Chapter 8
Microbial Proteins: A Potential Source of Protein

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8.1 Introduction

The difference between food availability and population growth is widening in recent years and population growth is surpassing food availability. This lack of equilibrium is mainly due to the increasing growth rate of the world population, longer life expectancy, constant rate of food production, along with uneven distribution. The increase in population growth is very high in twentieth century, while the sources and production rate of food supply are nearly at a constant level (Stephens et al. 2018). This problem has prompted initiation of strategies and research in recent years to limit the population growth rate. However the results of these approaches are not very promising. Even if these approaches are successful, the desired results are not going to be achieved in a few decades and then it would be too late to resolve the looming food crisis. Additionally, some available areas of land are not yet cultivated. Techniques and assets of modern technology and agriculture can be utilized to get a maximum exploitation of land and produce high yield of foodstuff. Yet, experts agree that the difference between population growth and conventional food supply is too big to bridge (Suman et al. 2015).

The minimum requirement for survival and health does not only depend on the amount of food but also on its nutritional value. Unfortunately half of the world's population live and survive below this minimum requirement. It is not only about lack of availability of food but the nutritional value of food is also critical. Nowadays significant part of the world population is malnourished, even in regions where raw material is available for microbial fermentation. The significance of microbes as possible resources of food originates from the concept that microbial cell content is particularly high in most B-group vitamins and in proteins that comprise vital amino acids. Thus, microbes can possibly augment an undersupplied diet in that proteins

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produced from microbes are very much similar to traditional protein sources such as meat, egg, fish and milk in terms of overall presence of nutrients (Upadhyaya et al. 2016).

8.2 Microbial Protein Synthesis

Proteins synthesis is carried out by the ribosomes along with messenger RNA, produced from the transcription of DNA in the nucleus of eukaryotes however there is no nucleus present in prokaryotes so both transcription and translation occur in the cytoplasm. In the DNA, a series of codes are transcribed and translated to synthesize proteins. Transcription proceeds with the opening of the DNA double helix with the help of an enzyme called DNA helicase. Another enzyme, RNA polymerase equates new bases to the DNA joining them in a long strand of mRNA. When the enzyme completes the transcription the mRNA strand will be detached. This mRNA strand is single stranded and only one side of the DNA strand is transcribed. mRNA does not have the same base pairs as DNA, one of the base is replaced. Thymine in DNA is replaced by Uracil (U) which bonds to Adenine, like thymine. The mRNA is organized in codons. Each codon codes for an amino acid that forms the building blocks of proteins. More than one codon can code for a single amino acid. Once transcription is completed, produced mRNA is transported out of nucleus into the cytoplasm to attach with a ribosome. Ribosome is an organelle which carries out protein synthesis. Some ribosomes are free in the cytoplasm while others are attached to the endoplasmic reticulum. The endoplasmic reticulum is an organelle which helps in the correct folding of proteins and delivers it to other areas. Proteins are synthesized by the process of translation. There are three steps to translation: Initiation, Elongation and Termination.

The ribosome binds around the mRNA and then a tRNA (transfer RNA) joins to a codon referred to as the start codon. In most cases, the start codon is AUG. tRNA are molecules residing in the cytoplasm, each with their particular codon which joins to corresponding codons on mRNA. tRNAs carry the amino acids which complement to that of the codon. The ribosomes are composed of two subunits, a larger subunit and another smaller one. There are three sites present where tRNA can be found at a given time while the protein is being synthesized. Next the ribosome starts synthesizing protein. An amino acid which complements the mRNA code begins to join in a strand, the ribosome moves on the strand in a direction of $5'–3'$ until a STOP codon is found. As the ribosome moves on the mRNA the amino acid from the first tRNA is released and binds to the amino acid on the next tRNA via a peptide bond. This is why a chain of amino acids is termed as a polypeptide chain. The ribosome achieves this with the aid of enzymes known as aminoacyl tRNA synthase. Once the ribosome finds a stop codon (UAG, UAA, and UGA) it will detach the polypeptide chain and fall off from the mRNA (Lewin et al. 2011).

8.3 Microbes for Microbial Protein Production

In this section, we describe the various microbes that are utilized for the production of microbial proteins (Fig. 8.1).

8.3.1 Bacteria

Properties which make bacteria appropriate for microbial protein production include fast growth and reproduction rate with minimum generation time. Bacteria can be grown on a variety of raw materials ranging from carbohydrates to gaseous and liquid hydrocarbons that comprise methane and petroleum fractions (Bamberg 2000) to petrochemicals like methanol and ethanol; nitrogen sources that are beneficial for bacterial growth comprise nitrates, ammonia, ammonium salts, urea, and the organic nitrogen present in wastes. It is recommended to provide mineral nutrient to the bacterial culture to fulfill shortage of nutrients that might be lacking in natural waters in amount enough to sustain growth. Generally phototrophic bacteria are suggested for microbial protein production. Some investigators also propose utilization of methanotrophic and other bacteria for microbial protein

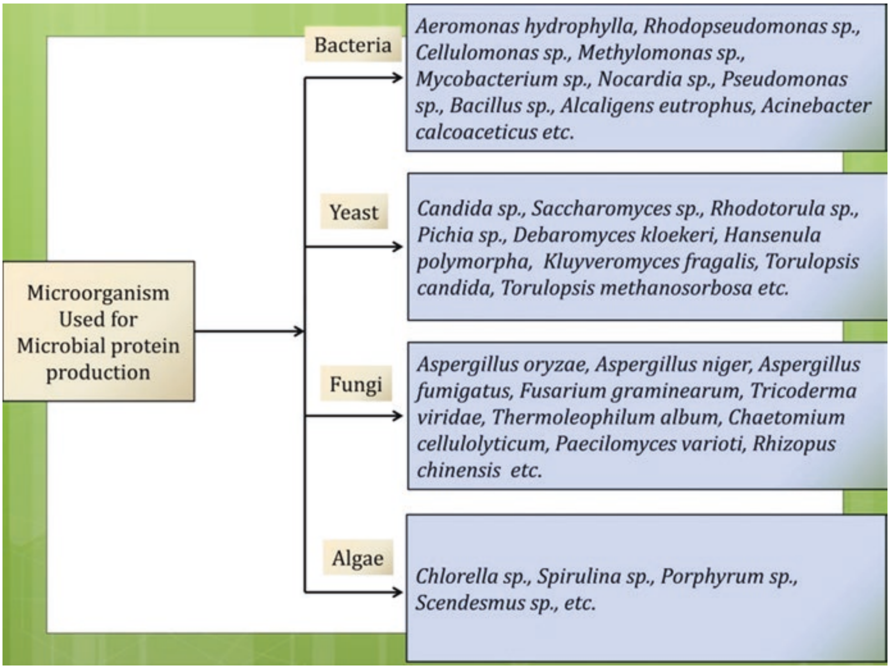

Fig. 8.1 Various microorganisms utilized for the production of microbial proteins

production. Generation time of *Methylophilusis* is about 2 h and it is utilized as animal feed and generally produces more favorable protein composition in comparison to fungi or yeast. Large amounts of microbial protein can be produced utilizing bacteria like *Methylophilus methylitropous*, *Acinetobacter calcoaceticus, Acromobacter delvaevate*, *Aeromonas hydrophilla, Bacillus megaterium, Bacillus subtilis, Brevibacterium, Lactobacillus species, Cellulomonas species, Methylomonas methylotrophu*s, *Pseudomonas fluorescens, Rhodopseudomonas capsulate, Flavobacterium species, Thermomonospora fusca* (Dhanasekaran et al. 2011; Suman et al. 2015).

8.3.2 Algae

Centuries ago *Spirulina* was used by people of Aztecs near Texcoco in Mexico and near lakechad in Africa. It was used as food after drying. *Spirulina* is the most commonly used algae as microbial protein. Even astronauts carried it to space during their space travels. Likewise, biomass gained from *Senedessmus* and *Chlorella* has been collected and utilized as food by tribal groups in some parts of the world. Algae are used as source of food in various ways and its benefits include relatively easy cultivation, efficient utilization of solar energy, high growth rate and rich protein content. The algae *Spirulina* is recommended as supplemental protein (Raja et al. 2008). It is blue green algae with strong antioxidant activity and incites a free radical scavenging enzyme system. A diet supplemented with *Spirulina* and other nutraceuticals can help to protect stem/progenitor cells. *Spirulina maxima* in particular, can help to avoid fatty liver development triggered by carbon tetrachloride. Evidence has shown that *Spirulina* can help patients with immune suppression, malnutrition, neural and hepatic compromise. Further inquiries on the antiviral effects of this alga and its clinical consequences are strongly needed (Suman et al. 2015).

8.3.3 Fungi

Various fungi are utilized as a source of protein rich food (Bhalla et al. 2007). Filamentous species of fungi are also utilized as microbial protein. In the late 1900s, filamentous fungi and *Actinomycetes* bacteria were noted to produce protein from several substrates. At the time of World War II, efforts were made to utilize *Fusarium* and *Rhizopus* grown in fermenters as a source of protein rich food. The strains of *Aspergillus oryzae* or *Rhizopus arrhizus* were chosen due to their nontoxic behavior. Saprophytic fungi can be grown on complex organic compounds and can render them into simple substrate. Large amount of fungal biomass is formed as a result of growth. Yield of mycelia varies greatly depending upon organisms and substrates. On the other hand, some species of moulds, for example, *Aspergillus niger, A. fumigates*, *Fusarium graminearum* which are reported to be dangerous to humans, must not be utilized or toxicological estimations should be performed before endorsing them as microbial protein (Suman et al. 2015). Lately, microbial protein technology using fungal species is utilized for the conversion of lignocellulose wastes. The filamentous fungi that have been used as microbial proteins comprise *Fusarium graminearum, Chaetomium celluloliticum, Aspergillus niger, A. fumigates, A. oryzae, Penicillium cyclopium, Cephalosporium cichorniae, Rhizopuschinensis, Tricoderma viridae, Scytalidum aciduphlium, and Tricoderma alba Paecilomyces varioti* (Jaganmohan et al. 2013).

8.3.4 Yeast

Yeast cells are high in proteins and nutrients (Burgents et al. 2004). Most popular yeast species utilized as microbial proteins comprise *Torulopsis, Candida, Hansenula, Pitchia*, and *Saccharomyces*. Microbial protein production utilizing *Saccharomyces cerevisiae, grown* on various fruit wastes has been done successfully. The typical oily yeasts genera including *Yarrowia, Candida, Rhodotorula, Rhodosporidium, Cryptococcus, Trichosporon and Lipomyces* have also been utilized as microbial proteins. Cucumber and orange peels were evaluated for the production of microbial protein using *Saccharomyces cerevisiae* by submerged fermentation (Sengupta et al. 2006).

8.4 Process of Microbial Protein Production

Microbial proteins can be produced by using waste materials as substrates, predominantly from agricultural waste like crumbs, wood cuttings, and corncobs among others. Few additional waste substances utilized as substrates are remnants from alcohol manufacturing processes, food processing, hydrocarbons, or even human and animal feces (Suman et al. 2015).

As described by Nasseri et al. (2011), the process of microbial protein production from any substrate or microorganism will necessitate some vital stages (Fig. 8.2):

(1) based on the condition of carbon source physical and/or chemical pretreatments may be needed.
(2) Together with the carbon source, nitrogen, phosphorus and few other nutrients are also needed to sustain microorganism's optimal growth.
(3) Contamination needs to be avoided by sustaining a sterile and aseptic environment. For this purpose, the constituents of the medium needs to be sterilized by heating or filtration and the apparatus used in the fermentation process needs to be heat sterilized.
(4) The desired microorganism should be inoculated in the pure form.

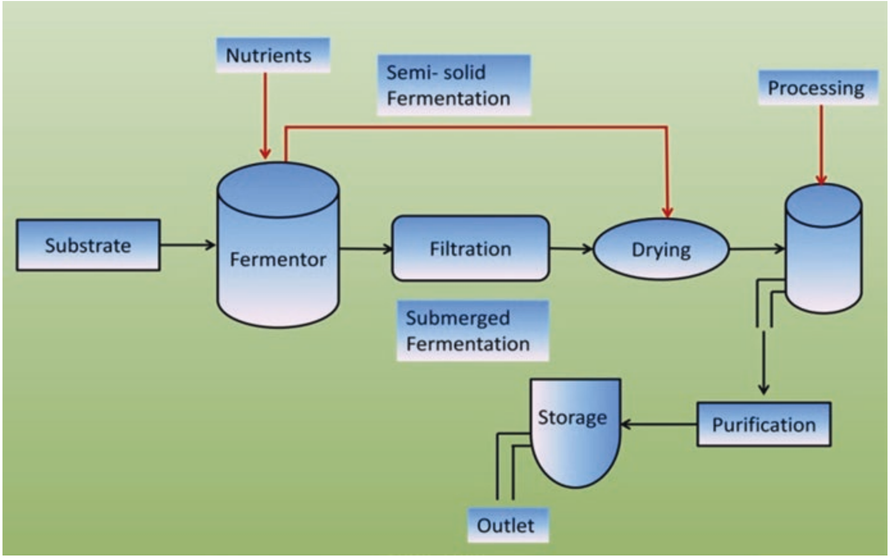

Fig. 8.2 Graphical representation of processes involved in the production of microbial proteins

(5) Procedures for microbial protein production are particularly aerobic (eliminating those using algae). Thus, adequate amount of oxygen needs to be provided. Furthermore, cooling systems are also vital as large amount of heat is produced.
(6) Recovery of the microbial biomass is achieved from the medium.
(7) In order to increase microbial protein use and/or storage time, processing of the microbiological biomass is necessary.

8.5 Nutritional Values of Microbial Proteins

The nutritional value of microbial proteins differs with the microorganisms used. The technique of drying, harvesting and processing affects the nutritional value of the end product. The composition of microbial protein depends on the microorganism and the substrate used to produce it (Suman et al. 2015).

Microbial protein generally includes proteins, fats, carbohydrates, water, and other elements like potassium and phosphorus. Aside from their nutritional benefits, microbial proteins can also be produced whole year round so supply may not be a problem. It can also help the environment as waste materials are utilized as substrates. Very less area of land is needed and microbial protein is produced in a very short time in comparison to other sources.

To evaluate nutritional value of microbial proteins, numerous factors should to be taken in consideration like composition of nutrients, amino acids and vitamins;

their effects on the gastrointestinal tract and the risk of allergies. The carcinogenic and toxicological potentials should also be evaluated so, feeding trials are also needed. The process of harvesting, drying and processing can also affect the nutritional values of the microbial protein. Microbial protein composition depends on the type of substrate and organism used. Proteins not only provide nutritional value but they are also involved in a number of other functions (Upadhyaya et al. 2016).

Microbial protein from yeast and fungi has 50–55% protein. It has high protein and carbohydrates ratio. It contains large number of lysine residues and less number of methionine and cysteine residues. It also provides good balance of amino acids and it contains high B complex vitamins. Few yeast strains have probiotic characteristics like *Saccharomyces cerisiae* and *Debaryomyces hansenii*. Microbial proteins produced by utilizing bacteria comprise more than 80% protein even though they contain a small amount of sulphur containing amino acids with high nucleic acid content (Upadhyaya et al. 2016).

Excellent nutrient profiles and the possibility of economical mass production make microbial proteins a potential solution for the ongoing food crisis. They are also used in aquaculture feeding as a replacement for fishmeal and for protection of rotifer and *Artemia.* Yeast proteins have revolutionized the aquaculture diets. Few yeast strains with probiotic characteristics, like *Debaryomyces hansenii* and *Saccharomyces cerevisiae* have the ability to boost survival of larvae either by residing in the fish gut, thus eliciting the early maturation of the pancreas, or through the immune exciting glucans derived from the yeast cell walls. The idea that microbial proteins can be the solution of food shortages in the less developed countries is gaining research interests among scientists. To assure future success of microbial proteins, first, food technology problems need to be resolved in order to make it comparable to the conventional foods and second, the production should equate favorably with other protein sources (Suman et al. 2015).

8.6 Harmful Effects of Microbial Proteins

The potentials that microbial proteins can do to human beings and the world's problem on hunger are gaining special interests but concerns regarding their acceptability, safety and potential toxicity are emerging. For one, high nucleic acid content in microbial proteins can be a problem which is noted to be beyond the acceptable level of 71%. To address this, nucleic acid can be removed or reduced with either one or all of the following methods: by the chemical treatment with sodium hydroxide, treating the cells with 10% sodium chloride, activating endogenous nucleases in last step of biomass production and thermal shock. Hueihsiung Yang developed a modest technique to reduce the nucleic acid content in *Brevibacterium NNJM98A* by incubating non-proliferating cells at a pH of 10.3 and 55 °C for 3 h (Upadhyaya et al. 2016).

Another issue is the presence of cell walls that cannot be digested. As with yeast and algae, there might be intolerable colour and flavours, and live cells of organisms need to be killed prior to consumption. There is a chance of skin reaction due to ingestion of foreign proteins and gastrointestinal reactions might occur, which may give rise to nausea and vomiting. There is high risk of contamination during the process of production and cell recovery is also problematic. All these detrimental factors lessen the acceptability of microbial protein as a potential global food (Suman et al. 2015).

8.7 Conclusion

Indeed, microbial proteins demonstrate very promising characteristics as a nutrient for humans. Microbial proteins possess various advantages in comparison to plant and animal proteins due to ease of reproduction and growth that is not seasonal or climate dependent. Microbial proteins have high protein content with wide spectrum of amino acids, low fat content and high protein to carbohydrate ratio. These can be produced using wastes as substrates and are therefore environment friendly. The utilization of microbial proteins as a nutrient source can resolve issues related with food shortage of rapidly growing population especially in underdeveloped countries. However with all of these benefits, microbial protein production has not gained much attention due to lack of popularity as a nutrition source among people. Furthermore, high nucleic acid content, non-digestible cell walls, unpleasant colors and flavors and risk of contamination further limit their acceptability as a global food. So efforts needs to be made to uncover alternate substrates and approaches which can minimize the drawbacks of microbial protein production which consequently can lead to the acceptance of this valuable nutrient source on a global scale (Upadhyaya et al. 2016).

Due to economic, political and practical issues, microbial protein has not become the major supply of foodstuff protein as initially envisioned. Developed countries do not see the need of utilizing microbial proteins because they have abundant stocks of high value proteins due to advances in agriculture. Microbial proteins can still be a supportive food source in tropical less developed countries where traditional food is high in carbohydrates and low in proteins. Continuing increase in population growth rate can eventually lead to shortage of food and nutrition even with agricultural advancement. Providing sufficient nutritious food to everyone is going to be a daunting task for future generations. Microbial proteins despite its current poor popularity can still be a promising solution for this problem in the future.

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References

Bamberg J (2000) British petroleum and global oil 1950–1975: the challenge of nationalism. Cambridge University Press, Cambridge

Bhalla TC, Sharma NN, Sharma M (2007) Production of metabolites, industrial enzymes, amino acids, organic acids, antibiotics, vitamins and single cell proteins. J Environ (6):34–78

Burgents JE, Burnett KG, Burnett LE (2004) Disease resistance of Pacific white shrimp, Litopenaeus vannamei, following the dietary administration of a yeast culture food supplement. Aquaculture 231(1–4):1–8

Dhanasekaran D, Lawanya S, Saha S et al (2011) Production of single cell protein from pineapple waste using yeast. Innov Rom Food Biotechnol 8:26–32

Jaganmohan P, Daas BP, Prasad SV (2013) Production of single cell protein (SCP) with Aspergillus terreus using solid state fermentation. Eur J Biol Sci 5:38–43

Lewin B, Krebs J, Kilpatrick ST et al (2011) Lewin's genes X. Jones & Bartlett Learning, Sudbury

Nasseri AT, Rasoul-Amini S, Morowvat MH (2011) Single cell protein: production and process. Am J Food Technol 6:103–116

Raja R, Hemaiswarya S, Kumar NA et al (2008) A perspective on the biotechnological potential of microalgae. Crit Rev Microbiol 34:77–88

Sengupta S, Bhowal J, Bhattacharya U (2006) The Association of official analytical chemists. The official methods of analysis of AOAC International. J Environ Issues 6:99–126

Stephens EC, Jones AD, Parsons D (2018) Agricultural systems research and global food security in the 21st century: an overview and roadmap for future opportunities. Agric Syst 163:1–6

Suman G, Nupur M, Anuradha S et al (2015) Single cell protein production: a review. Int J Curr Microbiol App Sci 4:251–262

Upadhyaya S, Tiwari S, Arora N et al (2016) Microbial protein: a valuable component for future food security. In: Singh JS, Singh DP (eds) Microbes and environmental management. Studium Press, New Delhi