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A New Selective Medium for Detecting *Acidovorax avenae* subsp. *avenae* in Rice Seeds

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A selective agar medium was developed and tested for the isolation of *Acidovorax avenae* subsp. *avenae*, the causal bacterial pathogen of bacterial brown stripe, from rice seeds. The new selective agar medium, designated sorbitol pyroglutamic acid agar (SPA) medium, contained 0.5 g of K_2HPO_4, 3.0 g of Na_2HPO_4, 2.0 g of D-sorbitol, 0.2 g of L-pyroglutamic acid, 10.0 ml of tween 80, 40.0 mg of victoria blue B, 15.0 mg of bromthymol blue, 15.0 g of agar, 150.0 mg of ampicillin and 25.0 mg of vancomycin per litter. Colonies of *A. avenae* subsp. *avenae* on SPA medium were smooth, round, convex, shiny, blue and 1.5-2.0 mm in diameter 4 days after incubation at 28°C. Blue colored colony having dark blue zone was typical type of *A. avenae* subsp. *avenae* colonies on the medium. Mean recovery of 8 isolates of *A. avenae* subsp. *avenae* on the selective SPA medium was 95.8% in comparison to that on KB medium. The saprophytic bacteria were reduced to 97.9% on SPA medium compared to those on KB medium. Most of other rice seedborne bacteria as well as reported pathogenic bacteria were failed to grow on SPA medium. This medium was highly selective for recovering *A. avenae* subsp. *avenae* from rice seed samples, and it could be used to enhance the recovery of this bacterium from rice seed samples, which may be contaminated with large numbers of competing microorganisms.

Keywords : selective agar medium, *Acidovorax avenae* subsp. *avenae*, bacterial brown stripe, rice.

Acidovorax avenae subsp. *avenae* is an important pathogen of several hosts including oat, corn, wheat, sugarcane and rice. Bacterial brown stripe of rice caused by *A. avenae* subsp. *avenae* is known to occur in most of the major rice growing countries (Cottyn et al., 1996; Tominaga et al., 1983). The symptoms of the disease are characterized by distinct brown stripes on the coleoptile, leaf sheath and leaf blade or bending symptom on rice seedling (Matzuda & Sato, 1983). Severely infected seedlings are stunted and often die. In Korea, it has been first reported to occur in nursery pots in 1980 (Shakya and Chung, 1980).

The bacterium was reported as a serious seedborne pathogen of rice (Shakya, 1987) and the infected seeds are an important source of primary inoculum. However, the typical symptom on affected seeds was not distinct and the bacterium has been isolated from apparently healthy as well as discolored seeds. Some methods such as blotter test (Shakya and Chung, 1983), serological test (Kadota et al., 1991; Shakya, 1987), and selective media (Kadota, 1996; Zeighler and Alvarez, 1989) have been developed for the detection of *A. avenae* subsp. av*enae*. A selective medium would be useful for quantitative recovery and monitoring the pathogen in seed lots. Some selective media (Kadota, 1996, Summer and Schaad, 1977; Zeigler and Alvare, 1989) had been developed and used but had limited success to detect the pathogen directly from rice seeds or differentiate the bacteria from other rice seedborne bacteria.

Therefore, the purpose of this study is to develope effective and selective medium that recovers the majority of *A. avenae* subsp. *avenae* but suppress other microorganisms present in rice seed samples.

Materials and Methods

Bacterial strains. Four isolated from rice in this study, 3 rice strains from Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF) and 1 maize strain from American Type Culture Collection (ATCC) were used to screen optimal nutritional sources and selective inhibitors of other bacteria (Table 1). The pathogenicity of the four rice strains of *A. avenae* subsp. *avenae* was tested by the modified methods of Sato et al. (1983). Other 8 reference strains pathogenic to rice were used to screen the selective components and evaluate the selectivity of designed medium. The bacteria were cultured and maintained on slants or plates of King's medium B (KB), yeast extract-dextrose-calcium carbonate agar and nutrient broth-yeast extract broth (NBY).

Development of a selective medium. Rice strains of *A. avenae* subsp. *avenae* in this study were used to screen the utilization of 95 carbon and amino acid sources on Biolog GN microplates (Biolog, Hayward, CA). Several carbon and nitrogen sources were selected from the results of Biolog test. The dilution plate

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Table 1. Origin of strains of *Acidovorax avenae* subsp. *avenae* and other species used to evaluate selective medium

Species & strain	Origin	Host	Source[a]
Acidovorax avenae subsp. *avenae*			
CAaa1, 2, 3, 4	Korea	Rice	This study
301507, 301508, 301511	Japan	Rice	MAFF
19860	U.S.A	Maize	ATCC
Burkholderia glumae			
COG1,12	Korea	Rice	W. Y. Song
33617	U.S.A	Rice	ATCC
B. plantarii			
301723	Japan	Rice	MAFF
Pseudomonas fuscovaginae			
301177	Japan	Rice	MAFF
P. syringe pv. apata			
301008	Japan	Rice	MAFF
P. marginalis pv. *marginalis*			
301173	Japan	Rice	MAFF
X. oryzae pv. *oryzae*			
CXO115	Korea	Rice	This study

[a]Sources: MAFF, Ministry of Agriculture, Forestry, Fisheries of Japan, Tsukuba, Japan; ATCC, American Type Culture Collection, Rockville, MD, U.S.A; W. Y. Song, Institute of Agricultural Science & Technology, Chonbuk National University, Chonju, Korea.

method was used to determine a candidate carbon and nitrogen source for selective growth of *A. avenae* subsp. *avenae*. The utilization of each selected carbon and nitrogen compounds was determined by using a defined agar medium (per litter, 0.5 g of K_2HPO_4, 3.0 g of Na_2HPO_4, 3.0 g of $MgSO_4 \cdot 7H_2O$ and 15.0 g of agar) with each combination of 0.2% carbon and 0.02% nitrogen source. Selected combination was used as a basal medium for choosing next inhibitors of other rice seedborne or pathogenic bacteria.

To make newly designed basal medium selective for *A. avenae* subsp. *avenae*, we screened compounds for the specific inhibition of other saprophytic bacteria from rice seed extracts and other bacterial pathogens reported. Twenty-eight antibiotics were screened for the selectivity by using paper disk method. The strains were cultured in NBY broth for 24 hr and the concentration of bacterial suspension was adjusted to approximately 1×10^5 cfu/ml. The bacterial suspension was sprayed onto the surface of basal medium. After 20 min, 0.1 ml absorbable paper disc was dipped in each antibiotics solution and the disc was placed on the agar with sterile forceps and gently pressed down to ensure contact. The plates were incubated at 28°C for overnight and diameter of the inhibition zone was measured. Compounds that failed to inhibit the growth of *A. avenae* subsp. *avenae* around the paper disc were selected and added to basal medium. The recovery of *A. avenae* subsp. *avenae* and reduction of saprophytic bacteria from rice seed extracts were determined on the medium.

To enhance the colony differentiation of *A. avenae* subsp. *avenae* from other bacterial colonies, selective dyes colorific to the colony of the bacterium and to lipid hydrolysis zone around the colony were screened and selected from several dyes on the combined final medium amended with tween 80.

Plating efficiency and selectivity of the newly developed selective media compared with KB. Eight strains of *A. avenae* subsp. *avenae* were used to evaluate growth on the newly designed, selective medium in comparison with KB. Cell concentrations of the cultures in NBY broth was adjusted to a turbidity of OD_{600} 0.1 and diluted to 10^{-5} with phosphate buffered saline (PBS). A portion of 0.1 ml of the dilute was inoculated on the new selective medium and KB. To determine the selectivity of new medium, other rice pathogenic bacteria as well as other seedborne bacteria were inoculated by dilution plating method on the new selective medium and KB.

To measure the degree inhibition of saprophytic bacteria from rice seed extracts, 30 g (approximately 1,000 seeds) of rice seeds from each of 11 seed lots was added to 50 ml of PBS containing 0.01% tween 20 and soaked at 4°C. After overnight shaking, 1 ml from each seed sample was removed and diluted to 10^{-2}, and 0.1 ml of each dilute was pipetted onto three plates of new medium. Plates were incubated at 28°C and colonies were counted after 4 days.

Recovery of *A. avenae* subsp. *avenae* from artificially inoculated rice seeds. To determine the efficiency of the selective medium in the recovery of *A. avenae* subsp. *avenae* from rice seed extracts, 10^{-5} dilution of *A. avenae* subsp. *avenae* standardized with spectrophotometer and 10^{-2} dilution of seed extract were mixed with same volume. 0.1 of the mixture was inoculated onto three plates of each agar medium by using agar plating method. The plates were incubated at 28°C for 4 days and then recovery percentages were determined.

Recovery of *A. avenae* subsp. *avenae* from naturally contaminated rice seed. The seed samples were harvested from 1993 to 1999 in Chonbuk province. 30 g of each seed samples were added to 50 ml of PBS containing 0.01% tween 20 in 100 ml erlenmeyer flask and shaken at 4°C for overnight. After overnight shaking, 1 ml was removed and diluted to 10^{-2}. 0.1 ml of 10^{-1} and 10^{-2} dilutions were pipetted onto three plates of each selective agar medium and spread with a L-shaped glass rod. The plates were incubated at 28°C for 4 days. Typical colonies of *A. avenae* subsp. *avenae* were cloned and presumptively identified by streaking onto KB and YDC. Representative colonies were tested for pathogenicity by injecting rice seedling with an inoculum of approximately 10^5 CFU/ml by using a 26-guage needle attached to a 1 ml syringe.

Results and Discussion

Development of a selective medium. Several carbon and nitrogen sources were chosen from the results of GN Biolog tests for rice strains of *A. avenae* subsp. *avenae*. In view of results, so far achieved, comparing morphology, size, uniformity and first emergence time, and plating efficiency of *A. avenae* subsp. *avenae* colonies and reduction recovery of other seedborne bacteria, $_D$-sorbitol and $_L$-pyro-

Table 2. Growth of *A. avenae* subsp. *avenae* strains on SPA medium supplemented with different concentrations of ampicillin, novobiocin, penicillin G, trimethoprim or vancomycin

Strains	Antibiotics (μg/ml)														
	Ampicillin			Novobiocin			PenicillinG			Trimethoprim			Vancomycin		
	50	100	150	25	50	100	25	50	100	25	50	100	25	50	100
CAaa2	+[a]	+	+	+	(+)	−	(+)	(+)	−	(+)	−	−	+	(+)	−
MAFF301507	+	+	+	+	(+)	−	(+)	(+)	−	(+)	−	−	+	(+)	−
ATCC19860	+	+	+	+	(+)	−	(+)	(+)	−	(+)	−	−	+	(+)	−
Other bacteria from rice seeds	(+)	(+)	−	+	+	+	+	+	+	(+)	(+)	(+)	−	−	−

[a] symbol : +, Growth; (+), slight inhibition; and −, no growth.

glutamic acid were selected as primary carbon and nitrogen sources, respectively, for the new selective medium. All 8 strains of *A. avenae* subsp. *avenae* readily utilize both substrates.

Attempts have been made to develop media for the isolation of *A. avenae* subsp. *avenae* and selective or differential media have been described (Kadota, 1996, Summer and Schaad, 1977; Zeigler and Alvarez, 1989). However, no medium has been proven to be effective for rice seed assay. Plating seed extracts on sorbitol-neutral red agar (SNR) or *Pseudomonas avenae* selective medium (PASM) has been used previously to assay rice samples for *A. avenae* subsp. *avenae*, but with limited success. D-Sorbitol have been used to carbon source in SNR medium that isolated *A. avenae* subsp. *avenae*, the causal agents of bacterial leaf blight of corn (Sumner and Shaad, 1977). However, SNR worked well with corn strains but not with rice strains. Furthermore, it could not inhibit large numbers of saprophytes from rice seeds. 2,3-Butanediol had been chosen for the carbon source of PASM for detecting rice strains of *A. avenae* subsp. *avenae*. However, rice strains did not readily utilize 2,3-butanediol in our GN Biolog results and grew slowly on PASM than on SPA or SNR (Data not shown). PASM had been designed for the isolation of rice *A. avenae* subsp. *avenae* strains but it showed retarded growth rate of the target bacterium and low selectivity from other seedborne bacteria.

Among all the antibiotics tested, ampicillin, novobiocin, penicillin G, trimethoprim and vancomycin were selected as candidates from the result of paper disc method (Table 2). These antibiotics were added to basal medium containing D-sorbitol and L-pyroglutamic acid and were further tested for the analysis of their ability to inhibit saprophytic bacteria but allow the growth of *A. avenae* subsp. *avenae* from rice seed extracts. The reduction of saprophytic bacteria and the recovery of *A. avenae* subsp. *avenae* on basal medium containing ampicillin and vancomycin were higher than those of other media containing other antibiotics. Trimethoprim was inhibitory to *A. avenae* subsp. *avenae* as well as other bacteria. To determine the concentration of ampicillin and vancomycin, each concentration of ampicillin and vancomycin were tested by comparing plating efficiency (Table 3). Ampicillin and vancomycin at 150 μg/ml and 25 μg/ml, respectively, suppressed the growth of most saprophytic bacteria from rice seed extracts without inhibiting the growth of *A. avenae* subsp. *avenae*.

To enhance the colony differentiation between *A. avenae* subsp. *avenae* and other bacteria from rice seeds, tween 80, victoria blue B and bromthymol blue were added to newly designed medium. Tween 80 was added for the final medium, because its utilization can be easily detected by appeared lipid hydrolysis zone and it is assimilated by few other bacteria from rice seeds. Two dyes, victoria blue B and bromthylmol blue at 4, 1.5 μl/ml, respectively, were added to improve coloring colony and lipid hydrolysis zone

Table 3. Reduction percentage of *A. avenae* subsp. *avenae* strains and other bacteria from rice seed extract on selective SPA medium supplemented with different concentrations of mixture of ampicillin and vancomycin compared with basal agar medium supplemented no antibiotics

Antibiotics (μl/ml)		CAaa2	MAFF 301507	ATCC 19860	Other bacteria from rice seeds
Ampicillin 100		5.3[a]	7.1	8.9	81.0
	125	5.7	6.7	8.5	86.1
	150	7.4	10.0	9.0	89.1
Ampicillin 100	Vancomycin 12.5	7.0	8.1	8.9	92.1
	Vancomycin 25.0	8.3	9.0	11.3	92.2
Ampicillin 125	Vancomycin 12.5	9.2	9.0	12.2	93.2
	Vancomycin 25.0	10.5	12.3	13.3	94.0
Ampicillin 150	Vancomycin 12.5	12.2	12.8	13.2	97.9
	Vancomycin 25.0	12.6	13.3	13.5	98.9

[a] Reduction percentage in each bacteria determined as follows; 100–(cfu of bacteria recovered on selective SPA medium × 100)/(cfu of bacteria recovered on basal medium added no antibiotics).

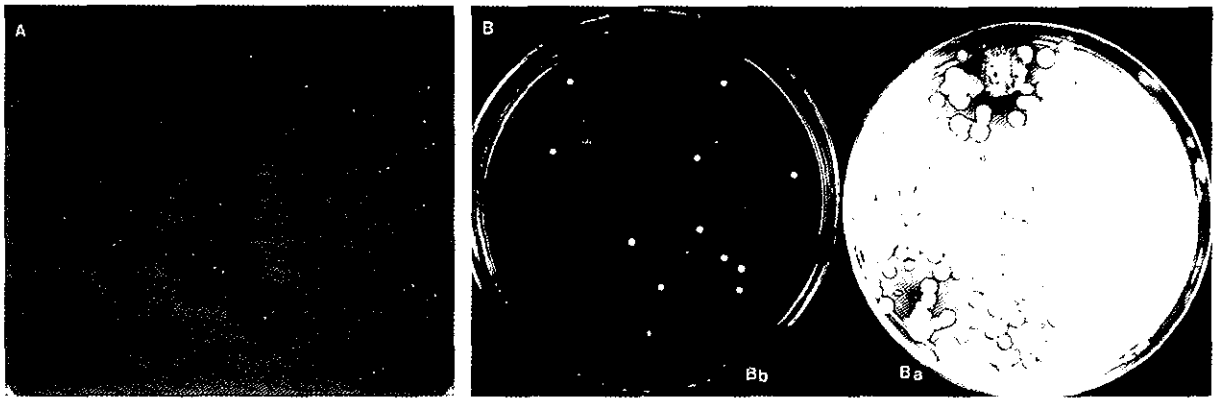

Fig. 1. Typical colonies of *Acidovorax avenae* subsp. *avenae* on SPA medium (A) and selectivity of the medium (Bb) in comparison to KB (Ba) from seed extract.

around the colony of *A. avenae* subsp. *avenae*, respectively. Victoria blue B had been reported as a selective coloring agent of lipase (Yadav et al. 1998) and bromthymol blue had been used in a differential medium for *Pseudomonas* spp. (Zeigler and Alvarez, 1989) but not for coloring *A. avenae* subsp. *avenae* colony selectively.

The final selective medium, designated sorbitol pyroglutamic acid agar (SPA) medium, contained 2.0 g of D-sorbitol, 0.2 g of L-pyroglutamic acid, 0.5 g of K_2HPO_4, 3.0 g of Na_2HPO_4, 3.0 g of $MgSO_4 \cdot 7H_2O$, 10.0 ml of tween 80, 40.0 mg of victoria blue B, 15.0 mg of bromthymol blue, and 15.0 g of agar per litter and pH of the medium was adjusted to 7.4 before autoclaving. After autoclaving, 150.0 ng of ampicillin and 25.0 mg of vancomycin were added.

Colony appearance and plating efficiency of *A. avenae* subsp. *avenae* on the selective medium. Colonies of *A. avenae* subsp. *avenae* were visible after 2 days. Colonies of *A. avenae* subsp. *avenae* were blue, shiny, round, smooth, convex and 1.5-2.0 mm in diameter after 5 days. Colonies on KB were typically 2.0-2.5 mm after 5 days. The colonies of *A. avenae* subsp. *avenae* were uniformed and dark blue-colored lipid hydrolysis zone around the colonies were formed on SPA (Fig. 1). The recoveries of 8 strains of *A. avenae* subsp. *avenae* on the medium were compared with

Table 4. Recovery of *Acidovorax avenae* subsp. *avenae* and other rice pathogenic bacterial strains on selective SPA media compared to growth on KB

Strains		Mean CFU grow on		Mean recovery[a] (%)
		KB	SPA	
A. avenae subsp. *avenae*	CAaa1	119.0 ± 8.0[b]	113.7 ± 1.7	95.6.
	CAaa2	105.0 ± 9.4	107.2 ± 3.5	102.1
	CAaa3	132.5 ± 11.3	117.9 ± 8.1	89.0
	CAaa4	114.7 ± 5.8	104.8 ± 5.6	91.4
	MAFF 301507	101.3 ± 2.5	106.8 ± 7.4	105.4
	MAFF 301508	127.6 ± 3.8	109.6 ± 4.2	85.9
	MAFF 301511	113.7 ± 6.4	103.7 ± 5.6	91.2
	ATCC 19860	104.3 ± 1.6	110.7 ± 2.3	106.1
Burkholderia glumae	COG1	78.0 ± 5.1	0	0
	COG12	88.6 ± 7.5	0	0
	ATCC33617	80.7 ± 5.1	0	0
B. plantarii	MAFF 301723	101.0 ± 9.4	0	0
Pseudomonas fuscovaginae	MAFF 301177	52.0 ± 1.5	0	0
P. syringe pv. *apata*	MAFF 301008	77.2 ± 3.3	0	0
P. marginalis pv. *marginalis*	MAFF 301173	112.3 ± 7.3	0	0
Xanthomonas. oryzae pv. *oryzae*	CXO115	138.3 ± 4.2	0	0

[a] Mean recovery=(cfu recovered on selective SPA medium/cfu of colonies on KB medium) × 100. Figures were calculated from the mean colony numbers per plates from 4 plates per strain.
[b] Average number of colonies per plate from four plates and standard deviation.

Table 5. Reduction percentage of other bacterial populations isolated from seed extracts of various rice cultivars on selective SPA medium[a]

Cultivar	Mean CFU of other bacteria[b]		Reduction[c] (%)
	KB	SPA	
Daeyabyeo	168.0 ± 14.2[d]	1.3 ± 0.3	99.2
Donganbyeo	204.0 ± 9.2	0.6 ± 0.6	99.7
Dongjinbyeo	348.0 ± 16.0	5.0 ± 1.0	98.6
Geahwabyeo	188.7 ± 8.0	2.3 ± 1.8	98.8
Heugnambyeo	102.3 ± 11.4	1.6 ± 0.6	98.4
Hyangnambyeo	156.8 ± 12.7	1.0 ± 1.0	99.4
Kumnambyeo	197.9 ± 14.8	3.0 ± 0.7	98.5
Shinseonchalbyeo	221.3 ± 14.6	5.6 ± 2.9	97.5
Mangembyeo	134.0 ± 8.7	3.0 ± 2.4	97.8
Nampyeongbyeo	249.3 ± 19.0	21.0 ± 3.7	91.6
Yangjobyeo	268.0 ± 11.0	4.6 ± 0.6	98.3
Mean			97.9

[a] Bacterial cells were extracted from 30 g of each seed lot in 50 ml of PBS contaning 0.01% tween 20 by overnight-incubation at 4°C.
[b] Mean cfu of other bacteria was calculated from the mean colony numbers per each plate by spetulating 0.1 ml of seed extract diluted to 10^{-2} onto each medium.
[c] Reduction percentage of saprophytic bacteria on SPA medium from rice seed extracts determined as follows; 100–(cfu of bacteria recovered on selective SPA medium × 100)/total cfu of bacteria recovered on King's medium B.
[d] average number of colonies per plate from four plates and standard deviation.

Table 6. Recovery percentage of *Acidovorax avenae* subsp. *avenae* on King's medium B and selective SPA medium from artificially inoculated rice seeds[a]

Seed lot	Mean CFU		
	KB	SPA	MRP[c]
Daeyabyeo	89.0	85.7	(96.7)
Donganbyeo	94.0	95.5	(101.6)
Dongjinbyeo	101.5	106.2	(104.6)
Geahwabyeo	117.5	113.0	(96.2)
Heugnambyeo	80.5	78.5	(97.5)
Hyangnambyeo	97.0	91.3	(94.1)
Kumnambyeo	109.0	106.9	(98.1)
Shinseonchalbyeo	123.5	120.7	(97.7)
Mangembyeo	112.5	105.0	(93.3)
Nampyeongbyeo	94.5	92.1	(97.4)
Yangjobyeo	109.5	108.2	(98.8)
Mean	102.6	100.3	(97.8)

[a] 30 g each seed lot was soaked in 50 ml PBS containing 0.01% tween 20 and shaken at 4°C for overnight. 10^{-5} dilution of *A. avenae* subsp. *avenae* standardized with spectrophotometer to OD_{600} 0.1 and 10^{-2} dilution of seed extract were mixed with same volume and 0.1 ml of this mixture were spetulated onto each media. Recovery percentage was determined after 4 day-incubation at 28°C.
[b] Mean cfu of *A. avenae* subsp. *avenae*-suspective colonies among all appeared colonies on each plate.
[c] Mean recovery percentage = cfu on the SPA medium per cfu on KB medium × 100.

those on KB. The SPA medium supported good growth of all 8 strains tested. Plating efficiencies for the 8 strians of *A. avenae* subsp. *avenae* were ranged 85.9-106.1% with an average of 95.8 percent compared to that of KB. None of other rice-pathogenic bacteria tested were grown on SPA medium (Table 4). 97.9% of saprophytic bacteria of rice seed extracts growing on KB failed to grow on SPA medium (Table 5).

Recovery of *A. avenae* subsp. *avenae* from artificially inoculated seeds. The mean recovery percentage of *A. avenae* subsp. *avenae* was ranged from 78.5 to 120.7% (Table 6). Some saprophytic bacteria on KB overgrew the colonies of *A. avenae* subsp. *avenae*, so that it developed to be confluent and uncountable. However, other seed extracted bacteria failed to grow, however *A. avenae* subsp. *avenae* grew normally on SPA.

Recovery of *A. avenae* subsp. *avenae* from naturally contaminated seeds. *A. avenae* subsp. *avenae* was isolated from eight of the 11 samples harvested from 1993 to 1999. Neither pathogens nor other saprophytic bacteria were detected on SPA medium in two of those seed lots harvested in 1993 and 1994. Populations recovered from each seed lots on SPA medium were ranged from 3×10^2 cfu per milliliter to 1.27×10^4 cfu per milliliter (Table 7).

Table 7. Isolation of *Acidovorax avenae* subsp. *avenae* and contaminating bacteria of rice cultivars harvested from 1993 to 1999 on KB and selective (SPA) medium[a]

Seed		CFU per plate[b]			
Cultivar	Year collected	KB		SPA	
		Aaa[c]	contaminating	*Aaa*	contaminating
Dongjinbyeo	93	UD[d]	84	0	0
Dongjinbyeo	94	UD	270	0	0
Donganbyeo	95	UD	C	227	66
Dongjinbyeo	95	UD	17,230	160	60
Dongjinbyeo	96	UD	15,600	196	22
Donganbyeo	97	UD	C	1,270	190
Dongjinbyeo	97	UD	125,700	1,000	0
Donganbyeo	98	UD	C	470	300
Dongjinbyeo	98	UD	64,500	0	100
Donganbyeo	99	UD	C	350	0
Dongjinbyeo	99	UD	11,520	30	0

[a] Figures are means of three samples of 30 g of seeds from each seed lot.
[b] CFU mean of three plates in 0.1 ml of dilution 10^0.
[c] Suspected colonies of *A. avenae* subsp. *avenae*.
[d] Abbreviation: UD, unable to differentiate the colony of *A. avenae* subsp. *avenae* from other bacteria; C, confluent.

The major advantage of this selective agar medium over SNR or PASM is the significant reduction in saprophytes and the enhanced colorific differentiation of *A. avenae* subsp. *avenae* colony from saprophytes being able to grow on the medium. SPA medium was proved to be very effective for isolating *A. avenae* subsp. *avenae* from rice seeds. This new selective medium SPA should prove to be well suited for use in detecting no symptomed seeds as well as discolored seeds. More importantly, SPA medium shows potential as an epidermiological tool for quantitatively monitoring populations of *A. avenae* subsp. *avenae* throughout rice growing regions. By using the medium, one could eliminate the most of other bacteria which so commonly appear on general medium and be expected to be useful in monitoring primary inoculum populations in rice seeds. Whereas the selective media improve the chances of successful isolation of the bacterium, they are not of definitive diagnostic value. Improvements on methods of extraction of the pathogen from the seeds coupled with rapid and specific means of identification of the bacterium isolated on the medium will be necessary for more efficient and reliable seed assays.

In conclusion, the simple composition of SPA makes it a suitable medium for routine monitoring purposes. Its selectivity could provide a differential tool for the recovery of *A. avenae* subsp. *avenae* in rice seed samples. The recovery on SPA suggest the practical use of SPA for enumeration of *A. avenae* subsp. *avenae* in routine monitoring of rice seed samples.

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References

Cottyn, B., Cerez, M., Van Outryve, M. F., Barroga, J., Swings, J. and Mew, T. W. 1996. Bacterial diseases of rice. I. Pathogenic bacteria associated with sheath rot complex and grain discoloration of rice in the philliphines. *Plant Disease* 80:429-437.

Kadota, I. 1996. Studies on the pathogen of bacterial brown stripe of rice and its ecology. The Bulletin of Hokuriku National Agricultural Experiment Station 38: 113-171.

Kadota, I., Ohuchi, A. and Nishiyama, K. 1991. Serological propeties and specificity of *Pseudomonas avenae* Manns 1909, the causal agent of bacterial brown stripe of rice. *Ann. Phytophath. Soc. Japan* 57:268-273.

Matsuda, I. and Sato, Z. 1983. Bending symptoms of young rice seedlings grown in nursery flat caused by *Pseudomonas avenae*, the causal agent of bacterial stripe of rice. III. Mode of infection. *The Bulletin of the National Institute of Agricultural Science* 38:169-180.

Sato, Z., Kawakubo, Y., Shirata, A., Matsuda, I., Tsuchiya. and Date, H. 1983. Bending symptoms of young rice seedlings grown in nursery flat caused by *Pseudomonas avenae*, the causal agent of bacterial stripe of rice. I. Symptoms, inoculation method, factors affecting symptom appearance and varietal differences. *The Bulletin of the National Institute of Agricultural Science* 38:149-159.

Shakya, D. D. and Chung, H. S. 1980. Bacterial brown stripe on rice seedling in Korea. *Annual Meeting of Korean Plant Protection Society* 3:125-126.

Shakya, D. D. and Chung, H. S. 1983. Detection of *Pseudomonas avenae* in rice seeds. *Seed Sci. & Technol.* 11:583-587.

Shakya, D. D., Chung, H. S. and Vinther, F. 1986. Transmission of *Pseudomonas avenae*, the cause of bacterial stripe of rice. *J. Phytopathology* 116:92-96.

Shakya, D. D. 1987. Rapid diagnosis of *Pseudomonas avenae* by pathogenicity and serology. *Korean J. Plant Pathol.* 3:300.

Sumner, D. R. and Schaad, N. W. 1977. Epidemiology and control of bacterial leaf blight of corn. *Phytopathology* 67:1113-1118.

Tominaga, T., Kimura, K. and Goh, N. 1983. Bacterial brown stripe of rice in nursery box, caused by *Pseudomonas avenae*. *Ann. Phytophath. Soc. Japan* 49:463-466.

Yadav, R. P., Saxena, R. K., Gupta, R. and Davison W. S. 1998. Rapid zymogram for lipase. *Biotechnology* 24:754-756

Zeigler, R. S. and Alvarez, E. 1989. Differential culture medium for *Pseudomonas* species causing sheath rot (ShR) and grain discoloration (GID) of rice. *Int. Rice Res. Newsletter* 14:27-28.