PLANT GROWTH PROMOTING POTENTIAL OF RHIZOBACTERIA ISOLATED FROM A CONTAMINATED AREA

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Aims and Scope

Plant growth promoting rhizobacteria (PGPR) is a group of rhizosphere-colonizing bacteria producing substances which increase the growth of plants and/or protect them against pathogens. These bacteria are significant to agricultural purposes, but they also play an important role in soil restoration by enhancing growth and successful establishment of plants in stressed soils. They also have the potential to be mycorrhizal helper bacteria (MHB), enhancing their importance on forest management.

In this work, we aimed to assess the ability of several rhizobacteria, retrieved from a metal contaminated area, to produce plant growth promoting substances. We also intended to evaluate the effect of those rhizobacteria on plant growth.

Methodology

✓ Isolates were screened for the production of plant growth promoting substances: ammonia (NH_3), cyanide (HCN), siderophores, indol acetic acid (IAA), ACC deaminase and the ability to solubilize phosphate

✓ White clover seedlings, growing in Jensen medium, were inoculated with 1 ml of each bacterial isolate (10^8 CFU/ml), and placed in a controlled growth chamber (22 ºC, 12h day, 75% relative humidity, 300 µmolm^{-2} s^{-1})

✓ After 6 weeks plants were harvested and root and shoot length were measured; biomass was determined after oven drying at 70 ºC for 48h

Results and Discussion

➢ Production of plant growth promoting substances

Table 1. List of isolates and *in vitro* screening for NH_3, HCN, siderophore production and phosphate solubilization
(–) negative; (+) positive; (++) good; (+++) very good; (++++) extremely good production.

Isolate	Closest relatives	Gram reaction	NH_3 production	HCN production	Siderophore production	Phosphate solubilization
3ZP2	*Microbacterium sp.*	+	+	++	++	-
1ZP3	*Microbacterium oxydans*	+	+	++	++	-
EC 16	*Microbacterium oxydans*	+	+	++	++	-
EC 29	*Microbacterium oxydans*	+	+	++	++	-
EAL	*Bacillus sp.*	+	+	++	+	-
EC 39	*Bacillus sp.*	+	+	+	++++	-
3AP1	*Bacillus megaterium*	+	+	++	++	-
EAMR	*Bacillus subtilis*	+	+	+	++++	-
EC 19	*Bacillus weihenstephanensis*	+	+	+++	++++	-
EC 10	*Arthrobacter sp.*	+	+	+++	+++	-
EC 28	*Arthrobacter sp.*	+	+	+++	++	-
EC 33	*Arthrobacter sp.*	+	+	+++	++	-
ECX 16	*Arthrobacter sp.*	+	+	++	++	+
3ZP3	*Arthrobacter sp.*	+	+	+++	++	+
3C5	*Arthrobacter sp.*	+	+	+++	++	+
EC 32	*Arthrobacter scmbi*	+	+	+++	++++	-
EAPAA	*Arthrobacter nicotinovorans*	+	+	+++	++++	+
EC 35	*Rhodococcus sp.*	+	+	+++	++++	+
1C3	*Rhodococcus sp.*	+	+	++	+++	-
1A2	*Rhodococcus wratislaviensis*	+	+	++	+++	-
EC 34	*Rhodococcus erythropolis*	+	+	++	+++	-
EC18	*Agrobacterium tumefaciens*	-	+	-	++	-
3A12	*Mesorhizobium sp.*	-	+	+++	+++	-
1C1	*Burkholderia sp.*	-	+	-	++	+
3A10	*Chryseobacterium palustris*	-	+	++	++	-
ECP37	*Chryseobacterium humi*	-	+	+	+++	-
1ZP4	*Sphingobacterium sp.*	-	+	++	++	-
1C2	*Cupriavidus sp.*	-	+	+	+++	-
EC 6	*Alcaligenes sp.*	-	+	+	+++	-
EAB	*Lysinibacillus fusiformes*	-	+	+	++++	-
EAV	*Pseudomonas sp.*	-	+	+	+++	+
EC 8	*Pseudomonas sp.*	-	+	+++	++++	-
S3X	*Pseudomonas fluorescens*	-	+	+++	++++	-
EAPC 8	*Pseudomonas putida*	-	+	+++	++++	-
EDP 28	*Pseudomonas reactans*	-	+	+	++++	-
1AP2	*Achromobacter sp.*	-	+	+++	++	-
1ZP7	*Variovax paradoxus*	-	+	-	+	-
EC 2	*Shewanella baltica*	-	+	++++	++++	-

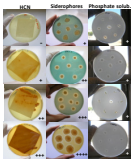

Figure 1. Cyanide (HCN) production; Siderophore production detected as halos surrounding colonies of isolates on CAS medium; Phosphate solubilizing bacteria; (–) negative; (+) positive; (++) good; (+++) very good; (++++) extremely good production.

✓ All isolates produced NH_3 and siderophores

✓ Isolates EC1B, 1C1 and 1ZP7 had negative results for HCN production

✓ Isolates EC19, EC10, EC32, EAPAA, EC35, 3A12, EC8, S3X, EAPC8 and EC2 produced high amounts of both HCN and siderophores

✓ Only 7 isolates had positive results for phosphate solubilization

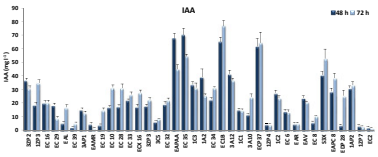

Figure 2. Production of indole acetic acid (mg l^{-1}) of screened isolates after 48 and 72h of incubation. Results are expressed as means ± SE (n= 6 to 12)

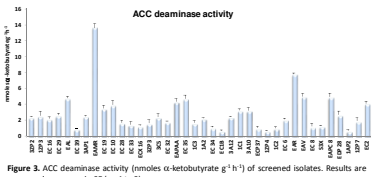

Figure 3. ACC deaminase activity (nmoles α-ketobutyrate g^{-1} h^{-1}) of screened isolates. Results are expressed as means ± SE (n= 4 to 8)

✓ IAA production was detected in all tested rhizobacteria, after 48 and 72h of incubation

✓ Isolates EAPAA, EC35, EC1B and ECP37 produced very high amounts of IAA (> 60 mg l^{-1})

✓ All isolates showed ACC-deaminase activity, however some of presented low levels (e.g. EC39, EC1B, 1ZP4)

✓ Isolate EAMR presented the highest activity for ACC-deaminase

➢ Effect of PGPR on *Trifolium repens* growth

Biomass

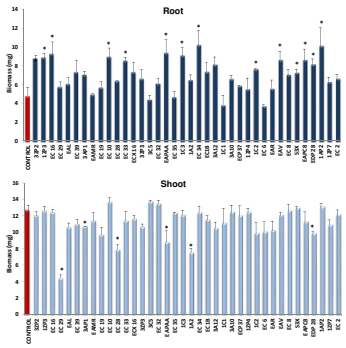

Figure 2. Influence of PGPR on root and shoot biomass (mg) of *Trifolium repens* plants. Results are expressed as means ± SE (n=3-5). * means are significantly different (P< 0.05) from control

✓ The root dry biomass was influenced by rhizobacteria

✓ Several isolates increased significantly (P<0.05) root biomass when compared to control non-inoculated plants. The isolates that better performed were 3ZP2, 1ZP3, EC16, EC10, EC33, EAPAA, 1C3, EC34, 1C2, EAV, S3X, EAPC8 and 1AP2.

✓ However, shoot dry biomass was not significantly affected by rhizobacteria (P>0.05)

Elongation

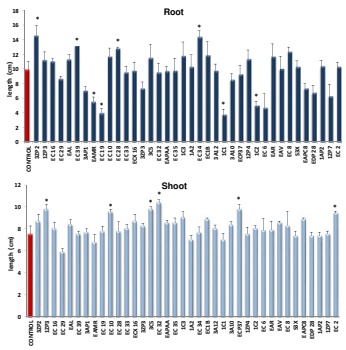

Figure 3. Influence of PGPR on root and shoot elongation (cm) of *Trifolium repens* plants. Results are expressed as means ± SE (n=3-5). * means are significantly different (P< 0.05) from control

✓ Isolates 3ZP2, EC39, EC28 and EC34 significantly (P<0.05) increased root elongation when compared with control plants

✓ Several isolates promoted (P<0.05) shoot growth (1ZP3, EC10, 3C5, EC32, ECP37, EC2)

➢ Plant growth/ bacterial traits

✓ Plant growth promotion was related to bacterial traits: IAA, HCN and siderophore production.

✓ IAA levels at 48 and 72h of incubation were positively correlated (r=0.411 and 0.511, P<0.05, respectively) to root biomass. Root elongation was also correlated to IAA levels at 72h (r=0.350, P< 0.05)

Conclusions

✓ Some of the inoculated rhizobacteria promoted *Trifolium repens* growth enhancing, particularly, root biomass and root elongation

✓ Isolate EC34 (*Rhodococcus erythopolis)* increased root biomass and root elongation by 112% and 44%, respectively. Other isolates, namely *Microbacterium* (3ZP2, EC16), *Achromobacter* (1AP2) and *Arthrobacter* (EC10, EAPAA) species have also showed good results, promoting white clover growth

✓ PGPR may constitute a biological alternative to increase yield and plant resistance on stress disturbed areas improving reforestation processes

Acknowledgements

Sofia Pereira thanks to Fundação para a Ciência e Tecnologia the grant SFRH/BPD/65134/2009. This work was supported by the FCT Project -PTDC-AGR-CFL-111583-2009.