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Research Article

Influence of saline tolerant biocontrol agent consortia on growth of Rice (*Oryza sativa* L.)

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Abstract

An influence of a mixture of biocontrol agents (BCAs) composed of *Agrobacterium larrymoorei*, *Alcaligenes* sp., *Bacillus amyloliquefaciens*, *B. cereus* and *Brevibacterium* sp on growth of rice in saline condition was assessed. The purpose of this research were to find out BCAs that were tolerant to high soil salinity and their possibility to support coastal agriculture and to assess the effect of bacterial BCAs consortia on rice growth under different kinds of fertilization and saline level conditions. The experiment was conducted in green house by randomized complete block design with factorial. The first factors were fertilizer treatments that consist of; 1. the mixture of the BCAs, 2. BCAs mixture + compost, 3. BCAs mixture + compost + NPK, 4.compost, 5. NPK, 6. control (without BCAs and fertilizers). The second factors were watering treatments, as follow 1. fresh water, 2. Fresh water + sea water (v/v 1:1), 3. Fresh water + NaCl 5%, 4. sea water, 5. sea water + NaCl 2%. The results showed that the BCAs consortia were able to survive up to level of salinity 12.43 d Sm^{-1} (watering with fresh water + NaCl 5%), while rice plant were not able to grow up to level of salinity 9,15 to 12.43 dSm^{-1} (watering with sea water, sea water +NaCl 2%, and fresh water + NaCl 5%), except for the treatment with addition of BCAs alone, BCAs + compost + NPK, and NPK alone.

Keywords: *Agrobacterium larrymoorei*, *Alcaligenes* sp., *Bacillus amyloliquefaciens, B. cereus, Brevibacterium* sp., compost, NPK, rice growth

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1. Introduction

Soil is replete with microscopic life forms including bacteria, fungi, nematodes, and algae. Over 95% of the bacteria exist in the plant roots and those plant obtain many nutrients through the soil bacteria (Dawe *et al*., 2000). Various species of bacteria like *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus* and *Serratia* have been reported to enhance the plant growth (Han and Lee, 2005). Plant growth-promoting Rhizobacteria genera: *Bacillus* (Idriss *et al*., 2002), have been reported to benefit plants by enhancing plant growth and improving plant health through various direct and indirect mechanisms. Plant growth promoting bacteria are commonly used as inoculants for improving the growth and yield of agricultural crops and offers an attractive way to replace chemical fertilizers, pesticides, and supplements (Ashrafuzzaman *et al*., 2009; Saharan and Nehra 2011). These bacteria significantly affect plant growth by increasing nutrient uptake, producing biologically active phytohormones and suppressing pathogens by producing antibiotics, siderophores, and fungal cell wall-lysing enzymes (Kuklinsky-Sobral *et al*., 2004;). Among these, auxin is one of the most vital hormones, primarily due to its pivotal functions in the initial processes of lateral and adventitious root formation (Gaspar *et al*. 1996; Idris *et al*. 2007) and root elongation (Yang *et al*. 1993). Indole -3- acetic acid (IAA) was detected in 80% of bacteria isolated from the rhizosphere (Loper and Schroth, 1986).

Salinity is a major cause of soil degradation with deleterious effects on crop production (Sumner, 2000), which concerns large area of cultivated land in the world. Nearly 40% of the world's surface has salinity problems (Jadhav *et al*., 2010). Salinization of soil is serious problem and is increasing gradually in many parts of the world, particularly in arid and semiarid area. At present out of 1.5 billion hectares of cultivated land around the world, about 77 million hectares is affected by excess salt content (Evelin *et al*., 2009). Soil salinization is often caused by natural phenomena (climate, rock salt deposition, and saline ground water) and human activities (irrigation methods and drainage condition). The importance of soil salinity for agricultural yield is enormous as it affects establishment, growth and developments of leading to huge loses in productivity (Marthur *et al*., 2007). Salinity poses several problems for plant growth and development by inducing physiological stresses (Said *et al*., 2005). It is also posese problem for microbial diversity and metabolic activities. For example, the abundance, composition, diversity, and metabolic functions of microbial communities are lower in saline and hypersaline terrestrial environments (Jiang *et al.*, 2007). Recently, soil biotechnology application can improve the potential of saline soils land use in agriculture. Active microbiological processes in soil enhance the rate of synthesis and mineralization of organic matter which then lead to better plant nutrition. Some of the microorganisms, particularly valuable bacteria and fungi can develop plant performance under stress condition and, therefore, improve yield (Evelin *et al*., 2009). *Agrobacterium tumefaciens* biotype I and III are tolerant to 2% NaCl (Smith *et al*.,1990), and *Bacillus subtilis* is able to adapt to wide variation in osmotic and saline strength (Palomino, 2009).

Fertilization is one of the major factors controlling the population densities and activity of soil organisms (Bünemann *et al*., 2006). Some studies have documented that fertilization has had significant impacts on the population, composition and function of soil micoorganisms, and that organic and inorganic fertilizer amendments have increased the soil microorganisms activity (Ge *et al*., 2008 and Mandal *et al*., 2007). However, other studies have demonstrated that inorganic and organic fertilizers have had relatively little or no effect on soil microbial activities and diversities (Okano *et al*., 2004 and Treseder, 2008).

The objectives of this research were to assess the effect of bacterial BCAs consortia on rice growth under different kinds of fertilization and saline level conditions and to find out BCAs that were tolerant to high soil salinity and their possibility to support coastal agriculture.

2. Materials and Methods

Bacteria consortia used in this study consist of five bacteria specie**s,** *Agrobacterium larrymoorei*, *Alcaligenes* sp., *Bacillus amyloliquefaciens*, *B. Cereus* and *Brevibacterium* sp. *A. larrymoore*, *B. amyloliquefaciens* and *Brevibacterium* sp were isolated from *Cucurbita maxima* (Pumpkin), *Brassica chinensis* (Chinese cabbage), *Citrus grandis* (pomelo) respectively, in organic farming in Sukabumi, West Java, Indonesia. *B. cereus* was isolated from *Fragaria vesca* (Strawberry) in organic farming in Bedugul, Bali, Indonesia and *Alcaligenes* sp. was isolated from *Ipomea aquatica* (water spinach) in Kepulauan Seribu in Java island, Indonesia.

2.1. Rice seeds selection and nursery (green house experiment)

This research was performed at green house of Microbiogy Division, Research Center for Biology, Indonesian Institute of Sciences, Cibinong, West Java, Indonesia. Rice seeds (Ciherang variety) was selected by entering them into plastic bucket containing salt water. Drowned seeds were selected, then the selected seeds were washed by using water. After that rice seeds were immersed in clean water for about 24-48 hours. Nursery was performed using a plastic tray. Bottom of plastic tray was lined with banana leaves, then soil and compost (1:1) were mixed and pour into its within 4 cm in hight. The selected rice seeds were spread on the surface of soil growing media, and covered with soil, and germinated them for about 10 days. One rice germ was planted into each holes in plastic pot contains 5 kg soil and 3 kg compost (1 germ for one hole). Inoculation biocontrol agent consortia was done by spreading 28 g inoculant granular (the density 1x 10^6 CFU/g^{-1} granular) on 5 days, and 1 month after planting.

The experiments were performed using a completely randomized design (CRD) with factorial pattern. Factor A was microbes consortia treatments (1. BCAs consortia alone, 2. BCAs+ Compost + NPK, 3.Compost alone, 4.Compost + BCAs consortia, 5. NPK, 6.Control (without BCAs, compost, and NPK). Factor B is watering treatments (1. Plants was watered with freshwater, 2. Plants were watered with fresh water and sea water (1:1), 3. Plants was watered with fresh water + 5% NaCl, 4.Plants was watered with sea water, 5. Plants were watered with sea water + 2% NaCl. Each of treatment was repeated 5 times and watering was performed every 2 days according to the treatment. Harvesting was done after a 3-month-old plants and the observed variables are plant height and number of tillers (1 month old and at harvest), shoot dry weight and number of panicle rice, and biomass.

2.2. Measurement of indole-3- acetic acidI (IAA)

Production of IAA by consortia of bacterial biocontrol agents (*Agrobacterium larrymoorei*, *Alcaligenes* sp., *Bacillus amyloliquefaciens*, *Bacillus cereus*, and *Brevibacterium* sp.) were measured spectrophotometrically by modified methods described by Gravel *et al*., 2007).

2.3. Data analysis

Data were subjected to analysis of variance (ANOVA) with SPSS software. Significance of mean differences was determined using the Duncan's test. And responses were judged significant at 5% level.

3. Results and Discussion

3.1.Influence of combination addition of BCAs consortia on total microbes population in saline soil

Total of microbes populations were significantly highest by addition of NPK alone combined with fresh and sea water watering, followed by addition of BCAs alone combined with fresh water watering, addition of NPK alone combined with sea water watering, addition of BCAS + compost + NPK combined with watering of fresh water + NaCl 5%, and addition of BCAS + compost + NPK combined with watering of fresh water + NaCl 2%, their population were 13.4, 10.0, 9.0, 8.5, and 7 × 10^{-6} g^{-1} soil, respectively (Table 1). The result was an agreement with Nakharo and Dkhar, (2010) who reported that the application of organic and inorganic fertilizers into soil increased population and biomas of microorganisms. Further, other researchers have found that incorporation of organic amendments increased microbial activity (Girvan *et al*., 2004 : Grayston *et al*., 2004), densities of bacteria (van Bruggen and Semenov, 2000), fluorescent *Pseudomonas* spp., fungi and nematodes (Abawi and Widmer, 2000).

Availability of nutrient elements in soil through addition of fertilizers such compost and NPK may enhance the population of total microbes in soil, including the BCAs. As Ge *et al*., (2008) reported that the composition and structure of microorganisms in paddy soils are diverse and complicated.

Table 1. Influence of combination addition of BCAs consortia on total of bacteria population (× 10^{-6} g^{-1}) in rhizosphere soil of rice

Treatments	Fresh water	Fresh and sea water (1:1)	Fresh water + NaCl 5%	Sea water	Sea water +NaCl 2%
BCAs	**10 l**	9.5 kl	6.5 fg	8.1 i	6 f
BCAs + Compost	5 e	5 e	1 bc	0.7	0.35 a
BCAs + Compost + NPK	8 i	7.4 h	**8.5 ij**	2 d	**7 gh**
Compost	8.5 ij	1.5 cd	1.1 bc	0.32 a	0.62 ab
NPK	8.5 ij	**13.4 n**	2 d	**9 jk**	0.75 ab
Control	6.5 fg	11 m	0.12 a	0.25 a	0.15 a

Means in any column with different letters are significantly different (*$P<0.05$*)

3.2.Influence of combination addition of BCAs consortia on number of rice tillers

Table 2 shows that number of rice tillers was significantly highest by addition BCAs alone with fresh water watering, addition combination of BCAs with compost or NPK or addition of compost alone and NPK alone also increased number of rice tillers significantly with fresh water watering. The addition of BCAs consortia alone and its combination in saline condition 5.93 dSm^{-1} (watering with fresh and sea water), 9.15 dSm^{-1}(watering with sea water), and 12.43 dSm^{-1} (watering with fresh water + NaCl5%) improved significantly the number of rice tillers. The number of rice tillers can be influenced by availability of microbes in the soil, therefore the addition of BCAs, may improve the number and diversity of soil microbes that may promote plant growth. Davison (1988) revealed that the main function of soil bacteria are (1) to supply nutrients crops, (2) to stimulates plant growth, (3) to control or inhibit plant pathogen, and (4) to improve soil structure. Besides Chivenge *et al*., (2011) and Herencia *et al*., (2008) stated that management of soil fertility through organic fertilizers has always been a pivotal principle of sustainable agriculture. Yet, the impacts of these fertilizers on soil microbial community structure and function as well as on nutrient availability can vary widely, having extremely different impacts on crop productivity.

Table 2. Influence of combination addition of BCAs consortia on number of rice tillers

Treatments	Fresh water	Fresh and sea water (1:1)	Fresh water + NaCl 5%	Sea water	Sea water +NaCl 2%
BCAs	**13 j**	**11 h**	**10 gh**	**6 e**	0 a
BCAs + Compost	9 fg	3 c	4.4 d	5 de	0 a
BCAs + Compost + NPK	12 i	8 f	8 f	0 a	0 a
Compost	10 gh	6 e	6 e	0 a	0 a
NPK	11 h	5 de	9 fg	4 d	0 a
Control	5 de	2 b	0 a	0 a	0 a

Means in any column with different letters are significantly different *($P<0.05$)*

3.3.Influence of combination addition of BCAs consortia on height of rice plants

Addition of BCAs alone, compost alone, and NPK alone, and combinations of BCAs + compost + NPK, increased significantly the height of rice plants by fresh water watering (Table 3). In saline soil 5.93 dSm-1 (fresh and sea water watering), the addition of BCAs consortia alone, NPK alone, and combination of BCAs + compost + NPK also resulted significantly increased the height of rice plants. By increasing the salinity soil up to 12.43 dSm^{-1} (fresh water + NaCl 5% watering), only addition of BCAs consortia alone gave significantly different of the height of rice, while the salinity soil 9.15dSm^{-1} (sea water watering) the addition of BCAs consortia alone, and NPK alone increased significantly the height of rice plants.

By addition of compost, and NPK may enhance soil elements such as nitrogen, phosphorus, sulfur and micro element for plant growth that may cause the increasing of rice height. Various yield components (maximum plant height and number of tillers per plant of rice) were affected positively and finally these components contribute towards improving rice yield. Other researchers such as Dixit and Gupta (2000), Selvakumari *et al*., (2000), Khoshgoftarmanesh and Kalbasi (2002), and Swarup and Yaduvanshi (2000) also observed increased of rice yield with the use of different organic materials alone and in combination with mineral fertilizer.

Table 3. Influence of combination addition of BCAs consortia on height of rice plants (cm)

Treatments	Fresh water	Fresh and sea water (1:1)	Fresh water + NaCl 5%	Sea water	Sea water +NaCl 2%
BCAs	85 ij	79 ghi	**74 efgh**	**59 bc**	0 a
BCAs + Compost	79 ghi	68 cdef	62 bcd	0 a	0 a
BCAs + Compost + NPK	**95 j**	**87 ij**	71 defgh	55 b	0 a
Compost	**93 j**	73 efgh	72 defgh	0 a	0 a
NPK	82 hi	77 fgh	62 bcd	**59 bc**	0 a
Control	69 cdefg	64 bcde	0 a	0 a	0 a

Means in any column with different letters are significantly different *($P<0.05$)*

3.4.Influence of combination addition of BCAs consortia on rice panicles number

Table 4 indicates that number of rice panicles increased significantly by addition of BCAs consortia alone, NPK alone, compost alone, and combination of BCAs + compost + NPK, by watering with fresh water. In saline condition 5.93 dSm^{-1} (fresh and sea water) addition of BCAs and BCAs + compost + NPK resulted significantly highest of rice panicles number. The other fertilizers addition and its combination also improved significantly different the number of rice panicles. At salinity level 9.15 dSm^{-1} (sea water watering), the addition of BCAs , BCAs + compost + NPK, and NPK alone also significantly improved rice panicles number. By increasing the soil salinity level up to 12.43 dSm-1 (fresh water + NaCl 5%), the addition of BCAs alone, BCAs + compost + NPK, and NPK alone increased significantly different of rice panicles. Addition of BCAs, compost, and NPK fertilizer may change structure of microbial community in soil, that may influence the increasing of rice panicles number. In addition Edmeades (2003) stated that shift in the structure and composition of the microbial community are strong indicators of soil biological activity, soil quality, and crop productivity.

Table 4. Influence of combination addition of BCAs consortia on number of rice panicles

Treatments	Fresh water	Fresh and sea water (1:1)	Fresh water + NaCl 5%	Sea water	Sea water +NaCl 2%
BCAs	**8 i**	**5 f**	2 c	**3 d**	0 a
BCAs + Compost	2 c	2 c	0 a	0	0 a
BCAs + Compost + NPK	7 h	**5 f**	1 b	1 b	0 a
Compost	5 f	2 c	0 a	0	0 a
NPK	**8 i**	4 e	**3 d**	2 c	0 a
Control	2 c	1 b	0 a	0 a	0 a

Means in any column with different letters are significantly different *($P<0.05$)*

3.5.Influence of combination addition of BCAs consortia on biomass of rice

Compared with control, biomass of rice was significantly different on all of treatments when the plants were watering with fresh water (Table 5). The highest one was found on the combination treatment of BCAs + compost + NPK with watering of either fresh water, fresh and sea water, fresh water + NaCl 5% and sea water, they were 51.7 g, 41.5, 20.0, and 9.1 respectively. These treatments combinations increased the biomass of rice plant for about 67 -100 %. Application of BCAs, compost, and NPK may improve soil physical and chemical properties as well as increased growth, biomass and yield crop, since such phenomenon have been proved by Lima *et al*., (2004). Meuchang *et al*., (2006) and Levy and Taylor (2003) reported that mature compost and sugar mill by-products compost application result in increase shoot and root growth in tomato.

Table 5. Influence of combination addition of BCAs consortia on biomass of rice (g)

Treatments	Fresh water	Fresh and sea water (1:1)	Fresh water + NaCl 5%	Sea water	Sea water +NaCl 2%
BCAs	45.0 n	35.2 k	17.5 g	5.4 b	0 a
BCAs + Compost	27.2 j	14.3 f	7.3 c	0 a	0 a
BCAs + Compost + NPK	**51.7 p**	**41.5 m**	**20.0 h**	**9.1 d**	0 a
Compost	42.3 m	25.8 i	10.7 e	0 a	0 a
NPK	49.5 o	38.1 l	13.7 f	7.4 c	0 a
Control	14.8 f	9.3 d	0 a	0 a	0 a

Means in any column with different letters are significantly different *($P<0.05$)*

3.6.IAA production by the the strains of BCAs consortia

Results of indole acetic acid measurement showed that each of strains of the BCAs consortia produced IAA, *Alcaligenes* sp. produced the highest one for about 8.0 mgL^{-1} (Figure). These results showed that IAA production might be responsible for enhancement of rice plant growth. Application of *Agrobacterium larrymoorei*, *Alcaligenes* sp., B. *amyloliquefaciens*, *B. cereus*, and *Brevibacterium* sp. increased height, number of seedlings and panicles of rice. These results were similar with Deshwal *et al.*, (2011) who observed that IAA producing *Pseudomonas aeruginosa* MR-9 enhanced maximum plant dry weight and plant height. IAA is essential for growth of root and shoots development. Many microbes including plant growth promoting rhizobacteria produce IAA. IAA is the main auxin in plants, controlling many important physiological processes including cell enlargement and division, tissue differentiation, and responses to light and gravity. Bacterial IAA producers have the potential to interfere with any of these processes by input of IAA into the plant's auxin pool. The consequence for the plant is usually a function of the amount of IAA that is produced. A root, for instances, is one of the plant's organ that is most sensitive to fluctuations in IAA and its response to increasing amounts of exogenous IAA extends from elongation of the primary root, formation of lateral and adventitious roots (Finnie and Van Staden, 1985). In addition Davies (1995) reported that production of IAA by microbial isolates varies greatly among different species and strains and depends on the availability of substrates. Different biosynthetic pathway for IAA production exist, sometimes in parallel in the same organism.

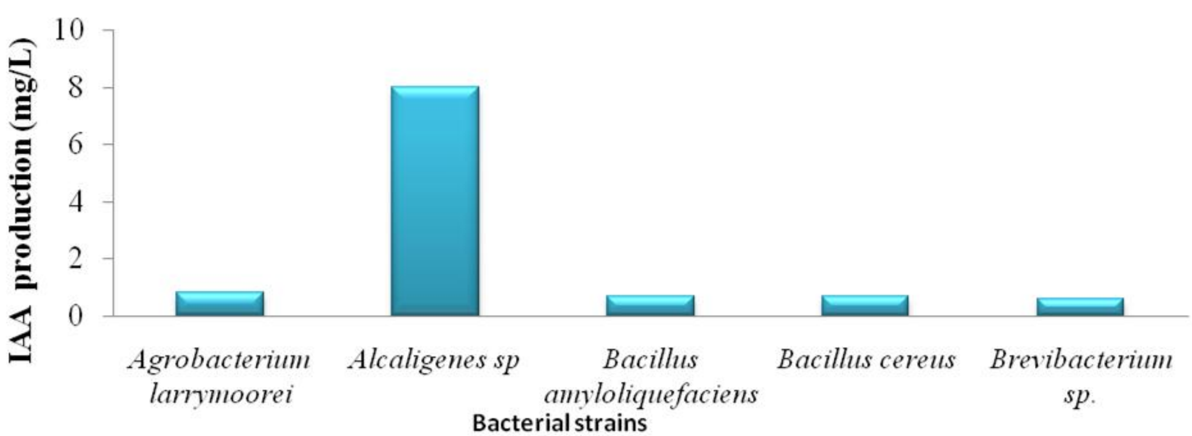

Figure. Ability of the strains to produce Indole Acetic Acid

4. Conclusions

From the above result, it can be concluded as follows;

1. Addition of BCAs alone and its combination with compost and NPK, compost alone, and NPK alone increased significantly the height and panicles of rice plants by fresh water watering .
2. Each of strains of the BCAs consortia (*Agrobacterium larrymoorei*, *Alcaligenes* sp., B. *amyloliquefaciens*, *B. cereus*, and *Brevibacterium* sp) produced IAA, its might be responsible for enhancement of rice height, number of tillers and panicles.
3. Rice plants were able to grow at the soil salinity level up to 12.43 dSm^{-1} (fresh water + NaCl 5% watering), when BCAs, compost, and NPK were applied to the soil. Application of BCAs, compost, and NPK increased the biomass of rice plant for about 67 -100 %.
4. Total of microbes populations were significantly highest by addition of NPK alone combined with fresh and sea water watering and number of rice tillers was significantly highest by addition BCAs alone with fresh water watering

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