US/Russia Collaboration for Optimization and Validation of Phytoremediation for *in situ* Oilfield Cleanup

Alexander M. Boronin* and Vera A. Dmitrieva**

* Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Russia

** Center for Ecological Research and BioResources Development, Russia

DOE –IPP Project

“Optimization and Validation of Bioremediation of Oilfield Contamination by an Integrated Microbial and Plant System”.

Russian Partners:

Center for Ecological Research and BioResources Development, Pushchino

Institute of Biochemistry and Physiology of Microorganisms of Russian Academy of Sciences, Pushchino

All-Russian Research Institute of Biological Plant Protection, Russian Academy of Agricultural Sciences, Krasnodar

Krasnodar Forestry Enterprise

State Research Center for Applied Microbiology, Ministry of Health of Russian Federation (MH), Obolensk

Research Center for Toxicology & Hygienic Regulation of Biopreparations (MH), Serpukhov

US Partners:

Argonne National Laboratory

Industrial Partners:

Chevron Texaco

Diversa

BP

The RETEC Group, Inc. (RETEC);

Center for

Ecological Research and BioResources Deveopment

(CERBRD)

CERBRD was established with the support of the DOE-IPP and legalized in 2000, as a non-commercial partnership.

CERBRD acts as the focal point of contact between Russian science and US business with interests in:

- microbial diversity,
- discovery of novel bioactive compounds and identification of useful commercial applications thereof,
- biomedicine,
- bioremediation,

Russia: Key Environmental Problem Areas

from Handbook of International Economic Statistics (CIA)

Oil Contamination in Western Siberia

The view of oil-contaminated site.

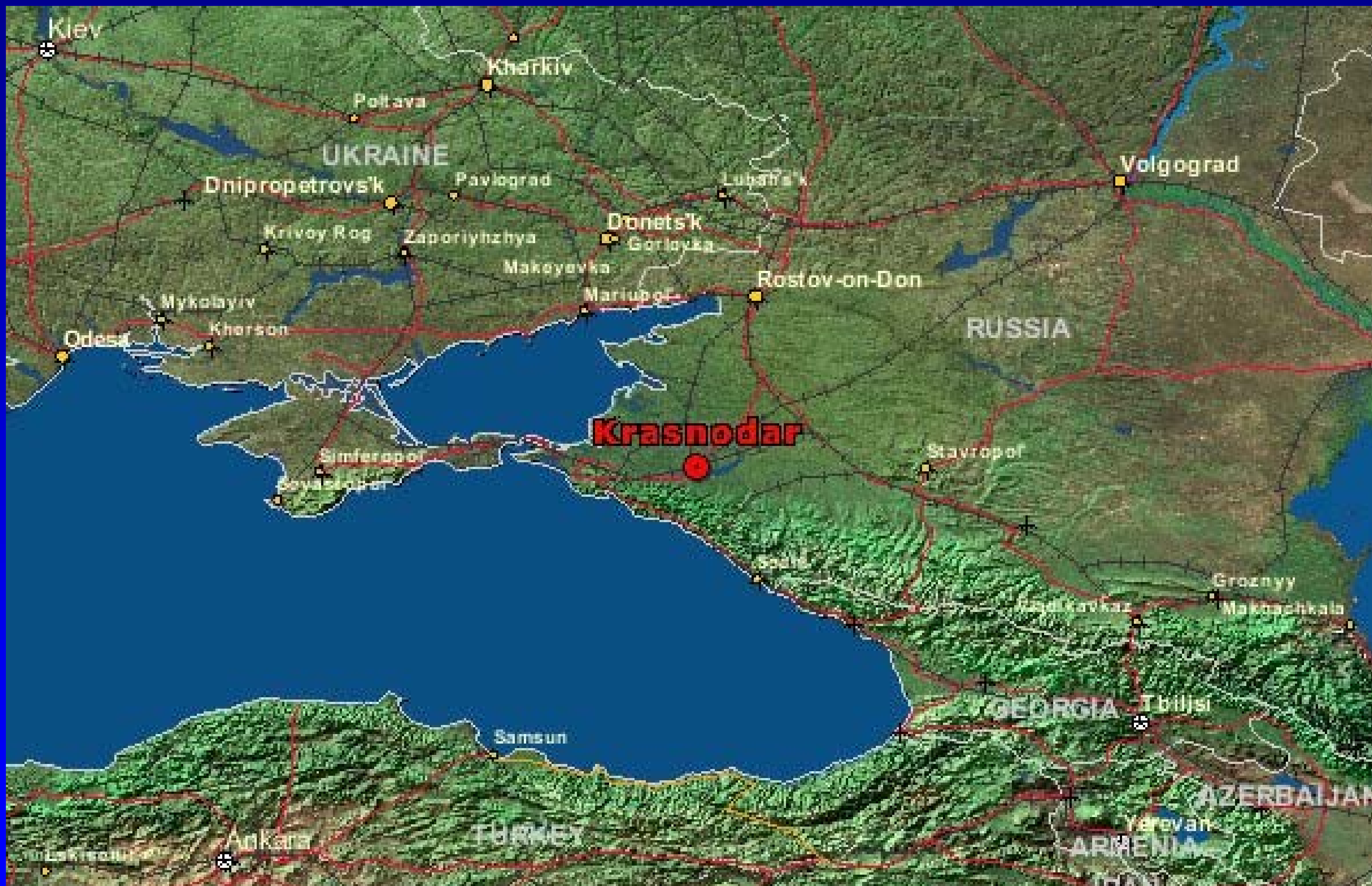
July, 1999.

Russian Federal Research and Development Program “Environmental Biotechnology”

1. Oil spills bioremediation

2. Biotechnology for destruction of chemical weapons

Map of Krasnodar Region



Project Goals:

Understand regulatory requirements for cleanup in Russia and compare them with pertinent US analogs.

Determine fate and transfer of petroleum hydrocarbons for a better understanding of phytoremediation processes and performance; provide realistic data for risk assessment.

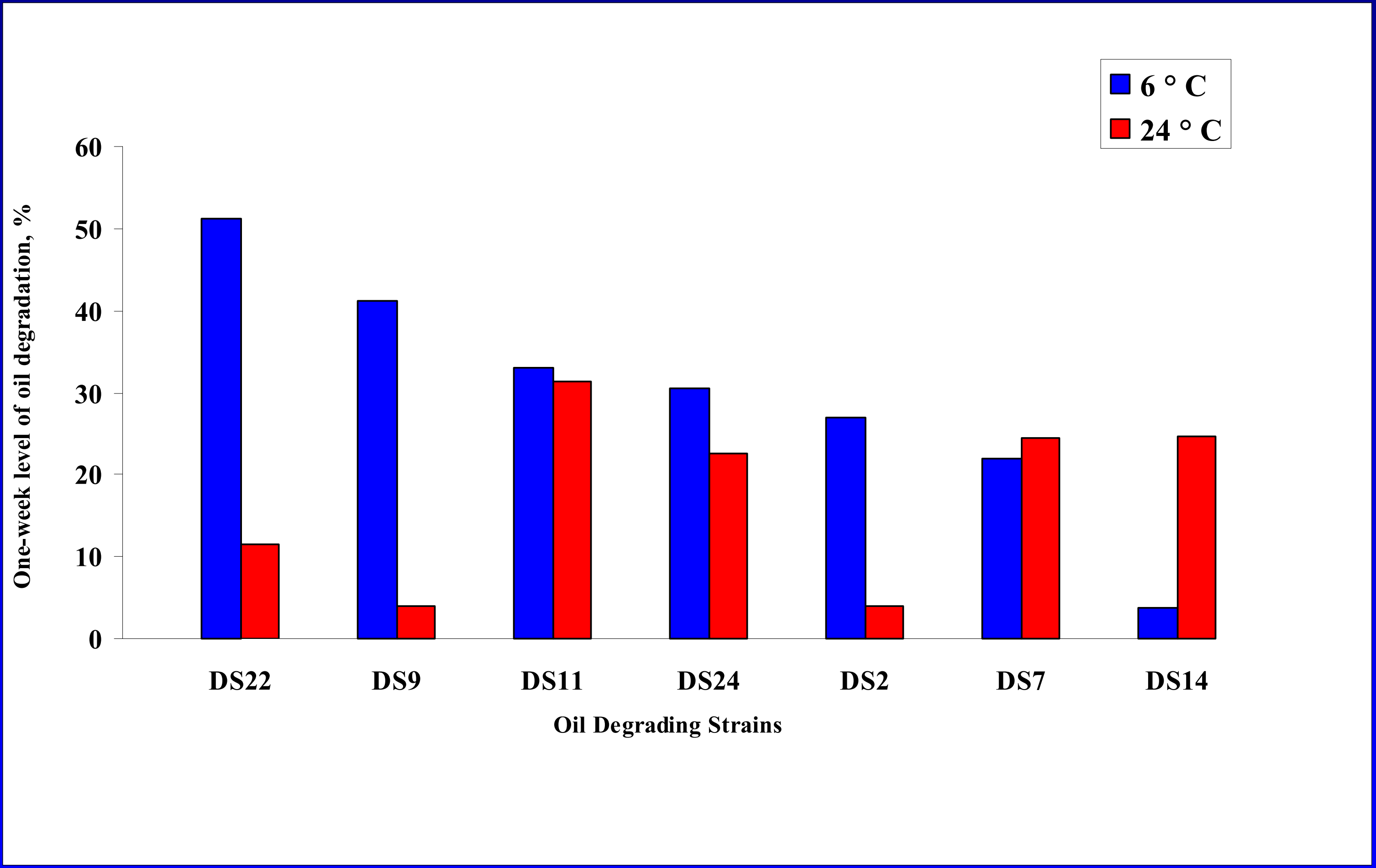
Collect statistically significant evidence of phytoremediation performance.

Optimize biodiversity in remediation and ecological restoration of impacted areas.

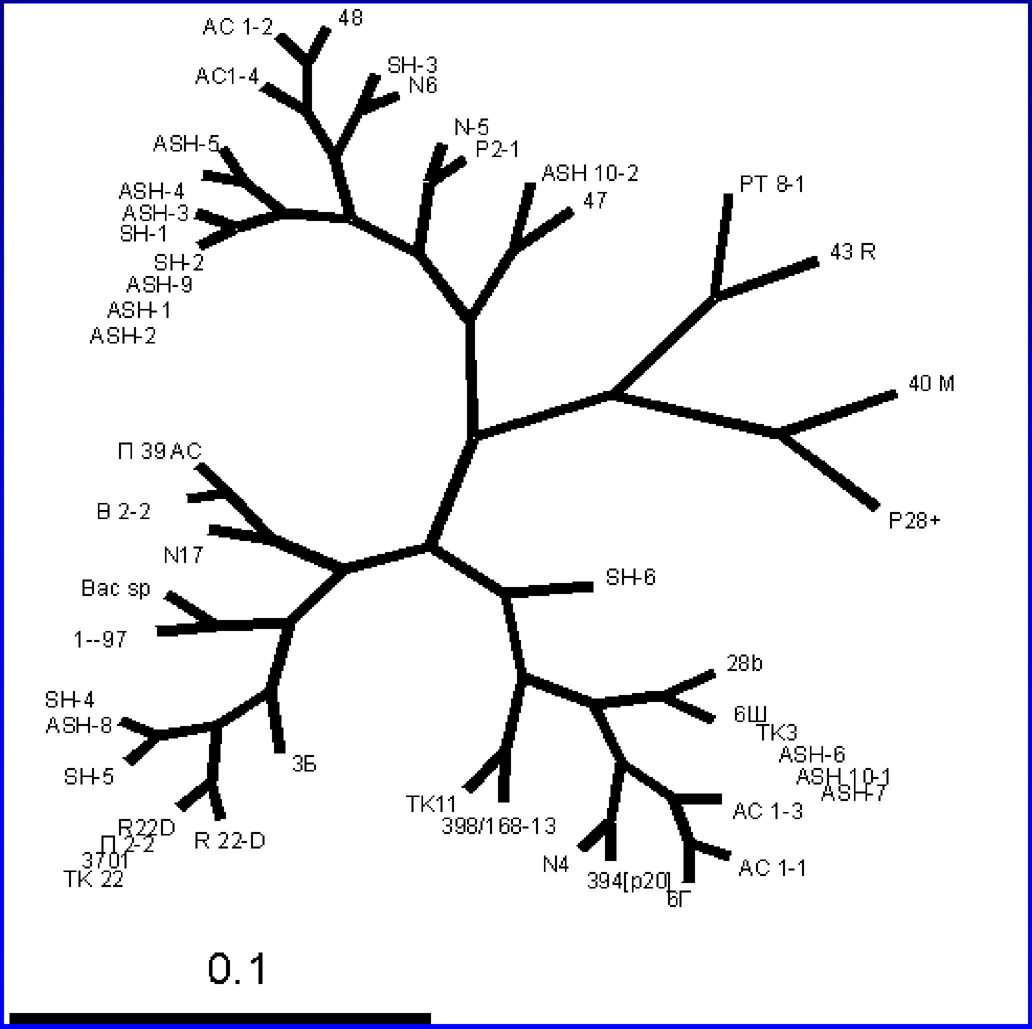
Expand rhizosphere and accelerate remediation, using microorganisms able to increase rooting density and plant growth-promoting ones able to degrade oil hydrocarbons.

Determine phytoremediation performance in sub-optimal conditions including cold climate.

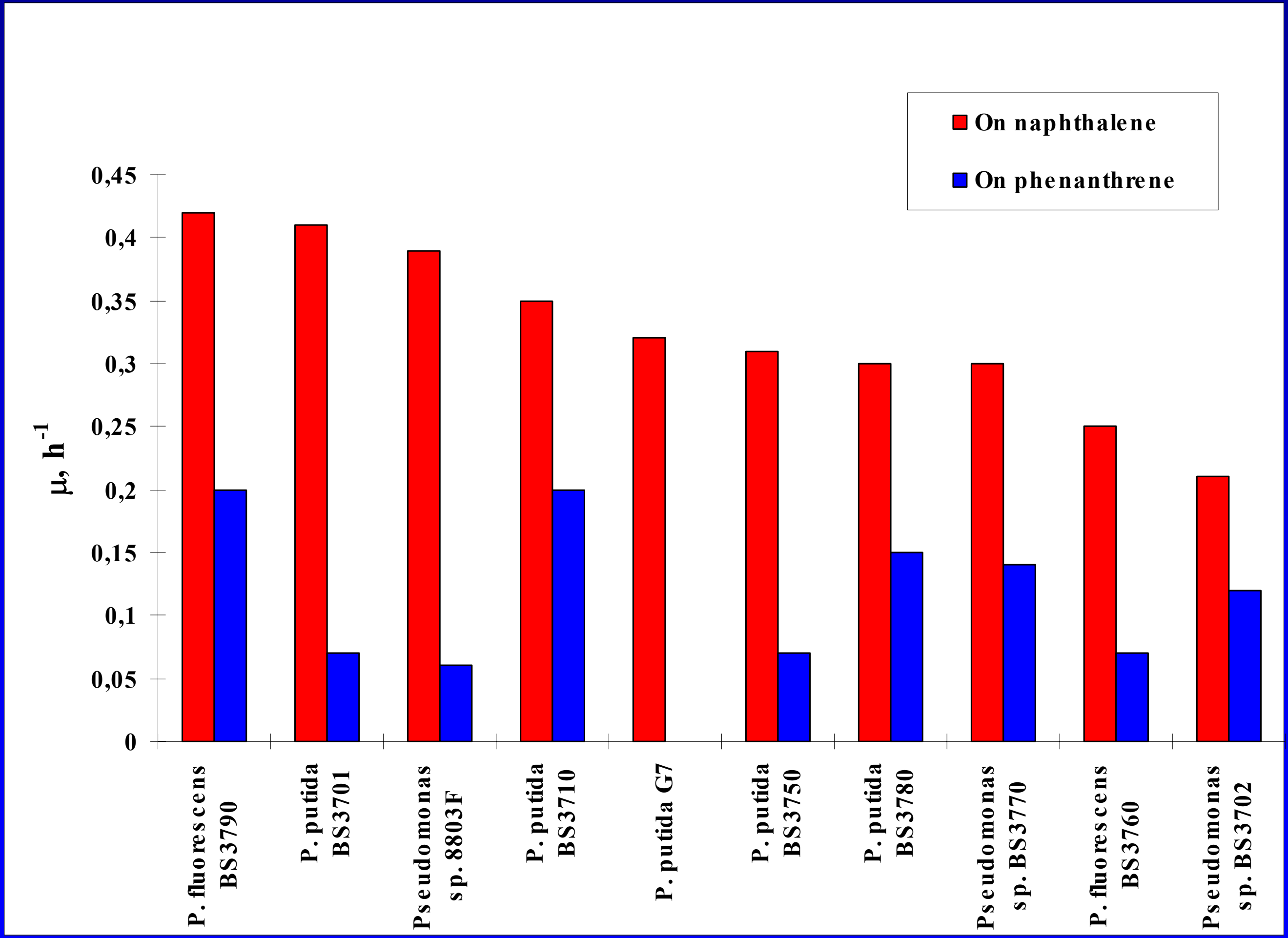
Degradation of Oil by Psychrotrophic Microorganisms under 6°C and 24°C



Dendrogram of Oil-degrading Strains Based on Their Catabolic and Physiological Properties



Specific Growth Rates of PAH-degrading Strains under Batch Cultivation on Naphthalene and Phenanthrene



Examples of plasmids encoding the degradation of predominantly naturally occurring organic compounds

(data from E.M. Top *et al*., 2000)

Strain	***Plasmid***	***Substrates***	***Size (kb)***	***Inc-group***
		Aliphatic compounds:		
Pseudomonas oleovorans PpG6	OCT	Octane, decane	500	P2
Pseudomonas putida PPU2	pSRQ	Acyclic isoprenoid (citronellol, geraniol)	75	-
		Monoaromatic compounds:		
P.putida R1	SAL1	Salicylate	85	P9
P.putida PpG1	CAM	Camphor	500	P2
P.putida PaW1	TOL	Xylene,Toluene	115	P9
Acinetobacter calcoaceticus RJE74	pWW174	Benzene	200	-
Pseudomonas sp. CIT1	pCIT1	Aniline	100	-
P.putida ST	pEG	Styrene	37	-
P.putida RE204	pRE4	Isopropyl benzene	105	-
P.putida NCIB9869	pRA500	3,5-xylenol	500	-
Pseudomonas sp CF600	pVI150	Phenol	-	P2
P.putida CINNP	pCINNP	Cinnamic acid	75	-
		Polyaromatic compounds:		
P.putida PpG7	NAH7	Naphthalene, Phenanthrene, Anthracene	83	P9
Pseudomonas sp. CB406	pWW100	Biphenyl	200	-
		Heterocyclic compounds:		
Pseudomonas convexa Pcl	NIC	Nicotine, Nicotinate	-	-
Pseudomonas alcaligenes DBT2	pDBT2	Dibenzothiophene	80	-

Incompatibility Groups of Degradative Plasmids

Incompatibility group	**Plasmid**	**Substrate**
P2	CAM	Camphor
	OCT	Octane
	pBS263, pBS264 pBS266, pBS271	ε-Caprolactam, ε-Aminocaproic acid
P7	pBS2, pBS3, pBS211, pBS213, pBS214, pBS217, pBS243, pBS4	Naphthalene
	pND50	*p*-Cresol
P9	NAH7, pND140, pND160, pWW60, NPL-1, NPL-41, pBS212, pBS216, pBS240, pBS244, pBS248, pBS2	Naphthalene
	pBS262, pBS265, pBS267, pBS268	ε-Caprolactam, ε-Aminocaproic acid
	pBS1004	*p*-Toluenesulfonic acid
	pWWO	Toluene

Plasmids Encoding the Degradation of Naphthalene

Plasmid	Properties	Incompatibility group	Transfer to *P.putida* frequency	Size (kb)
NPL-1	Nah^+	IncP-9	10^{-3}	100
pBS2	Nah^+Sal^+	IncP-7	10^{-4}	130
pBS101	Nah^+Sal^+	IncP-7(9)	10^{-3}	50
pBS240	Nah^+Sal^+	IncP-9	10^{-4}	160
pBS216	Nah^+Sal^+	IncP-9	10^{-3}	85
pBS244	Nah^+Sal^+	IncP-9	10^{-4}	150
pBS243	Nah^+Sal^+	IncP-7	10^{-4}	160
pBS213	Nah^+Sal^+	IncP-7	10^{-4}	150
pBS218	Nah^+Sal^+	IncP-7(2)	10^{-4}	160
pBS219	Nah^+Sal^+	IncP-7(2)	10^{-4}	180
pBS215	Nah^+Sal^+	IncP-? Not P-2, 7, 9	10^{-5}	150
pBS242	Nah^+Sal^+	IncP-? Not P-2, 7, 9	10^{-5}	150
pBS1191	Nah^+	IncP-9	10^{-7}	100
pBS1141	Nah^+	IncP-9	10^{-4}	100
pBS1181	Nah^+Sal^+	IncP-9	10^{-6}	110
pHK43	Nah^+Sal^+	ND*	ND	100
pHK72	Nah^+Sal^+	IncP-9	ND	85
p8C	Nah^+Sal^+	IncP-9	ND	120
p15C	Nah^+Sal^+	IncP-9	ND	120
p24C	Nah^+Sal^+	IncP-9	ND	120
p25C	Nah^+Sal^+	IncP-9	ND	120

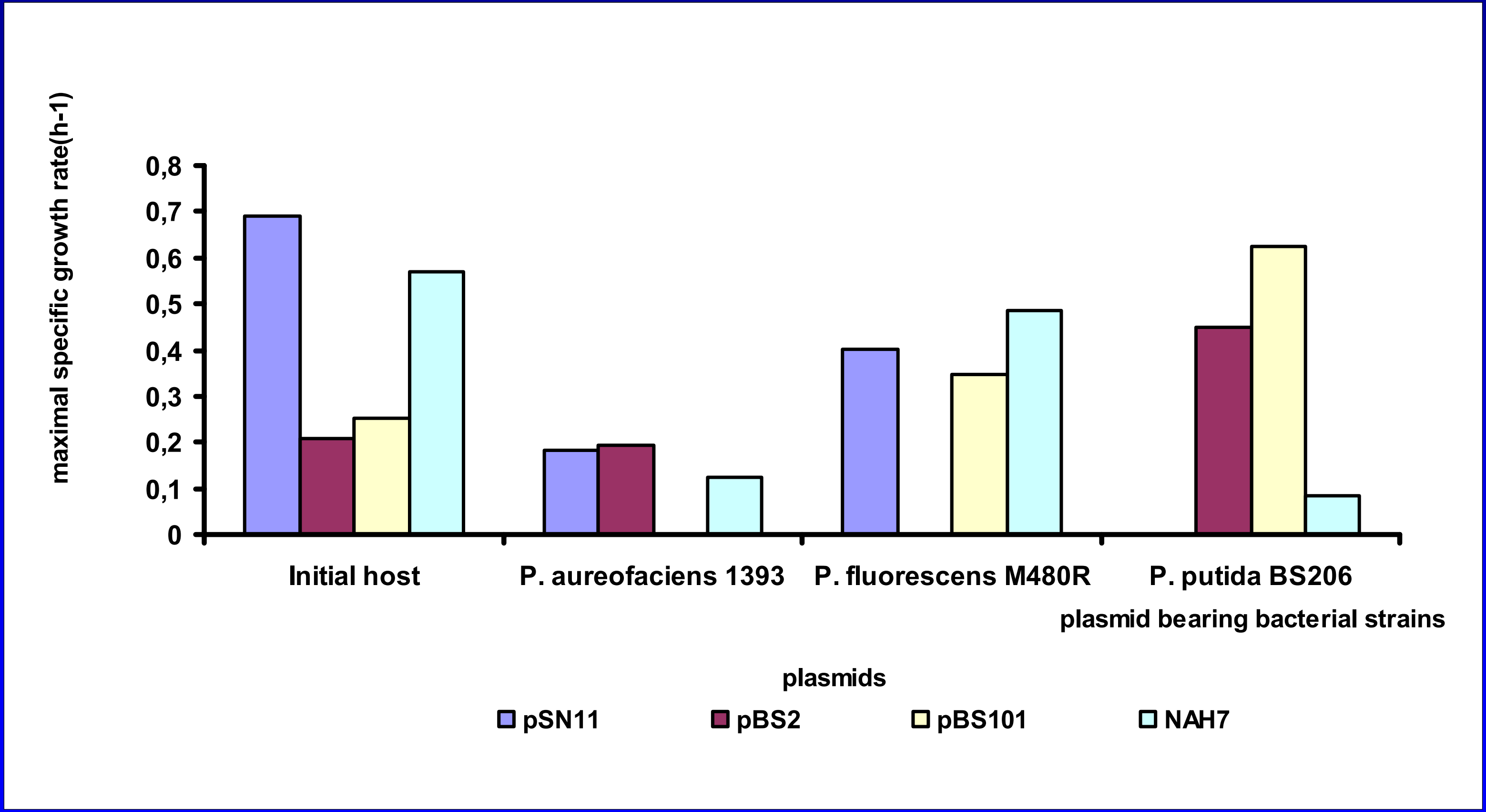
ND – not determined.

1. Is there a degradative (D) “superplasmid” capable of determining the most efficient degradation of a particular organic compound?

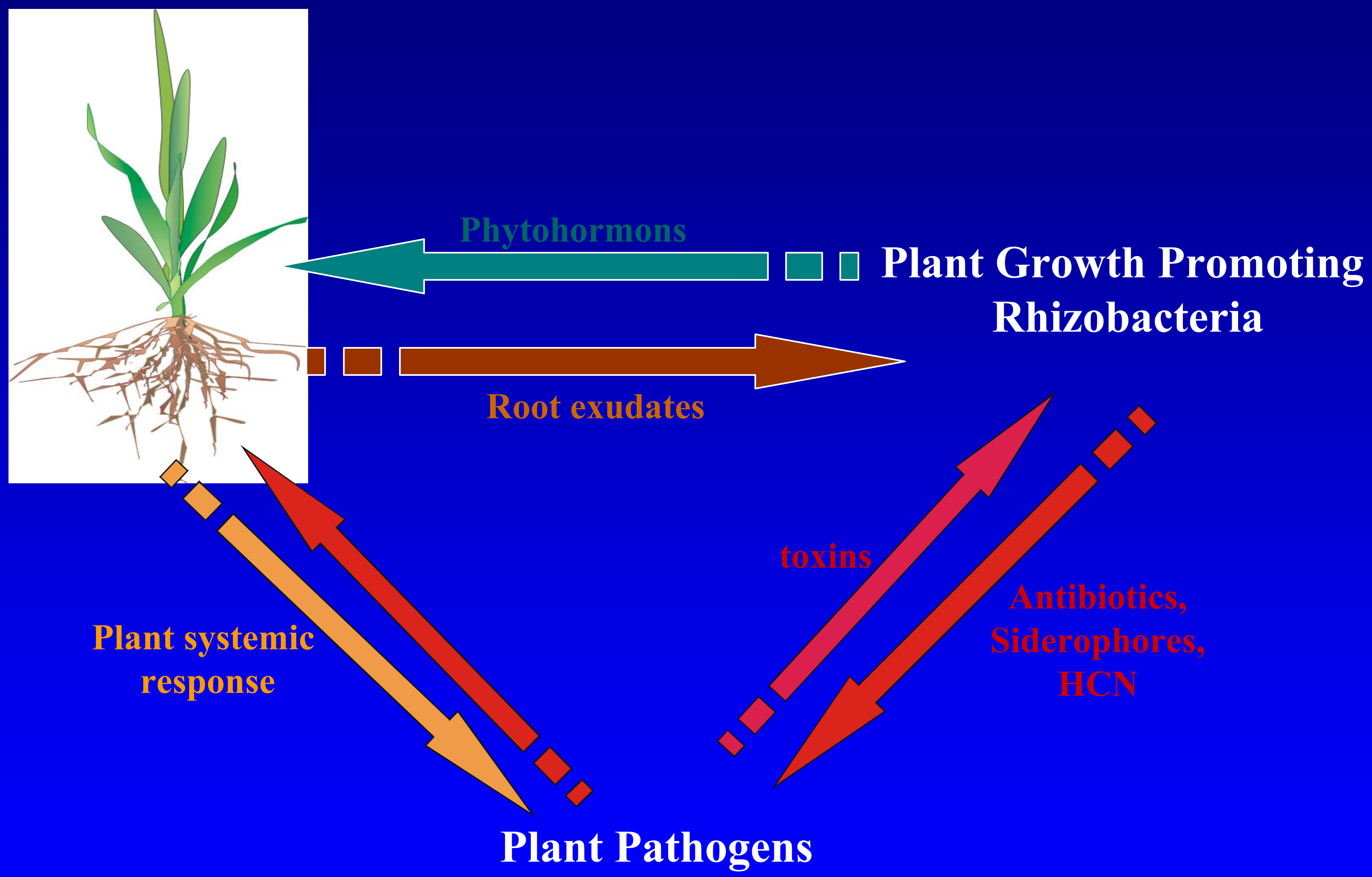
2. Is there a bacterium which is the optimal host for that plasmid from the viewpoint of the expression of degradative genes?

3. Is there an “ideal” combination of a D-plasmid and a bacterial host?

Specific Growth Rates of Plasmid Bearing Bacterial Strains in Batch Culture on Naphthalene

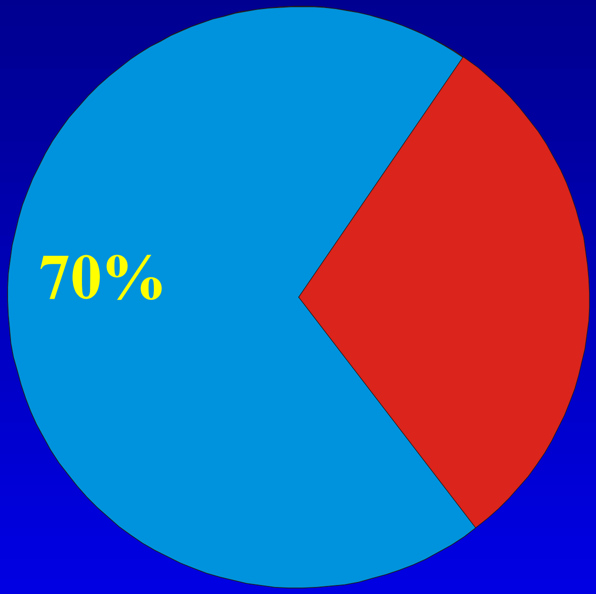


Plant-Microbial Interaction in Rhizosphere



Collection of Rhizobacteria

Over 1500 strains



Rhizospere
Rhisoplane

Potato
Cereals
Wild plant

Geography

Middle Russia
Ukraine
Caucasus
Sibiria

Pseudobacterin-2

Microbial plant-protecting biopreparation

- **is based on living cells of the *Pseudomonas aureofaciens***

(plant growth promoting rizobacterial strain)

- **has successfully undergone 3-years(State stand) test**

- **is highly efficient against many plant diseases and shows a plant growth promoting effect**

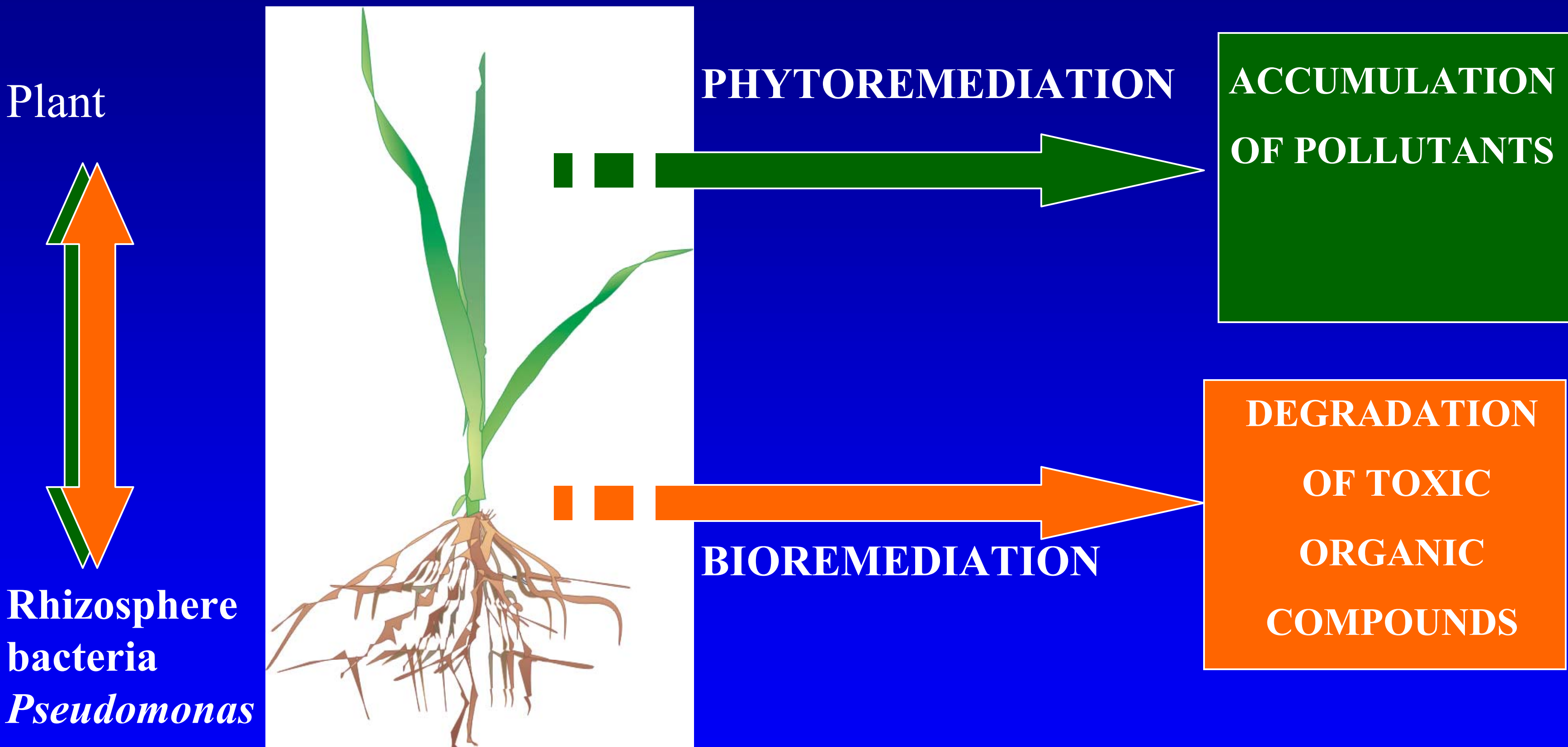
Examples of the increased productivity after the treatment with Pseudobacterin-2

	Centner(100 kg/hectare)
Cereals	2-15
Grapes	20-37
Vegetables	30-200

Microorganisms for bioremediation	Plant Growth Promoting Rhizobacteria
Burkholderia	*Burkholderia*
Pseudomonas	*Pseudomonas*
Comamonas	*Rhizobium*
Rhodococcus	*Azospirillum*

Burkholderia cepacia: Friend or Enemy?

Properties:	Examples:
Bioremediation agent;	PCB-degradation;
Biocontrol agent.	Plant growth promotion and inhibition of plant pathogens.
Phytopathogen.	Bulb rot of onions.
Pathogen.	Cystic fibrosis.


Plant
Rhizosphere bacteria *Pseudomonas*
PHYTOREMEDIATION
ACCUMULATION OF POLLUTANTS
BIOREMEDIATION
DEGRADATION OF TOXIC ORGANIC COMPOUNDS

Extreme Environmental Factors

- Toxic chemical agents
- Heavy metals
- Radionucleides
- UV-light
- Low or high pH values
- High salinity
- Water deficiency
- Oxygen deficiency

Effect of heavy metals on inhibition of plant pathogens

Co^{2+} 0,5 mM

BS1393 (pBS501)

BS1393

Control

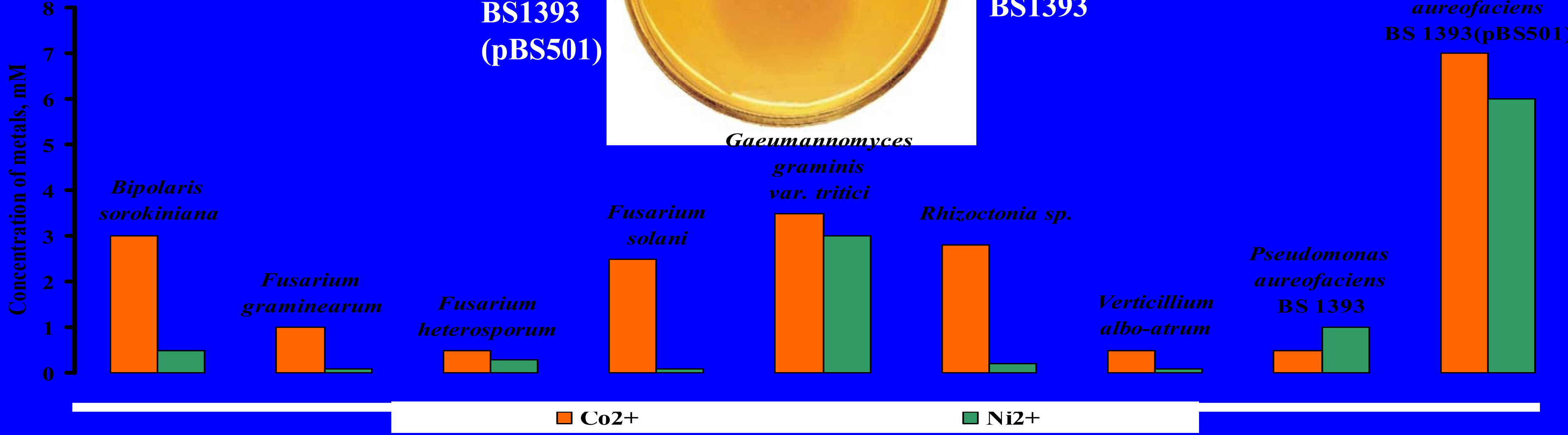
BS1393 (pBS501)

BS1393

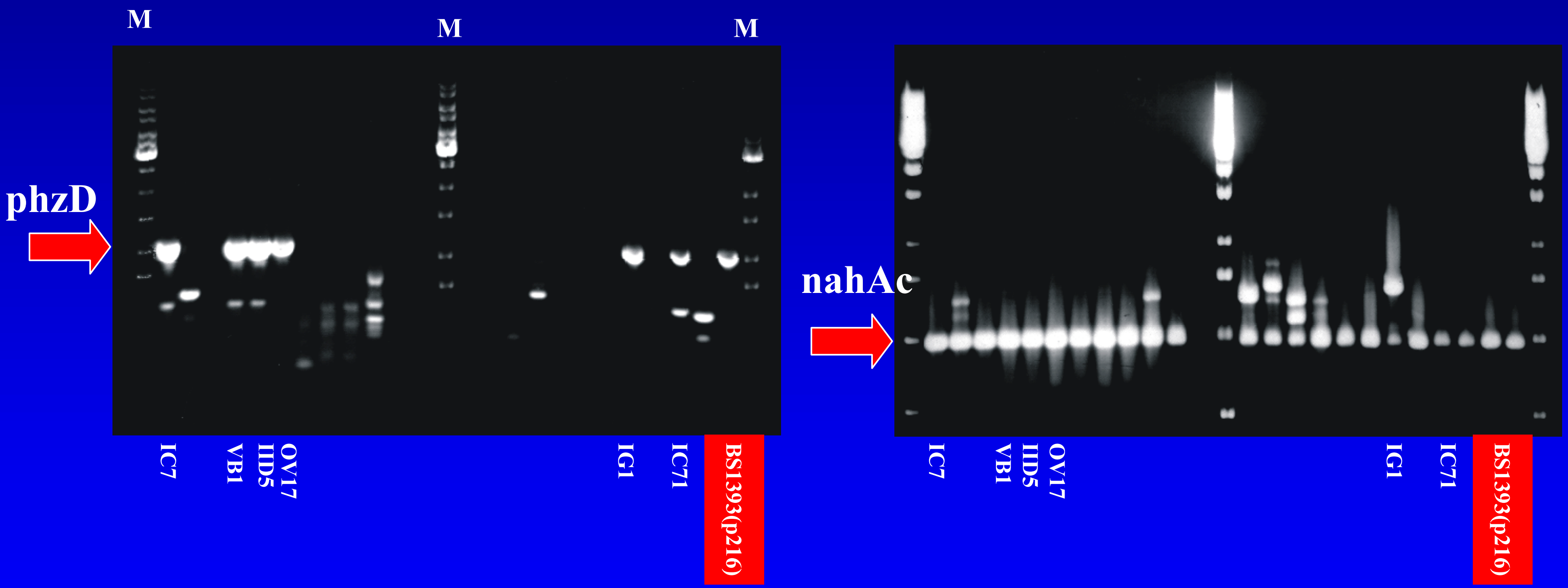
Ni^{2+} 0,5 mM

BS1393 (pBS501)

BS1393

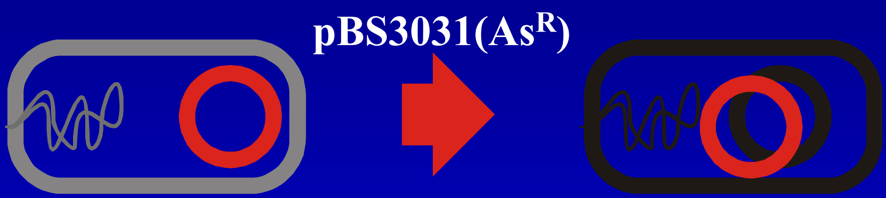


Natural rhizosphere strains combining both degradative abilities and plant growth promoting properties
(PCR analysis)



Strains harboring both phenazine antibiotic synthesis and polycyclic aromatic hydrocarbons degrading systems

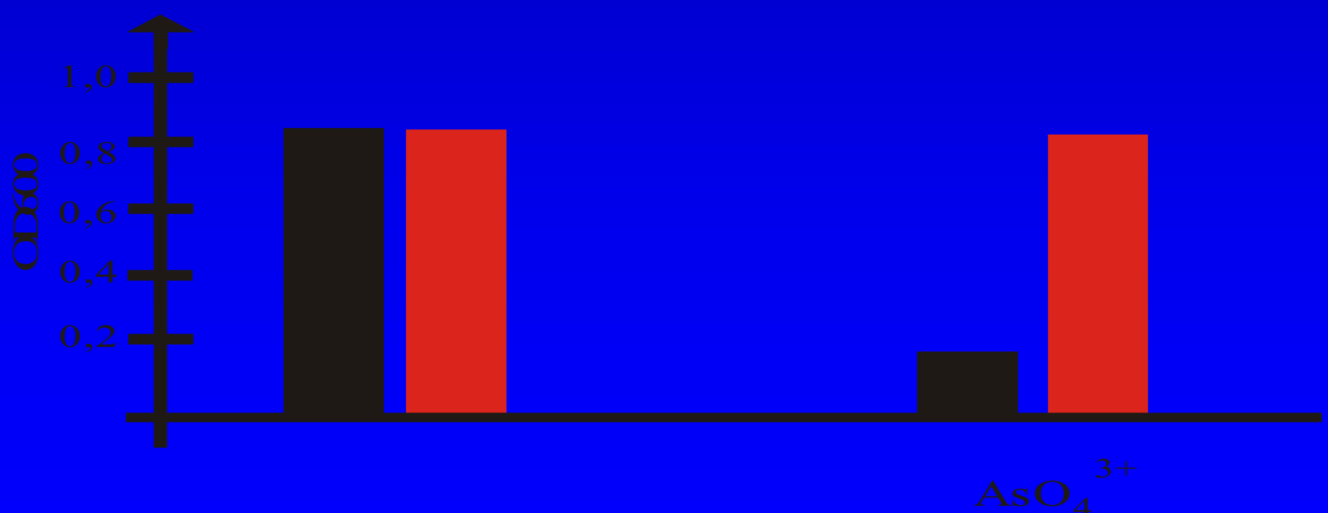
COMBINATION OF ABILITY TO UTILIZE POLYCYCLIC AROMATIC HYDROCARBONS AND RESISTANCE TO ARSENIC COMPOUNDS



Pseudomonas aeruginosa **BS3031(pBS3031)**

Pseudomonas putida **BS238(pBS2)**

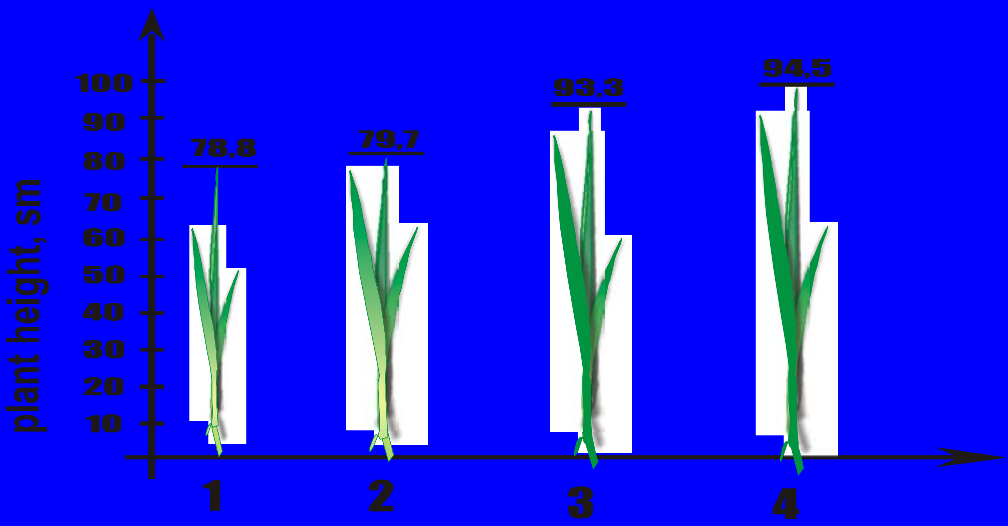
Growth on naphthalene

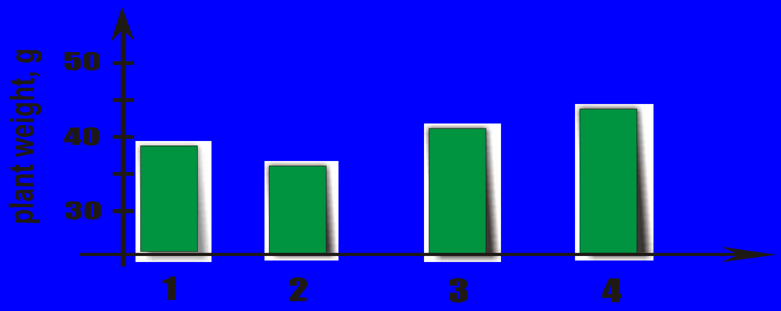


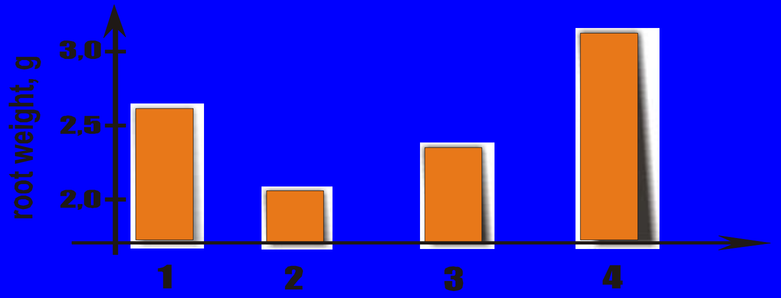
Pseudomonas putida **BS238(pBS2)**

Pseudomonas putida **BS238(pBS2+pBS3031)**

Influence of PGP *Pseudomonas* and endomycorryzal fungi *Glomus intraradices* on sorghum growth

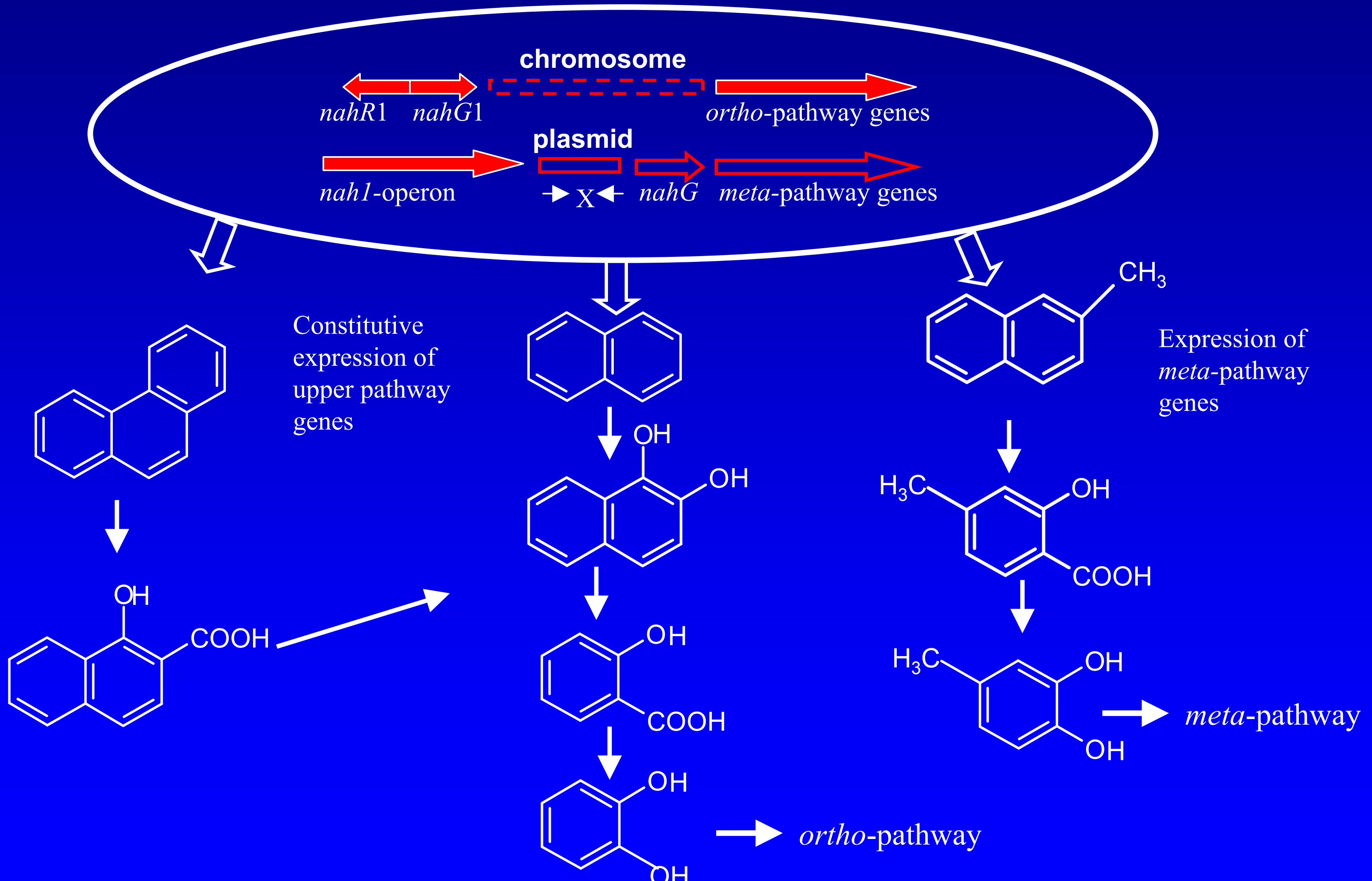




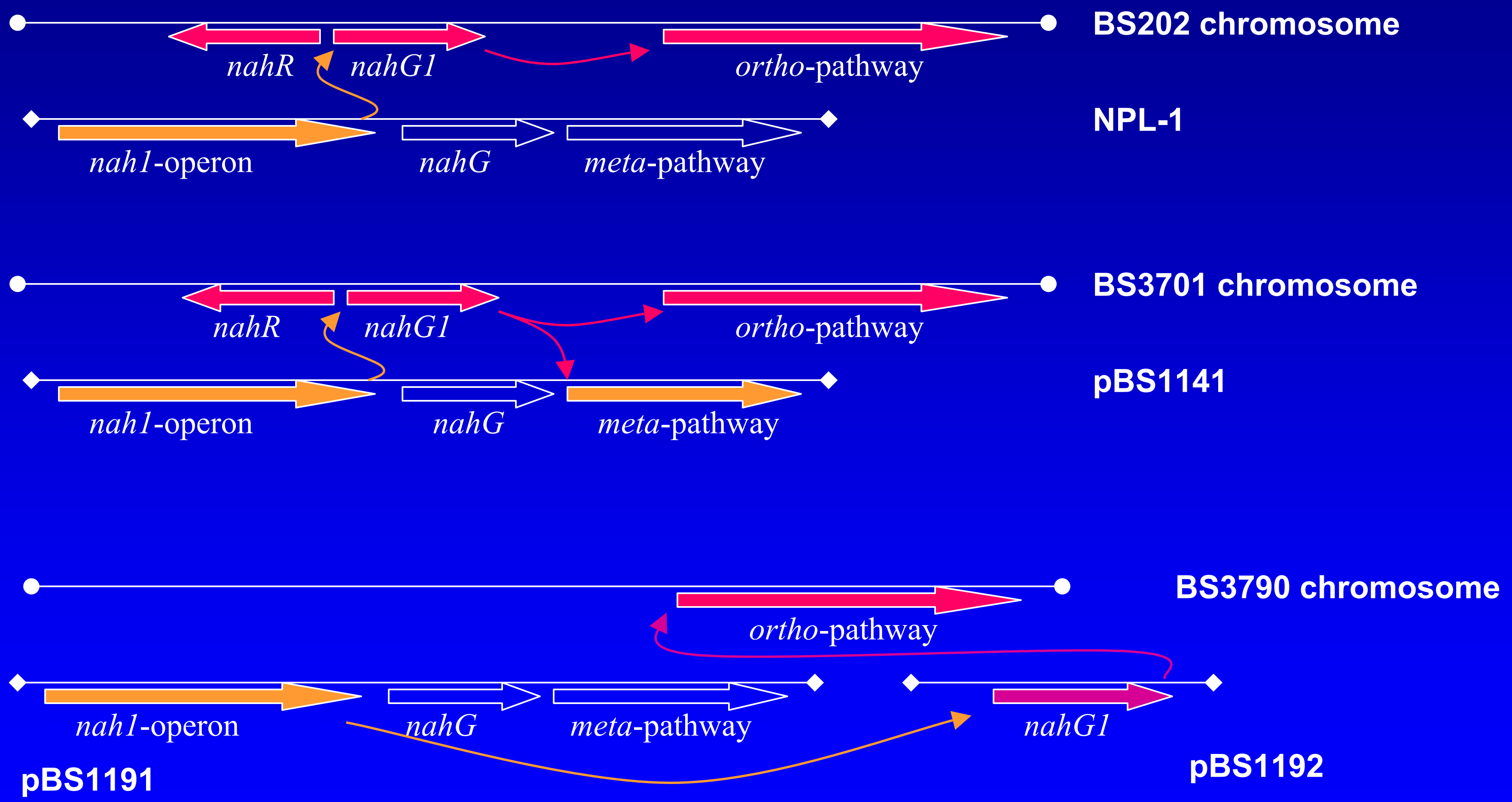


1. *G. intraradices*
2. *G. intraradices* + *P. fluorescens* 38a
3. *G. intraradices* + *P. fluorescens* 7H
4. *G. intraradices* + *P. aureofaciens* BS1393

Polycyclic Aromatic Hydrocarbons Biodegradation by *P. putida* BS202



Organization of PAH catabolic gene in some *Pseudomonas putida* strains



Use of terms according to Cartagena Protocol on Biosafety

- **Living modified organism (LMO)** means any living organism that possesses a novel combinations of genetic material obtained through the use of modern biotechnology.
- **Modern biotechnology** means the application of:
  - In vitro nucleic acid techniques, including recombinant DNA and direct injection of nucleic acid into cells or organelles, or
  - Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection.