SHORT COMMUNICATION

Isolation and Preliminary Characterization of an Iron-oxidizing Bacterium from an Ochre-polluted Stream

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An iron-oxidizing bacterium has been isolated from an ochre-polluted stream. Growth occurs optimally at pH 2.5 in a mineral medium containing 200 µg ferrous ions ml⁻¹ but lacking organic carbon. Manganese salts are not oxidized. The organism grows as long unbranched filaments encrusted with iron particles and is morphologically and physiologically distinct from other well characterized iron-oxidizing bacteria.

INTRODUCTION

Many bacterial species have been found in iron deposits (Cullimore & McCann, 1977) but only a few pure cultures have been isolated and used to study the physiology of iron oxidation. Iron deposition may result from the activities of both chemoautotrophic bacteria which use ferrous oxidation as an energy source, and heterotrophs which can cause the precipitation of ferric compounds either following utilization of iron-chelating compounds or by changing the oxidation–reduction potential of the environment.

An ochre-polluted stream near Sheffield was examined for the presence of iron-oxidizing bacteria. A small spring, rising through pyrite-rich rocks, was the main source of iron in the stream causing the pH to fall from 7.0 to 5.5 at the entry point of the spring into the stream and the formation of a bright orange precipitate on the stream bed and boulders for several hundred metres downstream.

This report describes the isolation of an autotrophic iron-oxidizing bacterium from this stream.

METHODS

Isolation and growth. The bacterium was isolated from Limb Brook which flows on the South West side of Sheffield (Ordnance Survey sheet SK38SW). Growth was in the mineral salts medium of Leathen *et al.* (1956) modified by the omission of K₂HPO₄. The autoclaved basal salts medium was supplemented with filter-sterilized FeSO₄ to a final iron concentration of 200 µg ml⁻¹. The final medium pH was 2.5, adjusted with 1 M-H₂SO₄ or 1 M-NaOH as required. The medium was inoculated with ochre samples from various parts of the stream and incubated without shaking at 20 °C. After 3 to 4 weeks, the orange deposit formed was subcultured and the process was repeated to obtain a pure culture.

Isolation of other iron-oxidizing bacteria was also attempted using the media of Kucera & Wolfe (1957) at pH 6.5 and Walsh & Mitchell (1972) at pH 4.1.

The ability of the isolate to oxidize manganese was tested on agar which contained 0.2% (w/v) MnCO₃ (Mulder, 1964) or 0.002% (w/v) MnSO₄·4H₂O (Tyler & Marshall, 1967).

Electron microscopy. Specimens were fixed in 5% (w/v) glutaraldehyde and either coated with 60% (w/w) gold/palladium and viewed in a Cambridge Stereoscan electron microscope Mark IIa or treated with 1% (w/v)

OsO₄, dehydrated and embedded in Spurr resin, sectioned on a Reichert OmU3 microtome, mounted on Formvar-coated copper grids and examined in a Philips EM300 microscope.

Iron determination. Total soluble iron and soluble ferric iron were measured by a spectrophotometric method (Sandell, 1959). Ferrous iron was calculated as the difference between these two values.

Incorporation of ¹⁴CO₂. Glass microfibre filters (Whatman GF/C) were suspended in growth medium containing 1 µg NaH¹⁴CO₃ ml⁻¹ (specific activity: 55.7 mCi mmol⁻¹, 2.061 GBq mmol⁻¹). Filters, with adhering bacteria, were washed under vacuum with either water (30 ml) or successive portions (10 ml) of 10% (w/v) trichloroacetic acid, 5% (w/v) trichloroacetic acid, and water. After drying at 80 °C, the ¹⁴C associated with the filters was determined by placing them in vials in 5 ml toluene containing 0.7% (w/v) 2,5-diphenyloxazole and counting in an Intertechnique SL30 liquid scintillation counter.

RESULTS

The iron-oxidizing bacterium was isolated, on two separate occasions, from ochre deposits near the main source of iron pollution. No other iron-oxidizing bacteria were isolated in any of the three media tested, although these gave a range of final pH values. Purity was assessed by the presence of a single colony type on iron-salts agar (Manning, 1975), by microscopic examination and the failure to obtain any growth on a variety of complex media. However, the presence of low numbers of other autotrophic iron-oxidizing bacteria cannot be ruled out.

During the exponential phase of growth in liquid medium the organism, as seen by phase contrast microscopy (Fig. 1*a*) and scanning electron microscopy (Fig. 1*b*), grew as long, iron-encrusted filaments, up to 200 µm in length. On solidified medium it formed small orange colonies surrounded by a diffuse zone of precipitated iron. Maximum population phase bacteria from liquid medium and bacteria grown on solidified medium were short rods (about 1–2 µm in length × 0.5 µm diameter, Fig. 1*c*). When inoculated into fresh liquid medium these cells grew as filaments.

Sectioning for transmission electron microscopy was difficult because of the high iron content of the samples. Longitudinal sections through filaments showed that the bacterium was aseptate and had a cell wall 20 nm thick (Fig. 1*d, e*). The filament walls appeared irregular with many surface indentations (Fig. 1*d*); these may represent distortions induced by the extracellular presence of large iron particles or a means of improving the efficiency of iron oxidation by an increased surface area.

The rate of iron oxidation in mineral salts medium plus various concentrations of ferrous ion was followed over a 10 d period. On inoculation into fresh medium the pattern of iron oxidation followed a typical bacterial growth curve. In uninoculated controls oxidation of ferrous ion was negligible. Iron oxidation occurred over a range of 50 to 500 µg ferrous ion ml⁻¹ and was optimal at 200 µg ml⁻¹. Growth occurred over a pH range of 2.0 to 4.5 and was optimal at pH 2.5. Above pH 4.5 chemical oxidation of ferrous ions and precipitation of the resultant ferric salts made the measurement of biological oxidation unreliable.

Iron oxidation was inhibited by chloramphenicol (100 µg ml⁻¹), slightly delayed by penicillin G (50 µg ml⁻¹) and unaffected by streptomycin (100 µg ml⁻¹). Iron oxidation was immediately stopped when sodium azide (10 mM) was added to the culture at any stage of growth.

The organism was found to incorporate ¹⁴CO₂ when supplied as NaH¹⁴CO₃. Iron oxidation initially occurred concurrently with incorporation of ¹⁴CO₂ into cell material by bacteria growing on glass fibre filters. To ensure that the ¹⁴C assimilation was not due to the deposition

Fig. 1. Morphological characteristics of the Sheffield isolate. (*a*) Phase contrast micrograph of an iron-encrusted filament. (*b*) Scanning electron micrograph of filaments grown *in situ* on coverslips suspended in the medium. (*c*) Scanning electron micrograph of maximum population phase cells from liquid medium; the arrow indicates an area of iron precipitation. (*d, e*) Transmission electron micrographs of a filament showing surface indentations. The bar markers represent 20 µm (*a*), 50 µm (*b*), 1 µm (*c*) 0.5 µm (*d*) and 0.1 µm (*e*).

of inorganic carbonate, but represented incorporation into cell material, the filters were washed with trichloroacetic acid or water. The difference in ^{14}C counts between the two treatments was not significant and indicated that the amount of inorganic material deposited was small. Further acid washes of the filters did not reduce the amount of radiolabelling. During later stages of iron oxidation, the assimilation of $^{14}\text{CO}_2$ was apparently not stoichiometric with the ferric iron formed; this may have been due to a scintillation quenching effect caused by the presence of precipitated ferric ions.

The bacterium did not oxidize thiosulphate, sulphide or manganese ions and no growth was observed on complex media. Growth was not stimulated by the addition of organic compounds to the medium. There was no appreciable change in the pH of the growth medium during growth.

DISCUSSION

The bacterium isolated from ochre deposits has several novel features which distinguish it from other known iron-oxidizing bacteria. The organism appears to be an obligate chemoautotroph in that it will fix CO_2 but does not utilize organic carbon sources. It grows as filaments and does not utilize sulphur compounds and therefore bears no resemblance to *Thiobacillus ferrooxidans* (Vishniac, 1974). Other autotrophic iron-bacteria which have been isolated to date are *Gallionella ferruginea* (Kucera & Wolfe, 1957), *Metallogenium* sp. (Walsh & Mitchell, 1972), and *Leptospirillum ferrooxidans* (Balashova *et al.*, 1974), which are morphologically very distinct and different from our isolate.

Several filamentous iron-bacteria have been reported, e.g. *Leptothrix* spp. and *Sphaerotilus* spp. (van Veen *et al.*, 1978). They are surrounded by a sheath on which large amounts of ferric and, in the case of the former, manganic compounds are accumulated. There is no evidence from the electron micrographs to suggest that the Sheffield isolate is sheathed and it does not oxidize manganese salts. Furthermore, both *Leptothrix* and *Sphaerotilus* are heterotrophic and are not acid-tolerant, unlike the Sheffield isolate.

This isolate appears to be a new iron-oxidizing bacterium, but before any further work can be carried out, several problems have to be overcome. Harvesting the bacterium presents difficulties. The bacterium strongly adheres to the walls of the growth vessel, irrespective of the material from which it is made. Obtaining energy by the oxidation of iron is not a very efficient process (Starkey, 1945). The large amounts of ferric precipitate produced as a result of growth is a particular problem in the determination of biomass. Attempts to remove the precipitate by acid washes have not been successful.

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