9 Poster

Salt tolerance of diazotroph *Alaligenes faecalis* and its salt-tolerant association with host rice

S.Z. PING, M. LIN, Y.N. LIU, C.B. YOU AND KAUSER A. MALIK

There are about 20 million hectares of saline land in North and East China, which causes a loss of approximately 30 million tons of crop yield annually. Some studies conducted in China showed that biofertilizer containing salt-tolerant nitrogen-fixing bacteria could promote growth of plants and increase the yield of crops (Ping *et al.*, 1996). *Alcaligenes faecalis* A1501, which was isolated from paddy soils in South China in 1980, could grow well in solid or liquid medium containing 0.8 mol/L NaCl (Table 1). Growth of bacteria was repressed in solid medium containing 1.2 mol/L NaCl. A1501 could grow very slowly even if the NaCl concentration reached 1.6 mol/L in liquid medium. A1501 could produce IAA under salt stress and maintain N-fixing activity until the NaCl concentration was higher than

Table 1. Effect of various concentrations of NaCl on growth of *A. faecalis* grown in solid medium

Na^+ (mol/L)	0.2	0.4	0.6	0.8	1.0	1.2
Solid medium	+++	+++	++	++	+	–

+++, grow well; ++, grow normally; +, grow slowly; –, no growth.

Table 2. Identification of IAA produced by *A. faecalis* A1501 under salt stress (0.4 M NaCl)

Time of incubation (h)	A1501	*Klebsiella* sp. 101	*Klebsiella* NIAB–I
24	++	+	–
48	++	+	–
72	++	+	–
96	++	+	–
120	+++	++	–

+, IAA production; –, no IAA production.

K.A. Malik et al. (eds.), Nitrogen Fixation with Non-Legumes, 87–89.

Table 3. Associative nitrogen fixation between host rice and *A. faecalis* A1501

	Concentration of NaCl (%)			
	0.01	0.5	1.0	3.0
Rice roots inoculated with A1501	1045.3*	1565.4	956.7	799.1
Pure culture of A1501	681.0	755.1	803.6	456.5

* Activity unit is 'nmol C_2H_4 produced/h. g f.w. roots'.

Table 4. Effect of various NH_4^+ concentrations on expression of *nifL–lacZ* fusion in conjugant A1501(pUK121.2) under microaerobic conditions

NH_4^+ concentration (mM)				
0.0.	2.0	6.0	10.0	15.0
2380.7*	1966.8	1800.2	1655.5	970.0

* β-galactosidase activity (Miller units).

Table 5. Effect of various NH_4^+ concentrations on N_2-fixing activity of A1501 and conjugant A15L1 (pUK121.2) under microaerobic conditions

	NH_4^+ concentration (mM)				
	0.0	2.0	6.0	10.0	15.0
A1501	100	76	54	15	2
A15L1	10	8	3	0	0

* N_2-fixing activity (%).

Table 6. Effect of various concentrations of nalidixic acid on expressions of *nifL-lacZ* fusion in conjugant A15L1 under microaerobic conditions

Nalidixic acid (μg/ml)					
0.0	1.0	2.0	3.0	4.0	5.0
3120.5*	2966.8	2657.0	1655.5	970.0	0.0

* β-galactosidase activity (Miller units).

1.0 mol/L (Tables 2 and 3). Southern hybridization of total DNA from A1501 grown under condition of different NaCl concentration with *Klebsiella pneumoniae nifHDK* DNA probe show that homologous hybridization bands under the normal condition were identifical to those of the cells grown under the salt stress.

By conjugation, *Azotobacter vinelandii nifL* or *nifL-lacZ* gene fusion (kindly provided by Professor Das) was introduced into *Alcaligenes faecalis* wild-type A1501. This is confirmed by physical mapping of plasmid purified from conjugant. A study on salt tolerance, chemotaxis, nitrogen-fixing activity,

Table 7. Effect of NH_4^+ and NaCl on the colonization of A1501 and conjugant A15L1 (pUK121.2) to rice roots

Stains	Treatment		
	$-NH_4^+ - Na^+$	$+NH_4^+$ (15 mmol/L)	$+Na^+$ (0.5%)
A1501	2.7×10^9*	2.75×10^8	9.62×10^9
A15(pUK121.2)	4.37×10^9	1.8×10^8	2.4×10^9

* Bacterial cells/g fresh root of rice.

β-galactosidase activity of A1501 and the conjugant was performed (Tables 4 and 5). The conjugant possesses the same salt tolerance as A1501, and the poor ability of chemotaxis, nitrogen-fixing activity and colonization on rice roots compared to A1501. Expression of *A. vinelandii nifL* in *A. faecalis* was regulated by NH_4^+ and O_2 and was inhibited by nalidixic acid, which is an inhibitor of DNA gyrase (Table 6). This suggested that the product of heterogeneous *nifL* in *A. faecalis* may possess the regulatory function.

Inoculation with A1501 could enhance salt tolerance of host rice, promote growth of rice plants and stimulate formation of lateral roots and root hairs under salt stress (Table 7). Study of the colonization of rice roots by histological assay indicated that (1) *A. faecalis nifH*, *A. brasilense nifA* and *A. vinalandii nifL-lacZ* gene fusions (Zhang *et al.*, 1996) could express well on the surface or in the endorhizosphere of rice roots under salt stress; (2) the sites and activities of expression of three gene fusions were quite different in endorhizosphere of rice.

References

Ping S Z, Lin M, You C B, Malik K A 1996. Salt tolerance of diazotroph *Alaligenes faecalis* and its interaction with host rice under the salt stress. J. Agric. Biotechnol., 4, 87–92.

Zhang H Y, Lin M, Ping S Z, You C B, Zhu Y X 1996. Construction *nifH::gusA* of *Alcaligenes faecalis* and expression of fusion gene in association with rice roots. J. Agric. Biotechnol., 4, 93–100.