Herellea (Acinetobacter) and Pseudomonas ovalis (P. putida) from Frozen Foods

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Seventeen strains of Herellea vaginicola (Acinetobacter anitratus) and 8 of Pseudomonas ovalis (P. putida), isolated from 23 (6.3%) of 364 samples of frozen, foil-pack foods, were identified and characterized morphologically and biochemically. Herellea was isolated from 17 foods (4.7%), P. ovalis from 6 (1.6%). No Mima were found. The food samples included precooked frozen meats, precooked and uncooked frozen vegetables, and uncooked frozen desserts. The bacteria were detected in the food with a procedure used generally for the detection of salmonellae. The pseudomonad simulated the characteristics of Herellea on Sellers differential agar, except for the fact that it fluoresced. From consideration of the habitat and pathogenicity of Herellea and Mima, it is concluded that, although the presence of these bacteria may not be desirable, their significance in food remains unanswered.

During a study of bacteria in frozen foods, Herellea vaginicola (Acinetobacter anitratus) and Pseudomonas ovalis (P. putida) were encountered. The pseudomonad simulated the characteristics of Herellea on Sellers differential agar, except for the fact that it fluoresced. This observation, along with the fact that Herellea has been rarely identified from foodstuffs (7, 34), prompted an investigation of the characteristics of these microorganisms which are discussed in this report.

The literature abounds in names for Debord's (9) H. vaginicola. This bacterium has been placed in many genera, such as Bacterium (19), Moraxella (28), Achromobacter (15, 16), and Acinetobacter (4–6, 37). Comprehensive reviews of the history and bewildering taxonomy of this bacterium are numerous (1, 5, 15, 17, 20, 32, 37). At present no designation is accepted taxonomically; since the term Herellea appeared in the 6th edition of Bergey's Manual of Determinative Bacteriology, this designation was used throughout this report.

Nomenclatural problems exist as well for the pseudomonads (2, 23, 35); the fluorescent species which do not liquefy gelatin have recently been grouped together and designated Pseudomonas putida strains (35). Although P. ovalis is considered a member of this group, its species identity was retained in this report.

MATERIALS AND METHODS

Food samples. Frozen, foil-pack meals packed in dry ice were shipped via air express weekly from F. E. Warren Air Force Base, Wyoming, to the School of Aerospace Medicine. Generally, 8 or 9 separate foods with 4 or 5 samples of each item were received and analyzed the same day; 182 food samples were examined in duplicate, for a total of 364. These samples included precooked frozen meats, precooked and uncooked frozen vegetables, and uncooked frozen desserts.

Method of isolation. Herellea and P. ovalis were detected with a procedure employed for the detection of Salmonellae in frozen foods. The method used was essentially that outlined for the Salmonellae in the 1966 supplemental issue of the Quarterly Bulletin of the Association of Food and Drug Officials concerning the microbiological examination of precooked frozen foods. Duplicate 25-g portions of food were weighed out in blender jars, and 225 ml of tetra-thionate broth was added to one and selenite-cystine broth to the other. The mixtures were blended (2 to 3 min), poured into flasks, and incubated at 35°C for 24 hr. After incubation, loopfuls of each of the broths were streaked onto MacConkey, Brilliant Green, and S S agar plates which were subsequently incubated 24 hr (the broths and plating media were Difco products). From these plates, the isolates of Herellea and P. ovalis were encountered, with the exception that no isolation of Herellea occurred on S S agar. Controls were incorporated in the experimental design for the distilled water and utensils (blenders, pipettes, etc.) with duplicate platings on the three media. Suspicous colonies were fished to Triple Sugar Iron agar (TSI;
Difco) slants; strains yielding an alkaline slant and unchanged butt were subjected to further study. Identifications were based on descriptions of these bacteria from several published sources (1, 12, 15, 16, 20, 21, 23, 27, 35) and from Sellers (unpublished data).

**Known cultures.** In addition to the isolated strains characterized in this study, two cultures of *Herellea* (ATCC 9955 and 9956) were obtained from Carmen Garcia and two of *P. ovalis* (ATCC 950 and 8209) from Walter Sellers. These strains were carried through all of the identification procedures.

**Morphological procedures.** Microscopic morphology was observed in smears (stained by Hucker's modification of the Gram reaction) from cultures incubated for 24 hr at 35 C. Smears were made of growth from Trypticase soy agar (TSA; BBL) with 5% human citrated blood, MacConkey agar (MAC), and *Herellea* agar (Difco). Colonial morphology was examined on blood-agar incubated for 24 hr at 35 C. Growth characteristics were observed on Sellers differential agar (SDA; Difco), S S agar, MAC agar, *Herellea* agar, Plate Count Agar (PCA; Difco), TSA, and Levine E M B agar (Difco). MAC agar was used for growth-temperature studies at 5 C. The improved motility medium of Ball and Sellers (3) was used to determine motility.

**Biochemical procedures.** The biochemical tests, reagents, and media employed included: acid production from glucose (oxidation), fluorescein production, nitrogen gas production, and anaerobic growth in the presence of nitrate (SDA); acid production from 0.5% dulcitol (Phenol Red Broth Base, incubated for 23 days); hydrogen sulphide production (TSI agar); decarboxylase activity (Decarboxylase Base Moeller, Difco, with 0.3% agar); citrate utilization (Simmons Citrate agar, BBL); indole production (1% Trypticase water; Kovacs reagent); nitrate reduction (Nitrate Broth; zinc dust added to negative tubes); oxidase activity (1% tetramethyl paraphenylenediamine dihydrochloride on filter paper with PCA growth); catalase activity (3% hydrogen peroxide on PCA growth); urease activity (Urease Test Medium, Difco); gelatin liquefaction (improved motility medium, incubated 3 weeks at 24 C), and activity in litmus milk (Difco) incubated for 30 days. The motility medium (3) and SDA (33) were employed as the original authors described. Other media were prepared and tests were performed essentially by use of the methods described by Edwards and Ewing (10).

**RESULTS**

**Morphological characterization.** Food-isolate strains of *Herellea* were predominantly pleomorphic gram-negative diplococci with coccoid, bacillary, and filament forms, agreeing in general with the morphology of *Herellea* ATCC 9955 and ATCC 9956. The food-isolate strains of *P. ovalis* also lacked uniformity, exhibiting pleomorphic gram-negative rods; some were short and ovoid; others were longer and more tapered, giving a fusiform appearance; still others were coccobacillary. These strains were somewhat more variable than *P. ovalis* ATCC 950 and strain ATCC 8209. If an ultraviolet light source (Ming-alight) were not used to detect fluorescence of the pseudomonads on SDA, *P. ovalis* isolates might be tentatively considered to be strains of *Herellea*, since on this medium both produced a blue (alkaline) slant, yellow (acid) junction, and green (unchanged) butt; no nitrogen gas was formed and no anaerobic growth occurred. In addition, the *P. ovalis* isolates, including strain ATCC 950, possessed a disagreeable putrid odor similar to that of *Herellea*, rather than the characteristic grape-like odor.

Colonies of all of the *Herellea* strains on blood-agar were circular, convex, entire, smooth, opaque, grayish-white to cream in color, glistening and butyrous. Strains of *P. ovalis* formed circular, convex, entire, smooth, opaque, grayish-white, and butyrous colonies on blood-agar. On MAC agar, the *Herellea* colonies were light pink to lavender in color, whereas the pseudomonads possessed a gray-green colonial color. On *Herellea* agar (24), *Herellea* colonies were pale lavender; the color of the medium and the colonies of *P. ovalis* were gray-green. Several workers (19, 27, 36) have noted that *Herellea* strains produce a distinctive blue colony on E M B agar; however, other bacteria, notably some *Salmonella* strains, may also produce a blue colony on this medium (E. S. Wynne, personal communication). In the present study, blue-colored colonies were formed by most of the *Herellea* strains, including the two ATCC cultures, after 24 hr of incubation on E M B. The blue color was more intense for some strains and was strongest after 48 hr of incubation; three strains did not appear to exhibit any blue coloration. The pseudomonads were all colorless on E M B agar. None of the *Herellea* strains grew on S S agar; in 24 hr the pseudomonads produced pinpoint colonies which became more distinct after 48 hr of incubation; however, *P. ovalis* ATCC 8209 was also negative for growth on S S. On TSA slants, both genera produced grayish-white growth; however, on continued incubation, the growth of the pseudomonads exhibited a pinkish pigmentation at the junction of the butt and slant. Concerning the broth cultures which were used in obtaining the isolates, it was found that all of the *Herellea* strains came only from selenite-cystine broth and, conversely, all of the *P. ovalis* strains were obtained only from tetraionate broth. No growth of *Herellea* occurred on MAC agar at 5 C, even after 30 days of incubation; all of the *P. ovalis* strains were psychrophilic and grew at 5 C. Growth at 5 C was observed in 4 days for four
strains, including the two ATCC cultures, with profuse growth occurring in 7 days; the remaining pseudomonad isolates showed growth within 2 weeks.

**Biochemical characterization.** Results of the diagnostic tests used for the characterization of these bacteria are shown in Table 1. *Herellea* ATCC 9955 was not included in the table, since it did not produce acid from glucose in SDA and was atypical for other *Herellea* characteristics. *P. ovalis* ATCC 8209 gave a similar biochemical pattern as demonstrated by the ATCC strain shown in the table, with the exception that the former produced a delayed positive citrate utilization test and gave only a weak positive test for fluorescein production. Approximately one-third of the *Herellea* isolates gave a weak positive test for urease production. Acid was produced from lactose in litmus milk by all *Herellea* strains in 2 to 21 days. Of the 17 food-isolate strains, 16 did not produce acidity until the 5th day of incubation. All of the pseudomonads produced an alkaline milk reaction within 2 days.

**Occurrence.** A total of 23 of the 364 (6.3%) samples of frozen, foil-pack foods yielded *Herellea* or *P. ovalis* organisms. All were found in only one of the duplicate samples for a given food. Every type of frozen food yielded these bacteria. *Herellea* was isolated from 17 foods (4.7%) and *P. ovalis* from 6 (1.6%). Controls were sterile.

**DISCUSSION**

When Sellers (33) devised his differential agar for nonfermentative gram-negative bacteria, he noted another organism which simulated the characteristics of *Herellea* on the medium, except for the fact that it fluoresced. He later keyed the bacterium to *P. ovalis* (Sellers, unpublished data). Recently, Farkas-Himsley (13) found that, with SDA, *P. putida* likewise was capable of oxidizing glucose in the presence of high peptone concentrations and gave the yellow band. Stanier, Palleroni, and Doudoroff (35) recently published an extensive taxonomic study of the pseudomonads and concluded that there was nothing in the published descriptions of *P. ovalis* which would permit its differentiation from *P. putida*. On this basis, strains of *P. ovalis* may be considered identical to strains of *P. putida*. The nomenclatural problems concerning both of these bacterial genera must be resolved, so that some degree of synonymy can be achieved in reporting results.

The psychrophilic pseudomonads are well-known and frequently occurring food-spoilage microorganisms (2, 14, 25). *P. putida* has been implicated as one causative agent of slimy chickens and refrigerated sliced beef and ofusty eggs (2). Rogers (31) reported one case of human infection with *P. putida*.

Little is known concerning *Herellea* in food. Few studies have shown the presence of these bacteria in food, although *Herellea*-like paired cocci may have been observed in many early food studies, but not identified or named as such (18). Mossel (26) found *Achromobacter anitratus* (*Herellea*) in 69% of bulk raw human milk samples. Cordaro, Ball, and Schmidt (7) identified *Herellea* from 49 of 56 samples of an experi-
mental space-flight liquid diet. They noted that Hereliea was absent from the feces of the subjects subsisting on the diet and suggested that the bacteria, when ingested, do not multiply to any great extent in the gastrointestinal tract. They further indicated that the utensils used in preparing the diet might have been contaminated by the food handler. Mima, the other member of DeBord’s tribe Mimeae (9), was also found in 31 samples. Koburger (22) isolated Mima from two samples of milk products. Snodgrass and Koburger (34) recently reported isolation of the tribe Mimeae from 25 of 96 retail food samples, including fresh, pasteurized, precooked, and frozen products. Mima was found in 16% of the food samples, Hereliea in 9%. In the present investigation, no Mima strains were found; the primary isolation method may not have been completely appropriate.

Increasing reports have been published concerning the finding of the tribe Mimeae in or on the human body, these bacteria seemingly existing as ordinary commensals in the genital and respiratory tracts, and on skin (8, 12, 15, 32, 36; J. E. Greer, G. R. Mikhail, and C. S. Livingood, Bacteriol. Proc., p. 49, 1962; C. L. Sklair, Bacteriol. Proc. p. 49, 1965). Their existence as commensals of the intestinal tract may be open to question, since they have rarely been recovered from normal human feces. Besides being endogenous to human hosts, these microorganisms have been isolated from exogenous environments such as soil and water, and stated to have a ubiquitous and worldwide existence (4, 15, 17, 20, 29). It is obvious that these bacteria may gain entrance to foods from other than a human source.

It is clearly established that, under certain conditions, Hereliea as well as Mima are pathogenic for humans (8, 29, 30). They apparently possess a low degree of virulence infecting hosts of altered or low resistance (11, 29). However, since Cordaro, Ball, and Schmidt (7) found that these bacteria, when ingested, do not multiply in sufficient numbers in the gastrointestinal tract to be isolated from feces, it might appear that their potential health danger from ingestion of food is minimal. Snodgrass and Koburger (34) indicated that these bacteria may present a potential health danger and may contribute to spoilage of foods, but suggested that further study was needed.

It is not possible at this time to suggest what the importance, if any, of these bacteria in food may be, except to recognize that their presence may not be desirable. Mosse1 (26) recognized the potential role of A. anitratus (Hereliea) as an indicator microorganism in food hygiene, but because of the paucity of knowledge regarding the ecology of this bacterium, declined to emphatically suggest such use.

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LITERATURE CITED