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NOTE

A new application for Coomassie Brilliant Blue agar: detection of *Aeromonas salmonicida* in clinical samples

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ABSTRACT: Coomassie Brilliant Blue (CBB) agar was examined to assess its applicability as a differential medium for *Aeromonas salmonicida*. The characteristics of 6 species of bacteria, including fish pathogens, on the CBB medium, in mixed culture, and upon isolation from asymptomatic carrier fish, were determined. Results indicate that this medium can be used to differentiate *A. salmonicida* in mixed bacterial populations from clinical samples.

INTRODUCTION

Aeromonas salmonicida, the etiological agent of furunculosis, infects a broad range of salmonid species and is responsible for substantial grow-out and post-release mortality in anadromous and resident stocks (Klontz 1968, Scallan & Smith 1985). Presumptive identification of *A. salmonicida* is based primarily on its ability to produce a soluble brown pigment when grown on tryptic soy agar. However, the use of this chromogenic characteristic for differential identification has been shown to be unreliable. Pigment production by *A. salmonicida* in mixed culture is apparently inhibited by the other bacteria (Austin & Austin 1987). In addition, several achromogenic strains of *A. salmonicida* (Smith 1963, Paterson et al. 1980) and a chromogenic strain of *Aeromonas hydrophila* (Ross 1962) have been isolated.

Coomassie Brilliant Blue (CBB) agar was developed by Udey (1982). This medium was used in modified form by Wilson & Horne (1986) for the differentiation of A+ and A− strains of *Aeromonas salmonicida* and has been successfully used for this purpose (Cipriano & Bertolini 1988). The goal of this study was to determine if the Udey form of this medium could be used as a differential medium for clinical identification of *A. salmonicida*.

METHODS

Coomassie Brilliant Blue agar preparation. 100 mg l^{-1} Coomassie Brilliant Blue dye (C.I. 42655) was added to 44.0 g tryptic soy agar. After autoclaving 15 min at 1.05 kg cm^{-2}, ca 18 ml were poured into 100 × 15 mm disposable culture dishes.

Pure culture analysis. Twenty-three bacterial isolates were examined for colony color on 3 CBB medium replicates following 48 h incubation at 17 and 21 °C (Table 1).

Mixed culture analysis. Individual tubes of tryptic soy broth were inoculated with selected bacterial colonies and incubated at 21 °C for 36 h. The *Aeromonas salmonicida* isolates selected for analysis were FHM 031 and FHM 034, along with representative isolates of other bacterial cultures (*A. hydrophila* – FHM 004, 014, 033, 036; *Yersinia ruckeri* – FHM 027; *Vibrio anguillarum* – FHM 009; *Enterobacter aerogenes* – BAC 01; and *Escherichia coli* – BAC 02). Two different broth cultures were mixed, streaked for isolation on CBB, and the individual colony types were streaked on new CBB plates. Selected colonies, identified by color as being *A. salmonicida*, were confirmed using direct immunofluorescence (FAT) (Klontz & Anderson 1968).

Tests with clinical samples. Asymptomatic carriers of *Aeromonas salmonicida* (FHM 034) were established via gastric intubation of juvenile spring chinook salmon (Markwardt & Klontz in press). The large intestine was removed from 50 randomly selected fish 4 d post-infection. Intestines from individual fish were weighed and homogenized in 0.5 ml sterile water with a 10 ml Wheaton motor-driven tissue homogenizer. The homogenized material was then diluted 10-fold, 4 times, in 0.85 % sterile saline. Three drops (0.10 µl) of

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Table 1. Bacterial isolates used for evaluating Coomassie Brilliant Blue medium. Sources: AL: Abbott Laboratories, Chicago, IL, USA; ATCC: American Type Culture Collection, Rockville, MD, USA; BC: Pacific Biological Station, Nanaimo, B.C., Canada; CST: Clear Springs Trout Co., Buhl, ID, USA; FHM: Fish Health Management Laboratory, Moscow, ID, USA; UI: University of Idaho, Dept. of Bacteriology, Moscow, ID, USA; WDF: Washington Department of Fisheries, Olympia, WA, USA

Bacterial isolate	Isolate no.	Source
Aeromonas salmonicida	FHM 008	BC
	FHM 026	ATCC 33659
	FHM 031	CST
	FHM 034	CST
	FHM 037	WDF
	FHM 038	WDF
	FHM 039	WDF
	FHM 040	WDF
	FHM 041	WDF
	FHM 042	WDF
Aeromonas hydrophila	FHM 004	FHM
	FHM 014	AL
	FHM 024	ATCC 14715
	FHM 033	CST
	FHM 036	CST
Yersinia ruckeri	FHM 007	BC
	FHM 020	CST
	FHM 021	CST
	FHM 027	ATCC 29473
Vibrio anguillarum	FHM 009	BC
Vibrio ordalii	FHM 010	BC
Enterobacter aerogenes	BAC 01	UI
Escherichia coli	BAC 02	UI

each dilution were placed in 3 locations on plates of CBB medium. The plates were then incubated for 48 h at 21 °C. Representative dark blue colonies were randomly selected for confirmation of identity by FAT.

RESULTS

Pure culture analysis. All of the *Aeromonas salmonicida* colonies were dark blue with the exception of achromogenic strain FHM 026. This isolate was originally medium blue; however, after several serial passes through chinook salmon, the colony color exhibited on CBB was dark blue (Table 2).

Mixed culture analysis. Isolation of *Aeromonas salmonicida* based on colony color was successful with both isolates (FHM 031 and FHM 034). The dark blue colonies from the following combinations were positive for *A. salmonicida* using FAT:FHM 031 with *Yersinia ruckeri* or with *A. hydrophila* (2 strains); FHM 034 with *Y. ruckeri* or with *A. hydrophila* (3 strains).

Tests with clinical samples from asymptomatic carriers. Two colony types were isolated from the asymptomatic carrier fish. The dark blue colonies, suspected to be *Aeromonas salmonicida*, were positively identified as such using FAT. The other colony type, medium blue, was identified as *A. hydrophila* using API 20 E bacteriological identification strips.

Table 2. Pure isolate colony color results when grown on the Coomassie Brilliant Blue medium

Bacterial species	Colony color
Aeromonas salmonicida	Dark blue
Aeromonas hydrophila	Medium blue
Yersinia ruckeri	Light blue (FHM 027-medium blue)
Vibrio anguillarum	Blue green
Vibrio ordalii	Light blue
Enterobacter aerogenes	Light blue
Escherichia coli	Light blue

DISCUSSION

The *Aeromonas salmonicida* isolates were the only cultures appearing dark blue when grown on CBB. The ability of this medium to differentiate *A. salmonicida* from other bacterial microflora present in fish is a valuable application of this medium. Rarely do samples from diseased or asymptomatic fish contain a single bacterial species. This ability to quickly identify *A. salmonicida* in mixed culture is even more powerful when one considers the ease of differentiation from the chromogenic strain of *A. hydrophila*.

The CBB medium is only effective for *Aeromonas salmonicida* differentiation when the isolate is A+ (Wilson & Horne 1986). The presence of the A+ layer protein is associated with the ability to autoagglutinate, to adhere to host tissue, and the ability to establish infection (Udey & Fryer 1978, Trust et al. 1983). Thus, the majority of the clinical isolates of *A. salmonicida* are A+. Although there are virulent A− strains (Johnson et al. 1985), these are the exception rather than the rule.

This medium is differential and presumptive. Thus, identification based on the colony color reaction on the CBB medium should be followed with definitive identification techniques, such as those based on serological and biochemical characteristics. This medium can be used in diagnostic and epizootiological work to presumptively identify *Aeromonas salmonicida* in samples from clinically ill or asymptomatic fish and from water samples; it can also be used to determine the prevalence of the pathogen in such samples.

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