SHORT COMMUNICATION

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

By BRIAN J. TINDALL,¹ ALLAN A. MILLS²

AND WILLIAM D. GRANT¹

¹Department of Microbiology and ²Department of Geology, University of Leicester, Leicester LE1 7RH

(Received 25 July 1979)

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; $MgSO_4 \cdot 7H_2O$, 2.0; KCl, 0.2; $Na_2CO_3 \cdot 10H_2O$, 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) $Na_2CO_3 \cdot 10H_2O$ and decreasing the $MgSO_4 \cdot 7H_2O$ 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 $MgSO_4 \cdot 7H_2O$ or with 0.1 or 2.0% (w/v) $MgSO_4 \cdot 7H_2O$. Varying amounts of sterile 20% (w/v) $Na_2CO_3 \cdot 10H_2O$ 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 $MgSO_4 \cdot 7H_2O$ 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) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ or without added $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. Strain SP-1 grew well at pH 9.5 with 0.1 % (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1 mM soluble Mg^{2+}). It also grew at pH 9.5 in media without any added $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, the concentration of soluble Mg^{2+} in the medium under these conditions being less than 0.05 mM. The Mg^{2+} 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 Mg^{2+} at pH 9.5). With 2 % (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (10 mM soluble Mg^{2+}) 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) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ at pH 7.5 but grew poorly with 0.1 % (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and not at all without added $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. 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 Mg^{2+} concentration, indicating that the requirement for high pH was not a consequence of the concentration of soluble Mg^{2+} . Growth of the known species of *Halobacterium* was good in 2 % (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 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 Mg^{2+} in this case, since high pH and high Mg^{2+} concentrations are mutually incompatible.

Strain SP-1 resembled the known species of *Halobacterium* in showing positive reactions for gelatinase, catalase, oxidase, S^{2-} from $\text{S}_2\text{O}_3^{2-}$, and negative reactions for amylase, reduction of NO_3^- , reduction of NO_2^- , production of gas from NO_3^- , and production of gas from NO_2^- . 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; Kushner & 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 % (T_m). 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 Mg^{2+} 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 Archaeobacteria remains to be determined (Woese *et al.*, 1978).

This work was aided by a grant to W.D.G. from The Royal Society and the Research Board of the University of Leicester, and by the award of a graduate studentship to B.J.T. by the Science Research Council. We are grateful to the Magadi Soda Company for providing hospitality and facilities on site, and to J. W. Kyte for performing the amino acid analysis. We would also like to thank the Government of Kenya for research permits and

the Botany Department, University of Nairobi, for hospitality and assistance during our recent visits.

REFERENCES

- BROWN, A. D. (1963). The peripheral structure of gram-negative bacteria. IV. The cation-sensitive dissolution of the cell membrane of the halophilic bacterium, *Halobacterium halobium*. *Biochimica et biophysica acta* **75**, 425–435.
- BROWN, A. D. (1964). Aspects of bacterial response to the ionic environment. *Bacteriological Reviews* **28**, 296–329.
- BROWN, H. J. & GIBBONS, N. E. (1955). The effect of magnesium, potassium, and iron on the morphology of red halophilic bacteria. *Canadian Journal of Microbiology* **1**, 486–494.
- COLWELL, R. R., LITCHFIELD, C. D., VREELAND, R. H. & GIBBONS, N. E. (1979). Taxonomic studies of red halophilic bacteria. *International Journal of Systematic Bacteriology* (in the Press).
- DUNDAS, I. E. D. (1977). Physiology of *Halobacteriaceae*. *Advances in Microbial Physiology* **15**, 85–120.
- GIBBONS, N. E. (1957). The effect of salt concentration on the biochemical reactions of some halophilic bacteria. *Canadian Journal of Microbiology* **3**, 249–255.
- GIBBONS, N. E. (1974). *Halobacteriaceae*. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 269–273. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- GOLTERMAN, H. L., CLYMO, R. S. & OHNSTAD, M. A. N. (1978). *Methods for Physical and Chemical Analysis of Fresh Waters*, I.B.P. Handbook no. 8, 2nd edn. Oxford: Blackwell Scientific Publications.
- GONZALEZ, C., GUTIERREZ, C. & RAMIREZ, C. (1978). *Halobacterium vallismortis* sp. nov. An amyolytic and carbohydrate-metabolising extremely halophilic bacterium. *Canadian Journal of Microbiology* **24**, 710–715.
- GRANT, W. D., MILLS, A. A. & SCHOFIELD, A. K. (1979). An alkalophilic species of *Ectothiorhodospira* from a Kenyan soda lake. *Journal of General Microbiology* **110**, 137–142.
- HARDIE, L. A. & EUGSTER, H. P. (1970). The evolution of closed-basin brines. *Mineralogical Society of America Special Paper* **3**, 273–290.
- IMHOFF, J. F., SAHL, H. G., SOLIMAN, G. S. H. & TRÜPER, H. G. (1978). The Wadi Natrun: chemical composition and microbial mass developments in alkaline brines of eutrophic desert lakes. *Geomicrobiology Journal* **1**, 183–195.
- KUSHNER, D. J. (1978). Life in high salt and solute concentrations: halophilic bacteria. In *Microbial Life in Extreme Environments*, pp. 317–368. Edited by D. J. Kushner. New York: Academic Press.
- KUSHNER, D. J. & ONISHI, H. (1966). Contribution of protein and lipid components to the salt response of envelopes of an extremely halophilic bacterium. *Journal of Bacteriology* **91**, 653–660.
- LARSEN, H. (1967). Biochemical aspects of extreme halophilism. *Advances in Microbial Physiology* **1**, 97–132.
- MANDEL, M., SCHILDKRAUT, C. L. & MARMUR, J. (1968). Use of CsCl density gradient analysis for determining the guanine plus cytosine content of DNA. *Methods in Enzymology* **12B**, 184–195.
- MARMUR, J. (1960). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *Journal of Molecular Biology* **3**, 208–218.
- MARMUR, J. & DOTY, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *Journal of Molecular Biology* **5**, 109–118.
- MOORE, R. L. & MCCARTHY, B. J. (1969). Characterisation of the deoxyribonucleic acid of various strains of halophilic bacteria. *Journal of Bacteriology* **99**, 248–254.
- MÜLLAKHANBHAI, M. F. & LARSEN, H. (1975). *Halobacterium volcanii* spec. nov., a Dead Sea *Halobacterium* with a moderate salt requirement. *Archives of Microbiology* **104**, 207–214.
- PAYNE, J. I., SEHGAL, S. N. & GIBBONS, N. E. (1960). Immersion refractometry of some halophilic bacteria. *Canadian Journal of Microbiology* **6**, 9–15.
- REISTAD, R. (1970). On the composition and nature of the bulk protein of extremely halophilic bacteria. *Archiv für Microbiologie* **71**, 353–360.
- SEHGAL, S. N. & GIBBONS, N. E. (1960). Effect of some metal ions on the growth of *Halobacterium cutirubrum*. *Canadian Journal of Microbiology* **6**, 165–169.
- STEENSLAND, H. & LARSEN, H. (1969). A study of the cell envelope of the halobacteria. *Journal of General Microbiology* **55**, 325–336.
- TALLING, J. F. & TALLING, I. B. (1965). The chemical composition of African lake waters. *Internationale Revue der gesamten Hydrobiologie und Hydrographie* **50**, 421–463.
- TOMLINSON, G. A. & HOCHSTEIN, L. I. (1976). *Halobacterium saccharovorum* sp. nov. a carbohydrate-metabolising, extremely halophilic bacterium. *Canadian Journal of Microbiology* **22**, 587–591.
- WOESE, C. R., MAGRUM, L. J. & FOX, G. E. (1978). Archaeobacteria. *Journal of Molecular Evolution* **11**, 245–252.