COLLECTION OF STRESS TOLERANT SOIL BACTERIA WITH PLANT GROWTH PROMOTING AND SOIL AMELIORATIVE PROPERTIES

Boglárka Rizó[1], Rita Kovács[1], Ildikó Puspán[1], Csilla Imre[1], Nikoletta Pék[2], Éva Kárpáti[2], József Kutasi[1]

[1]BioFil Microbiological, Biotechnological and Biochemical Ltd., Váci út 87, Budapest, H-1139; [2]Saniplant Ltd.,Raktár u. 19, Budapest H-1035

INTRODUCTION

Root-associated, or -colonizing soil bacteria which have a positive influence on plant growth and development by producing and secreting various bioactive substances are usually referred to as plant growth promoting rhizobacteria (PGPR). PGPR are commonly used as inoculants for improving the growth and yield of agricultural crops, however, screening for the selection of effective PGPR strains is very critical. We collected soil samples from natural habitats and arable lands of Hungary and isolated bacteria strains from them. Our aim was to assamble and preserve a strain collection, which contains effective microorganisms, and to create a database. The BioFil Ltd. has a collection of about 1500-2000 individual strains, selected by SBSS – Soil Bacteria Screening System.

MATERIALS AND METHODS

Collection of Soil Samples

Processing of Soil Samples

Isolation of strains based on different selective parameters

Testing the isolated strains

PGPR effects
- Polysaccharide and lipase production
- Potassium and phosphorus mobilization
- Biocontrol effect
- Production of plant growth promoting hormones

Growth in stress conditions
- High salt concentration (1-8 % NaCl)
- Cold conditions (4°C)
- Different pH level (between pH 4,5 and 9)

Creation of the BioFil Strain Collection

Preservation of strains
- Liophylization
- Deep Freezing

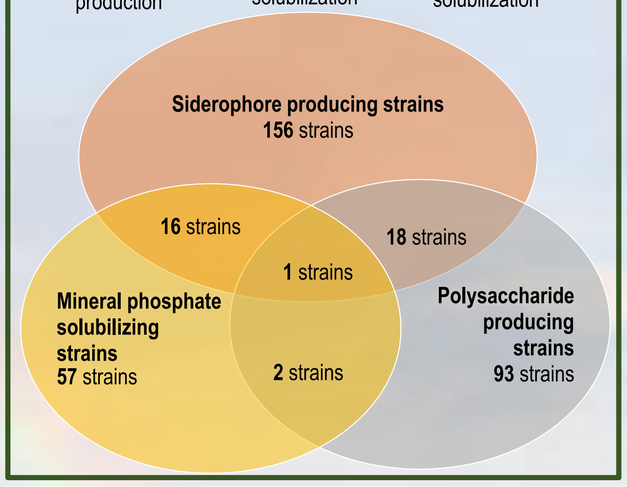
Selection of the effective strains
- PGPR effect
- Stress tolerance

Identification by 16s RNA sequence analysis

Creating strain **database**

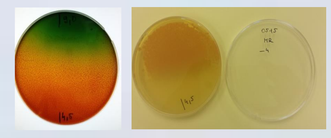
TEST RESULTS 1.

During the testing process individual strains were studied for various PGP features.

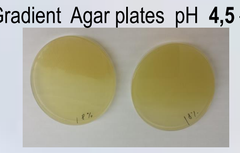


TEST RESULTS 2.

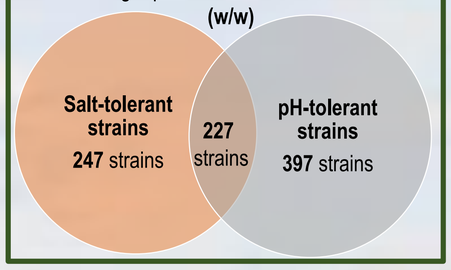
We have made quick slant Agar plate tests with NaCl concentration or pH gradients to select the salt and pH stress tolerant strains.



Gradient Agar plates pH **4,5 – 9,0**

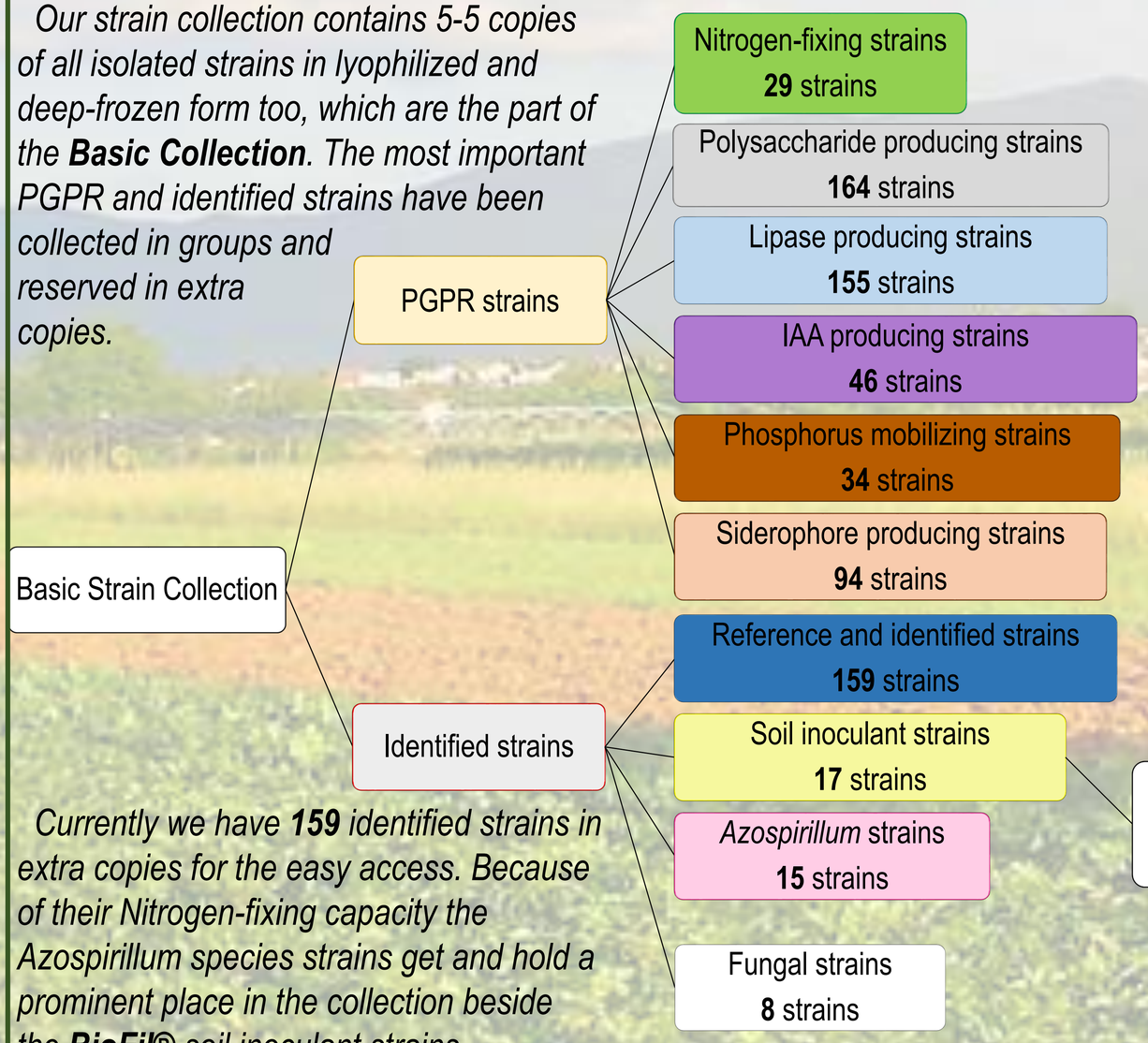


Gradient Agar plates NaCl concentration **1 – 8 %** **(w/w)**



BIOFIL STRAIN COLLECTION

*Our strain collection contains 5-5 copies of all isolated strains in lyophilized and deep-frozen form too, which are the part of the **Basic Collection**. The most important PGPR and identified strains have been collected in groups and reserved in extra copies.*



*Currently we have **159** identified strains in extra copies for the easy access. Because of their Nitrogen-fixing capacity the Azospirillum species strains get and hold a prominent place in the collection beside the **BioFil®** soil inoculant strains.*

PRESERVATION OF STRAINS

Deep Freezing
- *-80 °C*
- *Cryoprotective fluid: 40 % glycerol*
- *Samples can be stored for years*

Lyophilization
- *Freeze-drying protectant: 10 % Skim Milk solution*
- *Long-term storage*

BIOFIL STRAIN COLLECTION DATABASE

We created a collection database, which contains information about the exact place and number of the strains in the deep-freezer and other additional informations: strain code, identified species name, the origin of the strains etc.

Place in deep-freezer

Cryo tube

Cryo box

Strain code	Species name	Reference/ Identified		Soil inoc.	Nitrogen fixing		IAA production		Phosphorus mobilization		Siderophore production		Polisaccharide production		Lipase production		Species group	
1262	*Bacillus thuringiensis*	Ref.4	A6-B1															
13/4 B	*Pseudomonas caryophylli*	Ref.5	I4-I8	13/4B	N2	G7-H2									L1	H3-H7		
B13	*Enterobacter kobei*	Ref.1	F6-G1				IAA1	E5-E9	F1	B7-C2					L1	G7-H2		
B41	*Azospirillum largimobile*	Ref.6	B2-B6	B41	N1	A1-A5											Azos.2	G7-H7
Bac 01																		
Bac 02																		
Bac 11																		
Bj	*Bradyrhizobium japonicum*	Ref.6	B7-C2	Bj														
BM	*Bacillus megaterium*	Ref.2	C3-C7	Bact.1					F1	C8-D3	Sz1	G2-G6			L1	I4-I8		
BP	*Bacillus polymyxa*	Ref.3	B2-B6	Bact.2														
Brev. med	*Brevundmonas mediterranea*	Ref.3	I4-I8															
S 006	*Bacillus simplex*	Ref.7	I4-I8										P1	B7-C2				
S 007	*Bacillus pumilus*	Ref.2	F6-G1								Sz1	H8-I3						
TM 108	*Rhodococcus cerastrii*	Ref.9	C3-C7															

DISCUSSION

The BioFil Strain Collection contains about 1500-2000 individual strains. Most of them have some plant growth promoting properties and/or are able to grown in abiotic stress conditions. It can serve as PGPR screening source for development of new soil inoculants for agricultural utilization.

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