Desulfovibrio africanus sp. n., a New Dissimilatory Sulfate-reducing Bacterium

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Abstract

Campbell, L. Leon (University of Illinois, Urbana), Mary A. Kasperzycki, and John R. Postgate. Desulfovibrio africanus sp. n., a new dissimilatory sulfate-reducing bacterium. J. Bacteriol. 92:1122–1127. 1966.—The strains Benghazi and Walvis Bay can be distinguished from 40 strains of Desulfovibrio and from D. gigas on the basis of morphological and immunological studies. Electron microscopy revealed polar lophotrichous flagellation similar to that of D. gigas but different from the characteristic single polar flagellum of the 40 strains of Desulfovibrio. Immunological evidence shows that the two strains are related to members of the genus Desulfovibrio but possess several common antigenic components not present in the other strains tested. The deoxyribonucleic acid of both strains has a buoyant density of 1.724 g/cc and a guanine plus cytosine content of 60.2%. Cell-free extracts of both organisms show absorption bands of cytochrome C₃ and desulfoviridin, characteristic for Desulfovibrio. The two organisms carry out the sulfate-linked lactate fermentation and neither will grow in the absence of sulfate. Both strains contain the enzymes of the dissimilatory pathway of sulfate reduction. Therefore, these studies have demonstrated that the Benghazi and Walvis Bay strains should be regarded as taxonomically distinct from other species of Desulfovibrio.

In a previous study (16) it was shown that 30 strains of nonsporulating dissimilatory sulfate-reducing bacteria could be divided into three groups defined by their deoxyribonucleic acid (DNA) base composition. During the study we noted that two of the strains could be distinguished from the others by a consistent difference in their morphology. In this paper we show that these two strains represent a new species, for which the name Desulfovibrio africanus is proposed.

Materials and Methods

Organisms. The morphology of 42 strains of Desulfovibrio and one strain of D. gigas (8) was studied. Thirty of the strains were studied previously (16); the 12 additional strains were obtained from J. D. A. Miller. The two strains that could be distinguished morphologically from all other strains of Desulfovibrio and from D. gigas were Benghazi (NCIB 8401) and Walvis Bay (NCIB 8397). The Benghazi strain was originally isolated from well water from Benghazi, Libya. The Walvis Bay strain was originally isolated from a marine mud sample from Walvis Bay, S. W. Africa. Both strains were obtained from the National Collection of Industrial Bacteria, Aberdeen, Scotland. Unless noted otherwise, the use of the names Benghazi and Walvis Bay will refer to these two strains. All cultures were checked for purity by the agar shake-tube method described by Postgate (12). Media. Stock cultures were maintained in Baars’ medium (4) supplemented with yeast extract (0.1%, w/v) and, for strains of marine origin, with NaCl (2.5%, w/v). Walvis Bay, being of marine origin, was routinely grown in media containing 2.5% NaCl; the readiness with which this strain grows in freshwater media indicates, however, that NaCl is not required for growth.

Carbon source utilization studies were carried out in the medium C of Butlin, Adams, and Thomas (6) with lactate replaced by the appropriate carbon source at a concentration of 0.2% (w/v). Cells for other studies were grown in modified medium C (5), in medium N (16), or in Baars’ medium (4) supplemented with yeast extract (0.1%, w/v). Filter-sterilized (Millipore) Na₂S (1 mm) was added to poised the Eₘ except in the case of D. gigas, for which sodium ascorbate (0.5 mm) was used. For strains of marine origin, all media contained 2.5% (w/v) NaCl, except where otherwise mentioned.

Growth tests without sulfate were performed in Postgate’s sulfate-deficient medium (11); Na₂S was not added, thus avoiding contamination by the Na₂SO₄, normally present in commercial Na₂S.
Cultivation. Anaerobiosis was obtained with pyrogallol plugs made alkaline with a solution containing K₂CO₃ (15%, w/v) and NaOH (10%, w/v). After incubation for 24 to 36 hr at 30 C, cell yields were estimated in a Klett-Summerson photoelectric colorimeter with a 66 filter. Cell yields were converted to dry weight equivalents (per milliliter) of the Hildenborough (NCIB 8303) strain of *Desulfovibrio*.

Microscopy. Cell morphology was routinely monitored by phase-contrast microscopy. Cells were prepared for phase-contrast photography as described by Welker and Campbell (17). Photographs were recorded on Adox KB-14 film with a Zeiss phase photomicroscope.

Cells from a 12-hr culture grown in modified medium C were used for electron microscopy. A drop of cells was placed on carbon-coated Formvar stainless-steel grids; the grids were touched to water for 10 sec and then shadowed with gold at an angle of 27°. The grids were examined in a JEM T6-S electron microscope, and electron micrographs were made at initial magnifications of 5,000 X.

Enzyme assays. Cell-free extracts were prepared as described by Akagi and Campbell (1). Protein was estimated by the method of Lowry et al. (9) with bovine serum albumin as the standard. Hydrogenase was measured manometrically at 30 C with benzyl viologen as the electron acceptor (1). Adenosine triphosphate (ATP) sulfurylase activity was determined as described by Akagi and Campbell (2). Adenosine-5'-phosphosulfate (APS) reductase and 3'-phosphoadenosine-5'-phosphosulfate (PAPS) reductase were assayed by the methods of Peck (10). Inorganic pyrophosphatase was measured as described by Akagi and Campbell (3).

Cytochrome c₅ and desulfoviridin. Cell-free extracts were examined for cytochrome c₅ (13) with a Cary recording spectrophotometer. Reduced extracts were prepared by the addition of a small amount of solid sodium hydrosulfite, just prior to examination.

Desulfoviridin was detected by its characteristic absorption spectrum (13) and by the fluorescence test of Postgate (14).

Analytical methods. Lactate, acetate, carbon dioxide, and hydrogen sulfide were determined by the methods employed by Baker et al. (5).

Serology. The immunization procedures for obtaining rabbit sera against sulfate-reducing bacteria were those described previously (7). Ouchterlony immunodiffusion studies were carried out as described by Postgate and Campbell (15), except that the plates were incubated at 16 C. Cross-absorptions were performed by using 8 to 10 mg (dry weight equivalent) of frozen-and-thawed bacteria per ml of serum and removing the sediment by centrifugation after 20 to 30 min at ambient room temperature.

DNA base composition. DNA was isolated and the base composition determined by the methods used by Saunders et al. (16).

RESULTS

Morphology. Morphologically, Benghazi and Walvis Bay are different from the other 40 strains of *Desulfovibrio* studied and from *D. gigas*. Figure 1A shows the morphology of the Hildenborough strain, which is typical of the other strains examined. These strains are all small (3 to 5 X 0.5 to 1 μ) vibrios. Benghazi (Fig. 1B) and Walvis Bay (Fig. 1C) are slender, sigmoid rods, 5 to 10 X 0.5 μ. The morphology of these two strains does not change when they are grown in a variety of freshwater or saline media. *D. gigas* is a large spirillloid organism, 5 to 10 X 1.2 to 1.5 μ (Fig. 1D). Electron micrographs show that Benghazi and Walvis Bay possess polar lophotrichous flagella. Figure 2 shows the flagella arrangement of Benghazi. The flagellation pattern of these two strains is similar to that observed with *D. gigas* but differs from that characteristic of the other 40 strains of *Desulfovibrio* (single polar flagellum).
Immunological studies. Ouchterlony plates were prepared to examine the immunological relationships of Benghazi and Walvis Bay with *D. gigas* and with representatives of the three DNA base composition groups of *Desulfovibrio* (16). Figure 3 demonstrates that Walvis Bay shows some cross-reaction with the antisera of Hildenborough (DNA, 60.2% GC), Essex (DNA, 54.4% GC), British Guiana (DNA, 45.6% GC), and *D. gigas* (DNA, 60.2% GC), indicating a relationship at the genus level. (GC refers to the guanine plus cytosine content.) Walvis Bay reacted strongly with its homologous antiserum and with Benghazi antiserum, indicating a close relationship between these two strains. When Benghazi was used as the antigen in the above experiment, similar results were obtained. An examination of the cross-reactions of Benghazi and Walvis Bay with Benghazi antiserum revealed that the two strains possessed several common antigens but that Benghazi contains some antigenic components that are not present in Walvis Bay (Fig. 4). A similar experiment, with Walvis Bay antiserum, also showed that Walvis Bay contains some antigens that are not present in Benghazi (Fig. 5). These observations were confirmed by homologous and heterologous cross-absorption studies. Absorption of anti-Walvis Bay serum with Benghazi reduced the number of bands cross-reacting with Walvis Bay, and completely removed the bands cross-reacting with Benghazi (Fig. 5). Similarly, absorption of anti-Benghazi serum with Walvis Bay reduced the number of bands cross-reacting with Benghazi,
and completely removed the bands cross-reacting with Walvis Bay. Homologous absorption of either antiserum completely removed the bands cross-reacting with either antigen.

Substrate utilization. The range of substrates utilized by the various strains of *Desulfovibrio* is not well established because of divergencies in both published and unpublished observations from different laboratories. In this study, growth on a given substrate was accepted as positive only if it remained in excess of the yeast extract blank for two or more subcultures. Benghazi and Walvis Bay utilized lactate, malate, pyruvate, and ethyl alcohol, giving cell yields from 160 to 210 \( \mu g \) (dry weight equivalents) per ml of culture. Both strains grow through at least five successive transfers on the above substrates. Neither strain could grow in the absence of sulfate. Neither strain could grow on the following compounds: dulcitol, galactose, glucose, lactose, maltose, raffinose, salicin, sucrose, xylose, acetate, butyrate, citrate, formate, propionate, succinate, tartrate, oxamate, butanol, methanol, glycerol, and choline.

Respiratory pigments. Cell-free extracts of Benghazi and Walvis Bay show the characteristic absorption bands of cytochrome \( c_6 \), with maxima at 419, 525, and 553 nm (when reduced with sodium hydrosulfite), and of desulfoviridin at 630 nm. Cell suspensions of both strains give a positive fluorescence test for desulfoviridin (14). These two pigments are diagnostic of the genus *Desulfovibrio*. A purified preparation of Benghazi cytochrome \( c_6 \) does not show any cross-reaction with antisera tested against the cytochrome \( c_6 \) of the Hildenborough or "cholinicus" strains of *Desulfovibrio* (Drucker, Kasprzycki, and Campbell, unpublished data).

DNA base compositions. Benghazi and Walvis Bay DNA had identical buoyant densities of 1.724 g/cc, and a calculated GC content of 60.2\%, thus confirming the previous values reported by Saunders et al. (16). The DNA base composition of these two strains is identical to that of *D. gigas* and three other strains in the group I of Saunders et al. (16). Their morphological and immunological properties, however, clearly separate these two strains from the others; thus, the DNA base composition overlap may be regarded as coincidental.

Lactate-sulfate fermentation. Benghazi and Walvis Bay carry out the sulfate-linked fermentation of lactate characteristic of members of the genus *Desulfovibrio*. Table 1 shows that both strains effect this fermentation according to the equation:

\[
2 \text{ lactate} + \text{Na}_2\text{SO}_4 \rightarrow 2 \text{ acetate} + 2 \text{ CO}_2 + 2\text{H}_2\text{O} + \text{Na}_2\text{S}
\]

Enzymes of the dissimilatory pathway of sulfate reduction. Peck (10) has shown that members of the genus *Desulfovibrio* utilize the APS pathway of sulfate reduction. It was of interest, therefore, to determine the presence of the enzymes of this
pathway in Benghazi and Walvis Bay. Table 2 shows that cell-free extracts of both organisms contain high levels of the enzymes necessary for this pathway, and that PAPS reductase (characteristic of assimilatory sulfate-reducers) is not detectable.

**DISCUSSION**

The physiological and biochemical data show that Benghazi and Walvis Bay belong to the genus *Desulfovibrio*. The morphological and immunological data clearly distinguish them from the other strains of *Desulfovibrio* and from *D. gigas*. Owing to the fact that these two strains were isolated from sources in Africa, we propose the specific designation of *D. africanaus*.

### Desulfovibrio africanaus n. sp.

*africanaus*. L. adj. *africanaus* pertaining to Africa

**Morphology:** Nonsporulating, slender, sigmoid rods, 5 to 10 × 0.5 μ. Gram-negative. Shows rapid progressive motility and polar lophotrichous flagella.

**Culture:** Obligate anaerobe. Temperature, 28 to 40 C. Has wide salt tolerance.

**Carbon sources:** Utilizes lactate, malate, pyruvate, and ethyl alcohol if sulfate is present. Acetate accumulates as the end product of lactate fermentation, and sulfate is reduced. Does not utilize dulcitol, galactose, glucose, lactose, maltose, raffinose, salcin, sucrose, xylose, acetate, butyrate, citrate, formate, propionate, succinate, tartrate, oxamate, butanol, methanol, glycerol, or choline.

**Pigments:** Contains cytochrome $c$ and desulfovirdin.

**Enzymes:** Contains ATP sulfurylase, APS reductase, inorganic pyrophosphatase, and hydrogenase. Does not contain PAPS reductase.

**DNA base composition:** Contains 60.2% GC.

**Source:** Freshwater or salt water from Africa.

**Holotype:** Strain Benghazi. A salt water strain capable of growth in freshwater is Walvis Bay.

Subcultures of both strains are deposited in the National Collection of Industrial Bacteria, Aberdeen, Scotland. Benghazi (NCIB 8401); Walvis Bay (NCIB 8397).

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### LITERATURE CITED


