

Competition Between the Facultatively Chemolithotrophic *Thiobacillus* A2, an Obligately Chemolithotrophic *Thiobacillus* and a Heterotrophic *Spirillum* for Inorganic and Organic Substrates

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Abstract. Competition in a chemostat between the versatile *Thiobacillus* A2 and the specialized *T. neapolitanus* for thiosulfate as the sole growth-limiting substrate, led to dominance of the specialized over the versatile organism, at dilution rates $\geq 0.025 \text{ h}^{-1}$. Increasing concentrations of acetate or glycollate in the thiosulfate medium caused increased relative numbers of *T. A2* in steady states at $D = 0.07 \text{ h}^{-1}$. Eventually, with 10–12 mmol of organic substrate per litre, complete dominance of *T. A2* over *T. neapolitanus* occurred.

Mixed cultures of *T. A2* and a specialized spirillum-shaped heterotroph, competing for acetate as sole growth-limiting substrate resulted in complete dominance of the heterotroph at dilution rates of 0.07 and 0.15 h^{-1} . In this case increasing concentrations of thiosulfate in the acetate medium, up to 10 mM, eventually led to the elimination of the heterotroph.

These results have been interpreted as evidence that *T. A2* was growing mixotrophically. As the concentration of the second substrate was raised, the number of *T. A2* cells increased and as a result *T. A2* consumed an increasing portion of the common substrate.

In mixed chemostat cultures containing all three organisms, *T. A2* could maintain itself with all tested ratios of acetate and thiosulfate in the inflowing medium. The heterotroph was excluded from the culture below a relatively low acetate to thiosulfate ratio, whilst above a relatively high acetate to thiosulfate ratio *T. neapolitanus* was completely eliminated.

These results were discussed in relation to the ecological niche of *Thiobacillus* A2-type organisms.

Key words: *Thiobacillus* A2 — Mixotrophy — Competition — Mixed cultures — Facultative chemolithotroph — Ecological niche.

Since the first report by Nathanson (1902) on the isolation of organisms capable of autotrophic growth in an inorganic medium with reduced sulfur compounds as energy substrates, several different types of these organisms have been isolated. Later work by Starkey (1935) showed that this group of organisms, the genus *Thiobacillus*, does not only include obligately chemolitho(auto)trophic organisms but also facultative chemolitho(auto)trophs which possess the potential of heterotrophic growth in the absence of an inorganic sulfur containing substrate.

These facultative chemolithotrophs [also called versatile (Smith and Hoare, 1977) or mixotrophic (Rittenberg, 1969) thiobacilli] also can grow mixotrophically on mixtures of inorganic and organic (energy) substrates. Although some strains of facultatively chemolithotrophic thiobacilli have been isolated over the years, very little is known about their distribution and role in nature. The fact that they can be isolated from habitats (Starkey, 1935; Taylor et al., 1971; Swaby and Vitolins, 1968) which also harbour obligately chemolithotrophic thiobacilli, indicates that they must be able to maintain themselves in close proximity to the obligately chemolithotrophic thiobacilli in the natural environment. One of the characteristic properties of the versatile thiobacilli is their low maximum specific growth rate as compared to specialized heterotrophs and autotrophic thiobacilli. As growth rate must be one of the decisive selective properties for competing species, the survival of versatile thiobacilli would seem difficult to explain. However, it has been quite well established that the outcome of competition under substrate limiting conditions cannot always be predicted on the basis of maximum specific growth rates but also depends on many other parameters such as the actual specific growth rate (μ) (Veldkamp and Jannasch, 1972; Harder and Veldkamp, 1971; Meers, 1971), the substrate saturation constant (K_s) (Veldkamp and Jannasch,

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1972; Harder and Veldkamp, 1971; Meers, 1971), the presence of predators (Jost et al., 1973), the availability of other (limiting) substrates (Meers and Tempest, 1968; Megee et al., 1972) and probably a whole set of still unrecognized parameters. In considering the potential of a facultatively chemolithotrophic thiobacillus to survive in the competition with specialized heterotrophs and specialized chemolithotrophs it seems likely that metabolic versatility allows such organisms to survive in nature. As suggested by Rittenberg (1972) these organisms should be able to compete successfully with a „specialist“ thiobacillus under mixed substrate conditions. As a result of these considerations we investigated whether in chemostat mixed cultures of the very versatile facultatively chemolithotrophic *Thiobacillus* A2 (Taylor and Hoare, 1969) together with the specialized *Thiobacillus neapolitanus* and with a specialized heterotrophic spirillum, conditions could be created where *Thiobacillus* A2 could successfully compete for the available substrate(s). In this report it will be shown that such conditions can indeed be created. The implications of these observations will be discussed in relation to the ecological niche of a facultatively chemolithotrophic thiobacillus.

Materials and Methods

Organisms. *Thiobacillus* A2 was kindly provided by D. W. Smith, who originally obtained the organism from B. F. Taylor. Our culture of *T.* A2 has a maximum specific growth rate on acetate of 0.22 h^{-1} . This is relatively low compared to 0.36 h^{-1} as reported originally by Taylor and Hoare (1969). We observed that faster growing organisms sometimes appeared after prolonged cultivation in acetate-limited continuous culture.

The obligately chemolithotrophic thiobacillus used in this study was *Thiobacillus neapolitanus* (strain X).

A heterotrophic organism was isolated on glycollate from a ditch in the botanical garden, surrounding the Microbiology Department in Haren. A spirillum-shaped organism, preliminary called spirillum G7, was selected for its high maximum specific growth rate on glycollate and on acetate ($\mu_{\text{max}} = 0.43 \text{ h}^{-1}$). This organism is incapable of thiosulfate utilization.

T. neapolitanus was maintained by regular subculture on thiosulfate-minerals agar media (Kuenen and Veldkamp, 1973). *T.* A2 and spirillum G7 were maintained on the nutrient-rich agar medium described below.

Media. The basal medium for cultivation in continuous culture contained (% w/v): K_2HPO_4 , 0.4; KH_2PO_4 , 0.15; $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 0.04; NH_4Cl , 0.04; in deionized water, plus 2 ml per liter of a trace elements solution (Vishniac and Santer, 1957). The trace elements solution contained 2.2 g instead of the originally reported 22 g $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ per liter. The pH was 7.2. To this basal medium, different amounts of Na-thiosulfate, Na-acetate or Na-glycollate were added, according to the description in the experimental section. The medium was sterilized by autoclaving for 30 min at 118°C . Magnesium sulfate plus trace element solution and thiosulfate, acetate or glycollate were sterilized separately, each in 5% of the volume. The nutrient-rich agar medium contained (% w/v): K_2HPO_4 , 0.05; yeast extract, 0.5; peptone, 0.3; glucose, 0.2; Na-lactate, 0.1; agar (Difco), 1.5; in deionized water. Final pH 7.0–7.5.

Continuous cultivation was carried out at 28°C in the equipment described by Harder et al. (1974). Further conditions were as described previously (Kuenen and Veldkamp, 1973). The oxygen concentration in the culture was automatically controlled at 50% air saturation. Cultures were maintained at the desired pH = 7.5 by automatic addition of 1 M Na_2CO_3 or 1 M HCl, depending on the substrates being supplied to the culture.

Cultures were frequently checked for contaminants on plates and in liquid culture of the appropriate thiosulfate and nutrient-rich organic medium.

Viability and Cell Discrimination. Viability was measured on appropriate agar media by the method of Postgate (1969). By using the same method, the different organisms could be discriminated microscopically on the basis of their differences in morphology and their relative numbers counted. For each determination at least 400 microcolonies were counted.

Protein and Organic Carbon Determinations. After centrifugation at 12000 g for 10 min, cells were resuspended in deionized water. The protein concentration in these suspensions was measured using the method described by Lowry (1951). The organic cell carbon content of these suspensions was measured with a total organic carbon analyzer (Beckman). The contribution of *T.* A2 to the cell carbon of the mixed culture was calculated from its cell number. It was assumed that the volume of the cells in mixed culture was the same as that observed in pure cultures grown at the same dilution rate. The organic carbon in the supernatant of the culture was directly measured with this carbon analyzer.

Inoculation Mixtures of Different Organisms. Calibration curves were constructed for absorbance (E_{433}) versus cell-number in pure cultures. Volumes required to construct mixtures of known ratios (cell numbers) between different organisms were determined on the basis of E_{433} values. Total cell counts were estimated using microscopic counting chambers (depth = 0.01 mm).

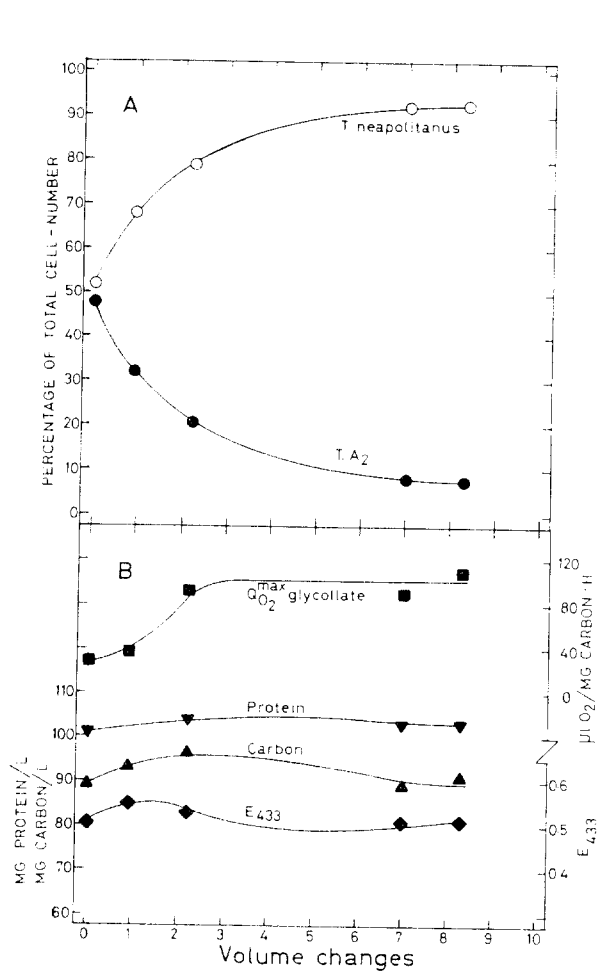
Miscellaneous Methods. Maximum oxygen consumption rates of cell suspensions were measured polarographically with a YSI Biological Oxygen Monitor. Thiosulfate concentrations were determined using the method described by Sörbo (1957). The acetate or glycollate concentration in the reservoir was measured with the Total Organic Carbon analyzer. Glycollate in the supernatant of cultures was also measured specifically with the method described by Calkins (1943).

Chemicals. All chemicals used were of analytical grade (Merck or BDH).

Results

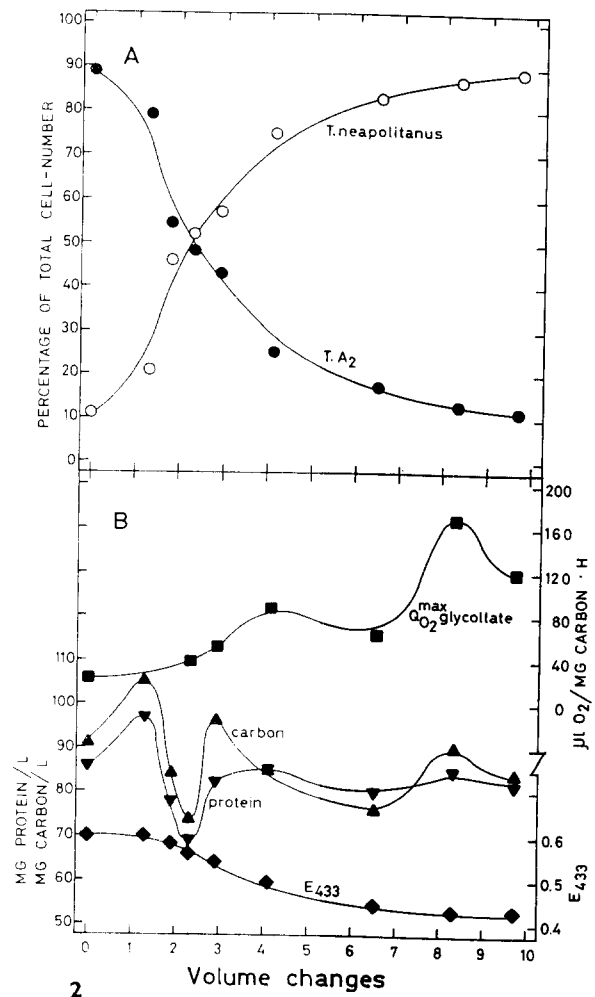
Competition for Thiosulfate by *Thiobacillus* A2 and *Thiobacillus neapolitanus*

Thiobacillus A2 and *Thiobacillus neapolitanus*, cultivated separately in continuous culture at a dilution rate of 0.05 h^{-1} with thiosulfate as the energy limiting substrate, were mixed in a 1:1 ratio (based on cell numbers) and cultivated in thiosulfate limited mixed culture at the same dilution rate. Figure 1A shows that the relative number of *T. neapolitanus* cells increased with time. The cell carbon and protein content of the culture remained almost constant during the experiment (Fig. 1B). In contrast to predictions based on mathematical analyses of this type of competition experiments for one growth limiting substrate, *Thiobacillus* A2 was not completely eliminated from the culture but maintained itself at a level of 5–10% of the



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Fig. 1A and B. Competition in continuous culture between *Thiobacillus A2* and *Thiobacillus neapolitanus* for thiosulfate as growth-limiting substrate. The chemostat was run at a dilution rate of 0.05 h^{-1} with a 40 mM thiosulfate concentration in the feed. Organisms were pregrown separately in continuous culture at $D = 0.05 \text{ h}^{-1}$ and at zero time mixed in a 1:1 ratio. **A** Relative cell number of *T. A2* (●—●) and *T. neapolitanus* (○—○) versus the number of volume changes. **B** Absorbance (◆—◆) E_{433} and maximum glycollate oxidizing capacity of *T. A2* (■—■) expressed per mg *T. A2* cell-carbon. This activity was determined by measuring the oxygen uptake rate with an excess of glycollate in samples taken from the culture



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Fig. 2A and B. Competition in continuous culture between *T. A2* and *T. neapolitanus* for thiosulfate as growth-limiting substrate. The chemostat was run at a dilution rate of 0.025 h^{-1} with a 40 mM thiosulfate concentration in the feed. Organisms were pregrown separately in continuous culture at $D = 0.025 \text{ h}^{-1}$ and at zero time *T. A2* and *T. neapolitanus* were mixed in a ratio of 9:1 respectively. Further details as described with Fig. 1A and B

total cell number. This is explained by assuming that *T. A2* is growing on organic compounds excreted by *T. neapolitanus*. Previous work in our laboratory has shown that *T. neapolitanus* excretes substantial amounts of glycollate (Y. Cohen et al., in preparation) which can serve as the only carbon and energy source for *T. A2*. Further evidence for this explanation comes from the observed increased capacity of the culture to oxidize glycollate (Fig. 1B). The capacity to oxidize glycollate can be induced easily in pure cultures of *T. A2* but not in *T. neapolitanus* cultures. Furthermore, glycollate could not be detected in the mixed culture

and the organic carbon content of the supernatant of the mixed culture was 4–6 mg C per liter as compared to about 10 mg C per liter in pure cultures of *T. neapolitanus*. This difference is sufficiently large to explain the observed number of *T. A2*.

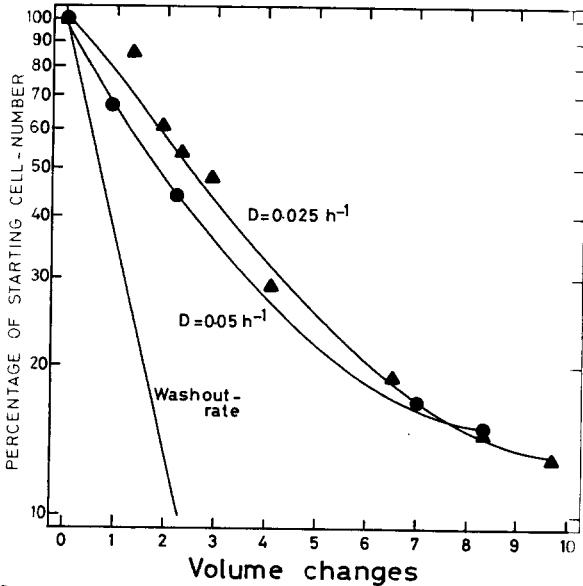
The next experiments were designed to study the effect of different dilution rates and the effect of different initial ratios of the two organisms in the inoculum on the outcome of the competition.

T. neapolitanus outcompeted *T. A2* not only at the higher dilution rate of 0.075 h^{-1} , but also at 0.025 h^{-1} , which is only 7% of the maximum specific growth rate

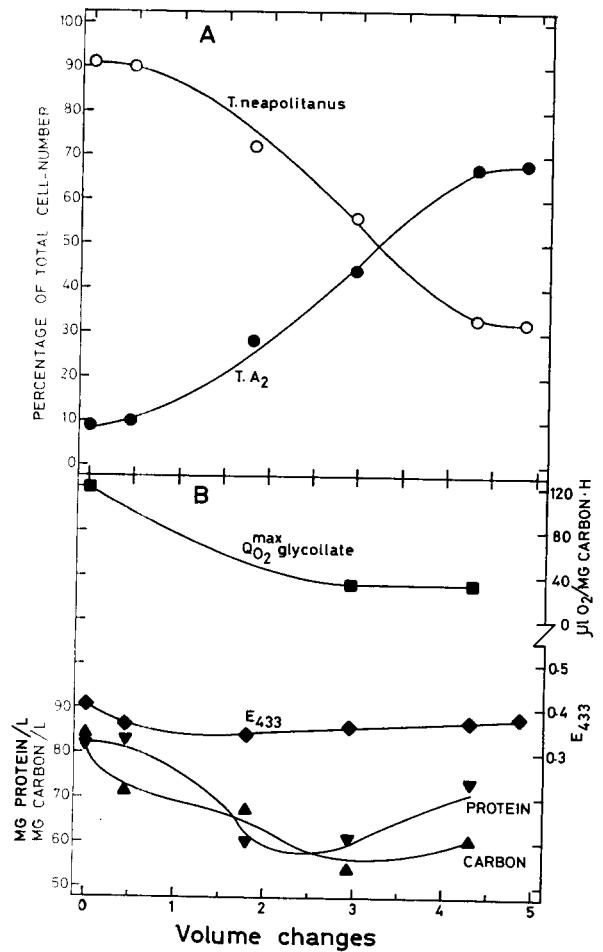
of *T. neapolitanus*. In both cases the residual *T. A2* population accounted for about 9% of the total cell number. The results at $D = 0.025 \text{ h}^{-1}$ are presented in Fig. 2A and B which also shows that a starting ratio of 9:1 in favour of *T. A2* did not change the outcome of the competition experiment. Figure 3 shows that the rate of disappearance of *T. A2* is clearly lower than the dilution rate. It could be calculated from the slope of these curves that *T. A2* initially grew with a growth rate equal to 60–75% of the applied dilution rates.

Furthermore, it became apparent that growth rate certainly is a factor of great importance for the outcome

of the competition. The results of a competition experiment at a dilution rate of 0.004 h^{-1} are presented in Fig. 4. The starting situation was the mixed culture in steady state with $D = 0.025 \text{ h}^{-1}$ described at the end of the previous experiment. At this very low dilution rate *T. A2* increased in relative cell number (up to 70% after 5 volume changes) compared to *T. neapolitanus*. Moreover, the viability had decreased to 40% for the *T. neapolitanus* cells, whereas more than 85% of the *T. A2* cells remained viable. It should be realized that the applied dilution rate was about 1% of the maximum specific growth rate of *T. neapolitanus* ($= 0.35 \text{ h}^{-1}$).



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Fig. 3. The rate of disappearance of *T. A2* from the mixed cultures described in Fig. 1 and 2. The number of *T. A2* cells has been expressed as the percentage of the *T. A2* cell-number present at the start of the competition experiment. These values have been plotted logarithmically versus the number of volume changes during the experiment at $D = 0.05 \text{ h}^{-1}$ (●—●) and at $D = 0.025 \text{ h}^{-1}$ (▲—▲). The solid line represents the expected decrease in relative *T. A2* cell-number if no growth would have occurred

Fig. 4A and B. Competition in continuous culture between *T. A2* and *T. neapolitanus* for thiosulfate at very low dilution rate. The chemostat was run at a dilution rate of 0.004 h^{-1} with a 40 mM thiosulfate concentration in the feed. The starting situation at zero time was the mixed culture at the end of the competition experiment at $D = 0.025 \text{ h}^{-1}$ as described above (Fig. 2A and B). Further details as described with Fig. 1A and B

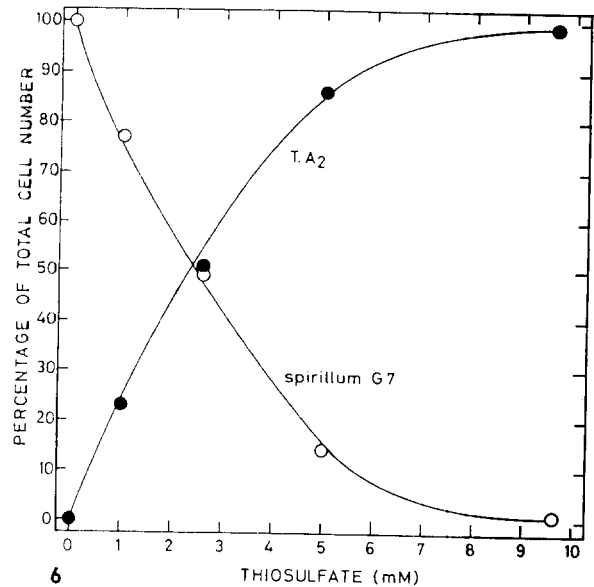
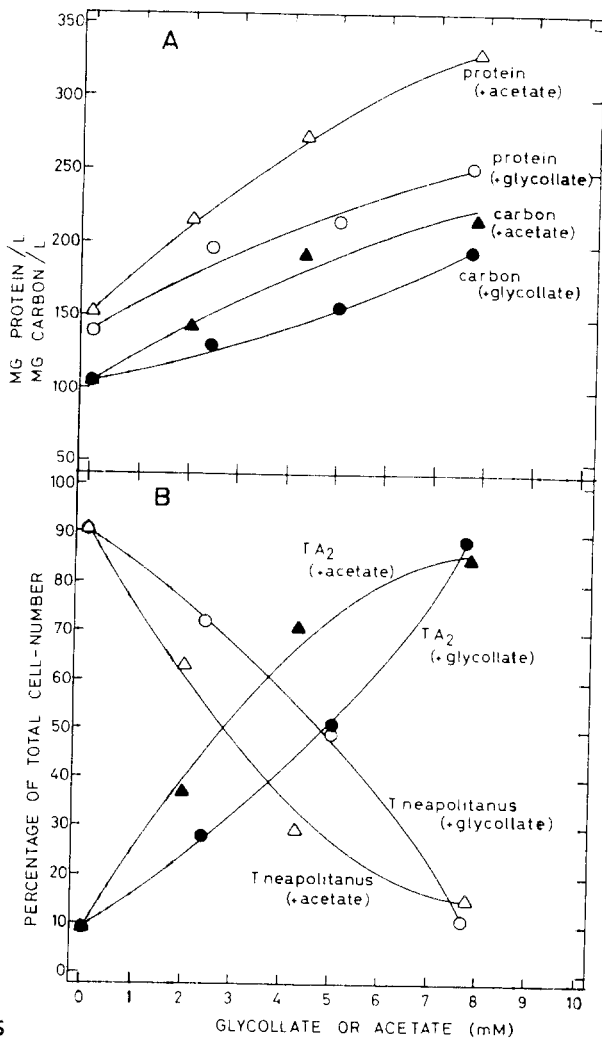


Fig. 5. A and B. Effect of different concentrations of organic substrate on the outcome of the competition between *T. A2* and *T. neapolitanus* for thiosulfate. The chemostat was run at a dilution rate of 0.07 h^{-1} . The inflowing medium contained thiosulfate (40 mM) together with either acetate or glycollate at concentrations ranging from 0–7 mM. Relative cell numbers, protein content and organic cell carbon in the culture were determined after steady states had been established. **A** Protein concentration in the cultures limited by thiosulfate plus acetate (Δ — Δ) or glycollate (\circ — \circ). Organic carbon content of cultures limited by thiosulfate plus acetate (\blacktriangle — \blacktriangle) or glycollate (\bullet — \bullet). **B** The percentage *T. A2* cells in cultures with thiosulfate plus acetate (\blacktriangle — \blacktriangle) or glycollate (\bullet — \bullet) in the feed. The percentage *T. neapolitanus* cells in cultures with thiosulfate plus acetate (Δ — Δ) or glycollate (\circ — \circ) in the feed.

Fig. 6. Effect of different concentrations of thiosulfate on the outcome of the competition between *T. A2* and spirillum G7 for acetate. The chemostat was run at a dilution rate of 0.07 h^{-1} . The inflowing medium contained acetate (10 mM) together with thiosulfate at concentrations ranging from 0–9.7 mM. After steady states had been established the percentage *T. A2* (\bullet — \bullet) and spirillum G7 cells (\circ — \circ) were determined.

Influence of Organic Matter on Mixed Cultures of T. A2 and T. neapolitanus

The experiments described showed the importance of organic matter (viz. excreted organic compounds) for the survival of the facultatively chemolithotrophic *Thiobacillus* in competition for thiosulfate with an obligately chemolithotrophic *Thiobacillus*. In Fig. 5 the results are shown of experiments aimed to explore this

point in more detail. Increasing amounts of glycollate or acetate were added to an otherwise inorganic thiosulfate medium (40 mM thiosulfate) which was fed to the chemostat containing a mixed culture of *T. A2* and *T. neapolitanus*. The dilution rate was 0.07 h^{-1} . As in the previous experiments steady states were reached in which *T. A2* and *T. neapolitanus* coexisted. These steady states were usually established after about 8–10 volume changes.

The observed proportional increase of *T. A2* cells with increasing amounts of organic substrate (until they account for 90% of the total cell number at 7.8 mM acetate or glycollate) necessarily means a relative decrease in *T. neapolitanus* cells. The absolute number of *T. neapolitanus* cells also decreased by a factor of 10. Figure 5A shows the increase in cell mass, as a function of the acetate or glycollate concentration in the medium. Such an increase must be mainly the result of growth of *T. A2*, because *T. neapolitanus*, when grown in the presence of single organic compounds, shows increases in cell yield of less than 20% (Kuenen and Veldkamp, 1973). Furthermore, the increase is much more than the predicted sum of heterotrophic growth of *T. A2* on acetate or glycollate and autotrophic growth of *T. neapolitanus*. From this it can be concluded that *T. A2* grows mixotrophically on acetate and thiosulfate, whereby the autotrophic CO_2 -fixation is repressed. Further details of this energy saving effect during mixotrophic growth of *T. A2* will be dealt with in another publication (J. C. Gottschal and J. G. Kuenen, in preparation).

Mixed Cultures of *Thiobacillus A2* and the Heterotrophic *Spirillum G7*

The experiments described above show just one side of the picture. A study of mixed cultures of *Thiobacillus A2* and a heterotroph should throw some light on the "fitness" of a facultatively chemolithotrophic thiobacillus when in competition for organic substrates. For this study we used a freshly isolated (see "Materials and Methods") heterotrophic spirillum which had been selected for its high maximum specific growth rate on glycollate and acetate.

Compared to *T. A2*, spirillum G7 exhibits a high specific growth rate on acetate (0.22 h^{-1} and 0.43 h^{-1} respectively). In batch culture (minerals-acetate medium), inoculated with a 1:1 mixture of both organisms, *T. A2* was rapidly outgrown by spirillum G7. Furthermore, it turned out that cultivation of these two organisms in mixed continuous culture, with acetate as growth limiting substrate both at $D = 0.07 \text{ h}^{-1}$ and at $D = 0.15 \text{ h}^{-1}$ resulted in complete elimination of *T. A2* from the chemostat (see Fig. 6 at 0 mM thiosulfate). This is compatible with the observation that in a pure culture of spirillum G7, growing on acetate, no organic excretion products could be detected. A competition experiment carried out at a very low dilution rate of $D = 0.0075 \text{ h}^{-1}$ showed that after 11 volume changes *T. A2* dominated and accounted for 90% of the total cell number. The viability of *T. A2* was greater than 80%. The viability of the spirillum could not be determined as it tended to form small clumps under these extreme conditions.

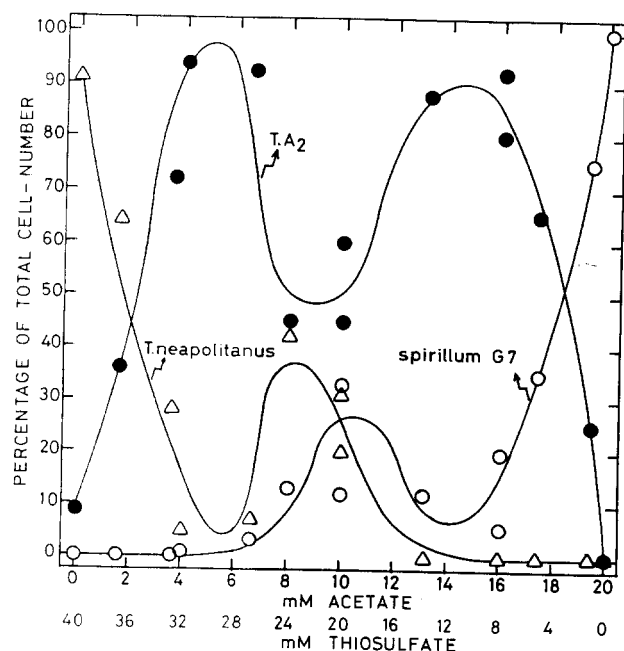


Fig. 7. Competition between *T. A2*, *T. neapolitanus* and spirillum G7 for thiosulfate and acetate as growth-limiting substrates in the chemostat at a dilution rate of 0.075 h^{-1} . Concentrations of these substrates in the inflowing medium ranged from 0–20 mM for acetate and 40–0 mM for thiosulfate. After steady-states with different ratios between acetate and thiosulfate concentrations in the feed had been established, relative cell numbers of *T. A2* (●—●), *T. neapolitanus* (△—△) and spirillum G7 (○—○) were determined.

Influence of Thiosulfate on Mixed Cultures of *T. A2* and Spirillum G7

Figure 6 depicts the effect of increasing concentrations of thiosulfate on the outcome of the competition for acetate at a dilution rate of 0.075 h^{-1} . The data show that above 10 mM thiosulfate added to the acetate medium (10 mM) more than 99% of the total cell number consisted of *T. A2* cells. Under these conditions the viability of both species remained above 90%. In a separate set of batch culture experiments it had been shown that the increasing concentration of Na_2SO_4 in the culture, as a result of the oxidation of thiosulfate by *T. A2* did not depress the maximum specific growth rate of spirillum G7.

Competition Between *T. A2*, *T. neapolitanus* and Spirillum G7 for Acetate and Thiosulfate as Growth Limiting Substrates in the Chemostat

From the above described competition experiments it became clear that the introduction of a second substrate, which could be metabolized by *T. A2* but could not be metabolized by the specialized organisms, offered a clear competitive advantage to the facul-

tatively chemolithotrophic thiobacillus. These results allowed no definite predictions of what would happen in a three-membered mixed culture. It might well be that with all three species together *T. A2* would be excluded from the culture, because now the organism would have to compete for both acetate and thiosulfate.

Figure 7 shows the results of such experiments. It can be observed that with all tested mixtures of acetate and thiosulfate in the inflowing medium, *T. A2* was able to maintain itself in the culture. In the left part of the graph we observe that as the [acetate]:[thiosulfate] ratio increased from 0/40 to 5/30 *T. A2* increased in relative numbers whereas *T. neapolitanus* decreased and spirillum G7 could not maintain itself in the culture. In the right part of the graph *T. A2* increased, this time with decreasing [acetate]:[thiosulfate] ratio from 20/0 → 15/10. The spirillum showed a concomitant decrease, whereas *T. neapolitanus* was unable to grow. So with these substrate ratios the outcome of competition is essentially the same as found for the competition between *T. A2* and only one "specialist" organism. However, the medium composition as shown in the middle part of this picture favours the coexistence of all three organisms together. This coexistence seems to represent a rather stable situation because during prolonged cultivation (20 volume changes) with a mixture of 10 mM acetate and 20 mM thiosulfate no changes in the relative numbers of the three species occurred during the last 8 volume changes.

Discussion

In the underlying study we have tested the hypothesis that a metabolically versatile *Thiobacillus* would be able to compete successfully with a specialized autotrophic *Thiobacillus* and a specialized heterotrophic bacterium under mixed substrate conditions (Rittenberg, 1972). Competition experiments were carried out in energy and/or carbon-limited continuous culture using two- and three-membered mixed populations. As expected the specialized organisms namely *T. neapolitanus* and spirillum G7 rapidly outcompeted the versatile *T. A2* in thiosulfate mineral medium and acetate mineral medium, respectively, over a large range of dilution rates. However, when mixtures of acetate and thiosulfate were supplied to these two-membered cultures, *T. A2* was able to maintain itself in the chemostat. When grown in mixed culture with *T. neapolitanus* the relative number of *T. A2* increased with increasing concentrations of acetate in the thiosulfate medium and eventually completely eliminated *T. neapolitanus* when more than 10–12 mM acetate was present in the feed.

These results can be explained when it is realized that *T. A2* is able to grow mixotrophically on thiosulfate

and acetate in this two-membered culture. It will consume all the substrate *not* utilized by the "specialist" organism (acetate), and some of the common substrate (thiosulfate). As the relative abundance of acetate increases, *T. A2* will increase in number. An increasing number of *T. A2* will lead to an increase in its consumption of the thiosulfate, irrespective of its affinity for thiosulfate. Thus increasing acetate concentration in the feed eventually will lead to complete dominance of *T. A2* over *T. neapolitanus*.

A similar explanation can be given for the dominance of *T. A2* over the heterotroph when increasing amounts of thiosulfate are added to the acetate medium.

In a recent publication (Laanbroek et al., in preparation) similar observations have been described for the competition of a specialized glutamate fermenting *Clostridium* and a more versatile *Clostridium* species. Thus it may be predicted that the observed pattern is a common principle in the competition between species. To further a better understanding of the role played by different growth parameters in these cultures, a mathematical model has been constructed based on simple Monod/Michaelis-Menten kinetics. A detailed description of this model, which enabled us to describe adequately the population changes observed in these mixed cultures, will be published elsewhere.

It should be stressed that during competition experiments both acetate and thiosulfate are present at growth limiting concentrations. Growth limiting concentration of the substrates is essential for the development of *T. A2* in the competition experiments. In batch culture the outcome of competition for a substrate depends on the maximum specific growth rates of the competing microorganisms for that particular substrate. Thus, the specialized organisms are expected to dominate over the more versatile bacteria in such a case because of their relatively high maximum specific growth rate. This has indeed been found for the mixed population of *T. A2* and spirillum G7 in a batch culture with acetate as the substrate (see results).

Our experiments further showed that the versatile *Thiobacillus* was also able to maintain itself under mixed substrate conditions in a three-membered culture with both the autotrophic and the heterotrophic specialist present at the same time. The coexistence of three species competing for two growth limiting substrates seems to contradict the results of recent mathematical analyses (Yoon et al., 1977; Taylor and Williams, 1974; de Freitas and Frederickson, 1978), predicting that only two organisms would be able to coexist under such conditions. However, if interactions other than pure competition for the growth limiting substrates are included in a mathematical model, a much greater species diversity is predicted (de Freitas

and Frederickson, 1978). Thus, the coexistence of the three species in the thiosulfate and acetate limited chemostats may perhaps be taken as an indication that until now unrecognized non-competitive interactions indeed play a role. It is questionable, however, whether the above mentioned mathematical models may be applied directly to our experimental system. It is assumed in these models that all organisms compete for all available substrates. This is not the case in our experimental model, in which competition for each substrate occurs only between two organisms. Whether in such a case stable coexistence can be predicted mathematically is currently being analyzed.

The above experiments with our model system have clearly demonstrated the ability of the versatile *T. A2* to survive in the presence of "specialists". Furthermore, our model provides a possible explanation for the survival of versatile chemolithotrophs among "specialist" heterotrophs and chemolithotrophs in the natural environment. Necessarily, our experimental conditions are a tremendous oversimplification of the actual ecosystem. Not only will, in most cases, the natural environment never create such stable conditions as existing in a chemostat but also the diversity of physiologically different organisms will be much greater in most natural habitats. Furthermore, "wash-out" of slowly and not growing organisms will certainly play a much less important role in nature than it does in the chemostat. Yet in our view the important principle of mixed substrate utilization will play a critical role in almost any natural habitat of versatile and specialized organisms.

In the chemostat the outcome of the competition is determined by the ratios of the two substrates. In order to translate this phenomenon to processes occurring in the natural environment, it should be realized that these changing ratios will lead to changes in the relative turnover rate of the two substrates. Thus one may predict that it will be this relative turnover rate of inorganic and organic compounds in the natural environment, which is of decisive importance in the competition between specialized chemolithotrophs and chemoorganotrophs and the versatile facultative chemolithotrophs. If these turnover rates have values of the same order of magnitude *Thiobacillus A2*-type organisms would be favoured, whereas with very high values of either one of these rates heterotrophs or autotrophs would predominate.

Recent publications (Hall and Berk, 1968; Schook and Berk, 1978; Trudinger, 1967; Tuttle and Jannasch, 1972) suggest the presence in many environments of heterotrophic organisms which also oxidize (in)organic reduced sulfur containing compounds in addition to their required organic substrates. It might well be that these organisms reduce the ecological niche of *T. A2*-

type organisms considerably. Therefore we feel that model experiments including these sulfur oxidizing heterotrophs are required. Furthermore other features of the mixotrophic physiology, like flexibility under transient state conditions, should be explored in relation to their survival value for this type of organism. This is currently being investigated.

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