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Rapid Method for Detection of *Pseudomonas aeruginosa* on MacConkey Agar Under Ultraviolet Light

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A simple screening technique for the detection of *Pseudomonas aeruginosa* colonies by their fluorescence on MacConkey agar under ultraviolet light is proposed. From 306 nonlactose fermenting cultures screened under the ultraviolet light, 108 fluorescent isolates were obtained. These were screened biochemically, with 103 (94.8%) being verified as *P. aeruginosa*. From the 198 nonfluorescing cultures, only one suspected *P. aeruginosa* was isolated.

Many members of the genus Pseudomonas are known to be pathogenic (3, 9, 10). Of the three fluorescent species associated with man, *Pseudomonas aeruginosa*, *P. fluorescens* and *P. putida* (8), *P. aeruginosa* is considered the primary pathogen (10–12) and consequently, its differential recognition is important. A wide range of biochemical tests is available to confirm the identity of this organism (9). Because special media were thought necessary to enhance fluorescence (7), this property has played only a minor role in the routine screening of suspected cultures.

In the course of an investigation on the isolation of *P. aeruginosa* from swimming pools, we observed that strains of this organism produced fluorescence on MacConkey agar when viewed under ultraviolet light. In this laboratory, the examination of bacteriological specimens for gram-negative bacilli routinely involves the use of MacConkey, Salmonella Shigella, and xylose-lysine-deoxycholate agars. The prospect of utilizing this routine MacConkey medium for the rapid detection of *P. aeruginosa* by fluorescence prompted us to assess the suitability of screening for this bacterial species by using this characteristic. This report covers our preliminary findings.

Initially, 250 nonlactose fermenting bacterial cultures were screened. These were obtained on MacConkey agar (B 75 Difco Laboratories, Detroit, Mich.) from the Clinical Bacteriology Laboratory and the Infection Control Section of the Environmental Bacteriology Laboratory of the Ontario Ministry of Health. All plates were examined under an ultraviolet lamp (Blak-Ray, long wave ultraviolet lamp, B-100A, Ultraviolet Products, San Gabriel, Calif.), fitted with a mercury reflector spot bulb (H 100 PSP44-4, General Electric Co., Cleveland, Ohio) enclosed in a black cabinet. Fluorescing colonies were checked for oxidase activity and then subcultured to fresh MacConkey agar plates, incubated at 37 C in a humidified incubator, and reexamined after 18 to 24 h.

The following fully identified bacterial cultures were received from the Special Bacteriology Laboratory of this Service and used as controls, the number of strains tested being shown in parenthesis: known pyocin-producing types of *P. aeruginosa* (8), *P. aeruginosa* ATCC 14207 (1), *P. fluorescens* (2), *P. putida* (3), *P. maltophilia* (3), *P. alcaligenes* (1), *Acinetobacter* sp. (15), *Achromobacter* sp. (6), *Alcaligenes* sp. (3), *Mima polymorpha* (1), and *Moraxella* sp. (1).

From this initial screening of 250 nonlactose fermenting cultures, 102 fluorescent isolates were obtained. All of these were oxidase-positive and presumably *P. aeruginosa* (2). Of the control cultures, only *P. aeruginosa* fluoresced after overnight incubation at 37 C.

Examination of an additional 306 nonlactose fermenting cultures from the aforementioned sources by both the fluorescent technique described and by biochemical screening confirmed the accuracy of the fluorescent screening procedure for the detection of *P. aeruginosa*. Out of 306 cultures examined, 108 were fluorescent. These fluorescing isolates were tested for the presence of cytochrome oxidase (6) and for their ability to grow on beef infusion-agar at 42 C

with or without the production of pigment (11), oxidize "Key" gluconate substrate (Key Scientific Products Co., Los Angeles, Calif.) (7), reduce triphenyltetrazolium chloride (11), only oxidize glucose (4), and peptonize bromocresol purple milk (1) (bromocresol purple milk hydrolysis is a modification of the milk-agar test referred to). Of the remaining 198 nonfluorescing isolates, 32 were oxidase positive and were biochemically screened as above. Tests were incubated at 37 C in a humid atmosphere unless otherwise indicated, and held for 48 h before being regarded as negative. All negative tests were repeated. The results are summarized in Table 1.

By applying established criteria for the biochemical screening of *Pseudomonas* species, close agreement was found between fluorescence on MacConkey agar at 37 C and the biochemical detection of *P. aeruginosa*. Out of 108 fluorescent isolates, 103 (94.8%) grew at 42 C and were able to oxidize gluconate or reduce triphenyltetrazolium chloride, indicating that these were *P. aeruginosa* isolates (3–5, 7). Of the five fluorescing isolates which did not grow at 42 C, none was able to oxidize gluconate, but two were able to reduce triphenyltetrazolium chloride. Only one of the 32 nonfluorescing, oxidase-positive isolates resembled *P. aeruginosa* by biochemical screening.

Although pigmentation, typically large rough colonial morphology, and distinctive odor of the majority of *P. aeruginosa* strains are prime characteristics on specified media, many authors (3, 7, 11) contend that lack of these specific features does not rule out the identification of a culture as *P. aeruginosa*. The ultraviolet screening technique described, which is both rapid and simple, may serve as a useful tool for the detection of *P. aeruginosa* colonies where other prime characters are not otherwise evident.

TABLE 1. *Biochemical characteristics of 109 suspected P. aeruginosa isolates*

Test	Positive cultures	
	No.	%
Oxidase	109	100
Glucose oxidation	109	100
Fluorescence on MacConkey agar	108	99.4
Growth at 42 C	104	95.7
Gluconate oxidation	99	91.1
Reduction of triphenyltetrazolium chloride	93	85.6
Peptonization of milk	100	91.7

Our preliminary findings deal with only a limited number of strains of *P. fluorescens* and *P. putida*. These two species do not grow well at 37 C (10). We propose that this lack of sufficient growth after overnight incubation at 37 C may explain their fluorescence being undetectable by our screening technique. To confirm our findings, we are currently conducting experiments with a large number of fully characterized strains of these species. The possible applications of this screening technique are also being investigated.

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