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Isolation of *Chromobacterium* spp. from Foods, Soil, and Water†

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Chromobacterium violaceum, a soil and water inhabitant, has been implicated in human disease with a high mortality rate, particularly in the southeastern United States. The psychrotrophic *Chromobacterium lividum* has been isolated from foods, water, and soil, but is not considered pathogenic. To determine the distribution of *Chromobacterium* spp. in soil, water, and foods in the Gainesville area, we evaluated Bennett, Ryalls and Moss, and *Aeromonas* membrane agars for their ability to recover these organisms from various samples when incubated at 25 or 35°C. Bennett agar was best for the isolation of both species when incubated at 25°C; however, at 35°C, *Aeromonas* membrane agar gave the highest recoveries of *C. violaceum*. *C. violaceum* was recovered only from soil and water, whereas *C. lividum* was frequently recovered from foods as well as soil and water.

Chromobacterium violaceum is recognized as an opportunistic pathogen of extreme virulence, with the greatest number of infections occurring in the southeastern United States (5). Florida alone has accounted for seven of the nine infections reported in the United States. The mode of infection is thought to be mainly soil-contaminated puncture wounds, although foods and water have been suggested as possible vehicles of transmission (3). The other member of the genus, *Chromobacterium lividum*, is a psychrotrophic nonpathogen and is easily distinguished from *C. violaceum* on the basis of a limited number of tests and by colonial morphology (1, 8). Both organisms are found mainly in soil and water, with occasional reports of their isolation from foods (2, 9). Because Florida has been often involved and so little is known regarding the distribution and preferred method of isolation of these organisms, a study was undertaken to determine the incidence of *Chromobacterium* spp. in the Gainesville area and to evaluate media used for their isolation.

Various media have been suggested for the isolation of *Chromobacterium* (4, 7). The formulations described by Bennett and by Ryalls and Moss are widely used in ecological studies dealing with these organisms. During a study in our laboratory on the distribution of *Aeromonas* in marine products, we observed that numerous purple colonies developed on *Aeromonas* membrane agar (6). Based on this observation, this medium without added pH indicator was also included in the study.

Subsurface (10 to 15 cm) samples of water were collected from various streams and lakes in the Gainesville area. Soil samples were also obtained from the same locations for comparative analyses.

Prepoured plates were allowed to dry at room temperature for 24 h before being used. Samples were analyzed within 2 h of collection. Generally, dilutions through 1:100 were prepared, and 0.1-ml portions were pipetted onto the surface of four plates of each medium. Initial 1:10 dilutions of the food samples were blended at 8,000 rpm for 2 min. Samples were spread over the agar surface with sterile glass hockey sticks and allowed to dry for 30 min, and duplicate plates of each medium were incubated at 25 and 35°C for 7 and 5 days, respectively. All purple colonies developing on the plates were isolated and identified by accepted methodology (1). Recovery of each organism was then calculated from plate counts.

Table 1 shows the recovery of *Chromobacterium* from soil and water samples on the three media. Data presented are for samples obtained in June and August. Bennett agar consistently yielded the highest recovery of both species of *Chromobacterium* at 25°C. However, at 35°C, *Aeromonas* membrane agar yielded the highest counts of *C. violaceum*, although recovery was slightly less than that found on Bennett agar at 25°C. When 35 retail food samples, mainly fresh vegetables as well as cheese, oysters, clams, and bakery products, were analyzed on the three media, only Bennett agar at 25°C recovered any

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TABLE 1. Comparison of Bennett, Ryall and Moss, and *Aeromonas* membrane agars for the recovery of *Chromobacterium* from soil and water

Sample	No. of organisms per g[a]								
	C. lividum			*C. violaceum*					
				25°C			35°C		
	B	RM	MA	B	RM	MA	B	RM	MA
Hogtown Creek water	930	220	160	1,800	1,800	940	80	1,100	2,400
Hatchet Creek water	3,800	1,100	160	2,700	1,800	1,400	300	900	1,200
Biven's Arm water	160	160	—[b]	—	—	—	—	—	—
Lake Alice water	200	160	—	160	—	—	—	—	—
Hogtown Creek soil	780	—	—	140	—	—	70	—	30
Hatchet Creek soil	660	130	80	4,200	2,600	3,300	280	700	4,300
Biven's Arm soil	390	160	—	—	—	—	—	—	—
Lake Alice soil	400	—	—	300	—	—	—	—	—
Average	915	241	50	1,162	775	705	91	338	991

[a] Results shown are the average of two experiments. Incubation for *C. lividum* was done at 25°C. B, Bennett agar; RM, Ryall and Moss agar; MA, *Aeromonas* membrane agar.
[b] —, None recovered.

TABLE 2. Recovery of *Chromobacterium* from various foods

Sample	No. of organisms per g[a]
Sliced peppered beef	—
Sliced dried beef	—
Cheddar cheese	—
Raw oysters	—
Raw clam	—
Walnut cluster	—
Strawberries	—
Red plum	—
Yellow plum	—
Potato salad	—
Pickle salad	—
Salad mix	>6,000
Watercress	>6,000
Green onion	>6,000
Mint	>6,000
Leaf lettuce	1,600
Celery cabbage	130
Parsnip	160
Endive	350
Parsley	160
Celery	2,700
Carrot	1,000
Artichoke	350
Green bean	—
Bok choy	—
Green cabbage	—
Red cabbage	—
Alfalfa sprouts	—
Mushroom	—
Okra	—
Ginger root	—
Boston lettuce	—
Bean sprouts	—
Green pepper	—
Radish	—

[a] Incubated on Bennett agar. —, None recovered.

Chromobacterium organisms. All isolates were identified as *C. lividum*. Twenty-three of the food samples were negative; however, *Chromobacterium* counts on the remaining 12 samples ranged from 130 per g to greater than 6,000 per g for such samples as mint, water cress, green onions, and a prepackaged salad mix (Table 2).

These results provide information on the incidence of *Chromobacterium* in environmental and food samples in the Gainesville area. Bennett agar with incubation at 25°C maximized the recovery of *Chromobacterium* organisms. This could be attributed to a number of factors, e.g., nutritional adequacy of the medium, enhanced pigmentation of the colonies, better control of competitive overgrowth due to the types and concentration of inhibitors used, as well as the ability of the medium to recover any injured cells. *C. violaceum* is present in the local soil and aquatic environment and can be recovered in high numbers, as is shown by the data in Table 1. The failure of certain other studies (4, 7) to recover *C. violaceum* from soil and water and our failure with foods may be related to the temperature sensitivity of this organism and to the limited number of food samples analyzed. *C. violaceum* dies rapidly at refrigeration temperatures (1) and is most frequently isolated in the tropics (5). Soil and water samples were collected during the summer months, and it would be of interest to observe whether distribution changes, if any, are associated with decreases in ambient temperature in the Gainesville area.

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