EFFECT OF DIFFERENT HEAVY METAL SALTS ON THE GROWTH OF PRE-SELECTED PGPR BACTERIAL STRAINS

Bálint Oldal[1], István Jevcsák[2], H.E.A.F. Bayoumi Hamuda[2], Mihály Kecskés[2]

[1]Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, Department of Soil Biology and Soil Biochemistry; [2]Szent István University, Agricultural, Environmental Microbiology and Soil Biotechnology Ph.D. Program and Environmental Protectoral Microbiological Research Group, Gödöllő–Budapest. E-mail: oldal@rissac.hu

Abstract

The effects of different heavy metal pollutants were studied on some selected bacteria isolated from Tisza River, its catch basin and the Westsik's long-term crop rotation experiment on sandy soil (Nyíregyháza, Hungary). Several representatives of bacteria (*Aeromonas*, *Arthrobacter*, *Acinetobacter, Cytophaga, Bacillus*, *Enterobacter*, *Escherichia, Klebsiella, Micrococcus, Paracoccus, Pseudomonas, Rhodococcus, Rahnella Stenofrophomonas*, *Streptomyces* and *Shewanella*) were identified. 13 strains were selected for further investigations, based on their plant growth promoting activity (OLDAL *et al.*, 2001).

Sensitivity tests against different heavy metal compounds were carried out with the use of the following chemicals at different concentrations: $ZnCl_2$, $Zn(NO_3)_2$, $CuCl_2$, $Cu(NO_3)_2$, $CuSO_4$, $Pb(NO_3)_2$, $(NH_4)_6Mo_7O_{24}$, $Fe^{(III)}Cl_3$, $Fe^{(II)}SO_4$, $Fe^{(III)}(NO_3)_3$. Among the investigated plant growth promoting bacteria, 7 strains proved to have a significant resistance against the heavy metal compounds compared to the other strains: *Aeromonas media* W.1, *Pseudomonas veronii* W.7, *Pasteurella* sp. W.30, *Pseudomonas* sp. W.36, *Bacillus thüringiensis* C.69, and III.118, III.119; those total mean sensitivity did not show a statistically reliable difference. The inhibitory effect of heavy metal compounds on the bacterial growth is further displayed.

Introduction

The groundwater contamination of Hungarian sandy soil lands requires an immediate intervention. An agricultural solution for the sustainable development is the low-input farming – which keeps fertilizer use as meagre, as possible –, e.g. Westsik's crop rotation experiment for sandy soil melioration (launched by Vilmos Westsik, 1929, Nyíregyháza, Hungary – now managed by University of Debrecen, Research Branch of Agricultural Scientific Centre, Nyíregyháza, Hungary).

Diazotrophic bacteria living in the plant rhizosphere can promote the biomass production of plants and stand for fertilizers first of all by fixing molecular nitrogen from the air. Diazotrophic bacteria however can help the plant growth not only by improving plant nutrition but through direct plant growth promoting (PGPR) effect. Physical and chemical characteristics of the rhizosphere environment are determined by the interaction of soils, plants, and organisms associated with the root, including bacteria, fungi, protozoa, and nematodes (ELLIOT et al., 1984; YANG & CROWLEY, 2000). Root exudates are providing the micro-organisms with the necessary nutrients; rhizobacteria on the other hand can improve the crop yields (FEDI et al., 1997; BUYSENS et al., 1999). Some rhizobacteria, which are commonly called as plant growth-promoting rhizobacteria (PGPR), can interact with plant

roots and can protect the roots against the pathogenic micro-organisms (KLOEPPER & SCHROT, 1978; LIGON et al., 2000). There are several mechanisms behind this beneficial behavior, such as the siderophore production, the fast-growing ability or the release of some antibiotic compounds. The PGPR effect therefore is the result of a complex interaction (BIRÓ et al., 1998). Under temperate climatic circumstances *Pseudomonads* are common and ubiquitous members of the rhizobacterial micro biota in the soils. These beneficial effects develop either as the suppression of diseases and deleterious effects caused by the soil-borne pathogens (WELLER, 1988; COOK,1993); or as a better growth and fitness due to the secondary metabolite production.

Fluorescent-putida type pseudomonades therefore are the most commonly studied bio-pesticides (SCHWYN & NEILANDS, 1987). Such beneficial bacteria – mainly belonging to *Pseudomonas* genus – which are resistant against natural and man-made ecological factors can play a well-founded role among the strains selected for plant inoculation.

Materials and Methods

43 Gram-negative and Gram-positive, aerobic, non-oligotroph rod-shaped bacterial strains were isolated from the **1/** rhizosphere and rhizoplane of lupine and vetch plants (26 strains, only *Pseudomonas*) grown in the Westsik's long-term crop rotation experiment on sandy soil (Nyíregyháza, Hungary), and from the **2a/** soil of flooded area (8 strains), **2b/** soil of river bank (9 strains) and **3/** surface water of the Upper-Tisza river (Hungary) at the following sampling places: Tivadar, Aranyosapáti, Záhony, Balsa (towns/villages along the river, in the order of North to South). Among them some places were free from pollutant chemicals, while the others were over-polluted.

Table 1
Code and origin of the investigated *Pseudomonas* sp. strains from Westsik's long term crop rotation experiment

Pseudomonas sp. types	Treatments in the Westsik experiment*			
	I. uncultivated	IV. straw (26.1 t/ha/3 year)	VIII. green manure (N.D.)	IX. farm-yard manure (26.1 t/ha/3 year)
P. aeruginosa	A10; AX	A5/2; A35/2; A20	A6; A9; A34	A16; A23/1; A28a; A30/2; A36
Fluorescens-putida type pseudomonades	F1; F2	F4; F8; F12	F15; F22; F38	F41; F44; F47; D65; D80

*Treatments were enriched with inorganic fertilisers, as follows: I = 32.5 kg·ha^{-1} N, 25 kg·ha^{-1} P and 20.5 kg·ha^{-1} K; IV = 50 kg·ha^{-1} N, 50 kg·ha^{-1} P and 16.2 kg·ha^{-1} K; VIII = 50 kg·ha^{-1} P and 16.2 kg·ha^{-1} K; IX = 50 kg·ha^{-1} N. N.D. = not determined

From the soil- and water samples different bacterial isolates were collected by using Ashby, King's B, Nutrient and Endo agar media with a simplified method (ANGERER *et al.*, 1998), for achieving the largest possible diversity of bacteria, which were identified by 16S-rDNA method (ALTSCHUL *et al.,* 1997), and the conventionally used BIOLOG identification assay (BOCHNER, 1989). Table 1 shows the list of investigated *Pseudomonas* strains from Westsik's experiment used for further tests; Table 2 shows the identified isolates among the investigated bacterial strains, which are the type representatives of each sampling places along the Tisza River.

Table 2
List of investigated strains, which were used for further tests (Note: pseudomonads were present at each of sampling places – see **bold-faced text**)

Place of origin and distance from the pollution	Species	Strain code
Tivadar	*Enterobacter agglomerans*	B 12
	Pseudomonas gassardi	B 83
	Pseudomonas marginalis	B 136
Aranyosapáti	*Acinetobacter johnsonii*	A 14
	Aeromonas hydrophila	A 106
	Rahnella aquatilis	A 211
	Acinetobacter johnsonii	A 212
	Klebsiella sp.	A 217
	Pseudomonas synxantha	A 308
	Aeromonas veronii	IV 313
Záhony	***Pseudomonas veronii***	D 11
	Pseudomonas fluorescens	D 65
Balsa	***Pseudomonas marginalis***	C 25
	Bacillus cereus	C 59
	Bacillus thüringiensis	C 69
	Pseudomonas sp.	C 115

In the first step the obtained individual strains were tested for plant growth promoting effect using the LETHAM's method (LETHAM, 1968) with a slightly modification. Based on their plant growth promoting activity (OLDAL *et al.,* 2001), 13 strains were selected for further investigations, as follows in a decreasing order: W.35, *Pseudomonas veronii* W.7, *Pasteurella* sp. W.30, *Pseudomonas gassardi* B.83, *Pseudomonas* sp. C.115, *Pseudomonas syringae* I.110, *Aeromonas media* W.1, *Pseudomonas veronii* D.111, III.119, VI.404, *Bacillus thüringiensis* C.69, III.108, *Pseudomonas* sp. W.36.

Sensitivity tests against different heavy metal pollutants were carried out by using the following chemicals at different concentrations: $ZnCl_2$, $Zn(NO_3)_2$, $CuCl_2$, $Cu(NO_3)_2$, $CuSO_4$, $Pb(NO_3)_2$, $(NH_4)_6Mo_7O_{24}$, $Fe^{(III)}Cl_3$, $Fe^{(II)}SO_4$, $Fe^{(III)}(NO_3)_3$; 50, 100, 200, 400, 800 and 1600 μM concentration scale for each compound. The effects of the compounds on the growth of bacteria were measured by spectral photometry (560 nm) after 24 hours of incubation at 28 ^oC in Nutrient broth. The sensitivity of strains is expressed in growth rate percentages compared to the non-treated control. Statistical analyses were made by Statgraphics 5.1 software (P5%).

Results

Among the bacteria investigated, *Aeromonas*, *Arthrobacter*, *Acinetobacter, Cytophaga, Bacillus*, *Enterobacter*, *Escherichia, Klebsiella, Micrococcus, Paracoccus, Pseudomonas, Rhodococcus, Rahnella Stenofrophomonas*, *Streptomyces* and *Shewanella* strains were identified by using BIOLOG and 16S-rDNA methodologies.

From the Westsik's experiment only the *Pseudomonas* sp. strains were kept for further cultivation. Regarding the Tisza River, *Pseudomonas* strains occurred at all of the sampling places, and the most numerous representatives were the *Pseudomonas, Aeromonas, Acinetobacter* and *Bacillus* species. According to the microbial isolates, Aranyosapáti city showed the highest value of microbial biomass and diversity.

Among the investigated plant growth promoting bacteria, 7 strains proved to have a significant resistance against the heavy metal compounds compared to the other strains: *Aeromonas media* W.1, *Pseudomonas veronii* W.7, *Pasteurella* sp. W.30, *Pseudomonas* sp. W.36, *Bacillus thüringiensis* C.69, and III.118, III.119; those total mean sensitivity did not show a statistically reliable difference. The inhibitory effect of heavy metal compounds on the bacterial growth is the following (in a decreasing order): $(NH_4)_6Mo_7O_{24}$, $CuSO_4$, $ZnCl_2$, $Cu(NO_3)_2$, $Zn(NO_3)_2$, $CuCl_2$, $Pb(NO_3)_2$, $Fe^{(II)}SO_4$, $Fe^{(III)}Cl_3$, $Fe^{(III)}(NO_3)_3$.

Heavy metal tolerance of the selected bacterial strains is shown through the investigational results of 7 representative strains affected by 9 heavy metal compounds (Figures 1–9; see next page). Bars in the figures are indicating the value of significant difference ($SD_{P5\%}$).

Discussion

In the present study the species composition of the river Tisza (at some remarkable polluted sites) and Westisk's long term crop rotation experiment on sandy soil (Nyíregyháza, Hungary) was established by some physiological and genetical studies (BIOLOG, 16S-rDNA methodologies). *Pseudomonas, Aeromonas, Acinetobacter* and *Bacillus* strains as well as representatives from other genera (*Arthrobacter, Enterobacter, Klebsiella, Micrococcus, Paracoccus, Streptomyces, Shewanella, Stenotrophomonas* – this genus became separated from *Pseudomonas* group recently) occur in the river of upper Tisza and soils of its banks and flooded area.

Regarding the metallic chemicals, compounds of lead had a weaker inhibition of strain growth than the compounds of copper and zinc, thus it is probable that the investigated bacteria may be adapted to the environmental pollution level of their site of origin, tolerating a higher lead stress. In the case of ironic compounds, the "microelement-effect" should be supposed, accordingly as the tested strains can use the iron in their metabolism, so the measure of inhibitory effect seems to be determined better by the anions dissociated in the electrolyte solution. The ecological study of bacterial micro-biota of Tisza River and related agricultural land fields has an essential importance; so by the selection of soil-borne beneficial bacteria directed to field use (seed/plant inoculation) both theoretically and

practically can prevent problems of environmental pollution, which have adverse effects on human health, such as accumulation of toxic chemicals, heavy metal compounds and high amounts of inorganic fertilizers reaching the groundwater.

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Figure 1

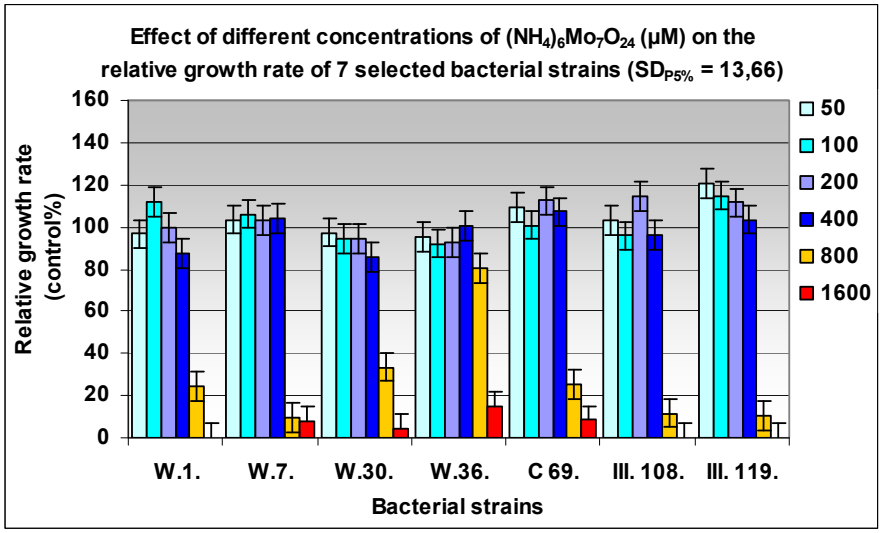
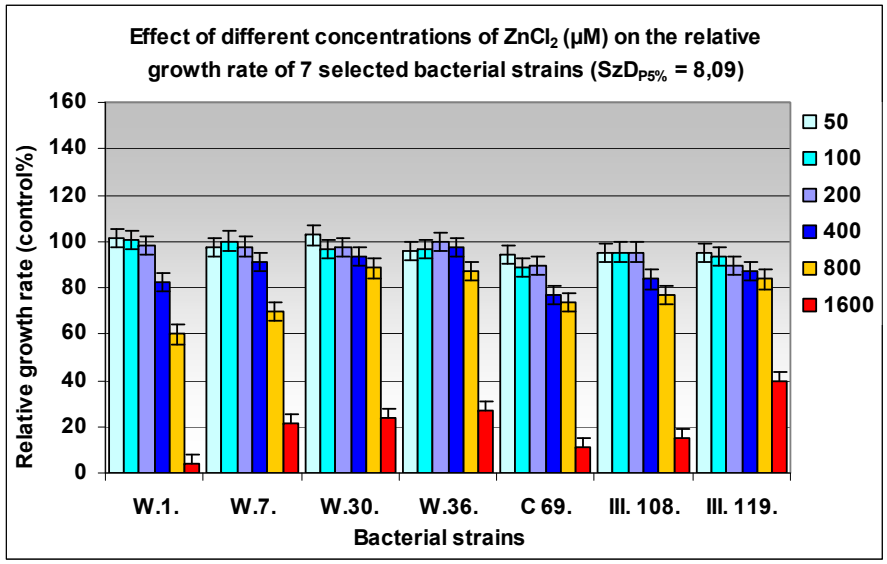


Figure 2



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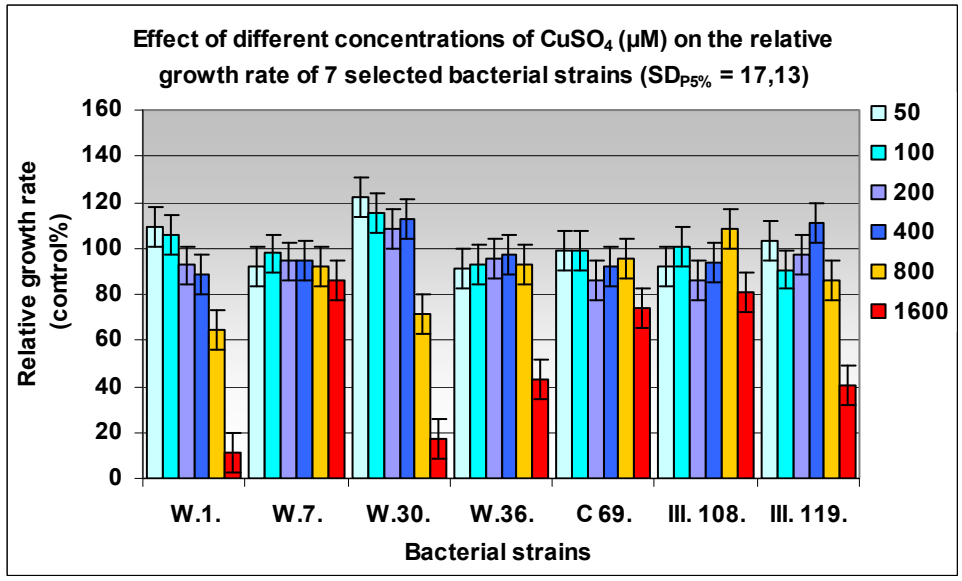


Figure 4

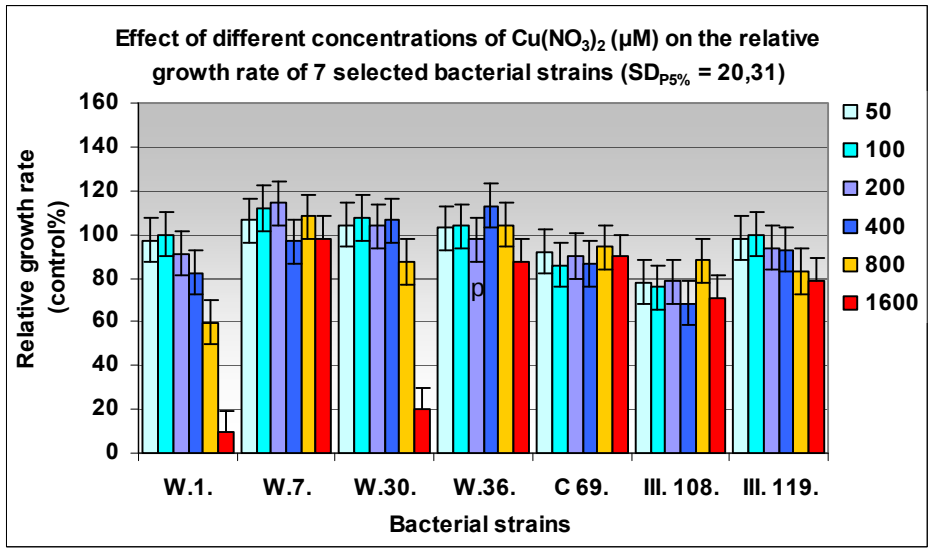
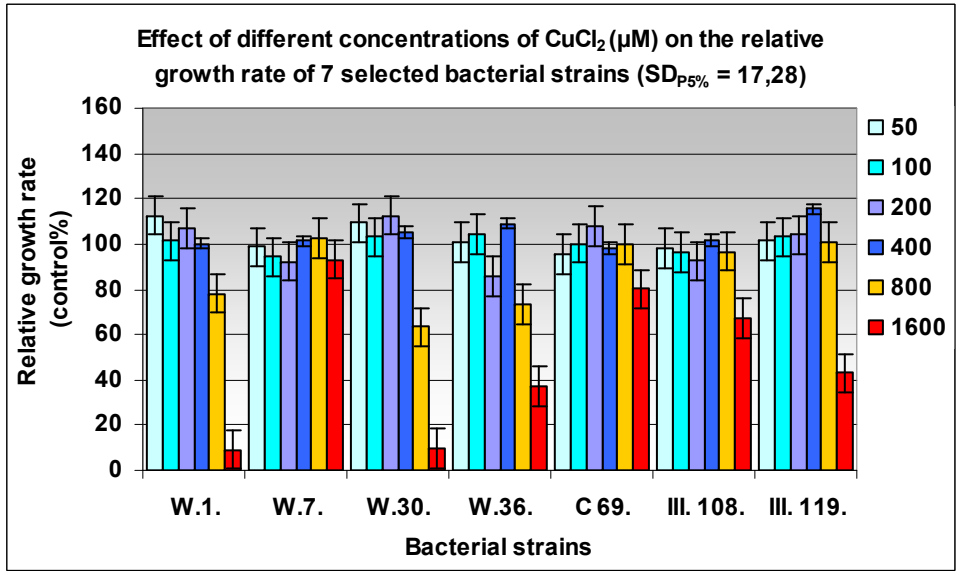


Figure 5



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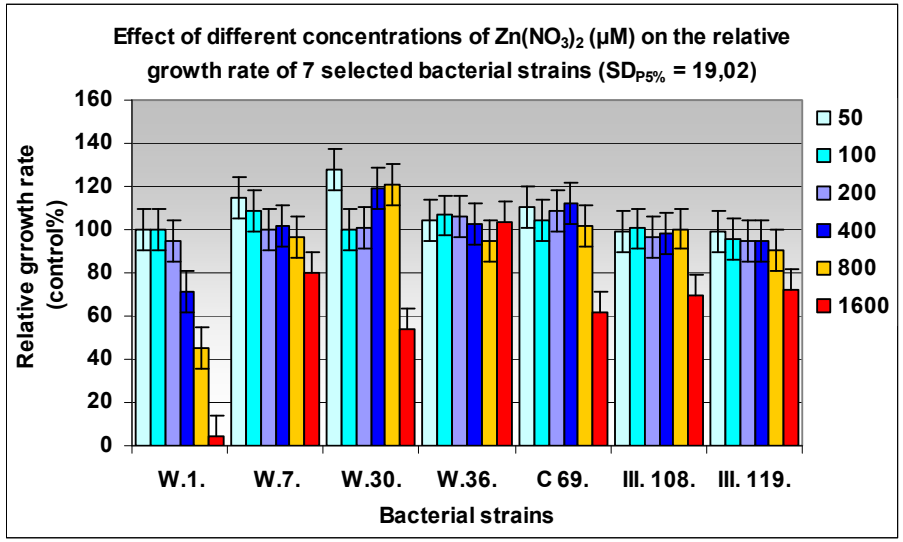

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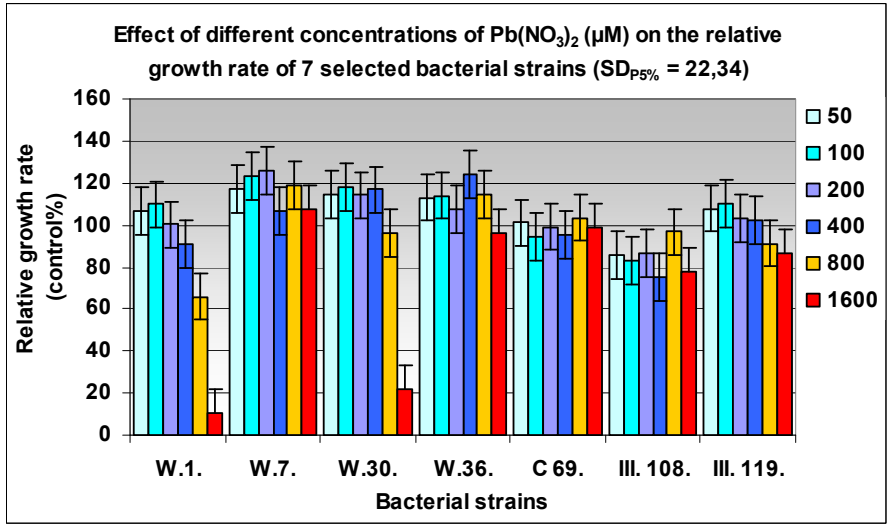

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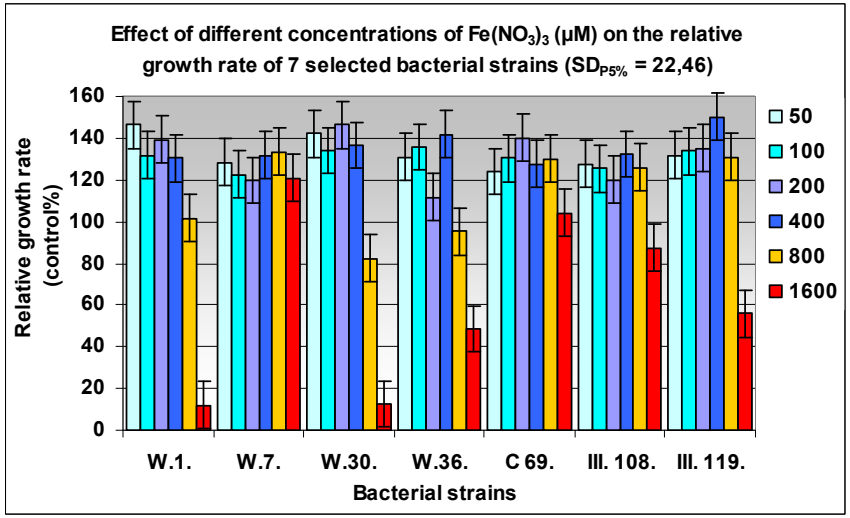

Figure 9