Cellular Stability of a Thermophilic, Acidophilic Mycoplasma

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SUMMARY

Thermoplasma acidophila is a free-living Mycoplasma which grows optimally at a temperature of 55 °C and a pH value of 2.0. *Thermoplasma acidophila* strains are resistant to lysis when suspended in distilled water. Organisms are also more resistant than other reported mycoplasmas to lysis by anionic and cationic surface active agents. Bacterial suspensions were unusually resistant to thermal lysis and were not affected even at temperatures of 100 °C. Although lysis did not occur at pH ranges of 1 to 7, rapid lysis occurred at pH values of 8 and above. These findings suggest that *T. acidophila* has an unusual membrane structure enabling this Mycoplasma to grow at high temperature and low pH.

INTRODUCTION

Thermoplasma acidophila was first isolated from a thermal and acidic region of a coal refuse pile (Darland, Brock, Samsonoff & Conti, 1970) and several other strains of this organism have been recently isolated and characterized in this laboratory. All isolates have a temperature optimum for growth of approximately 55 °C and a pH optimum of 2.0.

Although the possibility still exists that *Thermoplasma acidophila* is a stable L-form, it resembles members of the Mycoplasma group in several important characteristics: small size (0.1 to 3 µm), filterability through a 0.45 µm filter, the absence of a wall structure, the absence of detectable hexosamines and resistance to antibiotics which inhibit the growth of micro-organisms possessing walls. Furthermore, the low GC ratio of these organisms (24 to 28.5 %) and our inability to demonstrate reversion to a parent form also indicate that *T. acidophila* should be included in the Mycoplasma group of organisms.

Brock (1967) has suggested that the crucial thermostable structure allowing thermophiles to grow at high temperatures may be in their cell membranes. Evidence in support of this hypothesis has been obtained in several studies which indicate that protoplasts of thermophiles are extremely stable to heat and to osmotic shock (Abram, 1965; Golovacheva, 1967; Bodman & Welker, 1969; Ray & Brock, 1971) whereas protoplasts of mesophilic micro-organisms are more sensitive to osmotic shock (McQuillen, 1960) and lyse after heating at 60 °C for 60 min (Ray & Brock, 1971).

Although it is generally believed that acidophiles can grow at low pH because of their ability to exclude H⁺ ions (Brock, 1969), little work has been reported on the membrane properties or cellular stability of these organisms.

The following study of the effect of various physical and chemical agents on *Thermoplasma acidophila* was undertaken in an attempt to determine whether this organism was similar to

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other mycoplasmas and to determine whether membrane properties allow this organism to grow under such harsh environmental conditions as 55 °C and pH 2.0.

METHODS

Organism and growth conditions. *Thermoplasma acidophila* strain 122-1B3 was grown in 1 l flasks containing 100 ml of basal salts medium (Allen, 1959) adjusted to pH 2.0 with 10 N-H₂SO₄. Yeast extract was autoclaved separately and added to a final concentration of 0.1 % (w/v). After inoculation, the flasks were incubated at 55 °C, were harvested by centrifugation at 7000g for 30 min in the late exponential phase of growth (usually 2 days of incubation), washed twice with distilled water (pH 5.5), and resuspended in an equal volume of distilled water. The final pH of the resultant bacterial suspension was approximately 5.5.

Bacterial lysis. Bacterial lysis was measured spectrophotometrically using a Bausch & Lomb 'Spectronic 20' at a wavelength of 620 nm. To measure protein released in lysis, the unlysed bacteria were removed by centrifugation at 5000g for 10 min, and the protein in the resultant supernatant fluid was assayed as described by Lowry (Lowry, Rosebrough, Farr & Randall, 1951) using lysozyme standards.

RESULTS

Effect of changes in osmotic pressure. No lysis of *Thermoplasma acidophila* had occurred after 30 min when the cells were suspended in either distilled water or various concentrations of NaCl (Fig. 1). Furthermore, bacteria suspended in triple distilled or deionized water also remained stable for periods up to 36 h. This unusual stability in distilled water appears to contrast strongly with the reported rapid lysis of most Mycoplasma species under conditions of low osmotic pressure (Razin & Argaman, 1963).

Lysis by surface active agents. As shown in Fig. 1, *Thermoplasma acidophila* was lysed both by the cationic detergent cetyltrimethyl ammonium bromide (CTMB) and the anionic detergent sodium lauryl sulphate (SLS). However, the concentrations of these detergents required for lysis of *T. acidophila* was approximately eight times higher than that reported for other mycoplasmas by Razin & Argaman (1963). Non-ionic detergents such as Tween 80 or Triton X caused no detectable lysis under similar experimental conditions.

Lysis by enzymes. *Thermoplasma acidophila* was insensitive to lysis by lysozyme, trypsin or pronase. Pancreatic lipase at a concentration of 500 µg/ml did, however, cause about 10 % lysis after 1 h at 37 °C.

Lysis by primary alcohols and by digitonin. No detectable lysis of *Thermoplasma acidophila* occurred in the presence of primary alcohols (methanol, ethanol, *n*-propanol, *n*-butanol) or digitonin.

Lysis by heat. *Thermoplasma acidophila* was relatively resistant to heat even at temperatures of 90 or 100 °C (Fig. 2). For comparison, thermal lysis of *Streptococcus* protoplasts is also shown in Fig. 2 (these data are taken from Ray & Brock, 1971). The extreme thermal stability of *T. acidophila* contrasts strongly with the thermal sensitivity of *Streptococcus* protoplasts and of mycoplasmas (Razin & Argaman, 1963) since these mesophilic microorganisms readily lyse at temperatures above 60 °C when suspended in an osmotically protective sucrose medium.

Lysis by varying pH. The effect of varying the pH on the cellular stability of thermoplasmas is shown in Fig. 3. Buffers adjusted to various pH values were added to the bacterial suspensions at a final concentration of 20 µmol/ml. The suspensions were incubated at room

Fig. 1. Effect of surface active agents and changes in osmotic pressure on lysis of *Thermoplasma acidophila*. Cetyltrimethyl ammonium bromide (CTMB), sodium lauryl sulphate (SLS), or sodium chloride (NaCl) was added in the concentrations indicated. After 30 min at room temperature, lysis was measured spectrophotometrically.

Fig. 2. The effect of temperature on lysis of *Thermoplasma acidophila* and *Streptococcus* protoplasts. *Streptococcus* protoplasts were suspended in 0.6 M-sucrose containing 0.075 M-disodium malate (pH 7.3). *Thermoplasma acidophila* was suspended in distilled water (pH 5.5). Bacteria were heated at temperatures indicated for 30 min. After cooling, lysis was measured spectrophotometrically.

Fig. 3. Effect of changes in pH on lysis of *Thermoplasma acidophila*. Twenty μ mol of either: HCl-KCl buffer, pH 1 or 2; phthalate-HCl, pH 3 or 4; phthalate-KOH, pH 5; (2-*N*-morpholino) ethane sulphonic acid (MES), pH 6; *N*-tris (hydroxymethyl) methyl-2-aminoethane sulphonic acid (TES), pH 7 or 8; or KCl-borate, pH 9–10, were added to the cell suspension. After 30 min, lysis was measured either spectrophotometrically or by protein release.

temperature and lysis was measured after 30 min. Organisms were stable at pH values of 1 to 7. However, at pH values of 8 and above, lysis could be detected both spectrophotometrically and by measuring protein released during lysis.

To ascertain whether lysis demonstrated in this experiment might be due to an effect of the buffers rather than pH, a similar experiment was performed using either different buffers or an unbuffered system in which sodium hydroxide was used to adjust the pH. The results of this experiment also demonstrated bacterial lysis at pH values of 8 and above, indicating that lysis is dependent upon pH and not upon the buffer used. When various mono- or divalent cations or energy inhibitors (sodium azide, α -dinitrophenol, or potassium cyanide) were added, no detectable difference in lysis curves was obtained.

DISCUSSION

Our results indicate that *Thermoplasma acidophila* is more resistant than other reported Mycoplasma species to lysis by a variety of physical and chemical agents. In contrast to reports for many mycoplasmas (Razin, 1963; Razin & Argaman, 1963), *T. acidophila* does not lyse when placed in distilled water for periods up to 36 h. Organisms are also more resistant to lysis by surface active agents since the concentrations of SLS and CTMB required for lysis of *T. acidophila* were eight times greater than concentrations required for other mycoplasmas (Razin & Argaman, 1963).

As this organism lacks a wall structure, the extreme cellular stability to both high temperature and low pH is striking. After heating at 100 °C for 30 min only very slight lysis of *Thermoplasma acidophila* could be detected. This result contrasts strongly with reports for protoplasts of mesophilic bacteria (Ray & Brock, 1971) and mycoplasmas (Razin & Argaman, 1963) which reportedly lyse at 60 °C. Although *T. acidophila* was resistant to lysis at

pH values from 1 to 7, detectable lysis occurred at pH values of 8 and above, which would be considered rather mild conditions for most organisms.

Further experiments with the membrane stability of other acidophiles growing at lower temperatures (e.g. thiobacilli) to alkaline pH ranges might indicate whether membrane stability is one of the factors which determines the pH that an acidophilic organism can tolerate.

The molecular mechanisms which make possible the unusual stability of the *Thermoplasma* membrane to high temperature and low pH, and its sensitivity to slightly alkaline pH are not known. However, unusual fatty acid and lipid compositions of other thermophilic micro-organisms has been demonstrated (Ray, White & Brock, 1971 *a, b*). Studies on the lipids of *Thermoplasma acidophila* are now under way in the laboratory of Dr Paul Smith, University of South Dakota.

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