THE BACTERIA ADVANTAGE

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Revegetating soil can be challenging at the best of times, especially when using native species. Encounter some poor quality soil or naturally saline soil and it can be nearly impossible. Soil bacteria can be very beneficial for helping plants get established in challenging conditions. PGPR, or Plant Growth Promoting Rhizobacteria (ref. 1), are a group of soil bacteria that can colonize the roots of plants. The relationship is beneficial to both the plant and the bacteria, with the plant providing a favourable environment for the bacteria and the bacteria providing phytohormones and chemicals to facilitate plant growth.

GERMINATION TRIALS

Earthmaster conducted a number of laboratory germination studies to test the effect of adding bacteria to plant seeds. We used a variety of seed types and soil bacteria to determine the optimum combination to facilitate germination. The bacteria were isolated from soil, plant roots, or seed husks (Figure 1), and coated on to the surfaces of seed. The seeds were then grown in petri dishes on filter paper saturated with varying concentrations of produced water (PW, Table 1). PW is saline water generated during extraction of oil & gas from subsurface reservoirs.

MATCH MAKING

Not all PGPR are beneficial to all seed types. Figure 2 illustrates the effects different bacteria species can have on seed germination. For example, when compared to untreated (UT) seed, bacteria I3 can be beneficial to perennial ryegrass (PRG) as shown by the increase in % germination when grown in PW (expressed as mg/L chloride) and the increased concentration of chloride needed to inhibit germination in 50% of the seed (LC50). However, that same bacteria can be detrimental when coated on annual ryegrass (ARG) seed as shown by decreased % germination and a decreased LC50 when compared to UT seed. Bacteria CMH3 shows beneficial

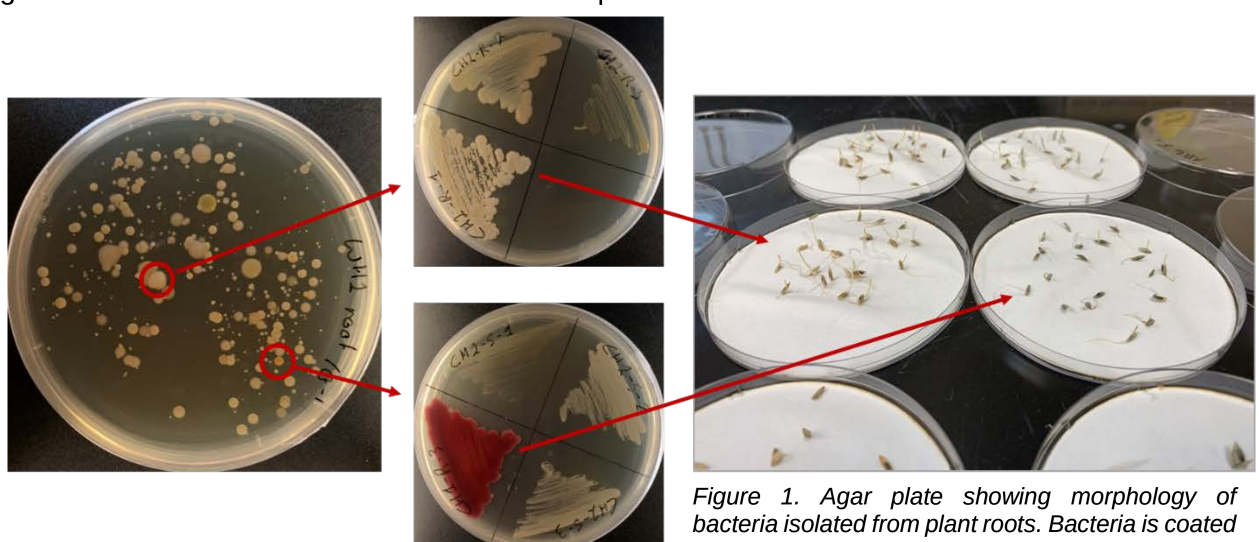

Figure 1. Agar plate showing morphology of bacteria isolated from plant roots. Bacteria is coated onto seeds and used in germination plate assays.

effects to all 4 seed types, facilitating increased germination and tolerance to increased concentrations of PW (LC50).

MORE IS NOT ALWAYS BETTER

The amount of bacteria added to the seed does matter, as too much can negatively affect germination. Figure 3 shows the effects of two concentrations of three types of bacteria on seed germination in PW. For example, lower concentrations (A) of CMH3 are beneficial to PRG which improves the seed's germination parameters; however, higher concentrations (B) are detrimental. In contrast, ARG appears to be minimally affected by the amount of bacteria used for the three types tested.

Table 1. Chemistry for two sources of produced water. The produced waters were diluted to provide the desired concentration, expressed as mg/L chloride.

Sample	Chloride (mg/L)	Calcium (mg/L)	Potassium (mg/L)	Magnesium (mg/L)	Sodium (mg/L)	Sulphate (mg/L)	pH -	EC (dS/m)
Produced Water Samples								
PW1	36775	4223	406	498	18430	1372	7.1	72.8
PW2	78870	9264	4054	1839	32970	997	6.5	125

REGULATORY REQUIREMENTS

Adding bacteria to soil is not permitted in Canada unless the product/bacteria have been approved by the Canadian Food Inspection Agency (CFIA). This applies to bacteria coated on to plant seeds or those added directly to the soil. Bacteria are considered to be supplements (even if they are isolated from the soil in which they are added back) and must be reviewed by the Pre-Market Application Submission Office (PASO) of the CFIA.

	CMH3		UW4		S.r.	
	A	B	A	B	A	B
ARG	1	0	1	1	1	1
PRG	1	-2	0	-1	-1	0
TF	-1	-2	-1	0	-1	-1
TWG	1	1	1	-	0	-1

Figure 3. Effect of bacteria concentration on seed tolerance to chloride. Four types of seed (Annual Ryegrass, Perennial Ryegrass, Tall Fescue and Tall Wheatgrass) were coated with different concentrations (A and B) of three types of bacteria and germinated in produced water PW2. Two parameters (total germination and the time to reach 50% germination) were analyzed and compared to untreated controls. For each parameter, the results were assigned a value or +1, 0 or -1 if they were statistically shown to have a benefit, no change or detriment, respectively, when compared to controls. The two values were added and color coded to produce a heat map of responses across species and treatments.

CONCLUSIONS

When specific bacteria concentrations and seed types are used in combination, the bacteria can greatly improve the germination of seed in challenging conditions, which can lead to improved success in revegetation projects.

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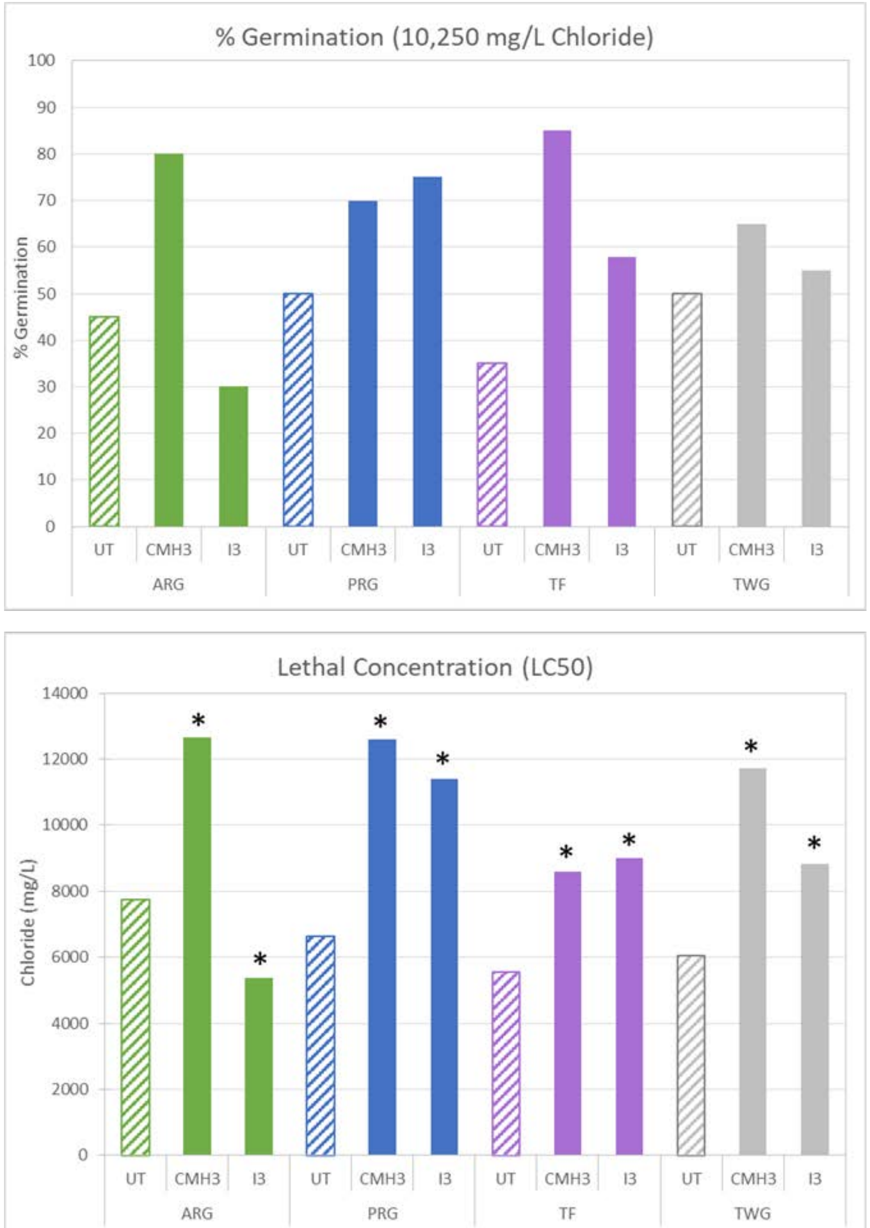

*Figure 2. The effect of bacteria on seed tolerance to chloride. Four types of seed (Annual Ryegrass, Perennial Ryegrass, Tall Fescue and Tall Wheatgrass) were germinated in varying concentrations of produced water (expressed as mg/L chloride, see Table 1). Seeds were untreated (UT) or were treated with one of two different types of bacteria (CMH3 or I3). Top chart shows the bacteria effect on the % of seeds that germinated in produced water (PW2) and the bottom chart shows the bacteria effect on the amount of produced water (PW1) required to prevent germination in 50% of the seeds. * indicates the LC50 values were statistically different from the UT control ($p<0.01$) (ref. 2).*