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Recovery of *Pseudomonas aeruginosa* Colonial Dissociants on a Protease Detection Medium

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Dialyzed brain heart infusion-skim milk agar medium facilitated the recovery of colonial dissociants of *Pseudomonas aeruginosa*. Differences in colonial morphology as well as in proteolytic activity were readily visualized, thus permitting facile isolation of segregating colony types for further biochemical, serological, and susceptibility studies.

The dissociation of *Pseudomonas aeruginosa* strains into multiple colony types has been a phenomenon noted for a number of years and has been suggested to result from the refluxing lysogenic state of the bacterium (8). Six different colonial types have been described based upon size, shape, surface, edge, elevation, and opacity of individual colonies (7). Particularly associated with this phenotypic phenomenon are strains derived from sputum specimens of patients with cystic fibrosis, in which up to 80% of the isolates display colonial heterogeneity (6).

To demonstrate and enumerate colonial dissociants of *P. aeruginosa*, a number of enriched and selective media have been used including nutrient, blood, chocolate, and deoxycholate agars (1, 4, 6). While each medium has certain inherent advantages (e.g., deoxycholate enhances recovery of mucoid strains), visualization and differentiation of multiple types on these media may be hampered since growth of certain morphotypes is restricted (dwarf strains on nutrient agar) and strains possessing low frequencies of multiple morphotypes may be overlooked.

In our studies assessing protease activity of numerous *P. aeruginosa* isolates (2, 3) on the medium of Sokol et al. (5) (dialyzed brain heart infusion—skim milk agar, DBHI-SMA), an enhanced discernment of different morphotypes of *P. aeruginosa* was achieved on the basis of colony morphology and degree of proteolytic activity. A loopful of individual strains of *P. aeruginosa* grown on blood agar was diluted in physiological saline 100-fold and plated to DBHI-SMA to achieve approximately 30 to 80 colonies per plate. After 48 h of incubation, plates were macroscopically viewed for colonial heterogeneity. By this technique, strains producing a single homogeneous type colony (Fig. 1A) were readily distinguishable from isolates producing several different morphotypes (Fig. 1B and 1C). The advantages afforded by this medium were considerable. Individual colonies grew more luxuriantly on DBHI-SMA (colonies of > 3 mm in diameter) than on nutrient agar. Discernment of colonial differences relating to texture, degree of pigmentation, or peripheral spreading were also augmented through the use of DBHI-SMA as contrasted to more routine media (e.g., tryptic soy, Mueller-Hinton, and nutrient agars). Colonial variants with significant differences in proteolytic activity, as assessed by the zone of proteolysis extending from the periphery of individual colonies, were readily detected. Even non-proteolytic variants (Fig. 1C, arrow) could be identified and isolated.

Strains suspected of harboring multiple morphotypes, as evidenced by spreading outgrowths peripheral to the original lawn (Fig. 2A), when plated to DBHI-SMA clearly resolved into two distinct morphotypes (Fig. 2B and C). Spot inoculation of these varieties onto substrate plate assays for the detection of exoenzymes revealed enzymatic differences between the smooth and rough variants (Table 1).

Colonial variants arising from individual strains of *P. aeruginosa* have been shown to display differences in pigmentation and in phage and antimicrobial susceptibility patterns (4, 6). The present data as well as those derived from 11 additional strains (E. J. Bottone and J. M. Janda, 12th Int. Cong. Chemother., Florence,

TABLE 1. Comparison of enzymatic properties of two distinct colonial morphotypes arising from a single strain of *P. aeruginosa*

Morphology on blood agar	Bioenzymatic profile[a]					
	Gel	Lip	DNase	Ela	Pro	Lec
Smooth	+	+	−	−	+	++
Rough	−	−	−	−	+	−

[a] Enzymatic assays were performed as previously described (3). Abbreviations: Gel, gelatinase; Lip, lipase; Ela, elastase; Pro, protease; Lec, lecithinase.

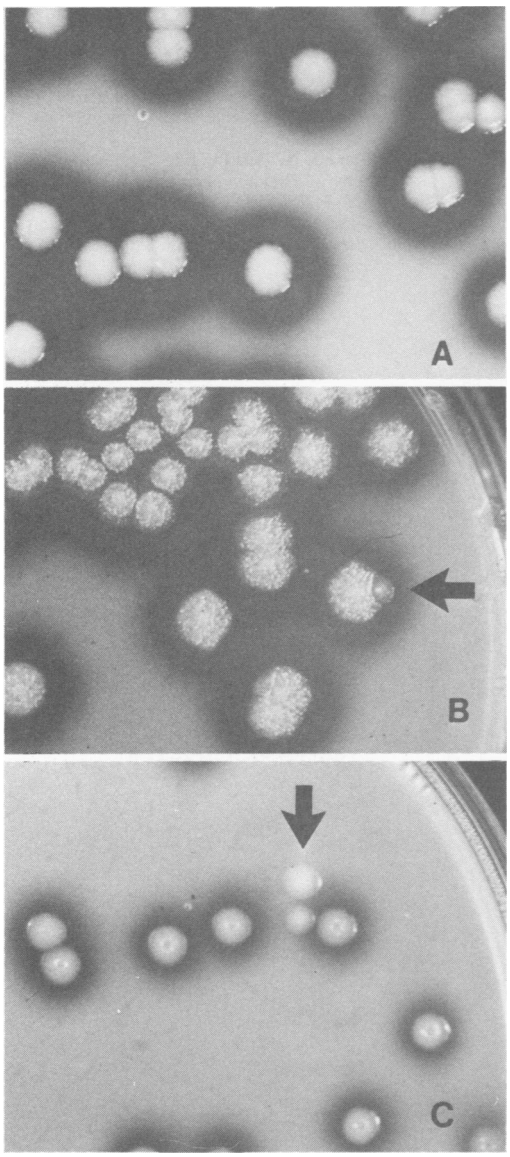

FIG. 1. *P. aeruginosa* strains plated onto DBHI-SMA displaying (A) homogeneous smooth colonies; (B) rough colonies with one smooth variant (arrow); (C) colonies with different proteolytic capacities including a non-proteolytic variant (arrow).

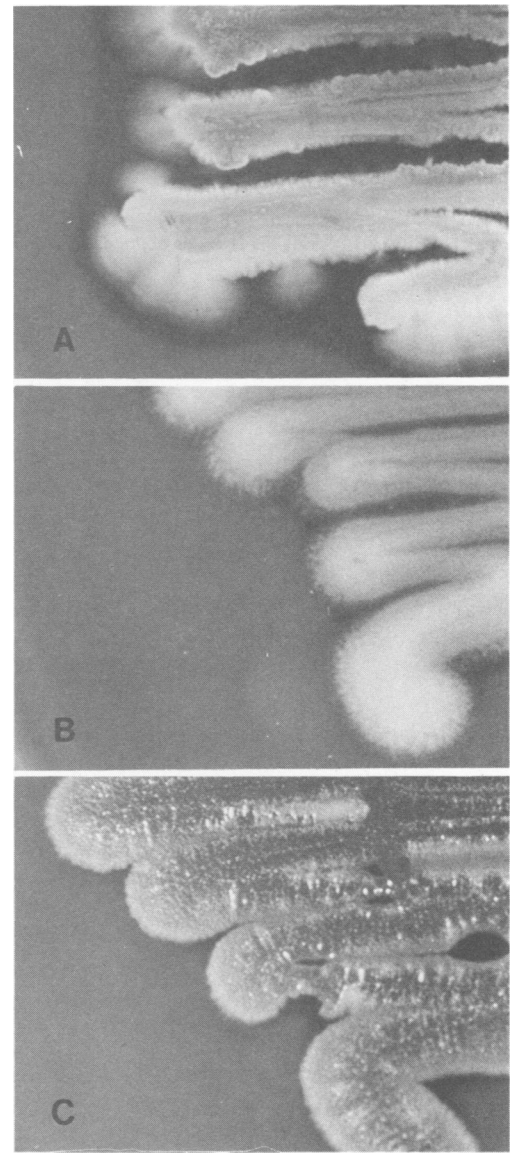

FIG. 2. *P. aeruginosa* strain on blood agar (A) displaying peripheral outgrowths to the original lawn streak after 72 h of incubation at room temperature. Smooth (B) and rough (C) varieties of this strain were recovered after plating the original isolate (A) onto DBHI-SMA.

Italy, abstr. 245, 1981) and from several published reports suggest that these differences may have clinical significance (1, 4, 6). In view of these findings, identification, isolation, and even characterization of colonial variants should be undertaken by clinical microbiologists. This endeavor can be facilitated by the use of DBHI-SMA medium. The plating medium was sensitive enough to detect the presence of a single variant among 80 (1.25%) or more isolated colonies.

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