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Acanthamoeba jacobsi sp. n. (Protozoa: Acanthamoebidae) from Sewage Contaminated Ocean Sediments

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ABSTRACT: A temperature-tolerant strain of *Acanthamoeba,* isolated from sewage contaminated ocean sediments, was characterized by isoenzyme analysis, mouse pathogenicity tests, and phase contrast microscopy and found to represent a new species. Intranasal inoculation of 10-g weanling mice killed 3/10 of them, and amebae were cultured from brain tissue. The new isolate, *Acanthamoeba jacobsi* sp. n., is described.

KEY WORDS: *Acanthamoeba,* Protozoa, sewage wastes.

Ocean sediments collected in 1974 from the discontinued New York 12-mile sewage disposal site yielded a temperature-tolerant strain of *Acanthamoeba* when cultured on freshwater agar medium (Sawyer et al., 1977). The new strain formed round cysts with an ectocyst wall that appeared to be smooth at low magnification, but rippled when viewed with an oil immersion objective, or with the electron microscope (Sawyer et al., 1987). At the time of original isolation, *Acanthamoeba culbertsoni* Singh and Das, 1970, was the only known species within the genus to form round cysts with a thin and delicate rippled ectocyst wall, to grow at mammalian body temperatures, and to kill experimentally infected laboratory animals (Culbertson et al., 1959; Singh and Das, 1970). Characteristics shared by the marine strain (Sawyer et al., 1977; Daggett et al., 1982) and *A. culbertsoni* led us to identify it tentatively as *A. culbertsoni.* Subsequent comparative studies using isoenzyme electrophoresis, morphology, and growth characteristics have shown sufficient differences between the Lilly A-1 type strain of *A. culbertsoni* (ATCC 30171) and the marine strain (ATCC 30732) to justify a new species designation for the latter. The new strain is designated *Acanthamoeba jacobsi* sp. n., in honor of Dr. Leon Jacobs (retired), former Director, Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland. Dr. Jacobs was one of the earliest investigators to recognize cysts of *Acanthamoeba* in cultures of monkey kidney cells (Jahnes et al., 1957).

Materials and Methods

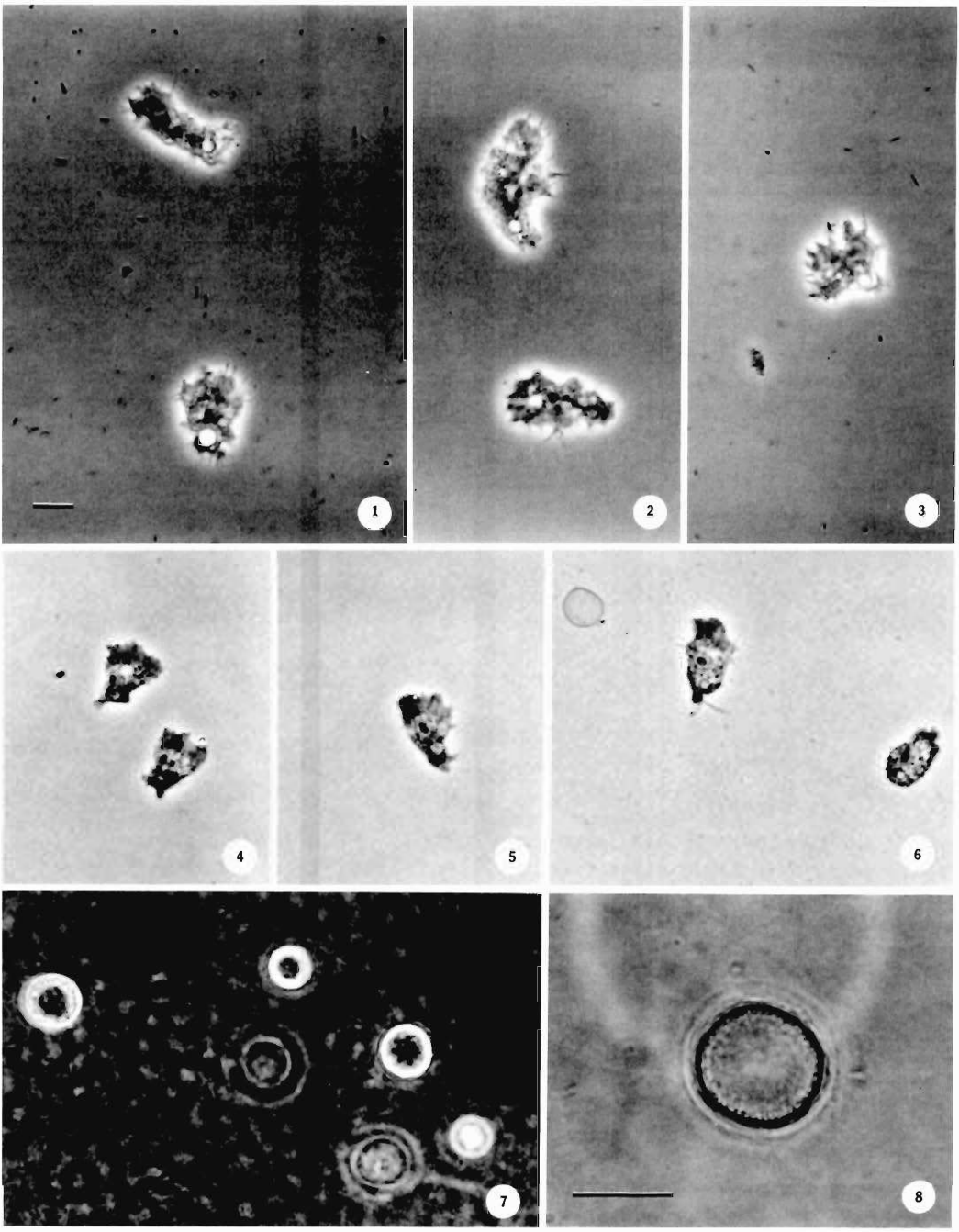
Bottom sediments were taken from the center of a near shore ocean sewage disposal site designated by the U.S. Environmental Protection Agency as the New York 12-mile Disposal Site. Sediments were taken with a Smith-McIntyre bottom grab and sampled by removing the top 1 cm with a sterile wooden tongue depressor as described earlier (Sawyer et al., 1987). Samples were taken in triplicate, stored in sterile plastic bags under refrigeration, and returned to the laboratory for culture studies. Six replicate cultures (2 each of 3 subsamples) were prepared by streaking one microbiological loopful of sediment on each of the 6 plates. Agar medium prepared by dissolving 0.1 g malt extract, 0.1 g yeast extract, and 15 g Difco agar in 1 liter distilled water, and sterilized by autoclaving, was used to discourage the growth of marine organisms otherwise requiring seawater medium. The surface of each agar plate was streaked with *Klebsiella pneumoniae* (ATCC 27889) as a food source for the amebae. Culture plates were inverted and stored in plastic boxes lined with moist paper towels; 3 cultures were incubated at room temperature and 3 at 38°–39°C.

One temperature-tolerant strain was isolated, cloned, and designated strain 31-B. Whole cell extracts of the new strain, and strain A-1 (ATCC 30171), were prepared and acid phosphatase (AP), leucine amino peptidase (LAP), and propionyl esterase (PE) isoenzymes were assayed using the method described by Nerad and Daggett (1979). Pathogenicity tests were carried out by instilling approximately 1×10^5 amebae suspended in 0.01 ml of Page's Saline (1988) intranasally into each of 25 20-g mice, 13 10-g mice, and 3 controls. Mice that died were necropsied and small fragments of brain tissue streaked on agar plates to test for the presence of amebae.

Living trophozoites and cysts were measured ($N = 25$) using phase contrast objectives. Trophozoites were also measured ($N = 25$) after staining with nuclear red (Kernechtrot) according to Page (1988). All measurements are given in micrometers.

Results

Cultures incubated at room temperature and at 38°–39°C were positive for cyst-forming amebae within 3 days of preparation. Encysted amebae were noted at all depths within the agar medium indicating that trophozoites had migrated



Figures 1–8. Trophozoites and cysts of *Acanthamoeba jacobsi* sp. n. Scale bar = 10 μm. For Figures 1–7, scale bar in Figure 1 applies. 1–3. Living trophozoites, phase contrast. Note phase halo effect and loss of nuclear detail due to contraction in response to light from microscope. 4–6. Trophozoites fixed in Nissembaum solution and stained with nuclear red, phase contrast. Note retraction of filose pseudopodia due to contraction during fixation, and uncollapsed empty cyst in Figure 6. 7. Living cysts on agar block, phase contrast. Note delicate rippled ectocyst and unfocused cysts at different depths in the agar medium. 8. Living cyst on agar, phase contrast. Photograph taken with 100× phase objective (oil), and phase ring in high dry position #2 to emphasize spherical contour of endocyst and delicate thin and rippled ectocyst.

throughout the agar matrix. Examination of cysts in wet mounts with 10×, 20×, and 40× phase contrast objectives indicated that there was little or no evidence of the irregular or rippled wall characteristic of amebae belonging to the genus *Acanthamoeba.* Examination with an oil immersion 100× objective, however, showed that a thin-rippled wall was present. Evidence of the wall was noted when small rectangular blocks of agar were removed from cultures and covered with a cover glass for study at 40×, probably due to slight pressure from the cover glass. Trophozoites had transitional acanthopodia characteristic of the genus when observed in wet mounts or hanging drops. Amebae contracted rapidly in the presence of light from the microscope and rarely could be photographed while undergoing typical locomotion (Figs. 1–3). Specimens measured under reduced light ranged 25.0–37.5 long × 10.0–17.5 wide (mean = 31.3 × 14.7). Stained specimens (Figs. 4–6) were smaller, probably due to fixation, ranging 15.0–27.0 long × 6.0–11.0 wide (mean = 21.0 × 8.6). Live cysts (Figs. 7, 8) had a spherical endocyst with a thin-rippled wall, and measured 12.5–17.5 in diameter. Cysts were present at all depths in the agar medium (Fig. 7).

The AP, LAP, and PE zymograms of *Acanthamoeba jacobsi* were distinct from the A-1 strain of *A. culbertsoni* (Fig. 9). With the possible exception of 1 PE isoenzyme band, no bands were shared between the strains.

None of the 20-g mice showed signs of infection during a 2-mo period of observation. Among the 10-g mice, however, 3/13 died within 5–24 days postinoculation. Control mice did not show evidence of infection. Cultures of brain tissue from 2/3 infected mice yielded *A. jacobsi.*

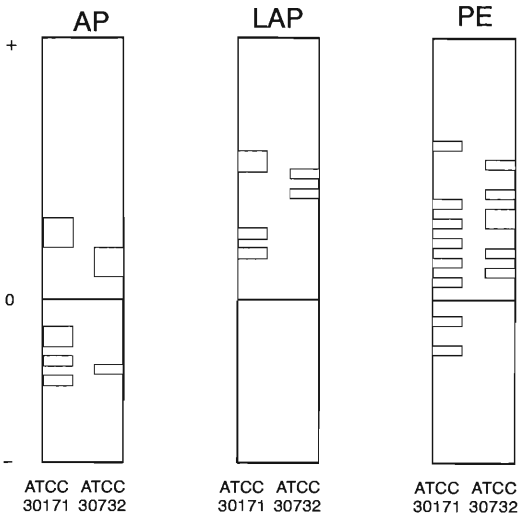


Figure 9. AP (alkaline phosphatase), LAP (leucine amino peptidase), and PE (propionyl esterase) zymograms of *A. culbertsoni* strain A-1 (ATCC 30171) and *A. jacobsi* sp. n. (ATCC 30732).

Description

Acanthamoeba jacobsi sp. n.

DIAGNOSIS: Trophozoites (Figs. 1–6) typical of the genus *Acanthamoeba,* 25.0–37.0 × 10.0–17.5 (mean = 31.3 × 14.7) in the living condition, and 15.0–27.0 × 6.0–11.0 (mean = 21.0 × 8.6) after fixation with Nissembaum's solution and staining with nuclear red. Amebae contract rapidly in the presence of light from the microscope lamp unless viewed with reduced or filtered light. Nucleus in stained specimens 4.0, nucleolus 2.5. Fine bristlelike pseudopodia transitional and arising randomly at all angles from the body surface. Pronounced posterior contractile vacuole. Cyst with a spherical endocyst closely appressed to the thin-rippled ectocyst wall. Cysts and trophozoites present at all depths within the agar medium. Excystment through an ostiole in the cyst wall; ostiole not evident by light microscopy. Growth at temperatures up to 38°–40°C. Amebae mildly pathogenic to weanling laboratory mice and culturable from brain tissue.

TYPE LOCALITY: Bottom sediments from an ocean sewage disposal site designated "New York 12-mile Site" in the New York Bight near Coney Island, approximately 40°27′ latitude and 73°45′ longitude.

TYPE SPECIMENS: Deposited at the American Type Culture Collection, Rockville, Maryland as strain ATCC 30732.

Discussion

Acanthamoeba jacobsi sp. n. was isolated in 1974 when *A. culbertsoni* and *A. palestinensis* were the only known members of the genus to form spherical cysts with a delicate sculptured or rippled ectocyst wall. The 2 species were readily distinguished by the more pronounced ectocyst wall and larger size of cysts of *A. palestinensis,* and its failure to grow at 37°C or higher. Strain 31-B seemingly had most of the features attributed to *A. culbertsoni* at the time, and was discussed and illustrated as such in 3 separate publications (Sawyer et al., 1977; Daggett et al.,

1982; Sawyer et al., 1987). Sawyer et al. (1987) designated strain 31-B simply as *Acanthamoeba* sp. 1 in 1 publication that illustrated the trophozoite and cyst as seen with the electron microscope. The cyst of *A. jacobsi* had a single ostiole, and the nucleus of the trophozoite was smooth-walled without evidence of pores in the nuclear membrane. Although *A. jacobsi* was pathogenic only to young 10-g mice, the recovery of amebae from brain tissue suggested that serial passage might lead to increased virulence.

Precise identification of strains of *Acanthamoeba,* especially those forming round cysts, has been complicated by the recent description of other new species, i.e., *A. lenticulata* Molet and Ermolieff-Braun, 1976, and *A. royreba* Willaert, Stevens, and Tyndall, 1978. Pussard and Pons (1977) proposed that all species of *Acanthamoeba* be placed within 1 of 3 groups on the basis of similar cyst morphology. Accordingly, *A. jacobsi* would be placed in group III, which includes those species with a round endocyst and a thinly rippled or wrinkled ectocyst wall. Careful study of all species included in group III has shown that cysts, except for those of *A. jacobsi,* may range from spherical to slightly angular or irregular, especially when densely crowded on agar culture plates. Isoenzyme studies on *A. jacobsi, A. culbertsoni, A. palestinensis, A. lenticulata,* and *A. royreba* have shown that they are distinct on the basis of zymograms and are valid species. Two other species of amebae, which also have round cysts, *A. glebae* and *A. invadens,* were previously included in the genus *Acanthamoeba.* Page (1988) stated that both species excyst by dissolution of the cyst wall rather than through an exit pore, and placed them within the genus *Protacanthamoeba* Page, 1981. The distinct pore, or ostiole, in the cyst wall of *A. jacobsi,* as seen with the electron microscope, clearly excludes this species from the genus *Protacanthamoeba.*

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