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# Semiquantitative Catalase Test as an Aid in Identification of Oxidative and Nonsaccharolytic Gram-Negative Bacteria

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A simple and rapid semiquantitative slide catalase test useful for the identification of oxidative and nonsaccharolytic gram-negative bacteria, i.e., "nonfermenters," is described. Using the intrepretative criterion of time of appearance of oxygen bubbles in 3% hydrogen peroxide, three categories of nonfermenters were established. The rapid catalase producers included *Achromobacter xylosoxidans* and *Achromobacter* species; *Acinetobacter anitratus* and *Acinetobacter lwoffii*; *Bordetella bronchiseptica*; CDC group IVE; *Pseudomonas aeruginosa*, *P. fluorescens*, *P. putida*, *P. diminuta*, and *P. acidovorans*; and *Moraxella urethralis* and M-6. The delayed catalase producers included *Bordetella parapertussis*, CDC group VA-1, *P. alcaligenes*, *P. cepacia*, *P. mendocina*, *P. pickettii* (VA-2), *P. pseudoalcaligenes*, *P. putrefaciens*, *P. stutzeri*, *P. testosteroni*, and *P. vesicularis*. The third group consisted of an additional 17 taxa of nonfermenters which were classified as moderate catalase producers.

The catalase reaction, as a bacterial identification test, offers many positive features, namely simplicity, low cost, reproducibility, and rapidity of results (direct testing of primary bacterial isolates, no incubation period). Thus, catalase detection remains a basic differential test, e.g., staphylococci (positive) and streptococci (negative). Catalase testing, with emphasis on semiquantitative interpretation of positive reactions, is a valuable identification test for the mycobacteria (1). In 1972, Taylor and Achanzar (5) reported on a slide catalase test in which semiquantitation of catalase activity was useful as an aid in the differentiation of members of the *Enterobacteriaceae*. Semiquantitative catalase data useful in the identification of oxidative and nonsaccharolytic gram-negative bacteria, i.e., "nonfermenters," is provided in a report by Weaver et al. (6). However, in their method, nutrient agar subcultures are necessary, causing a delay in obtaining results.

In the present report, a characterization of 41 species and groups of nonfermenters, based on a semiquantitative slide catalase test performed on primary isolates, is presented. Practical applications of the data are illustrated and discussed.

## MATERIALS AND METHODS

**Bacteria studied.** Table 1 lists the taxa and number of isolates of each nonfermenter examined and shows the number of isolates falling into each of the three semiquantitative categories of catalase activity. With the exception of the isolates of *Bordetella bronchiseptica*, *Bordetella parapertussis*, Center for Disease Control (CDC) groups IIJ, VE-1, and VE-2, and *Pseudomonas paucimobilis*, which are represented by stock cultures from the collections of R. E. Weaver (Special Bacteriology, CDC, Atlanta, Ga.) and G. L. Gilardi (Hospital for Joint Disease, New York, N. Y.), most of the bacteria tested were recent clinical isolates obtained from the V.A. Medical Center, Miami, Fla., and nine other hospital laboratories.

**Semiquantitative catalase testing and interpretation of results.** The catalase activity of 18- to 24-h isolates was determined by introducing a colony (several when colonies were pinpoint in size) into a drop of 3% hydrogen peroxide on a glass slide and observing the time required for the appearance of oxygen bubbles. Instant bubbling was classified as "rapid," whereas a 2-s or greater delay was classified as "delayed." Five percent sheep blood agar isolates were used for testing due to the poor or complete lack of growth on selective media (such as MacConkey agar) of many of the bacteria studied. Although catalase testing of blood agar isolates is not recommended due to the presence of erythrocyte and leukocyte peroxidases (2), the small and delayed bubbling resulting from these peroxidases in no way interferes with the interpretation of the semiquantitative catalase activity described.

## RESULTS

In Table 1, the following nonfermenter taxa are shown to belong to the category of rapid catalase bacteria: *Achromobacter xylosoxidans*; *Achromobacter* species (CDC group VD); *Acinetobacter anitratus*; *Acinetobacter lwoffii*; *B. bronchiseptica*; CDC group IVE; *Pseudomonas aeruginosa*, *P. fluorescens*, *P. putida*, *P. dimi-*

TABLE 1. *Comparison of the catalase activity of oxidative and nonsaccharolytic gram-negative bacteria using a semiquantitative slide test*

| Taxon | No. tested | Speed of catalase reaction | | |
|---|---|---|---|---|
| | | Rapid | Moderate | Slow |
| *Achromobacter xylosoxidans* | 27 | 27 | 0 | 0 |
| *Achromobacter* species (CDC group VD) | 25 | 20 | 5 | 0 |
| *Acinetobacter calcoaceticus* subsp. *anitratus* | 46 | 42 | 4 | 0 |
| *A. calcoaceticus* subsp. *lwoffii* | 17 | 14 | 3 | 0 |
| *Alcaligenes denitrificans* | 5 | 3 | 2 | 0 |
| *A. faecalis* | 7 | 3 | 4 | 0 |
| *A. odorans* | 23 | 16 | 7 | 0 |
| *Bordetella bronchiseptica* | 5 | 5 | 0 | 0 |
| *B. parapertussis* | 4 | 0 | 0 | 4 |
| CDC group: | | | | |
| IIF | 8 | 0 | 4 | 4 |
| IIJ | 5 | 1 | 4 | 0 |
| IIK-2 | 11 | 1 | 4 | 6 |
| IVC-2 | 6 | 4 | 2 | 0 |
| IVE | 7 | 7 | 0 | 0 |
| VA-1 | 8 | 0 | 0 | 8 |
| VE-1 | 9 | 1 | 1 | 7 |
| VE-2 | 8 | 1 | 1 | 6 |
| *Flavobacterium meningosepticum* | 10 | 1 | 9 | 0 |
| *Flavobacterium* species IIB | 10 | 6 | 4 | 0 |
| *F. odoratum* (M-4F) | 14 | 10 | 4 | 0 |
| *Pseudomonas aeruginosa* | 100 | 97 | 3 | 0 |
| *P. aeruginosa* (apyocyanogenic) | 27 | 26 | 1 | 0 |
| *P. acidovorans* | 11 | 11 | 0 | 0 |
| *P. alcaligenes* | 7 | 0 | 1 | 6 |
| *P. cepacia* | 16 | 0 | 0 | 16 |
| *P. diminuta* | 11 | 11 | 0 | 0 |
| *P. fluorescens* | 21 | 20 | 1 | 0 |
| *P. maltophilia* | 31 | 11 | 17 | 3 |
| *P. mendocina* | 7 | 0 | 2 | 5 |
| *P. paucimobilis* | 4 | 2 | 2 | 0 |
| *P. pickettii* (VA-2) | 6 | 0 | 0 | 6 |
| *P. pseudoalcaligenes* | 12 | 0 | 1 | 11 |
| *P. putida* | 27 | 26 | 1 | 0 |
| *P. putrefaciens* | 10 | 0 | 0 | 10 |
| *P. stutzeri* (Vb-1, Vb-2) | 22 | 0 | 4 | 18 |
| *P. testosteroni* | 8 | 0 | 1 | 7 |
| *P. vesicularis* | 7 | 0 | 0 | 7 |
| *Moraxella nonliquefaciens* | 3 | 2 | 1 | 0 |
| *M. phenylpyruvica* | 5 | 1 | 2 | 2 |
| *M. urethralis* (M-4) | 4 | 4 | 0 | 0 |
| M-6 | 5 | 5 | 0 | 0 |

*nuta*, and *P. acidovorans*; and *Moraxella urethralis* and M-6. Those taxa placed into the delayed catalase category are as follows: *B. parapertussis*, CDC group VA-1, *P. alcaligenes*, *P. cepacia*, *P. mendocina*, *P. pickettii* (VA-2), *P. pseudoalcaligenes*, *P. putrefaciens*, *P. stutzeri*, *P. testosteroni*, and *P. vesicularis*. The remaining nonfermenter species and groups studied consisted primarily of isolates which did not produce bubbles immediately but did so before 2 s had passed. These groups were referred to as "moderate" catalase producers. No significant differences were noted between stock and recent isolates of any given taxon.

Although profuse bubbling generally accompanied rapid catalase bacteria and limited bubbling was generally seen with delayed catalase bacteria, the criterion of time of appearance of bubbling rather than amount of bubbling was used for interpretation of results since the former provided a more clear-cut separation among the groups tested.

## DISCUSSION

In comparison with the identification of enteric bacteria, the task of identifying nonfermenters is a relatively difficult one. Reasons for this increased difficulty include the limited identification value of standard enteric biochemical tests with nonfermenters, the limited commercial availability of many of the tests specifically designed for the identification of nonfermenters, e.g., acetamide agar (3) and buffered single substrates (4), and the great number of taxonomically unrelated or poorly related members comprising the nonfermenter group.

One test which can be of great value in the identification of nonfermenters and which has been virtually ignored, except by Weaver et al. (6), is the semiquantitation of catalase activity. However, the procedure and interpretative criteria in the present report are based on the time of appearance of catalase activity (bubbling) as determined from the primary isolate rather than on the amount of catalase activity (accumulation of bubbles) produced by a nutrient agar slant subculture as in the method of Weaver et al. (6). In addition, the former method provides a more clear-cut differentiation among members of certain nonfermenter groups than the latter.

One group of nonfermenters in which semiquantitative catalase testing is valuable is illustrated in Table 2. In this group are oxidative pseudomonads which can be difficult to differentiate from apyocyanogenic isolates of *P. aeruginosa*, especially when encountering strains of the latter atypical for any of the key differential tests, e.g., cetrimide, Salmonella-Shigella agar, acetamide, or denitrification. A rapidly positive catalase test would serve to focus attention on the strong probability of an atypical *P. aeruginosa* rather than on any of the other five pseudomonads in this group, all of which produce delayed or occasionally moderate but never rapid reactions.

Table 3 illustrates another group of nonfermenters for which semiquantitative catalase testing is of identification value. This group consists of *Achromobacter* species, CDC group VA-1 and IVE, *P. pickettii*, and *B. bronchiseptica*, all weakly oxidative or nonsaccharolytic nonfermenters which are oxidase positive, grow on MacConkey agar, produce urease, and reduce nitrate to nitrite. A rapid catalase reaction separates the three peritrichous flagellate members, i.e., *Achromobacter* species, *B. bronchiseptica*, and CDC group IVE, from the two polar flagellate members, *P. pickettii* and CDC group VA-1, which produce delayed catalase reactions. Therefore, in this group, the catalase reaction can replace flagellar staining and polymyxin susceptibility testing.

A third example of the usefulness of the semiquantitative catalase test is furnished by a group of weakly oxidative or nonsaccharolytic pseudomonads which are difficult to differentiate from each other. Table 4 illustrates the separation of these five pseudomonads into two groups: (i) rapid catalase producers consisting of

TABLE 2. *Use of semiquantitative catalase testing as an aid in differentiation of apyocyanogenic P. aeruginosa from similar pseudomonads*

| Taxon | Catalase[a] | Cetrimide[b] | Acetamide[b] | No. of flagella | Arginine[b] | Salmonella-Shigella agar[b] | Denitrification[b] | Polymyxin[c] |
|---|---|---|---|---|---|---|---|---|
| *P. aeruginosa* | R | 90 | 89 | 1 | 99 | 87 | 60 | S |
| *P. mendocina* (Vb-2) | D, OM | 0 | 0 | 1 | 100 | 83 | 100 | S |
| *P. pseudomallei* | D | 0 | 20 | >1 | 100 | 0 | 100 | R |
| *P. stutzeri* (Vb-1, Vb-3) | D, OM | 0 | 6 | 1 | 2 | 72 | 100 | S |
| *P. pickettii* (VA-2) | D | 0 | 0 | 1 | 0 | 0 | 100 | R |
| *P. pseudoalcaligenes* | D | 45 | 0 | 1 | 16 | 65 | 0 | S |

[a] R, Rapid; D, delayed; OM, occasionally moderate.
[b] Numerical value represents percent of isolate positive for test.
[c] S, Susceptible; R, resistant.

TABLE 3. *Use of semiquantitative catalase testing as an aid in differentiation of P. pickettii and CDC group VA-1 from biochemically similar nonfermenters*

| Taxon | Catalase[a] | Xylose[b] | Nitrate reduction[b] | Phenylalanine[b] | Salmonella-Shigella agar[b] | Polymyxin[c] | Flagella arrangement[d] |
|---|---|---|---|---|---|---|---|
| *P. pickettii* (CDC group VA-2) | D | 100 | 100 | 0 | 0 | R | Pol. |
| CDC group VA-1 | D | 100 | 79 | 41 | 0 | R | Pol. |
| *Achromobacter* species (CDC group VD) | R, OM | 78 | 92 | 100 | 95 | S | Per. |
| *B. bronchiseptica* | R | 0 | 0 | 27 | 100 | S | Per. |
| CDC group IVE | R | 0 | 100 | 100 | 20 | S | Per. Lat. |

[a] D, Delayed; R, rapid; OM, occasionally moderate.
[b] Numerical value represents percent of isolates positive for test.
[c] R, Resistant; S, susceptible.
[d] Pol., Polar; Per., peritrichous; Lat., lateral.

TABLE 4. *Use of semiquantitative catalase testing as an aid in differentiation of weakly and nonsaccharolytic pseudomonads*

| Taxon | Catalase[a] | Nitrate reduction[b] | Fructose[b] | Mannitol[b] | Acetamide[b] | No. of flagella | Gelatinase[b] |
|---|---|---|---|---|---|---|---|
| *P. acidovorans* | R, OM | 90 | 100 | 95 | 100 | >1 | 3 |
| *P. diminuta* | R | 4 | 0 | 0 | 0 | 1 | 70 |
| *P. testosteroni* | D | 89 | 0 | 0 | 11 | 1 | 0 |
| *P. alcaligenes* | D | 57 | 0 | 0 | 0 | 1 | 3 |
| *P. pseudoalcaligenes* | D | 94 | 100 | 0 | 65 | 1 | 3 |

[a] R, Rapid; OM, occasionally moderate; D, delayed.
[b] Numerical value represents percent of isolates positive for test.

*P. acidovorans* and *P. diminuta* which can be separated from each other with acetamide and nitrate reduction tests and (ii) delayed catalase producers consisting of *P. testosteroni*, *P. pseudoalcaligenes*, and *P. alcaligenes*. The separation of *P. acidovorans* from *P. diminuta*, *P. testosteroni*, *P. alcaligenes*, and *P. pseudoalcaligenes* is more difficult than is indicated in Table 4 since the fructose and mannitol oxidations usually require 3 days to 1 week or more before becoming apparent. In addition, commercially prepared acetamide agar is not readily available. Therefore, the rapid separation of *P. acidovorans* and *P. diminuta* from the other pseudomonads in this group is made possible by catalase testing, in a quick and efficient manner.

Those laboratories utilizing one of the many commercial nonfermenter identification kits available may find semiquantitative catalase testing to be especially valuable since these kits generate identifications as small clusters of choices, with the specific identification depending upon one or more supplemental tests. In such cases, semiquantitative catalase testing may serve as a supplemental test which furnishes an immediate answer.

The three above examples represent only a few of the many uses to which semiquantitative catalase testing can be put in the identification of nonfermenters. It remains for the individual laboratory to find those specific instances in which semiquantitative catalase testing will complement its nonfermenter identification scheme.

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