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SHORT COMMUNICATION

Seedling growth promotion and nitrogen fixation by a bacterial endophyte *Paenibacillus polymyxa* P2b-2R and its GFP derivative in corn in a long-term trial

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Abstract A plant growth promoting endophyte, *Paenibacillus polymyxa* P2b-2R, originally isolated form a lodgepole pine seedling and its green fluorescent protein (GFP) derivative, P2b-2R*gfp*, were evaluated for their ability to survive, fix atmospheric nitrogen (N) and promote plant growth when inoculated into corn (*Zea Mays* L.) in a long-term trial. We were also interested to see the effects of GFP-tagging of P2b-2R on its ability to promote growth of corn seedlings in a long-term study. Corn seedlings were inoculated with either strain P2b-2R or P2b-2R*gfp* and non-inoculated seedlings were treated as controls. Seedlings were harvested after 3 months and evaluated for plant growth promotion (length and biomass) and N fixation (^{15}N foliar dilution assay). Colonization and survival of P2b-2R and P2b-2R*gfp* outside (rhizosphere) and inside (internal tissues) the inoculated seedlings were also determined. Both strains survived inside and outside corn seedlings forming rhizospheric and endophytic colonies in stem and root tissues. Inoculation by P2b-2R strain promoted corn plant growth via enhancing seedling length and biomass by 52 % and 53 %, respectively. Similarly, P2b-2R*gfp* inoculation enhanced seedling length by 68 % and biomass by 67 %. Corn seedlings inoculated with strain P2b-2R derived 30 % of foliar N from the atmosphere and seedlings inoculated with P2b-2R*gfp* derived 32 % of foliar N from the atmosphere. But there was no statistically significant difference between P2b-2R and P2b-2R*gfp* treated seedlings in terms of overall seedling length, biomass and amount of N fixed in this long-term trial. These results combined with the results from an earlier study suggest that *P. polymyxa* P2b-2R and its GFP-tagged derivative is capable of enhancing overall plant growth throughout the life cycle of corn plant.

Keywords Corn · *Paenibacillus polymyxa* · Bacterial endophytes · Plant growth promoting bacteria · Nitrogen fixation · Plant growth promotion

1 Introduction

The use of microorganisms with the aim of improving nutrient availability for plants is an important practice and in some parts of the world necessary for agriculture (Freitas et al. 2007). During the past couple of decades, the use of plant growth promoting bacteria (PGPB) for sustainable agriculture has increased tremendously in various parts of the world. PGPB include those that are free-living, those that form specific symbiotic relationships with plants (e.g., *Rhizobia* spp. and *Frankia* spp.), bacterial endophytes that can colonize some or a portion of a plant's interior tissues, and cyanobacteria (Glick 2012). Endophytic bacteria have been defined as 'bacteria that live within living plant tissues without doing substantive harm or gaining benefit other than securing residency' (Bressan and Borges 2004). Nutrient acquisition by endophytes from host plants also occurs. In contrast to free-living or rhizosphere microorganisms, bacterial endophytes are better protected from abiotic stresses such as extreme variations in temperature, pH, nutrient, and water availability as

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well as biotic stresses such as competition (Chanway et al. 2014). Endophytic bacteria enhance host plant growth and health via various direct and indirect mechanisms. The most important direct growth promotion mechanism is nitrogen (N) fixation (Puri et al. 2015). Döbereiner (1992) introduced the term "endophytic diazotrophic bacteria" to designate all N fixing endophytes that survive poorly in soil but colonize the interior tissues of roots of graminaceous plants, and fix N in association with them (Baldani et al. 1998).

Bal et al. (2012) isolated a potential endophytic diazotrophic bacterium, *Paenibacillus polymyxa* P2b-2R, from surface-sterilized tissues of lodgepole pine seedlings naturally regenerating at a nutrient-poor site near Williams Lake, BC, Canada (52°05′N latitude, 122°54′W longitude, elevation 1300 m, Sub-Boreal Pine Spruce, SBPSxc Zone). *P. polymyxa* P2b-2R was capable of growing on N-free medium, demonstrated significant acetylene reduction activity (Bal et al. 2012) and possessed the *nif*H gene that encodes nitrogenase (Anand and Chanway 2013c). These results provide strong evidence that P2b-2R is capable of fixing atmospheric N, which was confirmed in subsequent reports where this bacterial strain was inoculated into lodgepole pine (Bal and Chanway 2012a; Anand et al. 2013), western red cedar (Bal and Chanway 2012b; Anand and Chanway 2013b), corn (Puri et al. 2015), canola (Puri et al. 2016) and tomato (Padda et al. 2015). Significant plant growth promotion was also observed in these reports when seedlings were inoculated with P2b-2R. Strain P2b-2R significantly promoted plant growth and fixed N from the atmosphere in earlier stages of corn plant growth (10–30 days after sowing) (Puri et al. 2015), but can this bacterial strain keep on doing the same throughout its life, especially during its maturity stage?

A green fluorescent protein (GFP)-tagged derivative of P2b-2R, P2b-2R*gfp*, was generated to observe the endophytic colonization sites of this bacterial strain inside lodgepole pine seedlings (Anand and Chanway 2013a). Although GFP is the most popular autofluorescent protein system used for localization of endophytic bacteria (Chalfie et al. 1994; Zimmer 2002), very few studies have highlighted the effects of tagging GFP to an endophyte. Weyens et al. (2012) reported that GFP-labeling negatively affects the growth-promoting ability and colonization capacity of an endophyte, *Pseudomonas putida* W619, when inoculated into hybrid poplar. Other studies contrasting the plant growth-promoting capacity of wild-type and GFP-tagged endophytes in Jerusalem artichoke (Meng et al. 2014) and *Vitis vinifera* (Compant et al. 2005) found that there was no effect of GFP tagging on the performance of endophyte. Whereas a decade ago, Rodriguez et al. (2006) found that GFP-tagged *Azospirillum brasilense* 8-I strain showed increased N_2-fixation by approximately threefold, up to a twofold increase in exopolysaccharide production, and a significant decrease in indole-3-acetic acid and poly-β-hydroxybutyrate production over the parental strain. Similar findings were reported by Padda et al. (2015), where GFP tagged *P. polymyxa* strain P2b-2R outperformed the parental strain in terms of seedling growth promotion and nitrogen fixation in earlier stages of plant growth of canola (20–40 days after sowing). But, is this positive effect of GFP tagging persistent for longer durations of plant growth?

Thus, our main objectives were (i) to quantify plant growth promotion and N fixation by *P. polymyxa* P2b-2R and its GFP tagged derivative, P2b-2R*gfp*, and (ii) to test the effect of GFP tagging of P2b-2R on its efficacy to promote plant growth and fix N in corn grown for 3 months.

2 Materials and methods

2.1 Seed and bacterial strain

Corn seeds (*var.* Golden Bantam) were obtained from West Coast Seeds (Delta, BC, Canada). *P. polymyxa* strain P2b-2R and its GFP-tagged derivative, P2b-2R*gfp*, were used in this study. Details about the bacterial strain *P. polymyxa* P2b-2R have been described elsewhere (Bal et al. 2012). Transformation of *P. polymyxa* strain P2b-2R with GFP has also been described elsewhere (Anand and Chanway 2013a). Strain P2b-2R is resistant to rifamycin while P2b-2R*gfp* strain is resistant to rifamycin, tetracyline and chloramphenicol. Both strains, P2b-2R and P2b-2R*gfp*, were stored at −80 °C in combined carbon medium (CCM) (Rennie 1981) amended with 20 % (*v*/*v*) glycerol.

2.2 Seedling growth and inoculation

Seedling growth assays were performed in small pots (12cmx8cmx4cm) filled to 67 % capacity with a sterile sand-Turface mixture (69 % w/w silica sand; 29 % w/w Turface; 2 % w/w $CaCO_3$). Each pot was fertilized with 50 mL of a nutrient solution (Chanway et al. 1988) modified by replacing KNO_3 and $Ca(NO_3)$-$4H_2O$ with $Ca(^{15}NO_3)_2$ (5 % ^{15}N label) (0.0576 g/L) and Sequestrene 330 Fe with $Na_2FeEDTA$ (0.02 g/L). Other nutrients in the nutrient solution included (in g/L): KH_2PO_4, 0.14; $MgSO_4$, 0.49; H_3BO_3, 0.001; $MnCI_2$-$4H_2O$, 0.001; $ZnSO_4$-$7H_2O$, 0.001; $CuSO_4$-$5H_2O$, 0.0001; and $NaMoO_4$-$2H_2O$, 0.001. Corn seeds were surface-sterilized by immersion in 30 % H_2O_2 for 90s, followed by three 30s rinses in sterile distilled water. To confirm the effectiveness of surface sterilization, seeds were imprinted on tryptic soy agar (TSA) and checked for contamination 24 h later. Two surface sterilized seeds were aseptically sown in each pot. Corn seeds were inoculated with *P. polymyxa* strain P2b-2R and its GFP-tagged derivative; P2b-2R*gfp*. Non-inoculated seeds were treated with sterile phosphate

buffered saline (PBS) and were used as controls. This resulted in three treatment levels – P2b-2R, P2b-2R*gfp* and control, each replicated 15 times. Bacterial inoculums were prepared by thawing and streaking frozen cultures of strains P2b-2R and P2b-2R*gfp* onto CCM plates amended with 200 mg/L rifamycin and 200 mg/L rifamycin plus 5 mg/L chloramphenicol, respectively, and incubating at 30 °C for 2 days. After the colonies grew, a loopful of each strain was inoculated into 1 L flasks containing 500 mL of fresh CCM broth amended with rifamycin or rifamycin plus chloramphenicol as described previously. Flasks were then secured on a rotary shaker (150 rpm) and agitated for 48 h at room temperature. Bacterial cells were harvested by centrifugation (3000x g, 30 min), washed twice in sterile PBS (pH 7.4) and resuspended in the same buffer to a density of ca. 10^6 cfu/mL. Immediately after sowing the seed, 5 mL of the P2b-2R—PBS suspension or 5 mL of the P2b-2R*gfp* – PBS suspension was pipetted directly into each replicate pot designated for P2b-2R and P2b-2R*gfp*, respectively. Non-inoculated control seeds received 5 mL of sterile PBS. Pots were placed in a growth chamber (Conviron CMP3244, Conviron Products Company, Winnipeg, MB, Canada) under an 18 h photoperiod with an intensity of at least 300 μmol s^{-1} m^{-2} and a 25/18 °C day/night temperature cycle. Seedlings were thinned to contain the single largest germinant per pot once emergence was complete. Seedlings received modified nutrient solution without $Ca(^{15}NO_3)_2$ every 10 days and were watered with sterile distilled water as required. Corn seedlings were grown for 3 months before being harvested for analyses

2.3 Quantification of rhizospheric and endophytic population

For evaluation of rhizosphere populations, 3 randomly chosen seedlings of each treatment were harvested destructively 3 months after inoculation. Seedlings were removed from pots and loosely adhering soil particles were removed from roots with gentle shaking. Roots were then separated from shoots, placed in sterile Falcon tubes (50 mL; BD Biosciences, CA, USA) filled with 10 mL of sterile PBS and shaken on a vortex mixer at 1000 rpm for 1 minute. Serial dilutions were performed, and 0.1 mL aliquots were plated on CCM amended with rifamycin (200 mg/L) for P2b-2R inoculated and control seedlings, and on CCM plates amended with 200 mg/L rifamycin plus 5 mg/L chloramphenicol for P2b-2R*gfp* inoculated seedlings. Plates were incubated at room temperature for 7 days after which colonies were counted. Roots were oven-dried at 65 °C for 48 h before weighing. Rhizospheric bacterial populations were then calculated as colony forming units (cfu) per gram of dry root tissue.

Two randomly selected corn seedlings from each treatment were harvested destructively 3 months after inoculation to evaluate endophytic colonization. Seedlings were rinsed in a 2 L flask containing 1 L sterile distilled water for removal of loosely adhering growth media. Seedlings were then surface-sterilized in 0.6 % (w/v) NaClO for 2 min, rinsed three times with sterile distilled water and imprinted on TSA plates for 24 h to check for surface contamination. Samples of root, stem and leaf tissues were triturated separately in 1 mL of sterile PBS using a mortar-pestle. Triturated tissue suspensions of control and P2b-2R inoculated seedlings were diluted serially and 0.1 mL of each dilution was plated onto CCM supplemented with 200 mg/L rifamycin. Similarly, tissue suspensions of P2b-2R*gfp* inoculated seedlings were plated onto CCM supplemented with 200 mg/L rifamycin and 5 mg/L chloramphenicol. Plates were incubated at room temperature for 7 days and the number of cfu was evaluated. Data from seedlings that showed contamination after surface sterilization were excluded from further analysis.

2.4 ^{15}N foliar dilution analysis and seedling growth response

For ^{15}N foliar dilution analysis, 5 corn seedlings of each treatment were harvested destructively 3 months after inoculation. Oven dried foliage of each seedling from each treatment was ground to a particle size <2 mm using a mortar-pestle, mixed thoroughly and a 1 mg sample was sent to the Stable Isotope Facility in the Department of Forest and Conservation Sciences, Faculty of Forestry, University of British Columbia for determination of foliar N content and atom % ^{15}N excess. The amount of fixed N in foliage was estimated by calculating the percent N derived from the atmosphere (%Ndfa) (Rennie et al. 1978):

$$\%\ \text{Ndfa} = \left[1 - \left\{\text{atom}\ \%^{15}\text{N excess (inoculated plant)} \,/\, \text{atom}\ \%^{15}\text{N excess (control plant)}\right\}\right] \times 100$$

To evaluate seedling growth response, 5 corn seedlings from each treatment were harvested destructively 3 months after inoculation, and separated into roots and shoots. Shoot and root length of each seedling was then measured. Stem, leaves and roots of each seedling were then oven dried at 65 °C for 48 h before being weighed to evaluate dry weight of plant material.

2.5 Statistical analysis

A completely randomized experimental design with 15 replicates per treatment was used to assess the treatment effects of bacteria on growth of corn seedlings. Analysis of variance (ANOVA) was performed to determine treatment effects on atom % ^{15}N excess, foliar N content,

shoot, root and seedling dry weight, and shoot, root and seedling length. To test whether there are differences among the three treatments, F-test was performed and to check for difference between pairs of treatments we used *t*-test. The statistical package, SAS v9.4 (Copyright © 2013, SAS Institute Inc., Cary, NC, USA), was used to perform statistical analyses. The confidence level, α, was set to 0.05 to determine the significance of the model and treatment effects.

3 Results

3.1 Endophytic and rhizospheric colonization

P. polymyxa strain P2b-2R and P2b-2R*gfp* colonized internal tissues of corn roots with population densities of 2.11×10^6 cfu/g fresh weight and 7.52×10^6 cfu/g fresh weight, respectively. Endophytic colonization of stem tissues by P2b-2R and P2b-2R*gfp* was also observed with population densities of 2.34×10^3 cfu/g fresh weight and 5.67×10^2 cfu/g fresh weight. P2b-2R and P2b-2R*gfp* colonized the corn rhizosphere with population densities of 3.20×10^6 cfu/g dry root and 9.31×10^6 cfu/g dry root, respectively. No evidence of rhizospheric or endophytic colonization was found in control plants.

3.2 Seedling growth response and N-fixation

Seedling growth (length and biomass) was promoted significantly by inoculation with P2b-2R*gfp* or P2b-2R in comparison to control plants. Seedlings inoculated with P2b-2R*gfp* were 68 % longer than controls 3 months after inoculation (Fig. 1; Table 2). Similarly, P2b-2R inoculation led to the enhancement of seedling length by 52 % (Fig. 1; Table 2). P2b-2R*gfp* inoculation promoted seedling dry weight (biomass) by 67 % as compared to the controls after 3 months of inoculation (Fig. 2; Table 2). P2b-2R inoculation also increased the seedling biomass by 53 % as compared to the controls (Fig. 2; Table 2). It is important to mention that root dry weight of P2b-2R*gfp* inoculated seedlings was nearly 90 % greater than the controls and 24 % greater than P2b-2R inoculated seedlings (statistically significant). Although there was an 11 % difference in seedling length and 9 % difference in seedling biomass between P2b-2R*gfp* and P2b-2R inoculated seedlings, these were not statistically significant (Table 2).

Based on percent foliar ^{15}N atom excess data, corn seedlings inoculated with P2b-2R*gfp* were found to derive 32 % of foliar N from the atmosphere after 3 months of inoculation (Table 1). Similarly, P2b-2R inoculated seedlings derived

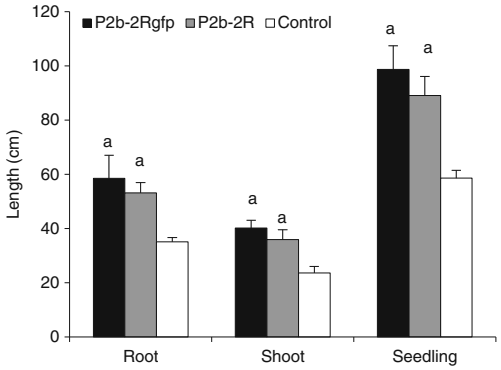

Fig. 1 Root length, shoot length and seedling length (means and standard errors; $n = 5$ seedlings per treatment) of corn seedlings inoculated with either *P. polymyxa* P2b-2R or its GFP-tagged derivative, P2b-2R*gfp*, harvested 3 months after inoculation. Seedling length was the sum of shoot length and root length measurements. [a]$P < 0.05$ (significantly different from control)

30 % of foliar N from the atmosphere (Table 1). Foliar N content of seedlings inoculated with P2b-2R*gfp* was 31 % higher than the control after 3 months of inoculation (Table 1; Table 2). In a similar way, P2b-2R inoculated seedlings had 27 % higher foliar N than the control (Table 1; Table 2). There was no statistically significant difference in foliar N content and atom $\%^{15}N$ excess in foliage values between P2b-2R*gfp* inoculated seedlings and P2b-2R inoculated seedlings.

4 Discussion

Since, corn tissues produced high autofluorescence when viewed under confocal laser microscope and the emissivity of GFP was low, we were not able to detect endophytic

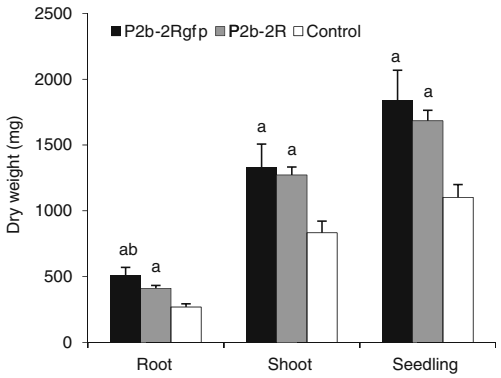

Fig. 2 Root dry weight, shoot dry weight and seedling dry weight (means and standard errors; $n = 5$ seedlings per treatment) of corn seedlings inoculated with either *P. polymyxa* P2b-2R or its GFP-tagged derivative, P2b-2R*gfp*, harvested 3 months after inoculation. Seedling dry weight was the sum of shoot dry weight and root dry weight measurements. [a]$P < 0.05$ (significantly different from control); [b]$P < 0.05$ (significantly different from P2b-2R)

Table 1 Atom percent ^{15}N excess in foliage, percent foliar N and percent N derived from the atmosphere (%Ndfa), developed from corn seeds inoculated with *P. polymyxa* strain P2b-2R and its GFP-tagged derivative, P2b-2R*gfp*, measured 3 months after inoculation

Treatment	Atom $\%^{15}N$ excess in foliage	%Foliar N	%Ndfa
P2b-2R*gfp*	0.43* ± 0.01[a]	0.58* ± 0.04	32.19
P2b-2R	0.44* ± 0.01	0.56* ± 0.02	30.19
Control	0.64 ± 0.01	0.44 ± 0.02	-

*$P < 0.05$ (significantly different from control)

[a] Mean ± standard error; $n = 5$ for atom $\%^{15}N$ excess and %Foliar N

colonies of GFP tagged *P. polymyxa* P2b-2R by confocal laser microscope. So, we used direct plating technique of surface-sterilized tissue extracts on CCM, which is a selective growth medium. *P. polymyxa* P2b-2R and its GFP tagged derivative, P2b-2R*gfp*, formed consistent endophytic populations in corn roots and stem confirming that the growth promotion detected in the inoculated seedlings was bacteria driven. The endophytic population size of P2b-2R and P2b-2R*gfp* in corn roots was comparable to those in some previous reports (Puri et al. 2015, 2016; Padda et al. 2015). Puri et al. (2015) reported that *P. polymyxa* P2b-2R is not able to form endophytic colonies inside stem tissues of corn in earlier stages of plant development (10–30 days after sowing). But, after 3 months of sowing and inoculation, we were able to detect endophytic colonies of both P2b-2R and P2b-2R*gfp* in stem tissues of corn. This clearly indicates that these bacterial strains might take some time to colonize and form a detectable population size inside stem tissues, which means that the growth promotion and N-fixation achieved in the earlier stages of plant growth is mainly due to the action of rhizospheric P2b-2R and endophytic P2b-2R in root tissues. Strains P2b-2R and P2b-2R*gfp* were also present in the rhizosphere of corn seedlings 3 months after inoculation and the population size was comparable to other studies reported about this bacterial strain (Anand et al. 2013; Puri et al. 2015, 2016). Thus, it can be concluded that *P. polymyxa* P2b-2R and its GFP-tagged derivative are able to survive inside and outside the corn plant throughout its life cycle.

Table 2 Percent differences of various growth parameters between different treatment combinations, developed from corn seeds inoculated with *P. polymyxa* strain P2b-2R and its GFP-tagged derivative P2b-2R*gfp* measured 3 months after inoculation

	% Difference between P2b-2R*gfp* and control[a]	% Difference between P2b-2R and control[b]	% Difference between P2b-2R*gfp* and P2b-2R[c]
Root length	66.86	51.63	10.04
Shoot length	70.57	52.42	11.91
Seedling length	68.35	51.94	10.80
Root dry weight	89.07	52.76	23.77
Shoot dry weight	59.73	52.70	4.60
Seedling dry weight	66.90	52.72	9.29
Foliar N	31.82	27.27	3.57

[a] Percent difference = {(growth parameter of P2b-2R*gfp* treated seedlings – growth parameter of control seedlings) / growth parameter of control seedlings} x 100 %

[b] Percent difference = {(growth parameter of P2b-2R treated seedlings – growth parameter of control seedlings) / growth parameter of control seedlings} x 100 %

[c] Percent difference = {(growth parameter of P2b-2R*gfp* treated seedlings – growth parameter of P2b-2R treated seedlings) / growth parameter of P2b-2R treated seedlings} x 100 %

Based on the differences in seedling length and biomass, we found that our endophyte *P. polymyxa* P2b-2R and its GFP-tagged derivative promote the overall growth of corn plant. *P. polymyxa* is well known for its plant growth promoting characteristics, viz., producing hydrolytic enzymes (Nielsen and Sørensen 1997) and bio-films (Timmusk et al. 2005; Haggag and Timmusk 2008) to antagonize pathogenic soil organisms; producing phytohormones like cytokinins (Timmusk et al. 1999), gibberlins (Lal and Tabacchioni 2009) and auxin-related compounds such as indole-3-acetic acid (IAA) (Lebuhn et al. 1997); fixing atmospheric N (Anand et al. 2013; Puri et al. 2015, 2016); solubilizing phosphorous in soil (Çakmakçi et al. 2006); mitigating negative effects of drought stress in plants (Figueiredo et al. 2008); increasing soil porosity (Gouzou et al. 1993). As opposed to the concerns reported by Weyens et al. (2012) that GFP-tagging of an endophyte could reduce its ability to promote plant growth by suppressing the colonization capacity and growth promoting traits, we didn't observe any reduction in growth promoting ability of *P. polymyxa* P2b-2R after tagging it with GFP. Padda (2015) reported that GFP tagging of *P. polymyxa* P2b-2R increased its ability to promote corn plant growth at earlier stages of plant development, but our results suggest that such enhancement is short term and would diminish as the plant grows and progresses in its life cycle.

Although *P. polymyxa* possess several traits that can result in plant growth promotion, results from our foliar ^{15}N dilution assay suggest that this growth promotion might have been caused by an increase in the amount of N derived from the atmosphere. As corn is an N demanding crop, reliability on biological N-fixation increases when it is grown under N-limited conditions. Significant foliar ^{15}N dilution has been observed previously and is thought to be due to a compensatory mechanism where less N is taken up from soil when fixed N is accumulated by the plant due to the N-fixing bacterial colonization (Anand et al. 2013; Anand and Chanway 2013b; Bal and Chanway 2012a, b). Since it has been reported that *P. polymyxa* P2b-2R and its GFP-tagged derivative can fix N when inoculated into corn during earlier stages (10–40 days

after sowing) of plant growth (Padda 2015; Puri et al. 2015), our results indicate that these bacterial strains keep on fulfilling the plant N requirements through biological N-fixation till the later stages of corn plant's life cycle. Increasing reliance on biological N-fixation with seedling age and concomitant decreasing dependence on soil N has been observed previously in sugarcane, where %Ndfa rose from 6 to 55 % during the interval of 100–250 days after emergence (Urquiaga et al. 1992).

To conclude, our results demonstrate that growth promotion and N-fixation characteristics of *P. polymyxa* P2b-2R and its GFP-tagged derivative benefit the corn plant throughout its life. More than 50 % increase in seedling length and biomass after 3 months of inoculation is considerable and shows that growth promotion is not limited to the earlier stages of plant development (Puri et al. 2015). In hopes to limit the detrimental effects of chemical fertilizers, the use of this plant growth promoting endophyte could pose a better option to act as biofertilizer, thus aiding sustainable agriculture with minimum impacts on the environment. Another main conclusion from this study is that the positive effect of GFP-tagging of *P. polymyxa* P2b-2R reported (Padda 2015) is only limited to earlier stages of corn plant growth and diminishes near the end of its life cycle.

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References

Anand R, Chanway CP (2013a) Detection of GFP-labeled *Paenibacillus polymyxa* in auto-fluorescing pine seedling tissues. Biol Fertil Soils 49:111–118. doi:10.1007/s00374-012-0727-9

Anand R, Chanway C (2013b) N_2-fixation and growth promotion in cedar colonized by an endophytic strain of *Paenibacillus polymyxa*. Biol Fertil Soils 49:235–239. doi:10.1007/s00374-012-0735-9

Anand R, Chanway CP (2013c) *nif* gene sequence and arrangement in the endophytic diazotroph *Paenibacillus polymyxa* strain P2b-2R. Biol Fertil Soils 49:965–970. doi:10.1007/s00374-013-0793-7

Anand R, Grayston S, Chanway CP (2013) N_2-fixation and seedling growth promotion of lodgepole pine by endophytic *Paenibacillus polymyxa*. Microb Ecol 66:369–374. doi:10.1007/s00248-013-0196-1

Bal A, Chanway CP (2012a) Evidence of nitrogen fixation in lodgepole pine inoculated with diazotrophic *Paenibacillus polymyxa*. Botany 90:891–896. doi:10.1139/b2012-044

Bal A, Chanway CP (2012b) ^{15}N foliar dilution of western red cedar in response to seed inoculation with diazotrophic *Paenibacillus polymyxa*. Biol Fertil Soils 48:967–971. doi:10.1007/s00374-012-0699-9

Bal A, Anand R, Berge O, Chanway CP (2012) Isolation and identification of diazotrophic bacteria from internal tissues of *Pinus contorta* and *Thuja plicata*. Can J For Res 42:807–813. doi:10.1139/x2012-023

Baldani JI, Olivares FL, Hemerly AS et al (1998) Nitrogen-fixing endophytes: recent advances in the association with graminaceous plants grown in the tropics. In: Elmerich EC (ed) Biological nitrogen fixation for the 21st century. Springer, The Netherlands, pp 203–206. doi:10.1007/978-94-011-5159-7_90

Bressan W, Borges MT (2004) Delivery methods for introducing endophytic bacteria into maize. Biocontrol 49:315–322. doi:10.1023/B:BICO.0000025372.51658.93

Çakmakçi R, Dönmez F, Aydın A, Şahin F (2006) Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. Soil Biol Biochem 38:1482–1487. doi:10.1016/j.soilbio.2005.09.019

Chalfie M, Tu Y, Euskirchen G, Ward W, Prasher D (1994) Green fluorescent protein as a marker for gene expression. Science 263:802–805. doi:10.1126/science.8303295

Chanway CP, Holl FB, Nelson LM (1988) Cultivar-specific growth promotion of spring wheat (*Triticum aestivum* L.) by coexistent *Bacillus* species. Can J Microbiol 34:925–929. doi:10.1139/m88-164

Chanway C, Anand R, Yang H (2014) Nitrogen fixation outside and inside plant tissues. In: Ohyama T (ed) Advances in biology and ecology of nitrogen fixation. InTech, Croatia, pp 3–23. doi:10.5772/57532

Compant S, Reiter B, Sessitsch A, Nowak J, Clément C, Barka EA (2005) Endophytic colonization of Vitis vinifera L. by plant growth-promoting bacterium Burkholderia sp. strain PsJN. Appl Environ Microbiol 71:1685–1693. doi:10.1128/AEM.71.4.1685-1693.2005

Döbereiner J (1992) Recent changes in concepts of plant bacteria interactions: endophytic N_2 fixing bacteria. Ciênc Cult 44:310–313

Figueiredo MVB, Burity HA, Martínez CR, Chanway CP (2008) Alleviation of drought stress in the common bean (*Phaseolus vulgaris* L.) by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. Appl Soil Ecol 40:182–188. doi:10.1016/j.apsoil.2008.04.005

Freitas A, Vieira CL, Santos C, Stamford N, Lyra M (2007) Caracterização de rizóbios isolados de Jacatupé cultivado em solo salino do estado de Pernambuco, Brasil. Bragantia 66:497–504. doi:10.1590/S0006-87052007000300017

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012:963401. doi:10.6064/2012/963401

Gouzou L, Burtin G, Philippy R, Bartoli F, Heulin T (1993) Effect of inoculation with *Bacillus polymyxa* on soil aggregation in the wheat rhizosphere: preliminary examination. Geoderma 56:479–491. doi:10.1016/0016-7061(93)90128-8

Haggag WM, Timmusk S (2008) Colonization of peanut roots by biofilm-forming *Paenibacillus polymyxa* initiates biocontrol against crown rot disease. J Appl Microbiol 104:961–969. doi:10.1111/j.1365-2672.2007.03611.x

Lal S, Tabacchioni S (2009) Ecology and biotechnological potential of *Paenibacillus polymyxa*: a minireview. Indian J Microbiol 49:2–10. doi:10.1007/s12088-009-0008-y

Lebuhn M, Heulin T, Hartmann A (1997) Production of auxin and other indolic and phenolic compounds by *Paenibacillus polymyxa* strains isolated from different proximity to plant roots. FEMS Microbiol Ecol 22:325–334. doi:10.1016/S0168-6496(97)00007-X

Meng X, Yan D, Long X, Wang C, Liu Z, Rengel Z (2014) Colonization by endophytic *Ochrobactrum anthropi* Mn1 promotes growth of Jerusalem artichoke. Microb Biotechnol 7:601–610. doi:10.1111/1751-7915.12145

Nielsen P, Sørensen J (1997) Multi-target and medium-independent fungal antagonism by hydrolytic enzymes in *Paenibacillus polymyxa* and *Bacillus pumilus* strains from barley rhizosphere. FEMS Microbiol Ecol 22:183–192. doi:10.1016/S0168-6496(96)00089-X

Padda KP (2015) Impact of GFP-modification of *Paenibacillus polymyxa* on its ability to enhance growth of corn, canola and tomato

seedlings. Master's Thesis, University of British Columbia, Canada. http://hdl.handle.net/2429/55019. Accessed 11 February 2016

Padda KP, Puri A, Chanway CP (2015) Effect of GFP tagging of *Paenibacillus polymyxa* P2b-2R on its ability to promote growth of canola and tomato seedlings. Biol Fertil Soils. doi:10.1007/s00374-015-1083-3

Puri A, Padda KP, Chanway CP (2015) Can a diazotrophic endophyte originally isolated from lodgepole pine colonize an agricultural crop (corn) and promote its growth? Soil Biol Biochem 89:210–216. doi:10.1016/j.soilbio.2015.07.012

Puri A, Padda KP, Chanway CP (2016) Evidence of nitrogen fixation and growth promotion in canola (*Brassica napus* L.) by an endophytic diazotroph *Paenibacillus polymyxa* P2b-2R. Biol Fertil Soils 52:119–125. doi:10.1007/s00374-015-1051-y

Rennie RJ (1981) A single medium for the isolation of acetylene-reducing (dinitrogen-fixing) bacteria from soils. Can J Microbiol 27:8–14. doi:10.1139/m81-002

Rennie RJ, Fried M, Rennie DA (1978) Concepts of ^{15}N usage in dinitrogen fixation studies. In: Isotopes in biological dinitrogen fixation. International Atomic Energy Agency, Vienna, pp 107–131

Rodriguez H, Mendoza A, Antonia Cruz M, Holguin G, Glick BR, Bashan Y (2006) Pleiotropic physiological effects in the plant growth-promoting bacterium *Azospirillum brasilense* following chromosomal labeling in the clpX gene. FEMS Microbiol Ecol 57:217–225. doi:10.1111/j.1574-6941.2006.00111.x

Timmusk S, Nicander B, Granhall U, Tillberg E (1999) Cytokinin production by *Paenibacillus polymyxa*. Soil Biol Biochem 31:1847–1852. doi:10.1016/S0038-0717(99)00113-3

Timmusk S, Grantcharova N, Wagner EH (2005) *Paenibacillus polymyxa* invades plant roots and forms biofilms. Appl Environ Microbiol 71:7292–7300. doi:10.1128/AEM.71.11.7292-7300.2005

Urquiaga S, Cruz KHS, Boddey RM (1992) Contribution of nitrogen fixation to sugar cane: nitrogen-15 and nitrogen-balance estimates. Soil Sci Soc Am J 56:105–114. doi:10.2136/sssaj1992.03615995005600010017x

Weyens N, Boulet J, Adriaensen D et al (2012) Contrasting colonization and plant growth promoting capacity between wild type and a gfp-derative of the endophyte *Pseudomonas putida* W619 in hybrid poplar. Plant Soil 356:217–230. doi:10.1007/s11104-011-0831-x

Zimmer M (2002) Green fluorescent protein (GFP): applications, structure, and related photophysical behavior. Chem Rev 102:759–781. doi:10.1021/cr010142r