Session 8. METAL IONS AND MICROORGANISMS

INVOLVEMENT OF THE BACTERIUM *AZOSPIRILLUM BRASILENSE* IN WHEAT TOLERANCE TO CADMIUM

[1] N.V. Bezverkhova, [2] V.I. Safronova, [1] L.P. Antonyuk, [2] A.A. Belimov

[1]Laboratory of Biochemistry of Plant-Bacterial Symbioses, Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov 410015, Russia; [2]Laboratory of Ecology of Symbiotic and Associative Rhizobacteria, All-Russia Research Institute for Agricultural Microbiology, Saint Petersburg 196608, Russia.

Cadmium is known to cause severe inhibition of plant growth. Some plant growth-promoting rhizobacteria were reported to be capable of decreasing partially the toxicity of Cd for barley. We studied the ability of the rhizobacteria *Azospirillum brasilense,* strains Sp7 and Sp245, to reduce Cd toxicity for wheat in hydroponic culture. Since one of the wheat lectins (wheat germ agglutinin, WGA) is involved in plant response to some kinds of environmental stress and is also essential for plant-bacterial interactions, the second aim of this work was to elucidate whether Cd stress could induce synthesis of WGA in wheat plants. The capacity of *A. brasilense* to decrease Cd toxicity for wheat was found to depend on the Cd concentration and on the wheat variety. Thus, when the $CdCl_2$ concentration in the plant growth medium was 2 mg/l, both strains diminished Cd toxicity for the wheat cultivar Saratovskaya 52; however, they gave no effect with the other three varieties used. At 8 mg/l $CdCl_2$, the inhibition of plant growth was significant (by 82–85%), and inoculation with bacteria did not decrease the toxicity of Cd. It was found that the presence of Cd in the plant growth medium caused an increase in the concentration of WGA in wheat roots. The enhanced level of WGA was retained during the whole period of plant cultivation with cadmium. The results obtained suggest that *A. brasilense* is capable of decreasing partially the toxicity of Cd for wheat, and WGA is probably involved in plant response to cadmium stress.

SENSITIVITY OF BACTERIAL BIOFILMS SETTLED ON COPPER TUBING TO TOXIC SHOCKS INDUCED BY COPPER IONS, WITH OR WITHOUT PREVIOUS EXPOSURE

[2] L.F. Jacquelin, [3] L.A. Khassanova, [2] M.P. Gelle, [1] C. Choisy

[1] Laboratoire d'Adhйsion Bactйrienne,UFR de Pharmacie, 51 rue Cognacq-Jay,51096 Reims Cedex, France; [2]UFR d'Odontologie, rue Gйnйral Koenig, 51100 Reims, France; [3] Bashkir State University, 32 Frunze Street, 450072 Ufa, Russia.

Antiseptic properties of copper have been known for centuries. Commitees dedicated to fight nosocomial infections recommend the use of copper pipes to avoid contaminations of hospital water-supplies. Whether this approach can be considered an efficient protection against bacterial multiplication in terminal portions of pipes remains unknown to date. Moreover, maintenance work using acids on these copper tubes, may free large amounts of copper ions. It is also possible that water acidity allows an almost permanent liberation of these ions, possibly leading to the selection of bacteria poorly sensitive to the effects of Cu^{++}.

To study the efficacy of Cu^{++}, we compared :

1. The attachment of biofilms on Tygon ^{R} (PVC) and on copper tubing. Monospecific biofilms were made with *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and a slime^{+} Negative Coagulase *Staphylococcus* (NCS).
2. The behaviour of these biofilms with and without previous exposure to copper ions in conditions of high and low toxic shock (150 and 50 mmoles).

Proliferation of these four species in the biofilms was sommewhat lower on copper than on Tygon^{R} tubings. *E. coli, Staphylococcus aureus* and NCS were sensitive to high toxic shocks, and previous exposition to copper ions sensitized them. Conversely, *Pseudomonas aeruginosa* biofilms, although less thick on copper than on Tygon tubing resisted better to toxic shocks, with or withour previous exposition to Cu^{++}.

BIOSORPTION OF METAL IONS BY MICROORGANISMS AND THEIRS CONSORTIA WITH AQUEOUS PLANTS

I.N. Gogotov, N.A. Zorin, K.G. Tikhonov

Institute of Basic Biological Problems RAS, Pushchino, 142290, Russia.

Phototrophic and chemotrophic microorganisms of different taxonomic groups and their consortia with water plants (aquatic fern *Azolla*, water hyacinth) are capable to accumulate metal ions of Ni, Pt, Ru, Cu, Cr,

Pb, Zn, Si and Au. This ability enables to use them for purification of agricultural and industrial waste water from toxic heavy metals and to obtain rare trace metals. For this purpose it is possible to use growing cultures and immobilized cells, their biopolymers or enzymes (hydrogenase). Purple non-sulfur bacteria (*Rhodobacter* spp., *Rhodopseudomonas* spp.) are able to accumulate Cu, Zn, Ni and Hg., showing various resistance to these metals. Biopolymer of purple sulfur bacterium *Ectothiorhodospira shaposhnikovii* accumulated more than 99 % Cu and Zn from diluted waste water of electrolysis. Green algae *Chlorella* spp. and water plants *Azolla*, duckweed and water hyacinth showed higher ability to a biosorption of metal ioins. Cyanobacteria (*Spirulina platensis*) and plants (amaranthus) may be recommend as producers of homeopathic drugs containing necessary metals (Se, Zn, Ni etc.) for microelemental diseases.

SPECTROSCOPIC ASPECTS IN STUDYING THE IMPACT OF HEAVY METALS ON RHIZOBACTERIA AND THEIR ROLE IN BACTERIAL METABOLISM

[1] A.A. Kamnev, [1] L.P. Antonyuk, [2] P.A. Tarantilis, [2] M.G. Polissiou, [3] L.A. Kulikov, [3] Y.D. Perfiliev

[1] Laboratory of Biochemistry of Plant-Bacterial Symbioses, Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, 13, Prosp. Entuziastov, 410015 Saratov, Russia; [2] Laboratory of Chemistry, Department of Science, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece; [3] Laboratory of Nuclear Chemistry Techniques, Department of Radiochemistry, Faculty of Chemistry, Moscow State University, 119899 Moscow, Russia.

Modern spectroscopic techniques are highly useful in studying diverse processes in bacterial cells related to their interactions with metal ions. Spectroscopic data for whole cells, supramolecular structures or isolated cellular constituents can reflect structural and compositional changes occurring in the course of metabolic responses induced by metal ions. This information on the molecular level is of importance for basic studies on mechanisms of bacterial tolerance to heavy metals and their impact on bacterial metabolism, as well as for applied multidisciplinary research in the fields related to biotechnology, bioremediation, agriculture, biogeochemistry, *etc*. In the present work, some examples are presented which illustrate the application of different spectroscopic techniques for monitoring metal-induced metabolic changes in soil bacteria, as well as for obtaining structural information for metal-activated bacterial enzyme. Thus, the effects of a range of heavy metals on the plant growth-promoting rhizobacterium *Azospirillum brasilense* are discussed as revealed using vibrational spectroscopy of whole cells and isolated cell walls. In particular, Fourier transform infrared (FTIR) spectroscopy provides information on the overall structure and state of functional groups, their involvement in metal binding and/or hydration. As its counterpart, FT-Raman spectroscopy gives complementary data on the state of less or non-polar functional groups. Emission (^{57}Co) Mцssbauer spectroscopy (EMS) can be used to monitor binding of cobalt(II) by bacterial cells at its trace (physiological) concentrations and its further metabolic transformations. These studies were performed in the presence of radioactive $^{57}Co^{II}$ in suspensions of *A. brasilense* cells rapidly frozen prior to measurements. Also, it has been shown for the first time how EMS can be used to probe the active sites of $^{57}Co^{II}$-activated enzyme. This is demonstrated using an example of glutamine synthetase, a key enzyme of nitrogen metabolism, isolated from *A. brasilense*, activated by $^{57}Co^{II}$.

EFFECTS OF HEAVY METALS ON THE PLANT-ASSOCIATED BACTERIUM *AZOSPIRILLUM BRASILENSE*: ENDOPHYTIC AND NON-ENDOPHYTIC STRAINS

[1] A.V. Tugarova, [1] A.A. Kamnev, [1] L.P. Antonyuk, [2] P.A. Tarantilis, [2] M.G. Polissiou

[1] Laboratory of Biochemistry of Plant-Bacterial Symbioses, Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, 13 Prosp. Entuziastov, 410015 Saratov, Russia; [2] Laboratory of Chemistry, Department of Science, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece.

The plant-associated soil bacterium *Azospirillum brasilense* attracts worldwide attention owing to its plant growth-promoting activities. Among hundreds of its strains known up to date, wild-type strain Sp245 is capable of colonising the root interior (i.e. facultative endophyte), whereas others are non-endophytes colonising the root surface only. Thus, the different ecological niches occupied by these strains suggest that their responses to environmental conditions might differ as well. In this study, we compared the metabolic responses of *A. brasilense* strains Sp245 and Sp7 to several heavy metal cations (Co, Cu, Zn) present in the medium in tolerable concentrations (up to 0.2 mM) taken up by the bacteria. Structural features of whole cells were studied using vibrational (Fourier transform infrared (FTIR) and FT-Raman) spectroscopic techniques. The results obtained show that all the heavy metals studied are significantly accumulated in the cells, albeit up to differ-

ent levels. In strain Sp7, the heavy metals induced noticeable metabolic responses revealed in vibrational spectra of whole cells, consisting in an enhanced accumulation of polyester compounds, as well as some effects on the state of certain functional groups. In contrast, the response of the endophytic strain Sp245 to heavy metal uptake was found to be much less pronounced. These dissimilarities in their behaviour may be caused by different adaptation abilities of the strains to stress conditions owing to their different ecological status.

REDOX REACTION OF BACTERIAL HYDROGENASE WITH METALS AND METAL IONS

N.A. Zorin, O.A. Zadvorny, I.N. Gogotov

Institute of Basic Biological Problems Russian Academy of Sciences, Pushchino, 142290, Russia.

Hydrogenase from phototrophic bacterium *Thiocapsa roseopersicina* accelerated anaerobic oxidation of some metals accompanied by hydrogen production. The most intensive enzymatic hydrogen production was observed in the presence of metallic aluminum or zinc in the system containing hydrogenase and methyl viologen as electron mediator between metal and enzyme. The hydrogen production from metallic nickel in such system occurred at low rate at pH below 5.0. At pH above 7.0 hydrogenase catalyzed the reverse reaction of Ni^{2+}reduction to metallic nickel in H_2 atmosphere. The direct electron transfer between metal and hydrogenase was demonstrated in the system containing metallic cadmium. The rate of non-mediator H_2 production from Cd^o reached 35% from maximal hydrogen production in the presence of methyl viologen. In control without enzyme H_2 production from Cd^o did not proceed independently of the presence of methyl viologen. The rate of metal oxidation decreased in the range of Al>Zn> Fe>Cd>Ni in accordance with redox potential of Me/Me^{2+} couple and depended on pH. The rate of H_2 evolution during oxidation of Cd^o in the system containing hydrogenase and methyl viologen at pH 4.0 was 5 times higher as that at pH 7.0.

The ability of hydrogenase for reduction of some metal ions is low due to the inhibition of enzyme activity. Inhibitory effect of Ni^{2+} and Cd^{2+} on the catalytic activity of this enzyme was reversible and competitive with respect to methyl viologen in the reaction of hydrogen oxidation. The affinity of these metal ions to the enzyme significantly increased with an increase of pH that indicated an electrostatic character of their interactions. Cu^{2+} and Hg^{2+} inhibited hydrogenase activity irreversibly and uncompetitively with respect to methyl viologen. In contrast to Ni^{2+} and Cd^{2+} ions which did not effect on process of activation, ions of Cu^{2+} and Hg^2 inhibited transfer to active state of hydrogenase from inactive form. The optical density of hydrogenase at 400 nm decreased in the presence of these metal ions suggesting the destruction of FeS cluster in the enzyme. This results indicate about two possible mechanisms of hydrogenase inhibition by metal ions.

APPLICATION OF ASSOCIATIVE BACTERIA FOR INOCULATION OF BARLEY GROWN IN SOIL CONTAMINATED WITH CD AND PB

[1] A.P. Kozhemiakov, [1] A.A. Belimov, [1] A.M. Kunakova, [1] V.I. Safronova, [2] A.Y. Alekseev, [3] V.V. Stepanok, [3] L.Y. Yudkin

[1] All-Russia Research Institute for Agricultural Microbiology (ARRIAM), 3, Podbelskogo Sh., Pushkin, 196608 Saint Petersburg; [2] Agrophysical Research Institute, 9 Gusarskaya, Pushkin, 196603 Saint Petersburg, Russia; [3] Research Institute for Agricultural Use of Ameliorated Lands P.O. Emaus, 171330 Tver, Russia.

Biopreparations based on plant growth-promoting rhizobacteria *Azospirillum lipoferum* 137, *Arthrobacter mysorens* 7, *Agrobacterium radiobacter* 10 and *Flavobacterium* sp. L30 are developed at the ARRIAM for increasing productivity of various agricultural crops. The aim of the present work was to investigate how application of these biopreparations affect plant growth and uptake of elements from soil contaminated with Pb and Cd. The studied strains showed a high tolerance to Pb and Cd *in vitro*. The sole exception was that the strain *Flavobacterium* sp. L30 was very sensitive to Cd. The Cd tolerant strains were capable of immobilizing Cd from the nutrient medium supplemented with this toxic metal The strains tolerant to Pb and/or Cd colonized actively the roots of barley cultivated in soil supplemented with 500 mg Pb kg^{-1} or with 75 mg Cd kg^{-1} in pot experiments. *Flavobacterium* sp. L30 showed weak survival on barley roots in Cd contaminated soil. Inoculation with bacteria stimulated plant growth and decreased Cd content in shoots at toxic Cd concentrations. In field trials, treatments of barley seeds with biopreparations had no effect or decreased the content of Pb and Cd in grain when the plants were grown in soil supplemented with these heavy metals. A beneficial effect of bacteria on barley was associated with an increase in the uptake of nutrient elements such as P, K, S and Ca by the inoculated plants. The results showed that biopreparations could be successfully applied for inoculation of barley cultivated in the presence of increased concentrations of heavy metals in soil.

DYNAMICS OF RECEIPT POTASSIUM AND RADIOCAESIUM IN PLANTS OF THE POTATO AT USE OF BACTERIA *KLEBSIELLA MOBILIS*

[1] V.O. Krasnyak, [2] A.A. Belimov, [3] V.F. Drichko

[1] Saint-Petersburg State Agrarian University, 2, Petersburg highway, 189620 Pushkin, Russia; [2] All-Russia scientific research institute of agricultural microbiology, 3, highway Podbel'skogo, 189620 Pushkin, Russia; [3] Agrophisics scientific research institute, 9, Hussar street, 189620 Pushkin, Russia.

At development of plants, in root zone layer of ground a variety of microorganisms is formed. Researches have shown, that some bacteria live in symbiosis with plants and render the certain influences on plants. It was noticed, that on growth and development of plants of a potato render positive influence of bacterium *Klebsiella mobilis.*

For revealing influence strains bacteria Klebsiella Mobilis on receipt of radiocaesium in plants of a potato vegetative test was incorporated.

Test was incorporated 9.06.2001 in vegetative vessels in volume 2 liters, on sandy soil. In test it was used early potato of grade "Pushkinets".

The circuit of test: 1. NPK; 2. NPK + bacteria. Each variant was carried out in 3 replicated on 3 time points.

In test it was used ^{134}Cs which was in regular intervals brought in ground two years prior to statement of the given test. Activity ^{134}Cs at the moment of realization of test made 8,2 Bk/g grounds.

Fertilizers were brought in ground as ecofoska and sulfate potassium, proceeding from recommended dozes under a potato for vegetative experience: N — 0.12 g/kg, P_2O_5 — 0.20 g/kg, K_2O — 0.28 g/kg.

Samples of plants selected: 2.07, 17.07 and 24.07. Duration of test — 45 days the First selection was carried out in 23 days after planting (for 12 day, after occurrence of shoots).

As a result of the carried out test the following conclusions were made:

1. Bacteria *Klebsiella mobilis* rendered positive influence on plants of a potato in an initial stage of development of plants. In variants with application of a bacterial preparation at the initial stages the root system and an elevated part of plants more intensively developed. At the further development of plants, action of a preparation is not observed.
2. The preparation renders weak (statistically doubtful) influence on increase of accumulation potassium and radiocaesium plants of a potato (a difference between variants less then least essential difference).
3. Potassium, caesium and weight plants of a potato within 45 day after planting it is possible to describe accumulation by rectilinear dependence with the identical tendency of growth. It concerns to an elevated part, roots and tubers.

COPPER TOXICITY FOR *ESCHERICHIA COLI* K-12 PLASMA MEMBRANE IN VITAMIN C PRESENCE

[1] A. Yu. Ivanov, [2, 6] L.A. Khassanova, [3, 6] Z. M. Khassanova, [4] A. V. Gavryushkin, [3] L.Yu. Markeliya, [1] E.E. Fesenko, [5] C. Choisy, [6] J.-C. Etienne, [6, 7] Ph. Collery

[1] Institute of Cell Biophysics, Russian Academy of Sciences, 142290 Pushchino, Russia; [2] Department of Environmental Protection, Bashkir State University, 32, Frunze Street, 450072, Ufa, Russia; [3] Department of Botany, Bashkir State Pedagogical University, 3a, October Revolution Street, 450000, Ufa, Russia; [4] State Research Institute of Applied Microbiology, 142279 Obolenck, Russia; [5] Laboratoire d'Adhйsion Bactйrienne, l'Universitй de Reims, 51, rue Cognacq-Jay, 51096 Reims, France; [6] Institut International de Recherche sur les Ions Mйtalliques 51687 Reims, France; [7] Service de Cancerologie, Polyclinique Maymard 20200 Bastia, France.

The influence of copper ions in the presence of vitamin C (ascorbate) on the plasma membrane (PM) of *Escherichia coli* K-12 cells was studied by the method of electroorientational spectroscopy. Disturbances in the barrier properties of PM were found to occur in the presence of a catalytic copper-ascorbate system. Addition of H_2O_2 to this system increased the degree of cell damage although the introduction of hydrogen peroxide to intact cells and cells preincubated with copper ions did not produce any change in membrane permeability. The inhibition of endogenic catalase by cell preincubation with sodium azide did not affect the degree of PM damage. The metal chelating agent EDTA suppressed completely the cell damage, whereas the well-known scavenger of OH^* radicals dimethyl sulfoxide, did not show protective properties under those conditions. When copper ions in the binary catalytic system were replaced with a large organic cobalt complex, hydroxycobalamine, ROS did not show the toxic effect for PM. It is suggested that the toxic effect of copper ions observed in the presence of ascorbate is provided by hydroxyl radicals generated by the catalytic system on the surface of cell PM, whereas the bulky hydroxycobalamine complex cannot penetrate the cell wall.

EFFECT OF FUMONISIN B_1 ON NA-A ZEOLITE EFFECTIVENESS TO ABSORB AFLATOXIN B_1

[1] C. Magnoli, [2] S. Chiacchiera, [1] A. Dalcero, [3] R. Miazzo, [4] A. Kikot, [4] E.I. Basaldella

[1] Dpto. de Microbiologna e Inmunologna; [2] Dpto. de Quнmica y Fнsica, Fac. de Ciencias Exactas, Fнsico Quнmicas y Naturales; [3] Dpto de Producciyn Animal, Fac. de Agronomна y Veterinaria. Universidad Nacional de Rнo Cuarto, AP#3(5800), Rнo Cuarto,Cyrdoba; [4] Centro de Investigaciyn y Desarrollo en Procesos Catalнticos CONICET-CIC-UNLP, Calle 47 N°257, (1900) La Plata, Argentina.

Previous works showed that mycotoxins: fumonisins and aflatoxins B_1 (AFB_1) found in poultry feeding act as co-contaminants in a high number of samples. One of the most economical and practical methods to prevent mycotoxicity in poultry rations is adding adsorbent substances to reduce the bioavailability of toxins in the gastrointestinal tract of the animals. *In vivo* and *in vitro* studies showed the efficacy of synthetic zeolites Na-A (metal aluminosilicates) to prevent aflatoxicosis. Physicochemical studies *in vitro* demonstrated that fumonisin B_1 (FB_1) and AFB_1 are markedly adsorbed at pH2 and 39°C. AFB_1 absorption is negligible at pHs 4 and 6, while FB_1 is absorbed at pH 4 but not at pH 6. The objective of the present work was to determine the magnitude in which co-contamination with FB_1 can modify Na-A zeolite capacity to reduce AFB_1 toxic effects. FB_1 did not modify the adsorption capacity of AFB_1, under equimolar concentrations of both toxins at pH 2 and 39°C. However, when concentrations of FB_1 were 5 to 30 times higher than those of AFB_1, a noticeable decrease of AFB_1 adsorption capacity was observed. A mathematical model that considered common sites and interactions between adsorbed molecules interpreted the experimental results. Model fitting to adsorption isotherms allowed to calculate association constants under co-adsorption conditions and a parameter related to interaction between adsorbed molecules. Under these conditions, FB_1-FB_1 was an attractive interaction and was statistically more significant than FB_1-AFB_1 interaction that was of repulsive nature. With regard to AFB_1, only the attractive interaction with itself resulted significant. Association constants for toxin mixes were 10 fold lower than those obtained when pure toxins were used. Extrapolated to *in vivo* conditions, these results indicate that at pH 2 fumonisin levels higher than AFB_1 ones decreased site availability on the absorbent. Even though FB_1 has a noticeable adsorption capacity, when pH reaches neutrality FB_1 is freed to the medium. At this pH, AFB_1 cannot be adsorbed. Thus, FB_1 presence should be taken into account since relative amounts of FB_1 might markedly decrease zeolite Na-A capacity to prevent aflatoxicosis.

ISOLATION, PURIFICATION AND CHARACTERIZATION OF A CHROMATE-REDUCTASE FROM AN *OCHROBACTRUM* spp. 5bvl-1

[1] R. Francisco, [1] P.V. Morais, [2] P. Verнssimo, [2] M.C. Alpoim

[1] Instituto do Ambiente e Vida, 3004-517 Coimbra, Portugal; [2] Departamento de Bioquнmica, Universidade de Coimbra, 3001-401 Coimbra, Portugal.

Hexavalent chromium Cr(VI)) is a strong oxidant and a toxic pollutant. Bacterial reduction of Cr(VI) to the less toxic and less water soluble Cr(III) has previously been reported and there is accumulated evidence that bacterial reduction of chromate can occur under both aerobic and anaerobic conditions. Such microorganisms can be a potential useful tool in the treatment of contaminated soils and waters.

Recently, we isolated several Cr(VI)-resistant bacteria strains from a Cr(VI)-contaminated activated sludge, belonging to bb-*Proteobacteria,* high G+C Gram-positive bacteria (1), and to the genus *Acinetobacter* (γ-*Proteobacteria)*. One of them, belonging to the genus *Ochrobactrum* (strain 5bvl-1), was found to show both high Cr(VI)-resistance and reduction capacities. The isolation and characterization of the enzyme(s) responsible for reducing Cr(VI) would afford information to implement or improve bioremediation strategies.

Aims: The cells of the strain 5bvl-1 were disrupted by sonication and each fraction obtained was tested for chromate-reductase activity. Several preliminary tests were realized on the fraction with Cr(VI) reduction capacity, preceding our attempt to isolate, purify and characterize the chromate-reductase.

Methods: The bacteria cells were grown to the late exponential phase in buffered mineral medium according to Branco *et al*. (2). The cell fractionation was achieved by sonication with 15W pulses during a total time of 5 minutes, and the cell extract obtained was ultracentrifuged. Each fraction obtained was tested for Cr(VI)-reduction activity. To measure the chromate-reductase activity, two methods were used. Diphenylcarbazide method was used to measure the chromate reductase activity in the experiments with cells and cell fractions. To determine initial rates of Cr(VI) reduction, we measured continuously the disappearance of the chromate yellow color at 410 nm. Each fraction was analyzed by electrophoresis, in a polyacrylamide gel, in denaturating conditions, and silver stained. To obtain partial purification of the enzyme present in the fraction

S2, the fraction was first lyophilized, and then concentrated by molecular exclusion chromatography and anion-exchange chromatography. An enriched fraction showing chromate-reductase activity was dialyzed and lyophilized before sequenciation.

Results: The cell extract S2 showed a high chromate-reductase activity at 20ϵC and a drastic decrease of activity above 40ϵC. The optimum initial Cr(VI)-concentration was of 2 mM, and the cell extract showed a decrease in the Cr(VI)-reduction activity for higher concentrations. The enzyme activity was dependent on NADH and the cell extract showed maximum activity at a NADH concentration of 4 mM. In the optimum conditions described above, the chromate-reductase activity was of 60.9 μM/min/mg. The presence of sulfate did not affect the chromate-reductase activity of the fraction.

Conclusion: This chromate-reductase has different physiological characteristics than the ones described by Park *et al* (3). The role that chromate reductase play in bacteria physiology has not been explored yet.

PHYSIOLOGIC STUDIES IN CR(VI)-RESISTANT AND CR(VI)-REDUCING *OCHROBACTRUM* spp. 5 bvl-1

[1] R. Branco, [2] M.C. Alpoim, [2] V.M. C. Madeira, [1] P.V. Morais

[1] Instituto do Ambiente e Vida, 3004-517 Coimbra, Portugal; [2] Departamento Bioquнmica, Faculdade de Cікncias e Tecnologia da Universidade de Coimbra, Apartado 3126, 3001-401 Coimbra Portugal.

Hexavalent chromium (Cr(VI)) is a strong oxidant, and this property is likely related to its toxicity for most organisms. Microrganisms are generally the first category to be exposed to environmental heavy metal contamination, therefore, they acquire a variety of mechanisms to detoxify metal ions. Bacterial resistance to Cr(VI) has been found in several bacterial species and recently we reported the isolation of several Cr(VI)-resistant bacteria belonging to the genus β-*Proteobacteria*, high G+C Gram-positive bacteria and *Acinetobacter*.

Aims: Strain 5bvl-1, isolated from a chromium-contaminated wastewater treatment plant from a tannery industrial area, identified as an *Ochobactrum* spp., was fully characterized for its ability to resist and to reduce Cr(VI) either in the presence or in the absence of other toxic metal contaminants, since there is an interest in the bioremediation potential of bacteria.

Methods: Strains were cultured in buffered mineral medium according to Branco *et al*. Bacteria characterisation was done by FAME analysis and API 50CH, G+C content,16S rDNA sequencing, and DNA-DNA hybridization. Bacteria growth, Cr(VI) reduction and total chromium were measured as already reported.

Results: Comparative analysis of the 16S rDNA gene sequence identified strain 5bvl-1 as *O. tritici* and DNA-DNA hybridization confirmed the identification (>70% homology). The bacterium was resistant to a broad range of antibiotics. In contrast to other strains of the genus *Ochrobactrum* this strain was able to grow, under aerobic conditions, in as much as 10 mM Cr(VI), and was also able to reduce Cr(VI). The strain Cr(VI)-resistance and Cr(VI)-reduction abilities were dependent on, the concentration of Cr(VI) in the culture medium, the starting cell density and the innocula growth conditions. The other strains of the genus *Ochrobactrum* were not Cr(VI)-resistant but were able to reduce Cr(VI). Strain 5 bvl-1 was also able to resist to 1 mM Ni^{2+}, Co^{2+}, Cd^{2+} and Zn^{2+}. The simultaneous presence of Cr(VI) and either Ni^{2+}, Co^{2+}, Cd^{2+} and Zn^{2+} did not affect the strain growth yield.

Conclusions: In the presence of 1 mM Cr(VI) were obtained the highest growth rate and reduction yield. The results obtained with the other strains of the genus *Ochrobactrum* allowed us to state that the Cr(VI) reduction ability does not confer resistance to Cr(VI).

PLANT GROWTH-PROMOTING RHIZOBACTERIA DECREASE CD TOXICITY FOR *BRASSICA NAPUS* PLANTS

[1] V.I. Safronova, [1] A.A. Belimov, [2] V.V. Stepanok, [3] C. Kluge, [3] A. Preisfeld, [3] K.-J. Dietz, [4] T. Mimura

[1] Research Institute for Agricultural Microbiology (RIAM), 3, Podbelskogo Sh., Pushkin, 196608 Saint Petersburg, Russia; [2] Research Institute for Agricultural Use of Ameliorated Lands, P.O. Emaus, 171330 Tver, Russia; [3] Bielefeld University, 33501 Bielefeld, Germany; [4] Nara Women's University, Nara, 630-8506, Japan.

Fifteen bacterial strains of plant growth-promoting rhizobacteria (PGPR) containing 1-aminocyclopropane-1-carboxylate (ACC) deaminase were isolated from soils and a long-standing sewage sludge contaminated with heavy metals. The isolated strains were characterized and assigned to various genera and species such as *Pseudomonas brassicacearum*, *P. marginalis*, *P. oryzihabitans*, *P. putida*, *Pseudomonas* sp., *Alcaligenes xylosoxidans*, *Alcaligenes* sp., *Variovorax paradoxus*, *Bacillus pumilus* and *Rhodococcus* sp. by determination of 16S rRNA gene sequences. The bacteria were quite tolerant to cadmium toxicity and stimulated root elongation of rape (*Brassica napus* var. *oleifera* L.) seedlings in the absence and presence of 300 μM $CdCl_2$ in the nutrient solution. The effect of ACC-utilising bacteria on root elongation correlated with the impact of chemical inhibitors of ethylene

biosynthesis aminoethoxyvinylglycine and silver ions. We propose that a beneficial effect of the studied PGPR on root elongation is associated with their ability to hydrolyse ACC, the immediate precursor of the plant hormone ethylene and thereby reduce ethylene levels within the plant. A significant improvement in the growth of rape caused by inoculation with certain selected strains was also observed in pot experiments, when the plants were cultivated in Cd-supplemented soil. The bacteria did not affect the content of Cd in plant shoots. The results suggest that PGPR containing ACC deaminase are present in various soils and offer promise as a bacterial inoculum for improvement of plant growth, particularly under unfavourable environmental conditions.

STIMULATION OF UPTAKE OF SOME METALS AND RADIO-NUCLIDES WITH THE HELP OF PHOSPHATE MOBILIZING BACTERIA

[1] I.V. Shtangeeva, [2] S. Ayrault, [3] T.B. Lissitskaya

[1] St.Petersburg University, Universitetskaya nab., 7/9, St.Petersburg Russia; [2] LPS CEA-CNRS 91191Gif sur Yvette Cedex France; [3] St.Petersburg Technical University, St.Petersburg, Russia.

Effects of two strains of bacteria on uptake of ions were studied. Seeds of oats *Avena sativa* L., barley *Hordeum vulgare* L. and wheat *Triticum vulgare* (Vill.) Horst were germinated on a moist filter paper for six days. A part of the seeds was germinated in ordinary conditions, and another part of the seeds was treated additionally by a culture of phosphate mobilizing bacteria *Cellulomas sp.*32 and *Mucobactrerium* sp.12. Germinated seedlings were transferred to pots containing clean soil, soil taken from contaminated sites and soil artificially contaminated by thorium. The seedlings have been grown in the soils for three weeks. During the time plants and soil (from surface of plant roots) were taken several times. Dynamics in soil pH and plant biomass were studied in the course of the experiment. Concentrations of exchangeable cations and mobile forms of metals in the experimental soil were determined by sequential leaching procedures and ICP-AES. Total amounts of 26 elements in soils and different parts of the plants were determined by instrumental neutron activation analysis.

Two species of bacteria affected the ion uptake rather differently. *Cellulomas* sp.32 stimulated transfer of some elements (Cr, Fe, Ni, Cu, Zn, Rb, Ag, Sb, Cs and Pb) from solid phase of the soil to more mobile and as a result, more available for plants forms. Concentrations of the elements in both roots and leaves of plants treated by the bacteria were higher than those in control plants. On the other hand, there was no significant effect on uptake of ions after treatment of plant seeds with *Mucobactrerium* sp.12. Moreover, it seems that excudates of the bacteria can decrease the mobility of some ions. It was found that concentrations of some elements in the plants treated before experiment with *Mucobactrerium* sp.12 were decreased. Addition of thorium to the experimental soil caused increase of Th content in all parts of the experimental plants. However, level of the increase depended on the previous treatment of plant seeds.

THE ROLE OF CATIONS IN THE FUNCTIONING OF *AZOSPIRILLUM BRASILENSE* GLUTAMINE SYNTHETASE

[1] V. E. Smirnova, [1] L.P. Antonyuk, [1] A.A. Kamnev, [2] L.A. Kulikov, [2] Y.D. Perfiliev

[1] Laboratory of Biochemistry of Plant-Bacterial Symbioses, Institute of Biochemistry and Physiology of Plants and Microorganisms, RAS 410015 Saratov, Russia; E-mail: micbio@ibppm.saratov.su; [2] Laboratory of Nuclear Chemistry Techniques, Department of Radiochemistry Faculty of Chemistry, Moscow State University 119899 Moscow, Russia.

Glutamine synthetase (GS) is a key enzyme of nitrogen metabolism of *Azospirillum brasilense*, a plant growth-promoting rhizobacterium. Molecules of all bacterial GSs studied up to now are dodecamers formed from two face-to-face hexameric rings of subunits with 12 active sites; divalent cations are absolutely necessary for their activity. Mg^{2+}, Mn^{2+} and Co^{2+} were each found to support the activity of the *A. brasilense* GS [1]. We studied (i) the effect of these divalent cations on the secondary structure of the *A. brasilense* GS, and (ii) binding of Co^{2+} at the GS active sites using emission ^{57}Co Мцссbauer spectroscopy (EMS). GS from *A. brasilense* (strain Sp245) was isolated and purified as described earlier [1]. This native enzyme contained strongly bound metal ions which could not be removed by dialysis. Circular dichroism (CD) analysis showed the *A. brasilense* GS to be a highly structured protein (59% of the residues as aa-helices and 13% as β-strands). Adding divalent metal cations (1 mM Mg^{2+}, or Mn^{2+}, or Co^{2+}) to the native enzyme caused only slight alterations in the CD spectra of the enzyme. On the contrary, a 30-min incubation of the *A. brasilense* GS with 5 mM EDTA leading to the removal of the cations from the protein molecule caused some noticeable changes in the CD spectrum. Calculations showed that the proportion of aa-helix in the cation-free enzyme diminished as compared to that in the native enzyme, whereas the proportion of β-strands increased. EMS studies of $^{57}Co^{2+}$ binding at the GS active sites have revealed that (i) the *A. brasilense* GS has two divalent cation-binding sites per

active site of the enzyme and (ii) the affinity of one site to Co^{2+} is higher than that of the other.

Acknowledgments: Supported in part by INTAS (EC), NATO (Grant LST.CLG.977664), and the Russian Academy of Sciences' Commission, Grant No. 205 under the 6th Competition-Expertise of projects.

EFFECT OF CADMIUM AND COBALT ON PGPR INOCULATED BARLEY IN ONTOGENY

S.V. Timofeeva, V.N. Pishchik, N.I. Vorobiov, A.P. Kozhemiakov

All-Russian Research Institute for Agricultural Microbiology, Russian Academy of Agricultural Sciences, Podbelsky Sh. 3, St. Petersburg, Pushkin 8, 196608, Russia.

The complex biopreparations are widespread in sustainable agriculture. The preparations based on the PGPR (plant growth promotion rhizobacteria) both increase plant mineral nutrition plants and suppress soilborne disease. *Arthrobacter mysorens* 7 and *Flavobacterium* sp. L-30 are base on complex biopreparation (mysorin and flavobacterin). We study the response of inoculated plants under cobalt- cadmium (Co-Cd) stress in ontogeny according the membrane filter technique. This approach allow to receive adequate information on the survive of PGPR in rhizosphere and rhizoplane in dynamic. Our data of experiments shown that the inoculation with *A. mysorens* 7 and *Flavobacterium* sp.L-30 increased significantly barley root weight by 124 % and by 380 % on the 3rd days of incubation in unpolluted soil. In our investigation barley growth on the soil polluted of Cd and Co nitrates (5–75 mg metals per kg soil).

Barley is one of the most tolerant plant to Co and Cd. However, we revealed the Co and Cd suppression of plant development in the ontogeny. It was found that the addition of Co and Cd into soil had a different toxic effect on barley. Negative effect of Co on the uninoculated plants was more significant in comparison with Cd-stress. Inoculation of PGPR reduced harmful influence of Co and Cd salts. A most benefit effect of plant inoculation in ontogeny we received with *Flavobacterium* sp.L-30.

We constructed a new non-linear mathematical model to describe population dynamics of PGPR during colonization of barley rhizosphere under Co-Cd stress. The coefficient of adaptive ability, characterizing a percentage of bacteria which are adaptive to soil conditions in the barley rhizosphere at the initial stage of time was calculated. It was revealed that the root growth of inoculated plants depended on coefficient of adaptive ability in the polluted soil. It was found that barley inoculation with *A. mysorens* 7 had the most effect under Cd-pollution. The addition of *Flavobacterium* sp.L-30 had a benefit effect on the barley root growth in ontogeny under Co-stress.