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Spirochaeta aurantia, a Pigmented, Facultatively Anaerobic Spirochete

JOHN A. BREZNAK AND E. CANALE-PAROLA

Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01002

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A strain of *Spirochaeta aurantia* was isolated from mud by a procedure involving migration of the organisms through cellulose ester filter discs (0.3-μm pore diameter) onto the surface of culture plates. The helical cells measured 0.3 by 10 to 20 μm during exponential growth. Electron microscopy showed the presence of two subterminally inserted axial fibrils partially overlapping in a 1-2-1 arrangement. An outer envelope, exhibiting a polygonal substructure, was observed. The spirochete grew either aerobically or anaerobically, with aerobic yields of 9.8×10^8 cells per ml and anaerobic yields of 3.0×10^8 cells per ml. The organism used carbohydrates, but not amino acids, as energy sources. Amino acids served as sole nitrogen sources, whereas inorganic ammonium salts did not. The presence of biotin and thiamine in the medium was required for growth. Growing cells fermented maltose mainly to carbon dioxide, hydrogen, ethyl alcohol, and acetic acid. Small amounts of formic and lactic acids, acetoin, and diacetyl were produced. Cells of *S. aurantia* growing aerobically produced a yellow-orange pigment. Chemical analysis indicated that the pigment was carotenoid in nature, its main component being lycopene or a similar compound. *S. aurantia* is not closely related to the leptospires, since it lacks both the hemolytic antigen and the hooked ends typical of the latter organisms. Furthermore, the guanine plus cytosine content in the deoxyribonucleic acid of *S. aurantia* (66.8 moles %) differs drastically from that of leptospires.

In 1926, Vinzent (25) briefly described a pigmented spirochete he isolated from polluted water. He named the organism *Spirochaeta aurantia* because of the yellow-orange color of its colonies. Since then, this spirochete has largely been ignored, no mention of it being made in classification keys (4), probably because the information included in the original description was not extensive.

In the course of a research project involving the isolation of free-living spirochetes from natural environments, we isolated an organism exhibiting characteristics identical with those of Vinzent's *S. aurantia*. The organism was especially interesting because, unlike other known spirochetes, it grew abundantly both aerobically and anaerobically. Furthermore, its ability to synthesize a pigment was a characteristic not observed in other spirochetes. The present report describes our studies on the morphology and physiology of this isolate designated *S. aurantia* strain J1.

MATERIALS AND METHODS

Isolation of S. aurantia. *S. aurantia* strain J1 was isolated by a method similar to that used by Canale-Parola et al. (7) for the isolation of *Spirillum gracile*. The isolation medium included (per 50 ml of distilled water): peptone and yeast extract, 0.1 g each; hay extract, 50 ml; agar, 1.0 g. The hay extract was prepared by boiling 0.5 g of dried barn hay in 100 ml of distilled water for 10 min. The boiled mixture was filtered through Whatman no. 40 filter paper and the filtrate was used for medium preparation. The *p*H of the isolation medium was adjusted to 6.5 before sterilization. The inoculum was a pond water-mud slurry from a duck pond in the vicinity of Urbana, Ill. The slurry was filtered through Whatman no. 40 filter paper to remove large particles. Approximately 0.1 ml of the filtrate was added to the center of each of six sterile cellulose ester filter discs (0.3-μm pore diameter; 47-mm disc diameter; Millipore Corp., Bedford, Mass.), each placed on the surface of a plate of isolation medium. After 24 hr at 30 C, the filter discs were removed aseptically from the plates, and incubation of the plates was continued. After 7 days, growth of spirochetes had developed in two of the plates. The growth appeared as a subsurface, semitransparent veil which diffused toward the periphery of the plate, away from colonies of other organisms. A pure culture of the spirochete, later identified as *S. aurantia* and designated strain J1, was obtained by conventional streaking procedures from the growth in one of the plates.

Culture media and nutrition studies. *S. aurantia* strain J1 was routinely grown, with or without aeration, in a maltose broth consisting of (g/100 ml of distilled water): maltose (C. P., Pfanstiehl), 0.2; peptone (Difco), 0.2; yeast extract (Difco), 0.4. The *p*H of the medium was adjusted to 7.5 before sterilization. Sterile 1 M potassium phosphate buffer, *p*H 7.0, was added to the sterilized medium to a final concentration of 0.01 M. The final *p*H of the medium was 7.2. Aerated cells were grown in 1-liter Erlenmeyer flasks containing 250 ml of maltose broth on a rotary shaker (New Brunswick Scientific Co., New Brunswick, N.J., model VS-100) operating at 120 rev/min. Maltose-agar medium was prepared by adding 1 g of agar per 100 ml of maltose broth.

Basal medium A, used in nutrition experiments, had the following composition (g/100 ml of distilled water): KH_2PO_4, 3×10^{-2}; Na_2HPO_4, 1.5×10^{-1}; $MgSO_4 \cdot 7H_2O$, 2×10^{-2}; $MnSO_4 \cdot H_2O$, 2×10^{-3}; adenine, guanine, and uracil, 10^{-4} each; thiamine·HCl, DL-calcium pantothenate, and nicotinic acid, 5×10^{-5} each; pyridoxine·HCl and riboflavine, 10^{-5} each; vitamin B_{12}, 5×10^{-6}; folic acid and biotin, 5×10^{-7} each. $MgSO_4$, $MnSO_4$, and the vitamins were sterilized separately. In experiments designed to study the vitamin requirements of *S. aurantia*, basal medium A was modified by adding vitamin-free casein hydrolysate (acid hydrolysis, salt-free; Nutritional Biochemicals Corp.) and maltose (0.5 g/100 ml each).

Basal medium B, used to study the utilization of carbon compounds by *S. aurantia* strain J1, had the following composition (g/100 ml of distilled water): vitamin-free casein hydrolysate, 0.5; thiamine·HCl, 5×10^{-5}; biotin, 5×10^{-7}; adenine, guanine, and uracil, 10^{-4} each; $MgSO_4 \cdot 7H_2O$, 2×10^{-2}; $MnSO_4 \cdot H_2O$, 2×10^{-3}; and potassium phosphate buffer, *p*H 7.0, to a final concentration of 0.01 M. To prepare this medium, concentrated solutions of the casein hydrolysate, vitamins, purines and pyrimidine, minerals, and buffer were sterilized separately and then combined.

In all nutrition experiments, the organisms were grown in test tubes (16 by 150 mm) each containing 10 ml of medium. The *p*H of the media used was adjusted to 7.2 to 7.4. Growth yields were determined turbidimetrically, 72 hr after the third transfer in the test medium, by means of a Klett-Summerson photoelectric colorimeter equipped with a 660-nm filter. Colorimeter readings were converted to cell numbers by using a standard curve relating the reading to direct cell counts. When a test medium did not support growth after 72 hr of incubation, the experiment was repeated by inoculating tubes containing the medium and observing the cultures up to 14 days. Incubation temperature was 30 C.

S. aurantia strain J1 was maintained on slants of maltose agar at 5 C. The cultures were transferred monthly.

Analyses of fermentation products. Cells were grown under N_2 in basal medium B to which 0.4 g of maltose per 100 ml was added. Gaseous fermentation products were determined by use of a fermentation train as described by Neish (19). The fermented medium was clarified (19) and the resulting solution was assayed for carbohydrates (maltose) by the anthrone reaction (1) and for acetoin and diacetyl (27). Samples of the neutral clarified fermentation solution were distilled (19), and the distillate was assayed for ethyl alcohol enzymatically (14). Ethyl alcohol was qualitatively identified by preparing the 3,5-dinitrobenzoate derivative (21) and by cochromatography of this derivative with standards (12). Volatile acids were separated from the fermented clarified medium by steam distillation (19) and isolated by partition chromatography on an acid-washed Celite 535 (Johns-Manville) column; the amount of each acid was determined by titration (19). Ether extracts (19) of neutral and acidic samples of the clarified fermentation solution were assayed colorimetrically for 2,3-butanediol (19) and lactic acid (2), respectively.

Ascending paper chromatography of the ether extracts (acidic) was employed for qualitative determination of fermentation acids. Washed no. 7 filter paper (Eaton-Dikeman Co., Mt. Holly Springs, Pa.) was used. The solvent systems were 95% ethyl alcohol-concentrated NH_4OH, 100:1 (v/v; reference 16), and 95% ethyl alcohol-concentrated NH_4OH-water, 80:5:15 (v/v; reference 3). Bromophenol blue was the indicator spray (16).

Pigment. All solvents used in extraction and chromatographic procedures were of reagent grade, redistilled and kept dry over anhydrous Na_2SO_4. Petroleum ether (boiling point, 30 to 60 C) was redistilled over concentrated H_2SO_4, and the 30 to 50 C fraction was collected and dried over anhydrous Na_2SO_4.

Cells were grown at 30 C in maltose broth, on a MicroFerm Laboratory Fermenter (New Brunswick Scientific Co.) operating at 200 rev/min, and aerated at a rate of 1.5 liters of air per min. Illumination was by means of a 40-w light bulb positioned 30 cm from the fermentor vessel. Cells were harvested when the culture reached a density of 7.5×10^8 cells per ml, and the pigment was extracted as described by Schmidt et al. (20). The combined acetone-methanol extracts were concentrated to a suitable volume under vacuum and saponified (13). The carotenoids were then transferred to petroleum ether, washed, dried (13), and stored under argon at −25 C.

For comparative purposes, carotenoids were extracted from tomato puree as follows. A 400-g amount of Progresso tomato puree (Uddo and Taormina Corp., Buena Park, Calif.) was lyophilized. Carotenoids were extracted from the dried material by the method mentioned above (20), except that a solution of acetone-methanol-carbon disulfide (7:3:3, v/v) was used. Subsequent concentration of carotenoids resulted in the formation of red crystals which were separated from the remaining solution by filtration, washed with 95% ethyl alcohol, and redissolved in carbon disulfide. No saponification step was included.

Separation of carotenoids was accomplished by multiple column chromatography. Neutral aluminum oxide (Calbiochem, Los Angeles, Calif., Neutral Alumina AG7, 100 to 200 mesh) was used as an adsorbant, as described by Weeks and Garner (26), except that the carotenoids were applied to the column as solutions in petroleum ether.

Estimation of the amount of carotenoid in hexane (Chromatoquality Reagent; Matheson, Coleman, and Bell, E. Rutherford, N.J.) solutions was made on a Beckman model DU-2 spectrophotometer by using the extinction coefficient for lycopene ($E^{mol}_{1cm} = 18.6 \times 10^4$ at 472 nm; reference 29).

Tentative identification of carotenoids was made on the basis of similarities in adsorption affinities, absorption maxima, and spectra to well-characterized carotenoids (15, 26, 28). Absorption spectra were determined in hexane with a Beckman recording spectrophotometer, model DK-1A.

Hemolytic (HL) antigen. Rabbits were injected in the marginal ear vein on the 1st and 6th days of immunization with 0.5 and 1.0 ml, respectively, of a living culture (5×10^8 cells/ml, grown in a modified maltose broth containing 0.05 g of yeast extract/100 ml). Cardiac bleedings (25 ml each) were taken 5 days before the first injection, and on the 4th, 5th, and 6th days after the last injection. The HL antigen was determined by the ability of a culture to induce synthesis (in rabbits) of antibody which reacted with known HL antigen, as described by Cox (9).

Microscopy. Cells for phase-contrast and electron microscopy were grown in maltose broth with aeration and harvested in the exponential phase of growth. Samples for negative staining were treated and examined as previously described (6), except that the cells were washed with and suspended in 0.01 M potassium phosphate buffer, *p*H 7.0, instead of distilled water.

Determination of guanine plus cytosine (GC) content in DNA. Cells of *S. aurantia* strain J1 were harvested by centrifugation and suspended in a solution containing NaCl (0.1 M) and sodium ethylenediaminetetraacetate (0.1 M) at *p*H 7.5. After lysis with sodium dodecyl sulfate (1%, w/v) and deproteinization with phenol (18), the GC content in the deoxyribonucleic acid (DNA) was determined by estimating the buoyant density in CsCl (18).

Other experimental procedures. Nitrite formation by cells grown anaerobically in maltose broth containing 0.1% (w/v) KNO_3 was detected as described by Conn et al. (8). Catalase tests were performed by observation of gas evolution from colonies and cell pellets flooded with a solution of H_2O_2 (3%, v/v).

Manometric experiments were conducted according to conventional techniques (23) at 30 C. Cells were suspended in 0.05 M potassium phosphate buffer, *p*H 7.0, (2.2×10^{10} cells/ml), and maltose was added to a final concentration of 16 μmoles/ml.

RESULTS

Morphology and growth characteristics. Most cells in cultures of *S. aurantia* strain J1 measured 0.3 by 10 to 20 μm during exponential growth (Fig. 1–3). Longer and shorter cells (5 to 50 μm) were also observed. The cells exhibited rather irregular and loose coils (Fig. 1, 3), sometimes with an angular appearance (Fig. 4). However, in young cultures, the coils in many cells appeared regular (Fig. 2). The cells were motile with a spinning motion around their longitudinal axis and with bending, undulating movements. Spherical bodies, similar to those formed by other spirochetes (6, 11, 24), were present in cultures (Fig. 5) either free or in association with cells. The spherical bodies generally measured from 0.5 to 2 μm in diameter, although they ranged up to 10 μm in diameter when the cells were incubated at 37 C (a temperature unfavorable to growth).

Electron microscopy showed that *S. aurantia* strain J1 exhibited morphological features common to all known spirochetes: (i) axial fibrils (Fig. 6–8), and (ii) a cell envelope or sheath surrounding both the protoplasmic cylinder and the axial fibrils (Fig. 6–8). *S. aurantia* strain J1 possessed two axial fibrils (Fig. 6, 7), each inserted at one end of the protoplasmic cylinder and overlapping with the other in the central portion of the cell (1-2-1 arrangement). The fibrils were inserted subterminally, approximately 0.35 μm from the end of the cell, by means of a disc-like insertion structure (Fig. 8). The sheath enclosing the protoplasmic cylinder appeared to be composed of polygonal substructural units (Fig. 8).

Colonies on maltose-agar plates were 2 to 4 mm in diameter, yellow-orange, round or nearly so, with slightly irregular edges, and growing mostly under the medium surface, but with a raised center. On plates of media low in carbohydrates (e.g., maltose-agar containing 0.01 g of maltose and 0.1 g of yeast extract/100 ml) the colonies tended to diffuse through the agar medium in the shape of almost perfect circles, probably because of chemotaxis towards the sugar. These colonies measured up to 10 mm in diameter, had a relatively low cell density, were semitransparent, and their pigmentation was not readily apparent. Anaerobic colonies on the surface of maltose-agar plates resembled those which developed aerobically on the same medium, except that they were not pigmented. Subsurface, anaerobic colonies in maltose agar were white, spherical, rather fluffy, approximately 1 mm in diameter. Growth in tubes of semisolid maltose-agar (0.2 g of agar/100 ml) developed 2 to 3 mm below the meniscus of the medium.

As previously mentioned, *S. aurantia* strain J1 grew either in the presence or absence of O_2. Aerobic cell yields in maltose broth were approximately 9.8×10^8 cells per ml, with a generation time of 3.8 hr at 30 C. Cultures grown anaerobically (in N_2) in the same medium yielded 3×10^8 cells per ml. Manometric studies indicated that cell suspensions of *S. aurantia* strain J1 consumed O_2 in the presence of maltose, yielding a respiratory quotient (CO_2 produced/O_2 consumed) of 1.01.

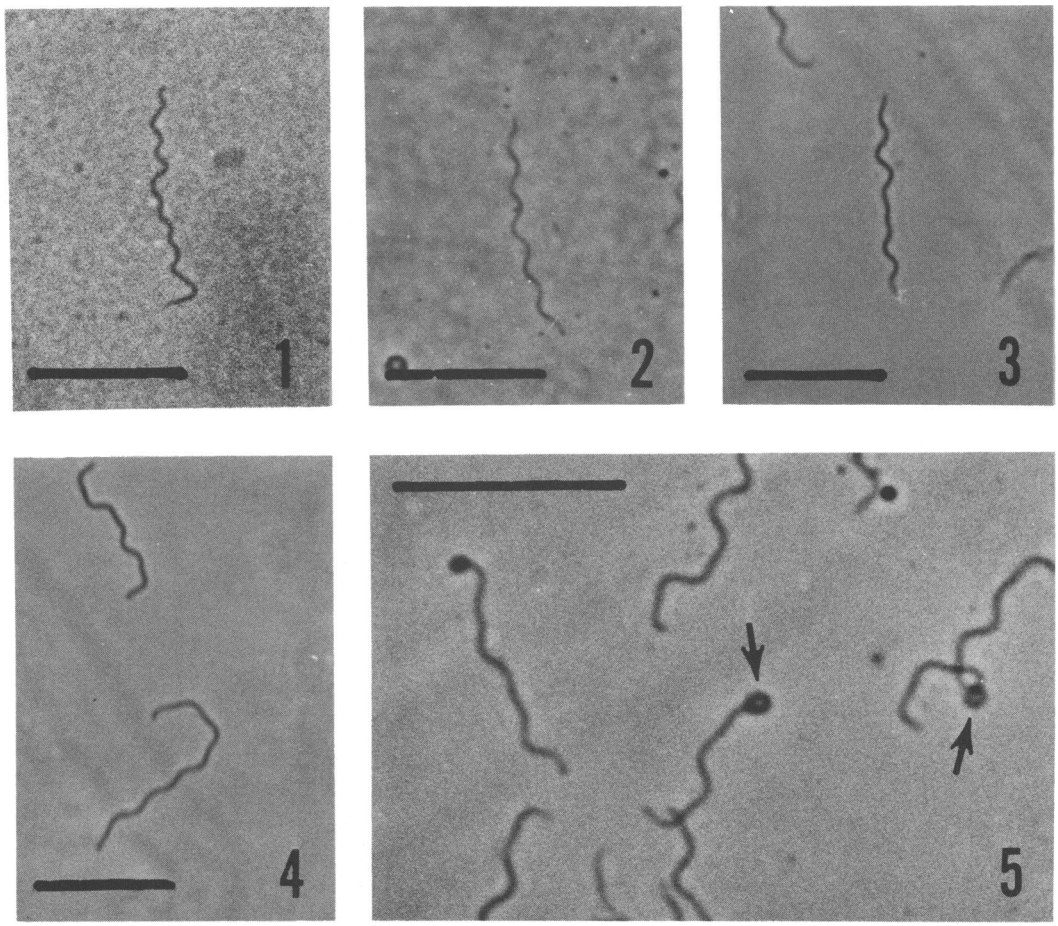

FIG. 1–5. *Phase-contrast photomicrographs of S. aurantia strain J1 (wet-mount preparations). Irregular coils (Fig. 1, 3), sometimes with an angular appearance (Fig. 4), are observed. Regularly coiled cells (Fig. 2) are common in young cultures. Spherical bodies (Fig. 5, arrows) are present in cultures. The marker bars represent 10 μm.*

Optimal growth was at 30 C. At 25 C, the rate of growth was slower and at 37 C the cells grew poorly or not at all. The highest growth yields were obtained when the initial *p*H of the medium was 7.0 to 7.3. *S. aurantia* strain J1 was gram-negative, catalase-positive, and reduced nitrate to nitrite when grown anaerobically.

Nutrition. *S. aurantia* strain J1 utilized carbohydrates, but not amino acids, as energy sources (Table 1). Amino acids served as sole sources of nitrogen, whereas inorganic ammonium salts or nitrate did not. Omission of all vitamins from the medium prevented growth (Table 1). Experiments in which individual vitamins were omitted from modified basal medium A indicated that an exogenous supply of thiamine and biotin was required for growth. Addition of adenine, guanine, and uracil to the media used in the nutrition studies resulted in slightly higher growth yields; however, the presence of these compounds in the medium was not required for growth.

To determine the nutritional versatility of *S. aurantia* strain J1, the ability of this organism to utilize various compounds was tested (Table 2). Carbohydrates were readily utilized, whereas other compounds tested, including intermediates of the Krebs cycle, generally were not.

Fermentation products. Because maltose supported the highest growth yields of all carbohydrates tested (Table 2), it was chosen as the substrate in studies on fermentation products. *S. aurantia* strain J1 growing anaerobically fermented maltose to the following products (expressed as μmoles per 100 μmoles of glucose fermented): CO_2, 165.3; H_2, 107.2; ethyl alcohol, 151.0; acetic acid, 69.2; formic acid, 5.2; lactic

acid, 1.0. Trace amounts (less than 1 μmole/100 μmoles of glucose) of acetoin and diacetyl were detected. The carbon recovery was 102% and the oxidation-reduction balance was 0.82. No detectable amount of 2,3-butanediol was present.

Pigment. Production of a yellow-orange pigment by *S. aurantia* strain J1 required aerobic growth conditions and was stimulated by illumination with white light (as observed visually). The cell-associated pigment exhibited solubility and absorption characteristics of carotenoids (15). Reaction of pigmented colonies with sulfuric acid yielded a blue color, an indication that the pigment was carotenoid in nature (22).

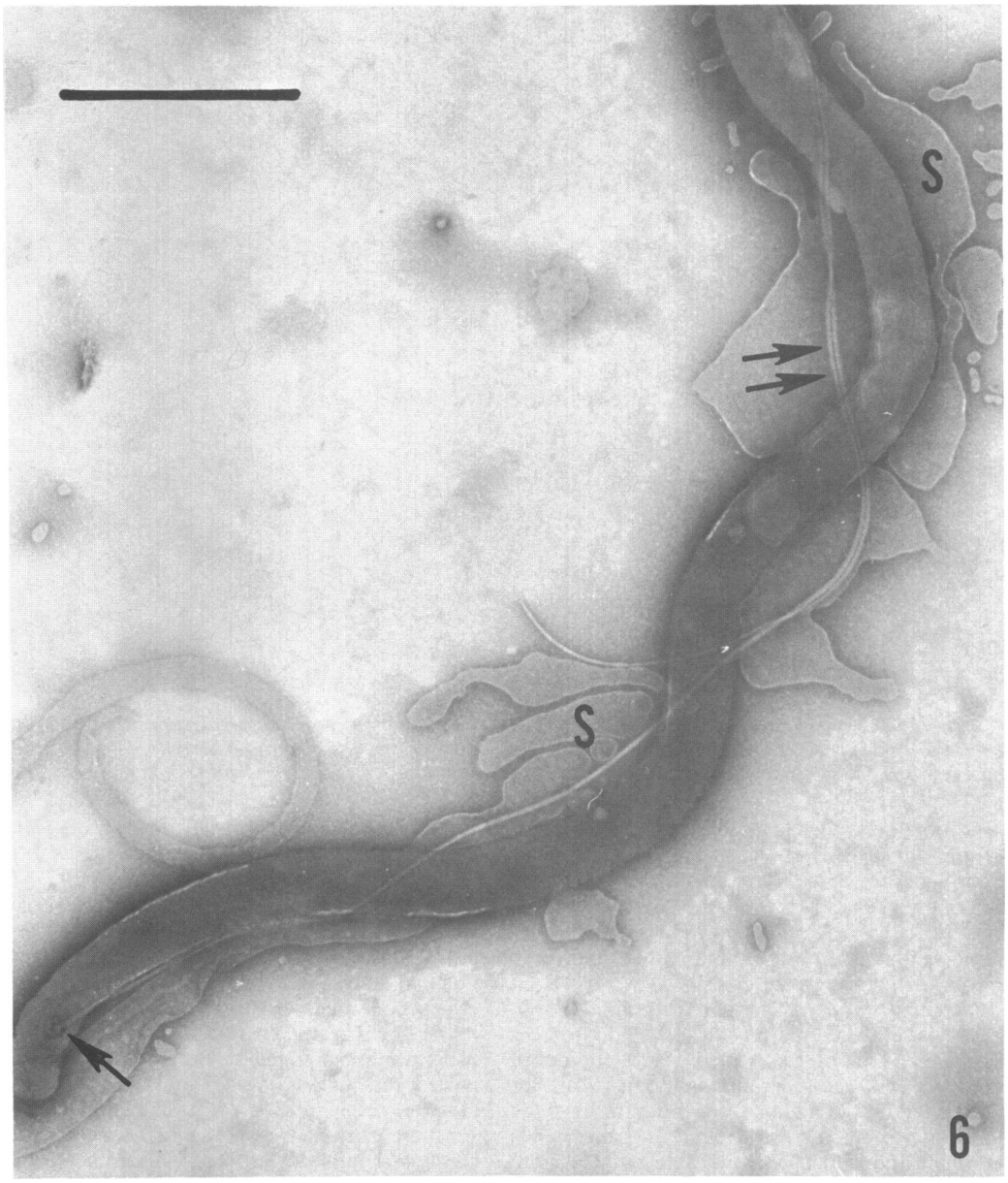

FIG. 6. *S. aurantia strain J1. Electron micrograph of approximately one-half of a cell. The insertion point (single arrow) of one of the axial fibrils is visible. The fibrils overlap in the central region of the cell (double arrows). The remains of the sheath or cell envelope (S), which disintegrated during preparation of the specimen, are apparent. Negative stain. The marker bar represents 1 μm.*

Preliminary purification procedures involving extraction of pigment with organic solvents, saponification, and column chromatography yielded numerous colored bands when neutral aluminum oxide was used as the column adsorbant. A successful procedure for isolating the major carotenoid components of *S. aurantia* strain J1 consisted of the column chromatographic systems outlined in Table 3. Fractions 3 and 4 comprised 30% and 62%, respectively, of the extractable colored carotenoids of *S. aurantia* strain J1. The absorption maxima of fraction 3 were 350 (shoulder), 363–364, 446–448 (shoulder), 470, and 501 nm. This pigment component was tentatively identified as an isomer of lycopene. Fraction 4, the major colored carotenoid, had absorp-

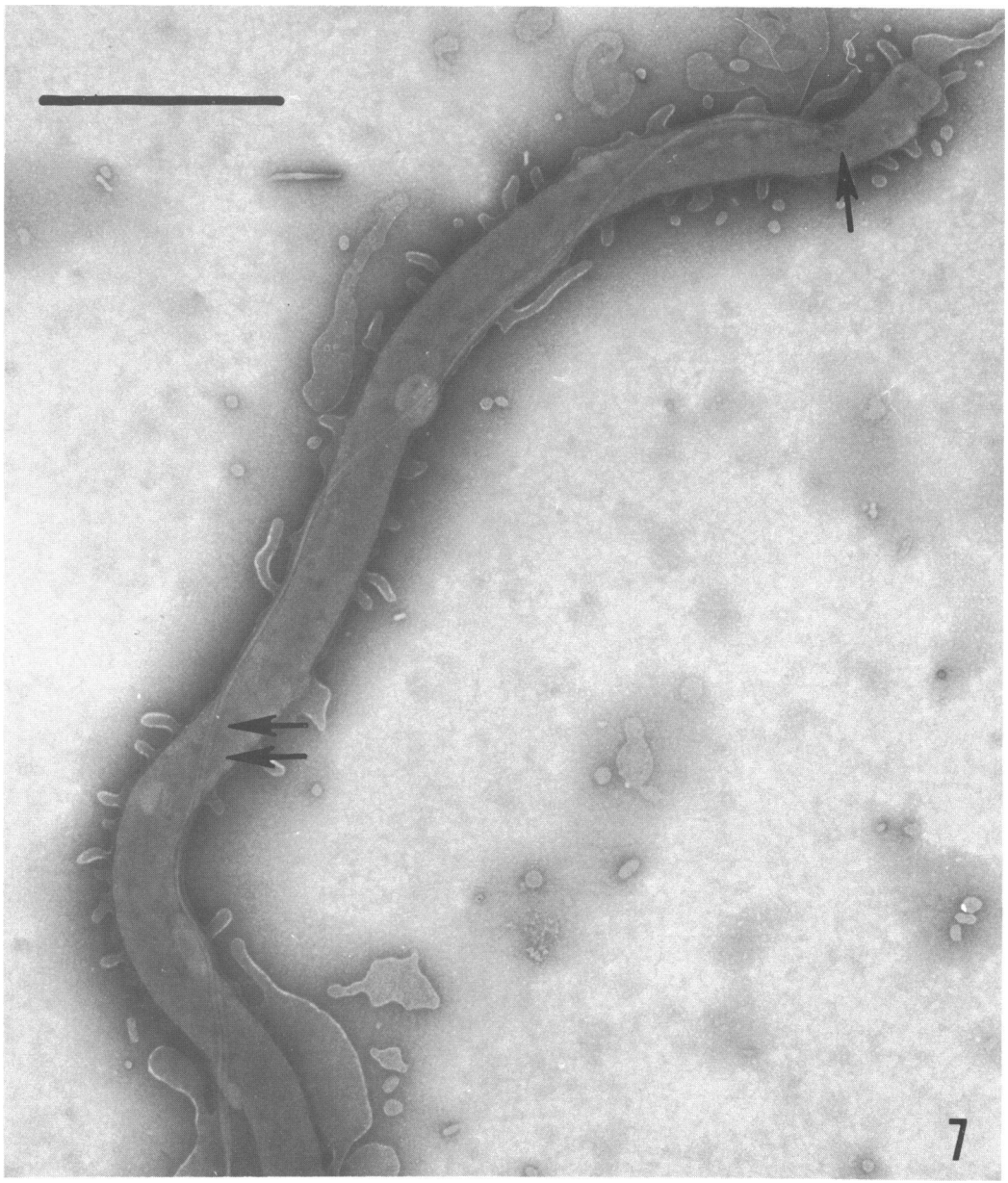

FIG. 7. *Electron micrograph of the remaining portion of the cell in Fig. 6. The axial fibril whose insertion is shown in Fig. 6 overlaps with the second fibril (double arrows). The single arrow indicates the insertion point of the latter fibril. Negative stain. The marker bar represents 1 μm.*

tion maxima at 447–448, 473, and 504 nm (Fig. 9) and was identified as *trans*-lycopene or a similar compound.

Tomato pigments, chromatographed in the same manner as the *S. aurantia* carotenoids, yielded a red band which eluted in the same position as fraction 2 in column 2 (Table 3). The absorption maxima of this carotenoid were 446–447, 473, and 504 nm (Fig. 9), which are typical of all-*trans*-lycopene (29).

The absorption spectrum of the pigment preparation from *S. aurantia* strain J1 was not as sharply defined as that of the *trans*-lycopene preparation from tomatoes (Fig. 9). This may be due either to a slight difference in chemical structure or to the presence of small amounts of *cis* isomer in the *trans*-lycopene preparation from the spirochete. Such isomerization may be of biological origin or may have arisen through accidental catalysis during preparation (28).

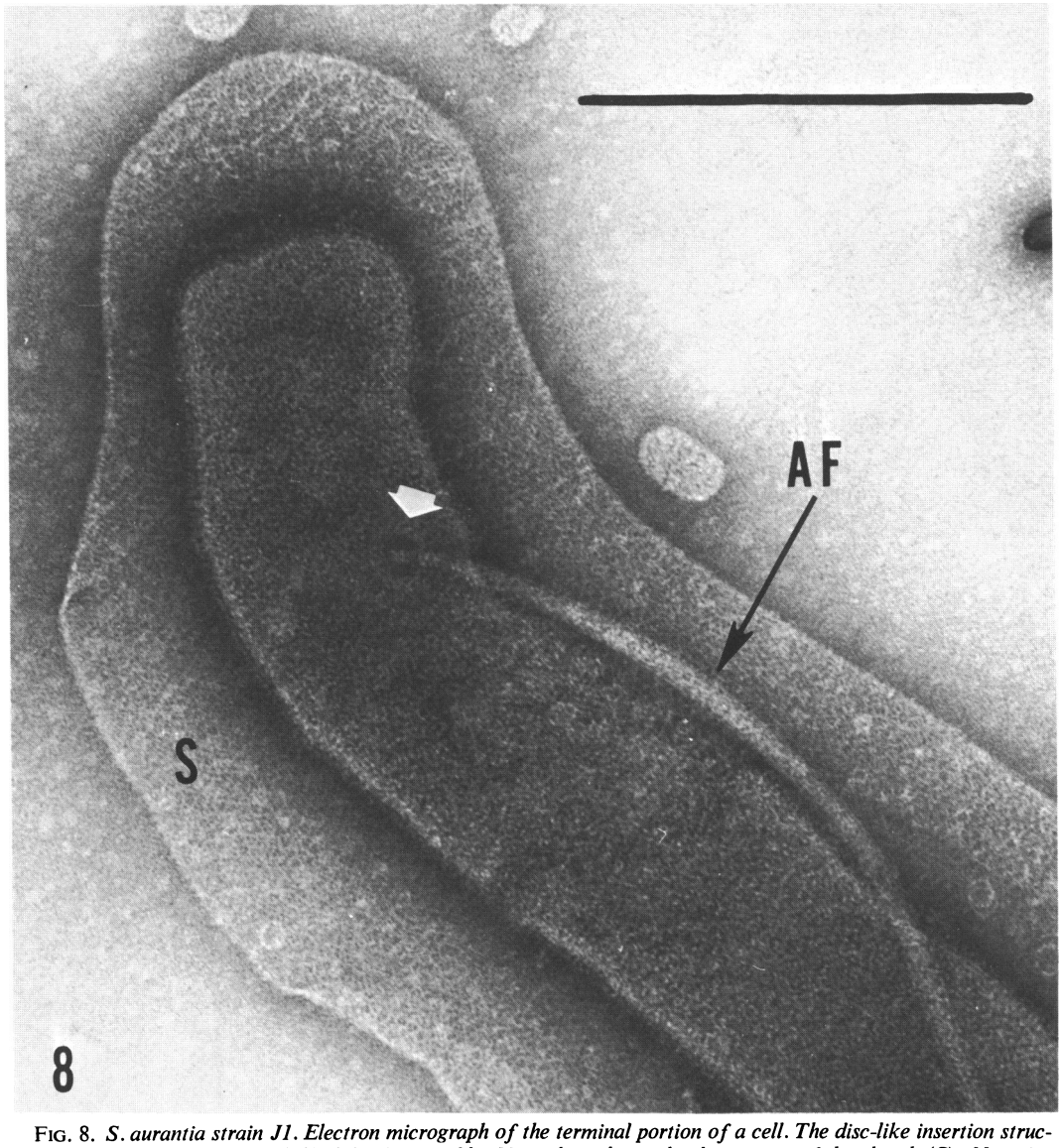

FIG. 8. *S. aurantia strain J1. Electron micrograph of the terminal portion of a cell. The disc-like insertion structure (white arrow) of an axial fibril (AF) is visible. Note the polygonal substructure of the sheath (S). Negative stain. The marker bar represents 0.5 μm.*

Relationship to other spirochetes. *S. aurantia* strain J1 had 66.8 moles % GC in its DNA. The HL antigen, characteristically found in species of *Leptospira* (9, 10), was not present in *S. aurantia* strain J1.

DISCUSSION

Comparison of the characteristics exhibited by Vinzent's strain of *S. aurantia* (25) with those of our isolate revealed marked similarities between the two organisms. Both spirochetes showed the same gross morphology, pigmentation, growth characteristics, and saccharolytic behavior, and both were isolated from similar environments. It was apparent that strain J1 possessed the general properties of *S. aurantia* described by Vinzent. On this basis, we concluded that our isolate was a strain of *S. aurantia*.

With the exception of the leptospires, known cultivable spirochetes generally do not use molecular oxygen as the final electron acceptor and grow best under anaerobic conditions. Although the ability of *S. aurantia* to grow aerobically suggests a relationship between this organism and the leptospires, pronounced differences exist between these strictly aerobic spirochetes and *S. aurantia*. The narrow, uniform coils and the hooked ends observed in cells of *Leptospira* are not present in *S. aurantia*. Nutritionally, the organisms are greatly dissimilar, since neither free-living leptospires isolated from water nor host-associated leptospires utilize sugars (J. B. Baseman, Ph.D. Thesis, University of Massachusetts, Amherst, 1968), whereas *S. aurantia* is a distinctly saccharolytic bacterium. Moreover, *S. aurantia* lacks the HL antigen characteristic of the genus *Leptospira*. Finally, the GC content in the DNA of *S. aurantia* (66.8 moles %) differs considerably from that in the DNA of leptospires, which, in strains that

TABLE 1. *Nutrition of S. aurantia strain J1*

Added[a] to basal medium A	Omitted from basal medium A	Growth yield (cells/ml)
No addition	—	NG[b]
Amino acids	—	NG
Amino acids, glucose	—	3.5×10^8
Amino acids, maltose	—	5.3×10^8
Amino acids, glucose (or maltose)	Vitamins	NG
NH_4Cl, glucose (or maltose)	—	NG
KNO_3, glucose (or maltose)	—	NG

[a] Amino acids were added as vitamin-free casein hydrolysate (0.5 g/100 ml). Final concentrations of other compounds added (g/100 ml): glucose and maltose, 0.5 each; NH_4Cl and KNO_3, 0.1 each. All compounds added were sterilized separately as individual solutions.

[b] No apparent growth.

TABLE 2. *Utilization of carbon compounds by S. aurantia strain J1*

Added[a] to basal medium B	Growth yield (cells/ml)	Added[a] to basal medium B	Growth yield (cells/ml)
No addition	NG[b]	L-Arabinose	3.4×10^8
D-Glucose	2.0×10^8	Glycerol	0.9×10^8
D-Fructose	4.4×10^8	Mannitol	1.0×10^8
D-Galactose	2.1×10^8	Sorbitol	NG
D-Mannose	1.7×10^8	Dulcitol	NG
L-Rhamnose	0.8×10^8	Potassium pyruvate	NG
L-Sorbose	NG	Sodium acetate	NG
Maltose	5.4×10^8	Sodium lactate	NG
Sucrose	4.5×10^8	Sodium succinate	NG
Lactose	1.2×10^8	Potassium α-keto-glutarate	NG
Cellobiose	3.5×10^8	Potassium fumarate	NG
Trehalose	3.2×10^8	Ethyl alcohol	NG
Raffinose	NG	Potassium gluconate	1.1×10^8
Dextrin	0.9×10^8	Allantoin	NG
Starch (soluble)	0.4×10^8	Uric acid	NG
Inulin	1.6×10^8	Orotic acid	NG
D-Ribose	NG	Sorbitan monooleate polyoxyethylene (Tween 80)	NG
D-Xylose	2.8×10^8		

[a] Sterilized separately and added at a final concentration of 0.2% (w/v). Tween 80 was added at a final concentration of 0.02% (v/v).

[b] No apparent growth.

TABLE 3. *Separation of major colored carotenoids of S. aurantia strain J1 by column chromatography*

Column no.	Neutral alumina activity grade[a]	Procedure	Eluant[b]	Eluted from column
1	III	Separation of major components from extract	0–4[c] 5	Minor yellow and yellow-orange bands Major orange band (fraction 1)
2	I	Purification of fraction 1	0–10[c] 11, 12	No colored bands Major orange band (fraction 2). Minor yellow band remained on column.
3	I	Purification of fraction 2	pure acetone	Yellow band (fraction 3), followed by red band (fraction 4).

[a] Brockmann and Schodder (5).
[b] Per cent acetone in petroleum ether.
[c] Stepwise increments of 1%.

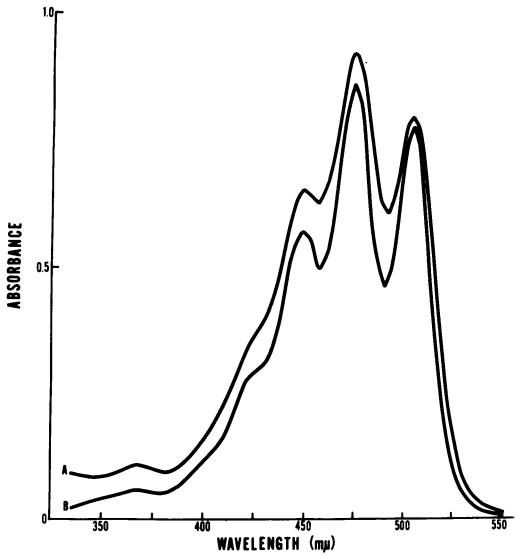

FIG. 9. *Absorption spectra in hexane of major carotenoid from S. aurantia strain J1 (A), and of a trans-lycopene preparation from tomatoes (B).*

were examined, ranges from 34.7 to 38.8 moles % (E. Canale-Parola et al., *submitted for publication*). Thus, *S. aurantia* may be a representative of a group of spirochetes which is phylogenetically distant from the leptospires.

The products of glucose fermentation by *S. aurantia* are similar to those of *Spirochaeta stenostrepta* (6; E. Canale-Parola et al., *submitted for publication*). Ethyl alcohol, acetic acid, CO_2, and H_2 are major products of both spirochetes, and both form small amounts of lactate and formate. However, *S. stenostrepta* is a strict anaerobe and its morphology is distinctly different from that of *S. aurantia*. It remains to be determined whether the similarity in fermentation products reflects a similarity in anaerobic energy-yielding pathways.

Polygonal subunits analogous to those present in the cell envelope of *S. aurantia* were observed by Listgarten and Socransky (17) in cell envelopes of oral spirochetes. This type of cell envelope substructure may have widespread occurrence among spirochetes.

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