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# Unusual Fermentative, Gram-Negative Bacilli Isolated from Clinical Specimens

## I. Characterization of *Erwinia* Strains of the "*lathyri-herbicola* Group"

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Five strains of gram-negative, yellow chromogenic bacilli were recovered from clinical specimens which fit the characteristics of the "*lathyri-herbicola* group" within the genus *Erwinia*. The strains were facultatively anaerobic, fermentative, anaerogenic bacilli with peritrichous flagella which grew at 37 C, reduced nitrate to nitrite, and failed to produce oxidase, pectinase, arginine dihydrolase, and decarboxylases for lysine and ornithine. Aggregations of bacteria (symplasmata) were observed in the syneresis water of slant cultures, and analogous granular aggregates and biconvex, spindle-shaped bodies developed in colonies on plate cultures. Awareness of these characteristics should result in more frequent identification of *Erwinia* species from human sources.

Graham and Hodgkiss (11) have shown that various gram-negative, yellow chromogenic, anaerogenic, fermentative bacilli with peritrichous flagella belong to the genus *Erwinia*. These bacteria include a variety of species formerly described under different names, such as the nonphytopathogenic *Erwinia lathyri* and *Bacterium herbicola* common on plants and in soil, and *B. typhi flavum* and "*Erwinia*-like microorganisms" isolated from animals and humans. As a result of the studies by Graham and Hodgkiss and similar comparative studies by Billing and Baker (3), the names "*lathyri-herbicola* group" of *Erwinia* and "*Erwinia herbicola*" have been proposed for these nonphytopathogenic bacteria isolated from plant, animal, and human sources.

This report describes the morphological and physiological characteristics of five *Erwinia* strains of the "*lathyri-herbicola* group" isolated from human sources, some of which apparently were responsible for the infectious process. The designations applied to these human isolates and the diagnostic features of this unusual organism are discussed.

## MATERIALS AND METHODS

**Cultures.** The five strains examined represent isolates recovered from clinical specimens between January and November 1969.

**Morphological studies.** All cultures were incubated for 24 hr at 37 C unless otherwise noted. Smears from Trypticase Soy Agar (TSA, BBL) cultures were stained by Gram's method (Hucker modification) and observed for microscopic morphology. Isolated colonies on TSA plates, containing 5% defibrinated rabbit blood (TSBA), were examined for colonial morphology. Colonies on TSA plates were examined for the presence of biconvex, spindle-shaped bodies. Growth on TSA with 5% sucrose was examined for mucoid colonies. Growth characteristics were also observed on Salmonella-Shigella Agar (SS Agar, Difco), Desoxycholate Agar (DC Agar, Difco), MacConkey Agar (Difco), and Kligler Iron Agar (KIA, Difco). TSA slants were used for growth-temperature studies in a 42 C incubator with a uniformity of ±0.5 C. Hanging drop preparations were made from the syneresis water in TSA slants to observe for sausage shaped aggregations of bacteria (symplasmata). Motility was determined by microscopic examination of a hanging drop of a Trypticase Soy Broth (BBL) culture. Gray's method for flagella stain as outlined by Bailey and Scott (1) was used.

**Biochemical studies.** The tests and media employed included: fermentation of 1% carbohydrates (OF Basal Medium, Difco); fermentation of 10% lactose (Purple Agar Base, Difco); gas production from carbohydrates (Purple Broth Base, Difco, with Durham tubes), $H_2S$ production (KIA); methyl red (MR) and Voges-Proskauer (VP) tests (buffered peptone-glucose broth); growth at *p*H 5.6 (Sabouraud's Dextrose Agar, Difco); production of *beta*-galactosidase (Differentiation Disc ONPG, Difco); casein hydrolysis (skim milk agar); lecithinase production (TSA with

10% egg yolk suspension); decarboxylase and dihydrolase activity (Decarboxylase Base Møller, Difco); citrate utilization (Simmons' Citrate Agar, Difco); malonate utilization (Leifson's Malonate Broth, Difco 0395); nitrate reduction (Trypticase Nitrate Broth, BBL); indole production (Tryptone Broth, BBL); urease activity (Christensen's Urea Agar, Difco); gelatinase activity (Nutrient Gelatin, Difco); oxidase reaction (Oxidase Discs, Difco); gluconate oxidation (Gluconate Substrate, Key Scientific Products); growth on cetrimide (Pseudosel Agar, BBL); growth on triphenyl tetrazolium chloride (TTC; TSA with 1% TTC); growth on 2.5, 6.5, and 10.0% NaCl (TSA with adjusted NaCl concentrations); phenylalanine deaminase activity (Phenylalanine Agar, Difco); deoxyribonuclease activity (DNase Test Agar, Difco); tyrosinase activity (TSA with 1% L-tyrosine); starch hydrolysis (TSA with 1% starch); aesculin hydrolysis (TSA with 0.1% aesculin and 0.05% ferric citrate); lipase activity (TSA with 1% polyethylene sorbitan monooleate); pectinase activity (pectate medium; 8); assimilation of organic compounds as the sole sources of carbon according to methods previously described (10). Sensitivity to antibiotics was determined by the Kirby-Bauer method (2) by using BBL Sensi-discs.

All cultures were incubated for 3 weeks at 37 C before tests were discarded as negative. The following tests, except the MR-VP tests, were performed after 24 hr of incubation. Gluconate oxidation was tested with Clinitest tablets (Ames Co.); starch hydrolysis was detected by flooding the plates with Lugol's solution; phenylalanine deaminase activity was tested with 1% (w/v) ferric chloride solution; deoxyribonuclease activity was determined by flooding the plates with 1 N HCl; indole production was tested with Kovacs' reagent; after 48 hr of incubation, acetylmethylcarbinol was detected by reddening after the addition of 1 ml of 40% (w/v) KOH containing 0.3% (w/v) of creatine to 1 ml of culture.

## RESULTS

All strains were facultatively anaerobic, gram-negative bacilli with peritrichous flagella. Aggregations of bacteria (symplasmata) were observed in all strains. Colonies on TSBA were 1 to 2.5 mm in diameter, circular, smooth, slightly convex, opaque, and yellow. Good growth was obtained on TSBA, TSA, and MacConkey Agar at room temperature and at 37 C. All but one strain grew at 42 C, and only one strain grew on SS Agar and DC Agar. Two strains produced mucoid colonies on 5% sucrose agar. The colonies exhibited characteristic granular aggregates (analogous to symplasmata) and biconvex, spindle-shaped bodies, both of which adhered to the agar surface when the colonies were removed with a wire loop. In KIA, an acid butt with no gas and an alkaline slant developed after 24 hr of incubation. One strain produced slight $H_2S$. The biochemical results (Tables 1 and 2) were detected after 24 hr

TABLE 1. *Common characteristics of Erwinia strains*[a]

| Characteristic | Presence | Characteristic | Presence |
|---|---|---|---|
| Motile | + | Gelatin | +[b] |
| Symplasmata | + | Casein | +[b] |
| Biconvex bodies | + | Oxidase | − |
| Yellow pigment | + | Arginine | − |
| Hemolysis | − | Lysine | − |
| MacConkey Agar | + | Ornithine | − |
| Gas glucose (PBB) | − | Lipase | − |
| Acid glucose (OFBM) | + | Amylase | − |
| | | Pectinase | − |
| Fructose | + | Deoxyribonuclease | − |
| Galactose | + | Lecithinase | − |
| Mannose | + | 2.5% NaCl | + |
| Rhamnose | + | 10.0% NaCl | − |
| Xylose | + | *p*H 5.6 | + |
| Maltose | + | TTC | − |
| Mannitol | + | Cetrimide | − |
| ONPG | + | Tyrosinase | − |
| Gluconate | − | Assimilation | |
| Nitrite | + | Glucose (BMM) | + |
| Nitrogen gas | − | Acetate | − |
| Voges-Proskauer | − | Lactate | + |
| Methyl red | + | Succinate | + |

[a] Abbreviations: PBB, Purple Broth Base; OFBM, OF Basal Medium; TTC, triphenyl tetrazolium chloride; BMM, basal mineral medium; ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

[b] Liquefaction required from 1 to 14 days of incubation.

TABLE 2. *Variable characteristics of Erwinia strains*[a]

| Characteristic | Strain no. | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Acid sucrose (OFBM) | + | + | + | − | + |
| Lactose | − | + | +(d) | +(d) | + |
| 10% Lactose (PAB) | − | + | + | + | + |
| Hydrogen sulfide | − | − | − | +(s) | − |
| Indole | − | − | − | + | − |
| Urea | − | − | − | + | − |
| Phenylalanine | + | − | − | − | + |
| Aesculin | + | − | + | + | + |
| SS Agar | − | − | − | + | − |
| Desoxycholate Agar | − | − | − | + | − |
| Mucoid colonies | + | − | + | − | − |
| Growth at 42 C | + | + | + | + | − |
| 6.5% NaCl | − | − | − | − | + |
| Citrate (Simmons) | − | + | + | − | + |
| Malonate (Leifson) | + | − | − | + | + |

[a] Abbreviations: (d), 48 hr of incubation; (s), slight reaction; OFBM, OF Basal Medium; PAB, Purple Agar Base.

TABLE 3. *Characteristics of Erwinia strains isolated from human sources adapted from reports in the literature*[a]

| Characteristic | Presence | Characteristic | Presence | Characteristic | Presence |
|---|---|---|---|---|---|
| Gas from glucose | −(+)[b] | Citrate | +(−) | Pectinase | − |
| Acid | | | | | |
| Glucose | + | Malonate | + | Deoxyribonuclease | − |
| Rhamnose | + | Acetate | + | Amylase | − |
| Xylose | + | Indole | −(+) | Lipase | −(+) |
| Sucrose | + | Nitrite | + | 10.0% NaCl | − |
| Maltose | + | Nitrogen gas | − | Symplasmata | +(−) |
| Lactose | V | Methyl red | V | Biconvex bodies | +(−) |
| Mannitol | + | Voges-Proskauer | V | Motile | + |
| 10% Lactose | + | Gelatin | V | Mucoid colonies | +(−) |
| ONPG[c] | + | Arginine | − | MacConkey Agar | + |
| Gluconate | V | Lysine | − | Desoxycholate Agar | + |
| Aesculin | V | Ornithine | − | SS Agar | − |
| Hydrogen sulfide | V | Phenylalanine | V | Growth at 42 C | − |
| Oxidase | − | | | | |

[a] Literature cited: Graham and Hodgkiss, 1967 (11); Muraschi, Friend, and Bolles, 1965 (17); Slotnick and Tulman, 1967 (19); von Graevenitz and Strouse, 1966 (21); Krishnan and Goldie, Bacteriol. Proc., p. 102, 1968.

[b] Symbols: −, negative; +, positive; (−), a few strains negative; (+), a few strains positive; V, variable.

[c] *o*-Nitrophenyl-β-D-galactopyranoside.

of incubation except that the MR-VP tests were performed after 48 hr of incubation, gelatin and casein hydrolysis were detected after 1 to 14 days of incubation, and acid production from lactose in OFBM was detected after 2 days of incubation with two strains. All strains were resistant to penicillin, novobiocin, erythromycin, and lincomycin, and sensitive to tetracycline, chloramphenicol, streptomycin, nitrofurazone, methenamine mandelate, nalidixic acid, kanamycin, neomycin, and polymyxin. One strain was resistant and four strains were sensitive to ampicillin, keflin, and nitrofurantoin.

## DISCUSSION

The first report of human isolation of yellow-pigmented, anaerogenic, fermentative bacilli was by Dresel and Stickl (6) who recovered the organisms from stool specimens and called them *B. typhi flavum*. Cruickshank's (4) survey of the literature showed that many other strains called *B. typhi flavum* were obtained by bacteriologists from urine and blood cultures as well as from water, air, and soil. The most recent isolation of *B. typhi flavum* was by Krishnan and Goldie (Bacteriol. Proc., p. 102, 1968) from blood cultures. Muraschi, Friend, and Bolles (17) recovered three strains of similar bacteria from throat cultures, which they called *Erwinia*-like microorganisms. Six strains called *Erwinia* species were isolated from human wounds, including four suppurative lesions and a clean postoperative leg wound by von Graevenitz and Strouse (21) and a skin infection over the medial mallous of a fractured ankle by Slotnick and Tulman (19).

Strain 1 in the present study was isolated in pure culture from a peritoneal dialysis fluid of an adult female who was suffering from chronic renal failure. There was no correlation between the organism and the patient's clinical condition. Strain 2 was isolated three times in association with *Staphylococcus aureus* and *Proteus rettgeri* from a gangrenous lesion of the left toe of an adult female diabetic. All three species were sensitive to chloramphenicol and the patient responded to this therapy. The significance of this strain is difficult to determine because of the mixed infection. Strain 4 was recovered in pure culture from an infected palm of the hand of an adult male following a hand laceration. The patient responded to saline irrigation and penicillin therapy in spite of in vitro penicillin resistance. Strain 3 was recovered in pure culture from a gunshot wound of the ankle of an adult male who responded to ampicillin therapy to which the strain was sensitive. In the fifth case, a neurologic examination of an adult male revealed a left parietal lobe mass which at surgery proved to be a well-encapsulated brain abscess from which *Erwinia* was isolated. The latter two strains appeared to be of clinical significance. Strain 5 apparently was a primary pathogen, and the role played by strain 3 would suggest that the organism is an opportunist which can cause infection when the hosts' conditions are appropriate such as when injury or trauma occurs.

The seventh edition of *Bergey's Manual of Determinative Bacteriology* describes the genus *Erwinia* as gram-negative bacilli pathogenic for plants, motile by peritrichous flagella, fermenting carbohydrates with or without gas, and producing variable lactose fermentation. *Bergey's Manual* recognizes two major groups: an anaerogenic group, with six species, which causes dry necrosis, galls, or wilts, and a pectinase-producing anaerogenic-aerogenic group, with eleven species, which causes soft rots. According to recent concepts of the classification of these bacteria (13, 14, 18, 22), the erwinias probably can be consolidated into two species with all the members that cause dry necrosis reduced to synonymy with *E. amylovora*, and all the members that cause soft rots, with the possible exception of the aerogenic strains, reduced to synonymy with *E. carotovora*. Waldee (22) was the first to suggest that the pectinolytic group be placed in a new genus, *Pectobacterium*, in the family *Enterobacteriaceae*. Ewing (9) followed this suggestion and revised the system of nomenclature of this family to include *Pectobacterium*.

Important comparative studies by Graham and Hodgkiss (11) demonstrated the relationship between the phytopathogenic *Erwinia* and the yellow chromogenic strains isolated from human sources. They concluded that the genus *Erwinia* consisted of two presumably phytopathogenic species and a nonphytopathogenic *E. herbicola*-group. The *E. herbicola*-group included the strains isolated by Muraschi and co-workers and the former *B. typhi flavum*, *B. herbicola*, *E. lathyri* (*E. trifolii*), and *E. ananas*. *E. lathyri* and *E. trifolii* were previously found (3, 7) to be similar to *E. herbicola*. Deoxyribonucleic acid base composition studies by De Ley (5) and Starr and Mandel (20) basically support the relationships of these bacteria and show their similarity to *E. amylovora*, the type species for the genus. As a result of these studies, the names "*lathyri-herbicola* group" of *Erwinia* (3) and "*E. herbicola*" (11) have been proposed for the nonphytopathogenic, yellow chromogenic bacteria.

The characteristics of the strains examined in the present study compare favorably with the descriptions of previous human isolates (Table 3) as well as nonphytopathogenic erwinias isolated from various plant (3, 11, 12) and nonplant hosts (17, 23). The proper identification of *Erwinia* in the diagnostic laboratory would appear to depend on the scrutiny of all yellow chromogenic, fermentative bacilli which generally are anaerogenic. All strains possess peritrichous flagella, are oxidase-negative, and reduce nitrate to nitrite (11, 17, 21). The development of symplasmata and biconvex bodies have previously been reported (3, 4, 11) and appear to be of diagnostic value. Pectinolytic activity has not been demonstrated with human isolates (17, 19), indicating that this is a negative feature of the *lathyri-herbicola* group. The lack of dihydrolase and decarboxylase activity in human isolates parallels the results obtained with phytopathogens (15, 16) and strains isolated from nonplant hosts (17, 23). Although the strains examined in this study produced a yellow pigment, some *Erwinia* species are known to be nonchromogenic (3, 15). Nonpigmented strains which fail to liquify gelatin may be confused with *Enterobacter hafniae* (*Hafnia*, *Paracolobactrum aerogenoides*) (21), but the latter species produces lysine and ornithine decarboxylase distinguishing it from *Erwinia*.

## ADDENDUM IN PROOF

Since the writing of this paper, six additional strains of *Erwinia* isolated from clinical specimens have been examined. Their features were comparable with those described except that the MR and VP reactions were strain-variable.

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