Competition in the Chemostat between an Obligately and a Facultatively Chemolithotrophic Thiobacillus

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The outcome of competition in thiosulphate-limited chemostat culture between the obligate chemolithotroph *Thiobacillus neapolitanus* and the versatile facultative autotroph *Thiobacillus* A2 was in part a function of pH. In pure culture *T. neapolitanus* grew faster than *Thiobacillus* A2 at pH values up to pH 7-6, but in competition *Thiobacillus* A2 dominated at pH 7-35 and 7-6. At pH 7-1, *T. neapolitanus* dominated, although a significant steady state population of *Thiobacillus* A2 persisted, apparently growing on organic nutrients excreted by *T. neapolitanus*. Coexistence of both organisms occurred under all chemolithotrophic growth conditions tested with the dominant organism comprising 85 to 99% of the population, indicating that competition was not the sole interaction between the species. At pH 7-1, the inclusion of glucose in the thiosulphate medium resulted in rapid domination of the culture by *Thiobacillus* A2, with the virtual elimination of *T. neapolitanus*. It is concluded that the capacity for mixotrophy is a selective advantage to a facultative thiobacillus in competition with an obligately chemolithotrophic species.

INTRODUCTION

The ability of facultatively heterotrophic (or 'versatile') thiobacilli to compete successfully in natural environments with obligately chemolithotrophic thiobacilli or heterotrophs was discussed by Rittenberg (1972) and Whittenbury & Kelly (1977). Rittenberg (1972) postulated that under mixed substrate conditions, the facultative organism might predominate as it can achieve greater reproductive power per unit of energy expenditure growing mixotrophically (or as a chemolithotrophic heterotroph) than can the obligately autotrophic organism. The ability of the facultative *Thiobacillus* A2 to outcompete both the obligate *T. neapolitanus* and a heterotrophic spirillum in chemostat culture on mixed substrates was subsequently demonstrated by Gottschal et al. (1979). They also demonstrated, however, that in autotrophic cultures subject to thiosulphate limitation, the 'specialist' chemolithotroph (*T. neapolitanus*) dominated over *Thiobacillus* A2 at all dilution rates above 0-025 h⁻¹ and suggested that, as a general principle, 'specialist' organisms would dominate over 'versatile' ones by virtue of the higher growth rates attainable by the former under specialist growth conditions. This principle has been the subject of several theoretical and practical treatments (Meers, 1971; Veldkamp & Jannasch, 1972; Taylor & Williams, 1974; Veldkamp, 1976; Kuenen et al., 1977; Laanbroek et al., 1979).

In this paper we report competitive and commensal interactions between the facultative *Thiobacillus* A2 (Taylor & Hoare, 1969) and the obligate *T. neapolitanus* (Kelly, 1967, 1970) showing that under some conditions the former can outgrow the latter even under the specialist conditions favourable to *T. neapolitanus*. 

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METHODS

Organisms and culture conditions. Thiosulphate agar media were used for culture maintenance as previously described for *Thiobacillus* A2 (Wood & Kelly, 1977) and *T. neapolitanus* strain C (Kelly, 1969; Tuovinen & Kelly, 1973). Liquid cultures (in shake-flasks and the chemostat) were grown in a medium containing (g l\(^{-1}\)): Na\(_2\)S\(_2\)O\(_3\), 5H\(_2\)O, 12.5; MgSO\(_4\), 7H\(_2\)O, 0.1; NH\(_4\)Cl, 0.4; KH\(_2\)PO\(_4\), 0 to 4.5; Na\(_2\)HPO\(_4\), 2H\(_2\)O, 3.95 to 9.86; NaOH as required; trace metal solution (Tuovinen & Kelly, 1973), 10 ml. Medium pH was adjusted to the desired value by altering the ratio of phosphates (maintaining the same total phosphate concentration) and amount of NaOH added.

Chemostat cultures were established in a LH modular type series 500 fermenter (LH Engineering, Slough, Bucks) with a culture volume of 750 ml, stirred (750 rev. min\(^{-1}\)) and aerated (200 ml min\(^{-1}\)) with air containing 5% (v/v) CO\(_2\). Temperature was maintained at 30 °C and pH at desired values by automatic addition of 1.6 M-K\(_2\)CO\(_3\), using an EIL model 91A or LH Engineering pH control unit. Culture purity was monitored by examination of growth on nutrient agar.

Discrimination of *Thiobacillus* A2 and *T. neapolitanus* in mixed culture. Appropriate serial dilutions of culture samples were spread on *Thiobacillus* A2 agar medium, pH 8.4 (Wood & Kelly, 1977) containing 0.1 M-sodium formate instead of thiosulphate. Only *Thiobacillus* A2 could grow on this medium and it could be discriminated from *T. neapolitanus*. Identical numbers of colonies of *Thiobacillus* A2 were produced on this medium and on thiosulphate agar, glucose agar or nutrient agar. Dilutions were also spread on to *T. neapolitanus* thiosulphate agar, on which both organisms could grow. *Thiobacillus neapolitanus* produced countable colonies more rapidly than *Thiobacillus* A2 and the morphology of colonies of the two species were quite distinct. *Thiobacillus neapolitanus* could thus be counted directly on these plates and good agreement was achieved by comparison with the total count and the count of *Thiobacillus* A2 obtained on formate agar.

Analytical procedures. Growth was monitored turbidimetrically using a Pye Unicam SP1700 spectrophotometer and absorbance at 440 nm was related to dry weight using calibration curves for pure cultures.

Organism dry weight concentrations and protein contents of cultures were determined in steady state conditions. For dry weight estimation, bacteria were harvested by centrifuging, washed and dried at 105 °C. For protein estimation, organisms from 4 ml samples were harvested by centrifuging, washed with distilled water and dissolved in 2.5 ml 0.5 M-NaOH at 100 °C for 10 min; protein was determined by the Lowry method.

Thiosulphate and polythionates were determined cyanolytically (Kelly et al., 1969) and glucose by the method of Somogyi (1945). Thiosulphate oxidation by washed suspensions of organisms harvested from growing cultures was measured with an oxygen electrode as described previously (Eccleston & Kelly, 1978).

RESULTS

Effect of pH on growth rates of *Thiobacillus* A2 and *T. neapolitanus*

Specific growth rates (μ) were determined from exponentially growing pure cultures on thiosulphate in cultures poised at different pH values. *Thiobacillus neapolitanus* exhibited a maximum growth rate (0.22 h\(^{-1}\)) at pH 6.6 to 6.8, at which pH *Thiobacillus* A2 did not grow. *Thiobacillus* A2 gave a maximum (0.1 h\(^{-1}\)) at pH 7.8 to 8.0, at which pH *T. neapolitanus* did not grow (Table 1).

Growth in batch and continuous culture

From measurement of washout kinetics (Pirt, 1975), maximum specific growth rates for the two strains were calculated to be 0.277 h\(^{-1}\) for *T. neapolitanus* and 0.148 h\(^{-1}\) for *Thiobacillus* A2. At dilution rates of 0.08 and 0.1 h\(^{-1}\) in the chemostat, growth yields were 4.5 and 4.5 g dry wt (mol thiosulphate oxidized\(^{-1}\)) for *T. neapolitanus* and 6.7 and 6.0 g dry wt (mol thiosulphate oxidized\(^{-1}\)) for *Thiobacillus* A2. When grown in mixed batch culture at pH 7.6, both organisms initially grew faster (μ = 0.23 h\(^{-1}\)) than they did alone, but within 10 h viable numbers of *Thiobacillus* A2 virtually ceased to increase while *T. neapolitanus* continued to grow at the same rate for a further 10 h.

Competition in mixed chemostat culture on limiting thiosulphate

The outcome of competition between the two species on limiting thiosulphate was dependent on the pH of the culture. Commencing with equal numbers of the two species growing together in batch culture at pH 7.6 in the chemostat vessel, *Thiobacillus* A2 was outcompeted
Table 1. Specific growth rates in autotrophic batch culture on thiosulphate for 
*Thiobacillus* A2 and *T. neapolitanus* at several pH values

<table>
<thead>
<tr>
<th>pH</th>
<th><em>T. neapolitanus</em></th>
<th><em>Thiobacillus</em> A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6.2</td>
<td>0.116</td>
<td>—</td>
</tr>
<tr>
<td>6.4</td>
<td>0.213</td>
<td>—</td>
</tr>
<tr>
<td>6.6</td>
<td>0.220</td>
<td>0</td>
</tr>
<tr>
<td>6.7</td>
<td>—</td>
<td>0.002</td>
</tr>
<tr>
<td>6.8</td>
<td>0.224</td>
<td>—</td>
</tr>
<tr>
<td>7.0</td>
<td>0.193</td>
<td>0.074</td>
</tr>
<tr>
<td>7.2</td>
<td>0.182</td>
<td>0.087</td>
</tr>
<tr>
<td>7.4</td>
<td>—</td>
<td>0.089</td>
</tr>
<tr>
<td>7.6</td>
<td>0.139</td>
<td>0.096</td>
</tr>
<tr>
<td>7.8</td>
<td>0</td>
<td>0.105</td>
</tr>
<tr>
<td>8.0</td>
<td>—</td>
<td>0.099</td>
</tr>
<tr>
<td>8.2</td>
<td>—</td>
<td>0.086</td>
</tr>
<tr>
<td>8.4</td>
<td>—</td>
<td>0.088</td>
</tr>
<tr>
<td>8.7</td>
<td>—</td>
<td>0.0035</td>
</tr>
<tr>
<td>9.0</td>
<td>—</td>
<td>0</td>
</tr>
</tbody>
</table>

—, Not determined.

Fig. 1. Effect of pH and dilution rate on the outcome of competition between *Thiobacillus* A2 (○) and *T. neapolitanus* (●) in continuous culture on limiting 50 mm-Na$_2$S$_2$O$_3$. Relative numbers (% of total population) of organisms and culture absorbance at 440 nm (△) were determined following the imposition of the following series of conditions at the times indicated by the arrows: (a) pH 7.6, batch culture; (b) pH 7.6, $D = 0.02$ h$^{-1}$; (c) pH 7.6, $D = 0.08$ h$^{-1}$; (d) pH 7.1, $D = 0.08$ h$^{-1}$; (e) pH 7.1, $D = 0.02$ h$^{-1}$. Absorbance values are proportional to dry weights of organisms; a value of 1.0 is equivalent to 300 mg *Thiobacillus* A2 l$^{-1}$ or 360 mg *T. neapolitanus* l$^{-1}$ when applied to pure cultures. Media at pH 7.6 or 7.1 contained (g l$^{-1}$): Na$_2$HPO$_4$.2H$_2$O, 7.9 or 6.0; KH$_2$PO$_4$, 1.5 or 2.94; adjusted with NaOH to the precise values.
and fell to 7% of the population (Fig. 1). A dilution rate of 0.02 h⁻¹ was then commenced and *Thiobacillus* A2 rapidly dominated and continued to constitute more than 95% of the total population after 10 volume changes at $D = 0.08$ h⁻¹ (Fig. 1). During this period *T. neapolitanus* was not eliminated, but remained at 1 to 6% of the population. A subsequent decrease in the steady state culture pH to 7.1 at $D = 0.08$ h⁻¹ (Fig. 1) resulted in takeover of the culture by *T. neapolitanus*, although *Thiobacillus* A2 persisted at about 5% of the total population for 19 volume changes. Decreasing the dilution rate to 0.02 h⁻¹ at pH 7.1 enabled *Thiobacillus* A2 to increase rapidly to about 14%, although *T. neapolitanus* remained dominant over a further 6 volume changes (Fig. 1). The higher absolute growth yield achieved by *Thiobacillus* A2 was reflected in the changes in mixed culture absorbance during these transitions (Fig. 1).

As pH 7.6 was at the upper limit for normal growth of *T. neapolitanus* (Table 1), the experiment was repeated at pH 7.35 commencing with a mixture of 25% *Thiobacillus* A2 and 75% *T. neapolitanus* allowed to grow as a batch culture for 16 h before imposing a dilution rate of 0.08 h⁻¹ (Fig. 2). During batch growth *T. neapolitanus* outcompeted *Thiobacillus* A2 to reach 97% of the total population, but following the switch to continuous flow culture it was rapidly outgrown by *Thiobacillus* A2 until after 6 to 7 volume changes the ratio of *Thiobacillus* A2 to *T. neapolitanus* was 94:6 (Fig. 2). This ratio did not vary significantly over a further 16 volume changes. The culture pH was then lowered from 7.35 to 7.1, which resulted in a slow but progressive takeover by *T. neapolitanus* (Fig. 2). Thiosulphate and polythionates were undetectable in these experiments within 1 (at $D = 0.08$ h⁻¹) to 3 (at $D = 0.02$ h⁻¹) volume changes after commencing continuous flow cultures. It is possible that in both series (Figs 1 and 2) the steady states attained, in which both organisms always coexisted, were not perfect, as some indication of regular oscillations of relative numbers is apparent.
Competition between thiobacilli

Fig. 3. Effect of glucose on the outcome of competition between *Thiobacillus* A2 (○) and *T. neapolitanus* (●) in chemostat culture on limiting 50 mm-Na$_2$S$_2$O$_3$ at pH 7·1, $D = 0.08$ h$^{-1}$. (a) Na$_2$S$_2$O$_3$ alone; (b) Na$_2$S$_2$O$_3$ plus 2·3 mm-glucose; (c) Na$_2$S$_2$O$_3$ alone. See Table 2 for comparative total viable numbers of the two species.

*Effect of glucose on competition between* *Thiobacillus* A2 and *T. neapolitanus* in thiosulphate-limited culture at pH 7·1

Commencing with a mixture of *Thiobacillus* A2 and *T. neapolitanus* in a ratio of 80:20 (Fig. 3), continuous culture at pH 7·1 on limiting thiosulphate alone at a dilution rate of 0·08 h$^{-1}$ led to rapid dominance by *T. neapolitanus*. After 4 volume changes, the culture contained about 1·3 × 10$^8$ *Thiobacillus* A2 ml$^{-1}$ and 9·7 × 10$^8$ *T. neapolitanus* ml$^{-1}$ (i.e. ca 13% *Thiobacillus* A2). At this time the 50 mm-thiosulphate medium feed was supplemented with 2·3 mm-glucose, which resulted in an extremely rapid takeover of the culture by *Thiobacillus* A2 to reach 60 to 70% of the total population within 2 volume changes and about 99·9% of the total population within 4 volume changes (Table 2, Fig. 3). Between 80 and 100 h the viable numbers of *T. neapolitanus* fell from 3 × 10$^8$ (at 80 h) to 10$^6$ (at 100 to 105 h). Since washout of non-growing organisms would only have reduced the numbers to about 4 × 10$^7$, *T. neapolitanus* must actually have lost viability during the takeover of the culture by *Thiobacillus* A2. This observation has not been investigated further. Subsequently reverting to a glucose-free medium resulted in a recovery of *T. neapolitanus* to reach 35·6% of the total population after 9 volume changes even though it had fallen to 0·05% of the total in the presence of glucose.

*Thiosulphate oxidation by washed suspensions of* *Thiobacillus* A2 and *T. neapolitanus*

The growth rates exhibited in batch culture at pH 7·6 by the two species (Table 1) would suggest that *T. neapolitanus* rather than *Thiobacillus* A2 might have dominated in mixed continuous culture at this pH. As the reverse was observed (Fig. 1), the oxidation of thiosulphate by non-growing suspensions was examined to see if differences in apparent $K_m$ values for thiosulphate oxidation might reveal some advantage enjoyed by *Thiobacillus* A2. Values for apparent $K_m$ were calculated from double reciprocal plots of thiosulphate concentration (14 concentrations between 0·1 and 10 mm) and oxidation rate for both strains at pH 7·6 and 7·1. The relation was biphasic for *T. neapolitanus* at both pH values, but *Thiobacillus* A2 gave a linear plot for all thiosulphate concentrations between 0·1 and 10 mm. Apparent mean $K_m$ values (mm-thiosulphate) from three determinations for oxidation by suspensions of *Thiobacillus* A2 were 0·24 ± 0·02 (pH 7·1) and 0·14 ± 0·02 (pH 7·6).
Table 2. Changes in numbers of Thiobacillus A2 and T. neapolitanus during continuous mixed culture at pH 7-1, \( D = 0.08 \, h^{-1} \), on limiting thiosulphate in the absence or presence of glucose

The initial increase in total numbers reflects the use of excess thiosulphate present at the time of switching from exponential batch growth to continuous flow culture. No significant levels of thiosulphate, polythionates or glucose were detected in any steady states.

<table>
<thead>
<tr>
<th>Limiting substrate(s) in medium input (mM)</th>
<th>Time (h)</th>
<th>Culture volume changes</th>
<th>10^4 × No. of viable organisms ml^-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(_2)S(_2)O(_3) (50)</td>
<td>1</td>
<td>0.1</td>
<td>Thiobacillus A2 210 T. neapolitanus 54</td>
</tr>
<tr>
<td></td>
<td>5-11</td>
<td>0.4-0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33-48</td>
<td>2.7-3.9</td>
<td></td>
</tr>
<tr>
<td>Na(_2)S(_2)O(_3) (50) + Glucose (2:3)</td>
<td>52-56</td>
<td>4.2-4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>61-72</td>
<td>4.9-5.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>78-85</td>
<td>6.2-6.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100-134</td>
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<td></td>
</tr>
<tr>
<td>Na(_2)S(_2)O(_3) (50)</td>
<td>144-149</td>
<td>11.6-11.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>242</td>
<td>19.4</td>
<td></td>
</tr>
</tbody>
</table>

With 0.2 to 2 mM-thiosulphate, the values for *T. neapolitanus* were 0.010 ± 0.05 (pH 7.1) and 0.12 ± 0.03 (pH 7.6); and 5.9 ± 3.5 and 1.00 ± 0.3 with 2 to 10 mM-thiosulphate. No firm conclusions can be drawn from these results, especially as the steady state cultures contained no detectable thiosulphate, so that the concentration to which the organisms were exposed was below these apparent \( K_m \) values. Apparent \( V_{max} \) values were very low since *T. neapolitanus* in particular rapidly lost activity in non-growing suspensions.

**DISCUSSION**

These experiments serve to demonstrate that a facultative thiobacillus like *Thiobacillus* A2 is likely to exhibit a marked survival advantage over obligately lithotrophic strains such as *T. neapolitanus* and that interactions other than solely competition can exist between such species. As expected, and as separately demonstrated by Gottschal et al. (1979), *T. neapolitanus* dominated over *Thiobacillus* A2 in thiosulphate-limited culture in our experiments at pH 7-1. However, in contrast to the view expressed by Gottschal et al. (1969) that the specialist organism would generally outcompete the versatile one under such conditions, we have shown that the outcome of competition was pH-dependent. Thus at pH 7.35 and 7.6, *Thiobacillus* A2 was the dominant organism even though separate batch culture of the two species showed *T. neapolitanus* to grow faster than *Thiobacillus* A2. At pH 7.35 or 7.6, *T. neapolitanus* fell to about 5% of the total number of organisms (Figs 1 and 2) but was never completely eliminated. Similarly, at pH 7-1 at which *T. neapolitanus* dominated, *Thiobacillus* A2 persisted at 5 to 14% of the total population (Fig. 1), being more abundant at a lower dilution rate than a high one. These observations of the establishment of seemingly stable mixed populations on a single limiting substrate are not consistent with the theory of competition between two species under such conditions. The organism of faster growth rate would be expected to exclude the second one completely (Jost et al., 1973; Veldkamp, 1976; Kelly, 1978). The key to explaining these phenomena is given in the fact that *Thiobacillus* A2 dominates over *T. neapolitanus* even at pH 7-1 when a small amount of glucose is added to the medium (Fig. 3). Under such conditions *Thiobacillus* A2 consumes all the available glucose, as *T. neapolitanus* strain C cannot incorporate glucose (Kelly, 1968), which would allow a steady state concentration of about 3.6 × 10^9 viable organisms ml^-1 in the absence of thiosulphate, compared with about 10^6 on 50 mM-thiosulphate alone. A large population could thus be produced that could compete with *T. neapolitanus* for the available thiosulphate. More significantly, the maximum specific growth rate of *Thiobacillus* A2 growing mixo-
trophically at pH 7.1 on glucose plus thiosulphate must exceed that of \( T. neapolitanus \) leading to the almost total exclusion of the latter (Table 2, Fig. 1). Dominance of \( \text{Thiobacillus A2} \) at pH 7.35 and 7.6 in thiosulphate-limited culture could result at least in part from its growth rate being increased to a value greater than that of \( T. neapolitanus \) by organic nutrients excreted by \( T. neapolitanus \). In pure culture on thiosulphate, \( T. neapolitanus \) excretes up to 27% of the carbon dioxide it fixes (Kelly, 1969), including considerable amounts of glycollate (Gottschal \textit{et al.}, 1979), which could enable the survival of small populations of \( \text{Thiobacillus A2} \) in mixed cultures dominated by \( T. neapolitanus \). The higher population at \( D = 0.02 \text{ h}^{-1} \) than at \( D = 0.08 \text{ h}^{-1} \) (Fig. 1) could reflect greater excretion of metabolites by \( T. neapolitanus \) at what might be a rather unfavourable growth rate (about 7% of \( \mu_{\text{max}} \)). Certainly cultures subject to growth inhibition, for example by phenylalanine, excreted far larger amounts of fixed carbon (Kelly, 1969). The persistence of significant numbers of \( T. neapolitanus \) in cultures dominated by \( \text{Thiobacillus A2} \) is less obviously explained but may indicate a complex interaction between the two species. If the advantage of \( \text{Thiobacillus A2} \) is a marginally greater growth rate than that of \( T. neapolitanus \) at pH 7.35 and 7.6, due to excreted metabolites from the latter organism, a decline of \( T. neapolitanus \) too low a level would reduce the growth rate of the \( \text{Thiobacillus A2} \), thus allowing \( T. neapolitanus \) to increase in numbers again. The stable ratio of the two could thus be a dynamic balance. The populations of both organisms may in fact show oscillations in the steady states (e.g. Fig. 1), which would be consistent with such dynamic metabolic interrelationships. Stable mixed cultures of heterotrophs on single limiting substrates in the chemostat can be maintained (Brunner \textit{et al.}, 1968; Slater & Somerville, 1979), but in many of these one or more members of the communities are growing on breakdown products of the primary substrate. Persistence of seemingly uncompetitive organisms has also been reported (Godwin & Slater, 1979). Mutual stimulation of growth rate when the two organisms were grown in mixed batch culture could indicate stimulation of \( \text{Thiobacillus A2} \) by \( T. neapolitanus \) metabolites, but the stimulation of the latter might be consequent on the removal of those metabolites, since \( T. neapolitanus \) is known to be sensitive to some organic metabolites (Kelly, 1969, 1971; Matin, 1978), and it and other chemolithothrophs may exhibit stimulated growth rates in dialysed cultures (Pan & Umbreit, 1972).

These results demonstrate that although one or other of a facultative or obligate chemolithotrophic autotroph may dominate in mixed cultures depending on substrate supply and pH, both may coexist under various conditions, showing that, in addition to competition, there can be synergism, commensalism or mutualism as has indeed been modelled or observed with purely heterotrophic systems (Megee \textit{et al.}, 1972; Taylor & Williams, 1974; de Freitas & Frederickson, 1978). The results demonstrate very clearly that the facultative organism \( \text{Thiobacillus A2} \) will dominate under mixotrophic nutrient conditions at pH values at which it would not compete successfully with \( T. neapolitanus \) under solely chemolithothrophic and autotrophic growth conditions. Facultative autotrophs exhibiting mixotrophic growth physiology would therefore be expected to be of greater abundance in natural environments than obligate autotrophs.

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**REFERENCES**


