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Research Article

EFFECT OF CADMIUM ON PLASMID PROFILE OF NITROGEN FIXING *RHIZOBIUM*

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ABSTRACT

Heavy metal pollution of the agricultural fields has become a big menace these days. This has affected the crops, cropping systems and agricultural production badly. The nitrogen fixing leguminous crops are very sensitive to the heavy metals especially cadmium (Cd). The present study reports the effect of Cd on the plasmids responsible for symbiotic nitrogen fixation in mungbean. The plasmid profile of *Rhizobium* sp. strain RG-1 indicated a concentration gradient dependent sensitivity of the bacterium to Cd. The heavier symbiotic plasmid disappears with the addition of 2 µM Cd to the growth medium. Both the plasmids were absent at higher concentration of 4 µM Cd in the cultures. The disappearance of symbiotic plasmids is a direct indicator of metal toxicity and absence of nitrogen fixing efficacy of the bacterial strain.

Keywords: *Rhizobium, Cadmium, Cd, Heavy Metals, Plasmids*

INTRODUCTION

The symbiotic nitrogen fixation is a very important aspect from human foods and soil fertility point of view. The crops, which fix atmospheric nitrogen with the help of soil gram negative bacterium *Rhizobium* have to face many natural and anthropogenic environmental conditions which directly or indirectly affect their growth, development, ability to fix nitrogen and finally the crop yields. Heavy metals are one of the most obnoxious environmental pollutants which severely affect all the plants which come in their contact. A comparative toxicity of heavy metals in a decreasing order of Cd>Ni>Cu>Zn>Pb has been shown on symbiotic nitrogen fixation (McGrath *et al*., 1995). Heavy metal cadmium (Cd) is extremely phytotoxic and adversely affects *Rhizobium* (Chaudri *et al*., 1992), morphological, physiological and biochemical processes (Thakur and Singh, 2012; Singh and Thakur, 2014) and plant productivity of nitrogen fixing leguminous crops. Cd is primarily released into the environment through human activities (Wagner, 1993).

The genes for nitrogen fixation in *Rhizobium* are located on two extremely large plasmids of about 1,400 kb (pSym-a or megaplasmid 1) and 1,700 kb (pSym-b or megaplasmid 2). These genes code for proteins of the nitrogenase complex, proteins involved in the assembly of nitrogenase with cofactors and for putative electron transport functions (MacLean *et al*., 2007). An attempt was made to study the effect of Cd on the plasmid profile of *Rhizobium* sp. strain RG-1 used for mungbean (*Vigna radiata* (L.) R. Wilczek *cv*. SML-32) during the phytotoxicity studies.

MATERIALS AND METHODS

The *Rhizobium* sp. strain RG-1 was obtained from Department of microbiology, Punjab Agricultural University, Ludhiana (India). It was cultured and maintained in yeast extract mannitol agar (YEMA) medium (Fred *et al*., 1932) in sterilized test tubes (25×150 mm) at 28±2 ºC in the incubator. The Rhizobia were re-grown in broth culture of the same medium containing 5 and 10 µM cadmium sulphate (2 and 4 µM Cd) for seven days. The plasmids containing nitrogen fixing genes were isolated using the method given in Kado and Liu (1981) to know the effect of Cd on the genes involved in nitrogen fixation and symbiosis. The bacterial cells were pelleted by centrifugation at 5,700 rpm in a refrigerated centrifuge for 7 minutes. The cell pellet was suspended in 1 ml of E buffer and 2 ml of lysing solution was added to it. The solution was thoroughly mixed by brief agitation and then heated at 50°C to 65°C for 20 minutes in a water bath. Two volumes of phenol-chloroform solution (1:1 v/v) were added and the solution was

emulsified by shaking. Thereafter, it was centrifuged at 6,000 rpm for 15 minutes at 4°C. The upper aqueous phase was transferred to a screw cap tube or directly used for electrophoresis immediately. The samples before loading were mixed with 0.25% bromocresol purple in 50% glycerol-0.05 M Tris-acetate (pH 7.9). 0.7% agarose was used to resolve the whole plasmids in horizontal apparatus (Pharmacia Biotech). A low salt buffer system composed of 40 mM Tris-acetate and 2 mM sodium EDTA was used to avoid overheating and to resolve high-molecular-weight plasmids. The Tris was adjusted to pH 7.9 with glacial acetic acid (E buffer). The agarose was melted for 3 to 5 minutes in E buffer either in an autoclave or in a microwave oven and mixed well before pouring. The electrophoresis was carried out at 12 V/cm which required about 2 hours for the bromocresol purple tracking dye in the sample to migrate 12 cm. The gel was stained with 0.5 pg of ethidium bromide per ml for 30 minutes at 23°C. The photograph of the gels was taken over a shortwave UV light source with a Polaroid camera.

RESULTS AND DISCUSSION

After careful observations of the photographs of electrophoresis (Figure 1), two large sized plasmids (pSym-a or megaplasmid 1 and pSym-b or megaplasmid 2) were reported to occur in control bacteria. It was observed that only one plasmid (lighter in molecular weight) was visible in *Rhizobium* cultured in broth medium containing 2 µM Cd.

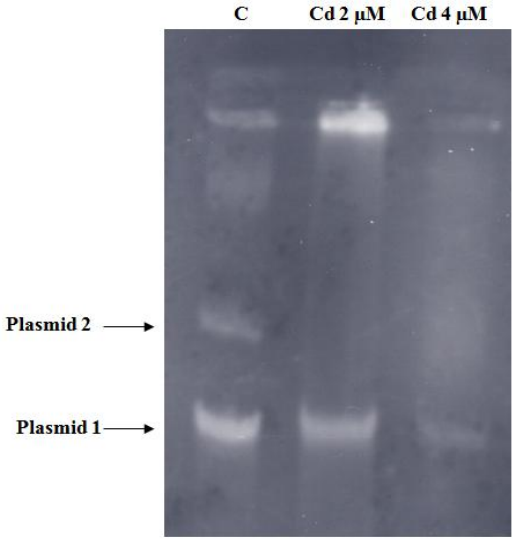

Figure 1: Plasmid profile of *Rhizobium* sp. strain RG-1 grown in 2 and 4µM Cd

The heavier plasmid disappeared at 2 µM Cd application, while both of them were lost when the rhizobia were cultured in broth containing 4 µM Cd. In both the cases, plasmids were lost while the bacteria were able to survive. The plasmid profile of this bacterial isolate indicates a Cd concentration gradient dependent sensitivity of the bacterium. Giller *et al.*, (1989) have reported the inability of white clover rhizobia to fix nitrogen in the presence of heavy metals. The nitrogenase activity is a collaborative function of rhizobia and legumes in the root nodules. Casella *et al.*, (1988) have reported that *Rhizobium leguminosarum* loses plasmids and symbiotic ability in the presence of Cd, Cu and Cr and acquire greater resistance to heavy metals, but, keep the option of reverting back to symbiotic relationships. Thus, the disappearance of symbiotic plasmids in the present research work is a direct indicator of metal toxicity and absence of nitrogen fixing efficacy of *Rhizobium* sp. strain RG-1.

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