/dahi/chaach during diarrhoea or dysentery is followed as a home remedy in all most all households. This is because of the presence of live lactic acid bacteria in these products which compete with the intestinal pathogens and neutralize their toxicity. This therapeutic effect of fermented milks was established long back in the early 20^{th} Century by a Russian Scientist Elie Metchnikoff in his book 'Prolongation of Life'. He wrote that the prolonged life of Bulgarian peasants was due to the consumption of fermented milk every day. Similarly, when any patient is prescribed an antibiotic, amoxicillin, after one or two doses he experiences loose and frequent stools. This is cured only after antibiotic dose is supplemented with another capsule containing large number of live lactobacilli (Figure 7). Such feed or food products containing live lactic acid bacteria or any other organism that exerts a beneficial health effect and improves intestinal microbial balance in animals and humans, are known as probiotics. This term has been derived from two Greek words *pro* and *bios* meaning 'for life'.

Types of Probiotics and Microorganisms

The most common organisms explored and extensively used as probiotics belong to the genera *Lactobacillus* and *Bifidobacterium*. Other species which have been tried as probiotics are *Saccharomyces boulardii* (yeast) and *Streptococcus* sps. These bacteria should be present in a dairy food at a minimum level of 10^6cfu/ml at the time of consumption. Hence, at least 10^8 cfu/ml of bacteria is added to the food material to compensate for any loss in viable number during production and storage. In general, probiotic bacteria are added to the fermented or non fermented foods once their processing is complete. There are certain properties that an organism must harbour to qualify as a probiotic candidate (Figure 8) .

Figure 7: Probiotic Foods and Supplements. a) Live cells of lactic acid bacillus with amoxycillin and dicloxacillin antibiotics to prevent antibiotic induced diarrhoea, b) Probiotic drinks and foods, c) Capsules containing live cells of lactic acid bacteria used as therapeutics.

Source: Author

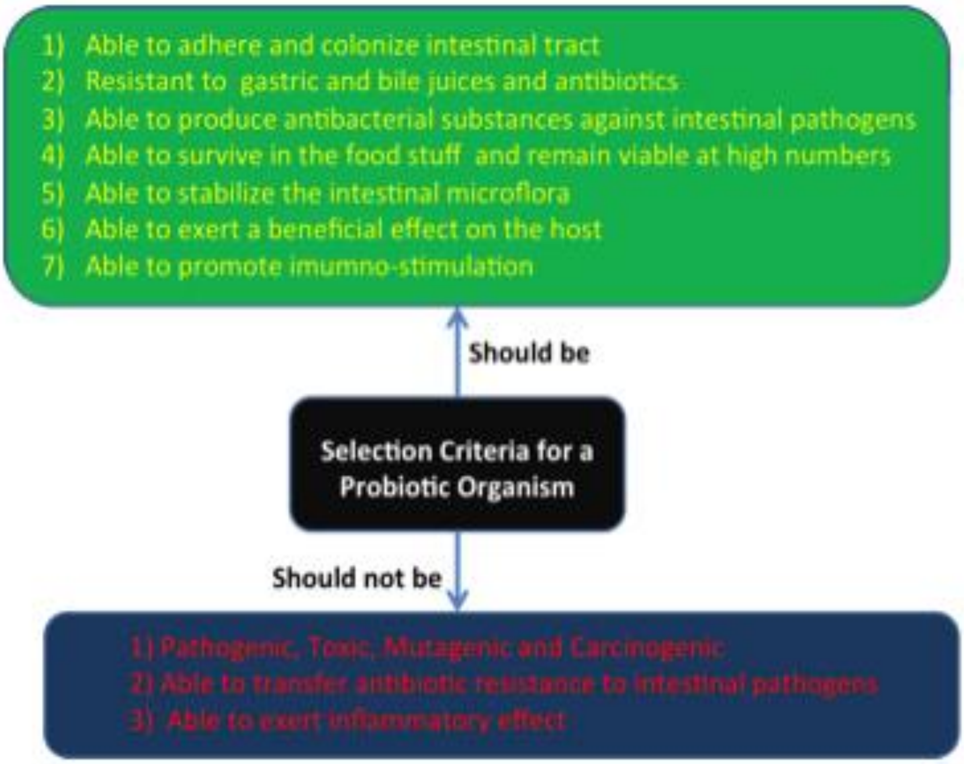

Figure 8: Characteristics of an organism to be used as probiotic supplement.

Source: Author

The dairy products like Yogurt, dahi, sweetened lassi are the most common probiotic carriers in the Indian Market (Table 7). Probiotic organisms are also being added to other dairy and non dairy products like ice-creams, cheeses, chocolates, frozen yogurt, fruit juices, etc.

Table 7: Probiotc Foods and associated Microorganisms.

Source: Author

Probiotic food/drink	**Microorganisms**
Probiotic Foods	
Probiotic Dahi Proactive – Mother dairy Prolife – Amul Actiplus - Nestle	Lactic acid bacteria: *Lactococcus lactis* subsp *lactis* *Lactobacillus* sps Probiotic bacteria: *Bifidobacteria longum/ Bifidobacteria lactis Bb-12/ Lactobacillus acidophilus la-5/ Lactobacillus rhamnosus.*
Probiotic Yogurt or Biogurt	Lactic acid bacteria: *Lactobacillus delbruekii subsp bulgaricus Streptococcus salivarius subsp thermophilus* Probiotic bacteria: *Bifidobacterialongum/ Bifidobacteria lactis/ Lactobacillus acidophilus la-5/ Lactobacillus rhamnosus* GG.
Icecream Prolife – Amul	Probiotic bacteria: *Bifidobacteria lactis Bb-12, Lactobacillus acidophilus la-5*.
Probiotic Drinks	
Yakult –Danone	Lactic acid and probiotic bacteria: *Lactobacillus casei* strain shirota
Nutrifit – Mother Dairy	Lactic acid and probiotic bacteria: *Bifidobacteria lactis Bb-12* and *Lactobacillus acidophilus la-5*.

Acidophilus Milk	*Lactobacillus acidophilus*

Prebiotics: Prebiotics are not the microorganisms, rather they are the food supplements that improve our health by selectively promoting the growth or activity of beneficial microorganisms (probiotic like) already present in the gastrointestinal tract. These substances are nondigestible by the host and pass through small intestine without hydrolysis or absorption. They are metabolized by bifidobacteria and lactobacilli present in large intestine. Prebiotic human milk oligosaccharides are digested specifically by *Bifidobacterium infantis* an inhabitant of intestines of breastfed infants. The most commonly used prebiotics are fructooligosaccharides like inulin, galactooligosaccharides, soybean oligosaccharides, psyllium etc.

Synbiotics: These are food supplements that contain both prebiotics and probiotics. It has been suggested from research on animal models and some human trials that synbiotic preparations provide additional benefits comapred with either prebiotics or probiotics alone.

Health Benefits and Therapeutic Effects of Probiotics

There are varieties of proposed health benefits of probiotics which have been proved and supported by extensive clinical trials. However, a large number of claims made in favour of probiotics is yet to be established clinically and require further research (Figure 9). There are conflicting reports about the use of probiotics in the treatment of cancers, hypertension, hypercholesterlemia, hyperlipidaemia, etc. In some cases probiotic use has been suggested to only supplement the therapy. Hence, lot more scientific efforts are needed to clearly recognise the potential of probiotics in the treatment of such life style diseases.

The use of prebioicts or probiotics or both in domestic animals cattle, pigs and poultry is currently acceptable and is being promoted by researchers and animal husbandry workers. The beneficial effects include improved general health, more efficient food utilization, faster growth rate and increased milk and egg production. Probiotic organisms when applied on the meat surface after slaugter has been shown to increase shelf-life by inhibiting contaminants entering the surface.

Figure 9: Health Benefits of Probiotic Foods.

Source: Author

Microbes as Foods

A continuous and sharp increase in population coupled with a reduction in cultivable area broadened the gap between total protein production and its requirement, resulting in worldwide malnutrition problems. In order to control this situation, alternative protein sources other than the cultivated ones have been explored. One of the novel ideas to address this problem is to use microbial protein. Microorganisms like algae, fungi, yeast and bacteria can be grown into a large biomass, harvested, dried and used as protein supplement in human foods or animal feeds. Such a protein constituting of microbial biomass as a whole or processed or extracted from microbial cells is called Single Cell Protein (SCP). Professor Carol L Wilson at Massachusetts Institute of Technology (MIT) coined this term Single Cell Protein in 1966.

We eat microorganisms knowingly or unknowingly, dead or alive as in fermented foods considering their health benefits, but never consume them as a protein source. When using microbial biomass or its proteins as SCP, it needs to have 1) satisfactory nitrogen and protein content, 2) satisfactory biological value, 3) satisfactory aroma, flavour, colour and texture, 4) low nucleic acid content, 5) no toxicity and allergic reactions, and 6) low production cost. Production of SCP has many advantages over the production of plant and animal proteins.

Benefits of SCP

1) Microorganisms can be cultivated in a large biomass in short time e.g. 50 kg of Yeast biomass will produce 250 tons of protein in 24 hrs and 20 tons of dry weight of algae can be generated per acre, per year in ponds.

2) SCP has better nutritive value as compared to plant proteins due to the high values of essential amino acids like lysine.

3) Protein content of microorganisms is high. Bacteria – 50-65%, Yeast – 45-55%, Fungi – 30-45% and Algae – 40-60%

4) SCP can be produced by growing microorganisms on a wide variety of substrates locally available as either solid waste, effluents or by-products of industries or agricultural practices, like whey, cellulose, natural gas, petroleum products like paraffin, molasses, sulphite waste liquor, ethanol, methanol, etc.

5) Requirements for SCP production by continuous culture like land, water and other resources are minimal with less amount of waste generation.

6) SCP production is independent of seasonal and climatic changes.

7) Microorganisms can be modified genetically to introduce desirable properties or to delete harmful traits.

However, there are some major concerns of SCP usage for human consumption.

1) Microorganisms are rich in nucleic acid content e.g. Bacteria – 8-12%, Yeast – 6-12%, Fungi – 7-10% and Algae – 3-8%. High intake of nucleic acids by humans will lead to metabolic disturbances such as gout, arthritic conditions and renal stone formation. Purines are metabolized into uric acid which tends to accumulate in body due to its poor solubility. Hence, the upper limit for nucleic acid consumption is 2 g per day which is equivalent to 10 g of yeast or bacterial protein. SCP is made suitable for human consumption only after levels of nucleic acids are reduced by various chemical processes. In animals, uric acid is converted into allantoin by enzyme uricase that is absent in man. Allantoin is a highly soluble compound and easily excreted in urine. Therefore, it is much safer to use SCP as animal feed than as human foods.

2) Poor digestibility of microbial cells particularly algae due to presence of cellulosic cell wall.

3) Concentration of heavy metals by microbial cells during growth from raw substrates.

4) Unsuitable for direct consumption due to poor aroma, taste or texture.

5) Microorganisms might produce toxins or harmful secondary metabolites.

6) Psychological barriers among consumers to accept any microbial cell as food due to lack of awareness about these products.

7) Production cost of SCP for human consumption is 10-12 times higher than of SCP for animal feed.

In view of the above, SCP usage has been restricted only to food supplements (as aroma carriers, emulsifying aids, etc.), cosmetics and animal feeds for poultry, fish and pigs (Figure 10). Their extensive use as human food is yet to find a place in the market.

Figure 10: SCP from Alga *Spirulina*: a) Capsules containing dried *Spirulina,* b) *Spirulina* filaments, c) *Siprulina* sold as super nutrient in Indian market.

Source: Author

Microorganisms used as SCP

Yeast was the first organism explored as food supplement during World War I in Germany when half of the protein requirement was replaced with it. It has advantage over other organisms as far as its acceptability as SCP is concerned because of its long use in traditional fermentation processes. SCP from oil (n-paraffin) using yeast was popularized by petroleum Industry (British Petroleum) and many pilot scale trials were conducted but could not be scaled up to industrial production. The main yeast genera being used as SCP are *Saccharomyces cerevisiae*, *Candida tropicalis*, *Candida utilis*, etc. 'Toprina' and 'Torutien' are the yeast dependent commercial SCP products available in market.

Pruteen was the first commercial bacterial SCP produced as animal feed additive by Imperial Chemical Industries (ICI), UK. Bacteria *Methylophilus methylotrophus* was grown on methanol and dried to 70% dry weight. Other bacterial species used as SCP are *Cellulomonas*, *Alcaligenes*, etc.

A Unicellular green alga *Chlorella* and a filamentous blue green alga *Spirulina* have been commercially cultivated for the purpose of SCP. Actually, use of alga as SCP in the diets of Aztecs of Mexico and natives of Chad in Africa has been a common practice since centuries. *Spirulina platensis* and *Spirulina maxima*dried biomass is available in the market in the form of cakes, capsules, powders or liquids for application in various health conditions (Figure 11).

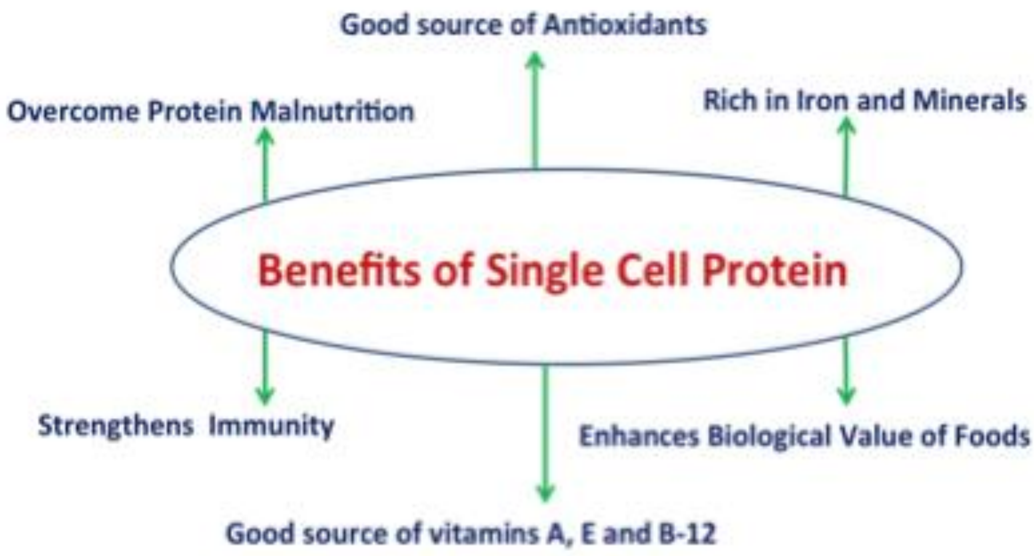

Figure 11: Health Benefits of Single Cell Protein

Source: Author

Mushrooms, the higher fungi, belonging to genera *Morchella* and *Agaricus* are the most important protein source constituents of worldwide diet. Similarly, other fungi like *Trichoderma*, *Fusarium*, *Rhizopus,* etc. have been researched for their potential to be used as SCPs. SCP from *Fusarium* has been commercially produced in the brand name Quorn.

Interesting Facts: Quorn, a mycoprotein extracted from *Fusarium venenatum* is being sold in UK, USA and other countries as a high protein, low fat, cholesterol-free meat alternative. It is used as a cooking ingredient in the form of cubes or grains and available on ready to eat meals like pizza, lasagne in the form of slices, granules or small pieces. Initially fungal protein was mixed with egg albumin to provide texture. Later, to address the concerns of vegetarians, it was mixed with potato protein as binder and used in 'quorn burgers' in USA making it a pure vegan i.e. vegetarian. By 2005, it has replaced about 60% of meat market in UK, with annual sales of around £95 billion.

Summary

Food being essence of all life is utilized and transformed by microorganisms, sometimes into desirable and at other times into undesirable and unsafe products. Hence, it is important to know and study the type, number and growth requirements of microorganisms present in food and food's biochemical properties before any treatment process is designed to control/eliminate them. Even the activity of beneficial microbes in food will be dependent on the food processing and the type of competing microorganisms existing in these foods. New reliable and rapid methods are needed to

identify the presence of food pathogens and spoilage microorganisms with low detection limits. Similarly, awareness among consumers about the changes in nutritive value and the possible harmful effects of some preservative methods warrants immediate attention to design new preservation techniques. Fermentation is a very popular process carried out by microorganisms in foods to impart certain significant changes in flavour, texture, taste, nutritive value and digestibility. However, no new fermented product has been developed and entered the market for a long time, leaving a great scope for such food product development. Ministry of Food Processing in India has begun this crusade to develop new food products by providing funds for research in this area. The field of Probiotics is growing at very fast rate with many issues regarding their therapeutic potential must be addressed by controlled and meticulous human trials. Use of microorganisms as human food in the form of SCP could solve some major challenges imposed due to protein malnutrition before the world. However, it needs more attention in terms of finding new microbes for this purpose and processing condition to reduce their nucleic acid content. The association of foods with microbes is vast and still remains to be fully explored by future food and fermentation microbiologists.

Exercises

1). What are the sources of microbial contamination of milk?

2). What type of microorganisms will enter into food from air?

3). Why is it important to clean utensils before storing food in them?

4). What are the advantages of packing foods in tetra packs? Find out the layers of materials present in the tetra pack containers.

5). Explain why the fruit juices are spoiled by yeast and molds?

6). What will happen to the shelf life if dried milk powder absorbs moisture?

7). Why do consumers favour processed foods without preservatives? Explain with the help of an example.

8). What are the two most common methods of milk preservation? Find out the differences between them.

9). Give two advantages and disadvantages of preservation of foods.

10). Differentiate between food borne infections and food intoxications with suitable examples.

11). What is traveller’s diarrhoea? What is its causative agent?

12). List the benefits of food fermentations.

13). How will you classify cheeses? Name few cheese varieties and their producing microorganism.

14). During yeast fermentation of bread CO_2 and alcohol is produced. What happens to alcohol?

15). What are the health benefits of Single Cell Protein?

16). Very small portions of SCP are prescribed as food supplements. Why?

17). Is it possible to replace all vegetable and animal food in the diet with SCP? Explain.

18). Probiotics can be harmful in some individuals. Explain.

19). Your doctor prescribed you amoxicillin for some infection. After taking two doses you experienced loose stools and abdominal discomfort. What is the reason and how will you get rid of this situation at home?

20). How can you see yourself as an entrepreneur after studying food microbiology?

Glossary

Antibiotic-induced diarrhoea : Loose and frequent stools on oral consumption of some antibiotics.

Bacterial soft rot : Soft, mushy and watery consistency of spoiled part of vegetable caused by bacteria.

Calf rennet : Dried powder obtained from calf stomach containing enzyme rennin.

Enterotoxin : Toxins produced by microorganisms, which adversely affect the intestine.

Extrinsic factors : External environmental factors surrounding food that affect microbial growth in food.

Gout : Inflammation and pain of joints; arthritis.

Intrinsic factors : Internal environment of food that affect microbial survival and growth.

Microbial rennet : Rennin like enzymes produced by microorganisms like *Mucor mehei* and used for milk coagulation during cheese production.

Moldiness : Bread spoilage by molds.

Neurotoxin : Toxin produced by microorganisms affecting nervous system.

O/R potential : Oxidation reduction potential of food that affects growth of bacteria.

Organoleptic : Property of foods that can be judged by taste, smell, touch, appearance.

Pasteurization : A process of heating liquid foods at temperatures lower than 100°C to kill all pathogenic and some spoilage microorganisms.

Pruteen : A Commercial Single Cell protein containing biomass of *Methylophilus methylotrophus* grown on methanol substrate.

Quorn : A mycoprotein having meat like taste produced from *Fusarium venenatum*.

Ripening of cheese : A process storing cheese blocks at 10°C for 1-12 months to enhance their nutritive value and flavour.

Shelf-life : The time for which any food product could be stored without spoilage in ambient temperature.

Sliminess : A sticky film found on meat surface when stored.

Spoilage : Stage when food becomes unacceptable to eat either due to microbial action or any other factor.

UHT : Treatment of milk at Ultra High Temperature of 135°C for few seconds to enhance shelf life.

Water activity : It is ratio of the water vapour pressure of food substrate (solution) to the vapour pressure of pure water (solvent).

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