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Pathogenicity of Nonpigmented Cultures of *Chromobacterium violaceum*

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Nonpigmented cultures of *Chromobacterium violaceum* have been found to be similar to pigmented cultures in their virulence for mice and the pathology of their infections. Clinicians and microbiologists should be prepared to consider nonpigmented *C. violaceum* in their differential diagnoses of infections caused by gram-negative bacteria. The laboratorian who is not aware of this possibility is likely to erroneously identify nonpigmented strains of *C. violaceum* as members of closely associated genera, particularly *Aeromonas*. It is known that violet pigmentation is not an essential feature or an exclusive character of the genus *Chromobacterium*. This study has also shown that pigmentation of *C. violaceum* is not related to its pathogenicity or to the pathology of its infections.

Twenty cases of *Chromobacterium violaceum* infection in humans and 17 reports of infection in animals are found in the literature (6, 7, 9, 13, 16). These infections were all caused by pigmented strains of *C. violaceum*. Although it has been known for some time that nonpigmented variants arise upon subculture of pigmented strains on artificial media (13), there have been no reports of infections due to nonpigmented strains of *C. violaceum*. The absence of such reports is even more conspicuous in the light of recent findings that nonpigmented strains of *C. violaceum* can be isolated from water (11).

It appeared of interest, therefore, to investigate the pathogenicity of nonpigmented cultures of *C. violaceum* for mice and the pathology of their infections.

MATERIALS AND METHODS

This study is based on the methods used by Sneath and Buckland (14), who were the first to report estimates of virulence of *C. violaceum* (pigmented strains) based on viable counts for laboratory animals. Male, white mice of 20-g weight bred in the laboratory animals section of the Veterinary Research Institute were used in the study. The mice were kept in plastic cages and were given a commercially available diet (Sin Heng Chan mice pellets) and water ad libitum. Twenty cultures of *C. violaceum* were used in this study. They consisted of 10 pigmented cultures and 10 nonpigmented cultures. Seven of these nonpigmented cultures were variants of pigmented strains, and these were designated by the same strain number as the parent followed by the letters NP. Three of the nonpigmented cultures (LSN3, LSN7, and LSN8) were isolated as nonpigmented strains from water. Sources of these strains were given in an earlier work (11), except for strain 294 which was isolated from water.

The bacterial cultures for inoculation into mice were prepared according to the method of Sneath and Buckland (14). The mice were inoculated intraperitoneally and were observed for 1 week. The death rate was scored and surviving animals were killed. All animals were autopsied. Organs that appeared abnormal or had gross lesions were selected for bacteriological culture and histopathology. The approximate 50% lethal dose was calculated by the method of Reed and Muench, as described by Davis et al. (2).

RESULTS

The pathogenicity of the nonpigmented cultures was found to be similar to our pigmented cultures and to those of Sneath and Buckland (14) (Table 1). The virulence of the nonpigmented and pigmented cultures varied widely. The more virulent cultures gave a 50% lethal dose for mice of about 10^6 viable organisms.

The pathology of the infections caused by nonpigmented cultures was similar to those caused by pigmented cultures. Lesions were often seen in the liver but less frequently in the lung, spleen, kidneys, and testes. The gross and histological appearances of the lesions were basically those of necrosis and were similar to those described in natural infections in humans and other animals and in experimental infections in laboratory animals described by other workers (6, 7, 9, 13, 15). *C. violaceum*, pigmented or nonpigmented as the case may be, was recovered from the lesions cultured.

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TABLE 1. *Pathogenicity tests in mice*

Pigmented cultures				Nonpigmented cultures	
Sneath and Buckland (13)		Present study			
Culture	LD_{50}[a]	Culture	LD_{50}	Culture	LD_{50}
FH & MW	Over 10^8	609	Over 2×10^8	609NP	Over 7×10^7
MK	10^6	886	2×10^6	886NP	2×10^6
BH	2×10^6	76	4×10^5	76NP	4×10^5
BHR/1	10^7	MDS9	3×10^5	MDS9NP	2×10^5
BN	10^6	25	4×10^5	25NP	4×10^5
BNR/1	10^7	617	6×10^5	617NP	8×10^5
AM	10^8	1,064	1×10^5	1064NP	2×10^5
TV	5×10^7	17	1×10^6	LSN3	Over 1×10^7
LG	10^7	510	5×10^6	LSN7	Over 2×10^8
LW, SH, RT, & NT	Over 10^7	294	8×10^6	LSN8	Over 2×10^8

[a] LD_{50}, 50% lethal dose.

DISCUSSION

It is possible that nonpigmented strains of *C. violaceum* have been isolated from cases of natural infection but have been misidentified as members of closely associated genera. Sneath had recognized this possibility and speculated that should an infection occur by a nonpigmented strain of *C. violaceum*, it would be difficult to identify (13, 15). *C. violaceum* (pigmented or nonpigmented) has not been used as a test organism in the proficiency testing program of the Center for Disease Control, and therefore no data are available on the ability of participants in the program to identify the organism (J. M. Barbaree, personal communication). We submitted two nonpigmented cultures (LSN3 and 76NP) of *C. violaceum* as bacterial cultures for identification to three local diagnostic laboratories (medical and veterinary). One laboratory identified both cultures as *Aeromonas hydrophila*, the second laboratory identified one as *Aeromonas* sp. and the other as *Pseudomonas* sp., and the third laboratory identified both as *Pseudomonas* sp. It is significant that nonpigmented cultures of *C. violaceum* were not correctly identified in a region that had reported the first case of infection in humans caused by pigmented strains (15). Therefore, such misidentification may indeed be a common occurrence.

Until recently there were no reports of the isolation of nonpigmented strains of *C. violaceum* directly from clinical material or from nature (11). The Center for Disease Control has two isolates of nonpigmented strains of *C. violaceum* from clinical material, but these are not from systemic infections (R. E. Weaver, personal communication).

Diagnostic bacteriologists should be aware that violet pigmentation is not an essential feature of *C. violaceum* (11), nor is it an exclusive character of the genus *Chromobacterium* (4). The oxidation-fermentation test (5) will separate *Chromobacterium*, which is a fermentative group, from oxidative *Pseudomonas*. Confusion with *Aeromonas* can be avoided by relying on a battery of biochemical tests and flagellar morphology (3, 10–12).

Identification schemas for gram-negative

TABLE 2. *Selected biochemical tests to differentiate C. violaceum from Aeromonas spp.*[a]

Test or substrate	% Positive[b]		
	C. violaceum[c] (35 cultures)	*A. hydrophila*[d] (113 cultures)	*A. shigelloides*[d] (54 cultures)
Lysine decarboxylase	0	0	100
Arginine dihydrolase	100	85	95
Ornithine decarboxylase	0	0	50
Indole	0	87	100
Methyl red	0	95	100
Glycerol	0	89	83
Gelatin	100	99	0
Nitrite reduction	43	0	0
Mannitol	0	99	0
Inositol	0	0	100
Maltose	0	99	55
Fructose	100	ND	ND
HCN production	100	ND	ND
Lecithinase activity	100	ND	ND
LSM liquefaction	100	ND	ND
Litmus milk reaction	100	ND	ND
MB reduction	100	ND	ND
β-Hemolysis	100	NA	0

[a] HCN, Hydrogen cyanide; LSM, Loeffler's serum medium; MB, methylene blue; ND, not done; NA, some cultures are positive but percentage data are not available.

[b] Including delayed reactions.

[c] Based on data in references 10, 11, and 13.

[d] Based on data in reference 3.

bacteria of medical importance initially identify *C. violaceum*, *Aeromonas* spp., and *Vibrio* spp. as a group on the basis of the following characteristics: motile rods, facultative anaerobes, good growth on ordinary isolation media, catalase positive, oxidase positive, and fermentative carbohydrate utilization. Violet pigmentation of *C. violaceum* is then used as the sole criterion for separating it from *Aeromonas* spp. and *Vibrio* spp. Such schemas cannot be expected to yield correct identifications of nonpigmented *C. violaceum*, and it would invariably be misidentified as an *Aeromonas* sp., particularly *A. hydrophila*. *C. violaceum* and *A. hydrophila* also share other common biochemical characteristics: reduction of nitrate to nitrite, Voges-Proskauer negative, and fermentation of glucose and trehalose but not xylose. Some biochemical tests likely to aid in the prompt differentiation of nonpigmented *C. violaceum* from *Aeromonas* spp. are given in Table 2. Unfortunately, data are not available on the reaction of *Aeromonas* spp. to some tests that constitute an essential part of the biochemical profile of *C. violaceum* (Table 2). Two recent techniques (1, 8) for the rapid examination of flagellar morphology make it feasible for laboratories to routinely determine this characteristic, which has great diagnostic value in the case of *C. violaceum*.

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