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# Biotypes of *Proteus rettgeri*

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Examination of 729 isolates of *Proteus rettgeri* showed that 674 reacted positively in tests for phenylalanine deamination, indole production, growth on citrate, and acid production from meso-inositol, and negatively for L-ornithine decarboxylation and acid production from lactose, maltose, D-xylose, and L-arabinose. Only 51 isolates differed in one, and four differed in two of these ten reactions, which were taken as the core characteristics of the species. On the basis of additional tests (acid production from salicin, L-rhamnose, D-mannitol, adonitol, and D-arabitol), the 729 isolates could be separated into five groups. Groups 1, 2, 3, and 4 could be further separated on the basis of the reaction with meso-erythritol, and group 5 could be subdivided on the basis of reaction with D-mannitol. Two metabolically distinct kinds of *P. rettgeri* were recognized. Isolates of the first kind (groups 1, 2, 3, and 4) each utilized both adonitol and D-arabitol and most utilized meso-erythritol. Isolates of the other kind (group 5) were negative with the three polyhydric alcohols but resembled, in their reactions, some strains of *Providencia stuartii*. These may be intermediates between *P. rettgeri* that catabolize these substrates and the Providencia.

One of the gram-negative species of bacteria that has increased in importance as an infectious agent is *Proteus rettgeri*. Its importance in causing outbreaks of hospital cross-infection has been observed by several groups of investigators (5, 21, 37) and its resistance to therapeutic antimicrobials has been reported (1, 3, 4, 5, 12, 13, 15, 22, 25-27, 35, 36). The epidemiology of the infections has not been resolved and, to a considerable degree, this has been due to the unavailability of schemes for typing strains. Typing on the basis of flagellar (H) and somatic (O) antigens has been investigated (19, 23, 24) and the application of O typing has been described in one report (37). In other studies the marker for identifying the cross-infecting strain was either its pattern of resistance to antimicrobials (5, 13) or its unusual biochemical reactions (35, 36). To our knowledge, strains have not been characterized according to biotypes defined for the species (19). In this study we examined isolates of *P. rettgeri* to determine reactions most characteristic for defining the species and the biochemical types within the species, and to determine the frequency distribution of the different types in our collection of 729 isolates.

## MATERIALS AND METHODS

**Bacterial strains.** Isolates of *P. rettgeri* were collected during the last 9 years from a variety of institutions, including 11 hospital laboratories in Canada and four in the United States. Some were obtained from hospital laboratories that reported cross-infections caused by *P. rettgeri* and some of these could be regarded as duplicates of the same strain. Strains were also obtained from a collection made in Japan by S. Namioka and R. Sakazaki (19). Most sources and contributors have been cited previously (24). Fifty-three isolates from frogs captured in Ontario were also included in the collection. Hence, from 23 separate sources, a total of 729 isolates were available to this study.

**Biochemical reactions.** The basal medium for the detection of acid production from carbohydrates and polyhydric alcohols was Andrade peptone water (Oxoid). Glucose, D-mannitol, salicin, adonitol, meso-erythritol, D-arabitol, L-rhamnose, and meso-inositol were added to the basal medium before sterilization. Lactose, maltose, L-arabinose, and D-xylose were sterilized by filtration and then added to sterilized basal medium. The concentrations of the compounds were according to Edwards and Ewing (7). Durham tubes for detection of gas production were placed in tubes containing glucose, D-mannitol, and maltose. All tests for acid and gas production were kept for 7 days at 37 C before a negative result was recorded. An intensive pink or red color was taken as a positive test for acid production. A positive test was recorded for gas production when the observed volume constituted a bubble equal or greater in diameter than the inside diameter of the Durham tube within a 48-h incubation period. Medium (Christensen [Difco]) was used in tests for urease activity. Tests were read at 24 h and positive tests were recorded on observation of a

pink color on the agar surface. Tests for phenyl pyruvic acid production from DL-phenylalanine were performed according to Edwards and Ewing (7) by using media supplied by Baltimore Biological Laboratory (BBL). A negative test at 24 h was repeated at 48 h. Production of blue color on Simmons' citrate (BBL) was taken as a positive test for growth on citrate as the sole source of carbon. Tubes were examined at 24 h and, if necessary, at daily intervals for another 6 days. Tests for indole production were performed in tryptone broth (Difco) at 24 h. If an isolate gave a negative test after this period the test was repeated after a 3-day period of incubation. Kovac reagent was prepared as previously described (7). Moeller decarboxylose broth base (BBL) was used for ornithine decarboxylase test media. L-ornithine dihydrochloride was added in the broth base and the medium was dispensed and sterilized according to the supplier's directions. Positive tests were indicated by the production of a violet color and tests were held for 4 days before a negative result was recorded. The incubation temperature of all biochemical tests was at 37 C.

**Serotyping.** All strains have been typed on the basis of the O antigens using antisera prepared in this laboratory and by methods described previously (24).

## RESULTS

**Biochemical reactions characteristic of most isolates of P. rettgeri.** A total of 729 isolates of *P. rettgeri* were examined. All produced acid and 18% produced gas from glucose. Each isolate was tested for deamination of phenylalanine, hydrolysis of urea, production of indole, growth on citrate, decarboxylation of ornithine, and acid production from inositol, lactose, maltose, xylose, arabinose, salicin, rhamnose, mannitol, adonitol, arabitol, and erythritol. Of the 729 isolates, 674 were positive on tests for phenylalanine deamination, urea hydrolysis, indole production, growth on citrate, and acid production from inositol but were negative for acid production from lactose, maltose, xylose, and arabinose, and for ornithine decarboxylation (Table 1). These 10 tests were taken as basic characters of the species. The 674 isolates were considered to be characteristic representatives of the species to be further examined for biogroups and to be used as a standard set for comparison in the examination of the remaining 55 isolates that varied in reaction from the rest in one or two of the 10 tests.

**Biogroups of P. rettgeri.** Namioka and Sakazaki described the division of 103 *P. rettgeri* into four types on the basis of acid production from salicin and rhamnose (19). In this study, their scheme was modified by the definition of a fifth group (Table 1). Each isolate of biogroups 1, 2, 3, and 4, but not 5, produced acid from each of the three polyhydric alcohols—adonitol, arabitol, and mannitol. Biogroup 5 consisted of isolates incapable of acid production from salicin, rhamnose, adonitol, and erythritol. Some group 5 isolates produced acid from mannitol and this test served to define two subgroups. The mannitol positives were designated 5a and the mannitol negatives, 5b. Each of groups 1, 2, 3, and 4 could be subdivided into two subgroups on the basis of acid production from erythritol. The erythritol positives were designated 1a, 2a, 3a, and 4a, and the erythritol negatives were designated 1b, 2b, 3b, and 4b.

The numbers of isolates in each subgroup are also shown in Table 1. The largest number was found in group 3 and most of these (177) were in subgroup 3a. Next in number with 133 isolates were the mannitol negatives of group 5. These were followed by the erythritol positives of group 2 with 103 isolates. In each of groups 1, 2, and 3, the erythritol positives outnumbered the negatives by a factor of 2.3 to 3.7 and, therefore, the ability and not the inability to produce acid with this polyhydric alcohol was characteristic of the majority in these groups. Only 1, 2, and 4 isolates were found in subgroups 4a, 4b, and 5a, respectively. These seven constituted less than 1% of the total, and this indicated that they may be rare types.

The 55 isolates that differed in one or two of the ten reactions characteristic of the 674 could all be assigned to one of the groups (and subgroups) on the basis of their reactions to salicin, rhamnose, adonitol, arabitol, mannitol, and erythritol. Sixteen biochemical types were recognized among the 55 (Table 2).

In some cases, the positive reactions with the polyols (adonitol, arabitol) were important, not only in assigning an isolate to a group or subgroup, but indeed, to the species. This was the case with the urease-negative isolates and the ability to produce acid from these polyols may be considered as a major defining characteristic of *P. rettgeri* since all isolates of groups 1, 2, 3, and 4 demonstrated this feature.

## DISCUSSION

Rettger's bacillus was first isolated by Rettger in 1904 from a cholera-like epidemic in chickens (14). It appears that the bacterium was not characterized by Rettger but was sent to P. Hadley who undertook a study of the organism. In a report by Hadley et al. (14), the organism was described as gram-negative, nonmotile, capable of acid production from glucose, mannitol, adonitol, salicin, xylose, and mannose,

TABLE 1. *Biochemical reactions of 674 isolates[a] of P. rettgeri*

| Biochemical reaction | Biogroups | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | |
| | a | b | a | b | a | b | a | b | a | b |
| Acid production from | | | | | | | | | | |
| Salicin | +[b] | + | + | + | −[c] | − | − | − | − | − |
| L-rhamnose | − | − | + | + | + | + | − | − | − | − |
| D-mannitol | + | + | + | + | + | + | + | + | + | − |
| Adonitol | + | + | + | + | + | + | + | + | − | − |
| D-arabitol | + | + | + | + | + | + | + | + | − | − |
| Meso-erythritol | + | − | + | − | + | − | + | − | − | − |
| Number of isolates | 109 | 39 | 103 | 28 | 177 | 78 | 1 | 2 | 4 | 133 |

[a] All 674 isolates were positive for phenylalanine deamination, urea hydrolysis, indole production, growth on Simmons' citrate, and acid production from meso-inositol. All were negative for ornithine decarboxylation and acid production from lactose, maltose, D-xylose, and L-arabinose. These 10 tests are the major defining "core" characters of *P. rettgeri*.

[b] Positive reactions in 2 days for most tests with most isolates. Less than 1% of inositol positives required 3 to 4 days and less than 2% of erythritol positives required 3 to 5 days.

[c] Negative reactions at 7 days.

TABLE 2. *Biochemical types of infrequently isolated P. rettgeri that vary in "core" characters*

| Biogroup[a] | Total no. of isolates | No. varying in "core" characters[b] | "Core" character which is different[c] |
|---|---|---|---|
| 1a | 116 | 7 | xyl$^+$ (1)[d]<br>ind$^-$ (1)<br>inos$^-$ (2)<br>ara$^+$ (2)<br>inos$^-$, man$^-$ (1) |
| 1b | 44 | 5 | inos$^-$ (2)<br>ara$^+$ (1)<br>xyl$^+$, lac$^+$ (2) |
| 2a | 103 | 0 | |
| 2b | 30 | 2 | xyl$^+$ (1)<br>ure$^-$, ind$^-$ (1) |
| 3a | 178 | 1 | xyl$^+$ (1) |
| 3b | 79 | 1 | ind$^-$ (1) |
| 4a | 4 | 3 | ure$^-$ (3) |
| 4b | 2 | 0 | |
| 5a | 4 | 0 | |
| 5b | 169 | 36 | lac$^+$ (1)<br>xyl$^+$ (14)<br>ind$^-$ (21) |

[a] Biogroups defined in Table 1.

[b] See text or Table 1 for 10 characteristic "core" reactions.

[c] Xylose (xyl), indole (ind), inositol (inos), arabinose (ara), mannitol (man), urease (ure), lactose (lac).

[d] Number of isolates with indicated variation.

and incapable of producing indole. Furthermore, they performed virulence tests in chickens but failed to demonstrate the anticipated virulence. Weldin (38) assigned the organism to the genus *Shigella* but later investigations by Edwards (6) established that the organism was motile, and by St. John-Brooks and Rhodes (32) who established that it produced indole, and it was subsequently rejected from the *Shigella* by Neter (20). It was later assigned to the genus *Proteus* by Rustigian and Stuart (29) after they found it capable of urea hydrolysis, a reaction most characteristic of *Proteus vulgaris* and *Proteus mirabilis*. F. Kauffmann, on the other hand, believed the organism should be separated from *Proteus* and proposed the genus *Rettgerella* for this group of bacteria (16). Both taxonomic designations are currently in use and agreement on final classification remains to be reached.

In this study, we have attempted to identify the definitive characteristic reactions of the species and to examine a group of isolates, many from geographically diverse sources, for variations in biochemical types and for reactions through which the different types may be readily defined. Of the 729 isolates, 674 reacted positively in five tests (phenylalanine deamination, urea hydrolysis, indole production, growth on citrate, and acid production from inositol), and negatively in five other tests (acid production from lactose, maltose, xylose, and arabinose, and decarboxylation of ornithine). Since reaction to the 10 tests was uniform for such a large portion (92.5%) of the isolates, the 10 reactions were considered basically definitive for the species, and the 674 isolates were re-

garded as characteristic representatives of the species. A strain showing these 10 reactions may therefore be diagnosed with confidence as *P. rettgeri*.

This representative group of isolates could be separated into five biogroups. Biogroups 1, 2, and 3 were defined according to Namioka and Sakazaki (19) who proposed the subdivision of the species on the basis of acid production from salicin and rhamnose. However, when the isolates were tested in mannitol, arabitol, adonitol, and erythritol, we observed that the fourth division, proposed by Namioka and Sakazaki for salicin-negative, rhamnose-negative strains, included two biochemically different types (19). Some were like those of groups 1, 2, and 3 which all produced acid from adonitol and arabitol and were assigned to group 4. Others that did not produce acid from the two pentitols were assigned to group 5. Each group could be further subdivided. Isolates of groups 1, 2, 3, and 4 could be separated into erythritol positives (subgroups 1a, 2a, 3a, and 4a) and erythritol negatives (subgroups 1b, 2b, 3b, and 4b) and group 5 could be separated into mannitol positive (subgroup 5a) and mannitol negative (subgroup 5b).

Adonitol, arabitol, and erythritol were important substrates in defining the groups. All isolates of groups 1, 2, 3, and 4 of the 674 that conformed uniformly to the first selected 10 tests and isolates of 13 types found among the 55 that differed in one or two reactions to these 10 characteristic tests produced acid with both adonitol and arabitol. This constituted 556 (76%) of the isolates and, therefore, an isolate positive for phenylalanine deamination and positive for acid production from these two pentitols is likely to be *P. rettgeri*. Although other *Proteae* may produce acid from one of the two pentitols, in our experience, none is positive on both. Furthermore, reactions to these two pentitols clearly separate *P. rettgeri* into two biochemically different kinds—those of groups 1, 2, 3, and 4, and those of group 5. Isolates of the latter differ from some *Providencia stuartii* only on urease agar and thus constitute a group intermediate between *Providencia* and the more frequently isolated *P. rettgeri* of the other groups. Erythritol was metabolized by 401 (55%) of the isolates and it is important to include this polyhydric alcohol in the identification of *Proteae* since *P. rettgeri* is the only member of the tribe that produces acid from this substrate. By its use, the subgrouping of groups 1, 2, 3, and 4 is possible and this assists in the characterization of strains that may be epidemiologically important.

Kauffmann pointed out the usefulness of erythritol and L-arabitol in differentiating *P. rettgeri* from the other *Proteae* (17, 18). In our laboratory, D-arabitol was used instead of L-arabitol after it was observed that 50 strains giving positive reactions with L-arabitol in 3 to 5 days also reacted with D-arabitol, but usually within 24 h. It was interesting to note that each isolate positive on erythritol was also positive on the two pentitols and, with one exception (see biogroup 1a, $ino^-$, $man^-$, Table 2), was positive on mannitol. Since no erythritol-positive, pentitol-negative isolates were found, it could be postulated that catabolism or transport (or both) of these acyclic 4- and 5-carbon polyhydric alcohols was related and that loss of activity commenced with the polyhydric alcohol of lower molecular weight. This was not investigated but two pertinent observations may be cited. The longer that cultures were maintained in the laboratory, the greater was the number of erythritol negatives. Among the 556 isolates of biotypes 1, 2, 3, and 4 collected over the past nine years, there were 155 (28%) erythritol negatives. Among these were 32 isolates obtained from two local hospitals during the past year, and these were all typed shortly after isolation as erythritol positives of groups 1, 2, or 3. Furthermore, the strains obtained from S. Namioka were examined. We were not able to demonstrate positive erythritol reactions for 11 (30%) of the 37 strains. These strains were all characterized in 1958 by Namioka and Sakazaki as erythritol positive (19). Erythritol was not used initially in our laboratory in characterizing strains and, therefore, changes from positive to negative reactions were not recorded. The reactions observed by us that differed from those of Namioka and Sakazaki (19) may have been due to differences in media or methods. Hence, retesting strains periodically would be necessary to document the loss of reactivity with time. Strains of groups 1, 2, 3, and 4 should receive further study in transport and catabolism of polyhydric alcohols, not only to determine the nature of the apparent relationship between utilization of erythritol and the pentitols and the apparent loss of activity with erythritol during maintenance in the laboratory, but also because they are capable of utilizing both a variety of acyclic polyhydric alcohols as well as the cyclic inositol.

An examination of the 55 isolates that differed in one or two reactions from the other 674 in the 10 tests taken as characteristic revealed 16 different biochemical types (Table 2). Three isolates differed from types characteristic of the large group of 674 (Table 1) in positive tests for

acid production from lactose. One (biogroup 5b, lac$^+$) was obtained from a hospital in Toronto and was characteristic of group 5b isolates except for the positive lactose reaction. Two isolates (biogroup 1b, xyl$^+$, lac$^+$) of the same O serotype were obtained from Traub who isolated them during a study of hospital cross-infections caused by *P. rettgeri* (36). Lactose-positive *P. rettgeri* have been known to occur since 1954 when one was reported in Israel by Singer and Bar-Chay (31). Sutter and Foecking described 53 isolates which were both lactose and xylose positive and indicated that they may have been isolates of an endemic strain (33). Suter et al. (34) reported that eight of their 48 strains were lactose fermenters, and eight were positive for xylose. From their tabulations of reactions, it could not be ascertained that the eight lactose positives were also the eight xylose positives. If this were the case, the occurrence of the lactose-positive, xylose-positive types in three geographically separated areas would be indicated. This leads to the view that these types may be more widely distributed and may occur more frequently than reported.

In our collection, four different types with the xylose-positive reaction were recognized in addition to the xylose-positive, lactose-positive type discussed above. Only one isolate was found for each of three types (biogroup 1a, xyl$^+$, biogroup 2b, xyl$^+$, and biogroup 3a, xyl$^+$), but 14 were obtained for the fourth (biogroup 5b, xyl$^+$). Of these 14 xylose-positive 5b types, nine isolates were of the same O serotype and from the same hospital, but the other five isolates were each of different O serotypes and thus, at least six different strains were included in this biochemical type. From some reports, the conclusion can be made that xylose-positive *P. rettgeri* are rare since they constitute a low proportion of the total isolates (2, 11, 19, 30), but in other reports, larger percentages of the isolates described are xylose positive (31, 33, 34). The original isolate of Rettger was shown by Hadley et al. (14) to be xylose positive and, according to its other reactions, it would appear to be a xylose positive of group 1 or 2. Only 19 (2.5%) of our isolates were xylose positive and, because of this low number, a negative test for acid production from this substrate was taken as a definitive characteristic reaction for *P. rettgeri*. Whether this choice was just a reflection of our present collection and does or does not reflect the distribution of these types in the species at large cannot be determined without large numbers of additional isolates from greater numbers of geographically separated sources than have been available to us in the past. Should the xylose-positive reaction be characteristic for large numbers of *P. rettgeri* rather than a few, it could be accepted as a definitive character and used in the same manner as erythritol in the further subdivision of the five biogroups.

Four types of indole-negative *P. rettgeri* were identified. In other respects one isolate was like the subgroup 1a isolates, one like the 3b isolates, and 21 like 5b isolates. Three of the latter were serotyped as O:26 and were obtained during a cross-infection in one hospital (37), 16 were serotyped as O:42 and were obtained from a second hospital reporting a cross-infection, and the remaining two, also O:42, were obtained from a third source. One strain, reported to have been isolated before 1954 and received labeled as a *Providencia*, was found to be both indole negative and urease negative. Three other strains, each of different O serotype and from two different sources, were classified by us as urease-negative *P. rettgeri*, although they were also received labeled as *Providencia*.

The positive urease reaction is a major defining character for the genus *Proteus* and a negative reaction is characteristic of *Providencia*. In the above cases, the reassignment from *Providencia* to *P. rettgeri* was based on reactions to 15 of the 16 biochemical tests, and the negative urease reaction was therefore ranked less in significance in classification than the other 15 reactions. In particular, the positive reactions with adonitol and arabitol and, in the case of the three strains, their positive reactions with erythritol, are criteria more characteristic of *P. rettgeri* than of the *Providencia* (9). Although the test for urease is an important defining character, too much attachment to its significance can result in misclassification.

Four isolates were inositol negative, but in other reactions were like those of group 1. One strain, isolated before 1958, was unique because it did not produce acid from inositol and mannitol, but was similar in other reactions to group 1 isolates.

Three isolates, two like those of subgroup 1a and one like those of 1b, produced acid from arabinose. Arabinose-positive *P. rettgeri* have been reported in other investigations (17, 30). Other workers did not use arabinose (11, 33) or reported no strains positive (7, 8, 10, 28, 34). To our knowledge, arabinose-positive strains have not been implicated in cross-infection as have xylose-positive, lactose-positive, and indole-negative strains (33, 36).

Since 674 (92.5%) of the 729 isolates were uniform in 10 tests, and only 51 (7%) differed by one reaction and only four (0.5%) differed by

two reactions in the 10 tests, a strain that conforms characteristically to the same 10 tests may be classified with confidence as *P. rettgeri*. Five biogroups and 10 subgroups have been defined among the 674 isolates reacting characteristically in the 10 tests. Furthermore, the other 55 isolates that differed from the 674 in one or, rarely, two reactions to the 10 characteristic tests, could each be assigned to one of the 10 subgroups on the basis of reactions to six tests used to define the subgroups.

On the basis of reactions to two pentitols, adonitol and arabitol, the species could be separated into two biochemically different kinds. The first (biogroups 1, 2, 3, and 4) include strains that may be referred to as metabolizers of polyols. These produce acid with inositol, mannitol, arabitol and, in most cases, with erythritol. The utilization of each strain of acyclic polyols of 4, 5, and 6 carbons is unique among the *Proteae* to four biogroups of *P. rettgeri* and, therefore, a *Proteus* isolate with this metabolic facility may be assigned to this species. This is advantageous in the identification of biochemical types that differ in one or two of the 10 reactions cited above as definitive characters of the species.

The second kind of *P. rettgeri*, found in biogroup 5, did not catabolize the two pentitols or erythritol, and only a few catabolized mannitol (subgroup 5a). Instead, they resembled some strains of *P. stuartii* in their reactions and may be a group intermediate to the polyol metabolizers of *P. rettgeri* and the *Providencia*. The relationship between the latter and the *P. rettgeri* of biogroup 5 is currently under investigation in our laboratory.

We anticipate that biotyping of strains according to the groups and subgroups defined in this study will serve advantageously in epidemiological investigations of infections caused by *P. rettgeri*. A finer resolution among strains will be achieved upon application of both this scheme for biochemical differentiation and the scheme for O serotyping proposed in a previous study (24).

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