Al binding to the EPS and DNA of the bradyrhizobial cells exposed to Al stress

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S. K.MUKHERJEE・浅沼修一：AIストレス下におけるダイズ根粒菌のEPSとDNAへのAIの吸着

Introduction

A quantitative comparison of Al binding to the DNA and to the exopolysaccharide (EPS) has been made with the selected Al-tolerant and sensitive strains of *Bradyrhizobium japonicum* exposed Al-stress conditions. Effect of Al binding to the DNA on the phenotypic expression of some antibiotic resistance trait was also studied.

1. Materials & Methods

To determine the amount of Al bound to the DNA or to the EPS, cells grown in a complex medium (Raina and Modi, 1969) for 5 days were harvested, washed with sterilized water by centrifugation, resuspended in Al-stress medium (pH 5.4; Keyser and Munns, 1979) and kept at 28℃.

DNA was extracted from the cells following Sambrook *et al.* (1989) after 48h of exposure to Al-stress. In another experiment EPS was extracted by ethanol precipitation method. Al present in DNA or EPS was determined in an ICP after acid digestion.

To determine the effect of Al on antibiotic resistance or sensitivity, Al (final conc. 50μM) was added to the culture of exponentially growing cells in low P medium (P 5μM, pH 5.4; Keyser and Munns, 1979). Growing cultures in the same medium without Al were considered as control. After 48h cultures were plated on the yeast extract mannitol agar (YMA) containing rifampicin (75μg ml^{-1}), streptomycin (100 μg ml^{-1}) or kasugamycin (75μg ml^{-1}) to enumerate resistant cells for respective antibiotic, or on YMA without antibiotic to count total cell number.

2. Results & Discussion

The amount of Al in the EPS varied among the test strains irrespective of their Al sensitivity or tolerance (Fig.1). Such binding of Al to this important

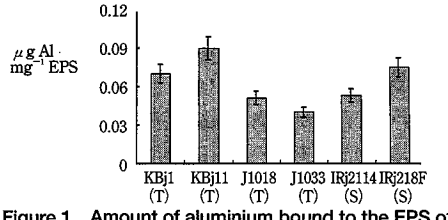

Figure 1. Amount of aluminium bound to the EPS of Al-tolerant (T) and sensitive (S) *B. japonicum* strains exposed to Al-stress

biopolymer may have a severe consequence on symbiotic recognition even in Al-tolerant strain, which could not be noticed during simple selection process. Thus, whether this binding of Al to the EPS of selected Al-tolerant strains has any adverse effect on the successful symbiotic processes is to be further delineated under acid-Al soil conditions. On the other hand, the amount of Al bound to the DNA was significantly higher in Al-sensitive strains than what in tolerant strains (Fig.2).

However, irrespective of Al tolerance or sensitivity

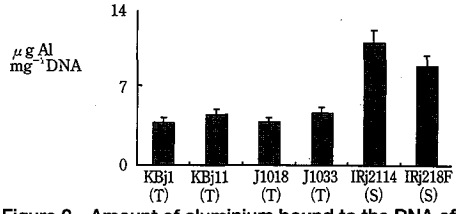

Figure 2. Amount of aluminium bound to the DNA of Al-tolerant (T) and sensitive (S) *B. japonicum* strains exposed to Al-stress

of the test bradyrhizobial strains there was no significant difference in the change of frequencies of antibiotic resistance traits due to Al binding to the DNA under described experimental set up (Table 1). The rate of such genetic alterations varied a little for three antibiotics (Streptomycin 10μg ml^{-1}, Kasugamycin 75μg ml^{-1} or Rifampicin 75μg ml^{-1}). The incidences of genetic alteration for antibiotic resistance or sensitivity in both direction due to Al binding to the DNA were not so significant to say Al at a concentration (50μM) of general use for selecting Al-tolerant rhizobial strains is severe for genetic alterations. The rate of such mutation in this study (ca < 1 in 10^6 cells) contradicts with the earlier claim that Al (50μM) at pH 5.5 caused mutation for the rifampicin resistance of Al-sensitive and tolerant rhizobial strains at a frequency of *ca* 1 in 10^3 and 10^5 cells respectively (Octive *et al.*, 1991). Thus, the manifestation of Al toxicity, at the concentrations more akin to those in acidic soils, depends whether on the site-specific genetic alterations caused by Al binding in the DNA or its involvement in certain metabolic processes requires further investigations.

Table 1. Rate of changes in antibiotic resistance or sensitivity among the Al-tolerant and sensitive *B. japonicum* strains due to Al-stress

Antibiotic used	Al-tolerant strain				Al-sensitive strain	
	KBj1	KBj11	J1018	J1033	IRj2114	IRj2118F
Streptomycin (100μg ml^{-1})	[a]$10^{6.3}$	IS	IS	$\underline{10^7}$	$\underline{10^6}$	IS
Rifampicin (75μg ml^{-1})	IS	$\underline{10^{7.5}}$	IS	$\underline{10^{6.8}}$	10^8	IS
Kasugamycin (75μg ml^{-1})	ND	IS	10^7	10^8	$\underline{10^{7.8}}$	10^6

[a] Frequency of changes in antibiotic resistance or sensitivity (1 cell in the total population)
IS=very insignificant; ND=Not obtained from the population
Underlined data are to denote alteration of antibiotic resistance to sensitivity

References

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