SGM SPECIAL LECTURE

Evolution of the Quorn® myco-protein fungus, *Fusarium graminearum* A3/5

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The 1994 Marjory Stephenson Prize Lecture

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I would like to begin by expressing my deep gratitude to the Society for inviting me to give the 1994 Marjory Stephenson Prize Lecture. I think that the invitation signals the importance the Society attaches to mycology as a component of microbiology.

It is appropriate that this lecture is being given in Cambridge because Marjory Stephenson spent most of her life here and indeed was born only ten miles away in Burwell. However, she taught for a while in London at University College and King's College of Household Science. Her connection with the latter College is of particular interest to me because King's College of Household Science eventually became Queen Elizabeth College, where Professor John S. Pirt founded the Department of Microbiology, which I joined in 1964. My apprenticeship with John Pirt has served me well throughout my academic career.

It is clear to anyone reading about Marjory Stephenson that she was a very remarkable person who successfully overcame the difficulties which confronted women of her generation who wanted to develop scientific careers. For example, she took a Part 1 Natural Sciences Tripos, but, as a woman, was excluded from university classes in two of her three subjects! Despite such disadvantages, in 1945, she became the first woman to be elected to Division B (Biological Sciences) of the Royal Society (the first request for women to be admitted to the Royal Society was made in 1902, but it was not until June 1944 that Fellows were balloted on the issue!). Marjory Stephenson also played a very active part in the formation of the Society for General Microbiology: she served on the Committee of the Society from its foundation, and in 1947 was elected President in succession to Sir Alexander Fleming, a post she held until her death in the following year.

Marjory Stephenson is perhaps best known for her monograph, Bacterial Metabolism (1949), and for her work on fermentations of anaerobic bacteria. Knowing this I wonder what she would have thought of Colin Orpin's (1975) discovery (also in Cambridge) of obligately anaerobic fungi in the rumen of sheep. I became fascinated by these fungi and, in collaboration with Dr M. K. Theodorou (AFRC Institute for Grassland and Environmental Biology), have studied them since 1983. Although, like most other fungi, anaerobic fungi have walls containing chitin, unlike other fungi they lack mitochondria, possess hydrogen-generating hydrogenosomes, and obtain energy by the kind of mixed acid fermentation (Trinci *et al.*, 1994a) familiar to Marjory Stephenson. However, in this lecture I will be describing our work with the myco-protein fungus, *Fusarium graminearum*, not the anaerobic fungi.

Evolution of the Quorn® myco-protein programme

In the late 1950s, forecasters predicted a worldwide shortage of protein-rich foods by the 1980s. Consequently in 1964, in response to these predictions, Lord Rank, the methodist and philanthropist, instructed Ranks Hovis McDougall (RHM) Research Centre to develop a way of converting starch into a protein-rich food. He stipulated that the new food must be highly nutritious, delicious and safe to eat. Today RHM can claim that the new food it eventually developed, Quorn® myco-protein, has been tested more rigorously than any other food consumed by man. RHM's ten-year myco-protein evaluation programme included feeding trials with 2500 human volunteers and 11 species of animal, and resulted in the submission to MAFF of a 26-volume, two-million-word report. In 1980, RHM was given permission to sell myco-protein for human consumption, and the first retail product (a Sainsbury's Savoury Pie) containing myco-protein was sold to the public in January 1985.

RHM decided to produce its new protein-rich food from the filamentous fungus *Fusarium graminearum* A3/5. A fungus rather than a yeast or bacterium was chosen for the project because (a) of the long history of man using fungi as food, (b) it is possible to formulate food products from filamentous fungi which have the appropriate smell, taste and texture, and (c) it is relatively easy to harvest fungal mycelia from culture broths. During the production process, the fungal biomass is RNA-reduced (from *ca.* 9%

to *ca.* 1 %, w/w) and then mixed with egg albumin, extruded and rolled (to rearrange the hyphae into a parallel configuration which resembles the arrangement of fibres in meat; Trinci, 1992), and finally heat-set with steam. As a result of this formulation process, myco-protein has the same 'chewiness' and succulence as meat. However, unlike meat, myco-protein retains colourings and flavours even when cooked.

Although myco-protein was originally conceived as a protein-rich food to supplement the world's flagging supply of conventional protein foods, by the early 1980s when the production techniques for myco-protein had been fully established, and when MAFF had approved it for sale to the public, the predicted global shortage of protein-rich foods in the West had not materialized. Consequently, Marlow Foods (the joint venture formed by RHM and ICI in 1984 to use the latter's expertise and fermenter capacity to scale-up the myco-protein process) adapted to the prevailing demands of the market place and decided to sell Quorn® myco-protein as a *new healthy* food which lacks animal fats and cholesterol, is low in calories and saturated fats, and is high in dietary fibre (it has more dietary fibre – fungal cell walls – than wholemeal bread). This dramatic change in marketing policy was fully justified by a survey in 1989 which showed that almost half the UK population was reducing its intake of red meats, whilst a fifth of young people were vegetarians. Today Quorn® myco-protein is sold as an ingredient in over 50 ready-made meals, and sold in an uncooked, unflavoured form (as chunks, strips or minces) which is used in the home as an ingredient in a wide range of cooked meals.

Use of continuous-flow culture in the production of *F. graminearum* A3/5 biomass

Dr Gerald L. Solomons (1983, 1985, 1986) led the RHM team responsible for developing the *F. graminearum* A3/5 fermentation. Today, *F. graminearum* A3/5 biomass is produced in an air-lift or pressure-cycle fermenter at Billingham. A continuous-flow culture system was chosen for the process because the growth conditions in such cultures, unlike those in batch culture, can be maintained constant throughout the production phase (an important consideration when producing single-cell-protein for human consumption), and because much higher productivities can be achieved in continuous culture than in batch culture (Pirt, 1975; Trilli, 1977). Up to the beginning of 1994, the air-lift fermenter used for myco-protein production was a fermenter originally built by ICI to grow the bacterium *Methylophilus methylotrophus* for the production of an animal feed (Pruteen). This 40000 l fermenter consists of an elongated loop, *ca.* 30 m tall, which is capable of producing 1000 tonnes of myco-protein per annum. However, Marlow Foods has recently commissioned a new 140000 l air-lift fermenter for myco-protein production. This purpose-built fermenter will enable myco-protein production to be increased to 5000–7000 tonnes per annum to satisfy increasing market demand for the product in the UK, Belgium, Germany and the Netherlands. Marlow Foods is constructing a second 140000 l fermenter at Billingham and has outline plans for the construction of myco-protein production plants in Europe and Japan, with a possible myco-protein launch in 1996 in the USA (following FDA approval). The two new Billingham fermenters should enable sales of Quorn® myco-protein to be increased to *ca.* £150 M per annum.

Evolution of *F. graminearum* in chemostat culture

In practice, industrial fermentations of *F. graminearum* are terminated 6 weeks or less after the onset of continuous flow. This premature termination of the fermentations, with consequent loss of productivity, is caused by the appearance of highly branched mutants in the culture (Fig. 1). These mutants can be detected at the 0·5–1 % level in industrial fermentations, which are then terminated. When grown in plate culture, the mutants form colonies which are much more compact than those of the parental strain and expand in radius much more slowly than the parental strain. However, in a chemostat culture, colonial mutants supplant the parental strain. Although the mycelia of colonial mutants possess a similar chemical composition and are as nutritious as those of the parental strain, their highly branched morphology is inappropriate to the formulation of Quorn® myco-protein of the correct texture (myco-protein containing colonial mutants is more crumbly) and their mycelia reduce the efficiency of the vacuum filters used to harvest the biomass. Thus, prevention or delay in the appearance of colonial mutants in myco-protein fermentations would enhance productivity and decrease the unit cost of the product. Similar highly branched (colonial) mutants have been observed in chemostat cultures of other filamentous fungi (Forss *et al.*, 1974; Righelato, 1976).

Mycelial fragmentation and production of macroconidia

Steady-state chemostat cultures of filamentous fungi can only be maintained provided that mycelia in the fermenter vessel fragment periodically. The observation that the concentration of mycelial fragments in chemostat cultures of *F. graminearum* A3/5 grown at a particular dilution rate remains approximately constant (Wiebe & Trinci, 1991) is certainly consistent with the hypothesis that mycelia fragment in a regular manner.

When *F. graminearum* A3/5 is grown in glucose-limited chemostat culture, it produces multinucleate, multi-septate, banana-shaped spores known as macroconidia, and more of these spores are formed at low (0·07 h^{-1}) than at high (0·19 h^{-1}) dilution rates (Wiebe & Trinci, 1991). Since macroconidia are formed from uninucleate spore-producing cells known as phialides, they are homokaryotic (Miller, 1946), and, based on the assumption that there is an equal probability of nuclei present in a coenocytic mycelium being incorporated into a phialide, the nuclei in macroconidia are representative of nuclei in the mycelial biomass.

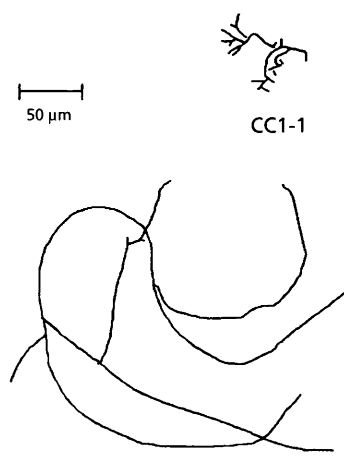

Fig. 1. Mycelia of *F. graminearum* A3/5 and a highly branched (colonial) mutant (CC1-1) which arose spontaneously in a glucose-limited culture of A3/5 (Wiebe *et al.*, 1991, 1992b).

To date, every colonial mutant of *F. graminearum* A3/5 isolated from chemostat culture has been found to be recessive to the wild-type (Wiebe *et al.*, 1992a). Consequently, a heterokaryotic mycelium containing both parental and colonial nuclei will only express the highly branched phenotype of the latter once colonial mutant nuclei have attained a sufficiently high concentration in the coenocytic mycelium (Wiebe *et al.*, 1992a). Mycelia containing colonial mutant nuclei may arise following separation of mutant from parental nuclei during sporulation (macroconidia germinate in chemostat culture and will give rise to homokaryotic mycelia) or during mycelial fragmentation (hyphae containing colonial mutant nuclei may be separated by fragmentation from other parts of the mycelium which are heterokaryotic).

Appearance of mutants in chemostat culture

Spontaneous mutants arising during chemostat culture of *F. graminearum* can be divided into three classes viz. (a) mutants which are at a selective disadvantage compared with the parental strain, (b) mutants which are neither at an advantage nor disadvantage compared with the parental strain (so-called, neutral mutants), and (c) mutants which are at a selective advantage compared with the parental strain. The first class of mutants will not accumulate in the fermenter (Powell, 1958). For neutral mutants, provided that the rate of the forward mutation, for example from cycloheximide sensitivity to cycloheximide resistance, is greater than the rate of back mutation, for example from cycloheximide resistance to cycloheximide sensitivity, accumulation in the population occurs at a linear rate. However, unless it becomes linked to an advantageous mutation, a neutral mutation never attains a high concentration in the population, e.g. the concentration of cycloheximide-resistant macroconidia in chemostat cultures of *F. graminearum* varies from *ca.* 0·001 to 0·1 % of the total population. The reason why neutral mutations do not accumulate to higher levels is because of the periodic appearance in the population of advantageous mutants which do not carry the neutral mutation and therefore cause a decrease in its concentration; this phenomenon (i.e. the periodic displacement of neutral mutants by advantageous mutants) is known as periodic selection (Dykhuizen & Hartl, 1983). By contrast, the third class of mutant, selectively advantageous mutants, may completely, or almost completely, supplant the parental population. The reason for the appearance of selectively advantageous mutants in chemostat cultures was suggested by Novick & Szilard (1950), Moser (1958) and Powell (1958), who recognized that micro-organisms adapt to the constant conditions prevailing in a chemostat by mutation and natural selection. Any mutation which confers a selective advantage to a member of a population will result in its accumulation to the exclusion or near exclusion of the parental strain. Thus, microbial populations in a chemostat are constantly evolving.

Measurement of the selection coefficient of advantageous mutants

The selective advantage of a mutant can be quantified (Dykhuizen & Hartl, 1981) by growing it in mixed culture with the parental strain and determining the selection coefficient. Thus:

$$s = \frac{\ln\left[\frac{p_{(t)}}{q_{(t)}}\right] - \ln\left[\frac{p_{(0)}}{q_{(0)}}\right]}{t} \qquad (1)$$

where s = the selection coefficient; $p_{(t)}$ = concentration of the mutant strain at time t; $q_{(t)}$ = concentration of the parental strain at time t; $p_{(0)}$ and $q_{(0)}$ = the initial concentrations of each strain.

Periodic selection in a glucose-limited chemostat culture of *F. graminearum* A3/5

As mentioned previously, although neutral mutants accumulate at a linear rate in a chemostat, their concentration decreases when an advantageous mutant arises which does not carry the neutral mutation. The phenomenon of periodic selection (Dykhuizen & Hartl, 1983) provides a means of determining when advantageous mutants appear in the population without needing to know the phenotype of the mutant. For example, the appearance of advantageous mutants in chemostat populations of *F. graminearum* A3/5 has been determined by monitoring increases and decreases in the levels of chlorate- (Trinci, 1992) and cycloheximide- (Wiebe *et al.*, 1993) resistant macroconidia in the population (Fig. 2); at least three advantageous mutants of unknown phenotype appeared in the glucose-limited chemostat population of *F. graminearum* A3/5 shown in Fig. 2. We have shown that, in glucose-limited chemostat cultures of *F. graminearum* A3/5 grown at a dilution rate of 0·19 h^{-1}, periodic selection occurred once every 124 h or 34 generations (Wiebe *et al.*, 1993).

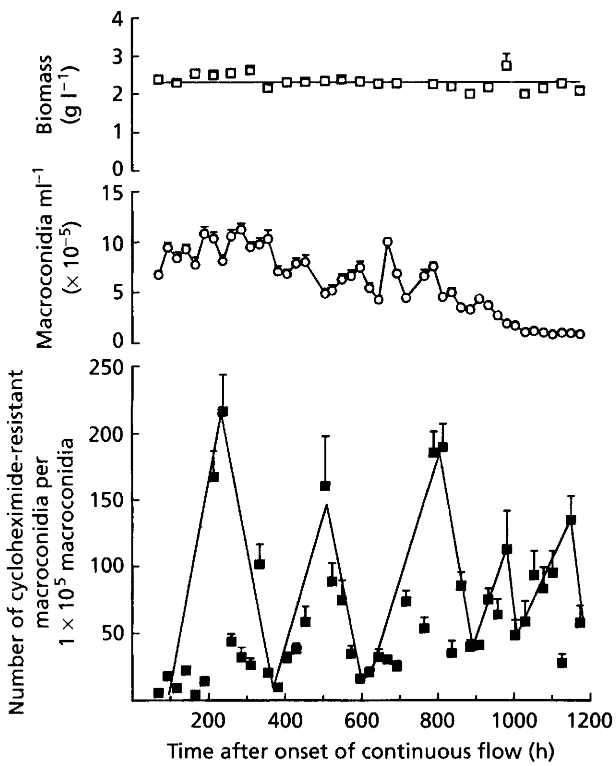

Fig. 2. Concentrations of biomass, total macroconidia and cycloheximide- (250 μM) resistant macroconidia in a glucose-limited chemostat culture of *F. graminearum* A3/5 grown at 25 °C and pH 5·8 at a dilution rate of 0·10 h^{-1} on modified Vogel's medium. The decreases in the concentrations of cycloheximide-resistant macroconidia in the population are associated with the appearance of advantageous mutants of unknown phenotype. This phenomenon is known as periodic selection (Dykhuizen & Hartl, 1981).

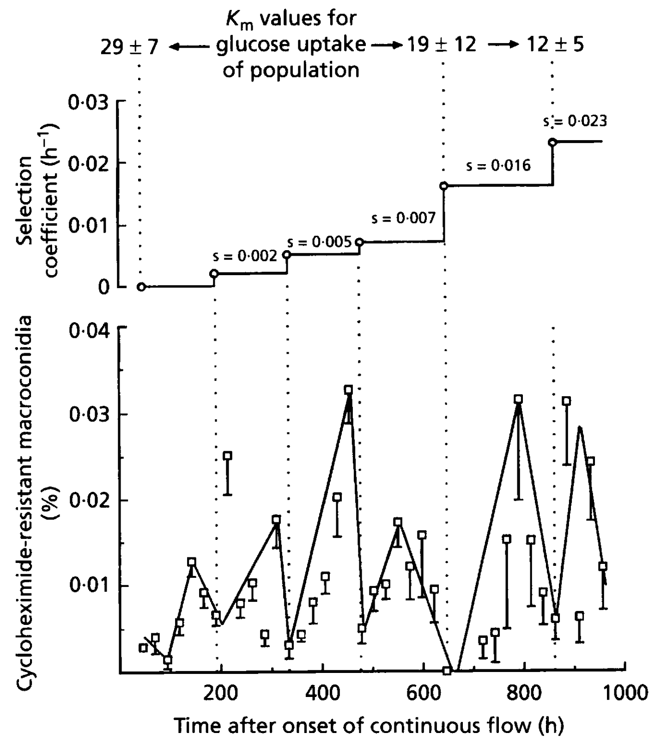

Fig. 3. Fluctuations in the proportion of cycloheximide-resistant macroconidia in a population of *F. graminearum* A3/5 grown at 25 °C and pH 5·8 in a glucose-limited chemostat culture at a dilution rate of 0·05 h^{-1}. The results are expressed as a percentage of the total macroconidia. The upper part of the figure gives selection coefficients, relative to strain A3/5, of populations derived from samples taken at 190, 334, 478, 646 and 862 h and the K_m values for glucose uptake of some of these populations. The error bars give the standard errors of the mean.

Appearance of advantageous mutants with reduced K_m values for glucose uptake in glucose-limited chemostats grown at a low dilution rate

When an organism is grown in a chemostat, the relationship between its specific growth rate (μ) and the concentration of the growth limiting substrate (S) is described by the Monod (1942) equation:

$$\mu = \frac{\mu_{max} S}{(S + K_s)} \tag{2}$$

where μ_{max} is the maximum specific growth rate of the organism in the absence of nutrient limitation and K_s, the saturation constant, is a measure of the organism's affinity for the limiting substrate and is the substrate concentration at which the organism grows at half μ_{max}. Where selective advantages have been identified for micro-organisms grown in chemostats at a low or high dilution rate, generally they have been classified into those which have a lower K_s value for the limiting substrate than the parental strain and those which have a higher μ_{max} than the parental strain.

Fig. 3 shows periodic selection in a glucose-limited chemostat culture of *F. graminearum* A3/5 grown at a dilution rate of 0·05 h^{-1} (doubling time of 13·9 h). The competitive ability of the population present at the start of each increase in the level of cycloheximide-resistant macroconidia was determined relative to *F. graminearum* A3/5, and the selection coefficient was found to increase progressively (from 0·002 to 0·023 h^{-1}). The K_m values of some of these populations were also determined and found to decrease with evolution of the culture (Fig. 3). Thus, growing *F. graminearum* A3/5 in a glucose-limited chemostat at a low dilution rate results in the selection of advantageous mutants of unaltered mycelial morphology which have more efficient uptake systems for glucose than the parental strain.

Appearance of advantageous mutants in glucose-limited cultures grown at a high dilution rate

Fig. 4 shows the appearance of advantageous colonial mutants in glucose-, ammonium- and Mg^{2+}-limited chemostat culture of *F. graminearum* A3/5 grown at dilution rates of 0·18 or 0·19 h^{-1}; under the prevailing

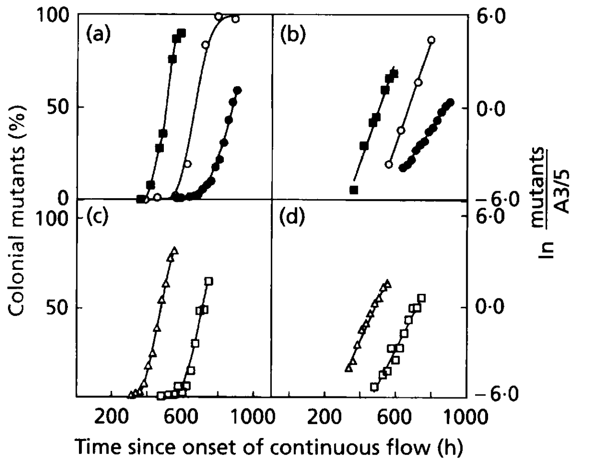

Fig. 4. Appearance of highly branched (colonial) mutants in nutrient-limited chemostat cultures of *F. graminearum* A3/5 grown at a dilution rate of 0·19 h^{-1} on modified Vogel's medium at 25 °C and pH 5·8. The colonial mutants are expressed as a percentage of the total population in (a) glucose-limited (■, ○, ●) and (c) Mg^{2+}- (△) and ammonium- (□) limited cultures, and as the ratio of ln of colonial mutant population to A3/5 population in (b) and (d); the slope of these lines gives the selection coefficient.

cultural conditions, the fungus is washed out of the fermenter at dilution rates of 0·23 h^{-1} (D_{crit}) and above. Colonial mutants were first detected in glucose-limited cultures at 360, 386 and 421 h (99, 106 and 115 generations) after the onset of continuous flow, and in ammonium- and Mg^{2+}-limited chemostat cultures at 447 h (115 generations) and 260 h (71 generations), respectively, after the onset of continuous flow. The colonial mutants replaced the parental strain with selection coefficients of 0·017–0·034 h^{-1} for glucose-limited cultures, 0·024 h^{-1} for the ammonium-limited culture, and 0·027 h^{-1} for the Mg^{2+}-limited culture. Whenever the evolution of these cultures was allowed to progress, the colonial mutants eventually formed more than 90 % of the total population. The variation observed for the appearance of colonial mutants in glucose-limited cultures (Fig. 4) confirms Powell's (1958) prediction that the timing of advantageous mutants will be variable, depending on the relative probability of the mutant being retained or washed out of the vessel after it has arisen.

In two perturbed glucose-limited chemostats in which steady-state cultures were not maintained (in one, the stirrer drive belt snapped and the culture went into stationary phase for a period, and, in the other, the air supply became blocked and the dilution rate varied for a period from 0·11 to 0·22 h^{-1}), appearance of advantageous colonial mutants was delayed for up to 648 h (177 generations) after the onset of continuous flow. Reasons why such perturbed conditions might delay the appearance of colonial mutants will be explained later.

Nine different colonial mutants were isolated from one (CC1) of the glucose-limited chemostat cultures, but eventually one of these (CC1-1; Fig. 1) formed *ca.* 97 % of the total colonial mutant population, suggesting that it had a higher selection coefficient than the others. Compared to the parental strain, A3/5 ($G = 232 \pm 11$ µm), the colonial mutants isolated from the CC1 fermentation formed more highly branched mycelia, varying in hyphal growth unit (G) length from 21 ± 1 µm (CC1-1) to 174 ± 13 µm (CC1-7); G is a measure of mycelial branching (Trinci, 1974).

Identification of the selective advantage of colonial mutants CC1-1 and MC1-1

Prolonged cultivation of an organism in a chemostat at a high dilution rate would be expected to result in the selection of mutants which have higher μ_{max} values than the parental strain. This is true for CC1-1, a colonial mutant selected in a glucose-limited chemostat, and MC1-1, a colonial mutant selected in a Mg^{2+}-limited chemostat. MC1-1 replaced A3/5 in all nutrient-limited conditions tested (glucose-, Mg^{2+}-, ammonium- and sulphate-limited chemostat cultures), suggesting that it is a 'general' μ_{max} mutant (Wiebe *et al.*, 1992b). However, as shown in Fig. 5, although CC1-1 replaced A3/5 in some nutrient-limited conditions (glucose-, maltose- and ribose-limited chemostat cultures) it failed to do so in others (Mg^{2+}-, ammonium-, sulphate-, phosphate-, fructose- and xylose-limited chemostat cultures). However, as expected for a μ_{max} mutant, the selection coefficient of CC1-1 in glucose-limited culture decreased with decrease in dilution rate (Fig. 6). Furthermore, no significant differences were detected between the K_m or V_{max} values of glucose uptake for CC1-1 and A3/5.

We concluded that CC1-1 behaved as a 'restricted' μ_{max} mutant, supplanting A3/5 under some nutrient-limitations, but not others; its behaviour in glucose-limited culture was consistent with that of a mutant with a μ_{max} of 0·26 h^{-1} compared with a μ_{max} of the parental strain of 0·22 h^{-1} (Wiebe *et al.*, 1992b). On the basis of its selection under various nutrient limitations, we suggested that the behaviour of CC1-1 could be explained if it was assumed that the mutation affected the activity of an enzyme (phosphoketopento-epimerase, which reversibly converts xylulose 5-phosphate to ribulose 5-phosphate) in the pentose phosphate pathway (Wiebe *et al.*, 1992b).

Importantly, as shown in Fig. 5, a highly branched phenotype does not itself give colonial mutants a selective advantage over the sparsely branched, parental strain (CC1-1 formed highly branched mycelia in both glucose- and sulphate-limited cultures but behaved as an advantageous mutant in the former culture and as a neutral mutant in the latter). Thus, CC1-1 depends upon its altered metabolism for its selective advantage under some cultural conditions, not on its morphology. Mutants of *Neurospora crassa* with altered glucose-6-phosphate dehydrogenase (Brody & Tatum, 1966) and phosphoglucomutase (Brody & Tatum, 1967) activities have highly branched phenotypes, and compounds (paramorphogens) which affect membrane or wall biosynthesis result in highly branched phenotypes without affecting μ_{max} (Trinci *et al.*, 1994b). Furthermore, a putative protein

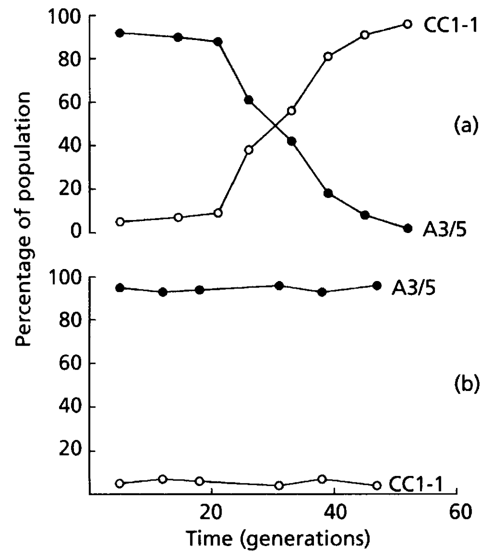

Fig. 5. Competition between A3/5 and colonial mutant CC1-1 in (a) glucose-limited and (b) sulphate-limited chemostat cultures grown at 25 °C and pH 5·8 at a dilution rate of 0·19 h^{-1}.

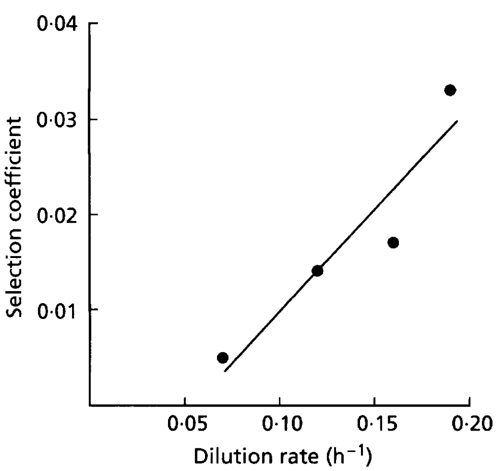

Fig. 6. Effect of dilution rate on the selection coefficient of colonial mutant CC1-1 measured against A3/5 in glucose-limited chemostat cultures at 25 °C and pH 5·8.

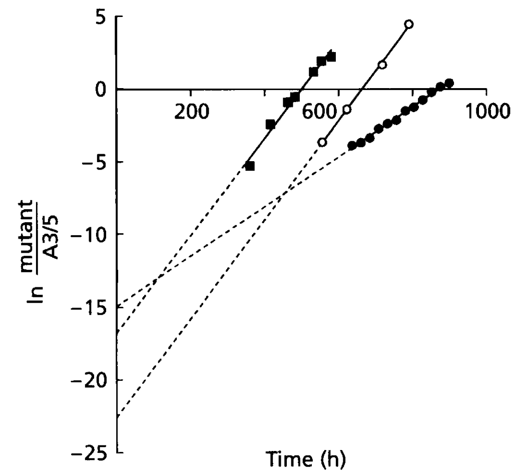

Fig. 7. Ratio (expressed as a natural logarithm) of colonial mutants to the parental strain, *F. graminearum* A3/5, at different times after onset of continuous flow in the glucose-limited chemostat populations shown in Fig. 4(a) and (b). The regression lines were extrapolated to $t = 0$ h (---); the point at which they crossed the y axis provides an estimate of the concentration of mutant propagules in the populations at the start of continuous flow.

kinase mutant of *N. crassa* forms highly branched mycelia (Yarden *et al.*, 1992). These observations suggest that highly branched phenotypes result from the pleiotropic effects of mutations whose primary effects are on the activities of enzymes involved in carbon metabolism, membrane biosynthesis or wall biosynthesis.

Origin of the advantageous colonial mutants

Advantageous, colonial mutants may either be present in the batch culture population prior to the onset of continuous culture, or may arise during continuous culture. For the three glucose-limited chemostat cultures in Fig. 4(a) and (b), extrapolation of the lines used to determine the selection coefficients to zero time (at the onset of continuous flow) suggests that colonial mutant propagules may already have been present by the end of batch culture (Fig. 7). Of the approximate 8×10^9 propagules (fragments or macroconidia) present at the end of batch, 1, 400, or 2600 may have been colonial mutants. For chemostat cultures of *Escherichia coli*, Dykhuizen & Hartl (1981) suggested that adaptation within the population within the first 200 h (*ca.* 80 generations) probably resulted from the selection of genetic variants, which were actually present in the inoculum. The sequence of selection of the advantageous mutants present in the inoculum would depend upon their initial concentration and their relative selective advantages (Adams & Oeller, 1986).

Strategies for preventing or delaying the appearance of colonial mutants in *F. graminearum* A3/5 chemostat cultures

The development of strategies to prevent or delay the appearance of colonial mutants in industrial myco-protein fermentations is of considerable economic importance. To date, three possible strategies have been identified viz. (a) operating the fermenter at a low dilution rate, (b) periodically changing the selection pressure, and (c) isolating sparsely branched strains of *F. graminearum* which are more stable than A3/5, at least as far as their mycelial morphology is concerned.

Effect of dilution rate on the appearance of colonial mutants in glucose-limited chemostat cultures

Table 1 shows that the appearance of colonial mutants in glucose-limited chemostat cultures can be delayed by reducing the dilution rate. This presumably is because

Table 1. First appearance of advantageous colonial mutants in glucose-limited chemostat cultures of *F. graminearum* A3/5, A23-S and A24-S

Strain	Dilution rate (h^{-1})	Timing of first appearance of advantageous colonial mutants	
		Time after onset of continuous flow (h)	Generations after onset of continuous flow
A3/5 (parental strain)	0·05	> 2207	> 159
A3/5	0·10	> 1173	> 169
A3/5	0·19	389	107
A23-S	0·18	480 (+23%)*	124
A24-S	0·185	600 (+54%)*	160

* Percentage increase compared with A3/5 population grown at the same dilution rate.

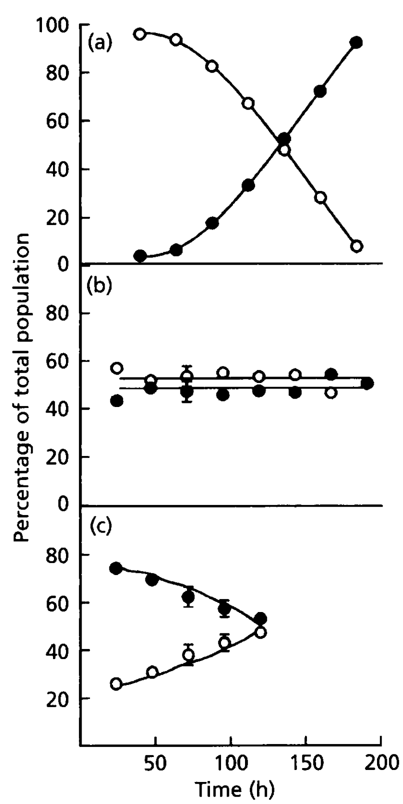

Fig. 8. Concentrations of A3/5 (○) and C106 (●) grown in competition in (a) magnesium-, (b) sulphate- and (c) phosphate-limited chemostat cultures at 25 °C and pH 5·8 at a dilution rate of 0·19 h^{-1} showing (a) positive, (b) neutral and (c) negative selection of C106 relative to A3/5. From Wiebe *et al.* (1992b).

mutants selected at low dilution rates differ from those selected at high dilution rates. However, in myco-protein fermentations, delay in the appearance of colonial mutants at low dilution rates would be partially or totally offset (depending on the dilution rate used) by the decreased productivity obtained.

Effect on the appearance of colonial mutants in chemostat cultures of varying the selection pressures

Fig. 8 shows that the prevailing selection pressure (nutrient-limitation) affects the outcome of the competition between colonial mutant C106 and the parental strain in chemostat culture. Colonial mutant C106 is positively selected in Mg^{2+}-limited culture (s = 0·038 h^{-1}), but not in glucose- (s = −0·006), ammonium- (s = 0·007), phosphate- (s = 0·010) or sulphate- (s = 0) limited culture. Thus, it may be possible to delay the appearance of colonial mutants in chemostat culture by periodically changing the limiting nutrient. Support for this hypothesis is provided by Fig. 9, which shows that changing from glucose- to magnesium-limitation stopped the parental strain being supplanted by the colonial mutant (CC1-1).

Isolation of variants of A3/5 which are more morphologically stable

Even when glucose-limited chemostats are maintained for very long periods, highly branched mutants never completely replace sparsely branched, A3/5-type mycelia. It is possible that the sub-population of sparsely branched mycelia which persists in such cultures may include evolved variants which are better adapted than A3/5 to compete with colonial mutants. We (M. G. Wiebe, G. D. Robson, S. G. Oliver & A. P. J. Trinci, unpublished results) have therefore used a series of glucose-limited cultures grown at a dilution rate of 0·18 h^{-1} for a total 'evolutionary' period of 2608 h or 677 generations to isolate two variants which have the same mycelial morphology as A3/5 but have a selective advantage over it when grown in mixed cultures with A3/5 in glucose-limited chemostats at a dilution rate of 0·18 h^{-1}; variants A23-S and A24-S selected in this way had selection coefficients of 0·013 h^{-1} and 0·017 h^{-1}, respectively, when grown in mixed culture with A3/5 in glucose-limited chemostat cultures at a dilution rate of 0·18 h^{-1}. Importantly, when these 'improved' variants with a μ_{max} of *ca.* 0·24 h^{-1} were grown in glucose-limited chemostat culture at a dilution rate of 0·18 h^{-1}, the appearance of advantageous colonial mutants was delayed compared with the appearance of these mutants in an A3/5 culture (Table 1). These results indicate that it is possible to select variants of *F. graminearum* which are morphologically more stable than A3/5.

Conclusion

Although it took a long time (21 years!) to bring Lord Rank's new food to the market place, Quorn® myco-protein has the distinction of being the sole survivor of the many single-cell-protein programmes initiated in the 1960s. The secret of this success is that myco-protein is a *high* value product used as a food rather than an animal feed.

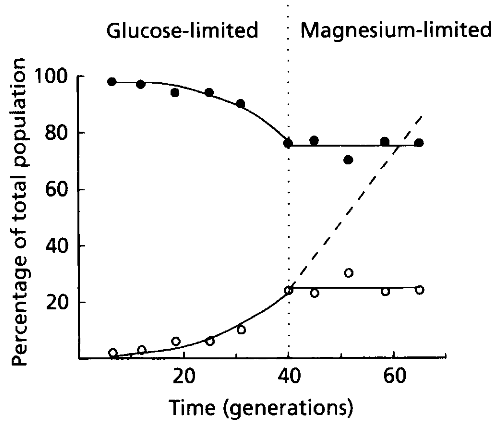

Fig. 9. **Effect on a mixed population of A3/5 (●) and CC1-1 (○) grown in a chemostat at 25 °C and pH 5·8 at a dilution rate of 0·19 h^{-1} of switching from glucose- to Mg^{2+}-limitation. The dashed line indicates the CC1-1 population expected if glucose-limited growth had been maintained throughout (modified from Wiebe *et al.*, 1992).**

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