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Khon Kaen University, Thailand**Review on alternative proteins: Marine macroalgae, yeasts and bacteria**Reddi S.S. Keerthi¹, Binod Pokharel^{1,*}, Prashant Mainali², Ziyad H.H. Abunamous¹ and Rajamahanti Vathsala¹¹Andhra University, Visakhapatnam, Andhra Pradesh, India²National University of Singapore, Singapore

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Abstract

The increasing global population and the decreasing crop production due to climate change threaten future food security. Providing adequate protein to the entire global population has become challenging since there are limited resources, such as land and water, for farming and agricultural purposes. Therefore, investigations on alternative protein sources have become critical. However, protein sources such as marine macroalgae, yeast, and bacteria appear to have been largely overlooked. Brown macroalgae contain a relatively low protein amount in their dry mass, while green and red macroalgae are rich protein sources. For example, *Porphyra* spp. are red macroalgae with protein content and quality similar to soybean. Their protein content ranges from 38% to 52%, and their production can use locally available substrates unrelated to human food sources. Similarly, Yeast is a richer source of protein which ranges from 40% to 55%. The industrial production of yeast can be done from locally available biomass that does not clash with human foods. Bacteria have a higher protein content that ranges from 50% to 83% of their dry mass. In addition, bacteria can grow more rapidly than other alternative protein sources. Significant achievements can be made through protein production technology with the proposed alternative protein sources to maintain global food security.

Keywords: Food-based dietary guidelines, Generation time, Genetic engineering, Microbial proteins**1. Introduction**

Uncontrolled global population growth and the estimated reduction in agricultural output due to altered climatic conditions raise questions about near-term food security [1,2]. In the current scenario, agriculture (except rubber, fibre, and narcotics) represents 43% of cultivable land [4] and 70% of freshwater use [5] worldwide. To provide the increasing population with adequate proteinaceous food, agricultural protein production needs to more than double by 2050 compared to 2005 [3]. However, there is a decline in available land and water for farming and agricultural purposes. Animal sources represent only one-third of worldwide protein consumption, although most are used to produce animal proteins [9]. In addition, due to urban sprawl, there is a huge demand for land and freshwater for forest conservation projects to produce biofuels, for wildlife conservation, and to mitigate climate change [6] by expanding forested areas. Since proteins are crucial macronutrients in diets, their abundance, biological value, and environmental impacts are key for estimating food security [7]. Minimal access and affordability to high biological value protein due to its uneven global distribution is the leading cause of malnutrition, especially in third world countries. For example, millions of small children rely upon inferior-quality protein sources with fewer essential amino acids (EAAs) [8]. In the near term, the availability and access to high-biological-value protein will become extremely challenging due to increased demand by the increasing population. Therefore, there is an urgent need for novel ideas and approaches to develop environmentally friendly, continual, climate-friendly protein production technologies with the lowest dependency on land and water resources [10].

Plant proteins have garnered significant attention in recent years since they can improve health markers such as the blood fat profile and glycemic control of diabetic patients when used as a substitute for animal protein [12]. Therefore, identifying alternative protein sources with beneficial effects similar to or better than plant proteins is

challenging. The coronavirus disease 2019 pandemic is a prime example since it has questioned animal protein distribution channels and food security worldwide, driving the importance of alternative plant-based protein sources [13]. In addition, plant proteins are rapidly being incorporated into emerging food-based dietary guidelines (FBDG). For example, a worldwide FBDG study estimated that 50% of countries recommend pairing plant and animal-based protein sources in their key food messages for protein intake [14]. Therefore, there are greater demands for production technologies providing sustainable, continual, climate and environment-friendly food protein sources that are vegetarian and less costly [11].

Industrial-scale microorganism production is achieved by culturing a particular strain in bioreactors supplied with adequate nutrient amounts under environmental conditions. The product created from culturing the desired microorganism is called biomass, which is further processed, purified, and sent for protein extraction. Various approaches are used to extract proteins from biomass, such as high-voltage electrical discharge, pulsed electric field, and ultrasound-assisted extraction methods. Therefore, proteins can be produced industrially in a sustainable, continual, and climate- and environmentally friendly manner.

Unfortunately, other potential protein sources, such as marine macroalgae, yeast, and bacteria, appear to have been relatively overlooked as protein sources. Therefore, this review focuses on marine macroalgae, yeast, and bacteria as alternative protein sources, highlighting their highly proteinaceous properties and high biological values.

2. Marine macroalgae

Marine macroalgae or seaweed are multicellular, photosynthetic plant-like protists. They have been categorized into red (Rhodophyta), brown (Phaeophyta), and green (Chlorophyta) macroalgae. Fucoxanthin pigment is responsible for the color of brown macroalgae. Similarly, phycobilin is responsible for the color of red algae. Moreover, pigments such as carotenes, xanthophyll, and chlorophyll a and b are responsible for the color of green macroalgae. They are richer protein sources, with proteins comprising up to 47% of their dry mass. However, insufficient research has examined the macronutrient content of macroalgae species. Moreover, extracting macroalgal proteins through raw biomass is challenging since they have rigid cell wall complexes.

The nutritional composition of a given macroalga varies based on its genetics, harvesting season, habitat, and growth conditions. In addition, the growth rate of macroalgae and their chemical composition may differ depending on the sunlight [18], harvest season [17], seawater salinity [19], sea depth [20], and nearby aquacultural plants. Brown macroalgae have a relatively low protein amount in their dry mass, with green and red macroalgae relatively more proteinaceous [22,23]. The proteinaceous content of their dry mass ranges from 10% to 30% in red macroalgae, 5% to 15% in brown macroalgae, and 3% to 47% in green macroalgae [25,26]. Research on marine macroalgae indicates that they become more proteinous during winter-early spring and less proteinous during summer-early autumn [27]. In addition, EAAs comprise almost half of all amino acids in macroalgal proteins [28] as shown in Figure 1 and Figure 2, meeting the EAA requirement of The Food and Agriculture Organization of the United Nations [29]. The EAA composition of macroalgal proteins aligns with those from other highly proteinaceous sources such as animal meat, poultry, fish, milk, egg, and soybeans [30], highlighting the great potential of macroalgae as an alternative protein source [31].

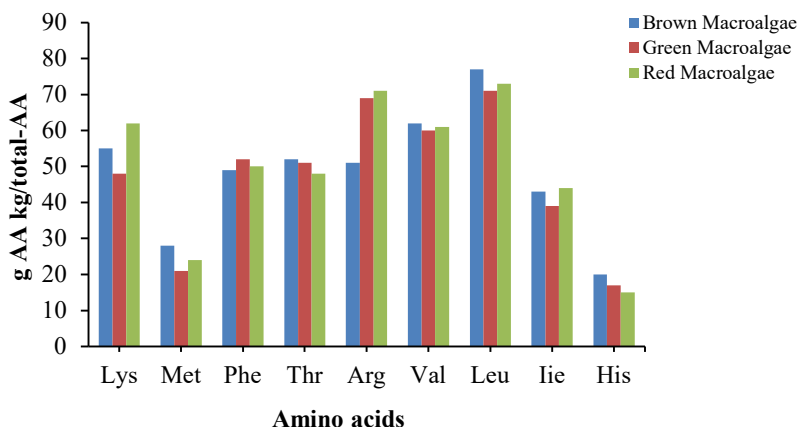


Figure 1 Amino acid composition of three types of marine macroalgae, expressed as (g AA kg/total-AA) [24].

Many macroalgae species have a ratio of EAA to total amino acid (TAA) greater than 450 g EAA kg of TAA [15]. The amino acid compositions of macroalgae show them to be high in glutamic acid and methionine but low

in histidine [16]. Their levels of biologically significant compounds in macroalgal proteins, such as taurine, carnosine, and glutathione, distinguish macroalgae [21].

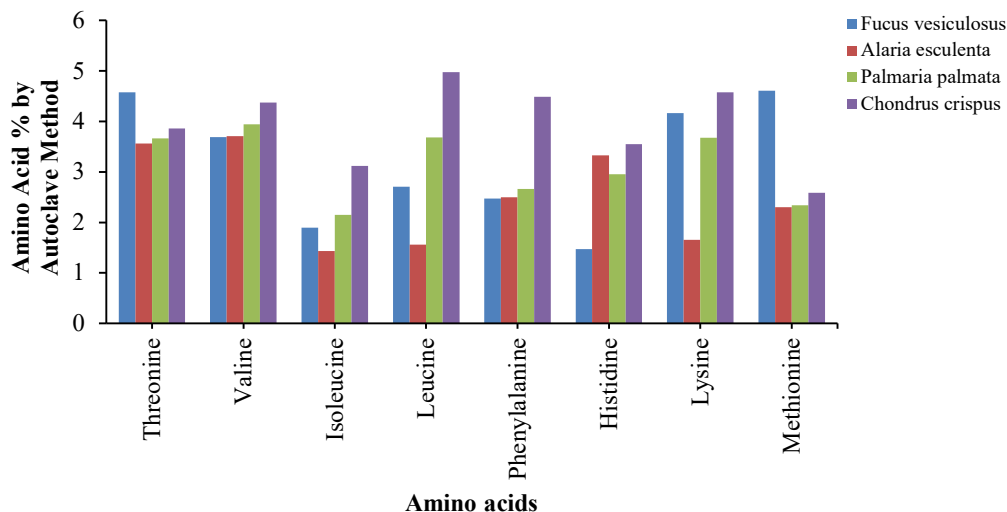


Figure 2 Amino acid composition of four strains of marine macroalgae, expressed as Amino acid % by Autoclave Method [37].

3. Yeasts

Yeasts are eukaryotic, single-celled microorganisms whose protein content ranges from 40% to 55%. For example, some yeast species, such as *Saccharomyces cerevisiae*, strictly undergo hexose fermentation. Similarly, the remaining yeast species ferment pentose sugars. In addition, the strict choice of yeast species for specific carbohydrate substrates can be altered by genetic engineering [33,34], using yeast strains that can ferment both sugar types [35], or by co-culturing two different yeast strains [36]. Moreover, growth conditions such as pH, temperature, and oxygen usually impact the biochemical constitution of yeast cells [37]. Indeed, proteins comprise 38% to 52% of the total mass of the five different yeast species whose EAA composition is shown in Figure 3. Yeasts are potential sustainable ingredients because of their value-adding capacity since they can transform non-food biomass into feed with lower land and water resource utilization and without impacting climate change [38].

Industrial yeast production can use locally available biomass that does not conflict with human foods through new technologies [39]. For example, *Cyberlindnera jadinii* grown on carbohydrate sources derived from lignocellulose have rich crude protein contents with EAA contents similar to soybean.

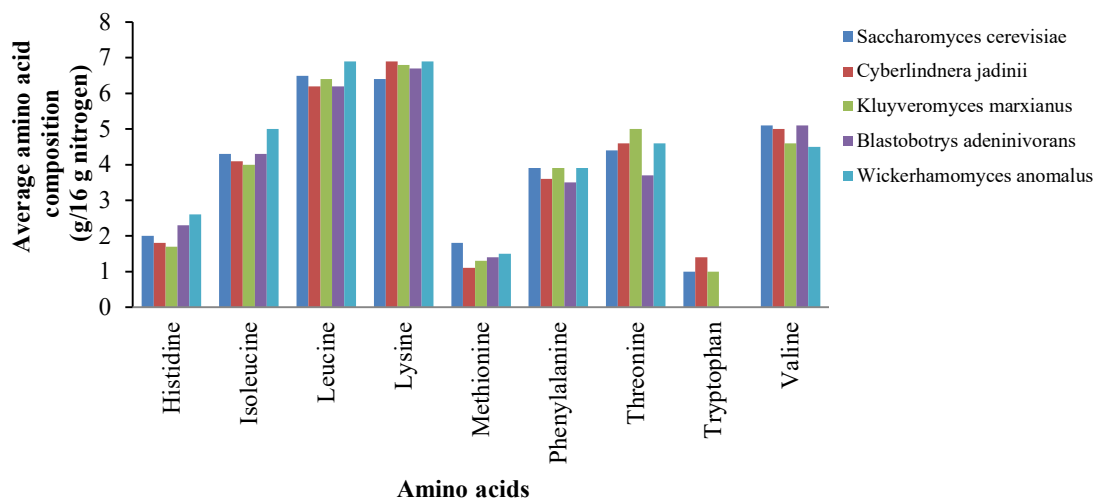


Figure 3 Amino acid composition of five yeast strains, expressed as Average amino acid composition (g/16 g nitrogen) [40].

4. Bacteria

Bacteria are small, single-celled prokaryotic organisms. To ensure food security with the smallest environmental impact through industrial high-quality protein production, microbial sources, such as bacteria, are potential alternatives to existing sources, such as animals and plants. For example, proteins from *Cupriavidus necator*, a Gram-negative bacteria found in soil, are relatively similar to traditional protein sources, such as soybeans, meat, and fish [41]. In addition, bacterial proteins have various beneficial characteristics over other protein sources. Bacteria are a rich protein source, ranging from 50% to 83% of their dry mass with significant EAA contents shown in Figure 4, and have higher growth rates than other alternative protein sources [42]. As the population doubling time of bacteria ranges from 20 minutes to 2 hours, multiplication can occur within a very short time [43]. In addition, they can grow on diverse substrates. Moreover, their bacterial proteins are more biologically significant than proteins from fungi.

Bacteria are more efficient in converting carbohydrates into proteinaceous biomass than other microbial protein sources, including fungi. Locally available and cheap carbon substrates, including waste materials, accessible in huge quantities, are mostly used for microbial protein production [44]. In addition, the industrial production process for microbial proteins can be made autonomous and stable, regardless of the environmental conditions. It can also effectively utilize substrates without any losses and does not require chemical agents such as herbicides or pesticides [45].

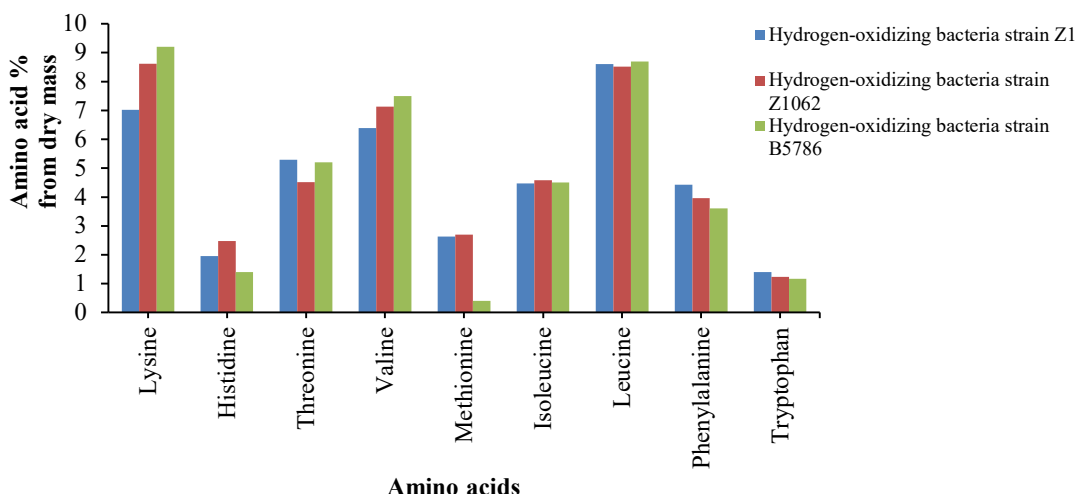


Figure 4 Amino acid composition of three strains of Hydrogen-oxidizing bacteria, expressed as % from the dry substance of a cell [41].

5. Summary

In conclusion, this review provided insights into alternative protein sources such as marine macroalgae, yeast, and bacteria. Green and red macroalgae, such as *Porphyra* spp., could be alternative protein sources since they are highly proteinaceous, with protein contents similar to soybean. While marine macroalgae can contain up to 47% protein, extracting macroalgal proteins from raw biomass is challenging since they have rigid cell wall complexes. Similarly, the protein contents of yeasts range from 38% to 52%, and their production can use locally available biomass that does not conflict with human foods. Finally, bacteria are rich protein sources, with proteins comprising 50% to 83% of their total dry mass and have higher multiplication rates than other alternative protein sources. However, marine macroalgae, yeast, and bacteria are not generally used as protein sources. Therefore, significant achievements can be made through protein production technologies using these alternative sources to maintain global food security. This review highlighted the possibility of industrial protein production through the near-term use of these alternatives.

Advancements in biotechnology herald an unprecedented change in food production. Therefore, they are expected to play pivotal roles in strengthening food security in the near future. For example, modern biotechnology has toolkits for growing *S. cerevisiae* with one carbon compound as its food source by integrating autotrophic carbon-fixing enzymes, making it possible to produce the necessary nutrients inside state-of-the-art bioreactors [46]. Moreover, advancements in animal/microbial cell culture in bioreactors, stem cell technologies, and the three-dimensional (3D) printing of proteins with meat-like textures have enabled the scientific community to grow cells in bioreactors and form them into products that look and taste like meat. These advancements are

reflected in the market since many animal-free protein companies (>100 globally) are burgeoning. Furthermore, other fields, such as synthetic and system biology, metabolomics, and artificial intelligence, will simultaneously aid in removing current bottlenecks in cellular agriculture for producing alternative proteins. Therefore, it is imperative to acknowledge that biotechnological advancements will continue to provide innovative solutions to diversify the sources of necessary alternative proteins in the future, not only for people living on Earth but for those on space missions [47].

However, current public perceptions of applying genetic engineering for alternative protein production and high-tech precision fermentation to produce animal meat are unfavorable. Therefore, classical biotechnology approaches leveraging non-modified cells, such as *Saccharomyces cerevisiae*, *Trichoderma reesei*, *Rhizopus oligosporus*, *Chlorella* sp., *Rhodospseudomonas* sp., and *Rhizopus oligosporus*, to produce proteins can be helpful. However, such processes will be sustainable only if their feed sources do not compete with normal human nutritional sources. There are many opportunities to use food and agricultural waste to grow these cells. Okara, seashell waste (rich in chitin), sugarcane bagasse, and palm oil waste are major food and agricultural wastes that could be harnessed worldwide. Enzymes could be used to convert this waste into simple sugars that could be used to grow these cells, creating a bio-circular economy, and meeting future nutritional demand [48]. Similarly, urban agriculture could aid alternative protein production. With rapid urbanization, food waste produced by cities will increase, which could be used as a feed source for growing insects. Black soldier flies, a promising alternative protein source, can easily feed on such food waste. Black soldier flies and their larvae are rich in proteins and chitin and could be integrated into human diets by initially using them as animal feed. The chitin from these insects could also be used as a food source to produce microbial single-cell proteins [49].

Science and technology are concrete and will only improve with time. The bottlenecks in using a diet integrating alternative protein stem mainly from cultural, nutritional/health, environmental, and awareness factors. Some cultures are keener to incorporate alternative proteins into their diets than others. Finally, changing consumer behavior is critical. Unless people are willing to give up or reduce their consumption of animal-derived meat and explore the available alternatives, these efforts will not bear fruit [50]. Therefore, proper government backing, policy intervention, and raising awareness should go hand-in-hand with the technological development of alternative proteins.

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