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Enzymatic Activities of *Legionella pneumophila* and *Legionella*-Like Organisms

FREDERICK S. NOLTE,* GARY E. HOLLICK, AND RICHARD G. ROBERTSON

Department of Microbiology, School of Medicine and Dentistry, University of Rochester, Rochester, New York 14642

Received 29 May 1981/Accepted 10 August 1981

The API ZYM system was used to investigate enzymatic activities of *Legionella pneumophila* and other *Legionella*-like organisms. Leucine aminopeptidase, alkaline and acid phosphatase, butyrate and caprylate esterase, and phosphoamidase activities were consistently detected in all strains tested. No evidence of myristate lipase, trypsin, chymotrypsin, or glycosidase activity was found.

Legionella pneumophila and *Legionella*-like organisms have emerged as significant respiratory pathogens in certain patient populations. There exist relatively few biochemical tests based on positive reactions for identification of this group of organisms (9, 11). Biochemical characterization is complicated by the fact that these organisms have an unusual metabolism characterized by the ability to use amino acids as the major source of energy and carbon (8). They also do not grow on standard laboratory media. Definitive identification of an isolate as one of the described species requires gas-liquid chromatography of cellular fatty acids (14) or direct immunofluorescent staining (3).

We examined the enzymatic profiles of nonproliferating cells by using the 19 chromogenic substrates comprising the API ZYM system (Analytab Products, Plainview, N.Y.). While this study was in progress, Müller (16) published the enzymatic profiles of four strains of *L. pneumophila*, using the API ZYM system. We expand upon his observation with *L. pneumophila* and extend it to other described species, considered by some to be in the genus *Legionella* (1, 2, 10, 12, 13) and by others to be in the genera *Tatlockia* and *Fluoribacter* (7).

Nine strains of *L. pneumophila*, designated Bellingham 1 (serogroup 1), California 1 (serogroup 1), Knoxville 1 (serogroup 1), Philadelphia 2 (serogroup 1), Togus 1 (serogroup 2), Bloomington 2 (serogroup 3), Los Angeles 1 (serogroup 4), Dallas I-E (serogroup 5), and Chicago 2 (serogroup 6), and one strain each of *L. micdadei* (Tatlock), *L. gormanii* (LS-13), *L. bozemanii* (WIGA), *L. dumoffii* (TEX-KL), and *L. longbeachae* (Longbeach 4), were studied. *L. longbeachae* was provided by R. M. McKinney, Centers for Disease Control, Atlanta, Ga.; all other strains were provided by R. D. Miller, School of Medicine, University of Louisville, Louisville, Ky.

All strains were plated on buffered charcoal yeast extract agar (BCYE) (17) and incubated for 48 h at 37°C in an atmosphere of 5% CO_2 in air. Strains of *L. pneumophila* were also plated on Mueller-Hinton medium (MH-IH) (5) supplemented with 5% (wt/vol) dried bovine hemoglobin and 1% (vol/vol) IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.) and incubated for 72 h. The other *Legionella*-like organisms failed to grow on MH-IH.

Dense cell suspensions were prepared in 4 ml of sterile saline from growth on these media and adjusted to a turbidity approximating a McFarland no. 5 standard. A sample of each suspension was plated onto 5% sheep blood agar to check for contamination.

Each cupule of the API ZYM strip was inoculated with 2 drops of the prepared bacterial suspension. The strips were then placed in a moist chamber and incubated for 4 h at 37°C in 5% CO_2 in air. After incubation, 1 drop each of reagent A (Tris, 250 g; HCl [37%], 110 ml; lauryl sulfate, 100 g; distilled water to 1,000 ml) and of reagent B (Fast Blue BB, 3.5 g; 2-methoxyethanol to 1,000 ml) were added to each cupule. The colors were allowed to develop for 5 min. The color intensity of the reactions was quantitated by assigning values of from 0 to 5 according to the color chart provided by the manufacturer. We translated the manufacturer's values as follows: 0 = negative, $\leq$1 = weakly positive, 2–3 = positive, and 4–5 = strongly positive reaction.

The enzymatic activities assayed and results are presented in Table 1. All strains tested gave similar enzymatic profiles. Alkaline (pH 8.5) and acid (pH 5.4) phosphatase, leucine aminopeptidase, butyrate and caprylate esterase, and phosphoamidase activities were consistently detected. The only exception was *L. bozemanii*, which showed no caprylate esterase activity.

TABLE 1. Enzymatic activities of *Legionellaceae* as tested by the API ZYM system

Enzyme	Result[a]
Acid phosphatase	++
Alkaline phosphatase	++
Butyrate esterase	+
Caprylate esterase	±[b]
Myristate lipase	−
Leucine aminopeptidase	++
Valine aminopeptidase	±[c]
Cystine aminopeptidase	±[d]
Trypsin	−
Chymotrypsin	−
Phosphoamidase	+
α-D-Galactosidase	−
β-D-Galactosidase	−
β-D-Glucuronidase	−
α-D-Glucosidase	−
β-D-Glucosidase	−
N-acetyl-β-D-glucoaminidase	−
α-D-Mannosidase	−
α-L-Fucosidase	−

[a] ++, Strongly positive; +, positive; ±, weakly positive; −, negative.

[b] *L. bozemanii* was negative.

[c] *L. pneumophila* and *L. bozemanii* only; all others were negative.

[d] *L. pneumophila* only; all others were negative.

Müller (16) reported that *L. pneumophila* has a wide range of aminopeptidase activity, including valine and cystine aminopeptidases. We found no convincing evidence of either valine or cystine aminopeptidase activity in the strains of *L. pneumophila* tested; however, there was some suggestion of weak action on these substrates. Leucine, valine, and cystine are among the amino acids required by some strains of *L. pneumophila* (8). The difference in relative aminopeptidase activity with regard to these three substrates is puzzling. Perhaps subtle variations in media composition, technique, or passage history of the strains are responsible for this apparent discrepancy between Müller's results and our own.

Of the *Legionella*-like organisms, only *L. bozemanii* showed weak valine aminopeptidase activity. An expanded aminopeptidase profile using other amino acids as substrates might provide a means of distinguishing the species.

The broad pH range of the phosphatase activity may indicate that phosphatase activity plays an essential role in metabolism of the *Legionellaceae*.

Müller (16) reported that *L. pneumophila* possesses esterase activity but no lipase. We found apparent butyrate and caprylate esterase activity. *L. pneumophila* has significant proteolytic activity (15; T. C. Thorpe and R. D. Miller, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, D3, p. 43), and it is possible that the esterase activity detected here was a result of proteolytic enzymes and not specific esterases, since most proteolytic enzymes also catalyze hydrolysis of ester bonds (18).

No myristate lipase, chymotrypsin, trypsin, or glycosidase activity was found. The lack of glycosidase activity demonstrated by this group of organisms is consistent with their reported inability to ferment carbohydrates (11).

The nine strains of *L. pneumophila* tested showed no qualitative or quantitative differences in enzymatic activity when inocula obtained from BCYE and MH-IH were compared.

The API ZYM strip has proved useful as an aid in the identification of other bacteria (4, 19, 20). The findings of strong phosphatase, strong leucine aminopeptidase, esterase, and phosphoamidase activities and no glycosidase activity, when coupled with the other phenotypic characteristics described by Hébert (9), provide additional criteria to identify an isolate as a member of the family *Legionellaceae*. The 19 substrates used in this assay system, however, do not differentiate the organisms at the species level. Additional strains of the *Legionella*-like organisms should be tested as they become available, to determine whether the other species are as homogeneous with respect to their enzymatic profiles as *L. pneumophila* appears to be. The similar enzymatic profiles obtained with this group of genetically heterogeneous free-living organisms (6) may be a reflection of the fact that they occupy a similar ecological niche.

Many fascinating questions remain unanswered concerning the physiology of this group of organisms. It remains to be seen whether an expansion of the enzymatic profile will provide a useful means of species identification.

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