Journal of Biological Researches: 19 (11-14) 2013

THE EFFECT OF SALT TOLERANT NITROGEN FIXING BACTERIA ON THE GROWTH OF PADDY RICE (*Oryza sativa*. L)

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ABSTRACT

*Nitrogen fixing bacteria (**Azospirillum** and **Azotobacter**) were isolated from coastal mangrove in Pulau seribu. The aims of this experiment was to find out isolates of nitrogen fixing bacteria which were tolerant to high salinity. The isolates can be used as a biofertilizer to support coastal agriculture. A total of 28 isolates (14 isolates of **Azospirillum** and 14 isolates of **Azotobacter**) were tested their tolerance to salt by growing them in their respective media containing 1% 2% and 3 % NaCl,. Salt tolerant isolates obtained, then used as inoculants in paddy. The experiment laid out factorial based randomized complete block design which was comprised of 7 fertilizer treatments and 5 watering treatments with 5 replicates for each treatment at green house of Microbiology Division, Research Center of Biology, Indonesian Institute of Sciences. The first factor was fertilizer treatments 1. Compost + mixed bacteria (K1), 2. Control without inoculant, 3. Compost, 4. NPK, 5. Compost + NPK + mixed microbial, 6. **Azotobacter** isolates; 7. **Azospirillum** isolates. The second factor was watering treatments, plant was watered by : 1. freshwater, 2. freshwater + sea water at mixture ratio 1:1, 3. Sea water, 4. sea water + 2% NaCl (20 g NaCl / l), 5. freshwater + 5% NaCl (50 g NaCl / l). The result showed that there were 9 isolates of **Azospirillum** and 4 isolates of **Azotobacter** which were tolerant to grow at media with 3% NaCl. The green house experimental result revealed that the plants were treated with bacteria can survive up to the level of salinity 12.43 dS^{-1}m.*

Keywords: nitrogen fixing bacteria, saline tolerant

INTRODUCTION

Soil salinity is a serious problem and increasing steadly in many part of the world. Nearly 40% of world's surface has salinity problems (Jadhav *et al*., 2010). In Indonesia, the salinity has long been a problem, there are the tidal areas along the coast, where paddy rice is the main component of farming systems, is strongly influenced by salinity. The importance of soil salinity for agricultural yield is enormour as it affects the establishment, growth and development plants leading to huge losses in productivity (Mathur *et al*., 2007). Recently, the application of soil biotechnology can improve the potential of saline soils land use in agriculture (Moradi *et al.*, 2011). The use of saline tolerant paddy rice varieties and microorganism is a better effort to improve land productivity and crop production.

Some of the microorganisms, particularly valuable bacteria and fungi can develop plant performance under stress condition and therefore, improve yield (Evelin *et al*., 2009). *Azospirillum* and *Azotobacter* are nitrogent fixing soil bacteria with great agricultural importance. They are associated with the roots of various plant and obiquitous in the soil (Young Ju Choi and Sang Wan Gal, 1998).

Some species of *Azotobacter* (*A. chroococcum, A. berijerinkii, A. vivelandii*) and *Azospirillum* (*A. lipoperum, A. brasilense, A. haloprefens, A. irakense*) tolerant of high salinity to 35 g / l and to improve germination and the growth of rice (Ravikumar *et al.*, 2004).

In this study, we screened salt tolerant *Azospirillum* and *Azotobacter* from sea water, sediments mangroves and other plant roots that grow around the sea. The isolates were used as a biological fertilizer in rice plants. Expected that these bacteria could be used as a biological fertilizer to support agriculture coast.

MATERIALS AND METHODS

Isolation of bacteria

Saline soil / water samples were collected from Pulau Laki, Pulau Seribu. Ten grams of each sample were suspended in 90 ml of sterile distilled water, then serial dilution was carried out. From each serial, 0.2 ml of aliquot was transferred to sterile petridish and over poured and dispersed swirling with respective selective media (500 C).

Media for *Azotobacter* used Mannitol Ashby agar (Subba Rao, 1994) and Okon agar media for *Azospirillum* (Okon *et al*., 1977). After 7 days incubation in room temperature, the forming colony were picked up with sterilized loop, transferred to selective media slants. The pure cultures obtained were stored in refrigerator at 4^{o}C. Subsequent sub-culturing was then made in selective media for salinity testing.

Salinity testing

A total of 28 isolates (14 isolates of *Azospirillum* and 14 isolates of *Azotobacter*) were further determined for their salt toleraces on Ashby and Okon media supplemented with different concentrations of 1% , 2% and 3 % NaCl. Salt tolerant isolates obtained, then used as inoculants in paddy.

Green House Experiment

Sorting paddy seed

Sorting seeds (Ciherang) is done by inserting a rice seed into the salt water. The seeds sink (good quality) was washed with water until clean, to further soaked with clean water for 24-48 hours. Then the seeds are drained.

Seedling

Seedling was done by using a plastic tray lined with banana leaves. As medium used soil mixed with compost in the ratio 1: 1, with a medium height of about 4 cm. About 500 seeds sown in the medium, then closed with thin soil.

Planting

The experiment laid out as complete factorial which was comprised of 7 fertilizer treatments and 5 watering treatments with 5 replication for each treatment. The pots were arranged in a randomized complete block design at green house of Microbiology Division, Research Center of Biology, Indonesian Institute of Sciences. It was carried out with the following treatments: The first factor is fertilizer treatments 1. Compost + mixed microbial (K1), 2. controls without inoculants 3. Compost, 4. NPK (10 g/pot), 5. Compost + NPK + mixed microbial, 6. *Azotobacter* sp. isolates; 7. *Azospirillum* sp. isolates.

The second factor is watering treatment, plant is watered by : 1. Freshwater, 2. Freshwater + sea water at mixture ratio 1:1, 3. Seawater, 4. Sea water + 2% NaCl (20 g NaCl / l), 5. Freshwater + 5% NaCl (50 g NaCl / l freshwater). Ten days-old rice seedling (3 seeds) were transplanted in pots containing 5 kg of soil mixed with 3 kg of compost at the same depth (approx 2.5 cm below the soil surface) in all pots. The seeds were thinned to single seedling per pot after emergence. Water depth was controlled at 1 to 2 cm during the first, and at 8 – 10 cm there after. 28 grams of inoculum (109 cell/g) were used for seedling inoculation. The same dose were added to each pot at flowering time. Rice plants were harvested on day 120 after planting. They were evaluated for plant height, tiller number, shoot wet weight, shoot dry weight, panicle number

RESULT

Table 1 shows the conditions of growing media in accordance with the treatment. Highest salinity obtained by planting medium with fresh water + 5% NaCl (M5) that is equal to 12.43 dSm^{-1}.

Table 1. The salinity of media

Treatment	Salinity (dS/m)
M1	0.74
M2	5.93
M3	9.15
M4	10.42
M5	12.43

Table 2. Shows the tolerance of both nitrogen-fixing bacteria (*Azospirillum* and *Azotobacter*) in media supplemented with different concentration of NaCl (1%, 2% and 3%). Out of 14 *Azospirillum* isolates scenned for salt tolerance. Almost of all isolates of *Azospirillum* were found to be tolerant to NaCl at a concentration 1-3%, except isolates L1, L4 and L5. While M1 and M2 isolates tolerated only at NaCl at a concentration 1%. For *Azotobacter* isolates only three isolates namely M2. M4 and M6 found to be tolerant to NaCl at a concentration l%, 2% and 3%, while the M1 and M7 isolates grew at medium supplemented with 1%, and 2% NaCl and L5 and M3 isolates grew only at 1% NaCl concentration.

Table 2. Salt tolerance of *Azospirillum* and *Azotobacter* at NaCl (1%, 2%, 3%)

Isolates	NaCl					
	Azospirillum			*Azotobacter*		
	1%	2%	3%	1%	2%	3%
L1	-	-	-	-	-	-
L2	+	+	+	-	-	-
L3	+	+	+	+	+	+
L4	-	-	-	-	-	-
L5	-	-	-	+	-	-
L6	+	+	+	-	-	-
L7	+	+	+	-	-	-
M1	+	-	-	+	+	-
M2	+	-	-	+	+	+
M3	+	+	+	+	-	-
M4	+	+	+	+	+	+
M5	+	+	+	-	-	-
M6	+	+	+	+	+	+
M7	+	+	+	+	+	-

Description: (+) = grow, (-) = Dead

Table 3. The average value of plant height in fertilizing and watering treatments

Treatment	Plant height (1 month) (cm)					Plant height (3 months) (cm)				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
K1	78 a (a)	62 a (a)	56 a (a)	-	57 a (a)	93 c (b)	87 b (b)	59 a (a)	-	66 a (ab)
K2	65 a (a)	59 a (a)	49 a (a)	-	47 a (a)	69 a (a)	64 a (a)	-	-	-
K3	84 a (c)	64 a (ab)	42 a (a)	-	68 a (ab)	93 c (b)	73 ab (a)	-	-	72 a (a)
K4	63 a (a)	64 a (a)	57 a (a)	-	64 a (a)	79 b (a)	68a (a)	-	-	62 a (a)
K5	85 a (b)	60 a (ab)	59 a (a)	-	66 a (ab)	90 bc (b)	77 ab (ab)	59 a (a)	-	71 a (ab)
I1	76 a (b)	62 a (ab)	51 a (a)	-	69 a (ab)	83 bc (b)	77 ab (b)	56 a (a)	-	65 a (a)
I2	71 a (a)	70 a (a)	53 a (a)	-	57 a (a)	71ab (ab)	74 ab (ab)	56 a (a)	-	77 a (b)

Description: letters without brackets indicate differences in columns and letters with the brackets indicate the difference in the lanes. The numbers followed by the same letter are not significantly different at 5% level of Duncan's test. K1 = compost + microbes; K2 = no microbial control; K3 = Compost; K4 = NPK; K5 = Compost + NPK + microbes; I1 = *Azotobacter* isolate; I2 = *Azospirillum* isolate; M1 = freshwater; M2 = freshwater + sea water ; M3 = seawater ; M4 = sea water + 2% NaCl; M5 = freshwater + 5% NaCl

Tables 3 and 4 showed the effect of salt tolerance inoculum cultivated in saline soil on rice growth and yield component. One month after planting, nearly all plants could grow in media with different watering and fertilization treatments, except plants treatments with M4 watering. In 3 months after planting, some plants without inoculated treatment on M3 and M4 watering treatments had died. The plants could not survive in salinity 9.15-12.43 dS^{-m}.

One monthafter planting, there were no the influence of inoculant on plant height at all levels of salinity. The plant height of paddy rice showed no significant differences among the treatments. However, three months after planting, the inoculant revealed significantly increase the plant height compare to control (non inoculated plants) under salinity condition (Table 3).

Table 4 showed that tiller number of paddy plants significantly were decrease by salinity treatments. Nevertheless, plants were inoculated with bacteria had more tiller number than the control.

Table 5 showed the effect of fertilization and watering treatment on shoot dry weight and panicle number. Fertilization with nitrogen fixing bacterial significantly increased shoot dry weight and panicle number compared to control under salinity conditions. The highest shoot dry weight was found by plant watering with fresh water and

Azospirillum treatment and the lowest shoot dry weight was obtained by plant control without inoculation.

In the M2 treatment with salinity values 5.93 dS/m, all the plants at different fertilization treatments were still alive and showed that shoot dry weight and panicle number lower than M1 treatment. Plant watering with seawater (M3 treatment) showed the lowest dry weight.

DISCUSSION

Some *Azospirillum* and *Azotobacter* bacteria isolated from marine waters and sediments of mangrove rhizosphere showed could grow in the media supplemented with the NaCl showed could grow in the media supplemented with the NaCl concentration of 10 - 30gr/ l. There are also several previous report, Zahran (1999) found that the nitrogen-fixing bacteria was able to grow in media containing 300 mM NaCl. Similarly Ravikumar *et al.*, 2004 reported that some types of *Azotobacter* isolated from mangrove sediments (*A. chroococcum, A. berijerinkii, A. vivelandii*) and *Azospirillum* (*A. lipoperum, A. brasilense, A. haloprefens , A. irakense*) tolerant to high salinity concentrations up to 35 g / l. Ravikumar *et al.* (2002), found *Azospirillum brasilense* could tolerate the concentration 3% in the medium.on media containing 3% NaCl.

Table 4. The average value of tiller number in fertilizing and watering treatments

treatments	Tiller number (1 month)					Tiller number (3 months)				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
K1	8 bc (b)	4 b (a)	3 ab (a)	-	7 c (b)	11 b (c)	6 b (ab)	4 a (a)	-	8 bc (b)
K2	3 a (a)	1a (a)	1 a (a)	-	2 a (a)	5 a (b)	1 a (a)	-	-	-
K3	9 c (c)	4 b (ab)	3 b (ab)	-	6 bc (b)	11 b (b)	6 b (a)	-	-	6 ab (a)
K4	6 b (b)	3 a (a)	3 ab (a)	-	4 ab (ab)	10 b (b)	3 a (a)	-	-	4a (a)
K5	12 d (d)	3 a (a)	3 ab (a)	-	5 bc (a)	12 b (b)	3 a (a)	4 a (a)	-	14 d (b)
I1	9 c (c)	6 bc (b)	4 b (a)	-	7 c (b)	12 b (b)	7 b (a)	6 a (a)	-	10 c (b)
I2	7 bc (b)	7 c (b)	3 ab (a)	-	8 c (b)	9 b (b)	11 c (b)	6 a (a)	-	9 bc (b)

Description: letters without brackets indicate differences in columns and letters with the brackets indicate the difference in the lanes. The numbers followed by the same letter are not significantly different at 5% level of Duncan's test. K1 = compost + microbes; K2 = no microbial control; K3 = Compost; K4 = NPK; K5 = Compost + NPK + microbes; I1 = *Azotobacter* isolate; I2 = *Azospirillum* isolate; M1 = freshwater; M2 = freshwater + sea water ; M3 = seawater ; M4 = sea water + 2% NaCl; M5 = freshwater + 5% NaCl

Table 5. The average value of shoot dry weight and number of panicles on fertilizing and watering treatment

Treatments	Dry weight (g)					Panicle number				
	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
K1	50.54c (b)	36.77c (b)	4.64ab (a)	-	19.99c (a)	7 b (b)	6 c (b)	1 a (a)	-	1 a (a)
K2	8.08a (b)	2.97a (a)	-	-	-	2 a (a)	1 a (a)	-	-	-
K3	42.27c (c)	20.01b (b)	-	-	9.65a (a)	5 b (b)	2 ab (a)	-	-	-
K4	27.19b (c)	14.34b (b)	-	-	7.30a (a)	2 a (a)	2 ab (a)	-	-	-
K5	49.48c (d)	38.13c (c)	4.69ab (a)	-	30.44d (b)	6 b (b)	4 bc (ab)	1 a (a)	-	3 a (a)
I1	39.21bc (d)	30.99bc (c)	2.69a (a)	-	11.05b (b)	6 b (b)	1 a (a)	-	-	1 a (a)
I2	50.65c (c)	32.99bc (b)	6.17 b (a)	-	7.92a (a)	6b (a)	4 bc (a)	-	-	1 a (a)

Description: letters without brackets indicate differences in columns and letters with the brackets indicate the difference in the lanes. The numbers followed by the same letter are not significantly different at 5% level of Duncan's test. K1 = compost + microbes; K2 = no microbial control; K3 = Compost; K4 = NPK; K5 = Compost + NPK + microbes; I1 = *Azotobacter* isolate; I2 = *Azospirillum* isolate; M1 = freshwater; M2 = freshwater + sea water ; M3 = seawater ; M4 = sea water + 2% NaCl; M5 = freshwater + 5% NaCl

Salinity can make the land unfit for cultivation because it affects the growth and development of plants. In this experiment shows that plants that grow on the medium with higher salinity levels are stunted which is characterized by a smaller number of shoots, plant stands are shorter with smaller leaves. Likewise disrupted their crops seen from the number of rice panicles of little or no growth at all. Keterji *et al.* (1998), Hoorn *et al.* (2002) and Rabie *et al.* (2005) who reported negative effects of salinity can cause a decrease in plant growth, reduction in biomass, plant stands are shorter, the leaves are smaller, the effect of osmotic stress and nutrient deficiencies.

In this experiment revealed that plants inoculated with bacterium can survive and show better growth to the level of salinity 12.43 dS/m. So, the adverse effect of salinity could be overcome by treatment with the bacterium. Some of the microorganisms, particularly valuable bacteria and fungi can develop plant performance under stress condition and, therefore, improve yield (Evelin *et al.*, 2009).

Ravikumar *et al.* (2002) reported an increase in the growth of roots and shoots of plants *Pennisetum americanum*, can also increase biomass and yield.

The study on salt influencing paddy plant growth found that plants were watered with sea water (M3 and M4) treatments cause the decrease in plant growth and the death of the plant.

This is due to the salt from sea water soluble in soil composed of cations Na, Ca, Mg and Cl and SO4 anions containing toxic to plant (Chapman, 1975). So, salinity is determined not only by NaCl but by different types of salts that influence and cause stress to the plant.

From the experimental results obtained nitrogen-fixing isolates were tolerant to high salinity can increase plant growth in growing media with high salinity conditions, so that the isolates are expected to be used as a biological fertilizer for coastal agriculture.

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