An Alkalophilic Species of *Ectothiorhodospira* from a Kenyan Soda Lake

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An *Ectothiorhodospira* sp. was isolated from an alkaline mud sample from Lake Hannington, Kenya. It closely resembled *Ectothiorhodospira shaposhnikovii* in its ultrastructure, GC content, photosynthetic pigments, flagella position and mode of sulphur deposition, but differed in exhibiting more extreme alkaliphily, the pH optimum being 9.0 to 9.5, and in being obligately phototrophic.

INTRODUCTION

The Gregory Rift Valley in East Africa contains a number of alkaline soda lakes, including Lakes Hannington (now known as Lake Bogoria), Elmenteita, Nakuru and Magadi (Gregory, 1921). The last is best known for the commercial exploitation of the mineral sodium sesquicarbonate (trona; $\text{Na}_2\text{CO}_3 \cdot \text{NaHCO}_3 \cdot 2\text{H}_2\text{O}$), which is dredged from the nearly dry lake (Baker, 1958). Individual lakes show total alkalinity measurements ranging from 200 to 1200 mequiv. l^{-1} (Talling & Talling, 1965; Melack & Kilham, 1974), implying a range of ($\text{CO}_3^{2-} + \text{HCO}_3^-$) from 1 to 10% (w/v), although the pH remains fairly constant at 11.0 for most of the soda lakes, with a higher value of 12.1 for the evaporation ponds used to extract NaCl at Lake Magadi. At these pH values Na_2CO_3 preponderates (Golterman & Clymo, 1971; Jones *et al.*, 1967).

The lakes have a restricted fauna and flora (Jenkin, 1929; Hecky & Kilham, 1973) but do support large numbers of flamingoes. Although the major species of phytoplankton have been recorded and their relationships to primary biomass production have been elucidated (Melack & Kilham, 1974), these organisms have not apparently been grown in pure culture. Moreover, the bacterial population of these lakes appears never to have been examined. Information on the microbiology of alkaline environments is scarce. There are a number of reports of organisms from extremely alkaline situations (Siegel & Giumarro, 1965; Deal *et al.*, 1975; Souza *et al.*, 1974; Souza & Deal, 1977; Isachenko, 1951) but there is much less data than that available on acidic environments (Kushner, 1971).

This paper describes the enrichment and isolation of an alkalophilic photosynthetic bacterium from Lake Hannington.

METHODS

Sample. A mud sample (50 ml) was collected from Lake Hannington by Dr M. A. Khan, Geology Department, University of Leicester, and kept in a sealed glass container for 3 months. When opened, the mud was blackish and had a definite odour of H_2S and a pH of 11.0.

Media. The basal medium for phototrophic growth had the following composition (g l^{-1}): NH_4Cl , 1.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.4; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.5; NaCl , 0.4; KH_2PO_4 , 1.0; sodium succinate, 2.0; yeast extract (Difco), 1.0; $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$, 125.0; agar, 15. A solution of trace elements [containing (g l^{-1}): $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 5.5;

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 5.0; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 1.0; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 5.5; CuSO_4 , 1.5; CoCl_2 , 1.5] was added to the medium at 1 ml l^{-1} . The normal procedure was to sterilize separately 10-fold concentrated basal medium without Na_2CO_3 and agar, a 20% (w/v) solution of $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$, and 10% (w/v) agar. These were then mixed together with sterile distilled water to give the required concentrations of salts and Na_2CO_3 . The final pH of the medium was 10.5 to 10.6.

In comparative growth experiments with various hydrogen donors for phototrophic growth, the pH was adjusted to 9.8 with HCl, sodium succinate was omitted and appropriate concentrations of sterile hydrogen donors were added. Liquid cultures containing 0.05% (w/v) ascorbic acid were routinely employed in testing hydrogen donors. Growth was determined turbidimetrically at 650 nm.

In comparative pH experiments, media containing sodium carbonate were not suitable because of the strong buffering capacity of the $\text{HCO}_3^-/\text{CO}_3^{2-}$ system. Liquid basal media with sodium carbonate replaced by 2% (w/v) NaCl were used, and the pH was adjusted with KOH.

Catalase. Bacteria grown on basal medium containing succinate were scraped from a plate and suspended in 3% (v/v) H_2O_2 . Formation of bubbles was taken as a positive reaction.

DNA base composition. Bacteria were grown phototrophically in liquid basal medium for 14 d, centrifuged at 5000g, and the cell pellet was frozen overnight at -20°C . After thawing the pellet under cold running water, DNA was extracted and purified (Hall *et al.*, 1975). Melting points were determined spectrophotometrically in standard saline/citrate buffer. A sample of DNA from *Escherichia coli* was processed simultaneously. The GC content was calculated according to Marmur & Doty (1962). A sample of the same DNA preparation was analysed by CsCl density gradient centrifugation.

Electron microscopy. Bacteria taken from agar plates were negatively stained by the procedure of Horne (1967). For embedding and sectioning, bacteria were fixed in 3% (w/v) glutaraldehyde for 1 h followed by post-fixing for 1.5 h in Millonig buffer pH 7.3 (Pease, 1964). Sections were stained with uranyl acetate and lead citrate.

Chemicals. All chemicals were analytical grade except $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ which was laboratory grade.

RESULTS

Enrichment and isolation

A 25 ml vial containing basal medium was inoculated with 5 to 6 ml of the original mud sample and incubated at 30°C for 7 d under 5000 lux (fluorescent strip lighting, Philips daylight). The sample was then deep red. After a further subculture on the same medium under the same conditions, bacteria were streaked on agar medium of the same composition and incubated anaerobically using the GasPak system in transparent polycarbonate jars (GasPak Anaerobic Systems; BBL), being held upright round the walls of the chamber by an inner glass sleeve. The light intensity at the surface of the jar was 5000 lux. Colonies were restreaked several times, the purity being checked microscopically and by inoculation into Difco AC medium and *Desulfovibrio* medium (Abd-el-Malek & Rizk, 1960) containing 12.5% (w/v) $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ at pH 10.6.

Morphology

Bacteria were Gram-negative and pleomorphic, ranging from relatively uniform slightly curved rods on media with organic compounds as hydrogen donors to a variety of cell shapes and sizes on media containing sulphide as hydrogen donor. Spiral cells were numerous in media containing propionate. Bacteria grown on succinate were 0.6 to $0.8 \mu\text{m}$ diam. and 2 to $3.5 \mu\text{m}$ long. Division was by binary fission and there was no evidence of budding. In cultures grown phototrophically with sulphide as the electron donor, globules of elemental sulphur were deposited outside the bacteria (Fig. 1). There was no evidence for internal sulphur deposition although dark granules in cells were common. Bacteria grown on acetate contained granules which stained with Sudan Black (Burdon, 1946). The bacteria were motile by a tuft of polar flagella (Fig. 2). Thin sections of the cells (Fig. 3) showed a type of ultrastructure described for some non-sulphur photosynthetic bacteria (Giesbrecht & Drews, 1966) and for sulphur photosynthetic bacteria of the genus *Ectothiorhodospira* (Raymond & Sistrom, 1967; Trüper, 1968; Cherni *et al.*, 1969).

The GC content was 61.2 mol % (T_m), 62.6 mol % (buoyant density).

Fig. 1. Phase contrast micrograph showing sulphur deposition in sulphide-containing medium. Bar marker represents 10 μm .

Fig. 2. Electron micrograph showing a negatively stained flagella tuft. Bar marker represents 1 μm .

Fig. 3. Electron micrograph showing lamellar stacks in sectioned cells. Bar marker represents 1 μm .

Photosynthetic pigments

The absorption spectrum of photo-organotrophically grown whole cells, prepared on glass fibre filters by the procedure of Trüper & Yentsch (1967), gave maxima at 375, 590 and 850 nm indicating the presence of bacteriochlorophyll *a*. The spectrum was similar to that described by Trüper (1968) for *Ectothiorhodospira mobilis*. The presence of additional absorption maxima between 435 and 535 nm indicated the possible presence of carotenoids of the spirilloxanthin series (Trüper, 1968). (A more detailed study of the carotenoids is in progress.)

Physiology

The organism only grew phototrophically under anaerobic conditions, either photo-organotrophically or photolithotrophically. Cultures more than 2 weeks old in liquid medium formed slimy aggregates. Some tolerance to oxygen was indicated by growth in agar to within 5 to 10 mm of the surface. Despite incubation periods of up to 2 months, the organism could not be induced to grow aerobically or anaerobically in the dark, or aerobically in the light or dark, under the conditions described by Uspenskaya & Kondrat'eva (1972) for *E. shaposhnikovii*. The organism was catalase-positive.

Growth could be maintained in basal medium in the absence of yeast extract, indicating no requirement for organic growth factors. However, as the growth rate was enhanced by the inclusion of 0.1% yeast extract, this was routinely incorporated. The ability to grow without exogenously supplied vitamins is shared by *E. halophila* (Raymond & Sistrom, 1969) and *E. shaposhnikovii* (Kondrat'eva, 1956) but not by *E. halochloris* (Imhoff & Trüper, 1977) or *E. mobilis* (Trüper, 1968).

After incubation for 6 to 7 d no growth occurred in culture media below pH 7.5 or above pH 11.0. The pH optimum was 9.0 to 9.5, and 50% of the maximum growth yield was obtained at pH 8.0 and 10.0.

The temperature range for growth was 20 to 37 °C with an optimum at 30 °C. Various hydrogen donors at the concentrations used by Trüper (1968) were tested in liquid media supplemented with 0.05% ascorbic acid. Na_2S , $\text{Na}_2\text{S}_2\text{O}_3$, Na_2SO_3 , acetate, propionate and

succinate all strongly supported growth, A_{650} reaching 0.8 to 1.4 after 10 d incubation. Elemental sulphur, malate and formate supported less growth, A_{650} reaching 0.2 to 0.8 after the same period. Butyrate, citrate, benzoate, ethanol, methanol, lactate, glucose, fructose, glycerol and Casamino acids did not support growth. Molecular hydrogen was also used as an electron donor for phototrophic growth.

Neither NaNO_3 nor NaNO_2 could be used as nitrogen sources in place of NH_4Cl .

Basal medium at pH 10.6 without Na_2CO_3 and with all other sodium salts replaced by potassium salts would not support growth. Growth was restored by the addition of either NaCl or Na_2CO_3 . Growth occurred under these conditions at NaCl concentrations between 0.1 and 8% and $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ concentrations between 0.5 and 15%. Growth was markedly better in the presence of Na_2CO_3 .

DISCUSSION

Like other phototrophic bacteria, *Ectothiorhodospira* species occur in aquatic environments where anaerobic conditions prevail. The genus is unusual amongst the purple sulphur bacteria in that members possess an extensive internal membrane system consisting of stacks of lamellae, and deposit sulphur extracellularly. The genus is also characterized by its common presence in high salt environments (Trüper, 1968), three of the four described species requiring salt for growth. Indeed two of the described species are among the few extreme halophiles outside the genera *Halobacterium* and *Halococcus* (Pfennig & Trüper, 1974). Additionally, the original strain of *E. mobilis* (a moderate halophile species) was isolated from an area of high gypsum concentration in the Crimea (Pelsh, 1937) and Butlin & Postgate (1954) may have observed members of the genus in CaSO_4 -saturated Libyan lake waters. Thus *Ectothiorhodospira* species are commonly found in situations where a variety of salts are present in high concentration.

The bacteriology of alkaline soda lakes has been little studied, although Isachenko (1951) carried out a survey of chlorous, sulphate and soda lakes of the Kulunda Steppe in the Crimea, including a microscopic examination of three alkaline soda lakes. The most alkaline of these, Tanatar III, had a predominant population of purple photosynthetic spiral bacteria which were referred to as *Thiospirillum* species, whereas the less alkaline lakes had a wide range of photosynthetic bacteria. It is possible that the former organisms, which were not grown in culture, could have been *Ectothiorhodospira* species.

The organism described here fits the generic description of *Ectothiorhodospira* in terms of ultrastructure, flagellation, photosynthetic pigments and sulphur deposition. Its low sodium requirement distinguishes it from three of the recognized species of *Ectothiorhodospira*, *E. halophila* and *E. halochloris* being extreme halophiles and *E. mobilis* moderately so (Imhoff & Trüper, 1977; Trüper, 1968). The fourth recognized species, *E. shaposhnikovii*, like the organism described here, has a low sodium ion requirement, but requires no added salt in the medium (Cherni *et al.*, 1969). The alkaliphily shown by our organism differentiates it from all the recognized species except *E. halochloris*. Although all the species have an alkaline pH optimum, only *E. halochloris* exhibits a similar tolerance (Chesnokov & Saposnikov, 1936; Cherni *et al.*, 1969; Imhoff & Trüper, 1977; Kondrat'eva, 1956).

The GC content of the DNA of the organism, its lack of requirement for significant amounts of NaCl and general morphology indicate a possible close relationship with *E. shaposhnikovii*. However, the organism described here exhibits several differences from the recorded strains of this species. *Ectothiorhodospira shaposhnikovii* is unable to use formate but can use lactate and butyrate as hydrogen donors for phototrophic growth (Kondrat'eva, 1956), whereas the organism described here appears to be unable to utilize lactate or butyrate but does utilize formate, albeit poorly. The former organism also uses nitrate as a nitrogen source (Kondrat'eva, 1956) and can grow aerobically both in the light

and the dark (Uspenskaya & Kondrat'eva, 1972), whereas the organism described here could only grow phototrophically under anaerobic conditions. Our organism is more strongly alkalophilic, whereas there have not been reports of *E. shaposhnikovii* being isolated from alkaline environments.

Organisms of this type are the predominant phototrophic bacteria in a wide range of alkaline lakes in the Gregory Rift Valley (W. D. Grant & B. J. Tindall, unpublished work). Recently, Imhoff & Trüper (1977) have shown that the halophilic species *E. halochloris* and *E. halophila* occur in large numbers in alkaline saline Egyptian lakes. *Ectothiorhodospira halochloris* is clearly different from the organism described here since it is a halophile, contains bacteriochlorophyll *b* and has a much lower GC ratio; but it does have the same features of alkalophily and growth enhancement in the presence of carbonate.

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