

BIOLOGICAL IMPORTANCE OF MICROBES IN AGRICULTURE, FOOD AND PHARMACEUTICAL INDUSTRY: A REVIEW

MARIA KALSOOM¹, FAZAL UR REHMAN^{2*}, TALHA SHAFIQUE², SANWAL JUNAID³, NIMRA KHALID⁴, MUHAMMAD ADNAN⁵, IRFAN ZAFAR³, MUHAMMAD ABDULLAH TARIQ², MUHAMMAD ADNAN RAZA⁶, AMBER ZAHRA⁶, HUSNAIN ALI⁵

¹Institute of Food Science and Nutrition, University of Sargodha, Pakistan. ²Department of Plant Pathology, College of Agriculture, University of Sargodha, Pakistan. ³Department of Entomology, College of Agriculture, University of Sargodha, Pakistan. ⁴Department of Zoology, University of Sargodha, Pakistan. ⁵Department of Agronomy, College of Agriculture, University of Sargodha, Pakistan.

⁶Department of Horticulture, College of Agriculture, University of Sargodha, Pakistan.

Email: fazalurrehman107@gmail.com

Received: 22 September 2020, Revised and Accepted: 16 October 2020

ABSTRACT

Biotechnology is the most prominent and rapidly growing segment of the biological sciences that is making its diversified application in sustainable agriculture. Biofertilizers, biopesticides, bioherbicides, bioinsecticides, and many of the other fungal based and viral based insecticides, obtained using microorganisms, are some of the outcomes of biotechnology playing a key role in sustainable agriculture. Many of other important food products are also obtained by microbial fermentation. Different microbes are added to get the desired effect of food at the specific stages of food production process. Pharmaceutical microbiology includes the manufacturing of different pharmaceutical and medicinal products. This review article has a wide overview of microbes mainly used in agriculture, food industries, and pharmaceutical industries.

Keywords: Sustainable agriculture, Biofertilizers, Biopesticide, Bioinsecticides, Whey, Baker's yeast, Vaccine.

INTRODUCTION

Biotechnology is making the use of microorganisms including fungi, bacteria, and viruses in fields of agriculture, food, and pharmaceutical products. Farmers and researchers are working to make the microbes as pests control agent that is destructive to their crops. Soil microbes, including bacteria and fungi, are essential for decomposing organic matter and recycling dead plant material [1]. In biotechnology and biomanufacturing, these tiny microbes and living cells are like miniature chemical factories that produce the vital products such as amino acids, enzymes, medicines, and food additives [2]. Naturally, microorganisms are used to carry out fermentation processes and for thousands of years man has been using yeasts, molds and bacteria to produce many of food products, that is, bread, vinegar, beer, wine, cheese, and yoghurt, as well as fermented fish, meat, and vegetables by making the use of microorganisms [3]. Microbes are used for the fermentation of different types of food to produce a variety of oriental food products. By making the use of microorganisms in large quantity, numbers of biological preparations have been prepared that have the great importance in field of medicine and pharmacy [4].

AGRICULTURAL INDUSTRY

In modern agriculture, along with the use of hybrid seeds, high yielding varieties, chemical fertilizers, and frequent irrigations, the utilization of microbes as natural fertilizer is becoming the most trending field in era of today. The unhealthy impacts and high cost of chemical fertilizers are making them unaffordable to use in agriculture. It is estimated that by 2020, there will be the requirement of 28.8 million tons of nutrients for the production of 321 million tons of production of grains of food. But still, the availability of nutrients is 21.6 million tons. There is a wide gap of 7.2 million tons between the nutrient supply and nutrient removal [5].

The agricultural productivity of soil can also be improved by microbes found in the soil. Today man is making use of naturally occurring microbes to produce the biological products that have ability to recycle the nutrients and are eco-friendly. These biological products are following;

Biofertilizers

The natural fertilizers of which the main constituents are living microbial inoculants including algae, fungi, bacteria alone, or in combination and have ability to enhance the availability of nutrients in soil for plants [6]. They have an ability to convert the nutritionally important component present in the soil from unusable form to the usable form by their microbial activities including phosphate solubilization, nitrogen fixation, excretion of plant growth hormones, and biodegradation in the soil. The use of biofertilizers is eco-friendly, productive, easily accessible to marginal farmers, and more efficient [7].

A lot of studies have been made to exploit the use of microorganisms and their associations for the production of biofertilizer. They can be grouped as following on the basis of their function and nature.

Rhizobium

Rhizobia are the soil habitat bacteria that have ability to clone in lines of roots of legume plants and can fix much of free nitrogen. In regard to the quantity of nitrogen they fix, they are the most efficient biofertilizers [8].

Arbuscular mycorrhizal fungi

The intracellular obligate microbes are present in association with the root cortex of higher plants and absorb the essential elements including phosphorus, zinc, and sulfur from the soil. They can work as biofertilizer and are mostly from the genus *glomus* [9].

Azotobacter

Other than rhizobium bacteria, the azotobacter also has an ability to fix nitrogen. The several species of azotobacter are present in the soil and can act as biofertilizer [10].

Cyanobacteria

Cyanobacteria is a blue green algae and found as free living or as symbiotic association with rice crop and can produce the fixed nitrogen

at the rate of 20–30 kg nitrogen per hectare under ideal conditions and nowadays it is being used as biofertilizer [11].

Aspergillus

They are present in rhizospheric zone of graminaceous plants and also present in intracellular spaces of root cortex of these plants. They act as biofertilizer for them by making them disease resistant, draught tolerance, and by growth promoting substance production [12].

Plant growth promoting rhizobacteria

The beneficial group of rice bacteria that is presents in rhizospheric zone of plant and analyze the root to improve the growth of the plant by acting as bioprotectant, biostimulant, and biofertilizers [13].

Biopesticides

The soil also has the plant pathogenic bacteria that are present in the rhizospheric zone and it can cause a lot of diseases in the plant. By making the use of these pathogenic microbes, scientist has made the biological tool to control the unwanted weeds and pests. These microbes possess the genes that are invasive and can attack on the weeds and kill them [14].

Bioinsecticides

Bioinsecticides has been developed to minimize the use of synthetic insecticides by making the use of microorganisms. Because of shortest shelf life, they do not persist in environment and are also eco-friendly. For example, 200 diseases are caused by fungi in insects that can control their population [15].

FOOD INDUSTRY

Beside agriculture, there are a lot of admirable applications of microorganisms in food industry. The microbes have a great influence on quality and quantity of food produced. Fermentation of milk is done by inoculation of pasteurized milk with specific culture of microbes. The different fermented products that are obtained from milk are yoghurt and cheese. They are actually used to change the nature of one substance to another which is safely used as food. For example, production of yoghurt and cheese from milk and production of wine and bread from sugar. Description of few important industrial food products is given below:

Yoghurt production by bacteria

Yoghurt is a product obtained from milk which is produced by inoculation of bacterial culture. It can be produced by making the use of any kind of milk but most commonly cow's milk is used. A variety of milk including whole, dried, skimmed, evaporated, or semi-skimmed milk can be used for yogurt production [16].

The lactose, milk sugar, is fermented and produces lactic acid by the bacteria including *Streptococcus salivarius*, *Streptococcus thermophiles*, and *Lactobacillus bulgaricus*. These bacteria are also called Lactic Acid Bacteria or LAB, collectively [17]. The bacteria feed on lactose present in milk and release lactic acid as their waste product of feeding process.

The lactic acid produced by feeding of bacteria causes the curdling of protein, that is, Casein present in milk is converted into a solid mass known as curd. The gelly texture and taste of yoghurt are imparted by the fermentation of lactose sugar into lactic acid [18]. The increase in acidity of yogurt due to lactic acid formation is also beneficial to prevent the proliferation of other potentially pathogenic bacteria. Both pasteurized and unpasteurized milk can be used for yoghurt formation. For complete fermentation, two or more bacterial cultures can be used together. Yoghurt is then flavored and sweetened or the fruits can be added at the bottom [19].

Cheese production by bacteria and fungi

Cheese is formed by inoculation of milk with a microbial culture containing specific microorganisms. Cheese is a solid food can be

produced from the milk of various animals but mostly cow milk is preferred. Milk from goat, sheep, reindeer, and water buffalo can also be used. Fermentation of milk by inoculation of bacterial cultures leads to the formation of lactic acid, which imparts the sour taste. It results in coagulation of casein, that is, milk protein. The solid portion of the coagulated milk is called curd and the liquid portion is called whey [20].

The curds is then separated and desired shape is given and whey, that is the liquid portion, is used as food source for yeasts, which is processed to produce the cattle feed and is also a rich source of proteins and vitamins. The cheese is ripened by the adding bacteria or fungi inoculum or both. These bacterial or fungal inoculums added, reduce the pH, alter texture, and develop a flavor [21]. Coagulation may be controlled adding the rennet tablets, which contains the rennin enzyme. Rennin enzyme is actually present in the stomach of calves. However, now its availability is made using genetically engineered bacteria [22]. Coagulation can also be done using vinegar and lemon juice.

There are several types of cheese [23]. On the bases of microbes added, cheese is of following types:

- Cheddar cheese is prepared by using bacteria to increase its flavor and texture
- Roquefort cheese and blue cheese is produced by using of mold fungi
- Camembert cheese is produced by using the combination of both bacteria and fungi
- Swiss cheese is prepared by using *Propionibacterium shermanii*.

The natural color of cheese is mostly from off-white to yellow. Herbs and spices can also be added to the cheese [24]. Other factors including different levels of milk fat, different processing treatments, changes in length of aging, and different breeds of mammals also contribute a lot to a different flavors and styles of cheese.

Bread production by yeast

Yeast is a single-celled, saprotrophic fungus. The yeast cells digest food that has sugar and minerals by secretion of enzymes. Yeast is used for production of bread [25]. When yeast culture is added to the flour and water, it results in the production of carbon dioxide which is trapped in the dough made from the flour. The bread is made by rising of dough by CO₂. The flour of wheat is mostly used and which contains starch. Energy source for the yeast is starch. The flour also has gluten protein, which produce the sticky stretchy threads as the yeast inoculum works on the starch. These threads trap the CO₂ and make the dough raised [26].

Since earlier times, the yeast is also use as leavening agent in baking. The mostly used species of yeast is *Saccharomyces cerevisiae* on the base of its ability to ferment sugar in the dough at very fast rate and to grow rapidly [27]. The CO₂ involved during the fermentation is responsible for the rising of the dough. The process of mass production of Baker's yeast is done under controlled conditions of pH, humidity, and temperature [28].

Chocolate production by yeast and bacteria

Chocolate is produced with the help of microbes. Chocolate is actually obtained from the seeds of cacao trees. These seeds are present in white fleshy pods of cacao trees [29]. For the removal of the seeds out of these pods, the pods are fermented first with naturally occurring microbes mainly includes yeasts and bacteria, that is, *Lactobacilli* and *Acetobacter*. The raise in temperature during fermentation process is occurred and products obtained by these microorganism, that is, the ethanol produced from yeast, kill the beans, and become contributor in flavoring of the chocolate [30]. In chocolate, fermentation is the process that produces aromas, flavor, and rich colors. There are two stages involved in this process, that is, alcoholic fermentation and acetic acid fermentation. In this process, first, the sugar is converted into alcohol in the cocoa pulp by yeast activity. Then, bacteria oxidize the alcohol and produce acetic acid [31].

Advantages of use of microbes in food industry

The benefits of using microbes as a food source are [32]:

- The growth of microbes is very fast and they do not need much space as the conventional ways require
- The protein content of microbe's cells is very high. They have a protein content that is about 40–50% in bacteria and 20–40% in algae
- They are also helpful in recycling the waste materials and therefore, clean up the waste products
- They have high yielding ability
- They are less effected by the environmental factors, such as climate does not affect them
- The microbe's proteins contain all the essential amino acids
- Their growth can be obtained on a wide range of cheap, Agricultural waste products and industrial by-products, that is, methanol, ethanol, other petroleum products, sugar, molasses, waste from paper mills, etc.
- Some microorganisms, mainly yeasts, have high content of vitamins.

PHARMACEUTICAL INDUSTRY

The role of microorganisms can be justified on the basis of the great association present between specific diseases and microbial activities. Because of microbiology, many advancements have led to the great outcomes in the field of both pharmaceutical and medical industries along with many discoveries and inventions. However, many of the microbes cause different microbial infections and infectious diseases, such as HIV but also have a great importance in immune system as well as digestive system. The microbiologist and pharmacists are working to make the drug therapies that must target the opportunistic infections diseases causing microbes rather than the host cell of the human body.

Many of important pharmaceutical products that are obtained by making use of microbes such as bacteria are mostly protein in nature, for example, the protein of *Halobacterium salinarum* plasma membrane, that is, Bacteriorhodopsin [33].

The beneficial products that are obtained by making the use of microbes in pharmaceutical industries are described below:

Vaccine production

A vaccine totally comprises biological productions from an agent that is disease causing microorganisms or resembles with disease causing microorganisms and have ability to provide immunity against that particular disease, from the causal organism, of which the biological preparation was actually made [34]. These biological preparations are mostly obtained from the killed or weekend form of microbes or may be made from surface protein or toxins of disease causing organism. The vaccination mostly do not have any kind of adverse reactions and by making sure the routine vaccination program, health department has protected millions of children from great number of opportunistic infections and diseases that has resulted in a lot of mortalities in past [35].

To minimize the risk and enhance the ability of vaccination, different types of vaccines are available.

Vaccine from killed microorganisms

In these types of vaccines, the microorganisms are first killed by different chemical, antibiotics, radioactivity, or by heat. These are vaccinations used against rabies, polio, hepatitis A, and influenza.

Vaccines from live microorganisms

Many of vaccines contain the living microorganism that is non-virulent and their virulency is destroyed by culturing them in the specific conditions that make disabled the new culture of microorganism to cause disease. The preparation of mostly attenuated vaccines is done from viruses and bacteria. These are the vaccines mainly used against typhoid, mumps, measles, yellow fever, and rubella [36].

Vaccines from the different products of microorganisms

Other vaccines may be obtained from toxoid compounds of microorganism, that is, toxoid based vaccination for diphtheria and tetanus, from protein subunits, that is, vaccination against hepatitis B virus and may be from conjugate compounds, that is, vaccination against high influenza type B (Hemophilina) [37].

Antibiotics

Antibiotics are antimicrobial agents that have ability to inhibit the growth of microbes or to kill the microbes, that is, bacteria and fungi. These are the component that are produced by the microorganism and have antagonistic activities against the growth of microbes. With the advancement in medical science, today the most of antibiotics are natural compounds obtained from the microorganisms, that is, penicillin some fungus *Penicillium* [38].

Probiotics

The live bacterial or other microbial supplements are also used that have beneficial effects on the host by improving the microbial balance of intestine [39]. They are the substances that are secreted by one microorganism and stimulate the other microorganisms. The most common used microbes as probiotic are lactic acid bacteria and biofidobacteria. In case of yoghurt, soya yogurt and dietary supplements, the active live culture of probiotic is added.

Other medical productions

Other than the above-mentioned products, there are large numbers of the products that are obtained from the microbes. Some of them are given as follow:

- Variety of enzymatic products is obtained from the large number of microbes. For example, protease enzyme is obtained from *Aspergillus flavus* [40]
- Using various enzymatic processes, the fat soluble vitamins and water-soluble vitamins are obtained from microbes. For example, Vitamin K2 is obtained from the conversion of quinone and precursor of chain reaction using *Flavobacterium* sp. [41]
- The alternatives of classic antibacterial compounds and small molecules that are peptides and are more readily engineered molecules are Bacteriocin. These are also prepared from the microbes mostly bacteria [42]
- Phage-therapy is another technique that makes the use of viruses to infect bacteria, that is, bacteriophage and widely used in medical field for cure of various bacterial infections [43].

REFERENCES

1. Shin SG, Han G, Lim J, Lee C, Hwang S. A comprehensive microbial insight into two-stage anaerobic digestion of food waste-recycling wastewater. *Water Res* 2010;44:4838-49.
2. Schmeisser C, Steele H, Streit WR. Metagenomics, biotechnology with non-culturable microbes. *Appl Microbiol Biotechnol* 2007;75:955-62.
3. Kharatyan SG. Microbes as food for humans. *Ann Rev Microbiol* 1978;32:301-27.
4. Kapoor D, Sharma P, Sharma MM, Kumari A, Kumar R. Microbes in pharmaceutical industry. In: *Microbial Diversity, Interventions and Scope*. Singapore: Springer; 2020. p. 259-99.
5. Kumar R, Kumawat N, Sahu YK. Role of biofertilizers in agriculture. *Pop Kheti* 2017;5:63-6.
6. Subba R. *Biofertilizers in Agriculture and Forestry*. 3rd ed. New Delhi: International Science Publisher; 1993.
7. Sandle T. Use of hazard analysis and critical control points. Part 2: Determining environmental monitoring locations. *Eur J Parenteral Pharm Sci* 2019;24:32.
8. Mia MB, Shamsuddin ZH. Rhizobium as a crop enhancer and biofertilizer for increased cereal production. *Afr J Biotechnol* 2010;9:6001-9.
9. Wu SC, Cao ZH, Li ZG, Cheung KC, Wong MH. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: A greenhouse trial. *Geoderma* 2005;125:155-66.
10. Abdel-Aziez SM, Eweda WE, Girgis MG, Ghany BF. Improving the productivity and quality of black cumin (*Nigella sativa*) by using azotobacter as N2 biofertilizer. *Ann Agric Sci* 2014;59:95-108.

11. Mishra U, Pabbi S. *Cyanobacteria*: A potential biofertilizer for rice. *Resonance* 2004;9:6-10.
12. Mehnaz S. *Azospirillum*: A biofertilizer for every crop. In: *Plant Microbes Symbiosis: Applied Facets*. New Delhi: Springer; 2015. p. 297-314.
13. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* 2003;255:571-86.
14. Hoagland RE, Boyette CD, Weaver MA, Abbas HK. Bioherbicides: Research and risks. *Toxin Rev* 2007;26:313-42.
15. Putter I, Mac Connell JG, Preiser FA, Haidri AA, Ristich SS, Dybas RA. Avermectins: Novel insecticides, acaricides and nematocides from a soil microorganism. *Experientia* 1981;37:963-64.
16. Dave RI, Shah NP. Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. *Int Dairy J* 1997;7:31-41.
17. Oyeleke SB. Microbial assessment of some commercially prepared yoghurt retailed in Minna, Niger state. *Afr J Microbiol Res* 2009;3:245-48.
18. Sandoval-Castilla O, Lobato-Calleros C, Aguirre-Mandujano E, Vernon-Carter EJ. Microstructure and texture of yogurt as influenced by fat replacers. *Int Dairy J* 2004;14:151-9.
19. Chollet M, Gille D, Schmid A, Walther B, Piccinali P. Acceptance of sugar reduction in flavored yogurt. *J Dairy Sci* 2013;96:5501-11.
20. Holmes DG, Duersch JW, Ernstrom CA. Distribution of milk clotting enzymes between curd and whey and their survival during cheddar cheese making. *J Dairy Sci* 1977;60:862-69.
21. Chapman HR, Bines VE, Glover FA, Skudder PJ. Use of milk concentrated by ultrafiltration for making hard cheese, soft cheese and yoghurt. *Int J Dairy Technol* 1974;27:151-5.
22. Desobry-Banon S, Richard F, Hardy J. Study of acid and rennet coagulation of high pressurized milk. *J Dairy Sci* 1994;77:3267-74.
23. Maubois JL, Mocquot G. Application of membrane ultrafiltration to preparation of various types of cheese. *J Dairy Sci* 1975;58:1001-7.
24. Roudot-Algaron F, Bars DL, Kerhoas L, Einhorn J, Gripon JC. Phosphopeptides from comté cheese: Nature and origin. *J Food Sci* 1994;59:544-7.
25. Edema MO, Sanni LO, Sanni AI. Evaluation of maize-soybean flour blends for sour maize bread production in Nigeria. *Afr J Biotechnol* 2005;4:71122.
26. Zhang L, Lucas T, Doursat C, Flick D, Wagner M. Effects of crust constraints on bread expansion and CO₂ release. *J Food Eng* 2007;80:1302-11.
27. Asyikeen ZN, Ma'aruf AG, Sahilah AM, Khan AM, Aida WW. A new source of *Saccharomyces cerevisiae* as a leavening agent in bread making. *Int Food Res J* 2013;20:967-73.
28. Baldo BA, Baker RS. Inhalant allergies to fungi: Reactions to bakers' yeast (*Saccharomyces cerevisiae*) and identification of bakers' yeast enolase as an important allergen. *Int Arch Allergy Immunol* 1988;86:201-8.
29. Motamayor JC, Risterucci AM, Lopez PA, Ortiz CF, Moreno A, Lanaud C. Cacao domestication I: The origin of the cacao cultivated by the Mayas. *Heredity* 2002;89:380-6.
30. Zhao J, Fleet G. Yeasts are essential for cocoa bean fermentation. *Int J Food Microbiol* 2014;174:72-87.
31. Schwan RF, Wheals AE. The microbiology of cocoa fermentation and its role in chocolate quality. *Crit Rev Food Sci Nutr* 2004;44:205-21.
32. Caplice E, Fitzgerald GF. Food fermentations: Role of microorganisms in food production and preservation. *Int J Food Microbiol* 1999;50:131-49.
33. Leader B, Baca QJ, Golan DE. Protein therapeutics: A summary and pharmacological classification. *Nat Rev Drug Discov* 2008;7:21-39.
34. Matthijs AM, Auray G, Jakob V, Garcia-Nicolás O, Braun RO, Keller I, et al. Systems immunology characterization of novel vaccine formulations for *Mycoplasma hyopneumoniae* bacterins. *Front Immunol* 2019;10:1087.
35. World Health Organization. International travel and health. In: *Vaccine-preventable Diseases and Vaccines*. Ch. 6. Geneva: World Health Organization; 2015.
36. Lartigue C, Timana YV, Labroussaa F, Schieck E, Liljander A, Sacchini F, et al. Attenuation of a pathogenic *Mycoplasma* strain by modification of the obg gene by using synthetic biology approaches. *Msphere* 2019;4:19.
37. Bonten MJ, Huijts SM, Bolkenbaas M, Webber C, Patterson S, Gault S, et al. Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults. *N Engl J Med* 2015;372:1114-25.
38. Gaynes R. The discovery of penicillin-new insights after more than 75 years of clinical use. *Emerg Infect Dis* 2017;23:849.
39. Sulakvelidze TL, Telli GS, de Carla DD, Gonçalves GS, Ishikawa CM, Cavalcante RB, et al. Effect of feeding strategy of probiotic *Enterococcus faecium* on growth performance, hematologic, biochemical parameters and non-specific immune response of Nile tilapia. *Aquac Rep* 2020;16:100277.
40. Reese ET, Maguire A. Surfactants as stimulants of enzyme production by microorganisms. *Appl Microbiol* 1969;17:242-5.
41. Mahdina E, Demirci A, Berenjian A. Production and application of menaquinone-7 (Vitamin K2): A new perspective. *World J Microbiol Biotechnol* 2017;33:2.
42. Delves-Broughton J, Blackburn P, Evans RJ, Hugenholtz J. Applications of the bacteriocin, nisin. *Antonie Van Leeuwenhoek* 1996;69:193-202.
43. Sulakvelidze A, Alavidze Z, Morris JG. Bacteriophage therapy. *Antimicrob Agents Chemother* 2001;45:649-59.