The Taxonomy of certain Thiobacilli

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SUMMARY

Numerical analysis has been applied to the taxonomy of certain Thiobacilli; 54 strains were examined, including both authentic cultures and new isolates, using 48 tests. The groups found corresponded to *Thiobacillus trautweinii*, *T. novellus*, *T. thioparus* and *T. neapolitanus*, and these were very well differentiated with no intermediate organisms. A check of the numerical method indicated that the results were independent of the tests used. A comparison of authentic strains from different laboratories showed their characteristics to be very stable, and any changes were within the error of the test method.

INTRODUCTION

During a study of the autotrophic bacteria responsible for the oxidation of reduced sulphur compounds, difficulty was experienced in identifying the various Thiobacillus species, since many of the criteria given by Parker & Temple (1957) appeared to be unsatisfactory. The most serious problem was that no strain could be found corresponding to *Thiobacillus thioparus* as described by these workers, since all the cultures examined produced polythionates. Similarly, the pH values given were of little use since no differentiation is made between the limits for the initiation of growth and the final pH value attained by the culture.

An examination of the literature of this field indicated that opinions differed on the taxonomy of this group. Some authors considered the genus to be a spectrum of types (Baalsrud, 1954), whilst others recognized distinct species, but these varied according to the author (Vishniac & Santer, 1957; Parker & Prisk, 1958). The latest comprehensive study of this group was made in 1953, and in this instance only the utilization of sulphur compounds was investigated and not all the species were examined. These facts led to the conclusion that a new taxonomic survey of the thiobacilli was required and that as many strains as possible should be investigated using a large number of differential tests.

This would involve the collection of a large amount of data and the programme was planned so that this could be subjected to a numerical analysis of the type described by Sneath (1957). At this point, however, no decision was made as to the method to be used in the final interpretation of the results, as no critical investigation of the numerical methods had been carried out.

A secondary aspect of this programme was the further investigation of the inter-species change which had been reported previously in this genus (Johnstone, Townshend & White, 1961). With a large number of strains under continuous observation, any change would be readily noted, the variants could be tested, and

the magnitude of the change determined. An additional assessment of the stability of these bacterial strains was made by the inclusion in the study of cultures of the same authentic strain from several sources. Since these cultures would have been maintained under different conditions in the various laboratories, any changes should have been obvious when they were examined by the same test scheme.

METHODS

Organisms. All the authentic cultures which could be obtained are given in Table 1 together with the worker or organization who supplied them. The attempt to collect cultures of the same authentic strain from several sources was most successful with *Thiobacillus thioparus*; cultures were obtained from three laboratories.

Table 1. *Authentic strains*

Collection or source	Name	Code number
ATCC 8098	<i>T. novellus</i> *	a1
ATCC 8158	<i>T. thioparus</i> *	a7
NCIB 8589	<i>T. neapolitanus</i>	b1
NCIB 9118	<i>T. novellus</i> *	b2
NCIB 8870	<i>T. thioparus</i> *	b5
P. A. Trudinger	<i>Thiobacillus</i> X (a)	c1
P. A. Trudinger	<i>Thiobacillus</i> X (b)	c2
T. Wiken	<i>T. trautweinii</i> (str. 1)	d1
T. Wiken	<i>T. trautweinii</i> (str. 2)	d2
F. C. Happold	<i>T. thioparus</i> *	11
F. C. Happold	<i>T. thiocyanoxidans</i> (1)	12
F. C. Happold	<i>T. thiocyanoxidans</i> (2)	13
F. C. Happold	<i>T. denitrificans</i> †	14
Mrs M. Townshend	—	m1
Miss E. S. Pankhurst	T3‡	p1
Miss E. S. Pankhurst	T4‡	p2
Miss E. S. Pankhurst	T2‡	p8
Miss E. S. Pankhurst	C‡	p4
Miss E. S. Pankhurst	T5‡	p5
T. G. Tomlinson	<i>T. thiocyanoxidans</i>	t1
T. G. Tomlinson	<i>T. thioparus</i> *†	t8

* These strains originated from R. L. Starkey.

† An autotroph could not be isolated from these cultures.

‡ Pankhurst (1964).

Since the number of authentic cultures was small, new strains were isolated from the sources given in Table 2. The variety of sources examined, together with the method of isolation used, also ensured that the strains examined were representative of the group as a whole. After suitable dilution, material from the source was inoculated into liquid and on to solid thiosulphate media of pH values from 5.0 to 7.8. At intervals the liquid enrichment cultures were plated out, and representative colonies selected from the initial and subsequent plates. The strains were purified by at least three single colony isolations.

Although many of the thiobacilli can be readily recognized by the inter-colonial deposition of sulphur, the only criterion used in the selection of isolates was the ability to grow on a thiosulphate plate. This ensured that the extent of the group investigated was not limited by the preconceived principle that all thiobacilli deposit sulphur.

Maintenance of organisms. The strains were maintained on plates of the S 6 medium (see below) and subcultured at 21-day intervals. The plates were incubated at 28° until growth had occurred and then stored at 5° for the remaining time interval. Before subculturing, each plate was checked under the microscope for purity and before testing, each culture was plated out on both S 6 and nutrient media. The latter plates were examined for atypical growth and any autotrophic strains which grew on the nutrient media were discarded.

Table 2. *New isolates*

Source	Code number of isolates
Barren soil	1 A
Marine mud	1 B, 2 B, 3 B, 4 B, 6 B, 7 B, 8 B, 10 B
Fertile soil	3 C, 5 C, 6 C
Barren soil	1 E
Estuarine mud	1 F, 2 F, 3 F
Lagoon system*	1 G, 2 G, 3 G, 4 G, 5 G
Laboratory isolates	1 H, 2 H, 3 H
Biological filter*	1 I, 2 I
Activated sludge*	1 J, 2 J
Biological filter	1 K, 2 K
Activated sludge*	1 L
Minewater (pH 7)	1 M

* These systems were treating carbonization liquors.

Planning the tests. Since at least 100 characters were required for the numerical analysis, some difficulty was experienced in devising a sufficient number of tests, and at the same time avoiding bias in any particular aspect. To overcome this the tests were designed to fall into four groups; substrate tests including carbon and nitrogen sources, physiological tests such as growth at selected temperatures and pH values, inhibition tests with both organic and inorganic compounds, and certain tests on organic media in view of the presence of certain facultative autotrophic strains.

For many of the tests a common substrate was required which could easily be measured to assess the amount of growth which had occurred. The only suitable energy source utilized by all the strains described in this paper was thiosulphate. In the preliminary experiments with this substrate it was found that even under the most rigidly standardized conditions many of the strains exhibited variable rates of thiosulphate oxidation when tested on different occasions. This variability was decreased as the test period was extended to 28 days, and this rather long test period was, therefore, used throughout the investigation. In many of the experiments this variability was further decreased by expressing the results as a percentage of the thiosulphate oxidized in a control series which was run alongside the test under 'standard conditions'.

The final selection of the tests was made on strictly logical grounds and only those were rejected which were either all positive or all negative or those which were obvious duplicates.

Tests. The basal mineral salts medium, S 0, was (g./l. distilled water): Na_2HPO_4 , 1.2; KH_2PO_4 , 1.8; MgSO_4 , 0.1; $(\text{NH}_4)_2\text{SO}_4$, 0.1; CaCl_2 , 0.03; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.02; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.02. The S 6 medium was prepared by adding $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, 10 g., to this basal medium and S 7 by the addition of NH_4CNS , 0.02 g. The more

acidic S5 contained KH_2PO_4 , 2 g. as the only phosphate, and NaCl , 1 g. to correct the ionic balance. The other energy sources tested were added to the S0 medium in the amounts given in Table 3. Where possible AR chemicals were used.

Table 3. *Tests*A. *Substrates and carbon and nitrogen sources*

Test compound	Concentration (%)	Comments	Characters
Sulphur	Excess	Sterilized by steaming on 3 successive days	2
NH_4 thiocyanate	0.02	Analytical determination using a colorimetric test	2
Hydrogen sulphide	—	See text	2
No carbon source	—	Carried out in restricted atmosphere using doubly distilled water and appropriate guard tube to prevent access of carbon dioxide or ammonia. Scored against control series, see text	3
Sodium carbonate	0.05		3
Glucose	0.02		3
No nitrogen source	—		4
K nitrate	0.02		3
K nitrite	0.02		3
Na glutamate	0.52		2
Nutrient media	—	Growth on plate	2
Thioacetamide	0.02	Titrated with iodine	3
S7 plate	—	Criterion: sulphur deposition	2

B. *Physiological tests*

Medium	Variable	Comments	Characters
S6	19°	Rate of thiosulphate oxidation	3
S6	29°	Rate of thiosulphate oxidation	3
S6	35°	Amount of thiosulphate oxidation	4
Modified S6	0.5 % thiosulphate	Amount of thiosulphate oxidation	3
	1.0 % thiosulphate		3
	2.0 % thiosulphate		3
	4.0 % thiosulphate		3
	6.0 % thiosulphate		2
S5	pH	Criteria: amount and nature of sulphur deposition	2
S6	None		4
S6	None	pH consistently attained in this medium	5
S6 + nutrient	None	Amount of thiosulphate oxidation	3
S6 plate	None	Criterion sulphur: deposition	2

C. *Inhibition tests*

Inhibitor	Concentration (%)	Comments	Characters
Ampicillin	—	These tests were done on S6 plates using 'Sentest' tablets. (Evans Medical Ltd.)	2
Bacitracin	—		2
Celbenin	—		2
Chloramphenicol	—		2
Novobiocin	—		2
Streptomycin	—	The results were scored as a percentage of the amount of thiosulphate oxidized relative to a parallel control series grown under the standard conditions	2
Mixed phosphate	4		4
Sodium chloride	5		2
Potassium nitrate	1		3
Ammonium chloride	2.5		3
NH_4 thiocyanate	200 p.p.m.		2
Phenol	25 p.p.m.		4
Phenol	100 p.p.m.		4
—	—	Number of occasions the final pH was alkaline rather than acid for the autotrophs in the above inhibition tests	3

Table 3 (cont.)

D. *Heterotrophic tests*

Gelatin	Liquefaction of charcoal gelatin discs, Oxoid	2
Nutrient broth	Putrefactive odour	2
Kosers citrate	Oxoid	2
Starch	Hydrolysis	2
Phenol	Utilization of 200 p.p.m. phenol	2
Tallow agar	Lipolysis	2
Tributyryn agar	Lipolysis Oxoid	3
Glucose peptone	Acid to phenol red	2
Nitrate peptone	Gas and nitrite formation	4
S6/1 % glutamate	Growth on plate, autotrophic or heterotrophic	2
S6/1 % glutamate	Inhibition of thiosulphate oxidation	2
S6/1 % glutamate	Yellow fluorescence	2

The inoculum used in the tests was 0.02 ml. of a 3-day-old culture grown under the standard conditions. The standard conditions, also used for the control cultures, were the S6 medium, 10 ml., in a 50 ml. conical flask at 28°.

Analysis. In the majority of tests, the result was obtained by titration of one ml. of the thiosulphate medium with 0.01 M-iodine. The final pH value of the tests was recorded using a Pye Universal pH meter with a glass electrode. The change in pH value was used as a criterion of growth in the sulphur and hydrogen sulphide tests.

Method of scoring tests. The results were scored according to the method of Beers & Lockhart (1962), and the S values calculated according to the method given by Sneath (1957).

RESULTS

The strains were arranged in order of similarity and the result is shown in Table 4. Four groups were clearly differentiated corresponding to *Thiobacillus trautweinii*, *T. novellus*, *T. thioparus* and *T. neapolitanus*. These have been numbered 0, 1, 3 and 4; group 2, comprising the anaerobic species will be described in a later paper. In addition to the two authentic strains of *T. trautweinii*, group 0 includes organisms which are very similar to other named species. Thus 5C is very similar to *Pseudomonas stutzeri* and 1F has been tentatively identified as *P. fluorescens*. These organisms are characterized by their ability to grow as facultative autotrophs, oxidizing thiosulphate to polythionates with a corresponding increase in the pH value of the medium.

Only three strains are present in group 1, two authentic strains of *Thiobacillus novellus*, and one new isolate (1L). The latter strain was lost shortly after being tested, and was thus one out of only three organisms which could not be maintained indefinitely. These organisms grow in both organic and thiosulphate media, and in the latter case decrease the pH value.

Of the 20 strains in group 3, ten are new isolates, four are authentic cultures of *Thiobacillus thioparus*, three are authentic cultures of *T. thiocyanoxidans* and three unnamed strains. The organisms of this group oxidize thiocyanate, and the final pH in thiosulphate media does not fall below 3.5. Although slight differences were found between the cultures received as *T. thioparus* and *T. thiocyanoxidans*, they were not sufficient to warrant a further subdivision of this group.

Group 4 is the most acid tolerant of the four species, the pH in thiosulphate media

Table 4. *Sneath diagram*

Group 0

1	3F	00
2	1F	94 00
3	2B	75 80 00
4	1A	75 80 93 00
5	5C	74 73 69 75 00
6	p4	71 71 71 73 80 00
7	d1	69 67 73 77 74 91 00
8	3C	63 64 74 76 66 76 80 00
9	d2	69 64 72 75 64 75 78 80 00
10	10B	69 69 70 72 63 66 70 78 84 00
11	4B	65 64 65 67 63 62 67 72 80 96 00
12	h4	64 52 62 61 63 65 70 70 79 81 79 00
13	t3	63 63 65 60 57 62 62 62 73 72 71 62 00

Group 1

14	a1	46 45 57 56 52 54 56 70 59 58 57 55 73 00
15	b2	49 48 56 56 53 56 58 69 61 59 57 55 73 96 00
16	1L	46 47 55 48 46 43 43 63 48 61 53 51 66 85 81 00

Group 3

17	2K	43 42 33 28 31 30 30 38 35 42 35 35 49 51 51 59 00
18	1K	45 43 34 29 33 32 32 39 34 41 34 33 46 54 55 62 91 00
19	1B	37 38 42 42 33 32 34 46 42 37 44 35 54 63 64 63 83 72 00
20	2F	40 38 37 37 34 34 39 46 44 43 42 43 54 63 64 68 88 79 86 00
21	1H	34 32 37 34 32 27 32 42 34 41 37 38 46 59 62 68 76 88 81 86 00
22	2H	34 38 44 37 32 29 32 49 34 43 39 40 49 63 64 73 73 78 83 88 91 00
23	6C	40 38 39 34 32 32 37 42 39 41 37 40 51 59 64 67 76 79 81 91 91 93 00
24	1E	40 35 46 39 32 29 34 46 37 41 37 43 49 63 64 73 71 74 81 86 88 93 91 00
25	2G	34 38 46 39 29 27 29 46 32 43 37 38 46 61 62 75 71 74 81 86 88 98 91 95 00
26	t1	34 32 39 32 30 27 32 44 34 41 34 38 49 61 57 70 83 86 76 83 88 86 83 83 83 00
27	3B	35 33 38 32 32 32 38 30 36 35 36 43 54 55 69 78 82 74 82 84 84 84 87 87 90 00
28	p2	40 38 34 34 38 32 42 44 39 43 44 43 49 61 62 65 83 86 81 88 81 81 83 79 79 86 90 00
29	p1	40 38 34 34 38 32 42 39 37 41 44 40 46 56 60 55 73 76 74 83 76 79 81 76 76 79 87 91 00
30	h3	34 32 32 34 38 32 42 34 39 39 39 44 59 61 62 76 79 79 83 88 83 83 81 81 81 82 88 83 00
31	h2	29 30 29 32 35 24 32 39 29 38 42 38 39 54 57 58 76 79 74 81 86 83 81 79 81 81 82 88 86 93 00
32	p3	26 27 29 27 30 20 27 32 24 35 39 30 39 45 45 53 71 74 71 69 81 74 76 69 71 79 82 79 81 83 86 00
33	b5	30 31 30 33 36 25 33 38 30 39 45 38 41 51 51 54 66 68 73 73 73 76 68 71 73 81 83 83 83 83 90 00
34	h1	29 30 29 32 35 24 32 37 29 38 44 35 39 49 50 52 63 67 74 76 74 76 79 71 74 71 79 81 86 83 86 91 98 00
35	b5	24 24 27 27 24 15 24 27 21 33 39 27 38 44 44 50 59 62 74 71 71 74 77 74 74 68 80 77 82 79 83 91 97 00 00
36	a7	26 27 24 29 33 24 32 32 24 30 37 28 28 39 41 43 61 64 64 67 69 67 69 62 64 67 68 71 76 73 81 86 88 91 91 00

Group 4

37	8B	29 30 34 37 29 22 27 27 22 27 27 25 33 46 45 42 51 53 60 45 52 48 43 50 50 40 53 50 52 53 55 55 51 50 56 50 00
38	7B	28 31 28 26 26 18 21 28 15 31 31 24 32 46 43 55 62 63 48 43 53 48 43 45 50 50 53 53 45 53 55 58 49 48 50 48 75 00
39	1G	35 33 35 33 30 23 28 35 25 39 43 31 40 48 49 49 45 46 44 37 42 42 37 44 44 39 51 44 51 44 44 54 48 49 52 44 71 78 00
40	5G	26 19 32 27 27 22 33 27 23 24 29 28 28 37 38 43 61 52 41 43 52 43 43 50 45 50 61 48 52 44 50 55 44 45 50 50 60 60 73 00
41	m1	26 27 24 27 22 15 20 20 15 19 24 18 18 22 21 25 44 43 36 29 38 33 29 36 36 38 40 36 38 38 41 48 39 38 44 45 67 60 66 69 00
42	2J	29 24 24 24 30 20 24 27 20 24 32 23 28 37 38 38 46 48 45 41 48 43 41 43 41 43 50 52 50 49 50 60 56 57 59 62 64 58 68 76 83 00
43	p5	26 22 20 22 23 10 15 20 12 19 24 18 23 29 29 43 51 50 41 36 45 38 36 43 41 45 50 43 45 49 50 57 49 48 53 50 67 65 71 74 88 76 00
44	4G	24 22 20 22 20 12 17 15 12 14 20 15 15 22 21 25 37 36 29 24 33 26 24 31 29 29 37 29 33 34 36 45 42 41 47 48 57 55 83 74 84 74 81 00
45	2I	21 19 24 20 20 14 19 20 14 24 27 20 28 36 35 43 49 48 41 41 48 43 41 43 45 45 50 45 48 51 50 57 56 55 59 55 64 73 69 76 84 83 79 71 00
46	3G	18 16 24 20 17 10 15 17 10 19 24 18 21 29 29 33 39 31 36 31 38 33 31 38 36 33 45 36 41 42 41 50 49 48 56 48 60 63 73 76 71 86 81 83 88 00
47	1I	30 28 30 30 28 15 20 23 20 22 28 23 24 30 29 31 43 42 34 29 39 32 29 37 34 37 43 37 42 43 44 54 45 44 46 49 59 63 68 71 83 76 81 85 78 81 00
48	6B	26 19 30 30 27 23 33 30 23 27 33 31 28 40 39 45 43 42 45 39 48 42 39 48 45 39 44 42 42 47 45 52 47 45 52 48 68 73 77 74 84 77 81 84 87 85 90 00
49	1M	25 19 26 26 26 19 26 26 19 22 29 27 23 36 34 43 45 44 44 38 47 41 38 47 44 38 46 41 41 45 44 50 45 44 50 44 75 77 84 75 84 84 84 94 91 94 94 00
50	3H	37 37 21 26 21 11 16 21 16 26 26 16 33 45 45 44 55 55 40 40 40 40 40 40 40 45 40 45 45 50 50 50 55 55 58 60 60 74 75 60 65 77 85 75 90 80 65 90 90 00
51	b1	24 19 17 20 20 10 15 17 10 16 22 15 21 32 31 35 44 43 38 33 41 36 33 41 38 33 42 38 38 44 43 48 46 45 53 48 64 68 73 71 74 86 88 83 86 91 76 87 94 95 00
52	c1	24 19 18 20 21 10 15 18 10 17 23 15 21 32 32 36 44 44 39 34 42 37 34 42 38 34 43 39 39 44 44 49 46 46 53 49 63 67 90 73 73 88 88 81 85 90 76 87 94 95 00 00
53	c2	24 19 15 17 23 12 17 20 12 16 24 18 23 34 33 35 46 45 41 36 43 38 36 36 36 40 41 41 46 45 50 49 48 53 60 62 65 88 69 72 88 86 81 83 88 73 84 91 95 98 98 00
54	1J	32 27 28 28 15 20 22 15 22 27 20 23 34 33 38 46 45 41 36 43 38 36 43 41 36 45 41 41 46 45 50 49 48 53 50 67 68 71 61 71 88 81 81 83 83 78 87 94 85 93 93 91 00

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54

falling to 2.8. The only other outstanding feature of this group is the resistance to inhibitors, particularly inorganic salts such as sodium chloride and ammonium chloride. Three of the 18 strains are authentic cultures of *Thiobacillus neapolitanus*, and the remainder new isolates.

The ten S values from the comparison of duplicated strains range from 88 to 100, with a mean value of 95.7. These figures include both the differences which may have arisen between the strains and the errors of testing. An indication of the error involved in testing is given by the S value of 97 between b5 and b5a which are the same strain divided immediately prior to testing.

Considerable doubt has been expressed about the validity of *Thiobacillus neapolitanus* (Starkey, 1956; Vishniac & Santer, 1957; Vishniac & Trudinger, 1962) and six further sets of S values were calculated to ensure that the differentiation between this species and *T. thioparus* was not caused by the particular set of tests selected. For this purpose 21 strains were statistically selected and S values calculated using the tests in sections A, B and C. Similar calculations were then made using these tests arbitrarily split into two, and the tests divided into three on the basis of the original design, substrates, physiology, and inhibition.

Inspection of the resulting six Sneath diagrams indicated that a separation between the groups corresponding to *Thiobacillus thioparus* and *T. neapolitanus* had been achieved in all cases. However, a more objective assessment of the data was required which would correct for the small population sample on which the study was based. Groups may be compared in terms of an imaginary central organism, but it was thought that the selection of a real central strain was closer to bacteriological practice as this allows comparative testing for the purposes of identification. The central organism was obtained by calculating the mean and standard deviation of the S values of each strain of the *T. thioparus* group, to the other members of the group, and selecting the central strain which had the smallest standard deviation. This procedure gives the strain which is most typical of the group as a whole. From the mean and standard deviation of the S values of the central strain it is possible to construct a probability curve as shown in Fig. 1. This gives the probable distribution of the S values of the *T. thioparus* group if an infinitely large number of strains had been examined. The mean and standard deviation of the *T. neapolitanus* strains to the central organism of the *T. thioparus* group were then calculated and the curve drawn on the same axes.

DISCUSSION

Despite the fairly wide application of multivariate analysis to bacterial taxonomy very few tests of the validity of the method have been reported. The results given in this paper fully support the concept that the classification of bacteria by overall similarity is independent of the tests used, and suggest that the number of tests required to give an adequate classification may have been overestimated (Sokal & Sneath, 1963). These conclusions, however, only apply to thiobacilli and in view of the unusually well-defined groups found within this genus should not be extended to other bacterial groups without verification.

The very well-defined species and the absence of intermediate forms in this genus found here are unusual as compared with similar studies which have been made

of heterotrophic organisms (Sokal & Sneath, 1963). The most probable explanation of this is that classification in other groups has been carried much further, and that one of the *Thiobacillus* species would be regarded in other groups as a genus, or an even higher taxonomic rank. Alternatively, the rather extreme

Fig. 1. The distribution of S values for *Thiobacillus thioparus* and *T. neapolitanus*. Group 3, *T. thioparus*, and group 4, *T. neapolitanus*. A: complete set of tests, 111 characters; B: split tests (i), 55 characters; C: split tests (ii), 56 characters; D: physiological tests, 40 characters; E: substrate tests, 34 characters; F: inhibition tests, 37 characters. The arrows indicate the numerical limits of each group.

environment occupied by these organisms may exert a relatively high selection pressure and the genetic constitution which corresponds to an intermediate type may not be viable.

Starkey (1935*b*) and Baalsrud (1954) were of the opinion that *Thiobacillus trautweinii* should be excluded from the genus *Thiobacillus* on the grounds that the oxidation of thiosulphate to tetrathionate did not release sufficient energy for these organisms to live autotrophically. Although the present results support the exclusion

of this group from the genus *Thiobacillus*, some of the test results suggest that inability to fix CO₂ may be an alternative criterion for this division. The tests, however, were not designed to yield specific information on this point, and further detailed studies would be required before this concept could be established.

Independently of the criterion used, there appears to be little doubt that *Thiobacillus trautweinii* should be excluded from the thiobacilli, particularly when other strains within this group belong to other named genera. It is unfortunate in this respect that the evidence from numerical analysis does not assist in making this decision. Although the division between the different groups is well marked, there does not appear to be obvious inter-group division at which to separate the thiobacilli from the facultative heterotrophs.

The results for *Thiobacillus thioparus* and *T. neapolitanus* fully support the work of Parker & Prisk (1953), and there now appears to be little doubt about the validity of these two species. Observations on their behaviour in thiosulphate media indicate that the differences between them may be sufficiently large to suggest differing metabolic pathways. This might account for some of the present differences of opinion regarding the thiosulphate metabolism of these organisms, as in some cases no distinction has been made between these two species.

The evidence from the S values of the duplicated authentic strains indicates that these organisms are relatively stable over long periods of time, even when subjected to varying conditions of culture. In the authors' opinion the amount of variation which was found can be almost wholly attributed to differences which are found in these organisms when tested on different occasions.

The inclusion of the duplicate strains, particularly those of *Thiobacillus thioparus*, gave the impression of a subgroup within the *T. thioparus* group on the Sneath diagram. This was obviously an artifact, but does raise the far more general problem about the frequency of organisms with very high S values in numerical analysis, and whether one should eliminate such strains before inspecting the data for subgroups.

After careful consideration of the original literature the authors have concluded that the most appropriate names for the species described are those given in this paper. The major decision which led to this conclusion was that Beijerinck's original papers (1904*a, b*) do not give sufficient detail to identify the aerobic species which he described. The first adequate description was given by Starkey (1935*a, b*) who believed his organism was the same as that of Beijerinck and therefore called it *Thiobacillus thioparus*. This strain is still available in the various national collections and its characteristics correspond with the original description. The evidence given in this paper has shown that *T. neapolitanus* is distinct from *T. thioparus*, and that the name given by Parker & Prisk (1953) is therefore valid. The validity of *T. novellus* does not appear to have been questioned in the literature.

A diagnostic table for the identification of these species will be given in a later paper.

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