The Cytochromes of *Thermoplasma acidophilum*

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INTRODUCTION

Respiratory chain systems containing bound dehydrogenases, flavoproteins, lipoquinones and various cytochromes are common in cell wall-synthesizing bacteria and their L-forms. With the mycoplasmas and related organisms, the determination of respiratory chain components which are presumably present in small amounts has often had to compete with contaminating constituents from the highly complex media. This is probably the reason for the equivocal reports so far presented on the cytochromes of the mycoplasmas (Van Demark, 1969; Van Demark & Smith, 1964). Members of the *Acholeplasma*, *Mycoplasma* and *Spiroplasma* group contain very small amounts of menaquinones when grown in peptone media supplemented with horse serum, but these probably have no respiratory function (Holländer *et al.*, 1977). However, *Thermoplasma acidophilum* synthesizes menaquinone-7 in amounts similar to those of respiring bacteria (Langworthy *et al.*, 1972; Holländer *et al.*, 1977) and also *c*-type cytochromes (Belly *et al.*, 1973). This communication reports the occurrence of additional cytochromes in two strains of *T. acidophilum*.

METHODS

The strains of *T. acidophilum*, 122-1b2 and 112-1b3, were grown in a mineral salt medium containing 0.1% (w/v) yeast extract and 1% (w/v) glucose (Christiansen *et al.*, 1975). 'Aerobic' cultures were grown in Fernbach flasks (no. 20511; Schott & Gen, Mainz, F.R.G.) containing 500 ml medium and aerated by shaking in a rotary shaker at 200 rev. min⁻¹. Oxygen-limited cultures were grown statically in Fernbach flasks filled with 2 l medium. After about 72 h incubation at 57 °C, cells, then in the early stationary phase of growth, were harvested by centrifuging (11 000 g, 4 °C) and washed twice in 0.1 M-Tris/HCl buffer, pH 7.2.

Cytochromes were determined by difference spectroscopy of turbid suspensions of untreated cells at liquid nitrogen temperature (–196 °C) with 0.5 M-sucrose in the sample (optical path length 0.1 cm; band width 1.65 nm) (von Jagow *et al.*, 1973). The characteristic spectral properties of *d*(a₂), *b*- and *c*-type cytochromes were recognized by the difference spectrum of Na₂S₂O₄-reduced minus H₂O₂-oxidized samples from 390 to 650 nm. Cytochrome *o* was detected by the difference spectrum of Na₂S₂O₄/CO-reduced minus Na₂S₂O₄-reduced samples. The spectra were identified according to Bartsch (1968) and Smith (1968).

RESULTS AND DISCUSSION

The difference spectrum (reduced minus oxidized) of strain 122-1b3 grown aerobically showed alpha-peaks at 554 and 619 nm with shoulders at 559 and 549 nm, beta-peaks at 534 and 526 nm and a gamma-peak at 424 with a shoulder at 434 nm (Fig. 1*a*). A similar spectrum was obtained with cells of strain 122-1b2 (not shown) except that the 549 nm shoulder was not so pronounced. This might be due to slight differences in culture conditions since the relatively long incubation time made it difficult to reproduce the aeration conditions exactly.

By examining comparable amounts of thermoplasma protein, the cytochrome contents under different culture conditions could be compared. When strain 122-1b3 was grown in oxygen-limited conditions, the *c*-type cytochrome content was decreased especially at 555 nm and the shoulders at 549 and 560 nm were more pronounced (Fig. 1*b*). The shift of the

Fig. 1. Cytochrome difference spectra at -196°C of *Thermoplasma acidophilum* strain 122-1b3: (a) cells grown aerobically (optical path length 0.1 cm ; $12\text{ mg protein ml}^{-1}$); (b) cells grown in oxygen-limited conditions (optical path length 0.1 cm ; $10\text{ mg protein ml}^{-1}$). A: $\text{Na}_2\text{S}_2\text{O}_4$ -reduced minus H_2O_2 -oxidized. B: $\text{Na}_2\text{S}_2\text{O}_4/\text{CO}$ -reduced minus $\text{Na}_2\text{S}_2\text{O}_4$ -reduced.

peak at 554 nm and the shoulder at 559 nm by 1 nm to longer wavelength, compared with aerated cultures, might be due to the relative increase of the *b*-type cytochrome and the decrease in the amount of the *c*-type cytochromes. Strain 122-1b2 exhibited essentially the same cytochromes. In both strains grown under oxygen-limited conditions, the peaks at 619 nm indicating the cytochrome oxidase $d(a_2)$ were not so pronounced, corresponding to the diminished function of the oxidases under these conditions. The difference spectra of the CO-treated cells of both strains grown aerobically showed the characteristics of cytochrome *o* at 558 nm , and a shift of 7 nm to longer wavelength in the absorption of cytochrome $d(a_2)$; these were not detected with cells grown in oxygen-limited conditions.

The presence of cytochromes, cytochrome oxidases and menaquinones (Langworthy *et al.*, 1972; Belly *et al.*, 1973; Holländer *et al.*, 1977) in amounts similar to those in respiring, cell wall-synthesizing bacteria indicated the presence of intact, oxidative, terminal electron transport systems in the *Thermoplasma* strains investigated. These properties distinguished the *Thermoplasma* strains from other members of the class *Mollicutes* in which quinones and cytochromes in comparable amounts have not been detected (Rodwell & Rodwell, 1954; Gale *et al.*, 1964; Smith *et al.*, 1963; Holländer *et al.*, 1977). The presence of cytochromes in *Mycoplasma arthritidis* strain 07 and *M. bovis* seemed doubtful since cytochrome contamination by medium constituents (Smith, 1955) could not be ruled out. Using equipment of normal sensitivity for difference spectroscopy, we could not detect cytochromes in *M. arthritidis* strain 07 grown in PPLO-broth (Difco) supplemented with 20% (v/v) horse serum and 1% (w/v) yeast extract (Holländer *et al.*, 1977).

Thus, amongst the *Mollicutes*, *T. acidophilum* appears to be unique in producing cyto-

chromes and lipoquinones. One might therefore argue that the thermoplasmas are closely related to the L-forms of thermophilic and acidophilic, respiring bacteria.

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