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Influence of Root Exudate Carbon Compounds of Three Rice Genotypes on Rhizosphere and Endophytic Diazotrophs

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ABSTRACT

Root exudates play an important role in microbial colonization of the rhizosphere. An *in vitro* experiment was conducted to study the root exudate sugars and production of amino acids of three different rice (*Oryza sativa*) genotypes, as well as the influence of these compounds on *Rhizobium* sp. (Sb16) and *Corynebacterium* sp. (Sb26) colonization. Using HPLC, a total of 7 carbohydrate sugars and 16 amino acids were identified from the Mahsuri, Mayang Segumpal and MR219 rice root exudates. A significant ($p<0.05$) relationship was observed between diazotrophic population growth and root exudates sugar and amino acid consumption of the three rice varieties. Higher bacterial population was found in the plant rhizosphere, as compared to the endosphere. *Rhizobium* sp. consumed more sugar and produced higher rhizosphere population as compared to *Corynebacterium* sp. The *Rhizobium* sp. consumed 100% of mannose, xylose, arabinose and sucrose in the root exudates of three rice genotypes. The differences in sugar consumption by *Corynebacterium* sp. were observed between the rice varieties. *Corynebacterium* sp. consumed 100% mannose, xylose and fructose in Mahsuri rice, 100% xylose and arabinose in Mayang Segumpal and 100% arabinose and sucrose in MR219 variety. The identification of the preferred carbon sources by the diazotrophs and the selection of genotypes which produce these compounds may increase the root colonization and subsequently N fixation in the rice plants.

Keywords: Amino acids, carbon sugars, *Corynebacterium* sp., diazotrophic population, *Rhizobium* sp., rice genotypes, root exudates

INTRODUCTION

Diazotrophs are nitrogen fixing bacteria which depend on their environmental carbon sources for their metabolic activity and nitrogen fixation. Besides soil organic matter, plant root exudates are a vital carbon source for microbes. Several plants allocate up to 40% of recently assimilated carbon to the root zone (Degenhardt *et al.,* 2003). Microbes in the vicinity of plant root consumed about 64-86% of the carbon released from the roots (Hutsch *et al*., 2002). The presence of organic compounds, released by the plant roots, stimulates the microbial activity in the rhizosphere (Bacilio-Jiménez *et al.,* 2003). The microbial activity is generally higher in rhizosphere, and the population size of 10 to 100 fold higher was found in this zone as compared to the surrounding bulk soil (Weller and Thomashow, 1994). Sugar, organic acids and amino acids are the major component of the plant root exudates. Soil bacteria perceive and actively move toward niches which are

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optimal for the survival and attached to the plant root surface. This activity plays a key role in the establishment of associative and symbiotic relationships between plants and micro-organisms (Yost and Hynes, 2000).

Root exudate component and concentrations vary with plant genotypes. He *et al.* (2004) observed that the quantity, content and chemical composition of the root exudates of two rice accessions were different. The amount and composition of root exudates entering the soil can be affected by multiple factors such as light intensity, temperature, nutritional status of the plants, activity of retrieval mechanisms, various stress factors, mechanical impedance, sorption characteristics of growth media and microbial activity in the rhizosphere. The differences in the preference of diazotrophs for root exudate carbon compounds are also observed. *Azospirillum brasilense* utilizes fructose and grows poorly on glucose and amino acids as sole carbon and energy sources (Hartmann *et al*., 1988). *Escherichia coli* is attracted by proline, glycerol and succinate and its growth is dependent on the oxidation of these substrates. Root colonization and rhizosphere competence of *Pseudomonas fluorescens* WCS365 is dependent on the synthesis of amino acid. Lectin-specific carbohydrates such as glucose, glucuronic acid, glucosamine, and galactosamine could function as receptors for the lateral surface lectines (Karpunia *et al*., 2003). Different amounts of sugar are consumed by the bacteria when there were more than one preferred sugar present in the root environment.

At present, not much information is available on the types of root exudates and their production by diazotrophs. The search for the natural associative and endophytic diazotroph establishment with the rice is important in increseaing the efficiency of nitrogen fixation. The application of preferred carbon compounds may enhance inoculum growth and provide a conducive environment for nitrogen fixation as well as promotion of direct plant growth. The present study focused on the rhizosphere and endophytic populations of two inoculated diazotrophs strains and their subsequent utilization of sugars and amino acids, released from the root exudates of three different rice genotypes.

MATERIALS AND METHODS

The root exudates of three rice (*Oryza sativa*) varieties, Mahsuri, Mayang Segumpal, and MR219, and the population growth of two diazotrophic strains, *Corynebacterium* and *Rhizobium* spp. (previously isolated from the rice rhizosphere of Tanjong Karang rice irrigation project area, Malaysia) were studied. Mahsuri and MR219 were high yielding rice varieties, while Mayang Segumpal was a local accession. The sugar and amino acid concentrations of the root exudates and the total bacterial population in the growth culture media, rhizosphere and root endophytic were determined at 3, 6, 12, and 18 days after inoculation. The experiment was carried out in a factorial completely randomized design (CRD) with 3 replications. Data were analyzed using the SAS (9.1 version) statistical software.

Seed Surface Sterilization

The seed surface sterilization method was adopted from Amin *et al.* (2004) and Elbeltagy *et al*. (2001). Rice seeds were dehusked and agitated in 70% ethanol for 5 seconds. The ethanol was discarded and the seeds were washed in hypochlorite solution, comprising 3% Chlorox ™ (2.6% NaOCl), with a few drops of Tween20. The seeds were rinsed (three times) with sterile water, followed by 2% sodium thiosulphate solution to neutralize the chloramine residue. The efficacy of the sterilization procedure was checked by germinating the seeds on nutrient agar (NA) plates.

*Preparation of Diazotrophs (*Rhizobium *and* Corynebacterium *spp.) Inoculums*

Rhizobium and *Corynebacterium* spp. (isolated from MR219 rice rhizosphere) were grown in the ATCC broth for 48 hours. The bacterial cells were harvested by centrifugation at 13500 rpm for 10 min in an eppendorf tube and washed with 0.85% sterilized phosphate buffer saline (Bacteriological Analytical Manual, 2001). Optical density (OD_{600}) of the washed cells were checked and adjusted accordingly. The population was confirmed by cell enumeration in drop plate method on NA.

In vitro Growth of Rice Seedlings

The surface sterilized rice seeds were germinated on the sterile petridishes and eight rice seedlings of 5 days old were grown in the growth culture tubes, where a stainless steel sieve was placed above 50 mL of nutrient solution. After that, the seedlings were placed on the sieve in such a way that only the roots were in touch with the nutrient solution. Carbon and nitrogen free plant nutrient solution, modified from Egener *et al.,* (1999), was used for each glass tube. The solution contained (1L): KH_2PO_4, 1.5 g; K_2HPO_4, 0.33 g; K_2SO_4, 0.2g; ferric citrate, 13 mg; $CaCl_2.2H_2O$, 0.4 g; $MgCl_2$., 0.4g; $Na_2MoO_4.2H_2O$, 2 mg; H_3BO_3, 3mg: $MnSO_4.H_2O$, 2 mg; $ZnSO_4.7H_2O$, 0.2 mg; $CuSO_4.5H_2O$, 0.1 mg. Before transplanting, the seedlings were gently washed with sterile distilled water to remove plant metabolites formed during growth on petridishes. Each growth tube was subsequently inoculated with 5 mL of 10^9 cfu / mL of live bacterial cells. The plants were grown for 20 days in a growth chamber with 12 hr. light/dark cycle at 28 ˚C.

Collection of the Root Exudates

The root exudates of the non-inoculated and inoculated plants were collected from the respective growth tubes at 5, 8, 14 and 20 days after transplanting. The collected plant growth culture solutions of all the glass tubes (50 mL) were filtered through a 0.2 µm Millipore filter and kept in screw-top vials at -20 ˚C for determination of sugar and amino acids.

Determination of Sugars

Sugar concentrations were determined using high performance liquid chromatography (HPLC) with a refractive index (RI) detector. Galactose, arabinose, xylose, fructose, and sucrose were determined on NH_2-carbohydrate column using acetonitrile (75%), as the mobile phase with a flow rate of 1mL min^{-1}. Mannose and glucose were determined by Supel cogel column using phosphoric acid (1%) as the mobile phase at a flow rate of 0.8 mL min^{-1}.

Determination of Amino Acids

The concentrations of amino acid were determined using the HPLC by a modified method proposed by Strydon and Cohen (1994), following the pre-column derivatisation with AQC reagent (6-aminoquinolyl-N-hydroxysuccinimdyl carbamate, Waters, USA). The content of tryptophan was determined using alkaline hydrolyses. Cysteine and methionine were not determined.

Total Sugar and Amino Acids Production

Total productions of sugar and amino acids were estimated as $TP = TC + ER$ where, TP = total sugars and amino acids production by the plant, TC = total sugar and amino acids consumption/utilization by the diazotrophs over period, ER = Extra sugar and amino acids remaining in the inoculated plant root exudates.

Sugar and Amino Acids Utilization by Diazotrophs

The total sugar consumption/utilization (TC) was determined as $TC = S_t - S_{t1}$ where, S_t is the total sugar production by the control plant over time, and S_{t1} is the total sugar remaining in the culture solution by the inoculated plant over time.

Determination of Diazotroph Population in Growth Culture Solution

At each sampling date, 1.0 mL of plant growth culture solution from each glass tube was diluted 10 folds up to 10^{-11} dilution. Aliquotes of 0.1 mL from each dilution was dropped onto NA plates and the populations were determined following the drop plate count method.

Determination of Rhizosphere Population

At each sampling date, 2 plants were harvested, and roots were gently washed with sterile water and placed in conical flask containing 99 ml distilled water. The contents were then shaken for 15 minutes and a series of 10 fold dilutions were prepared, while the bacterial populations were determined as described previously.

Determination of Root Endophytic Population

The roots (1.0 g) were washed, blotted dry and surface sterilized with 70% ethanol for 5 min and then treated with 3% Clorox for 30 sec. The roots were checked for the efficacy of surface sterilization by rolling them on the NA plates. Using a sterilized mortar and pestle, the roots were macerated (Gyaneshwar *et al.,* 2001). A 10 fold dilution series of was prepared up to 10^{-10} and the diazotroph populations were determined as described previously.

RESULTS

Production and Utilization of Sugar by the Diazotrophs

The Mahsuri rice, inoculated with *Rhizobium* sp., was found to produce and utilize more total sugar (2989 µmol g^{-1} root dry wt) as compared to *Corynebacterium* sp. (2853 µmol g^{-1} root dry wt) during the 20 days of growth (Table 1). *Rhizobium* sp. utilized more arabinose, mannose and xylose, while *Corynebacterium* sp. utilized higher amounts of xylose, mannose and fructose (*Fig. 1a*). Among the sugar, the highest amount of fructose (791 µmol g^{-1} root dry

TABLE 1
Production and utilization of sugar (µmol g^{-1} root dry wt.) in inoculated and non-inoculated Mahsuri rice (*Oryza sativa*) root exudates during 5, 8, 14 and 20 days of growth period in an axenic condition

Sugar	5 days seedling			8 days seedling			14 days seedling			20 days seedling		
	Control (TP)	Sb16 (R)	Sb26 (R)	Control (TP)	Sb16 (R)	Sb26 (R)	Control (TP)	Sb16 (R)	Sb26 (R)	Control (TP)	Sb16 (R)	Sb26 (R)
Glu	112.03	--	--	177.03	--	15.2	244.03	--	30.5	265.73	--	30.7
Man	329	--	--	430	--	--	534	--	--	534	--	--
Xyl	290	--	--	585	--	--	591	--	0.9	591	--	--
Arab	204	--	--	346	--	--	346	241	294	346	--	--
Fruc	--	--	--	122	--	233	441	--	--	558	--	--
Galac	--	--	--	--	--	--	--	33	0.61	--	--	--
Suc	--	--	--	300	120	--	300	--	--	300	--	6.6
Total Production (TP)	935	-	-	1960	-	-	2456	-	-	2595	-	-
Total Utilization (TC)	-	935	935	-	1840	1823	-	2302	2363	-	2989	2853

Here, Control = non-inoculated plant, Sb16 = Plant inoculated with *Rhizobium* sp., Sb26 = plant inoculated with *Corynebacterium* sp. (--) = no sugar residue detected. R = residual sugars in the inoculated plant root exudates, TP = total sugar production by the plant, TC = Probable sugars utilization by the diazotrophs ($TC = S_t - S_{t1}$). Where, S_t is the total sugar production by the control plant over time, and S_{t1} is the total sugar remaining in the culture solution by the inoculated plant over time.

wt) was utilized by *Corynebacterium* sp. Both diazotrophs utilized 100 % of released mannose and xylose in the Mahsuri root exudates.

The total sugar production and utilization by the diazotrophs was lower in Mayang Segumpal rice, as compared to the other two varieties. Plants inoculated with *Rhizobium* sp. produced 2686 µmol g^{-1} root dry wt of sugar, while *Corynebacterium* sp. inoculated plants produced half of the sugar during 20 days of growth (Table 2). In Mayang rice root exudates, *Rhizobium* sp. consumed significantly higher amounts of mannose, xylose, arabinose and sucrose, while *Corynebacterium* sp. consumed more xylose and arabinose (*Fig. 1b*). The *Rhizobium* sp. utilized the most released mannose (959 µmol g^{-1} root dry wt) in the Mayang Segumpal. Xylose and arabinose seemed to be completely utilized by both diazotrophs. Nevertheless, glucose was not detected from this rice root.

The MR219 rice plant, inoculated with *Rhizobium* sp., generally exhibited higher sugar exudation (3127 µmol g^{-1} root dry wt), as compared to *Corynebacterium* sp. inoculated

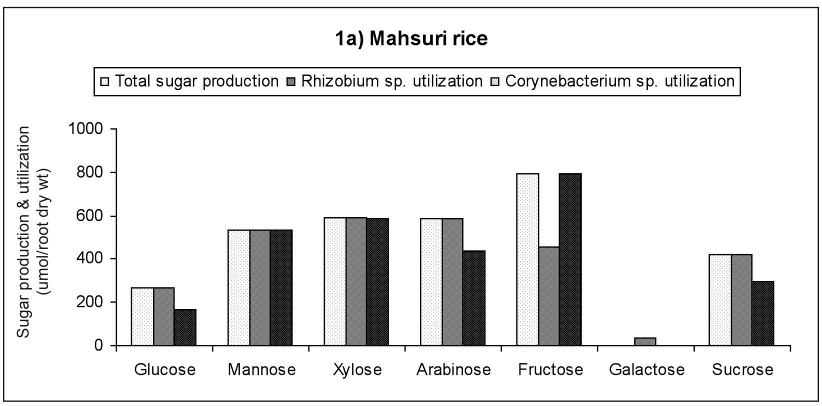

Fig. 1a: The root exudate sugar production and utilization by Rhizobium *and* Corynebacterium *spp. in Mahsuri rice during 18 days of inoculation period (Mean values of 3 replications)*

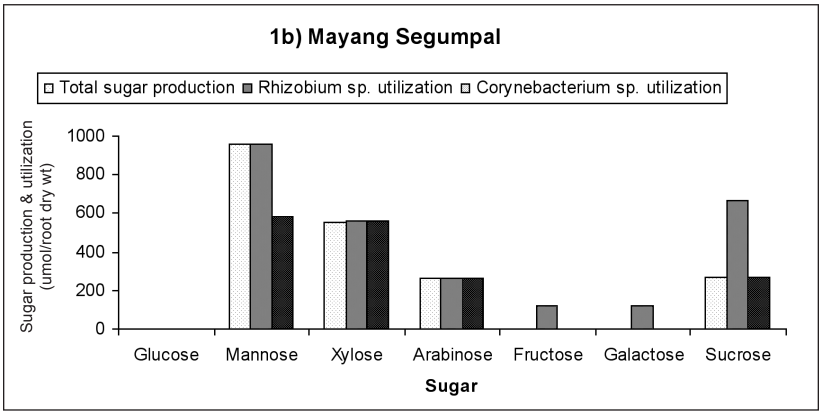

Fig. 1b: The root exudate sugar production and utilization by Rhizobium *and* Corynebacterium *spp. in Mayang Segumpal rice during 18 days of inoculation period (Mean values of 3 replications)*

TABLE 2
Sugar production and utilization (µmol g^{-1} root dry wt.) of inoculated and non-inoculated Mayang Segumpal rice (*Oryza sativa*) root exudates during 5, 8, 14 and 20 days of the growth period in an axenic condition

Sugar	5 days seedling			8 days seedling			14 days seedling			20 days seedling		
	Control (TP)	Sb16 (R)	Sb26 (R)	Control (TP)	Sb16 (R)	Sb26 (R)	Control (TP)	Sb16 (R)	Sb26 (R)	Control (TP)	Sb16 (R)	Sb26 (R)
Glu	--	--	--	--	--	--	--	--	--	--	--	--
Man	285	--	155	601	--	216	772	--	221	959	--	376
Xyl	196	10	10	551	--	--	551	--	--	551	--	--
Arab	--	--	--	259	--	--	259	--	--	259	--	--
Fruc	--	119	--	--	--	--	--	466	--	--	466	--
Galac	--	--	--	--	--	--	--	122	--	--	--	--
Suc	--	--	--	--	--	--	266	400	--	266	--	--
Total Production (TP)	481	-	-	1411	-	-	1848	-	-	2035	-	-
Total Utilization (TC)		471	316		1540	1205		1977	1637		2686	1669

Here, Control = non-inoculated plant, Sb16 = Plant inoculated with *Rhizobium* sp., Sb26 = plant inoculated with *Corynebacterium* sp. (--) = no sugar residue detected. R = residual sugars in the inoculated plant root exudates, TP = total sugar production by the plant, TC = Probable sugars utilization by diazotrophs ($TC = S_t - S_{t1}$), where S_t is the total sugar production by the control plant over time, and S_{t1} is the total sugar remaining in the culture solution by the inoculated plant over time.

plant (Table 3). In particular, *Rhizobium* sp. utilized higher amounts of mannose, fructose, sucrose and galactose, whereas *Corynebacterium* sp. utilized higher amounts of mannose, fructose and sucrose (*Fig. 1c*). Mannose was highly utilized (697.5 µmol g^{-1} root dry wt) by *Corynebacterium* sp. Arabinose and sucrose produced by plants was almost completely utilized by both the strains.

Production and Utilization of Amino Acids in the Root Exudates

The production of amino acids was higher in Mahsuri, as compared to the other two varieties. Most of the amino acids produced were utilized by both bacteria. Mahsuri and MR219, inoculated with *Corynebacterium* sp., utilized higher amount of amino acids as compared to *Rhizobium* sp., while *Rhizobium* sp. utilized most of the amino acid from the Mayang rice root exudates.

In Mahsuri rice, *Rhizobium* sp. utilized higher amounts of glycine and isoleucine, while *Corynebacterium* sp. utilized serine, glutamine, glycine, isoleucine and leucine (*Fig. 2a*). In the Mayang Segumpal rice root exudates, *Rhizobium* sp. was found to consume more than 70% of aspartic, glutamine, serine, glycine, threonine and isoleusine, while *Corynebacterium* sp. consumed more than 80% of released leucine and tryptophan (*Fig. 2b*). In MR219 rice root exudates, *Rhizobium* sp. was indicated to utilize higher amounts of glycine, alanine and *Corynebacterium* sp. utilized higher amounts of glycine, alanine, arginine, and isoleucine (*Fig. 2c*).

TABLE 3
Sugar production and utilization (μmol g^{-1} root dry wt.) of inoculated and non-inoculated MR219 rice (*Oryza sativa*) root exudates during 5, 8, 14 and 20 days of the growth period in an axenic condition

Sugar	5 days seedling			8 days seedling			14 days seedling			20 days seedling		
	Control (TP)	Sb16 (R)	Sb26 (R)	Control (TP)	Sb16 (R)	Sb26 (R)	Control (TP)	Sb16 (R)	Sb26 (R)	Control (TP)	Sb16 (R)	Sb26 (R)
Glu	25	--	--	52.3	--	--	77.3	--	--	150	--	24.8
Man	32	--	--	46	--	--	474	--	--	834.9	--	--
Xyl	20	--	--	20	2.9	--	20	--	1.5	20	--	10.6
Arab	186	--	--	186	--	--	198	--	--	198	--	--
Fruc	--	444	--	139	--	--	326	--	--	326	--	14
Galac	48	--	--	48	30	--	258	--	--	591	--	26
Suc	333	--	--	522	9	--	522	--	9	522	--	--
Total Production (TP)	644	-	-	1013	-	-	1875	-	-	2642	-	-
Total Utilization (TC)	-	644	644	-	1415	1013	-	2361	1874	-	3127	2577

Here, Control = non inoculated plant, Sb16 = Plant inoculated with *Rhizobium* sp., Sb26 = plant inoculated with *Corynebacterium* sp. (--) = no sugar residue detected. R = residual sugars in the inoculated plant root exudates, TP = total sugar production by the plant, TC = Probable sugars utilization by the diazotrophs ($TC = S_t - S_{t1}$), where S_t is the total sugar production by the control plant over time, and S_{t1} is the total sugar remaining in the culture solution by the inoculated plant over time.

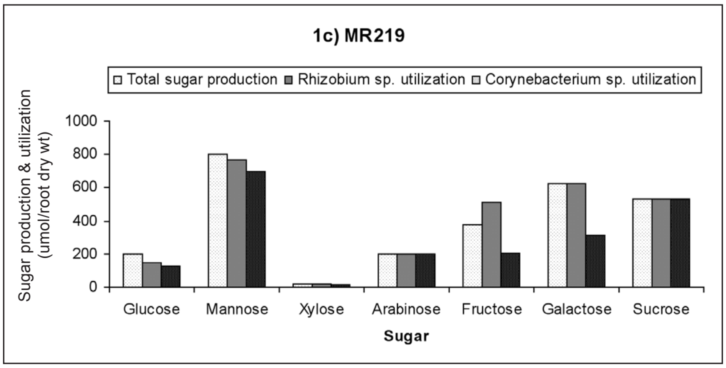

Fig. 1c: The root exudate sugar production and utilization by Rhizobium *and* Corynebacterium *spp. in MR219 rice during 18 days of inoculation period (Mean values of 3 replications).*

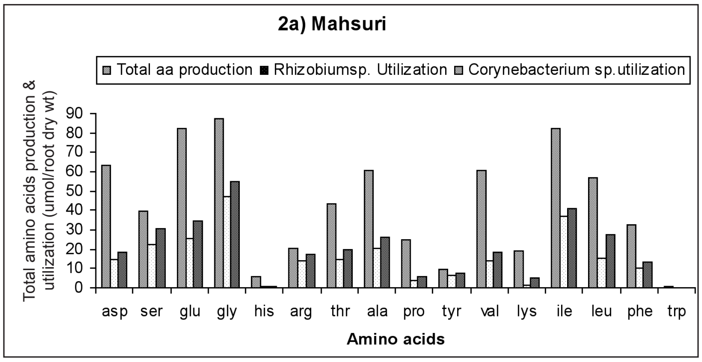

Fig. 2a: The root exudate amino acids production and utilization by Rhizobium *and* Corynebacterium *spp. in Mahsuri rice during 18 days of inoculation period (Mean values of 3 replications)*

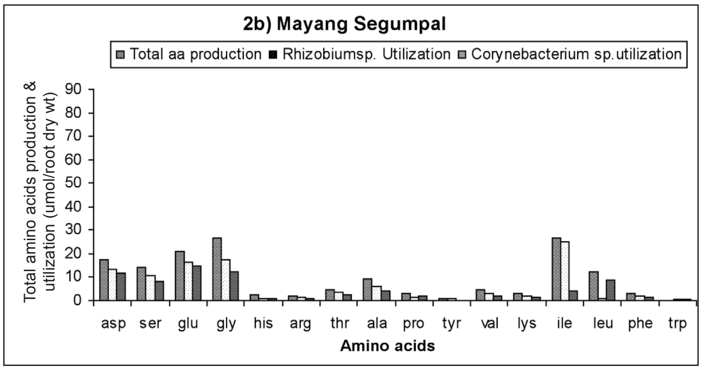

Fig. 2b: The root exudate amino acids production and utilization by Rhizobium *and* Corynebacterium *spp. in Mayang Segumpal rice during 18 days of inoculation period (Mean values of 3 replications)*

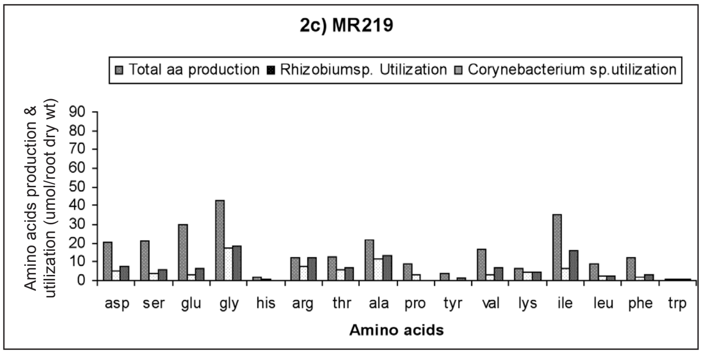

Fig. 2c: The root exudate amino acids production and utilization by Rhizobium *and* Corynebacterium *spp. in MR219 rice during 18 days of inoculation period (Mean values of 3 replications)*

Relationship between Root Exudates Sugar and Amino Acids Utilization and Diazotrophs Colonization

A significant (P≤0.05) relationship was found between the root exudate sugar and amino acid utilization and diazotroph population growth in the growth culture solution, rhizosphere and root endophytes of the rice varieties (Tables 4 and 5). The population of rhizosphere was significantly higher than in the growth culture solution and root endosphere populations (*Fig. 3*). The population of rhizosphere ranged from 10^8 to 10^{11} cfu g^{-1} root dry weight. As indicated earlier, *Rhizobium* sp. utilized more sugar than *Corynebacterium* sp., and this subsequently produced higher rhizosphere population.

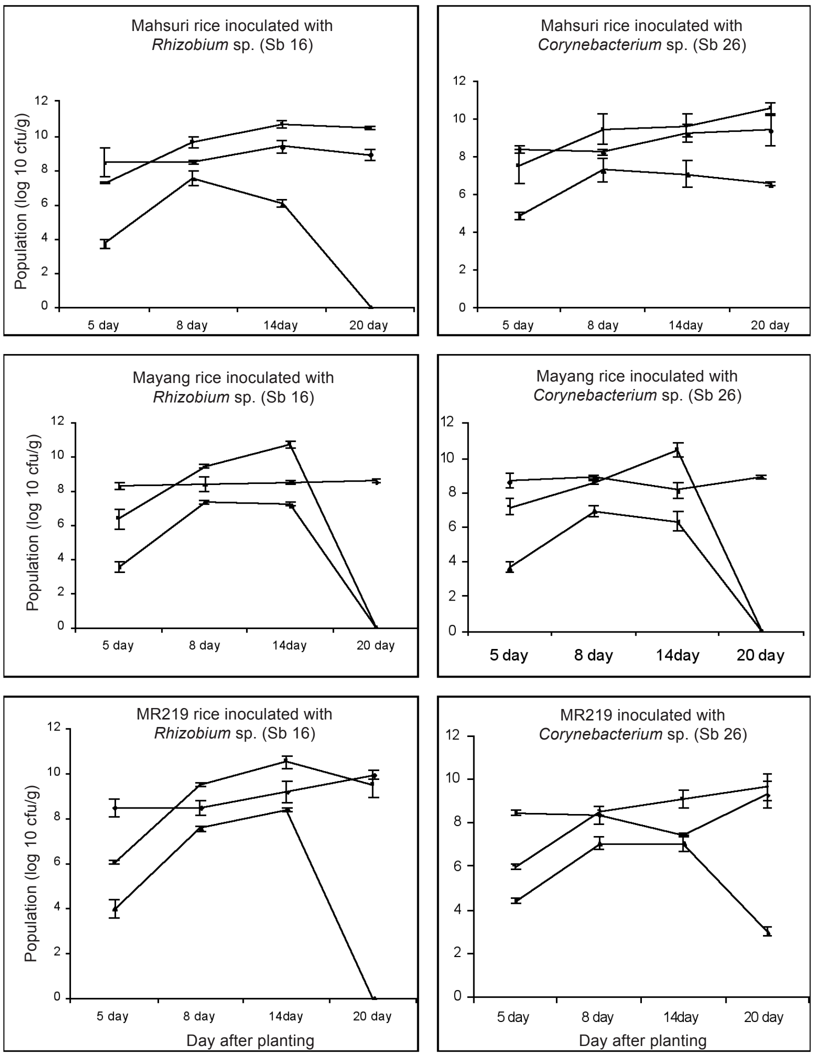

Fig 3: Changes in growth of diazotrophs population in the rice varieties inoculated with Rhizobium *and* Corynebacterium *spp. Populations of* Rhizobium *sp. and* Corynebacterium *sp. in culture solution (◆), rhizosphere (■), root endophytes (▲)of different rice varieties*

TABLE 4

The correlation between the root exudate sugar consumption and the population growth of *Rhizobium* and *Corynebacterium* spp. in Mahsuri, Mayang and MR219 rice varieties. CS = Growth culture solution, Rhi = Rhizosphere, and Root = root endophytes. Negative (-) indicates the absence of sugar. Significance levels are *, 0.05, **, 0.01, ***, 0.001, respectively. NS= not significant from the untreated control

The correlation result of root exudate sugar consumption and population growth in Mahsuri, Mayang and MR219 rice varieties inoculated with *Rhizobium* and *Corynebacterium* spp.

Sugar	Mahsuri						Mayang Segumpal						MR219					
	Rhizobium sp Population			*Corynebacterium sp* population			*Rhizobium sp* population			*Corynebacterium sp* population			*Rhizobium sp* population			*Corynebacterium sp* population		
	CS	Rhi	Root	CS	Rhi	Root	CS	Rhi	Root	CS	Rhi	Root	CS	Rhi	Root	CS	Rhi	Root
Glu	***	NS	NS	NS	***	NS	0	0	0	0	0	0	***	NS	- ***	NS	NS	NS
Man	NS	NS	NS	NS	NS	NS	- *	NS	NS	NS	*	**	NS	- ***	NS	- ***	NS	***
Xyl	- ***	NS	*	- ***	NS	NS	- *	NS	NS	- *	NS	NS	NS	NS	NS	NS	NS	NS
Arab	***	NS	NS	NS	- ***	- *	- *	NS	NS	- *	NS	NS	**	NS	NS	- **	NS	NS
Fruc	NS	NS	*	NS	- ***	- *	***	NS	NS	NS	NS	NS	NS	- ***	NS	***	- ***	- ***
Galac	***	NS	*	NS	*	***	NS	*	NS	NS	NS	NS	- **	NS	NS	***	- ***	- ***
Suc	NS	- ***	NS	NS	*	***	*	NS	NS	NS	NS	NS	- *	NS	NS	- ***	NS	NS

TABLE 5
The correlation between the root exudate amino acids utilization and the population growth of *Rhizobium* and *Corynebacterium* spp. in Mahsuri, Mayang and MR219 rice varieties. CS = Growth culture solution, Rhi = Rhizosphere and root = root endophytes. Negative (-) indicates the absence of sugar. Significance levels are *, 0.05, **, 0.01, ***, 0.001, respectively. NS = not significant from the untreated control

The correlation result of root exudate amino acid consumption and population growth in Mahsuri, Mayang and MR219 rice varieties inoculated with *Rhizobium* and *Corynebacterium* spp.

Amino acids	Mahsuri						Mayang Segumpal						MR219					
	Rhizobium sp. Population			*Corynebacterium* sp. population			*Rhizobium* sp. population			*Corynebacterium* sp. population			*Rhizobium* sp. population			*Corynebacterium* sp. population		
	CS	Rhi	Root	CS	Rhi	Root	CS	Rhi	Root	CS	Rhi	Root	CS	Rhi	Root	CS	Rhi	Root
Asp	NS	- ***	NS	NS	- ***	NS	NS	**	**	NS	NS	NS	NS	NS	*	- ***	NS	*
Ser	NS	- ***	NS	NS	- ***	NS	NS	**	**	NS	NS	NS	NS	NS	*	- ***	NS	*
Glu	NS	- ***	NS	NS	- ***	NS	NS	***	**	NS	NS	NS	NS	*	NS	- ***	NS	NS
Gly	NS	- ***	NS	NS	- ***	NS	*	*	*	NS	NS	NS	NS	- ***	NS	NS	- **	NS
His	NS	NS	*	- *	NS	**	NS	*	NS	NS	NS	NS	NS	- ***	NS	NS	- ***	NS
Arg	NS	- ***	NS	NS	- ***	- *	NS	NS	NS	***	- **	NS	**	NS	- ***	*	*	NS
Thr	NS	- ***	NS	NS	- ***		NS	NS	NS	*	NS	NS	NS	- *	NS	- **	- *	NS
Ala	NS	- ***	NS	NS	- ***	- *	NS	NS	NS	*	NS	NS	NS	- ***	NS	- *	NS	NS
Pro	- *	- ***	NS	- *	- ***	NS	NS	NS	NS	**	NS	NS	NS	- ***	NS	NS	NS	NS

Table 5 (continued)

Tyr	NS	- ***	NS	NS	- ***	NS	NS	NS	NS	***	- ***	- ***	NS	- ***	NS	- ***	NS	NS
Val	NS	- ***	NS	NS	- ***	NS	NS	NS	NS	**	NS	NS	NS	- ***	NS	- ***	NS	NS
Lys	NS	- ***	NS	NS	- ***	- **	NS	NS	NS	**	NS	NS	NS	NS	NS	- ***	NS	NS
Ilu	NS	- ***	NS	NS	- ***	NS	- *	- *	- *	NS	NS	- **	NS	NS	*	- ***	NS	NS
Leu	NS	- ***	NS	- *	- *	NS	NS	NS	NS	***	- ***	- ***	NS	*	NS	- ***	NS	NS
Phe	NS	- ***	NS	- *	- ***	NS	NS	NS	NS	*	NS	NS	NS	- ***	NS	- ***	NS	NS
Trp	NS	NS	- ***	NS	NS	**	NS	- ***	- ***	***	- ***	- ***	NS	- ***	NS	NS	- ***	NS

Meanwhile, fructose, sucrose, xylose, arabinose, and mannose were significantly correlated with the population of *Rhizobium* sp. On the other hand, sucrose, mannose, glucose, fructose and galactose were significantly correlated with the *Corynebacterium* sp. population.

The population of *Rhizobium* sp. in the Mahsuri rice rhizosphere did not correlate with the concentrations of histidine and tryptophan. In Mayang rice, the concentrations of aspartic, serine, glutamine, glycine and histidine were correlated with the increased *Rhizobium* populations. In MR219 rice, serine, glutamine, isoleusine, arginine and leucine were significantly correlated with the population of *Rhizobium* sp. Meanwhile, the concentrations of histidine, and tryptophan were significantly correlated with the population of *Corynebacterium* sp. in Mahsuri rice. In Mayang rice, the population significantly increased by arginine, threonine, alanine, proline, tyrosine, valine, lysine, leucine, phenylalanine, and tryptophan. In MR219 aspartic, serine and histidine were significantly correlated with the population of *Corynebacterium* sp. The populations of endophytic in all the three rice genotypes were found to decrease after 14 days of inoculation.

DISCUSSION

Inoculation and Stimulation of Sugar and Amino Acids

Root exudates provide a favourable niche for diazotrophic association and roots are prerequisite for the establishment of the symbiotic association between plant and bacteria (Alexandre and Zhulin, 2001). The attraction towards the exudation component is dependent on the concentration and configuration of the compounds (Reinhold *et al*., 1985). In the present study, a significant correlation was found between the utilization of sugar and amino acids in the root exudates and the growth of the diazotrophic population. The populations of rhizosphere were shown to be significantly higher than the populations of non-rhizosphere and root endophytic. The site of exudation may provide suitable environment for colonization on the rhizosphere. The nutrients released in the root exudates were readily consumed by the diazotrophs and consequently, established themselves on the root surface. Conditions provided by the host plants significantly contributed to the initiation of the association process (Reinhold *et al*., 1985).

Root exudates sugar, amino acids production and composition were different between the rice cultivars which might influence the population growth of diazotrophs. Kumar *et al*. (2007) also found differences in the root exudates between cotton cultivars which influence the chemotactic responses in *Azotobacter* spp. The absence of glucose in Mayang Segumpal root exudates, may be due to the re-absorption of plant, as plants were grown in strictly carbon-free condition (Guckert *et al.,* 1991). Another probable cause may be due to the very low amount of glucose present in the root exudates, which could not be detected.

The rice varieties, which were inoculated with *Rhizobium* sp., produced higher rhizosphere population than *Corynebacterium* sp. The higher *Rhizobium* sp. population resulted in more utilization of total sugars from the root exudates of all the three rice varieties. A previous study showed that several compounds, including sugar, and amino acids, attracted *Rhizobium* spp. to the roots (Aguilar *et al*., 1998). The *Corynebacterium* sp. consumed higher amounts of amino acids as compared to *Rhizobium* sp. from Mahsuri and MR219 root exudates. In more specific, amino acids were found to be weak attractants for the bacteria which fixed nitrogen under microaerophilic conditions (Alexandre and Zhulin, 2007). The slightly higher consumption of amino acids observed by *Rhizobium* sp. in Mayang Segumpal may be due to the low sugars in root exudates and compels this strain on amino acids for their physiological activities. Decreased diazotrophs population in the growth culture solution was probably caused by the accumulation of high levels of toxic substances in the vicinity of the root which inhibited growth.

Correlation analyses revealed that the *Rhizobium* sp. significantly consumed almost all sugar, while *Corynebacterium* sp. consumed glucose, mannose, xylose, arabinose, and sucrose from the root exudates of rice varieties. The concentration of fructose significantly correlated the diazotrophs population in both Mahsuri and Mayang Segumpal rice. Our previous study also showed higher population growth of these diazotrophs in fructose substrate, and this is analogous to the findings of the present study (Naher *et al*., 2008).

Rhizobium sp. seemed to utilize all detected amino acids. Another previous study showed that glutamate, aspartate, alanine, and arginine were significant attractants to *Bradyrhizobium japonicum* (Barbour *et al*., 1991). The attraction to proline was found in many bacteria including *E. coli* and *Sinorhizobium meliloti* (Clancy *et al*., 1981; Götz *et al*., 2000). In the present study, the population of *Corynebacterium* sp. was positively correlated with the concentrations of histidine, tryptophan, arginine, tyrosine, threonine, alanine, proline, valine, lysine, leusine, and phenylanine. In general, it can be concluded that the utilization of sugar and amino acids in root exudates is not only dependent on the concentration of the compounds, but also the presence of the other compounds and the preferences of the diazotrophs to those specific compounds.

In the present study, 7 sugars and 16 amino acids were determined in the root exudates. The utilization of sugar and amino acid by the diazotrophs were assumed to be based on the residual component, which was determined at different dates and deducted from the non-inoculated plants. However, this indirect determination could provide fundamental information on the production of sugar and amino acid, as well as the utilization and growth of diazotrophs in the rice plant system.

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