Convergent Characters of Extremely Thermophilic Acidophilic Bacteria

Particularly notable among those bacteria which require both high temperature (60–90 °C) and low pH (1–3) for growth are the mycoplasma-like *Thermoplasma acidophila* of DARLAND et al.[1], the pleiomorphic Fe or S autotroph of BRIERLEY and BRIERLEY[2], and the pleiomorphic *Sulfolobus acidocaldarius* of BROCK et al.[3], which is a facultative S autotroph. In our own studies of the bacterial flora of extremely hot acid sources at Pisciarelli (Agnano) near Naples, above the temperature range at which the spore-forming *Bacillus acidocaldarius* predominates[4], we have encountered a range of organisms, here designated MT, which is very similar to *Sulfolobus*; we believe that the study of the MT series has a considerable relevance to the problem of relating and classifying all of these species.

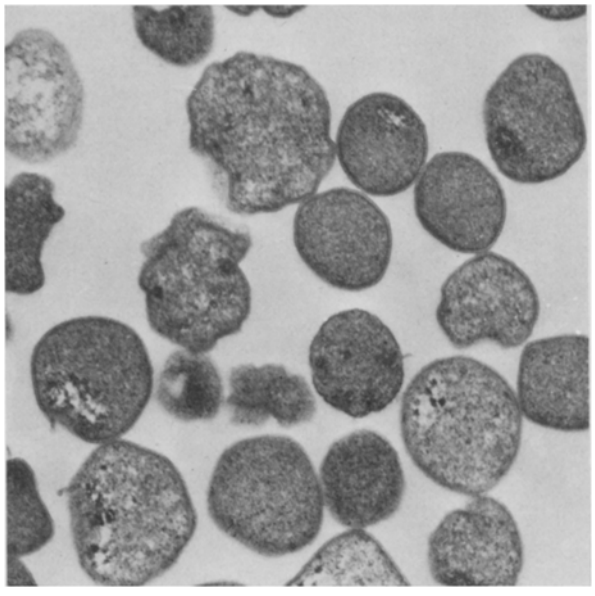

Fig. 1. Microorganisms of the strain MT-3. The cells appear almost spherical and are surrounded by a plasma membrane and an extracellular coat. Their cytoplasm is rather dense and granular. The larger cells are aged organism. Glutaraldehyde and osmium tetroxide fixation at pH 6.8. ERL embedding medium. ×19,200.

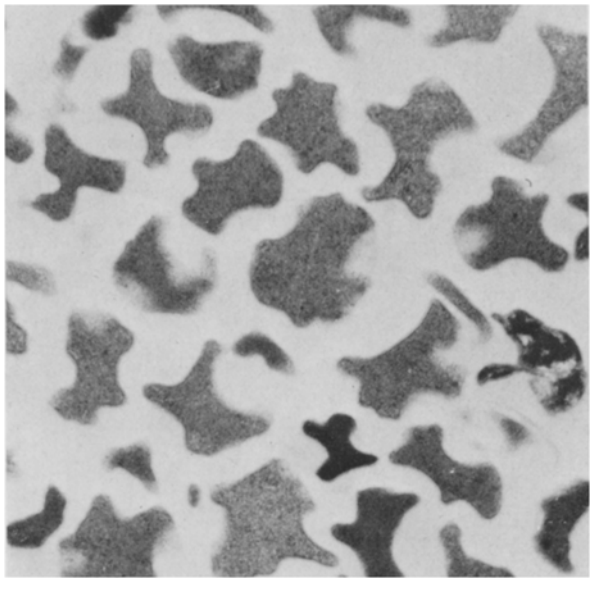

Fig. 2. Before processing for electron microscopy the organism were centrifuged at about 3000 *g* to form a pellet. The bizarre lobulated forms have been artificially produced by compression during centrifugation. Same fixation procedure as in Figure 1. ×18,000.

The physiological, chemical, and ultra-structural data for the MT series will be described in full accounts elsewhere, but the most important characteristics are summarized in the Table in parallel with the comparable data, where available, for the organisms already mentioned. In particular it is clear from this list that the MT organisms are on most criteria identical with *S. acidocaldarius*. One apparent difference was that the conspicuously 'lobed' morphology ascribed to the latter[3] is absent in the MT organisms, which are almost spherical (Figure 1).

Direct comparison under phase contrast (using the 98-3 strain of *S. acidocaldarius* supplied by J. L. MOSSER) confirms that these cells are indeed somewhat more irregular, but on the other hand the very irregular appearance seen in published electron micrographs[3] is in our view an artefact, since it can also be induced in MT cells, e.g. by centrifugation at *g* forces superior to 3000, as shown, for example, in Figure 2.

In Figure 3 we show one of a series of electron micrographs demonstrating the mode of division of MT cells, by a process of median constriction, during which a bipolar localization of nuclear material is also observable; at all other times the DNA strands are dispersed throughout the cytoplasm (as in *S. acidocaldarius*, for which the mode of division is unknown). In Figure 4 we show the unit membrane and the structured extra-membrane layer, which we have examined extensively in both MT and *Sulfolobus* without finding any significant differences.

The MT isolates also form numerous rather fragile pili, when grown on yeast extract. These pili exactly resemble those described for *S. acidocaldarius* grown on sulphur in the presence of yeast extract[5], and supposedly associated with autotrophic nutrition, but these authors did not record any observations for *S. acidocaldarius* growing heterotrophically on yeast extract alone. A feature of the MT isolates, not so far described for *S. acidocaldarius*, is that, in addition to growing heterotrophically on simple media and autotrophically on sulphur, they will also grow, albeit slowly, autotrophically on ferrous iron.

Essentially, therefore, we should have been content to regard the MT series and *S. acidocaldarius* merely as different isolates of 'the same' organism. Given the degree of identity observed, we were therefore very surprise when measurements of guanosine-cytosine% in high-molecular-weight homogeneous DNA from 2 MT strains by 2 different methods (quantitative base separation[6,7]; preparative ultracentrifugation with labelled marker DNA[8]) gave

[1] G. DARLAND, T. D. BROCK, W. SAMSONOFF and S. F. CONTI, Science *170*, 1416 (1970).
[2] C. L. BRIERLEY and J. A. BRIERLEY, Can. J. Microbiol. *19*, 183 (1973).
[3] T. D. BROCK, K. M. BROCK, T. R. BELLY and L. R. WEISS, Arch. Microbiol. *84*, 54 (1972).
[4] M. DE ROSA, A. GAMBACORTA and J. D. BU'LOCK, G. Microbiol. *19*, in press (1974).
[5] R. L. WEISS, J. gen. Microbiol. *77*, 501 (1973).
[6] J. WYATT and S. S. COHEN, Biochem. J. *55*, 774 (1953).
[7] A. BENDICH, in *Methods in Enzymology* (Eds. P. S. COLOWICK and O. N. KAPLAN, Academic Press, New York 1957), vol. 3, p. 716.
[8] D. D. BROWN, P. C. WENSINK and E. JORDON, Proc. natn. Acad. Sci., USA, *68*, 3175 (1971).

results in the range 39–45%, as compared with the reported values obtained for *S. acidocaldarius* (by analytical ultracentrifugation) of 60–80%. Accordingly we submitted high-molecular-weight DNA from separately-grown cultures of an MT strain and of *S. acidocaldarius* strain 98-3, under coded designations, to Dr. S. AYAD (Dept. of Biochemistry, University of Manchester) who by analytical ultracentrifugation obtained the essentially confirmatory values of 47.9% and 70.4% GC respectively.

Since the two GC% ranges are so much farther apart than would be expected for a single genus, let alone for the same species, we could only conclude that in the MT isolates and in *S. acidocaldarius* we are dealing with an extreme case of convergent evolution, under drastic environmental pressure and from quite diverse origins.

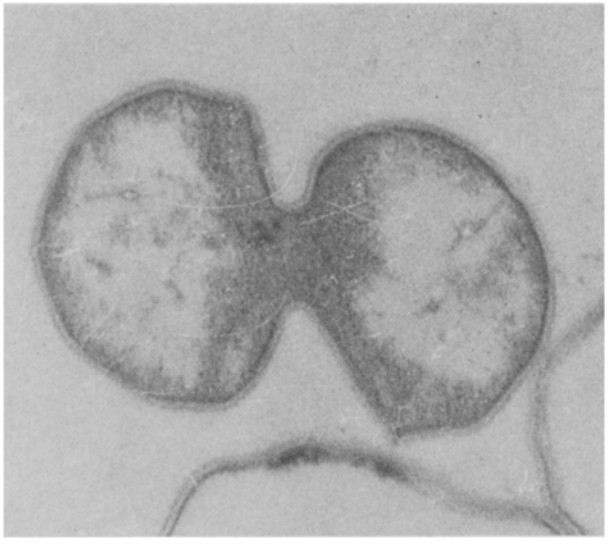

Fig. 3. A replicating microorganism. In the dividing cell 2 nuclear clear areas have been formed in which DNA strands are evident. The constriction occurs in the cytoplasmic area. Fixation with 1% osmium tetroxide buffered at pH 6.8 ×42,000.

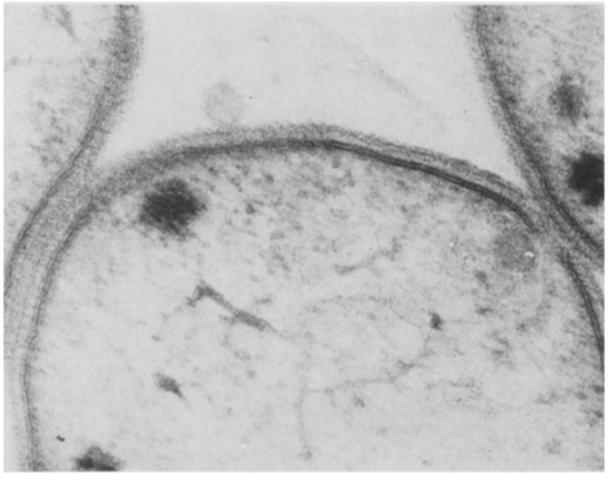

Fig. 4. Part of a cell depicting the dense plasma membrane which shows the unique membrane structure and the extracellular coat. Within this coat, a clear interior and a dark exterior layer can be distinguished. In the cytoplasma, a dense cluster of unknown chemical composition, small ribosomes and strands of DNA are visible. Fixation with 1% osmium tetroxide, buffered at pH 6.8. ×155,000.

The case approaches the limit at which the 'adaptive' features totally dominate the range of observable characters. In our own case, using the data now available, the attempt to separate out 'adaptive' features, so as to be able to assign organisms to monophyletic groups on the remaining data, must fail – unless the single datum of GC content is made the absolutely primary criterion of classification. On the other hand, if the totality of characters is taken into account, differences which we must accept as indicating different phyletic origins, i.e. the GC ratios, must be accepted within a single group or even a single 'species'.

With such an approach in mind, we have also included in the Table summarized literature data for the isolates of BRIERLEY and BRIERLEY[2], and for *T. acidophila*[1]. For the latter we have included a revised value[9] for the GC content, as well as that originally given. It is apparent that the main distinction between the organism of BRIERLEY and BRIERLEY and the pair MT/*S. acidocaldarius* is that the former cannot grow heterotrophically and the extra-membrane coating is amorphous. The differentiation of *T. acidophila* is somewhat more marked since it is somewhat less thermophilic, incapable of autotrophic growth, and has no extra-membrane coating at all (cf. the probably equivalent datum, lack of glucosamine). Even so, it would be rather difficult to distinguish between *T. acidophila* and a wall-less form of the MT series.

There is a further important link between the MT isolates, *S. acidocaldarius*, and *T. acidophila*. LANGWORTHY et al.[10] have shown that ester lipids are totally absent in the latter organism, and all the major lipids are based upon a 1,2-diether of glycerol (stereochemistry unspecified) with a C_{40} isoprenoid moiety, which on cleavage (HI followed by reduction) afforded a mixture of hydrocarbons, $C_{40}H_{80}$ and $C_{40}H_{82}$. In a detailed study of the lipids from MT strains and from *S. acidocaldarius* we have found that these are qualitatively identical with each other and probably with those of *T. acidophila*. The glycerol has the L-$\alpha\beta$- or *sn*-2,3-configuration, as in the diphytanyl glycerol ether lipids of extreme halophiles[11], and the alkyl group is bidentate (i.e. linked to both glycerol oxygens); on cleavage it affords 3 isoprenoid alkanes, viz. $C_{40}H_{82}$ (acyclic), $C_{40}H_{80}$ (monocyclic) and $C_{40}H_{78}$ (bicyclic). These studies will be elaborated in a fuller account.

These cyclodiether lipids are unique; whether or not their formation is regarded as an 'adaptive' character, their presence clearly demonstrates a close relationship between *T. acidophila* and the pair MT/*S. acidocaldarius*.

In conclusion we would note that, as described, both *Sulfolobus* and *Thermoplasma* are new genera each containing but one species. In our view the perpetuation of such a procedure is bound to conceal, rather than to illuminate, the taxonomic problem. We should prefer that all 4 isolates of the Table should be grouped together, at least for the time being, and the differences between them relegated to species level (or lower for the pair MT-*Sulfolobus*), while admitting that any such group is a 'form/habitat' assembly and most probably polyphyletic. We would suggest that new isolates of the same general

[9] E. A. FREUNDT, in *Pathogenic Mycoplasmas*. CIBA Symposium (Eds. K. ELLIOT and J. BIRCH, Elsevier, Amsterdam 1972), p. 10.

[10] T. A. LANGWORTHY, P. F. SMITH and W. R. MAYBERRY, J. Bact. *112*, 1193 (1972).

[11] M. KATES, in *Ether Lipids, Chemistry and Biology* (Ed. F. SNYDER, Academic Press, New York 1972), p. 351.

Parallel characteristics of extremely thermophilic acidophilic bacteria

	Sulfolobus acidocaldarius[3]	MT series[a]	BRIERLEY and BRIERLEY[2]	*Thermoplasma acidophila*[1]
Source (°C)	76–90	74–89	66–69	56
Source (pH)	1.5–2.5	1.4–2.6	2.6	2.0
Optimum (max)°C	70–75 (75–85)	75–87 (80–89)	60 (75)	60 (65)
Optimum pH	2.0–3.0	3.0–4.5	2.0	2.0
Nutrient tolerance	<0.25%	<0.20%	n.d.	<0.20%
Size (μm)	0.8–1.0	1.0–1.5	1.0–1.5	0.1–3.0
Form	Irregular spheres, (lobed), plastic	Irregular spheres, plastic	Irregular spheres, plastic	Spheres, plastic
Nuclear region	No	Only in dividing cells	No	No
Pili	Present, numerous	Present, numerous	n.d.	Present[9]
Coat	Sub-unit array	Sub-unit array	Amorphous	Absent
Peptidoglycan	Absent	Absent	Absent	Absent
Glucosamine	Present (low)	Present (low)	n.d.	Absent
Vancomycin	Resistant	Resistant	n.d.	Resistant
Novobiocin	Sensitive	Sensitive	n.d.	Sensitive
Lysis, Na dodecyl sulphate	Rapid	Rapid	n.d.	Rapid
Lysis, lysozyme	No	No	n.d.	No
Autotrophy utilizing	S, facultative	Fe, S, facultative	Fe, S, obligate	No
Heterotrophy utilizing	Aminoacids ±, sugars ± (strain-variable)	Aminoacids −, sugars +	Obligate autotroph	Requires complex media
Lipids based on	Glycerol diether of isopranoid C_{40}[a]	Glycerol diether of isopranoid C_{40}	n.d.	Glycerol diether[a] of isopranoid C_{40}[10]
GC content (%)	60–66, 70[a]	39–45, 48	54–60	24–29[1], 46[9]

n.d. = no data. [a] Present work.

character should be included in this group and not assigned to still further new genera; however, we have refrained from a formal assignment of nomenclature either to or within this group, taking the view that unless a change of nomenclature proves generally acceptable, it is better not proposed. Informally we propose the group-name *Caldariella*.

Riassunto. Si riporta l'isolamento di un nuovo microorganismo acidotermofilo per molti aspetti identico al *S. acidocaldarius*, ma nettamente diverso nella composizione in basi del DNA. Si discute dell'opportunita di classificare in un unico gruppo oltre ai due microorganismi gia menzionati, anche altri microorganismi acidotermofili simili.

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Effect of Testes Removal and Androgen Replacement Therapy on Enzyme Levels in Hypothalamus and Pituitary of Frog (*Rana esculenta*)

The vertebrate hypothalamus and pituitary are intimately related, in as much as the hypothalamus controls many of the hypophyseal functions, and as such these two organs (hypothalamo-hypophyseal system = HHS) constitute the central feedback system[1–3]. The biochemical events which characterize the various phases in the regulatory mechanisms of the vertebrate HHS are, however, poorly known[4]. About the enzymic profile of this system under normal and experimental conditions, the literature is still more scanty[4,5].

Since castration has been found to produce remarkable morphological and metabolic changes in the HHS of green frog, *Rana esculenta*[6,7] it was our aim to study the effects of castration upon the enzyme activity in the hypothalamus and pituitary of this anuran and to establish if these changes could be corrected by testosterone propionate (TP).

[1] C. B. JØRGENSEN, in *The Hypothalamus* (Eds. L. MARTINI, M. MOTTA and F. FRASCHINI; Academic Press, New York & London, 1970), p. 649.

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