JOURNAL OF BACTERIOLOGY, Feb. 1969, p. 589-593
Vol. 97, No. 2

Characterization of *Actinomyces israelii* Serotypes 1 and 2[1]

DARRELL W. BROCK[2] AND LUCILLE K. GEORG

Health Services and Mental Health Administration, National Communicable Disease Center, Atlanta, Georgia 30333

Received for publication 7 October 1968

In a previous serological study, we compared 14 isolates of *Actinomyces israelii* serotype 2 with 13 serotype 1 cultures. The present study reports the morphological, physiological, and biochemical characteristics of these same 27 cultures. All of the isolates exhibited similar cellular morphology, and all but one produced the typical spider type microcolony on solid media. Twelve of 13 serotype 1 isolates produced the molar tooth type macrocolony, whereas only 2 of 14 serotype 2 cultures produced this type of rough colony. All of the serotype 1 isolates fermented arabinose with the production of acid; none of the serotype 2 cultures fermented this carbohydrate. All 27 cultures produced the greatest amount of growth when cultured under anaerobic conditions and grew poorly or not at all in air. Both groups of organisms produced similar reactions on other biochemical test media; these findings suggested that *A. israelii* serotype 2 should not be given a species designation.

Several investigators (1, 4, 5) have reported the existence of serotypes of *Actinomyces israelii*. In a previous study, we (2) reported on the serological analysis of 27 isolates of *A. israelii* serotypes 1 and 2. This paper reports the results of morphological and biochemical characterization studies on the same 27 cultures. These studies were performed to (i) confirm culture identifications, (ii) evaluate the various procedures used to identify this species in the diagnostic laboratory, and (iii) determine whether morphological, physiological, and biochemical variations could be detected that would relate to the demonstrated serological differences.

MATERIALS AND METHODS

Cultures studied. The 13 *A. israelii* serotype 1 cultures studied included National Communicable Disease Center (NCDC) cultures W855, W726, A601, X522, X372, X373, W721, A905, W909, W911, W912, W1066, and W1067. The serotype 2 cultures studied were NCDC cultures W838, X695, W748, W940, W1009, W1010, W1011, W1122, W1123, W1124, W1125, W1126, W1128, and W1129. The sources of these cultures and other identifying information were given in the accompanying publication (2).

Morphology. Colonial morphology was determined by streaking inoculum from 3- to 5-day Reinforced Thioglycollate (RT) Broth (1.5 g of dehydrated Trypticase Soy Broth and 1.25 g of Tryptose Broth per liter of Thioglycollate Broth) cultures on Brain Heart Infusion (BHI) Agar plates. The plates were incubated at 37 C in an anaerobic jar with a Gaspak (BBL) envelope activated according to the manufacturer's directions. After 12 to 36 hr of incubation, the plates were examined microscopically (100 ×) to determine the morphology of microcolonies. Mature colonies were examined with a dissecting microscope (40 ×) after 7 to 10 days of incubation. Cellular morphology was determined by examining (970 ×) Gram-stained smears of 3- to 5-day RT Broth cultures.

Oxygen requirements. The oxygen requirements of the various isolates were determined by inoculating six BHI Agar slants with a 3- to 5-day RT Broth culture and incubating them aerobically, microaerophilically (CO_2 seal; 5 drops each of 1 M Na_2HPO_4 and 10% Na_2CO_3 in a cotton pledget under a rubber stopper), and anaerobically (anaerobic seal; 5 drops each of saturated pyrogallol and 10% Na_2CO_3 in a cotton pledget under a rubber stopper) according to the method of Georg et al. (3).

Biochemical tests. The following biochemical tests were performed: catalase, indole, nitrate reduction, gelatin liquefaction, H_2S production, esculin hydrolysis, urease production, and acid production from starch and various carbohydrates.

Catalase production was determined by flooding one of the BHI slants showing maximal growth with fresh

[1] A portion of a dissertation submitted by the first author to the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Public Health in the School of Public Health. The laboratory research was performed at the Laboratory Program, National Communicable Disease Center, under the supervision of the second author.

[2] Present address: Idaho State Health Department, Boise, Idaho.

3% hydrogen peroxide solution. Tubes of Indole Nitrite Medium (BBL) were inoculated in duplicate, and tests were performed for nitrite after 3 days of incubation and for indole after 10 days. Gelatin liquefaction was determined with Thiogel (BBL). Triple Sugar Iron (TSI) Agar and Heart Infusion (HI) Agar slants with lead acetate paper were used to detect H_2S production. Infusion broth with 0.1% agar and 0.1% esculin was used to demonstrate esculin hydrolysis. This was detected by adding a drop of 1.0% aqueous ferric citrate solution to a sample of the culture after 3 days of incubation; if negative, a second sample was tested after 10 days of incubation. The development of a brown-black color indicates hydrolysis.

The basal medium for fermentation studies consisted of Thioglycollate Broth (without dextrose or indicator) with added yeast extract (0.2%) and bromocresol purple indicator (0.1%). The glucose fermentation tubes were sterilized by autoclaving. All other carbohydrate media were prepared by adding Seitz-filtered aqueous solutions to the basal medium. The final carbohydrate concentration was 1.0%, except for starch which was 0.4%. Urease production was determined by incubating a tube of fermentation base medium with added Urea Broth (Difco).

All media except the agar slants were placed in a boiling-water bath for 10 min and were rapidly cooled immediately before inoculation. The inoculum for all tests was taken from 3- to 5-day RT Broth cultures. The media were incubated for 10 days at 37 C unless otherwise indicated. All biochemical test media were incubated under aerobic conditions except the TSI and HI Agar slants, which were incubated in an anaerobic jar with a Gaspak envelope.

RESULTS

Morphological characteristics. Twelve of the 13 serotype 1 isolates produced the typical "spider" type of microcolony (Fig. 1) and the "molar tooth" type of macrocolony (Fig. 2). The X522 (ATCC 10048) serotype 1 isolate produced a flat granular microcolony and a smooth convex macrocolony (Fig. 3). All of the serotype 2 isolates produced the spider type of microcolony (Fig. 4); however, only two serotype 2 isolates (W1123 and W1129) produced typical molar tooth macrocolonies. The other serotype 2 isolates yielded white, convex macrocolonies which were relatively smooth. The two types of macrocolonies observed with serotype 2 isolates are shown in Fig. 5 and 6.

Gram stains of RT Broth cultures of both serotype 1 and 2 isolates revealed gram-positive branched filamentous organisms which fragmented to produce diphtheroidal forms.

Physiological and biochemical characteristics. The results of physiological and biochemical tests on this group of organisms are shown in Table 1.

DISCUSSION

All 13 serotype 1 isolates fermented arabinose with the production of acid, but none of the serotype 2 strains fermented this carbohydrate. In addition, 12 of 13 serotype 1 cultures produced the rough, molar tooth type of colony on BHI Agar, whereas only 2 of 14 serotype 2 isolates produced this type of macrocolony. There were no apparent differences in cellular morphology, and 26 of the 27 cultures studied produced the spider type of microcolony on BHI Agar. This finding suggests that production of spider colonies is a useful diagnostic character for both serotypes of *A. israelii*. However, similar microcolonies are produced by other members of the genus *Actinomyces* (*A. naeslundii* and *A. propionicus*). The morphology of macrocolonies was variable. Some of the isolates listed as producing molar tooth colonies did not yield this type of colony every time the culture was streaked out on BHI Agar. *A. israelii* serotype 1 isolate X522 failed to produce either the microcolony or macrocolony characteristic of this species. Previously, this particular isolate had been shown to have important antigenic differences when compared with other serotype 1 isolates (2).

Isolates of serotypes 1 and 2 have similar oxygen requirements. Cultures of both serotypes produced the greatest amount of growth when incubated under anaerobic conditions and grew poorly or not at all in air.

The results of other biochemical tests were similar for both groups of organisms and consistent with the published descriptions for this species. All cultures were negative for catalase, indole, urease, and gelatin liquefaction. In addition, none of the isolates studied fermented glycerol.

It has been shown previously that serotype 1 and serotype 2 isolates share a common antigen as evidenced by the fact that low dilutions of serotype 1 fluorescent antibody (FA) conjugate stain serotype 2 antigens. This finding, coupled with the fact that both groups of organisms have similar morphological and biochemical characteristics, suggests that serotype 2 should not be designated as a separate species.

The accurate identification of *A. israelii* requires the use of morphological, biochemical, and serological criteria. In our experiments, the FA procedure provided the most accurate and rapid diagnostic method for identifying this species. On the basis of these studies on *A. israelii* (2 and this paper), it is apparent that a polyvalent conjugate incorporating antisera to both serotypes 1 and 2 of *A. israelii* and made specific by adsorption with

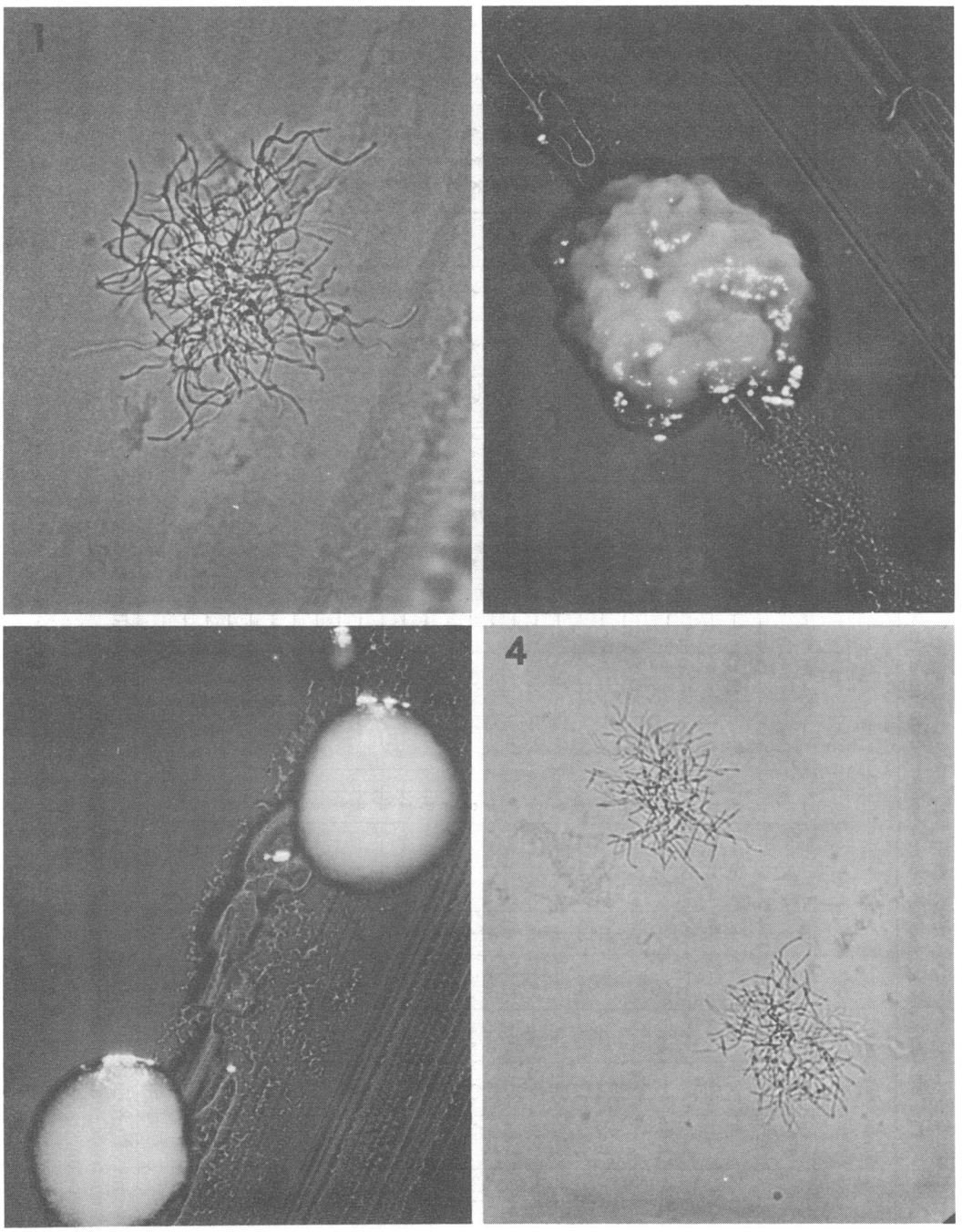

FIG. 1. *Typical "spider" type of microcolony from A. israelii serotype 1 (W855). × 210.*
FIG. 2. *"Molar tooth" type of macrocolony from A. israelii serotype 1 (W726). × 11.*
FIG. 3. *Smooth convex macrocolony of A. israelii serotype 1 (X522). × 18.*
FIG. 4. *"Spider" type microcolony of A. israelii serotype 2 (X695). × 275.*

TABLE 1. *Morphological and biochemical characteristics of A. israelii serotype 1 and 2 isolates*

Isolate	Serotype	Spider colony	Molar tooth colony	Oxygen[a] requirement			Biochemical reactions								Fermentations of										
				Aerobic	CO_2	Anaerobic	Catalase	NO_3	Indole	Gelatin	H_2S on TSI	H_2S on HIA	Esculin	Urea	Glucose	Mannose	Lactose	Sucrose	Maltose	Salicin	Glycerol	Starch	Arabinose	Xylose	Inositol
W855	1	+	+	—	3+	4+	—	+	—	—	3+	—	+	—	A[b]	a[c]	A	A	A	A	—	A	A	A	A
W726	1	+	+	—	3+	4+	—	+	—	—	4+	—	+	—	A	a	A	A	A	A	—	A	A	A	A
A601	1	+	+	1+	2+	4+	—	+	—	—	4+	—	+	—	A	A	A	A	A	A	—	A	A	A	A
X522	1	—	—	1+	2+	4+	—	+	—	—	4+	—	+	—	A	a	A	A	A	A	—	A	A	A	A
X372	1	+	+	—	1+	4+	—	+	—	—	3+	—	+	—	A	A	A	A	A	A	—	A	A	A	A
X373	1	+	+	—	1+	4+	—	+	—	—	3+	—	+	—	A	a	—	A	A	A	—	A	A	A	A
W721	1	+	+	1+	3+	4+	—	+	—	—	4+	—	+	—	A	A	A	A	A	A	—	A	A	A	A
A905	1	+	+	1+	3+	4+	—	+	—	—	4+	1+	+	—	A	a	A	A	A	A	—	A	A	A	A
W909	1	+	+	2+	4+	4+	—	—	—	—	2+	—	+	—	A	A	A	A	A	A	—	A	A	A	A
W911	1	+	+	—	—	1+	—	+	—	—	4+	—	+	—	A	a	A	A	A	A	—	A	A	A	A
W912	1	+	+	1+	2+	4+	—	+	—	—	4+	1+	+	—	A	A	A	A	A	A	—	A	A	A	A
W1066	1	+	+	1+	2+	4+	—	+	—	—	4+	—	+	—	A	a	A	A	A	A	—	A	A	A	A
W1067	1	+	+	—	1+	4+	—	+	—	—	2+	—	+	—	A	a	A	A	A	A	—	A	A	A	A
W838	2	+	—	—	2+	4+	—	+	—	—	4+	—	+	—	A	A	A	A	A	A	—	A	—	A	A
X695	2	+	—	—	1+	4+	—	—	—	—	3+	—	+	—	A	A	A	A	A	A	—	A	—	A	A
W748	2	+	—	—	2+	4+	—	+	—	—	4+	—	+	—	A	A	A	A	A	A	—	A	—	A	A
W940	2	+	—	—	3+	4+	—	—	—	—	4+	—	—	—	A	A	A	A	A	A	—	A	—	A	A
W1009	2	+	—	—	3+	4+	—	—	—	—	4+	—	+	—	A	A	A	A	A	A	—	A	—	A	A
W1010	2	+	—	—	—	4+	—	+	—	—	4+	—	+	—	A	A	A	A	A	A	—	A	—	A	A
W1011	2	+	—	1+	3+	4+	—	+	—	—	4+	—	+	—	A	A	A	A	A	A	—	A	—	A	A
W1122	2	+	—	—	3+	4+	—	+	—	—	3+	—	+	—	A	A	A	A	A	A	—	a	—	A	A
W1123	2	+	+	1+	3+	4+	—	—	—	—	4+	—	+	—	A	A	A	A	A	A	—	A	—	A	A
W1124	2	+	—	—	3+	4+	—	—	—	—	4+	—	+	—	A	A	A	A	A	A	—	A	—	A	A
W1125	2	+	—	—	3+	4+	—	—	—	—	3+	—	+	—	A	a	A	A	A	A	—	a	—	A	A
W1126	2	+	—	1+	4+	4+	—	+	—	—	3+	—	+	—	A	A	A	A	A	A	—	A	—	A	A
W1128	2	+	—	—	3+	4+	—	—	—	—	4+	—	+	—	A	A	A	A	A	A	—	a	—	A	A
W1129	2	+	+	—	2+	4+	—	—	—	—	2+	—	—	—	A	a	A	A	A	A	—	A	—	A	A

[a] Expressed by amount of growth: no growth, —, maximal growth, 4+, under different conditions of oxygen tension. Aerobic, growth in air; CO_2, under "CO_2 seal"; anaerobic, under "anaerobic seal."

[b] Strong acid production.

[c] Weak acid production.

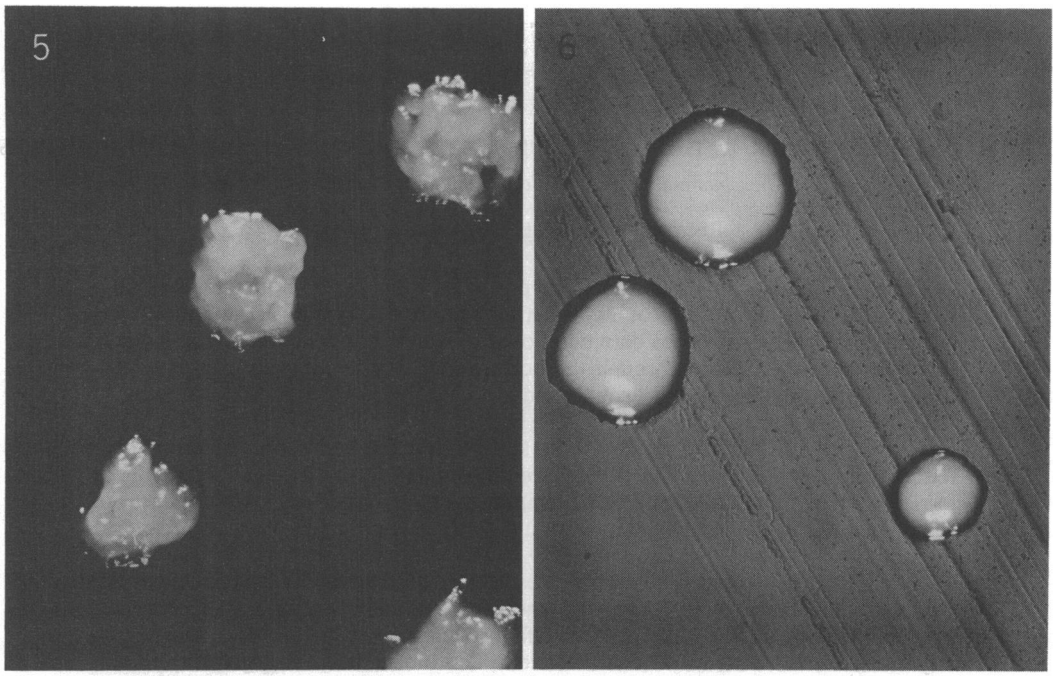

FIG. 5. *Macrocolony of A. israelii serotype 2 (W1129).* × *3.5*
FIG. 6. *Macrocolony of A. israelii serotype 2 (W1122).* × *4.8.*

A. naeslundii cells must be used for adequate detection or accurate identification of this organism in clinical materials or culture.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service training grant 5T01 GM 567 from the National Institute of General Medical Sciences.

We thank John P. Gust, Jr., National Audiovisual Center, for the photographic work.

LITERATURE CITED

1. Blank, C. H., and L. K. Georg. 1968. The use of fluorescent antibody methods for the detection and identification of *Actinomyces* species in clinical material. J. Lab. Clin. Med. 71:283–293.
2. Brock, D. W., and L. K. Georg. 1968. Determination and analysis of *Actinomyces israelii* serotypes by fluorescent-antibody procedures. J. Bacteriol. 97:581–588.
3. Georg, L. K., G. W. Robertstad, and S. A. Brinkman. 1964. Identification of species of *Actinomyces*. J. Bacteriol. 88: 477–490.
4. Holm, P. 1930. Comparative studies on some pathogenic anaerobic *Actinomyces*. Acta Pathol. Microbiol. Scand. Suppl. 3:151–158.
5. Lambert, F. W., Jr., J. M. Brown, and L. K. Georg. 1967. Identification of *Actinomyces israelii* and *Actinomyces naeslundii* by fluorescent-antibody and agar-gel diffusion techniques. J. Bacteriol. 94:1287–1295.