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Sulphur oxidation by a *Streptomyces* sp. growing in a carbon-deficient medium and autoclaved soil

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Abstract. *Streptomyces* colonies, apparently all of the same species, were isolated from a range of soils using a polysulphide medium lacking an organic carbon source. Growth on this medium, and clearing of the otherwise white, opaque overlay, suggested that the organisms were capable of growing autotrophically. However, investigation of one of these isolates showed that it was unable to fix $^{14}CO_2$ and did not possess the enzyme ribulose bisphosphate carboxylase, showing that it was incapable of autotrophic growth. The isolate oxidized elemental sulphur, thiosulphate and tetrathionate to sulphate in vitro in carbon-deficient medium, and also oxidized elemental sulphur to sulphate when inoculated into autoclaved soil supplemented with sulphur. It also oxidized polysulphide when growing on Czapek Dox and plate count agars. The isolate can therefore grow heterotrophically in both carbon-rich media and in media lacking organic carbon – presumably by scavenging organic carbon from the laboratory atmosphere. The possible role of these organisms in sulphur oxidation in soils is commented upon.

Key words: Heterotrophic sulphur oxidation – Oligotrophy – Sulphur cycle

The ability of actinomycetes to oxidize sulphur has long been recognised (Guittoneau 1927; Starkey 1950; Vitolins and Swaby 1969). More recently Yagi et al. (1971) reported that 33 of the 36 species of *Streptomyces* which they screened had the ability to oxidize elemental sulphur to thiosulphate when growing heterotrophically with glucose as the carbon source. Oi and Yamamoto (1977) also showed that a heterotrophic *Streptomyces* sp. possessed thiosulphate oxidase and rhodanese activities and could oxidize thiosulphate to sulphate.

Wieringa (1966) isolated sulphur oxidizing actinomycetes from soil using an opaque, polysulphide medium developed to isolate thiobacilli. He concluded that these organisms were autotrophic since they grew on this carbon-deficient medium.

During recent studies on sulphur-oxidation in soils (Killham and Wainwright 1984; Skiba and Wainwright 1984) we also isolated apparently autotrophic sulphur-oxidizing actinomycetes when using Wieringa's (1966) medium. The autotrophic nature of these isolates subsequently became questionable however. Here we report studies on the ability of one of these isolates to oxidize sulphur in vitro and when inoculated into elemental sulphur amended, autoclaved soils; evidence is also presented to show that these organisms are incapable of autotrophic growth.

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Materials and methods

Isolation of presumptive S-oxidizing Streptomyces sp. using a carbon-deficient medium. Samples (10 g) of soils and sands (Table 1) were shaken in ¼ strength Ringer's solution (90 ml) and serially diluted. The final dilution (0.1 ml) was then spread onto the surface of Wieringa's (1966) medium containing actidione (50 μml^{-1}); nystatin (10 $\mu mg\ ml^{-1}$); polymyxin B sulphate (5 $\mu g\ ml^{-1}$) and sodium penicillin (1 $\mu g\ ml^{-1}$) as suggested by Williams and Davies (1965). Colonies which grew on the medium after 6 days incubation at 25° C were replica-plated onto fresh medium using sterile velvet. After a further period of incubation for 6 days at 25° C colonies producing clear haloes in the otherwise white-opaque medium were counted and representative colonies were transferred to fresh medium lacking organic carbon and maintained for further use.

S-oxidation in vitro in carbon-deficient media. An isolate, later identified as a *Streptomyces* sp. was inoculated into the following liquid medium (25 ml in 100-ml Erlenmeyer flasks), containing a source of reduced sulphur, but not sulphate: K_2HPO_4, 0.5 g; NH_4HCO_3, 1 g; $MgCl_2$, 0.25 g; $CaCO_3$, 0.05 g; trace elements 10 ml (Wieringa 1966); water 1 l, adjusted to pH 7.5 with 1 N HCl.

The following sulphur sources were added to separate series of flasks: S°; $Na_2S_2O_3 \cdot 10\ H_2O$ and $Na_2S_4O_6$ to produce respectively sulphur concentrations of 3.1; 1.2 and 3 mM. Elemental sulphur was sterilized by steaming for 1 h on three successive occasions, while the other sulphur sources were membrane filter (0.22 µm) sterilized. Flasks were inoculated with a liquid starter culture (2 ml) and incubated (slowly at 60 throws min^{-1}) on a reciprocal shaker for 7 days. The contents were then membrane filtered (0.22 µm) and the dry weight determined. Chromic acid washed glassware was used throughout.

Determination of sulphur ions. Thiosulphate and tetrathionate were determined colorimetrically (Nor and Tabatabai

Table 1. Numbers of presumptive sulphur-oxidising *Streptomyces* in soils and sands

Sampling site	pH	Organic C (% w/w)	Total N (% w/w)	Counts $\times 10^3$ g sand, soil^{-1}
Loam soil	6.7	2.1	0.3	86.2 (8.1)
Agricultural soil	5.9	5.5	0.7	26.0 (3.7)
Unpolluted deciduous woodland soil	4.6	9.8	0.8	25.6 (4.0)
Sulphur polluted deciduous woodland soil	3.3	14.6	0.9	1.8 (1.9)
Dune soil	7.8	0.54	0.1	3.2 (3.0)
Rhizophere of:				
Hippophaë rhamnoides	8.5	0.037	0.0062	5.5 (2.0)
Ammophila arenaria	8.4	0.056	0.0063	8.3 (4.2)
Uncolonized dune sand	8.6	0.026	0.0034	5.1 (1.6)

Means of counts from 5 plates (± S.D.)

Table 2. The effect of temperature on presumptive sulphur-oxidizing *Streptomyces* counts in a fertile loam soil

Temperature of incubation (°C)	Counts $\times 10^3$ g soil^{-1}
15	0
25	64.4 (4.3)
30	40.6 (9.1)
37	0.8 (0.84)
41	0.6 (0.55)

Means of counts from 5 plates (± S.D.)

1976) and sulphate by turbidimetry (Hesse 1971). All values are expressed as excess over non-inoculated flasks or soils to account for the occurrence of the auto-oxidation of the reduced forms of sulphur.

Determination of ribulose bisphosphate carboxylase activity in crude cell-free extracts. A crude cell-free extract prepared from cells growing in the minimal salts medium described above was assayed for ribulose bisphosphate carboxylase using the method of Anderson (1975). The medium (700 ml in 2-l Erlenmeyer flasks) containing $NaS_2O_3 \cdot 10\,H_2O$ (1.2 mMS) was inoculated with starter culture (50 ml) and incubated on a reciprocal shaker (60 throws min^{-1}) at 25° C. After 10 days, glycine (1% v/v) was added and the culture was incubated for a further 24 h. Cells were harvested by centrifugation (4,500 *g* for 30 min at 2° C) washed once with K_2HPO_4 buffer (50 mM, pH 7.2) and suspended in 3 volumes of buffer and sonicated (MSE, 60 W) for 10 × 1-min intervals, interspersed with a period of cooling in ice. The cell debris was then removed by centrifugation (38,000 × *g* for 20 min at 2° C). Both the clear supernatant and the cell-debris were assayed. Protein was determined by the method of Lowry et al. (1951), using bovine serum albumin as standard.

Measurement of ^{14}carbon-incorporation. To monitor the incorporation of $^{14}CO_2$, mineral salts medium containing $NaS_2O_3 \cdot 10\,H_2O$ (1.2 mMS; 10 ml in 50-ml conical flasks) were sealed with sterilised Suba Seal vaccine stoppers and injected with $NaH^{14}CO_3$ (0.1 ml) containing 20 μCi ^{14}C ml^{-1}. This provided a total of 158 μg C ml^{-1}. After incubation at 40° C for 1 h to allow for equilibration of liquid and gaseous $^{14}CO_2$ within the medium and gas phase *Streptomyces* sp. starter culture (1 ml, equivalent to 1 mg dry weight cells ml^{-1}) was injected into the flasks. The flasks were then incubated at 25° C, and at intervals during growth three flasks were removed and their contents filtered through membrane filters (0.22 μm), which had been washed once with distilled water (adjusted to pH 1.7 with H_2SO_4) and once with unadjusted distilled water (10 ml of each). Membranes were dried under an infra-red lamp and then transferred to scintillation vials containing scintillation fluid (5 ml). All samples were counted using a Philips PW 4540 liquid scintillation counter. Growth during the incubation period was determined by repeating the above experiment without $^{14}CO_2$ addition and measuring the dry weight produced using a precision balance (Stanton Instruments).

Growth of Streptomyces sp. in a methanol-saturated atmosphere. Triplicate samples of liquid medium (25 ml) containing $NaS_2O_3 \cdot 10\,H_2O$ (1.2 mMS) were inoculated with starter culture, and then placed in a desiccator containing methanol (100 ml). Controls were set up in desiccators with distilled water in the place of methanol. The dry weight of cells was determined after 6 days incubation at 25° C.

Sulphur-oxidation by a Streptomyces isolate growing in sterile loam soil. Loam soil (100 g) in 500-ml Erlenmeyer flasks was autoclaved at 120° C for 30 min on three successive occasions, with a period of cooling in between. Samples (100 g) of the autoclaved soil were amended in triplicate with steam sterilized S° (1% w/w); water (9 ml) and *Streptomyces* culture (5 ml) grown on organic carbon-deficient medium containing thiosulphate. Uninoculated controls were also included. The samples were incubated at 25° C and at weekly intervals the pH and sulphate was determined, using a soil:water suspension (1:10, shaken for 15 min) and lithium chloride (1:10 soil extraction ratio, shaken for 15 min) respectively.

Results

Isolation from soils. Colonies growing on Wieringa's medium were white and formed concentric rings, with a powdery surface texture. Clearing of the otherwise opaque polysulphide medium indicated sulphur oxidation. All colonies isolated appeared to be the same and were identified as a species of *Streptomyces*. The chosen isolate grew on arginine

Table 3. Oxidation of elemental sulphur by a *Streptomyces* isolate growing on organic carbon-deficient medium

Length of incubation (days) at 25° C	pH of the inoculated medium	pH of the uninoculated control medium	Concentration of sulphur ions (mMS)		
			Thiosulphate	Tetrathionate	Sulphate
0	8.7	8.7	0	0	0.05 (0.009)
4	8.7	8.7	1.8 (0.11)	0	0.3 (0.009)
8	8.4	8.7	0.75 (0.14)	0	0.62 (0.09)
11	8.2	8.5	0.05 (0.04)	0	0.6 (0.13)
14	8.4	8.6	0	0	0.55 (0.22)
17	8.3	8.5	0.03 (0.005)	0	2.27 (0.07)

Means of triplicates (± S.D.)

Table 4. Oxidation of thiosulphate by a *Streptomyces* isolate growing in organic carbon-deficient medium

Length of incubation (days) at 25° C	pH of inoculated medium	pH of uninoculated control medium	Sulphate (mMS) formed	Dry weight (mg) produced
0	8	8 (0)	0.11 (0.02)	1.5 (0.6)
4	8.6	8.6 (0.06)	0.11 (0.03)	4.8 (3.1)
8	8.5	8.6 (0.06)	0.39 (0.21)	3.5 (0.5)
11	8.6	8.4 (0.1)	0.72 (0.36)	10.7 (4.8)
14	8.3	8.2 (0.17)	0.78 (0.1)	10.3 (5.2)
17	7.9	8.1 (0.06)	1.56 (0.02)	11.1 (1.5)

Means of triplicates (± S.D.)

Table 5. Oxidation of tetrathionate by a *Streptomyces* isolate growing in organic carbon-deficient medium

Length of incubation (days) at 25° C	pH of inoculated medium	pH of uninoculated control medium	Sulphate (mMS) formed	Dry weight (mg) produced
0	8.0	8 (0)	1.8 (0.19)	1.5 (0.6)
4	8.4	8.6 (0.06)	0.49 (0.01)	6.1 (5.1)
8	8.2	8.5 (0.1)	1.14 (0.11)	6.4 (1.6)
11	7.2	8.3 (0.1)	1.71 (0.08)	9.7 (3.6)
14	6.8	8.3 (0.21)	1.92 (0.17)	12.1 (1.1)
17	5.9	8.2 (0.15)	2.64 (0.25)	16.5 (1.2)

Means of triplicates (± S.D.)

glycerol medium as well as Plate Count (Oxoid) and Czapek-Dox media, both in the presence and absence of a source of reduced sulphur. The isolate was Gram-positive, non-acid fast and was incapable of growing anaerobically.

Presumptive-sulphur-oxidizing *Streptomyces* sp. were isolated from all the soils examined (Table 1). Highest counts were obtained from the loam soil, followed by the agricultural and relatively unpolluted deciduous woodland soil. Low counts were obtained from the heavily polluted deciduous woodland soil, which is known to contain reduced sulphur (Killham and Wainwright 1984) and from samples taken from a sand-dune succession (Table 1). The effect of incubation temperature on the isolation of the sulphur-oxidizing *Streptomyces* sp. is shown in Table 2. *Streptomyces* sp. were obtained from samples incubated at 25° C; while no colonies appeared at 15° C, and numbers declined markedly at temperatures above 30° C.

Sulphur-oxidation in vitro. The isolate oxidized elemental sulphur to sulphate in vitro (Table 3), with the highest concentration of the ion being formed after 17 days. No tetrathionate was detected, and thiosulphate was found only after 4 and 8 days. Thiosulphate and tetrathionate were oxidized to sulphate (Tables 4, 5). The dry weight of the *Streptomyces* sp. increased over the incubation period, while a pH decrease occurred only in tetrathionate amended medium.

Sulphur oxidation in soil. The isolate oxidized elemental sulphur to sulphate when inoculated into autoclaved garden loam amended with the element (1% w/w). As sulphur-oxidation proceeded the pH of the inoculated soil decreased only slightly compared with the control (Table 6).

Growth characteristics of the isolate. The isolate was capable of limited growth in the carbon-deficient medium both in the presence and absence of elemental and reduced sulphur. The amount of cell mass produced doubled in the former [from 1.2 (± 0.25) mg to 2.4 (± 0.16) mg after 6 days] when the cultures were grown in a methanol-saturated atmo-

Table 6. Oxidation of elemental sulphur (1% w/w) by a *Streptomyces* isolate inoculated into autoclaved soil

Length of incubation (days) at 25° C	Soil pH	Sulphate formed (μgS g soil^{-1})
0	6.8 (0)	0
4	6.7 (0)	30 (10.7)
7	7.0 (0.1)	20 (8.6)
11	6.7 (0)	80 (20)
15	6.6 (0.08)	45 (6.6)
22	6.6 (0.06)	65 (8.8)
29	6.5 (0.06)	60 (1.1)
35	6.5 (0.06)	120 (20.8)
42	6.4 (0.06)	160 (18.5)

Means of triplicates ($\pm$ S.D.)

sphere. No ribulose bisphosphate carboxylase was detected in either a crude cell-free extract containing 0.1 mg ml^{-1} protein, or in dissolved cell debris, independent of whether the organism was grown in the presence or absence of a source of reduced sulphur.

During a 3 day incubation at 25° C less than 10% of the newly-synthesised carbon originated from ^{14}C labelled CO_2; a calculation based on the assumption that the cells consist of 40% w/w C. There was no evidence therefore for CO_2 fixation and autotrophic growth.

Discussion

Colonies of sulphur-oxidizing *Streptomyces* sp. capable of growing on a carbon-deficient medium were readily isolated from a variety of soils and coastal sands of differing pH, and carbon and nitrogen contents. The colonies were apparently all of the same species and were initially considered to be autotrophs, as suggested by Wieringa (1966). We were unable to substantiate this view however, as the isolate which we studied did not possess ribulose bisphosphate carboxylase, and was incapable of fixing $^{14}CO_2$. The organism was able to grow in the presence or absence of reduced sulphur source in carbon-deficient media and did not appear to nitrify the ammonia sulphate added as nitrogen source. It seems therefore that the organism is capable of satisfying its energy and carbon needs by scavenging carbon from the medium or the atmosphere, a view enhanced by the fact that the dry weight of the organism doubled when exposed to a methanol atmosphere.

Growth of actinomycetes in media lacking an added C-source has been previously reported by Beijerinck and Van Delden (1903); Hirsch and Engel (1956), while Hirsch (1958) showed that the growth of *Nocardia petroleophila* was stimulated by exposure to petroleum vapour. The ability of bacteria to scavenge carbon from the laboratory atmosphere was also demonstrated by Hirsch (1964) and more recently by Geller (1983), who showed that carbon-deficient media in flasks closed with cotton can accumulate 0.5 mg l^{-1} dissolved carbon per week.

The rate of sulphur-oxidation shown by the isolate in carbon-deficient medium containing a sulphur-source was very slow when compared with that shown by thiobacilli; or of actinomycetes, other heterotrophic bacteria and fungi when growing in media supplemented with both organic carbon and sulphur (Pepper and Miller 1978; Wainwright and Killham 1980; Yagi et al. 1971). Similarly, the maximum amount of sulphate formed by this organism when inoculated into autoclaved soil amended with the element (after 40 days incubation) was about half that produced by *Thiobacillus thiooxidans* growing in similar soil, under similar conditions (350 μg S g soil^{-1}) (Pepper and Miller 1978); although the rate of sulphur oxidation by the *Streptomyces* isolate compares more favourably with that achieved by an heterotrophic bacterium when inoculated into autoclaved soil (40 μg S g soil^{-1}) (Pepper and Miller 1978). However, as autoclaving releases carbon and other nutrients from soils, the growth of the *Streptomyces* sp. would not be carbon-limited under these conditions.

A wide range of heterotrophic micro-organisms can oxidize reduced forms of sulphur (Wainwright 1984), but their role in the process may be limited by the fact that most non-rhizosphere soils appear carbon-deficient. This study shows however, that certain soil *Streptomyces* can grow and oxidize sulphur, albeit slowly, under such limitation. Sulphur-oxidation by these, and similar microorganisms, may therefore be significant in soils which are deficient in both thiobacilli and easily available organic carbon.

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