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M. Darkazanli, I. C. Kiseleva

Ural Federal University
named after the first President of Russia B. N. Yeltsin,
620002, Russia, Yekaterinburg, Kuybeshiva St., 48,
mdarkazanli@urfu.ru

THE EFFECTS OF INOCULATION BEANS BY ENDOPHYTIC BACTERIA *METHYLOBACTERIUM* SP., AND *BACILLUS SUBTILIS**

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Food is the energy source for everyone around the world, we live in an age where we are growing and producing more food than ever before. Generally, the food price crisis has led to the assumptions that food price rises are due to inadequate food production and that such food insecurity is linked to seed insecurity [1]. One of the many technics which used to improve the quality of the seeds is by inoculating its by plant growth-promoting (PGP) bacteria. Some of these bacteria inhabit soil just in the rhizosphere, another – inside plants and seeds called endophytes. Endophytic bacteria have been isolated from many different plant species and almost being found in every plant worldwide [2].

Heavy metals (HM) and metalloids have become one of the major environmental concerns which pose a serious threat to plants, animal, and human health. In this context, endophytic bacteria could play an important role in understanding the HM uptake mechanism by plants and their resistance to HM [3].

In our study, we have tested *Bacillus subtilis* and *Methylobacterium* sp. ability to promote plant growth and to reduce heavy-metal toxicity. The preliminary sterilized bean seeds (by using 70 % ethanol for 1 min, 2 % sodium hypochlorite for 6 min, and 0.2 % mercuric chloride for time 1 min) were: inoculated with *B. subtilis* and *Methylobacterium* sp. culture (standard), at 7.23 log cfu/mL, prepared under sterile laboratory conditions, and irrigated with sterile water. Seeds were germinated for 9 days on filter paper. The primary barley leaf was used for the evaluation of leaf photosynthesis (CO_2 assimilation rate was recorded by LiCor 6400XT, 23 °C, PAR 1500 μmol/m^2*s); level of lipid peroxidation (LPO) in control conditions and under heavy metal stress with protocol (Health & Packer, 1997) [4].

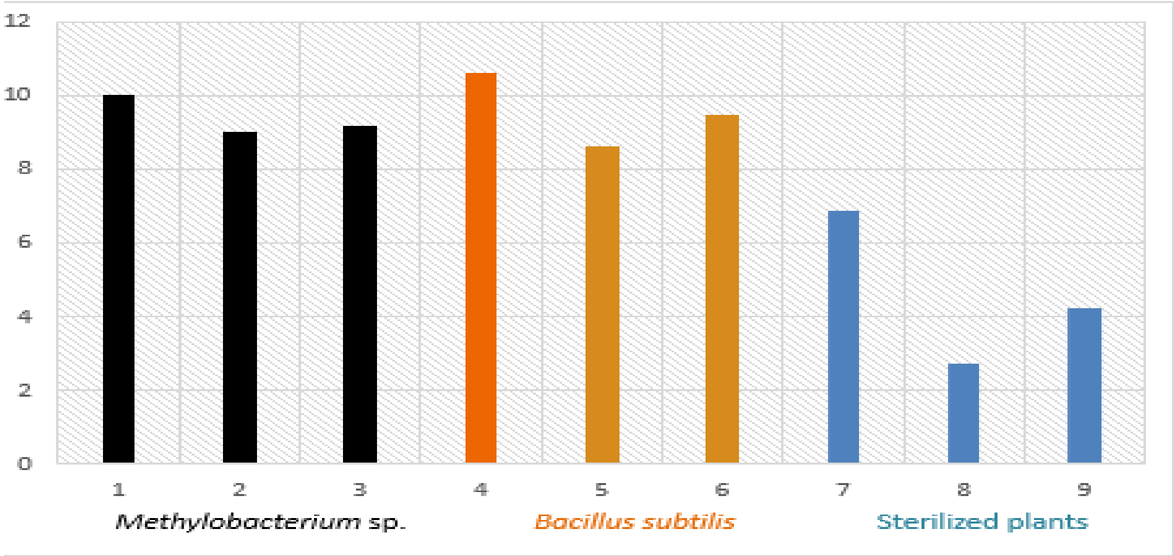

Figure 1. Photosynthesis (μmol $CO_2/m^{-2}{*}s^{-1}$) in inoculated and sterile plants

From fig. 1. the results have shown that the level of CO_2 assimilation rate was higher in inoculated plants compared to those grown from sterile seeds. The level of CO_2 assimilation rate was higher in seeds inoculated with *B. subtilis* than seeds inoculated with *Methylobacterium* sp.

The mean value CO_2 assimilation rate was 9.5±0.07 in inoculation plants with *B. subtilis* and was 9±0.05 in inoculation plants with *Methylobacterium* sp. Total chlorophyll content was higher in inoculated plants compaered with sterilized plants. Thus we suppose that bacteria have stimulated the photosynthetic function in beans.

Our results have also revealed that *Methylobacterium* sp. were able to promote beans resistance to HM stress.

The increase of the LPO level under heavy metal stress was shown in all plants, both inoculated by bacteria and sterilized compared to normal conditions, but sterilized plants were stressed higher then inoculated (fig. 2). So PGP bacteria allow plants to resist HM impact.

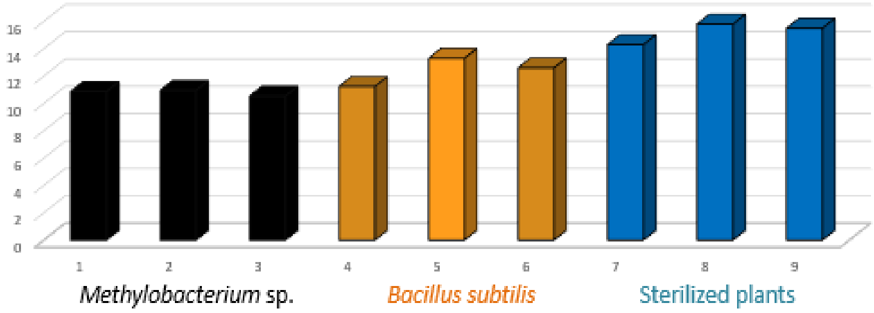

Figure 2. The rise of LPO level (%) under stress conditions (0.05M $CuSO_4$, 2 hours) in inoculated and sterilized plants

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Thus PGP bacteria can increase the growth and development of the plants either indirectly by reducing the toxic effects of metals or directly by producing the phytohormones [5]. Formerly it was shown that most of the studied endophytes revealed resistance to multiple HM [6] and our results agree with those studies. Another study in 2010 showed that the light response curves of beet showed that photosynthetic capacity was significantly increased in endophyte-infected plants. Promotion of photosynthetic capacity in sugar beet was due to increased chlorophyll content, leading to a consequent increased carbohydrate synthesis. It is possible that the increased maximum yield of photosynthesis in sugar beet was promoted by phytohormones produced by the bacteria [7].

In general, endophytic microorganisms are found in smaller quantities than rhizospheric bacteria or bacterial pathogens.

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