SHORT COMMUNICATION

An Alkalophilic Red Halophilic Bacterium with a Low Magnesium Requirement from a Kenyan Soda Lake

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A Halobacterium species isolated from solar evaporation ponds and sodium sesquicarbonate deposits at Lake Magadi, Kenya, differs from known species of Halobacterium in its GC content, in being obligately alkalophilic with a pH optimum between 9.0 and 10.0, and in having a Mg2+ requirement of between 0.1 and 2.0 mM for optimum growth.

INTRODUCTION

Extremely halophilic bacteria of the genus Halobacterium characteristically occur in highly saline environments, such as solar evaporation ponds, salt lakes, and on various salted food products, turning them from orange/red to pink or even purple (Dundas, 1977; Gibbons, 1974; Larsen, 1967). The currently described species of Halobacterium are characterized by the presence of carotenoids and by a minimum requirement of NaCl for growth of 2 to 3 M. All the recorded isolates have an additional requirement for relatively large amounts of Mg2+ (Brown & Gibbons, 1955) which might be related to the stability of the cell wall and/or of the protein-synthesizing apparatus (Kushner, 1978). Mg2+ above 2 M inhibits most species of Halobacterium, although the recently described H. volcanii tolerates these concentrations, reflecting its natural habitat, the Dead Sea (Mullakhanbhai & Larsen, 1975). The habitats described so far for Halobacterium species have been mainly neutral to acidic, highly saline environments, where divalent cations such as Mg2+ are readily soluble, none of the recorded isolates growing in semi-defined media in the absence of added Mg2+ (Brown & Gibbons, 1955).

The African Continent contains a number of alkaline soda lakes where the main ions are Na+, CO32−, HCO3− and Cl−, with varying amounts of SO42− and K+ (Talling & Talling, 1965). The more dilute lakes have a characteristic population of phototrophic bacteria (Grant et al., 1979). Concentrated soda lakes exist, such as the Wadi Natrun, Egypt, and Lake Magadi, Kenya, where the total dissolved salts may exceed 35% (w/v) at a pH of 10-5 to 11-0 (Imhoff et al., 1978). Under these conditions divalent cations are present in only trace amounts, since precipitation occurs as carbonates during formation of the lake waters (Hardie & Eugster, 1970). Parts of one such lake, Lake Magadi, are solid sodium sesquicarbonate (trona, Na2CO3·NaHCO3·2H2O) for most of the year. The crystalline trona, which is mined by the Magadi Soda Company as a source of soda ash, has an orange/pink crust which is similar in colour to the local solar evaporation ponds used to produce common salt. The solar evaporation ponds are unusual in that Na2CO3 and NaCl are present in equally large amounts in the liquor (16%, w/v) at a pH of 10-8. Microscopic examination of the liquor reveals large numbers of bacterial rods. The Mg2+ concentration is less than 0.5 mM both in the pan liquor and in Lake Magadi surface waters (Talling & Talling, 1965).
Recently Imhoff et al. (1978), as part of an ecological and microbiological survey of the lakes in the Wadi Natrun, a similar environment to Lake Magadi, reported red halophilic bacteria growing in the highly saline, alkaline waters where the concentration of Mg\(^{2+}\) was below the level of detection. Isolates obtained from this environment were by implication alkalophilic or alkalotolerant and grew at Mg\(^{2+}\) concentrations of less than 10 mM.

We report here the isolation of a red obligately halophilic and alkalophilic bacterium from both the trona crust and the solar evaporation ponds at Lake Magadi. We believe that this organism and others like it are responsible for the orange/pink coloration. The organism differs from previously described members of the _Halobacteriaceae_ in its GC content, alkalophily and ability to grow in media containing less than 0.5 mM-Mg\(^{2+}\).

**METHODS**

_Halobacterium cutirubrum_ (NCMB 763), _H. volcanii_ (NCMB 2012), _H. halobium_ (NCMB 736), _H. salinarium_ (NCMB 764) and _H. trapanicum_ (NCMB 767) were obtained from the National Collection of Marine Bacteria and maintained on the medium of Payne et al. (1960). The NaCl content was lowered to 2 M for _H. volcanii_.

Enrichments were originally performed in a modification of the medium of Brown (1963) of the following composition (% w/v): bacteriological peptone (Oxoid), 1.0; trisodium citrate, 0.3; MgSO\(_4\), 7H\(_2\)O, 2.0; KCl, 0.2; Na\(_2\)CO\(_3\), 10H\(_2\)O, 5.0; NaCl, 25; pH 9.5. Samples from the trona crust and the solar evaporation ponds were inoculated into this medium and incubated at 37 °C under fluorescent lighting (Philips daylight). Isolates were purified by streaking on to media solidified with 2–0% (w/v) agar. The isolates were subsequently maintained in the medium of Payne et al. (1960) modified by adding 5% (w/v) Na\(_2\)CO\(_3\), 10H\(_2\)O and decreasing the MgSO\(_4\), 7H\(_2\)O content to 0.1% (w/v); the final pH was 9.5.

The related effects of pH and Mg\(^{2+}\) concentration were studied in media prepared either without MgSO\(_4\), 7H\(_2\)O or with 0.1 or 2.0% (w/v) MgSO\(_4\), 7H\(_2\)O. Varying amounts of sterile 20% (w/v) Na\(_2\)CO\(_3\), 10H\(_2\)O or NaHCO\(_3\) were added to produce pH values of 7.5, 8.5 and 9.5.

To determine the optimal concentration of Mg\(^{2+}\) for growth in media at pH 9.5, media of different MgSO\(_4\), 7H\(_2\)O contents were prepared and the concentration of Mg\(^{2+}\) in solution was then determined with a Varian Tectron atomic absorption spectrophotometer, using media passed through a 0.45 μm Millipore filter (Golterman et al., 1978).

The GC content of the cells was determined by the buoyant density (Mandel et al., 1968) and the melting point (Marmur & Doty, 1962) methods using DNA prepared by the method of Marmur (1960).

The amino acid analysis of the bulk protein prepared by the method of Reistad (1970) was performed on a Locarte Mark IV Floor Model automatic amino acid analyser. Growth tests were done as previously described (Colwell et al., 1979; Gibbons, 1957; Gonzalez et al., 1978; Sehgal & Gibbons, 1960; Tomlinson & Hochstein, 1976).

**RESULTS AND DISCUSSION**

A motile Gram-negative rod, designated strain SP-1, was isolated from a sample of solar evaporation pond liquor after enrichment in the modified medium of Brown (1963) for 2 to 3 weeks. A similar organism was isolated from the trona crust of the lake.

The cells were 0.7 μm by 1.5 to 3.0 μm in medium containing 4.5 M-NaCl and 2 mM soluble Mg\(^{2+}\) at pH 9.5. Orange–red colonies were obtained after 2 weeks incubation at 37 °C on the modified medium of Payne et al. (1960); these were convex with an entire edge, 3 to 4 mm in diameter, translucent and mucoid.

Cells became spherical in medium containing 1 M-NaCl and lysed as the salt content was lowered further; cells suspended in distilled water lysed rapidly. Growth occurred between 3.0 and 5.5 M (saturation) NaCl, with an optimum at 4.0 M at 37 °C, pH 9.5.

Absorption spectra of whole cells showed maxima at 475, 505 and 545 nm, characteristic of the bacterioruberins found in halobacteria. The characteristics of the isolate, therefore, indicated a close relationship to the genus _Halobacterium_.

Growth of strain SP-1 and known _Halobacterium_ species was tested at pH 7.5, 8.5 and...
9.5 in media containing 2.0 or 0.1% (w/v) MgSO₄·7H₂O or without added MgSO₄·7H₂O. Strain SP-1 grew well at pH 9.5 with 0.1% (w/v) MgSO₄·7H₂O (0.1 mM soluble Mg²⁺). It also grew at pH 9.5 in media without any added MgSO₄·7H₂O, the concentration of soluble Mg²⁺ in the medium under these conditions being less than 0.05 mM. The Mg²⁺ optimum for strain SP-1 at pH 9.5 was between 0.1 and 2 mM (more precise determinations of the optimum were not possible due to the difficulty of constructing media with a precise range of soluble Mg²⁺ at pH 9.5). With 2% (w/v) MgSO₄·7H₂O (10 mM soluble Mg²⁺) at pH 9.5, some inhibition of growth of strain SP-1 occurred and the cells became coccoid in morphology. All the recognized species of *Halobacterium* showed good growth in media containing 2% (w/v) MgSO₄·7H₂O at pH 7.5 but grew poorly with 0.1% (w/v) MgSO₄·7H₂O and not at all without added MgSO₄·7H₂O. Strain SP-1 grew well at pH values between 9.0 and 10.0, but not below pH 8.5. Cells were elongated at pH 8.5. It survived inoculation into medium of pH 11.0, although the pH dropped to 9.5 to 10.0 as growth occurred. It did not grow at pH 7.5 irrespective of the Mg²⁺ concentration, indicating that the requirement for high pH was not a consequence of the concentration of soluble Mg²⁺. Growth of the known species of *Halobacterium* was good in 2% (w/v) MgSO₄·7H₂O at pH 7.5 but poor and variable at pH 8.5; no growth occurred at pH 9.5, agreeing with other reports (Colwell *et al.*, 1979). It was not possible to separate the effects of pH and Mg²⁺ in this case, since high pH and high Mg²⁺ concentrations are mutually incompatible.

Strain SP-1 resembled the known species of *Halobacterium* in showing positive reactions for gelatinase, catalase, oxidase, S²⁻ from S₂O₅²⁻, and negative reactions for amylase, reduction of NO₃⁻, reduction of NO₂⁻, production of gas from NO₃⁻, and production of gas from NO₂⁻. The antibiotic sensitivity of strain SP-1 also resembled that of known species of *Halobacterium* in that inhibition of growth was recorded in the presence of novobiocin, but not in the presence of penicillin G, polymixin B, ampicillin, tetracycline or dihydrostreptomycin. Strain SP-1 did, however, differ from known species of *Halobacterium* in being sensitive to vibriostat O/129.

Further similarities between *Halobacterium* spp. and strain SP-1 were indicated by amino acid analysis of the bulk protein. Comparison of *H. halobium* with strain SP-1 indicated a similar pattern of amino acids, the total acidic amino acid content being greater than the basic amino acid content by 14.4 mol % and 13.9 mol %, respectively (not corrected for amide). These values are characteristic of the genus *Halobacterium* (Brown, 1964; Kushmer & Onishi, 1966; Mullakhanbhai & Larsen, 1975; Steensland & Larsen, 1969).

Buoyant density centrifugation indicated no satellite bands, in contrast to most species of *Halobacterium* (Moore & McCarthy, 1969). The GC content was 59.0 ± 1.0 mol % (buoyant density) and 59.5 ± 1 mol % (Tm). These values are considerably lower than those found for the major DNA bands of recognized *Halobacterium* species (Gibbons, 1974).

Strain SP-1 appears to differ significantly from the recognized species of *Halobacterium* by virtue of its alkalophily and low Mg²⁺ requirement. Highly saline, alkaline environments are relatively rare in the world compared with highly saline, neutral environments and, possibly, such environments harbour a unique microbial population. Strain SP-1 may be similar to the *Halobacterium* sp. described by Imhoff *et al.* (1978) from the Wadi Natrun.

Further work is in progress on a comprehensive comparison of strain SP-1 with other isolates from highly saline, alkaline environments and type strains of *Halobacterium*. Whether strain SP-1 is also a member of the Archaeabacteria remains to be determined (Woese *et al.*, 1978).

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REFERENCES


