

The Use of Chromogenic Media for α -Glucosidase Determination and Presumptive Drug Susceptibility Profiles in the Fish Pathogen *Nocardia seriolae*

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ABSTRACT—The utility of chromogenic media for determination of α -glucosidase (α -glu) activity in 116 *Nocardia seriolae* isolates was investigated. In all isolates, the results obtained using chromogenic media were identical to those of the API ZYM test. All the α -glu-positive isolates ($n = 27$) were erythromycin (Em)-sensitive, and more than half of them ($n = 18$) were oxytetracycline (OTC)-resistant. On the other hand, all the α -glu-negative isolates ($n = 89$) were OTC-sensitive, and most of them ($n = 81$) were Em-resistant. These results supported the relationship between the α -glu activity and drug susceptibility profiles in *N. seriolae*. Therefore, chromogenic media can be used as a simple and reproducible one-step test to determine the α -glu activity and presumptive drug susceptibility profiles in *N. seriolae*.

Key words: *Nocardia seriolae*, chromogenic medium, α -glucosidase, drug susceptibility, oxytetracycline, erythromycin

In recent epidemiological studies, *Nocardia seriolae* isolates have been typed based on both phenotypic characteristics using α -glucosidase activity, and genotypic characteristics using biased sinusoidal field or pulsed-field gel electrophoresis. *N. seriolae* isolates can be divided into α -glu-positive and -negative isolates^{1,2}. Many Japanese isolates were α -glu-nega-

tive^{1,3,4}, whereas many Taiwanese isolates were α -glu-positive². The α -glu-positive and -negative phenotypes show different electrophoretic patterns, and belong to epidemiologically unrelated clusters^{1,2}. Therefore, the α -glu test is considered an important marker for investigating the epidemiology of *N. seriolae* strains.

The use of chromogenic media has become a key method for the rapid identification of microorganisms and the diagnosis of clinical samples⁵. ChromID MRSA (bioMérieux, Marcy l'Etoile, France) is a chromogenic medium that was designed for the isolation and presumptive identification of *Staphylococcus aureus*. A chromogen in ChromID MRSA medium serves as a substrate for *S. aureus* α -glu; the reaction results in the formation of green-colored colonies⁶.

The purpose of the present study was to evaluate the utility of ChromID MRSA for determining the α -glu activity of *N. seriolae*. In addition, we further explore the epidemiological relationship between α -glu activity and oxytetracycline (OTC)/erythromycin (Em) susceptibility profiles in *N. seriolae* isolates collected from six prefectures in Japan between 2000 and 2007.

Materials and Methods

Bacterial strains

One hundred and sixteen *N. seriolae* isolates were collected from six prefectures in Japan between 2000 and 2007. The *N. seriolae* isolates were isolated from diseased amberjack *Seriola dumerilli* ($n = 81$) and yellowtail *Seriola quinqueradiata* ($n = 35$). Primary isolation was performed using Ogawa medium (Nissui, Tokyo, Japan), Brain Heart Infusion agar (BHIA; Difco, Michigan), or Todd Hewitt agar (THA; Difco, Michigan). After being sub-cultured for isolation of pure colonies, all isolates were cultured in Todd Hewitt broth, and were maintained at -80°C until use. Pure colonies obtained on the agar plates were subjected to Gram and Ziehl-Neelsen staining. All isolates were Gram-positive, acid-fast filamentous or branching bacilli as observed by light microscopy. They showed a positive reaction in the species-specific PCR targeting of the 16S rRNA gene, as previously described⁷.

Alpha-glu activity

The API ZYM test (bioMérieux, Marcy l'Etoile, France) was used to detect α -glu activity as previously described⁴. Two types of agar plates were used, namely, ChromID MRSA, which is a ready-to-use commercially available medium, and an in-house-developed differential X-Glu medium consisting of Mueller-Hinton agar (Difco, Michigan, USA) supplemented with 0.1 g/L X-alpha-D-glucoside (Glycosynth Ltd, Warrington, UK). All isolates ($n = 116$) were inoculated on both types of agar plates, and were incubated at 25°C for seven days.

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The development of coloration was observed daily.

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) for OTC and Em were determined using the broth microdilution method as previously described⁴. The type strains *N. seriolae* ATCC43993 and *N. seriolae* JCM3361 were used as controls for the validation of the identification scheme. The MIC was defined as the lowest concentration of the antimicrobial agent that inhibited visible growth.

Results and Discussion

The α -glu activity of the 116 *N. seriolae* isolates was determined using the API ZYM test (Table 1), and 27 isolates (27.3%) isolated from amberjack were α -glu positive (Fig. 1A), whereas 89 isolates (76.7%) were α -glu negative (Fig. 1B). Of the 89 α -glu-negative isolates, 35 were isolated from yellowtail and 54 were from amberjack. With respect to the year of isolation, the α -glu-negative isolates were collected in all 8 years of the study. The α -glu-positive isolates were collected in 2002, 2004, 2006, and 2007, and the number of isolates collected in these years was eight, six, three, and ten, respectively. These results support the previously reported prevalence of the negative α -glu phenotype in Japan^{1,3,4}.

On the ChromID MRSA medium, α -glu-positive *N. seriolae* isolates appeared as green colonies, whereas α -glu-negative isolates formed yellowish-white colonies

(Fig. 1C). On the X-Glu medium, α -glu-positive isolates appeared as blue-green colonies, whereas α -glu-negative isolates formed yellowish-white colonies (Fig. 1D). For all isolates tested, the results obtained using the ChromID MRSA and X-Glu media were identical to those obtained using the API ZYM test. On the chromogenic media, rapid color development indicative of α -glu-positive activity was observed within 2 to 3 days of culturing. No color change of the media observed during the same period was indicative of α -glu-negative activity. For the API ZYM test, as recommended by the manufacturer, the bacteria must be cultured for at least 6 days on BHIA or THA because a highly concentrated bacterial suspension with a turbidity of McFarland no. 5–6 is required to obtain reproducible results. Therefore, chromogenic media are useful and simple culture-based tests for laboratories and farms that screen the α -glu activity of *N. seriolae*.

In antimicrobial susceptibility tests, the isolates were classified into sensitive and resistant to the tested antibiotics according to the criteria previously described⁴. Table 1 shows the detailed OTC/Em susceptibility profiles of all isolates along with their α -glu activities and year of isolation.

Irrespective of the year of isolation, the *N. seriolae* isolates belonged to three OTC/Em susceptibility profiles as follows: (1) nine α -glu-positive and eight α -glu-negative isolates were both OTC-sensitive and Em-sen-

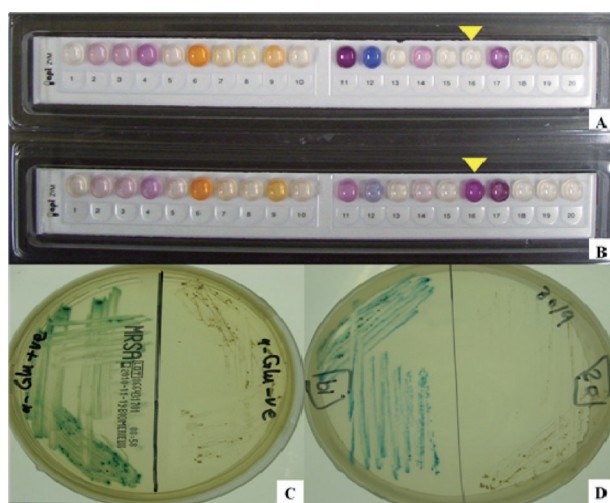


Fig. 1. Alpha-glucosidase activity of *N. seriolae*: (A) API ZYM panel with arrow pointing at colorless cupule no. 16 indicating negative activity, (B) API ZYM panel with arrow pointing at violet cupule no. 16 indicating positive activity, (C) ChromID MRSA medium showing green colonies (positive) and yellowish-white colonies (negative), and (D) X-Glu medium showing blue-green colonies (positive) and yellowish-white colonies (negative).

Table 1. Oxytetracycline (OTC)/erythromycin (Em) susceptibility profiles of all *Nocardia seriolae* strains, with reference to their α -glu activities and the year of isolation

Year	Fish	α -Glu	OTC/Em susceptibility profile		
			S/S	S/R	R/S
2000	Amberjack	–	2	12	
	Yellowtail	–		4	
2001	Yellowtail	–		1	
2002	Amberjack	+			8
		–		1	
2003	Amberjack	–		3	
	Yellowtail	–		2	
2004	Amberjack	+	6		
		–		5	
2005	Amberjack	–		3	
	Yellowtail	–	1	17	
2006	Amberjack	–	1	6	
		+	3		
2007	Amberjack	–		8	
		–	2	6	
	Amberjack	+			10
	Yellowtail	–	2		
	Yellowtail	–		7	

S/S, sensitive/sensitive; S/R, sensitive/resistant; R/S, resistant/sensitive

sitive (S/S) with MICs of 2–4 and $< 0.125 \mu\text{g/mL}$, respectively; (2) 18 α -glu-positive isolates were OTC-resistant and Em-sensitive (R/S) with MICs of 32–64 and $< 0.125 \mu\text{g/mL}$, respectively; and (3) 81 α -glu-negative isolates were OTC-sensitive and Em-resistant (S/R) with MICs of 2–4 and $> 128 \mu\text{g/mL}$, respectively. No α -glu-positive or -negative isolates were resistant to both antibiotics tested. All the α -glu-positive isolates ($n = 27$) were Em-sensitive, and more than half of them ($n = 18$) were OTC-resistant. On the other hand, all the α -glu-negative isolates ($n = 89$) were OTC-sensitive, and most of them ($n = 81$) were Em-resistant. In our previous study⁴, the same susceptibility profiles were observed except for one difference. In the previous study, the S/S susceptibility profile was only observed in two α -glu-negative isolates isolated from amberjack (2/110, 1.8%); however, in the present study, the S/S susceptibility profile was observed in nine α -glu-positive isolates isolated from amberjack in 2004 and 2006 and in eight α -glu-negative isolates isolated from amberjack and yellowtail in 2000 and 2005–2007 (17/116, 14.7%). The small number of the S/S susceptibility profile isolates in 2008 may support the environmental selection pressure that was proposed in our previous study⁴. These results confirm the relationship between the α -glu activity and susceptibility profiles to OTC and Em in *N. seriolae*.

This study showed that chromogenic media could be used as simple and reproducible one-step epidemiological tests to determine the α -glu activity for presumptive drug susceptibility profiles in *N. seriolae*.

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