Genetic modification strategies in Rhizobia to combat abiotic stress in legumes

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Abstract

PGPR is one of the most important tools used in the modern agriculture practice. PGPR can be used as biofertilizers as well as biocontrol agents. Rhizobium is an important PGPR, which is has been used as biofertilizer for legumes since last two decades. Besides biofertilizers it has been also employed as bioremediation agent, for remediation of heavy metal polluted soil. Rhizobium is an important PGPR which is capable of symbiosis with legumes for nitrogen fixation, so it can work in soil as well as plant. Any genetic modification in rhizobium bacteria would have an amplified effect due to its ability to form nodules in the legume roots. A single nodule could contain ab out 10^9 bacteria which is 100 times more than the entire rhizosphere of a single crop like fenugreek. Also, while living in non-symbiotic state, it can perform all the duties of any other PGPR. Because of this rhizobium especially is used in biofertilizer formulations for legumes.

Aim of our study was to check the effects of genetically modified rhizobium on the growth of fenugreek seedlings in Arsenic and Cadmium contaminated soil. *E. coli* DH10B *ybdK* gene, which encodes a carboxylate- amine ligase was cloned in a low copy number plasmid pBBR1MCS2 plasmid under a constitutive lac promoter, which yielded a 6.2 kb recombinant plasmid, pPAT. Since it is a carboxylate- amine ligase, it possesses the gamma glutamyl cysteine ligase activity. The *gshA* gee in bacteria encodes this enzyme which catalyses the first step in glutathione biosynthesis. Many studies have stated that the rhizobium devoid of *gshA* synthesizes very less glutathione, as well as loses the ability to form symbiotic nodules with legumes, but when complemented with *gshA* containing plasmid, it regains its ability to produce sufficient glutathione and symbiosis. Many studies have also reported the enhanced synthesis of glutathione by cloning *gshA* gene using plasmid. Similarly, our study also reported enhanced glutathione synthesis by GMO rhizobium compared to the wild type rhizobium. It was observed that M3 and M5 accumulated significantly higher levels of glutathione compared to M2 and M4 respectively. M2 and M4 are the wild type counterparts of M3 and M5 respectively. This was observed in rich as well as minimal media.

To determine their effect on fenugreek seedlings, seeds of fenugreek were coated with M1-M5 bacteria and sown in Arsenic and Cadmium spiked soil. M1 was used as a control for all experiments. Seedlings were allowed to grow for 16 days and its morphology, growth parameters, oxidative stress parameters and antioxidant enzyme profile was measured in order

to check the effect of glutathione overproducing rhizobium on the growth of fenugreek. It was observed that M3 and M5 treated seedlings showed enhanced growth parameters, reduced oxidative stress and reduced antioxidant enzyme levels in shoots as well as roots compared to M2 and M4 treated seedlings respectively. Glutathione reductase GR showed increase in GMO bacteria treated seedlings which justifies that more glutathione is present in the tissue, despite of low antioxidant enzyme levels, which means that the source of glutathione is external. This concludes that the GMO rhizobium exhibits protective effect towards fenugreek growing in Arsenic and Cadmium polluted soil.

Similar experiments were performed with the bacterial consortia. Fenugreek seeds coated with C2 (M1+M3+M5) and C1 (M1+M2+M4) consortium were grown in Arsenic and Cadmium contaminated soil for 25 days. C2 bearing M3 and M5 is a GMO consortium. It was observed that C2 treated seedlings showed increased growth parameters, reduced oxidative stress and reduced antioxidant enzyme levels. Rather the interplay was more complex as the crosstalk between different bacterial species and plants differ in consortia compared to single bacteria. In our conclusion this GMO consortium also exhibits protective effect towards fenugreek growing in Arsenic and Cadmium polluted soil compared to wild type consortium.

PGPR employs varieties of methods to capture/detoxify heavy metals. They are known to form intracellular as well as extracellular nanoparticles. It is also established that glutathione has the capability to synthesize and stabilize the cadmium sulphide nanoparticles. In our study the ability of GMO rhizobium was checked for the *invitro* formation of extracellular cadmium sulphide nanoparticles. We observed that M3 and M5 produced significantly higher amount of CdS NPs compared to M2 and M4 respectively, due to higher secretion of glutathione in the growth media. Also, the NPs synthesized by GMO rhizobium showed smaller aggregation compared to wildtype rhizobium, which was confirmed by SEM. FTIR was performed to get an idea about the functional groups of the molecules attached to the surface of nanoparticles, to confirm the presence of glutathione on them. Amide I band in the FTIR spectra of CdS NPs produced by M3 and M5 showed reduced transmittance of the concerned peak compared to M2 and M4 respectively. Finally, XRD analysis was done to confirm that the material synthesized by bacteria were nanoparticles. Biosynthesized CdS nanoparticles were in cubic phase which was confirmed by analysis of the prominent 2 theta peaks of diffractogram. Thus, GMO rhizobium are capable of biosynthesis of more extracellular CdS nanoparticles.

Therefore, clubbing the above observations, we can conclude that rhizobium has the ability to reduce the bioavailability of cadmium metal by converting it into nanoparticle aggregate. Both GMO as well as wild type of forming aggregates. But the only difference is that the GMO rhizobium are capable of formation of smaller aggregates, which was confirmed by SEM. Smaller aggregates have large surface to volume ratio which gives it better chance of detoxification by further modification by other PGPR present in the rhizosphere/vicinity. Thus, we conclude that GMO PGPR producing enhanced levels of glutathione could be used as a PGPR for fenugreek growing in Arsenic and Cadmium polluted soil.