# *Erwinia* and yellow-pigmented *Enterobacter* isolates from human sources

G. L. GILARDI AND E. BOTTONE

*Department of Laboratories, Hospital for Joint Diseases and Medical Center, New York, New York 10035, and Microbiology Department, Mt. Sinai Hospital, New York, New York 10029, U.S.A.*

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Sixty-five strains of gram-negative, yellow-pigmented bacilli, including four chromogenic *Enterobacter* strains and 55 anaerogenic and six aerogenic *Erwinia* strains, were isolated from human sources. The genus *Erwinia* contained two groups; an anaerogenic group which produced aggregates of bacteria (symplasmata) in the syneresis water of slant cultures and biconvex bodies in colonies on agar medium, and an aerogenic group which lacked these characteristics. *Erwinia* was differentiated from *Enterobacter*, since the latter possessed dihydrolase and decarboxylase activity and demonstrated resistance to cephalothin.

## INTRODUCTION

Several accounts in the literature describe the isolation from human sources of yellow-pigmented, fermentative, gram-negative bacilli of the genus *Erwinia* (Muraschi, Friend and Bolles, 1965; von Graevenitz and Strouse, 1966; Slotnick and Tulman, 1967; Gilardi, Bottone, and Birnbaum 1970; von Graevenitz, 1970; Wechsler et al., 1971). The following is a report of the morphological and physiological features of 65 strains of yellow chromogenic bacilli isolated from human sources. Included among these isolates were four pigmented *Enterobacter* strains and 61 strains of the *Erwinia* group.

## MATERIALS AND METHODS

The source of the strains is listed in Table 1. The tests and media employed were previously described (Gilardi, 1969) except for the following: gas production from glucose, Purple Broth Base (Difco) with Durham tubes; methyl red

Table 1. Source of *Erwinia* and yellow-pigmented *Enterobacter* isolates

| | *Erwinia* | Pigmented *Enterobacter* | | *Erwinia* | Pigmented *Enterobacter* |
|---|---|---|---|---|---|
| Wounds, abscesses | 15 | 3 | Skin | 2 | 0 |
| Blood | 13 | 0 | Ear | 2 | 0 |
| Eye | 10 | 0 | Sputum | 2 | 0 |
| Nasopharynx, nose | 8 | 0 | Intravenous catheter site | 2 | 0 |
| Cerebral spinal fluid | 3 | 0 | Peritoneal fluid | 1 | 0 |
| Urine | 2 | 1 | Pleural fluid | 1 | 0 |

(MR) and Voges-Proskauer (VP) tests, buffered peptone-glucose broth; growth at pH 5.6, Sabouraud's Dextrose Agar (Difco); production of β-galactosidase, Differentiation Disc ONPG (Difco); casein hydrolysis, Trypticase Soy Agar (TSA, BBL) with 50% skim milk; lecithinase production, TSA with 10% egg yolk suspension; growth on Desoxycholate Agar (DC Agar, Difco); development of mucoid colonies, TSA with 5% sucrose; pectinase activity, pectate medium (Edwards and Ewing, 1962). Sensitivity to antibiotics was determined by the method of Bauer et al. (1966) utilizing BBL Sensi-discs. MR-VP tests were performed after 48 hr of incubation; gelatin liquefaction and casein hydrolysis required 1 to 14 days of incubation; with the exception of occasional delayed reactions of 48 hr for acid production from carbohydrates, all other reactions were detected after 24 hr of incubation. Cultures were incubated for 3 weeks at 37 C before tests were discarded as negative.

## RESULTS AND DISCUSSION

*Cellular morphology.* The unusual bacterial arrangement consisting of aggregates of individual cells in chain formation (symplasmata), as previously noted by Kathe (1931) and others (Hirsch, 1934; Mack, 1936), was observed in all anaerogenic *Erwinia* strains. The granular, elongated segmented masses were demonstrated after 24 hr of incubation by observing a hanging drop preparation of the condensate of an agar slant culture, a broth culture, or an emulsified colony (Fig. 1). The individual cells comprising the symplasmata are embedded in some supporting matrix (Lev,Alexander and Sobel, 1969) and can be discerned when stained with safranin. These formations apparently are not an exclusive property of *Erwinia* since we also observed them in several unidentified yellow-pigmented oxidative bacilli.

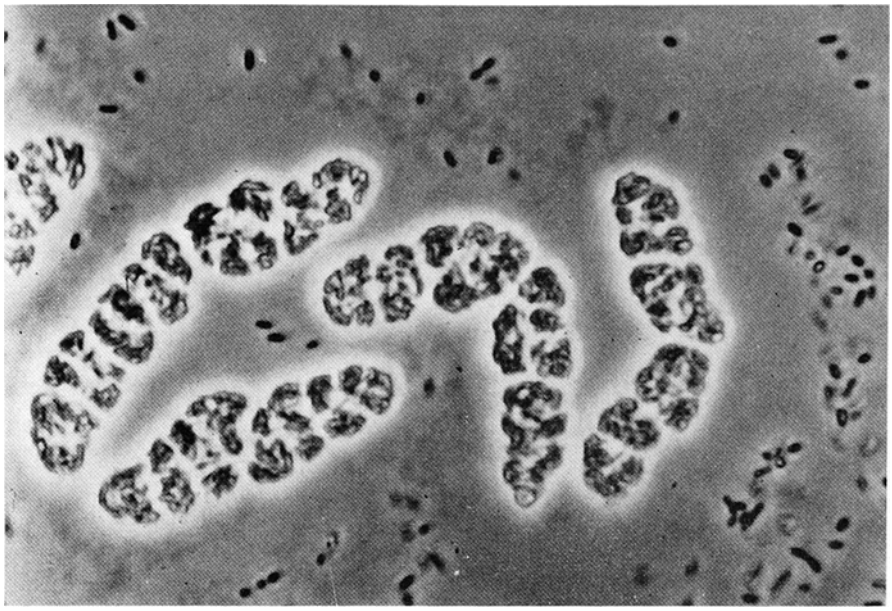

Fig. 1. *Erwinia* showing symplasmata. Hanging drop preparation of the syneresis water of a 24 hr slant culture. 400 ×.

*Colonial morphology.* Most *Erwinia* strains gave rise to predominately round, smooth, convex colonies 1–2 mm in diameter and less frequently to smaller, rough, irregular colonies. Twenty-seven of the isolates displayed larger mucoid colonies resembling *Klebsiella* in appearance and consistency.

Another unusual structure observed in all anaerogenic *Erwinia* strains was the distinct, spindel-shaped, biconvex body first observed by Hirsch (1934) and subsequently described by Cruickshank (1935) as a down-growth of the colony into the agar medium. Examination of colonies after 24 hr of incubation with the low-power objective revealed centrally situated bodies varying from one to several per colony, particularly in rough and smooth colonies and less frequently in mucoid colonies (Fig. 2). The bodies remained adherent to the agar surface when the colonies were removed with a wire loop. Granular aggregates analogous to symplasmata were occasionally observed in association with the biconvex bodies. The biconvex bodies appear unique to this group of bacteria and may be regarded as a diagnostic attribute.

*Anaerogenic group.* The biochemical features of the strains examined are listed in Table 2. The anaerogenic group of strains represents a well defined entity within the genus *Erwinia* (von Graevenitz, 1970). The organisms are faculta-

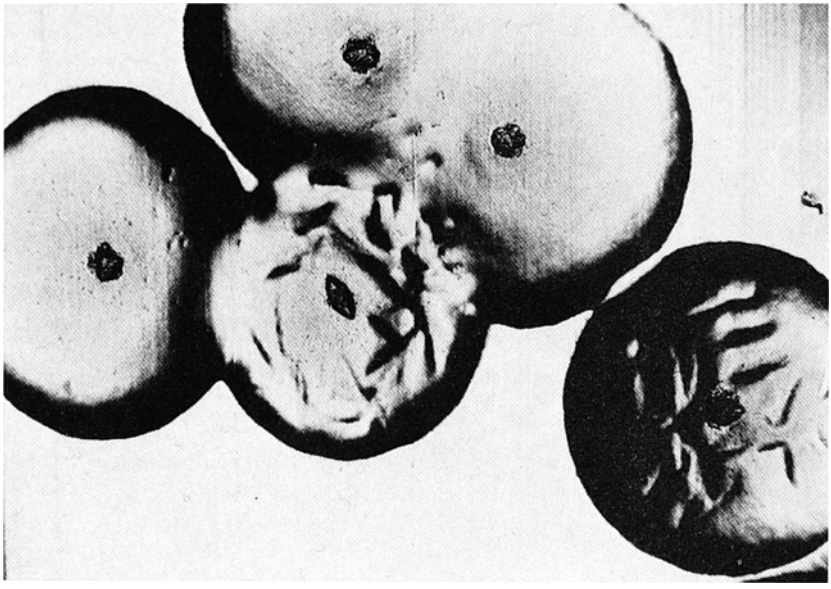

Fig. 2. *Erwinia* colonies showing centrally situated biconvex bodies and granular aggregates analogous to symplasmata after 24 hr of incubation. 27 · .

tively anaerobic, fermentative anaerogenic bacilli with peritrichous flagella which grow at 37 C, liquefy gelatin slowly, generally reduce nitrate to nitrite, and fail to produce oxidase, arginine dihydrolase, and decarboxylase for lysine and ornithine. Of the 11 strains examined none produced pectinase.

*Aerogenic group.* Some of the aerogenic members of *Erwinia* described in the 7th edition of Bergey's Manual of Determinative Bacteriology have been examined by several investigators (Graham, 1964; Brisou, Tysset and Jacob, 1960) and on the basis of decarboxylase activity were found to more closely resemble pigmented *Enterobacter cloacae*.

Seven aerogenic strains examined in this study possess the features of *Erwinia*. In addition to gas production from carbohydrates, the strains differ from the anaerogenic group by their failure to produce symplasmata and biconvex bodies (Table 3). The aerogenic group is tentatively considered by von Graevenitz (1970) as belonging to *Erwinia* because of the lack of dihydrolase and decarboxylase activity.

*Yellow-pigmented Enterobacter.* Thomas and Elson (1957), using the IMViC profile as a criterion, classified yellow chromogenic, gram-negative, aerogenic and anaerogenic bacilli isolated from plants and soil as either *Escherichia coli*,

Table 2. Characteristics of yellow-pigmented bacilli[1]

| | *Erwinia* anaerogenic group (55 strains) | *Erwinia* aerogenic group (6 strains) | Pigmented *Enterobacter* (4 strains) | | *Erwinia* anaerogenic group (55 strains) | *Erwinia* aerogenic group (6 strains) | Pigmented *Enterobacter* (4 strains) |
|---|---|---|---|---|---|---|---|
| Gas from glucose (PBB) | 0 | 6 | 4 | pH 5.6-tolerance | 55 | 6 | 4 |
| Acid: Glucose (OFBM) | 55 | 6 | 4 | TTC-tolerance | 0 | 0 | 0 |
| D-Fructose | 55 | 6 | 4 | Growth on cetrimide agar | 0 | 0 | 0 |
| D-Galactose | 55 | 6 | 4 | Tyrosinase | 0 | 0 | 0 |
| D-Mannose | 55 | 6 | 4 | Growth at 42 C | 43 | 3 | 3 |
| Rhamnose | 55 | 6 | 4 | Motile | 55 | 6 | 4 |
| Xylose | 55 | 6 | 4 | Symplasmata | 55 | 0 | 0 |
| Sucrose | 50 | 5 | 4 | Biconvex bodies | 55 | 0 | 0 |
| Maltose | 55 | 6 | 4 | Yellow pigment | 55 | 6 | 4 |
| Lactose | 54 | 6 | 4 | Hemolysis | 1 | 0 | 0 |
| D-Mannitol | 55 | 6 | 4 | Growth on MacConkey | | | |
| 10% Lactose (PAB) | 55 | 6 | 4 | Agar | 55 | 6 | 4 |
| ONPG | 55 | 6 | 4 | Growth on SS Agar | 15 | 4 | 1 |
| Gluconate oxidation (GS) | 0 | 0 | 0 | Growth on DC Agar | 15 | 4 | 3 |
| Aesculin hydrolysis | 39 | 6 | 4 | Mucoid colonies | | | |
| Nitrate to nitrite | 50 | 6 | 4 | (5% sucrose) | 22 | 5 | 4 |
| Nitrogen gas | 0 | 0 | 0 | Acetate assimilation | | | |
| Voges-Proskauer | 29 | 2 | 3 | (BMM) | 0 | 0 | 0 |
| Methyl red | 25 | 3 | 1 | Glucose assimilation | 55 | 6 | 4 |
| Hydrogen sulfide (KIA) | 2(s) | 0 | 0 | Citrate (Simmons) | 45 | 5 | 4 |
| Indole | 1 | 2 | 0 | Malonate (Leifson) | 15 | 3 | 1 |
| Urea (Christensen) | 2 | 1 | 0 | Sensitive to: | | | |
| Phenylalanine deaminase | 22 | 2 | 0 | Penicillin | 0 | 0 | 0 |
| Gelatin | 55 | 6 | 4 | Novobiocin | 0 | 0 | 0 |
| Gasein | 46 | 2 | 1 | Erythromycin | 44 | 4 | 1 |
| Oxidase | 0 | 0 | 0 | Lincomycin | 0 | 0 | 0 |
| Arginine dihydrolase | 0 | 0 | 2 | Ampicillin | 36 | 4 | 2 |
| Lysine decarboxylase | 0 | 0 | 2 | Tetracycline | 55 | 6 | 4 |
| Ornithine decarboxylase | 0 | 0 | 4 | Chloramphenicol | 55 | 6 | 4 |
| Lipase | 0 | 0 | 0 | Streptomycin | 52 | 5 | 4 |
| Amylase | 0 | 0 | 0 | Cephalothin | 48 | 6 | 0 |
| Pectinase | 0[2] | NT | NT | Nitrofurantion | 47 | 6 | 3 |
| Deoxyribonuclease | 0 | 0 | 0 | Nitrofurazone | 51 | 6 | 4 |
| Lecithinase | 0 | 0 | 0 | Nalidixic acid | 55 | 6 | 4 |
| 2.5% NaCl-tolerance | 55 | 6 | 4 | Neomycin | 55 | 6 | 4 |
| 6.5% NaCl-tolerance | 26 | 3 | 2 | Kanamycin | 55 | 6 | 4 |
| 10.0% NaCl-tolerance | 0 | 0 | 0 | Polymyxin | 55 | 6 | 4 |
| | | | | Gentamicin | 55 | 6 | 4 |

[1]) PBB = Purple Broth Base; OFBM = OF Basal Medium; PAB = Purple Agar Base; ONPG = ortho-nitrophenyl-β-D-galacto-pyranoside; GS = Gluconate Substrate; KIA = Kligler Iron Agar; TTC = triphenyl tetrazolium chloride; BMM = basal mineral medium; NT = not tested; (s) = slight reaction. [2]) Eleven strains tested.

Table 3. Differentiation of *Erwinia* and yellow-pigmented *Enterobacter*[1]

| Test | *Erwinia* (anaerogenic) | *Erwinia* (aerogenic) | Pigmented *Enterobacter* |
|---|---|---|---|
| Gas from glucose | − | + | + |
| Dihydrolase/decarboxylase activity | − | − | + |
| Symplasmata | + | − | − |
| Biconvex bodies | + | − | − |
| Cephalothin susceptibility | V | V | − |

[1]) − = negative; + = positive; V = variable (89% of the *Erwinia* strains cephalothin-susceptible).

*Citrobacter freundii*, or *Enterobacter* species. Leclerc (1963) examined similar strains and proposed that they be designated as *Escherichia adecarboxylata* since they lacked decarboxylase activity. Yellow-pigmented bacilli designated coliforms recovered from human sources have been incriminated in septicemia (Pangalos, 1929), multiple abscesses (Schütz and Laun, 1933), and meningitis (Urmenyi and Franklin, 1961). However, insufficient characterization of these plant-, soil-, and human-isolates makes it difficult to accurately place them into a particular genus, but some of the strains appear to fit the description of *Erwinia*.

Four yellow chromogenic strains identified in this study as *Enterobacter*, including two strains each of *E. aerogenes* (acid produced from inositol) and *E. cloacae*, were differentiated from *Erwinia* by virtue of their dihydrolase and decarboxylase activity (Edwards and Ewing, 1962) and their resistance to cephalothin (Koch and Rose, 1966) (Table 3). Koch and Rose (1966) noted that this latter characteristic may also be of value in distinguishing *Enterobacter* species from other Enterobacteriaceae.

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