Media-Dependent Oxidase Reaction in a Strain of *Aeromonas hydrophila*

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A strain of *Aeromonas hydrophila* isolated from bile was oxidase positive only when grown on nonselective media. The isolate was oxidase negative when grown on gram-negative selective and/or differential media. It is proposed that oxidase tests on gram-negative rods be performed on colonies grown on nonselective media to assure valid results.

Members of the genus *Aeromonas* are defined as gram-negative, nonsporeforming rods (1.0 to 3.5 μm long $\times$ 0.4 to 1.0 μm wide) which may be motile by means of polar flagella. They ferment glucose and other carbohydrates and produce indophenol oxidase (2). The positive oxidase test is used to separate aeromonads and vibrios from the glucose-fermenting, oxidase-negative *Enterobacteriaceae*. If the oxidase test is not routinely performed on isolates of glucose-fermenting gram-negative rods, it is quite possible to misidentify *Aeromonas hydrophila* as *Escherichia coli* or *Serratia* and *Aeromonas shigelloides* as *Shigella sonnei* (2).

We would like to report on the isolation from bile of a strain of *A. hydrophila* which is variable in its ability to produce indophenol oxidase. The organism was identified as *A. hydrophila* using the API 20E (Analytab Products, Inc., Plainville, N.Y.) identification system. The biochemical characteristics of this organism are listed in Table 1. These reactions are consistent with those previously reported for *A. hydrophila* (2). The identification of this organism as *A. hydrophila* was confirmed by the Division of Laboratory Services, Kentucky Department of Human Resources.

During the identification process, it was noted that the oxidase reaction of this organism was either strongly positive or completely negative depending upon the medium from which the colony was taken. The oxidase test is that of Kovacs (5) except that 0.5% (wt/vol) tetramethylphenylenediamine dihydrochloride (TPD) is used (7). The organism has a negative oxidase reaction (no reaction within 10 s) when picked from selective and/or differential media, but is strongly positive (positive within 2 s) when picked from nonselective media. Oxidase tests were performed on isolated colonies of the organism after 24 h of growth on various media.

The results are listed in Table 2.

To determine if the oxidase variability was due to insensitivity of the modified Kovacs method (5, 7), additional oxidase procedures were performed on the isolate. Twenty-four-hour-old colonies grown on the selective media listed in Table 2 with the exception of SS agar and on 5% sheep blood agar were tested for oxidase by PathoTec Strip (General Diagnostics Div., Warner-Lambert Co., Morris Plains, N.Y.), Kovacs (5) method using 1% (wt/vol) dimethylphenylenediamine hydrochloride (DPD), and dropping TPD, DPD, and the reagents used by Ewing and Johnson (3) (EJR) directly on the plates. Colonies grown on selective media had negative oxidase reactions. The tests were considered to be negative at the following time intervals: 30 s for PathoTec strip, 10 s for Kovacs, 2 min for TPD, 2 min for DPD, and 2 min for EJR. Colonies grown on 5% sheep blood agar were rapidly oxidase positive with all methods tested.

False-positive oxidase tests can result if an iron-containing wire loop is used (6), but precautions concerning false-negative tests have not been published. The existence of oxidase-negative aeromonads has been proposed (1), but this theory has not gained acceptance as it is not mentioned in more recent descriptions of the genus *Aeromonas* (2, 4). Hugh (4) alluded to the existence of *A. hydrophila* strains which have positive oxidase reactions only after 3 or more days of incubation. Those strains do not appear to be the same as the one described here since the oxidase-variable isolate is oxidase positive within 24 h if grown on nonselective media.

The percentage of *A. hydrophila* strains exhibiting oxidase variability is not known. Such strains are probably not too prevalent since their existence has not been previously de-

TABLE 1. *Biochemical characteristics of the isolate*

Test	Result
ONPG ^a	+
Arginine dihydrolase	+
Lysine decarboxylase	—
Ornithine decarboxylase	—
Citrate	—
H ₂ S	—
Urea	—
Tryptophan deaminase	—
Indole	+
Voges-Proskauer	—
Gelatin	+
Glucose	+
Mannitol	+
Inositol	—
Sorbitol	—
Rhamnose	—
Sucrose	+
Melibiose	—
Amygdalin	+
Arabinose	+

^a ONPG, *o*-nitrophenyl- β -D-galactopyranoside.

TABLE 2. *Oxidase reactions of A. hydrophila from bile after growth on various media*

Medium	Oxidase reaction
5% Rabbit blood agar	+
5% Sheep blood agar	+
Tryptic soy agar	+
Heart infusion agar	+
Mueller-Hinton agar	+
EMB agar	—
MacConkey agar	—
XLD agar ^a	—
Brilliant green agar	—
SS agar	0 ^b

^a XLD, xylose lysine deoxycholate agar.

^b 0, No growth.

scribed. This hypothesis is supported by the fact that our six most recent *A. hydrophila* isolates have been oxidase positive on all media tested.

Possible explanations for oxidase variability are that this organism may be less metabolically active on selective media than on nonselective media or that cytochrome oxidase is inhibited by some component of selective media. Until the extent and/or cause of this phenomenon is determined, we propose that oxidase tests on gram-negative rods be performed only on colonies from nonselective media to assure valid results.

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