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# Quorn™ Myco-protein – Overview of a successful fungal product

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Fungi have provided food for man, primarily in the form of fruit bodies of basidiomycetes and a few ascomycetes, for thousands of years. Similarly, yeasts have provided dietary supplements (e.g. vitamins) in the form of beer and bread. However, it has not been until the last 18 years that a filamentous fungus has been deliberately cultured for use as a primary source of protein for people. This is the filamentous fungus *Fusarium venenatum* A3/5 (ATCC PTA-2684), which is grown in continuous flow culture to produce myco-protein, which is sold under the trade name Quorn™ in the UK, the USA and at least 8 other European countries, including Belgium, Denmark, France, Germany, Ireland, the Netherlands, Sweden and Switzerland.

The process of bringing myco-protein into the market place began long before the first product was marketed in 1985, however. The decision to develop a meat substitute based on fungal mycelium was made by the British company Rank Hovis McDougall (RHM) during the 1960s and was followed by more than 15 years of research, development and toxicity testing before approval for the sale of the new food product was obtained from the British Ministry of Agriculture, Fisheries and Food (Edelman *et al.*, 1983; Angold *et al.*, 1989).

At the start of the period of research and development, more than 3000 fungal isolates from around the world were analysed for protein content, toxin production and suitable growth and morphology. Strain ATCC PTA-2684 (initially identified as an *F. graminearum*, but subsequently re-identified as *F. venenatum*, O'Donnell *et al.*, 1998) was selected as the best organism for myco-protein production. Once the production organism had been chosen, fermentation and product development could proceed alongside safety testing. The initial safety testing involved 12 years of research, and demonstrated that myco-protein could be consumed by lab animals or human volunteers without harmful effects if the RNA content of the cells was reduced (Solomons, 1987). Testing during this time also demonstrated the nutritional value of myco-protein, which was found to be comparable to eggs in amino acid composition (Miller & Dwyer, 2001), but contained no cholesterol and had a substantial fibre content. After receiving MAFF approval, toxicity and allergen testing of Quorn™ have continued (Miller & Dwyer, 2001; Tee *et al.*, 1993).

Because of its high fibre content, Quorn™ has been found to help decrease blood cholesterol levels (Turnbull *et al.*, 1992) and may encourage reduced energy intake (Turnbull et al., 1993; Burley et al., 1993). It has been suggested that myco-protein could facilitate healthier eating habits if people were encouraged to eat it in place of high fat, low fibre foods (Wheelock, 1993).

In development of the process, the decision was made that a continuous flow process would be the most appropriate culture system (Edelman *et al.*, 1983), since a continuous flow process operated at a high dilution rate is the most economic production system for a biomass related product (Pirt, 1975). Initially, a 300 L stirred tank bioreactor was used to produce the myco-protein for health and safety testing, but once approval was gained for sale of the myco-protein, an agreement was made between RHM and ICI to produce myco-protein in a 40,000 L airlift (pressure cycle) reactor. Subsequently, two 150,000 l pressure-cycle reactors (Fig 1) have been custom built for the process. Initially, the culture was operated as a nutrient-excess glucose-stat, i.e. the flow rate was set such that all nutrients would be in excess and the concentration of excess glucose was kept approximately constant, so that the medium flow rate could be adjusted to the fungus' specific growth rate. The current process, however, uses the $CO_2$ evolution rate instead of glucose concentration to control medium flow rate (Rodger, 2001). The use of

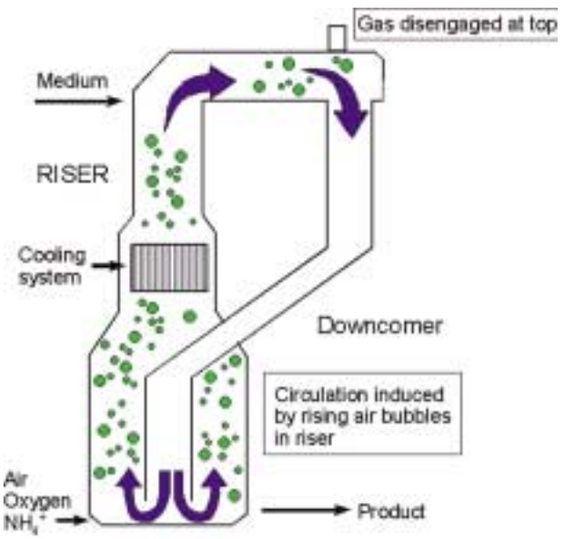


**Fig 1** Diagrammatic representation of the Quorn™ pressure cycle fermenter used by Marlow Foods at Stokesly, UK, for the production of myco-protein in continuous flow culture.

$CO_2$ evolution rate to control medium flow offers the advantage that $CO_2$ evolution is measurable on-line, so that finer, more regular adjustments to the flow rate can be made.

A completely defined medium, based on glucose and ammonium (and supplemented with biotin), is used for the production of myco-protein. Cultures are maintained at 28-30°C, pH 6.0. Under these conditions, *F. venenatum* A3/5 has a specific growth rate ($\mu$) of 0.17 to 0.20 $h^{-1}$ (i.e. a doubling time between 3.5 and 4.1 h), which enables the production of 300 to 350 kg biomass $h^{-1}$. There is regular testing (at 6 h intervals) for mycotoxins and potentially harmful contaminants, although no harmful products have ever been detected under production conditions.

After being produced, the *F. venenatum* A3/5 biomass is subjected to a heat treatment to reduce the RNA content of the biomass (Edelman *et al.*, 1983). This is achieved by heating the mycelia in a separate tank to temperatures above 68°C (optimum 72-74°C) for 30 to 45 minutes (Ward, 1998). This treatment allows RNA to be degraded into monomers which diffuse out of the cells. Some loss of proteinaceous and other materials also occurs during this treatment. Following RNA treatment, the biomass in heated to 90°C, and concentrated by centrifugation to give a paste containing greater than 20% (w/v) solids (Wiebe, 2001). The liquid extracted from the biomass can be


**Fig 2** A selection of Quorn™ products currently available in the UK. (Photograph provided courtesy of Marlow Foods)

further concentrated to yield the flavouring product, Quessent™ (Marlow Foods, 2001). The mycelial paste generated is called myco-protein and can be further combined with a binding agent such as egg albumin and various spices or flavourings, depending on the desired final product. Standard food processing technology is used to shape the final products, which include unflavoured chunks, mince, sausages, burgers, fillets and steaks (Fig 2).

Quorn™ has been commercially available in the UK since 1985 and has seen considerable expansion of the market since that time, with retail sales expected to have been approximately US$150 million in 2001. Studies have shown that Quorn™ compares well with meat, tofu and textured vegetable protein (TVP, derived from soya flour) in terms of appearance, texture, aroma and flavour (McIlveen *et al.*, 1999; Rodger, 2001), although not all Quorn™ products have been equally well received (*Which?*, 1991). This is no doubt also reflected in the marketing strategy, where the range of products available in different countries has been developed to suit the targeted population.

Since 1994, the marketing of Quorn™ has been largely focused on the choice of myco-protein as a healthy food choice, rather than as a vegetarian product. The expansion of the myco-protein market into the USA (in 2001) and an increasing number of European countries has demonstrated the general acceptability of the product. It has been suggested that myco-protein could now also be used in the production of breakfast cereals and puffed snacks, or be added to yoghurt and ice-cream products as a fat replacer (Rodger, 2001).

From the biological perspective, one of the more interesting aspects of myco-protein production is the contribution of hyphal morphology to the final product and the effect that cultivation in long term, submerged, continuous flow cultures has on hyphal morphology. A filamentous fungus was chosen for the production of this meat substitute, since it was believed that the mycelia would add a fibrous texture, comparable to that of meat, to the final product (Edelman *et al.*, 1983) and an optimal branch length was determined for the production process (Marlow Foods Limited, 1997). However, as with several other filamentous fungi (Forss *et al.*, 1974; Righelato, 1976), when *F. venenatum* A3/5 is grown in a continuous flow system for long time periods, mutants which have altered branching patterns (usually increased branching) arise and eventually displace the parental strain. Such mutants were considered to be unsuitable for formation of the final product (Trinci, 1992). Their appearance in these populations is a reflection of selection pressures in the continuous flow system. The highly branched mutants usually have small, but significant growth rate advantages over the parental A3/5 strain (Simpson *et al.*, 1998), and nutrient excess glucose- or $CO_2$-stats are the ideal environment for selecting mutants with growth rate advantages over the parental strain. Thus, once the mutation occurs (randomly as the thousands of nuclei in the mycelia replicate), the increase of these mutants in the population is inevitable.

Although the appearance of highly branched mutants was primarily a problem when myco-protein was produced in the 40,000 l reactor, a number of strategies were suggested to prevent or delay the appearance of these mutants. Periodic changes to the environment delay the appearance of the highly branched mutants by temporarily changing the selection pressure that enables them to increase in the population (Trinci *et al.*, 1993; Wiebe *et al.*, 1996). It has also been possible to isolate strains with the wild-type branching pattern which have improved morphological stability in long term continuous flow cultures (Wiebe *et al.*, 1994; Naylor *et al.*, 1999a). Naylor *et al.* (1999b) suggest that diploids are more morphologically stable than haploids. Although none of these strategies has as yet been implimented in the production process, they have considerably expanded our understanding of how fungal populations respond to selection pressures and evolve.

Filamentous fungi which are grown to produce other products (e.g. citric acid) may be used as animal feed supplements, but Quorn™ remains the only source of myco-protein for human consumption which is on the market today. It required nearly two decades of research and development before it was introduced to the market, but since its introduction it has been able to establish itself as one of Europe's leading meat-free products and there are hopes that it will be equally successful in the USA now that it is available there.

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