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A Selective Medium for the Isolation of *Vibrio damsela* from Marine Environments

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Keywords : *Vibrio damsela, Vibrio vulnificus,* selective medium, Z/SPT agar, halo production

Abstract

A new medium termed as Z/SPT was developed for the differential isolation of *Vibrio damsela* and *V. vulnificus* after a modification of SPS agar originally described by Kitaura *et al.* Z/SPT agar consisted of sodium dodecyl sulfate (SDS) 1.0 g, polymyxin B 100,000 units, bromothymol blue (BTB) 0.04 g, trehalose 10 g and Z-BII agar 1 liter (pH 7.6). *Vibrio damsela* strains produced green colonies with a large opaque halo as grown on Z/SPT agar. On the other hand, *V. vulnificus* strains formed yellow colonies with a small halo on the agar plates. The recovery rates of viable counts for the representative strains of *V. damsela* and *V. vulnificus* grown on Z/SPT agar plates to those on non-selective Z-BII agar plates ranged from 44% to 106%.

Vibrio damsela has been isolated from skin ulcers of damselfish (*Chromis punctipinnis*)[1] and octopuses (*Octopus* spp.)[2], wound infections of human[3], and necrotic tissues of a brown shark (*Carcharhinus plumbeus*)[4] and a leatherback turtle (*Dermochelys coriacea*)[5], and marine fish[6]. However, the damage of aquacultured fish caused by *V. damsela* had never reported until it was isolated from deseased fish of young yellowtail *Seriola Quinqueradiata* cultured in Miyazaki Prefecture in 1987[7,8].

Vibrio damsela is known to produce acid and gas from the carbohydrates such as glucose, fructose and mannose but not from arabinose, sucrose and trehalose. In fact, the organism forms green colony on both TCBS and TCBS/T agars (TCBS supplemented with trehalose)[8]. Furthermore, the organism as well as *V. vulnificus* and *V. cholerae* serogroup O1 was reported to produce an opaque halo (sulfatase zone) on the medium containing SDS (SPS agar) by Kitaura *et al*[9].

In this paper, we utilized these characteristics in order to develop a selective medium which is convenient to differentiate *V. damsela* strains among marine vibrios. Namely, *V. damsela* strains are supposed to produce green colonies with an opaque halo on the agar plate containing trehalose, BTB and SDS, while *V. vulnificus* strains to produce yellow colonies with an opaque halo and most of other vibrios green or yellow colonies without an opaque halo.

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Materials and Methods

Bacterial strains

The bacterial strains used in this study and their sources are listed in Table 1.

Media

Bacteria were grown and maintained on Z-BII agar containing polypeptone 5 g, yeast extract 1 g

Table 1. The bacterial strains used in this study

Genus (species)	Source	No.	Strains
Vibrio sp.	Intestine of yellowtail	8	YI-1, -9, -b2, -b4, -b7, YF-1, -15, -25
	Stomach of yellowtail	1	YS-12
	Intestine of skipjack	6	KI-a1, -a2, -j1, -j6, -j7, -j11
	Intestine of tuna	2	TI-5, -11
Vibrio sp.	Viscera of diseased yellowtail	8	Yp-1, -5, -7, -12, -13, -14, -17 Yp-16 (*V. damsela*) Dy-1, -3, -6, (*V. damsela*)
Vibrio sp.	Sea water of aquaculture area	39	DR-1, -2, -3, -5, -7, -24, -25, -30 DK-10, -15, DN-21 DS-21, -23, -24, -25, -26, -27,-28, -30, -31, -32, -45, -49, -50, -58, -66, -67 VR-1, -2, -3, -4, -5 VK-8, -9 VT-11 VS-56, -70, -70, -76
Vibrio sp.	Muscle of diseased prawn	17	Pv-s1, -s2, -s3, -s4, -s5, -s6, -s7, -s8, -s9, -s10, -s11, -s12, -s13, -s14, -s15, -s16, -s17
Vibrio alginolyticus	sea water	11	VA-1, -2, -3, -6, -7, -9, -10, -13, -15, VA-IIb, -II4r
	K. Takagi	3	VA-F1 (NIH 154-78), VA-F2 (NIH 155-78), VA-F3 (NIH 156-78)
Vibrio parahaemolyticus	K. Takagi	5	VP-HK6 (NIH 128-71), VP-FK46 (NIH 156-71). VP-HK32 VP-K20, VP-K36
Vibrio fischeri	NCMB	2	VF-74 (NCMB 1274), VF-81 (NCMB 1281)
Vibrio anguillarum	IFO & K. Tajima	2	Va-B (IFO 1366), V-7 (NCMB 6)
Vibrio vulnificus	Liver of diseased tilapia & ATCC	4	TV-5, -6, -7t, VV-62 (ATCC 27562)
Vibrio damsela	ATCC	1	VD-39 (ATCC 33539)
Alteromonas haloplanktis	M. Sakai	1	1055-1
Pseudomonas sp.	Sea water	2	I-6, C-25
Total number		112	

in one liter of half strength of artificial sea water (1/2ASW) (pH 7.6). SDS-polymyxin B sucrose (SPS) agar was prepared as described by Kitaura *et al*[9]. Z/S agar was made by the addition of sodium dodecyl sulfate (SDS) 1 g/l to Z-BII agar. Z/SPT agar consisted of SDS 1.0 g, polymyxin B 100,000 units, trehalose 10 g and Z-BII agar 1 l.

Colony and halo production

Liquid cultures of test strains in Z-BII broth were diluted and spread on various agar plates. Inoculated plates were incubated at 25°C for 3 days. The number of colonies grown on each plate was counted and halos produced around the colonies were measured in diameter.

Results

In order to determine the optimal concentration of SDS for halo production by *Vibrio damsela* strains, the number of colony and the diameter of halo produced on Z/S agar media with different concentrations of SDS were compared. As shown in Table 2, viable counts of two isolates identified as *V. damsela* decreased gradually as the concentration of SDS in Z/S agar was increased. On the other hand, an opaque halo produced around colonies grown on Z/S agar with

Table 2. Effect of SDS concentration in Z/S agar on colony and halo formation of *V. damsela* strains

SDS Conc.	Yp-16				DR-1			
	Colony		Halo		Colony		Halo	
	Count ($\times 10^7$)	RPE*1 (%)	Size*2 (mm)	Ratio*3 (H/C)	Count ($\times 10^7$)	RPE (%)	Size (mm)	Ratio (H/C)
Z-BII	14.0	100	—	—	14.0	100	—	—
Z/S (0.1%)	8.8	63	9.5	3.4	9.0	65	10.4	4.7
Z/S (0.2%)	7.8	56	7.8	3.3	8.6	62	9.2	4.6
Z/S (0.5%)	6.3	44	3.2	1.5	7.2	51	5.2	2.6

*1 RPE; relative plating efficiency (Z/S versus Z-BII).

*2 Mean diameter of 5 halos.

*3 Ratio of halo diameter to colony diameter after 3 days.

Table 3. Colony and halo formation of *V. damsela* strains on various media containing SDS

Media	Yp-16				DR-25			
	Colony		Halo		Colony		Halo	
	Count ($\times 10^8$)	RPE*1 (%)	Size*2 (mm)	Ratio*3 (H/C)	Count ($\times 10^8$)	RPE (%)	Size (mm)	Ratio (H/C)
Z-BII	4.3	100	—	—	2.1	100	—	—
Z/S	2.0	47	14.3	4.3	1.3	62	11.5	3.7
Z/SPS	2.2	51	15.2	4.3	1.5	71	13.4	3.7
SPS	3.4	79	15.1	3.8	1.6	76	12.9	3.5

*1 RPE; relative plating efficiency of SDS media to Z-BII.

*2 Mean diameter of 5 halos.

*3 Ratio of halo diameter to colony diameter after 3 days.

Table 4. Colony and halo formation of *Vibrio* strains on Z/SPT plates

Strains	Colony			Halo	
	Count ($\times 10^8$)	RPE*1 (%)	Color*2	Size*3 (mm)	Ratio*4 (H/C)
Yp-16	1.7	106	Gr	14.8	5.3
DN-21	1.5	79	Gr	9.8	4.9
DS-67	4.8	96	WY	5.4	1.4
VD-39	0.57	44	Gr	12.5	4.2
TV-7t	3.6	77	Y	5.0	2.7
VV-62	6.6	66	Y	7.5	3.0

*1 RPE; relative plating efficiency of Z/SPT to Z-BII.
*2 Gr, green; Y, yellow; WY, whitish yellow.
*3 Mean diameter of 5 halos after 3 days.
*4 Ratio of halo diameter to colony diameter.

0.1% SDS was the largest in diameter and the most distinct in appearance among those on Z/S agar with different concentrations of SDS. No significant difference was found in viable counts and halo production of two isolates grown on Z/SPS and SPS agar plates as shown in Table 3. *Vibrio damsela* strains such as Yp-16, DN-21 and VD-39 (ATCC 33539) formed green colonies with a large opaque halo which was over four times as larger in diameter as the colonies grown on Z/SPT agar, while *V. vulnificus* strains such as TV-7t and VV-62 (ATCC 27562) did yellow colonies with a small halo as shown in Table 4 and Fig. 1.

The ability of halo production for test strains isolated from fish and marine environments was determined by use of Z/SPT agar. As Table 5 shows, test strains to be identified as *V. damsela* produced green colonies with a large halo as grown on Z/SPT agar. *Vibrio* strains including isolates from the gut content of marine fish, *V. parahaemolyticus, V. alginolyticus, V. fischeri* and *V. anguillarum* could not produce an opaque halo around the colonies as far as examined in this study. *Vibrio vulnificus* and some unidentified vibrio strains showed yellow colonies with a small halo on Z/SPT agar plates.

Discussion

Sodium dodecyl sulfate-polymyxin B sucrose (SPS) medium was developed originally by Kitaura *et al*[9] for the differential isolation of *Vibrio vulnificus* from marine shellfish. They confirmed the liberation of the dodecyl alcohol from SDS molecules in the SDS-containing broth cultures of *V. vulnificus* or *V. cholerae* by use of gas liquid chromatography and suggested that the organisms excreted alkylsulfatase extracellularly to give an opaque zone around the colonies on SPS agar plates. Bryant *et al*[10] also evaluated the potential use of SPS agar in the selective isolation and enumeration of *V. vulnificus* from marine shellfish. However, the differentiation among the organisms such as *V. vulnificus* and *V. damsela,* which have the inability of sucrose fermentation and the ability of halo production, is difficult if SPS agar is used as the selective medium.

In this study, we developed Z/SPT agar consisting of SDS, BTB, polymyxin B, trehalose and

Table 5. Colony and halo formation of fish and marine isolates on Z/SPT agar

Strains	Colony	Halo	Strains	Colony	Halo	Strains	Colony	Halo
YI-1	Y*[1]	−*[2]	DS-21	Gr	+++	Pv-s12	−	
YI-9	−		DS-23	Gr	−	Pv-s13	−	
YI-b2	−		DS-24	Gr	−	Pv-s14	Y	−
YI-b4	−		DS-25	Gr	−	Pv-s15	Y	++
YI-b7	−		DS-26	Gr	−	Pv-s16	Y	−
YF-1	−		DS-27	Gr	+++	Pv-s17	Gr	++
YF-15	−		DS-28	Gr	−	VA-1	Y	−
YF-25	−		DS-30	Gr	−	VA-2	Y	−
YS-12	−		DS-31	Gr	−	VA-3	Y	−
KI-a1	−		DS-32	−		VA-6	Y	−
KI-a2	−		DS-45	Gr	−	VA-7	WY	−
KI-j1	WGr	−	DS-49	Gr	−	VA-9	Y	−
KI-j6	−		DS-50	Gr	−	VA-10	WY	−
KI-j7	−		DS-58	Gr	−	VA-13	WY	−
KI-j11	−		DS-66	Gr	−	VA-15	Y	−
TI-5	WGR	−	DS-67	WY	++	VA-IIb	Y	−
TI-11	−		VR-1	−		VA-II4r	−	
Yp-1	Y	−	VR-2	−		VA-F1	Y	−
Yp-5	Y	−	VR-3	Y	−	VA-F2	Y	−
Yp-7	Y	−	VR-4	−		VA-F3	Y	−
Yp-12	Y	−	VR-5	Y	−	VP-HK6	Y	−
Yp-13	Y	−	VK-8	Y	−	VP-FK46	Y	−
Yp-14	Y	−	VK-9	−	−	VP-HK32	Y	−
Yp-17	Y	−	VT-11	Y	−	VP-K20	Y	−
Yp-16	Gr	+++	VS-56	−		VP-K36	WY	−
Dy-1	Gr	+++	VS-70	−		VF-74	−	
Dy-3	Gr	+++	VS-71	−		VF-81	−	
Dy-6	Gr	+++	VS-76	Y	−	Va-B	−	
DR-1	Gr	+++	Pv-s1	Gr	+++	V-7	−	
DR-2	Gr	+++	Pv-s2	Y	−	TV-5	Y	++
DR-3	Gr	+++	Pv-s3	Y	−	TV-6	Y	+
DR-5	WGr	−	Pv-s4	Gr	+	TV-7t	Y	++
DR-7	WGr	−	Pv-s5	−		VV-62	Y	++
DR-24	Y	++	Pv-s6	Y	−	VD-39	Gr	+++
DR-25	Y	++	Pv-s7	−		1055-1	−	
DR-30	Y	−	Pv-s8	−		I-6	−	
DK-10	−		Pv-s9	−		C-25	−	
DK-15	−		Pv-s10	−				
DN-21	Gr	+++	Pv-s11	−				

*[1] Y, yellow; WY, whitish yellow; Gr, green; WGr, whitish green; −, no growth.

*[2] Ratio of halo diameter to colony diameter after 3 days at 25°C +++ >4≧++ >2≧+ >1≧−.

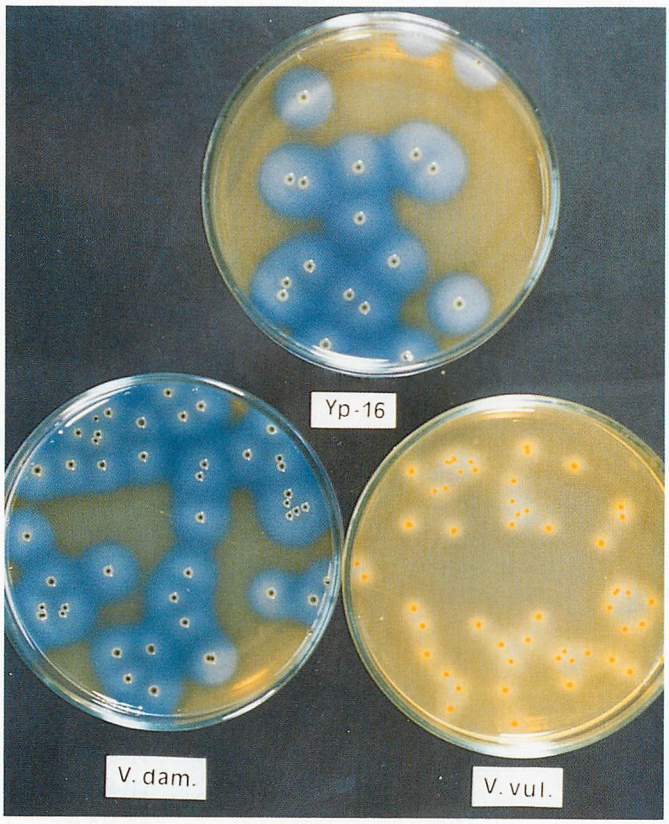

Fig. 1. Colony and halo formation of *Vibrio* strains on Z/SPT agar. Yp-16, *Vibrio damsela* isolated from a diseased yellowtail; V. dam., *Vibrio damsela* ATCC 33539; V. vul., *Vibrio vulinificus* ATCC 27562.

Z-BII agar for the purpose of the differential isolation of *V. damsela* and *V. vulnificus* strains. It was confirmed that *V. damsela* strains produced green colonies with a large opaque halo, and while *V. vulnificus* strains do yellow colonies with a small halo on Z/SPT agar. Although the representative strains of *Vibrio* belonging to *V. parahaemolyticus, V. alginolyticus, V. fischeri* and *V. anguillarum* could not produce halo on Z/SPT agars, a considerably large number of unidentified strains were found to produce a halo around the colonies grown on Z/SPT agar. However, the halos produced around the colonies of these strains were generally smaller than that of *V. damsela* strains. Consequently, the results obtained in this study suggest the effectiveness of Z/SPT agar for the differential isolation of *V. damsela* and *V. vulnificus* strains. The efficiency of Z/SPT agar for direct isolation and enumeration of *V. damsela* strains from marine animals and environments, and the detail identification of the strains producing an opaque halo around the colonies grown on Z/SPT agar are currently under study..

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