

## Use of Microorganism in Biotechnology

### Mycoprotein:

Fungi have a high protein content and grow rapidly, so offer considerable potential as a source of protein in human diet or as a supplements of animal feeds. They can grow on a wide range of substrates, including waste materials from industrial or other processes. Some edible large fungi are already well-known for their eating qualities- these include the common edible mushroom (*Agaricus biosporus*), other oyster mushroom (*Psalliota* sp.) and truffles (*Tuber melanosporum*). During the 1950s to 1970s, there was active research into ways of utilizing microorganisms as a source of food, to produce Single Cell Protein (SCP). The term SCP is used to describe protein derived from microbial cells such as (yeasts, other fungi, algae and bacteria), though the microorganism producing the protein is not necessarily 'single celled'. The whole organism is harvested and consumed, rather than using the products of their fermentation or other processing. Exploitation of SCP production offers a way of increasing the available protein for consumption by humans and by livestock, and could be valuable particularly in areas where the land infertile or the climate inhospitable. While SCP production may have potential for feeding the ever increasing world population, in practice only a few schemes have proved to be commercially successful-the most successful for human consumption being mycoprotein, marketed under the name of Quorn.

Mycoprotein is obtained from the growth of the fungus *Fusarium gramineum*. Glucose syrup is used as the carbon source, gaseous ammonia supplied the nitrogen and salts are added. Wheat or maize starch is used as a source of glucose, though other starchy crops can be used. Choline is added to encourage growth of long hyphae and biotin (A vitamin) is also required. The *Fusarium gramineum* is grown in 1300 liter continuous culture fermenter at

30°C and pH 6. The ammonia gas helps to maintain the pH and oxygen gas is supplied to keep conditions aerobic.



*Agaricus biosporus* (edible mushrooms)

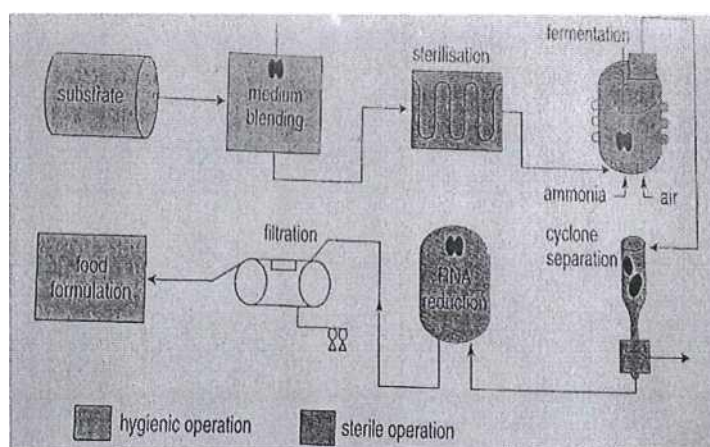
The fast growth rate of microorganisms leads to a high RNA content which is unsuitable for consumption by human and other animals. In humans, excess nucleic acids are converted to uric acid which is not excreted by the kidneys, resulting in the accumulation of uric acid crystals in the joints giving gout-like symptoms. In the normal production of mycoprotein, after fermentation the RNA content is around 10 percent which is too high, but this can be reduced to about 2 percent by using thermal shock and the action of ribonuclease. After RNA reduction, the mycelium is harvested continuously on horizontal filter bed and the filter cake which is recovered can be stored at 18°C for long periods.

The harvested mycoprotein is a mat interwoven fungal hyphae which can then be formulated into a range of food products. Its filamentous nature gives it a texture and 'bite' similar to that of meat. Mycoprotein itself tastes bland but can

be flavoured to resemble chicken and is added to pies, burgers and cold slicing meats. Its composition compared to that of lean beef it's given in table 1.

Table (1): Comparison of Mycoprotein and Beef

Feature	Mycoprotein	Beef
Protein	44.3%	68.2%
Dietary Fiber	18.3%	0.0%
Fat	13.0%	30.2%



Other SCP products are represented by *Fusarium graminearum* (filamentous fungi), *Candida lipolytica* (yeast), *Spirulina* sp. (blue green bacterium) and *Methylophilus methylotrophus* (bacterium). Ultimately, the success depends on the economics of microbial production compared with protein production from animals and plants in conventional agriculture and horticulture table 2. listed some of the perceived advantage of SCP production and some of the disadvantage that have come to light with industrial schemes already attempted.

Table (2): Some Advantage and Disadvantage of Single Cell Protein (SCP)

Advantage of SCP	Disadvantage of SCP
<ul style="list-style-type: none"> <li>• Fast growth rate, high yield in relative short time.</li> </ul>	<ul style="list-style-type: none"> <li>• May be deficient in certain amino acid, such as methionine or other sulphur containing amino acids, which are essential for humans or other animals.</li> </ul>
<ul style="list-style-type: none"> <li>• Production throughout the year regardless of season</li> </ul>	<ul style="list-style-type: none"> <li>• Microbial cell walls indigestible by humans and non-ruminant mammals.</li> </ul>
<ul style="list-style-type: none"> <li>• Range of substrates can be utilized, including waste materials from industrial processes.</li> </ul>	<ul style="list-style-type: none"> <li>• The high RNA content in microbial cells unsuitable for humans because they lack the enzyme which would break it down</li> </ul>
<ul style="list-style-type: none"> <li>• High protein content compared with some other sources such as (soya bean or fish meal).</li> </ul>	<ul style="list-style-type: none"> <li>• Concern that toxins may persist in the growth medium when using waste from industrial process.</li> </ul>

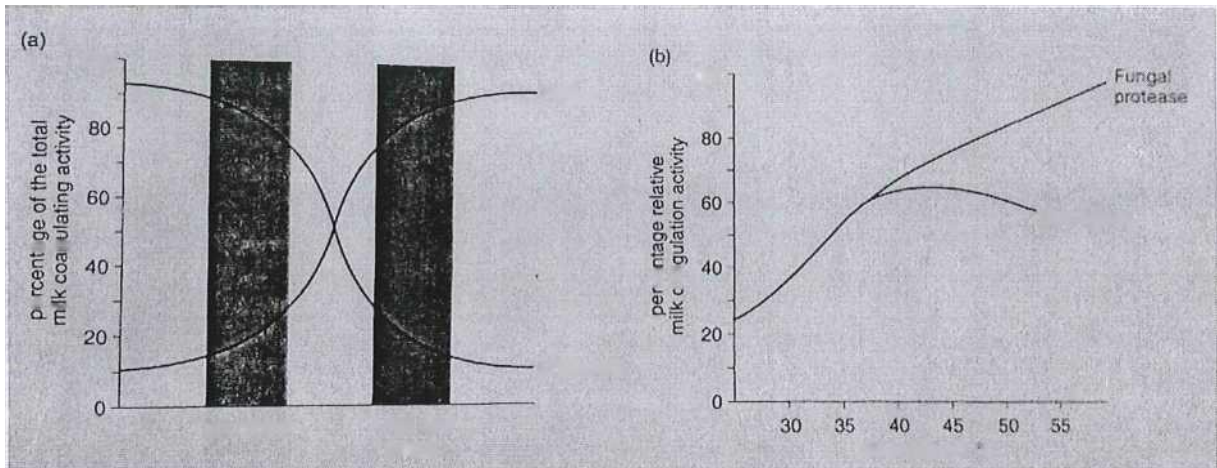
### **Production of Chymosin (Microbial Rennin):**

In cheese making, chymosin (rennin) is the main enzyme involved in the coagulation of casein, the protein in milk. Traditionally, the source of chymosin was rennet, an extract from the abomasum (stomach) of young calves, or sometimes from kids or lambs. In the 16<sup>th</sup> century, rennet was prepared by cutting strips of the stomach of young calves and steeping these in warm milk or brine to extract the rennet. By the late 19<sup>th</sup> century the first industrial preparation of calf rennet was established by a Danish chemist. Calves destined for consumption as veal were used, so they were not sacrificed specially for the extraction of the enzyme. More recently, in 1960s, because of changing eating patterns, there was concern that there would be a world- wide shortage of rennet for commercial cheese production. This led to pressure to find alternative source of rennet and to develop substitutes to keep up with the demand.

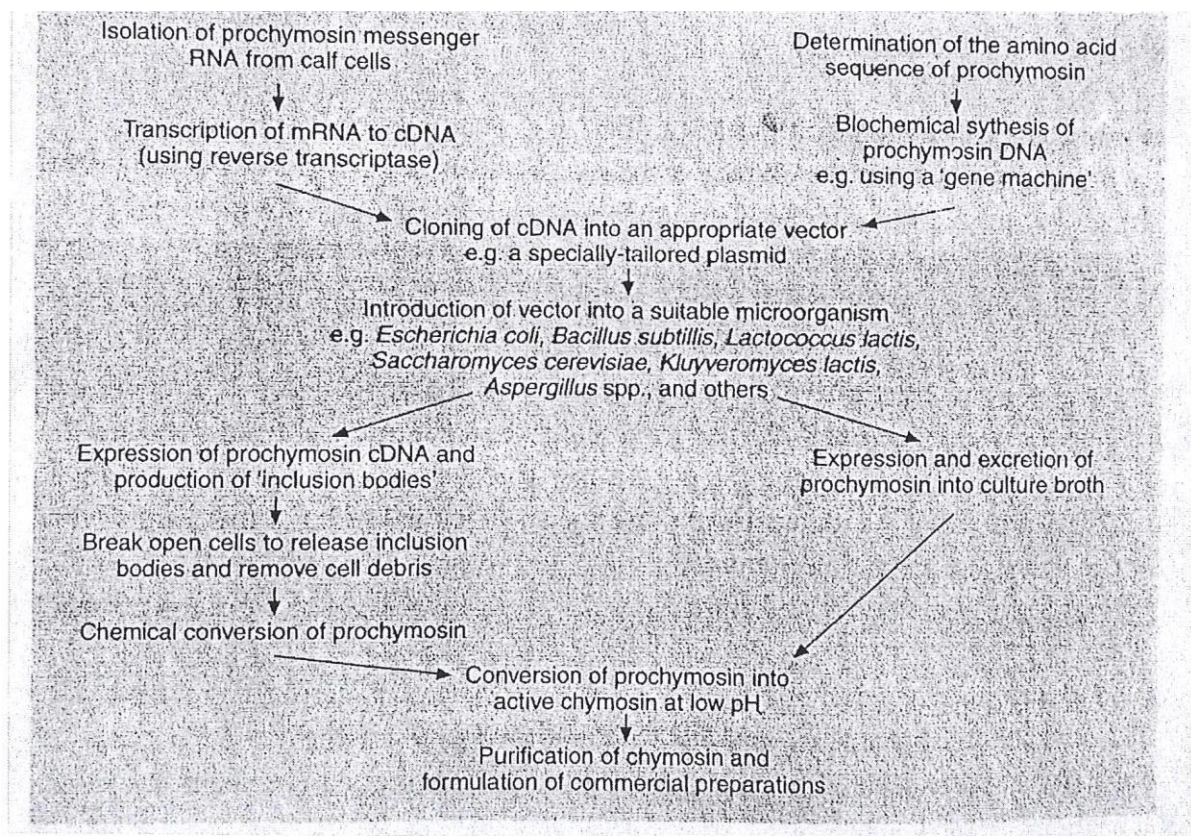
Bovine rennet from adult cattle can be used as an alternative to calf chymosin, but the bovine extract contains a higher proportion of **pepsin** and gives a lower yield of cheese. Certain fungi produce proteases which can clot milk proteins. Fungal enzymes are now used in more than one third of cheese produces world-wide. Three fungal used for production of the enzymes are *Mucor miehei*, *M. pusillus* and *Endothia parasitica*. Compared with calf chymosin, the fungal enzymes are **more stable**, but this can be a disadvantage in cheese which have a **long maturing stage** (e.g. Cheddar cheese) because degradation of the milk protein continues. **To counteract this, these enzyme can be destabilized, using oxidizing agents, so that they behave in a way similar to the more successful calf chymosin.** Fungal enzymes are used widely in production of cheese for vegetarians.

DNA technology has provided further substitutes for calf rennet. The first microorganism capable of making chymosin were produced in 1981, using *Escherichia coli*. Now chymosin were produces from genetically modified yeasts, including *Kluyveromyces lactis* and *Saccharomyces cerevisiae*. Precisely the some DNA code as in the calf is incorporated into the microorganism, so the enzyme produced is identical to that from calves. Expert tasters can detect no differences between the cheeses produced using chymosin from genetically modified organism and that from extracted calf rennet. The enzymes actually have fewer impurities and their behavior is more predictable. At first there was resistance to accepting cheese made with the involvement of genetically modified organism (GMOs). Before being release for general consumption, there was rigorous tasting of the products. The enzyme used for cheese produced in this way have been proved by the relevant regulatory bodies and by the vegetarian society. Such cheese is on sale in several countries, including the UK.





- (a) Changing in relative proportions of chymosin and pepsin in rennet from young calf to adult cow, showing relative chymosin to calves and higher pepsin in adult bovine rennet;
- (b) Difference in behavior of calf rennet and fungal protease- influence of temperature



Stage in the production of calf chymosin by genetically modified microorganism. Prochymosin is an inactive precursor of chymosin