Metabolism of *Spirochaeta aurantia*

II. Aerobic Oxidation of Carbohydrates

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Summary. 1. Growing cells of *Spirochaeta aurantia* performed an incomplete aerobic oxidation of glucose to CO₂, acetate, pyruvate, and lactate. Approximately half of the glucose carbon metabolized was incorporated into cell material. Slightly more than one-third of the assimilated sugar carbon was recovered in cell lipids which comprise a large proportion (29 to 36%) of the dry weight of *S. aurantia*.

2. Aerobic and anaerobic molar growth yield determinations indicated that *S. aurantia* derives more energy from the aerobic oxidation of maltose than from fermentation of this sugar and suggested the presence of an oxidative phosphorylation mechanism in this bacterium.

3. O₂-dependent NADH dehydrogenase activity, cytochrome b₅₆₃, and cytochrome c were associated primarily with the particulate portion of cell extracts of *S. aurantia*. The latter two pigments may be one and the same hemoprotein. No a or c-type cytochromes were detected in this spirochete. Protoheme, but not heme a or mesoheme, was detected in *S. aurantia*.

*Spirochaeta aurantia*, when growing anaerobically, utilizes the Embden-Meyerhof pathway, coupled with a clostridial-type pyruvate decarboxylation reaction, for the fermentation of glucose to ethanol, acetate, CO₂ and H₂ (Breznak and Canale-Parola, 1969, 1972). However, *S. aurantia* is a facultative anaerobe and uses O₂ as terminal electron acceptor (Breznak and Canale-Parola, 1969). Thus, this bacterium has the ability to exist in aerobic natural environments and, therefore, possesses an ecological advantage with respect to the strictly anaerobic species of *Spirochaeta*.

Since it seemed possible that *S. aurantia* derives more energy from the aerobic oxidation of carbohydrates than from their fermentation, we have studied the aerobic metabolism of this saccharolytic spirochete. Efforts were directed at obtaining an *in vivo* assessment of aerobic versus anaerobic energy yields, and at characterizing biochemically some of the components of the aerobic electron transport system in *S. aurantia*.

Materials and Methods

**Organism and Growth Conditions.** *Spirochaeta aurantia* strain J1 was routinely grown aerobically at 30°C in 2-liter Erlenmeyer flasks containing 1 liter of MTY medium (Breznak and Canale-Parola, 1972) and aerated on a rotary shaker (model...
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VS-100; New Brunswick Sci. Co., New Brunswick, New Jersey, U.S.A.) operating at 250 rev/min. Cells were also grown in maltose broth (Breznak and Canale-Parola, 1969) under the same conditions.

Escherichia coli strain B (ATCC 11308) was grown at 37°C, under similar aeration conditions, in medium C (Roberts et al., 1957) containing 1% glucose.

Products of Sugar Oxidation. Products of sugar oxidation by growing cells were determined using a "respiration train" modeled after a fermentation train described by Neish (1952) except that sterile CO₂-free air was used as carrier gas. Erlenmeyer flasks containing one-half their volume of growth medium (see below) were used as culture vessels. Aeration was by means of a gas dispersion tube inserted into the vigorously stirred medium. A volume of carrier gas, equivalent to the volume of the culture fluid, was passed through the culture every minute. At the end of growth, HCl was added and the culture was swept with carrier gas for an additional 12 h before Zn(OH)₂ clarification (Neish, 1952).

The semi-defined growth medium used in the respiration train was basal medium B (Breznak and Canale-Parola, 1969) to which 0.4 g maltose (C. P., Pfannstiehl), or glucose (Difco), per 100 ml was added as energy source. A 1% (v/v) inoculum, containing 6 × 10⁸ cells/ml was used and the culture was acidified after 24 h incubation at 30°C. At this time most of the cells had lost their motility, and numerous spherical bodies (Breznak and Canale-Parola, 1969) were present in the culture. The cell density prior to acidification was 9 to 10 × 10⁸ cells/ml.

Sugars, and end products of sugar oxidation, were assayed by methods described previously (Breznak and Canale-Parola, 1969 and 1972).

2,4-Dinitrophenylhydrazone derivatives of keto acids were prepared by the method of Le Page (1950), and identified by co-chromatography with standards on Whatman #4 paper using n-butanol-ethanol-0.5 N NH₄OH, 7:1:2 (v/v; Block et al., 1959) as solvent system. To confirm identification, hydrazone derivative spots were cut from chromatograms, eluted with Na₂CO₃ and hydrogenated to the corresponding amino acid by the procedure of Meister and Abendschein (1956). The resulting amino acids were then chromatographed in acetic acid-n-butanol and hydrochloric acid-i-propanol solvent systems (Fink et al., 1963).

Purification of Lipids. Cells were harvested by centrifugation and washed twice with 0.05 M N-2-hydroxyethylpiporazine-N'-2-ethanesulfonic acid (HEPES) solution adjusted to pH 7.2 with KOH. Total lipids in the cell pellet were extracted according to the method of Takaes and Holt (1971), except that an initial acetonemethanol (7:3, v/v) extraction was performed and pooled with the total extractable lipids. Pooled extractable lipids, and bound lipids, were purified by Sephadex G-25 column chromatography (Wells and Dittmer, 1963).

Preparation of Cells and Extracts. Cells used for manometric experiments were harvested by centrifugation and suspended in 0.1 M potassium phosphate buffer (pH 7.0). Late exponential phase cells used for extract preparation were washed once with, and suspended in, the above-mentioned phosphate buffer containing 5 × 10⁻³ M MgCl₂ (Mg-PO₄ buffer). Extracts, prepared by French pressure cell treatment or sonic disruption (Breznak and Canale-Parola, 1972), were fractionated by differential centrifugation (Fig. 1). Fraction S₁ was routinely used for cytochrome spectra and enzyme assays. Fractions S₂ (pale yellow) and P₂S (red-orange) constituted the "soluble" and "particulate" portions of fraction S₁, respectively, and were used to locate electron transport components of S. aurantia.

Radioactive Measurements. The procedure used was described previously (Breznak and Canale-Parola, 1972). Radioactivity of cells, which had incorporated ¹⁴C-labeled compounds, was determined after the cells were washed three times with distilled water, or with 0.05 M HEPES (pH 7.2).