Annual Report, 2011

to

Alberta Pulse Growers

Potato Growers of Alberta

Irrigated Cropping Systems for Sustainable Management

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INTRODUCTION

Irrigated cropping offers a dual challenge of producing high value crops while maintaining soil quality. Common irrigated crops (e.g. potatoes, beans, sugar beets) produce little crop residue for return to the soil and tight rotations may have long-term detrimental effects on our soil resource in terms of diminished soil quality and increased erosion risk.

An irrigated rotation study was initiated in 2000 to examine the impact of conventional and sustainable rotations for potatoes, sugar beets, beans, soft wheat and timothy. The merits of each of six rotations are judged using data on crop yield and quality, weed, insect, and disease pressures and soil quality.

Our objectives were to devise crop sequences and tillage management systems for irrigated land that: (1) optimize crop response; (2) reduce soil erosion, enhance soil quality and promote long-term sustainability; and (3) minimize weed, insect and disease pressures.

EXPERIMENTAL TREATMENTS

Crop rotations (Table 1) were established in spring 2000 at the Vauxhall Sub-station of Agriculture and Agri-Food Canada. The 2010 growing season represented the 11th growing season of this study and, and as such completed two full cycles of the 5-yr rotations. Each phase of each rotation was represented resulting in 26 treatments. These were replicated four times to give 104 plots. The plot dimensions were 10 x 18.3 m with a 2.1 m interplot area between each plot.

The sustainable rotations are built around four specific management practices:

(1) direct seeding or reduced tillage where possible
(2) fall-seeded cover crops where possible
(3) composted cattle manure as a substitute for inorganic fertilizer
(4) straight cutting of solid seeded rather than undercutting of wide-row seeded beans

Table 2 gives an outline of the cropping history of each of the 26 rotation phases.

WEATHER CONDITIONS, 2011

The 2011 growing season (April 1– Sept. 30) precipitation was right on the 30-yr (1981-2010) normal of 265 mm. June precipitation was 146% of normal (115 vs. 78 mm) which led to flooding of some plots. Seeding dates were as follows: Wide-row beans, May 14; Narrow-row beans, May 18; Potatoes, May 17; Sugar beets, May 17 and Wheat, May 16.

Harvest dates were as follows: beans, September 22, potatoes, September 16; sugar beet, September 20 and wheat, September 14, 2012.

CROP PERFORMANCE, 2011

Bean Yields

Bean yields in 2011 were highest in the 12 years of the study. There was a significant rotation effect on bean yield with the 4-yr conventional rotation yielding significantly lower than all others except the 3-yr conventional rotation (Table 1). There were significant differences between rotations for the agronomic characters such as plant stand, maturity and *Sclerotinia* (Table 2). Plant height and bacterial blight incidence were not significantly affected by rotation (Table 2). Sustainable rotations averaged significantly higher yields than conventional (3886 vs. 2791 kg/ha, Table 3). However, plant stand was lower with sustainable and maturity was faster (Table 3). *Sclerotinia* incidence was significantly higher on sustainable narrow-row rotations than conventional wide row (8.5 vs. 1.6%, Table 3). All contrasts for previous crops (after potatoes vs. after sugar beet) were non-significant for beans in 2011 (Table 4).

Potato Yields
In 2011, potatoes (Russet Burbank) were grown on 24 plots (6 rotations x 4 replicates). There were significant differences in yield between the 6 potato rotation treatments in 2011 (Table 5). Yields on the longer 5 and 6 yr sustainable rotations were significantly higher than the 4-yr conventional rotation (Table 5). For potato agronomic parameters, significant rotation effects were found for date to emergence, vigour and total yield, but not stand count, marketable yield, oversize yield , undersize yield, marketable yield/plant and oversize yield/plant (Table 6). For quality and disease characteristics, there was no rotation effect on marketable deformities or oversize deformities (Table 7). However, hollow heart and specific gravity were affected by rotation (Table 7).

All conventional vs. sustainable contrasts for potatoes were non-significant except for marketable yield (sustainable significantly higher than conventional, Table 8). Only two parameters were significant in previous crops contrasts (after wheat vs. after beans, Table 9). Vigour was higher after beans while hollow heart was higher after wheat.

Sugar Beet Yields
Each plot was approximately 33 ft wide and 60 ft long, with 7-ft borders between plots within the replication and 60-ft borders between each of the four replications. Exact measurements were conducted on each individual plot. The experiment was conducted as a randomized complete block design. Table 10 details experimental and cultural methods for the sugar beet plots. Individual sugar beet plots were 18 rows wide, and were planted to a final stand. Commercial sugar beet seed (variety BTS 47RR65) was used to plant the sugar beet plots in 2011. This was the third year that a Roundup Ready® variety was planted in this rotation trial, as well as being the third year Roundup Ready® sugar beets were planted commercially in Alberta.

At harvest, the centre 14 rows of each plot were harvested for yield, as measured with a weigh scale on the harvester. The outside two rows on each edge of the plot were not included in the data due to border effects. From each plot, three two-row samples were collected for quality and tare dirt analysis. The selection of the quality rows varied from plot to plot to avoid rows with crop damage, typically occurring on the centre rows from the side roll irrigation system. All sub-sampled beets were washed, weighed and passed through a multi-saw rasp to provide brei for immediate determination of sugar, amino-nitrogen, sodium and potassium. Individual quality analyses from each plot were averaged together to provide one overall quality dataset per plot.

Results from the 2011 rotation experiment are found in Tables 11 and 12. The vast majority of the 2011 Alberta commercial harvest occurred in October, so a comparison between the Vauxhall research station plots harvested in September and commercial yield and quality is somewhat irrelevant. Because of the earlier harvest date for the Vauxhall station research plots, yield and quality was somewhat lower than commercial fields in the area.

Significant treatment differences occurred in beet yield (Table 11). Beet yield for the 6-year treatment (after timothy) was significantly lower than 4-year sustainable and 5-year treatments. Beet yield for the 4-year conventional treatment was significantly lower than the 4-year sustainable treatment. It is noted that spring waterlogged soil in the 6-year treatment in replication 4 likely was a factor in reducing the average yield of this treatment in 2011. Average yields in 2011 were higher than in the 2010 rotation trial; however, the trend of the 4-year sustainable and 5-year treatments being higher in yield than the 4-year conventional and 6-year treatments were similar between years. There were no significant yield or quality differences between replications in this 2011 trial. Amino nitrogen, sodium and potassium levels were not significantly different between treatments or between replications (Table 12).

Beet yield for the 2011 research station plots at Vauxhall were 21% higher than the 2010 plots primarily because the 2011 plots had an extra 15 growing days and summer temperatures were warmer. Plant stand in 2011 was within the target range of 125 to 150 beets per 100 feet of row. In 2001 significant treatment differences occurred in extractable sugar per tonne and sugar (%). No differences were observed in 2002 to 2008 among treatments for any of the measured parameters. Extractable sugar per acre was significantly different between treatments in 2009 and 2010. Significant treatment differences in root yield were observed in 2009, 2010 and 2011.

Wheat Yields

The soft white spring wheat variety AC Andrew was planted in 2011. The continuous wheat rotation (12 years duration in 2011) had significantly ($P = 0.05$) lower yield than the other rotations (Table 13). Wheat grown on the 3-5 yr rotations yielded on average 56% higher than continuous wheat. However, wheat grown in all other rotations varied from 6.54 to 6.73 Mg/ha, a difference of only 3% (Table 13). Wheat maturity, plant stand and take-all rating were significantly affected by rotation (Table 14) but plant height and test weight were not. Take-all rating was significantly higher on continuous wheat.

Protein content was highest on the 5-yr sustainable rotation (10.23%), being significantly higher than the 3-yr conventional rotation (9.33% [Table 15]. Grain hardness (NIRI), sedimentation, P and K concentration were unaffected by rotation (Table 15). However, hardness (PSI), Ca and S concentration were affected significantly by rotation.

Conventional vs. sustainable practices were non-significant for all wheat parameters except S concentration where sustainable (0.115%) was higher than conventional (0.108%, Table 16). The preceding crop contrasts (after beans vs. after potatoes) were non-significant for all wheat parameters (Table 17).

SOIL PROPERTIES, 2011

Soil Organic Carbon

Soil organic carbon measured in fall 2011, showed that levels were significantly higher in the 5-yr sustainable rotation (36.9 Mg/ha, 0-15 cm) than in the 3-yr (26.8 Mg/ha) and 4-yr conventional) 24.6 Mg/ha) rotations (Table 18). Fig. 1 shows the trends in soil organic C levels over time since baseline samples were taken in fall 1999. Substantial increases are seen in the sustainable rotations, especially the 5-yr), whereas conventional rotations initially declined and then levelled off. Continuous wheat stayed more or less flat over the 12 yr and therefore acts as a good benchmark.

Soil Microbiological Parameters

Microorganisms are sensitive to soil disturbance and carbon additions in the form of compost, cover crop residues and are therefore good indicators of soil quality changes. β-glucosidase is important in the soil C cycle because of its role in degrading cellulose, the most prevalent polysaccharide in soil and its activity is another indicator of biological soil quality. Because it catalyzes the decomposition of cellobiose is β-glucosidase sensitive to inputs of cellulose in the form of compost or cover crop biomass. Wheat and oat plots were sampled for micribiological measurements on July 28, 2011.

For non-rhizosphere (bulk) soil, rotation had a significant effect on microbial biomass carbon (MBC), Shannon index and β-glucosidase activity (Table 19). The 5-yr sustainable phase 2 (wheat after sugar beet) had the highest MBC (623 mg/kg soil) which was significantly higher

than the continuous wheat (477 mg/kg soil), 3-yr (372 mg/kg soil) and 4-yr (435 mg/kg soil) conventional wheat and 5 yr sustainable phase 1 (wheat after potatoes) [424 mg/kg soil]. The Shannon index was significantly lower on the two 4 yr rotations (1.76-1.77) than on all other rotations (2.21-2.54) [Table 18]. For rhizosphere soil (adhering to wheat roots) rotational effects were less distinct with MBC and Shannon index being non-significant (Table 20). However, β-glucosidase activity showed a rotational effect for rhizosphere soil, showing it is a more sensitive indicator of rotational management than either MBC or Shannon index Activity on the 4-yr conventional rotation was significantly lower (94 mg nitrophenol/kg soil/hr) than all others (152-243 mg nitrophenol/kg soil/hr), while the 3 yr conventional rotation was significantly lower than all others except continuous wheat.

For conventional vs. sustainable rotation contrasts, all six parameters compared (MBC, Shannon index, and β-glucosidase activity for both non-rhizosphere and rhizosphere soil) showed significantly higher values for sustainable vs. conventional rotations (Table 21). MBC on sustainable rotations was 26% higher for non-rhizoshphere and 22% higher for rhizosphere than conventional rotations. Shannon index was 15-17% higher on sustainable rotations while β-glucosidase activity was ~2 times higher on sustainable rotations.

For the previous crop contrasts only Shannon index on the non-rhizosphere soil showed a significant effect, being significantly lower on wheat following potatoes (2.01) than beans (2.38) [Table 22].

Summary

2011 marked the end of the regular rotation as such. In 2012, all plots will be seeded to narrow-row beans as a bioassay of historical management over 12 years (2000-11). The attached PowerPoint file (saved in pdf format) summarizes the longer-term results from the 12 yr study. This presentation was given at the Alberta Soil Science Workshop, Edmonton, AB, February 14-16, 2012

Achievements

Pageni, B.B., Lupwayi, N.Z., **Larney, F.J.**, Pearson, D.C. and Blackshaw, R.E. 2011. Soil microbial biomass and diversity in irrigated crop systems. Abstr. Poster Presentation, Soil Ecology Society Meeting, May 24-27, 2011, Kelowna, BC.

Larney, F.J. 2011. Changes in cropping and tillage practices in Alberta: A recent revolution. Pages 78-84 *in* Proc. Int. Conf. No-till and Crop Diversification as Base for Soil Conservation and Achieving National Food Security, M.K. Suleimenov et al. (eds.), July 23-24, 2011, Shortandy, Kazakhstan.

Larney, F.J., Pearson, D.C., Blackshaw, R.E., Lupwayi, N.Z. and Regitnig, P.J. 2011. A soil conservation package for irrigated rotations in southern Alberta. Abstr. Poster Presentation. ASA-CSSA-SSSA-CSSS Annual Meetings, October 16-19, 2011, San Antonio, TX.

Larney, F.J., Pearson, D.C., Blackshaw, R.E., Lupwayi, N.Z. and Regitnig, P.J. 2011. Potato performance in the Vauxhall irrigated rotation study over 12 growing seasons (2000-11). Abstr. Poster presentation. Ann. General Meeting, Potato Growers of Alberta, November 15-18, 2011, Calgary, AB.

Pageni, B.B., Lupwayi, N.Z., **Larney, F.J.**, Kawchuk, L.M., Pearson, D.C., Blackshaw, R.E. and Gan. Y.T. 2011. Preceding crop, rotation length and soil management effects on bacterial endophytes. Abstr. Pulse Science Cluster Scientific Meeting, November 17-18, 2011, Saskatoon, SK.

Larney, F.J., Pearson, D.C., Blackshaw, R.E., Lupwayi, N.Z. and Regitnig, P.J and Balasubramanian, P. 2011. Dry bean performance in the Vauxhall irrigated rotation study over 12 growing seasons (2000-11). Pulse Science Cluster Scientific Meeting, November 17-18, 2011, Saskatoon, SK.

Larney, F.J., Pearson, D.C., Blackshaw, R.E., Lupwayi, N.Z. and Regitnig, P.J and Balasubramanian, P. 2012. Irrigated crop rotation research: Findings from the Vauxhall rotation study over 12 years (2000-11). Abstr. Irrigated Crop Production Update, January 31-February 1, 2012, Lethbridge, AB.

Larney, F.J., Pearson, D.C., Blackshaw, R.E., Lupwayi, N.Z. and Regitnig, P.J. 2012. Building soil quality on irrigated rotations in southern Alberta. Abstr. Oral Presentation. 49th Alberta Soil Science Workshop, February 14-16, 2012, Edmonton, AB.

Pageni, B.B., Lupwayi, N.Z., **Larney, F.J.**, Kawchuk, L.M., Pearson, D.C., Blackshaw, R.E. and Gan. Y.T. 2012. Preceding crop, rotation length and soil management effects on bacterial endophytes. Abstr. Poster presentation. 49th Alberta Soil Science Workshop, February 14-16, 2012, Edmonton, AB.

Larney, F.J. and Lupwayi, N.Z. 2011. Irrigated cropping systems for sustainable production: The Vauxhall Irrigated Rotation Study. Annual Report to Pulse Science Cluster, submitted April 4, 2011, 16 pp.

Larney, F.J., Pearson, D.C., Blackshaw, R.E., Lupwayi, N.Z., Regitnig, P.J. and Forge, T.A. 2011. Soil properties of an irrigated rotation study with sustainable and conventional practices. Handout (PowerPoint) provided at Lethbridge Research Centre Open House, July 20, 2011.

Pearson, D.C. and **Larney, F.J.** 2011. Irrigated crop rotation study. Visit by Leanne Fischbuch, newly-appointed Executive Director, Alberta Pulse Growers (Leduc, AB), August 9, 2011, Vauxhall, AB.

Table 1. Effect of rotation management on bean yield, 2011.

Rotation	Previous Crop	Fall preparation	Spring Preparation	Yield, kg ha^{-1}
3 yr Conv.	Potatoes	Cultivate + crazy harrow	Triple K	3080ab*
3 yr Sust.	Potatoes	Disc/crazy harrow. Fall rye cover	Direct seed into fall rye burnoff	3992a
4 yr Conv.	Sugar beet	Cultivate + crazy harrow	Triple K	2501b
4 yr Sust.	Sugar beet	Cultivate + crazy harrow	Direct seed	4056a
5 yr Sust.	Wheat	Shred stubble	Direct seed into shredded stubble	3769a
6 yr Sust.	Sugar beet	Cultivate + crazy harrow	Direct seed	3725a

*Means followed by the same letter are not significantly different from each other. (P = 0.05).

Table 2. Effect of Rotation Management on other Bean Agronomic and Disease Characteristics. Vauxhall 2011

Rotation	Plant Stand n/8m, 17th June	Height - cm	Maturity - Days from seeding	Sclerotinia % disease of 30 plants	Bacterial Blight scale 0-5
3 yr Conv.	107a	50.99a	108.0a	1.54b	1.96a
3 yr Sust.	61c	50.88a	102.5c	9.58a	1.75a
4 yr Conv.	108a	46.65a	105.6ab	1.67b	1.75a
4 yr Sust.	77b	49.74a	104.2bc	9.20a	2.16a
5 yr Sust.	70bc	50.77a	101.6c	6.43ab	1.82a
6 yr Sust.	63c	49.23a	104.2bc	8.75a	1.50a

*Means followed by the same letter are not significantly different from each other (P= 0.05)

Table 3. Contrast for Beans: Conventional vs Sustainable:

Characteristic	Conventional	Sustainable	P value
Yield (kg/ha)	2791	3886	0.003 **
Plant stand (n/8m)	108	68	<0.0001***
Maturity (days)	106.8	103.1	0.001 ***
Height (cm)	48.8	50.2	0.355 *NS*
Sclerotinia (%)	1.60	8.49	0.001 ***
Bacterial Blight	1.9	1.8	0.887 *NS*

Table 4. Contrast for Beans: After Potatoes vs After Sugarbeet:

Characteristic	After potatoes	After Sugarbeet	P value
Yield (kg/ha)	3536	3428	0.719 *NS*
Plant stand (n/8m)	84	83	0.778 *NS*
Maturity (days)	105.2	104.7	0.517 *NS*
Height (cm)	50.9	48.5	0.123 *NS*
Sclerotinia (%)	5.56	6.54	0.533 *NS*
Bacterial Blight	1.9	1.8	0.879 *NS*

* significant at the 5% level; ** significant at the 1% level.
*** significant at the 0.1% level, NS not significant

Table 5. Effect of rotation management on potato yield, Vauxhall, 2011.

Rotation	Previous crop	Fall preparation	Nutrient input, kg ha^{-1}	Yield*, Mg ha^{-1}
3 yr Conv.	Wheat	Plough,	112 N, 67 P, 67 K	36.2ab**
3 yr Sust.	Wheat	Dammer diker	62 N, 28 P, 67 K 28 t/ha compost	36.4ab
4 yr Conv.	Beans	Plough	112 N, 67 P, 67 K	30.7b
4 yr Sust.	Beans	Dammer diker	37 N, 0 P, 67 K 42 t/ha compost	38.3ab
5 yr Sust.	Beans	Dammer diker	37 N, 0 P, 67 K 42 t/ha compost	42.7a
6 yr Sust.	Beans	Dammer diker	37 N, 0 P, 67 K 42 t/ha compost	41.6a

*Yield = marketable + oversize yield.
**Means followed by the same letter are not significantly different from each other (P = 0.05).

Table 6. Agronomic parameters for potatoes, 2011.

Rotation	Julian date 50% Emergence	Vigour scale 10-50	Stand count/36m row	Total yield Mg ha^{-1}	Marketable Yield T/ha	Oversize Yield T/ha	Undersize Yield T/ha	Marketable yield/plant kg	Oversize yield/plant kg
3 yr Conv.	161.50ab	31.3ab	117.0a	41.03b	20.78a	15.46a	2.67a	0.595a	0.442a
3 yr Sust.	161.23ab	30.0b	119.8a	40.95b	22.68a	14.16a	2.46a	0.623a	0.406a
4 yr Conv.	162.50a	31.9ab	107.1a	39.71b	19.12a	11.60a	3.53a	0.658a	0.412a
4 yr Sust.	161.50ab	33.8a	117.8a	46.21a	22.86a	15.43a	3.53a	0.648a	0.439a
5 yr Sust.	160.84b	34.6a	120.3a	48.61a	25.84a	16.31a	3.58a	0.730a	0.443a
6 yr Sust.	161.75ab	34.4a	119.4a	47.46a	26.27a	15.77a	2.41a	0.726a	0.453a

Table 7. Quality and Disease Characteristics for Potatoes, 2011.

Rotation	Marketable deformities T/ha	Oversize deformities T/ha	Oversize Hollow Heart^	Specific Gravity
3 yr Conv.	0.29a	1.46a	0.50a	1.0937a
3 yr Sust.	0.50a	1.16a	0.32ab	1.0844ab
4 yr Conv.	1.22a	3.70a	0b	1.0811b
4 yr Sust.	0.74a	3.11a	0b	1.0857ab
5 yr Sust.	0.42a	1.66a	0.03ab	1.0838ab
6 yr Sust.	1.09a	1.88a	0b	1.0904ab

Table 8. Potatoes, Conventional vs Sustainable Contrasts:

Characteristic	Units	Conventional	Sustainable	P value
Emergence	Julian days	162.0	161.3	0.148 *NS*
Vigour	scale 10-50	31.6	33.2	0.144 *NS*
Stand count	36m row	112.0	119.3	0.062 *NS*
Yield (M + O)^	T/ha	33.48	39.74	0.027 *
Marketable yield	T/ha	19.95	24.41	0.051 *NS*
Oversize yield	T/ha	13.53125	15.418775	0.273 *NS*
Undersize yield	T/ha	3.10	2.99	0.809 *NS*
M Yield/plant	Kg	0.626	0.682	0.238 *NS*
O Yield/plant	Kg	0.427	0.435	0.879 *NS*
M deformities	T/ha	0.75	0.69	0.861 *NS*
O deformities	T/ha	2.58	1.95	0.539 *NS*
O hollow heart	n/10 tubers	0.25	0.09	0.266 *NS*
Specific Gravity	G	1.08743	1.08605	0.637 *NS*

Table 9. Potatoes, After Beans vs After Wheat Contrasts:

Characteristic	Units	After wheat	After beans	P value
Emergence	Julian days	161.4	161.6	0.544 *NS*
Vigour	scale 10-50	30.6	33.6	0.012 *
Stand count	36m row	118.4	116.1	0.533 *NS*
Yield (M + O)^	T/ha	36.31	38.33	0.448 *NS*
Marketable yield	T/ha	21.73	23.52	0.418 *NS*
Oversize yield	T/ha	14.81	14.78	0.984 *NS*
Undersize yield	T/ha	2.56	3.26	0.132 *NS*
M Yield/plant	kg	0.609	0.690	0.104 *NS*
O Yield/plant	kg	0.424	0.437	0.813 *NS*
M deformities	T/ha	0.39	0.87	0.232 *NS*
O deformities	T/ha	1.31	2.59	0.243 *NS*
O hollow heart	n/10 tubers	0.41	0.01	0.018 *
Specific Gravity	g	1.08904	1.08525	0.206 *NS*

^M = marketable; O = oversize

Table 10. Cultural and experimental details of the sugar beet plots at Vauxhall - 2011

Legal Description	SE 4-13-16 W4	
	Following timothy	
	Fall	Glyphosate application
		Plough and disc plus diamond harrows
	Spring	Fertilize
		Triple K cultivator (1x)
		Packers and harrows (1x)
	Following wheat	
	Fall	Glyphosate application
		Shred wheat stubble
		Disc and diamond harrows (1x)
	Spring	Fertilize
		Triple K cultivator (1x)
		Packers and harrows (1x)
Fertilizer Applied	Timothy - 150 lbs N/ac; 25 lbs P_2O_5 (broadcast)	
Planting Date	May 17	
Seed/Row Spacing	6"/22"	
Inter-row Cultivation	None	
Herbicides (Liters of product/acre broadcast)	June 20	Start Up *glyphosate - 540g ae/l* (1.0)
	July 5	Vantage Plus Max *glyphosate – 480 g*
Insecticides	Planting time	Cruiser seed treatment
Stand Count	September 19 & 20	
Harvest Date	September 20	
Growing days	126	

Table 11. Yield and quality results for sugar beet, Vauxhall rotation test - 2011.

Treatment	Extractable Sugar		Sugar	Molasses Loss	Beet Yield	Stand
	kg/acre	kg/t	%	%	t /acre	pl/100 ft
4-yr c	3050a	156.9a	17.51a	1.82a	19.45a	125a
4-yr s	3279a	148.6a	17.05a	2.19a	22.15a	125a
5-yr	3306a	154.9a	17.56a	2.07a	21.37a	131a
6-yr	2881a	157.7a	17.77a	2.01a	18.24b	124a

Table 12. Amino-N, Na and K results for sugar beet, Vauxhall rotation test – 2011.

Treatment	Amino-N	Na	K	Amino-N	Na	K
	meq/100g fresh wt.			ppm fresh wt.		
4-yr c	0.75a	1.08a	4.93a	106a	249a	1928a
4-yr s	0.91a	1.39a	5.64a	128a	320a	2206a
5-yr	0.82a	1.04a	5.66a	114a	240a	2213a
6-yr	0.69a	0.90a	5.66a	97a	207a	2213a

Table 13. Effect of rotation on wheat yield, Vauxhall, 2011.

Previous Crop		Fall preparation	Yield, Mg ha^{-1}
1 yr Cont.	Wheat	Disc and crazy harrow	4.28b*
3 yr Conv.	Beans	Cultivate + crazy harrow	6.73a
3 yr Sust.	Beans	Disc + crazy harrow. Fall rye cover crop	6.68a
4 yr Conv.	Potatoes	Cultivate + crazy harrow	6.71a
4 yr Sust.	Potatoes	Disc + crazy harrow, Fall rye cover crop	6.73a
5 yr Sust.	Potatoes	Disc + crazy harrow, Fall rye crop	6.58a
5 yr Sust.	Sugar beet	Compost, Disc + crazy harrow	6.54a

*Means followed by the same letter are not significantly different from each other (P = 0.05).

Table 14. Wheat agronomic factors, 2011.

Rotation	Maturity - Days from seeding	Height (cm)	Plant Stand (n/8m)	Take All Rating	Sawfly %	Test Weight Kg/hl
1 yr Cont.	103.5b	81.33a	304b	1.74a	Not available	77.07a
3 yr Conv.	108.0ab	91.18a	345ab	1.23b		78.69a
3 yr Sust.	106.8ab	93.65a	358ab	1.24b		77.96a
4 yr Conv.	107.8ab	95.45a	328ab	1.09b		78.59a
4 yr Sust.	110.0a	94.40a	337ab	1.14b		78.38a
5 yr-P Sust.	108.5a	91.48a	338ab	1.18b		78.32a
5 yr-Sb Sust.	106.0ab	96.80a	374a	1.17b		78.48a

* Means followed by the same letter are not significantly different from each other. ($P = 0.05$)

Table 15. Wheat Quality, 2011.

Rotation	Protein Cont %	Hardness PSI	Hardness NIRI	Sediment-ation	Ca %	P %	K %	S %
1 yr Cont.	9.85ab	73.15b	26.75a	24.25a	0.075a	0.315a	0.373a	0.115ab
3 yr Conv.	9.33b	76.00ab	28.00a	23.75a	0.065b	0.330a	0.363a	0.108b
3 yr Sust.	10.00a	79.75a	28.75a	26.75a	0.070ab	0.333a	0.368a	0.118a
4 yr Conv.	10.03ab	75.25ab	26.75a	23.25a	0.063b	0.333a	0.370a	0.108b
4 yr Sust.	9.93ab	76.25ab	27.75a	27.75a	0.068ab	0.343a	0.363a	0.113ab
5 yr-P Sust.	10.23a	75.75ab	28.25a	29.67a	0.068ab	0.323a	0.355a	0.118a
5 yr-Sb Sust.	9.80ab	75.45ab	28.00a	26.00a	0.070ab	0.335a	0.365a	0.113ab

Protein content corrected to 13.5% moisture

PSI>80, indicates a soft wheat.

For NIRI, a soft wheat has an average value of 25.

Sedimentation: A soft wheat has an average value of 20.

Table 16. Wheat, Conventional vs Sustainable Contrasts.

Characteristic	Conventional	Sustainable	P value
Yield(T/ha)	6.72	6.63	0.784 *NS*
Plant Stand (n/8m)	336.4	351.6	0.353 *NS*
Test Wt. (kg/hl)	78.64	78.28	0.503 *NS*
Height (cm)	93.3	94.1	0.721 *NS*
Maturity (days)	107.9	107.8	0.966 *NS*
Take All (Rating)	1.16	1.18	0.668 *NS*
Sawfly %			
Protein Conten %	9.68	9.99	0.153 *NS*
Hardness PSI	75.63	76.80	0.416 *NS*
Hardness NIRI	27.38	28.19	0.401 *NS*
Sedimentation	23.5	27.5	0.096 *NS*
Ca %	0.064	0.069	0.064 *NS*
P %	0.331	0.333	0.844 *NS*
K %	0.366	0.363	0.534 *NS*
S %	0.108	0.115	0.010 **

Table 17. Wheat, After Beans vs After Potatoes Contrasts.

Characteristic	After potatoes	After beans	P value
Yield(T/ha)	6.67	6.71	0.922 *NS*
Plant Stand (n/8m)	334.3	351.3	0.325 *NS*
Test Wt. (kg/hl)	78.43	78.33	0.856 *NS*
Height (cm)	93.8	92.4	0.549 *NS*
Maturity (days)	108.8	107.4	0.377 *NS*
Take All (Rating)	1.14	1.23	0.104 *NS*
Sawfly %	Not available		
Protein Content %	10.06	9.66	0.076 *NS*
Hardness PSI	75.75	77.88	0.165 *NS*
Hardness NIRI	27.58	28.38	0.437 *NS*
Sedimentation	26.9	25.3	0.511 *NS*
Ca %	0.066	0.068	0.540 *NS*
P %	0.333	0.331	0.901 *NS*
K %	0.363	0.365	0.699 *NS*
S %	0.113	0.113	1.000 *NS*

*** significant at the 0.1% level; ** significant at the 1% level; * significant at the 5% level; *NS* not significant

Table 18. Soil organic carbon levels (0-15 cm depth), fall 2011

Rotation	Mg ha^{-1}
1c	28.97abc
3c	26.82bc
3s	32.46ab
4c	24.62c
4s	32.88ab
5s	36.93a
6s	31.96ab

Fig. 1. Soil organic carbon changes over duration of study, Vauxhall 1999 (baseline) to 2011.

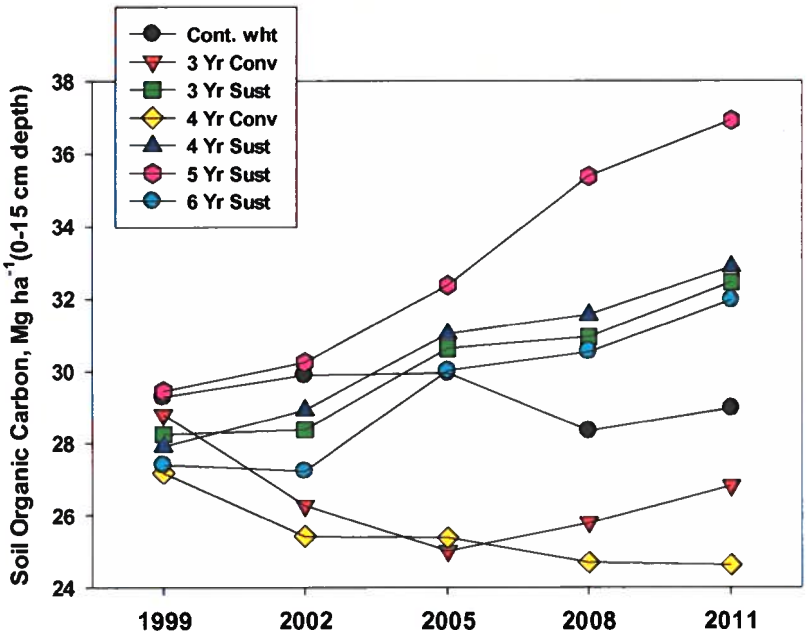

Table 19: Microbiological parameters for non-rhizosphere soil, wheat plots, July 2011

Rotation	Microbial biomass C mg kg^{-1} soil	Shannon index of diversity	β-glucosidase mg^{-1} nitrophenol kg^{-1} soil hr^{-1}
1YrC	477bc	2.22a	200a
3YrC	372c	2.21a	103b
3YrS	514ab	2.54a	189a
4YrC	435bc	1.76b	86b
4YrS	507ab	1.77b	190a
5YrS1	424bc	2.24a	184a
5YrS2	623a	2.33a	228a
6YrS*	477bc	2.27a	184a

*Oats

Table 20: Microbiological parameters for rhizosphere soil, wheat plots, July 2011

Rotation	Microbial biomass C mg kg^{-1} soil	Shannon index of diversity	β-glucosidase mg^{-1} nitrophenol kg^{-1} soil hr^{-1}
1YrC	484a	2.51a	194ab
3YrC	430a	2.11a	152b
3YrS	573a	2.71a	237a
4YrC	420a	2.12a	94c
4YrS	462a	2.30a	204a
5YrS1	507a	2.41a	212a
5YrS2	576a	2.24a	243a
6YrS*	476a	2.46a	204a

*Oats

Table 21. Soil Microbiological Parameters Wheat, Conventional vs Sustainable Contrasts.

Characteristic	Conventional	Sustainable	P value
Non-Rhizosphere soil			
MBC, mg kg^{-1} soil	403	509	0.008**
Shannon index	1.99	2.33	0.05*
β-glucosidase, mg^{-1} nitrophenol kg^{-1} soil hr^{-1}	95	195	<0.001***
Rhizosphere soil			
MBC, mg kg^{-1} soil	425	519	0.05*
Shannon index	2.11	2.42	0.03*
β-glucosidase, mg^{-1} nitrophenol kg^{-1} soil hr^{-1}	123	220	<0.001***

Table 22. Soil Microbiological Parameters Wheat, After Beans vs. After Potatoes Contrasts.

Characteristic	After beans	After potatoes	P value
Non-Rhizosphere soil			
MBC, mg kg^{-1} soil	443	461	0.64NS
Shannon index	2.38	2.01	0.007**
β-glucosidase, mg^{-1} nitrophenol kg^{-1} soil hr^{-1}	146	161	0.30NS
Rhizosphere soil			
MBC, mg kg^{-1} soil	501	466	0.46NS
Shannon index	2.41	2.32	0.52NS
β-glucosidase, mg^{-1} nitrophenol kg^{-1} soil hr^{-1}	194	178	0.30NS

*** significant at the 0.1% level; ** significant at the 1% level; * significant at the 5% level;
NS not significant

What have we learned about potatoes in the 12-yr Vauxhall Irrigated Rotation Study?

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Background

Common irrigated crops in southern Alberta such as potatoes, sugar beets and dry beans produce little crop residue for return to the soil. Growing these crops in tight rotations may have long-term detrimental effects on our soil resource in terms of diminished soil quality and increased erosion risk.

An irrigated rotation study was initiated in 2000 at Vauxhall, Alberta to examine the impact of rotation length and conventional (CONV) and conservation (CONS) management for potatoes, sugar beets, beans and soft wheat. The CONS rotations were built around four specific management practices:

1. direct seeding/reduced tillage where possible
2. fall-seeded cover crop (fall rye)
3. feedlot manure compost application (28 or 42 t/ha every third year) and
4. where beans occurred in the rotation, solid-seeded narrow-row (20 cm) beans vs. conventional wide row (60 cm) beans.

There was a total of six rotations (Table 1) with each of the 26 phases appearing every year and replicated 4 times (104 plots).

Table 1: Irrigated rotation treatments at Vauxhall, Alberta

Length, yr	Rotation	Management
1	W	Cont. wheat
3	P-B-W	Conventional
3	P-B-W	Conservation
4	P-W-SB-B	Conventional
4	P-W-SB-B	Conservation
5	P-W-SB-W-B	Conservation (cereal breaks)
6	P-O(t)-T-T-SB-B	Conservation (forage break)

W: wheat; P: potatoes; B: beans; SB, sugar beet; O(t): silage oats harvested July, timothy seeded Aug.; T: timothy.

This poster will focus on the results from the potato crop averaged over 12 yr (2000-11). All yields are expressed as marketable yield (>48 mm diam.).

Results

Trends in potato yield, averaged over the 12 yr (Fig. 1), showed that the 3-Yr CONV rotation (35.4 t/ha) was significantly lower than all others (38.6-42.0 t/ha), being 12% lower than the 3-Yr-CONS rotation. Simply lengthening the rotation to 4 yr while maintaining CONV practices increased yield by 11% (4-Yr-CONV vs. 3-Yr-CONV). There was no significant yield effect of imposing conservation practices if the rotation length was 4 yr (4-Yr-CONV vs. 4-Yr-CONS).

The 5-Yr CONS rotation yielded 18% higher than the 3-Yr-CONV rotation, and 10% higher than the 4-Yr CONS. However, stretching potatoes to one yr in six did not lead to a significant yield increase except over the 3-Yr-CONV rotation (14%).

There was no effect of rotation on specific gravity (SG) averaged over 12 yr (Fig. 2). However there was a declining trend in SG as rotation lengthened, from 1.08475 on the 3-Yr-CONV to 1.07954 on the 6-Yr-CONS.

A contrast of management groupings (3- and 4-Yr CONV vs. 3-, 4-, 5- and 6-Yr-CONS, Fig. 3a) showed an overall significant 7% increase in yield (t/ha) with CONS management, but a significant decline in SG (from 1.08353 to 1.08053). Also comparing the shortest 3-Yr rotations (3-Yr-CONV and 3-Yr-CONS vs. 4-Yr CONV, and 4-, 5- and 6-Yr-CONS), showed a significant 7% yield increase but a decline in SG (from 1.08375 to 1.08042), when moving away from 3-yr rotations (Fig. 3b).

Summary

- Based on our results, growing potatoes in 3-yr rotations (potatoes-beans-wheat) without conservation practices is not recommended due to significant yields declines of 12-18% over the long-term.
- Overall, CONS practices increased potato yield by 7% but lowered specific gravity.
- Longer rotations ($\geq$ 4 yr) led to 7% higher potato yield but lower specific gravity.
- CONS practices also improved soil quality parameters such as organic carbon, microbial biomass and aggregate stability.

Acknowledgements

We acknowledge funding support from the Potato Growers of Alberta, Alberta Pulse Growers and Rogers Sugar/Lantic Inc. over the entire length of the study, the Alberta Agricultural Research Institute-Farming for the Future Research Funding Program (2001-06), Agriculture and Agri-Food Canada's Matching Investment Initiative (2000-09); and the Pulse Science Cluster (2010-13). Jim Sukeroff and Dale Reynolds provided field help.

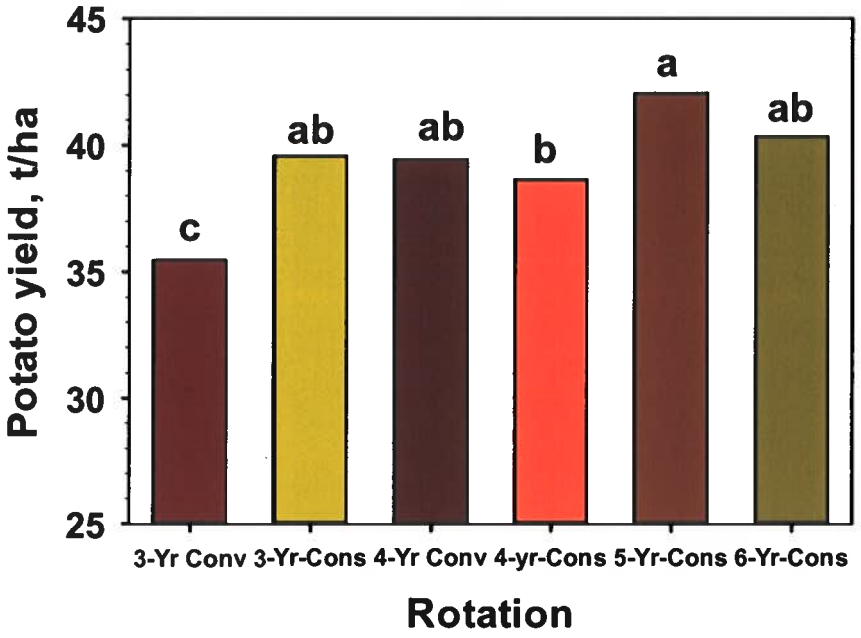

Fig. 1. 12-yr average potato yields. Bars with different letters are significantly different from each other 19 times out of 20.

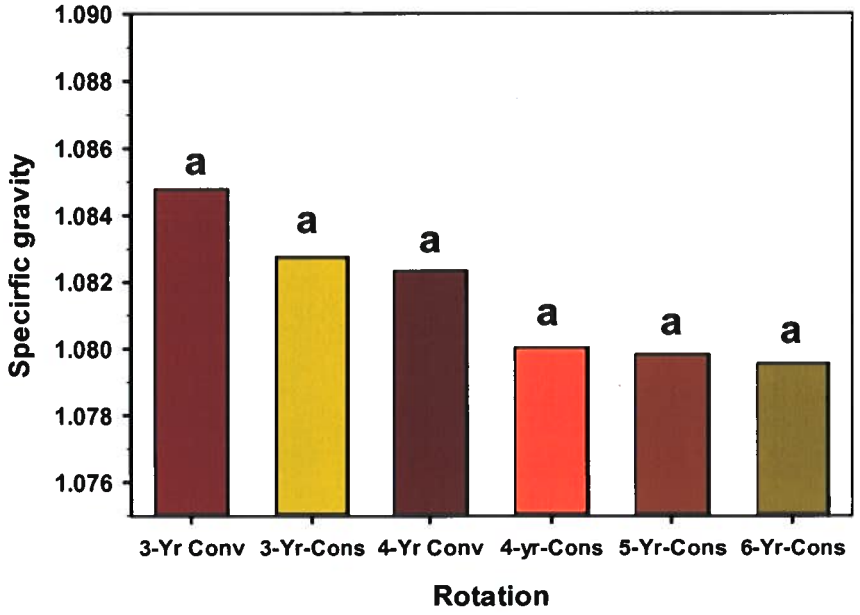

Fig. 2. 12-yr average specific gravity. Bars with the same letters are not significantly different from each other 19 times out of 20.

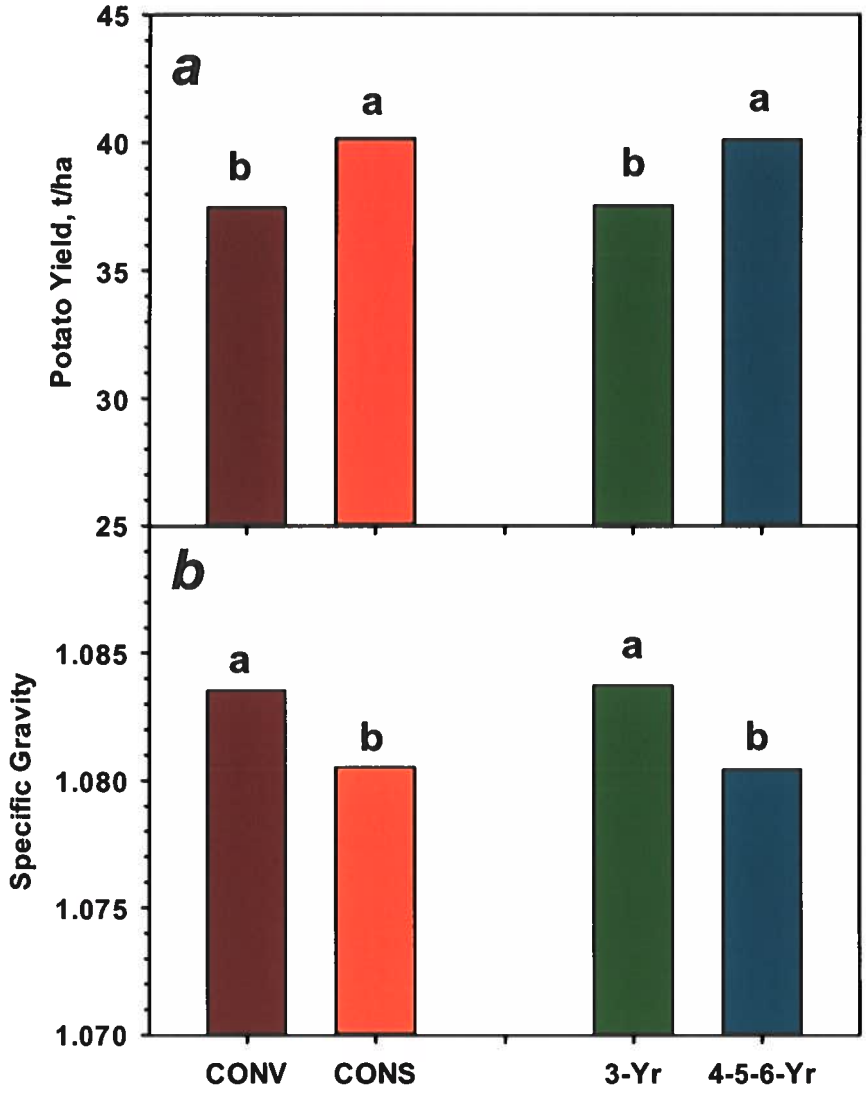

Fig. 3. Effect of rotation management and short vs. longer rotation length on (a) 12-yr average potato yield; (b) 12-yr average specific gravity. Bars with different letters are significantly different from each other 19 times out of 20.

Populations, diversity and identities of bacterial endophytes in potato (*Solanum tuberosum* L.) cropping systems

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ABSTRACT

Most plants host endophytic bacteria, but their identities and functions are usually unknown. Bacterial endophytes associated with potato grown after dry bean (*Phaseolus vulgaris* L.) or wheat (*Triticum aestivum* L.) were isolated, quantified and identified in a field study that compared crop rotations (3- to 6-yr in length) and soil management (CONV, conventional; CONS, conservation) for dry bean, potato, sugar beet (*Beta vulgaris* L.) and spring wheat. Populations of culturable endophytes ranged from 2.83×10^3 to 7.65×10^3 colony forming units (cfu) g^{-1} of root dry matter. The populations and diversity of the endophytes were greater with CONS than CONV soil management, and tended to be greater in longer than shorter rotations. The community structures of the endophytes were different between CONV and CONS soil management. A terminal-restriction fragment length polymorphism (T-RFLP) assay targeting the 16S rRNA gene, and its sequencing, showed that CONS management systems contained more *Proteobacteria* than CONV management systems, and vice-versa for *Acidobacteria*. *Bacteriodetes* were found only in long CONS rotations. This phylogenetic characterization of potato endophytes is important for further studies on their effects on the host plants.

Keywords: Crop rotations, endophytic bacteria, soil management, T-RFLP.

Short title: Bacterial Endophytes in Potato

Endohphytes are microorganisms that live within a plant in a mutualistic relationship (Schulz and Boyle 2006; Wilson 1995). They are believed to support plant processes that include growth

promotion, nutrient uptake, tolerance to abiotic stresses, and inhibition of infection by pathogens (Ryan et al. 2008). Culturable bacterial endophytes can be isolated after surface sterilization of plant material. Endophytes are found in roots, stems and leaves of plants and are ubiquitous in a range of different plant species (Garbeva et al. 2001). Bacterial endophytes have been isolated from sweet potato (*Ipomoea batatas*) (Khan and Doty 2009), cottonwood (*Populus deltoids*) (Xin et al. 2009), grapevine (*Vitis vinifera*) (West et al. 2010), poplar (*Populus alba*) (Doty et al. 2005), soybean (*Glycine max*) (Hung et al. 2007), tomato (*Solanum lycopersicum*) (M-Santacruz et al. 2010) and potato (Andreote et al. 2009; Manter et al. 2010; Sessitsch et al. 2002; Garbeva et al. 2001). Greater soil microbial counts, biomass and diversity have been observed in legume-based crop rotations (Biederbeck et al. 2005; Lupwayi et al. 1998). Some of the soil bacteria develop endophytic associations with non-legumes (Lupwayi et al. 2004), probably resulting in nutritional, crop protection and stress tolerance benefits to the host plants. Therefore, endophytic microorganisms can play important roles and offer environmentally-friendly methods to increase productivity while reducing chemical inputs.

While culturable endophytes have been isolated in the studies cited above, endophytic populations in potato monitored by denaturing gradient gel electrophoresis (DGGE) (Garbeva et al. 2000) revealed the presence of several distinct phylogenetic groups of organisms, suggesting that non-culturable endophytes are also present in potato. Despite the difficulty of obtaining taxonomic information on the organisms from particular terminal-restriction fragments (TRFs), the T-RFLP, a semiquantitative fingerprinting method, is extensively used for comparative community analysis. In the method, fluorescent end-labelled polymerase chain reaction (PCR)-amplified markers (commonly the small-subunit rRNA gene) are digested with one or more

restriction enzymes, resulting in the production of fluorescent labelled TRFs of different lengths. The TRFs can be separated and detected as peaks on an automated sequence analyser. The T-RFLP technique has the advantage of using an internal size marker with each sample, which greatly facilitates automatic sizing of peaks as compared to other molecular fingerprinting methods like DGGE. This method has been widely used to investigate bacterial community structures in various environments (Conn and Franco 2004; Dunbar et al. 2000; Liu et al. 1997; Luna et al. 2006; Osborn et al. 2000) including plant interiors.

The objective of this study was to isolate, quantify and identify endophytic bacteria from roots of potatoes grown in various crop rotations under irrigation, as a first step towards understanding their effects on potato growth. The rotation study had been running for 12 yr (2000-11) when sampled. The diversity and identities of the endophytes were determined using T-RFLP analysis, and the identities were confirmed using 16S rRNA gene sequencing. To our knowledge, this is the first report of microbial analysis by T-RFLP of 16S rRNA genes to characterize endophytic bacterial communities of potatoes grown in irrigated cropping systems.

MATERIALS AND METHODS

Treatments

An irrigated cropping study was initiated in 2000 at Vauxhall, Alberta, (50° 06' N; 112° 13' W) to compare the effects of 3- and 4-yr rotations under CONV or CONS soil management, and 5- and 6-yr under CONS soil management on yields of dry bean, potato, sugar beet and spring wheat. The CONS rotations represented a package built around five specific soil conservation practices: 1) Direct seeding/reduced tillage where possible, to maintain surface residue and

reduce wind and water erosion risk; 2) Fall-seeded cover crop (fall rye, *Secale cereale* L.) to reduce wind erosion risk in fall and early spring; 3) Feedlot cattle manure compost application (28 or 42 t ha^{-1} every third year) to replace inorganic fertilizer inputs and improve soil organic matter; 4) Solid-seeded narrow-row (20 cm) beans direct-cut at harvest *vs.* conventional wide row (60 cm) beans which are undercut at harvest leaving loose soil and non-anchored stubble; 5) Longer rotations of 5- and 6-yr under CONS soil management. The CONV rotations had none of the above management practices. There were a total of six rotations with all 26 phases appearing every year, arranged in a randomized complete block design (RCBD) and replicated 4 times (104 plots, each 10.1 x 18.3 m) (Table 1). Rotations varied in length from 3 to 6 yr with 2 yr of timothy (*Phleum pratense* L.) included in the longest rotation. Soil management systems (CONV, CONS) and length of crop rotations were considered the different treatments.

Isolation of Endophytes

Potato roots and adhering rhizosphere soil were sampled in all 24 potato plots (6 rotations x 4 replicates, Table 1) at the flowering stage in August, 2011 and kept at -20 °C until required. Roots were washed with distilled water and surface-sterilized using 10% (v/v) sodium hypochlorite for 8 min, followed by 1% iodophor for 5 min, and then washed 10 times with sterile water. About 0.5 g of surface-sterilized roots was macerated to fine powder using liquid nitrogen in a sterilized and precooled mortar. The powder was suspended and serially diluted with sterile water to isolate bacterial endophytes. Aliquots of 100 µL were plated in tryptic soy agar (TSA) medium plates containing 100 µg per ml cycloheximide (antifungal chemical), and the plates were incubated at 28 °C for 48 h (Garbeva et al. 2001; Khan and Doty 2009). Samples of root tissue from the surface sterilization process were also plated into the same medium to

check the success of the sterilization. Bacterial colonies were counted after 48 h and these colonies were further screened in TSA medium. Different morphological characteristics (number, colour and size of colonies) of endophytes were observed, counted, and different colonies were chosen for further analysis. Endophyte populations were estimated as cfu g^{-1} potato root tissue. Culture broths of screened endophytes were kept at -80 °C with 30% glycerol solution as stock solution.

Nucleic Acid Extraction and 16S rRNA Gene Amplification

Some of the distinct colonies were grown in tryptic soy broth (TSB) liquid media and chromosomal DNA isolated using the QIAamp DNA Mini kit (Qiagen, Gaithersburg, MD, USA). The concentration and purity of chromosomal DNA were checked with Nano Drop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Eubacterial 16S rRNA gene sequences were amplified by universal bacterial primers (8F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-GGTTACCTTGTTACGACTT-3') using the chromosomal DNA as template for identification of 16S rRNA, while 341F: 5'-CCTACGGGAGGCAGCAG-3' and 1492R were used for amplification of 16S rRNA gene for T-RFLP analysis (Khan and Doty 2009; Liu et al. 1997). PCR was carried out with a thermocycler (Eppendorf, Hamburg, Germany) using an initial denaturation step of 7 min at 95 °C followed by 40 cycles of 30 s at 95 °C, 1 min annealing at 56 °C and 1 min extension at 72 °C, followed by final extension at 72 °C for 7 min. The PCR products of 16S rRNA genes were visualised with ethidium bromide in 1% agarose gel.

Endophyte Community Analysis by 16S rRNA-Based T-RFLP

Genomic DNA from potato root tissue was used for T-RFLP analysis. Eubacterial universal forward primer 341F (Liu et al. 1997) was labelled at the 5' primer end with 6-carboxyfluorescein (6-FAM) (Integrated DNA Technologies, Coralville, IA, USA) and reverse primer 1492R (Khan and Doty 2009) were used to amplify the 16S rRNA gene. Reactions were carried out in a thermocycler (Eppendorf, Hamburg, Germany) as described above. The PCR reactions (50 µl) contained 5 U HotStarTaq DNA polymerase (Qiagen, Hilden, Germany), PCR Buffer (with 3 mM $MgCl_2$), and 400 µM of each dNTP (Qiagen, Hilden, Germany), 800 µM of each primer and 15-25 ng template DNA. The PCR products were purified using QuickA PCR Purification Kit following the manufacturer`s protocol from Qiagen. About 1200 ng of purified-16S rRNA PCR products were individually digested for 4 h at 37 °C with 4-bp recognition sites *Hinf*I, *Rsa*I, *Mbo*I and a combination of *Hae*III and *Mbo*I (New England Biolabs Inc., Ipswich, MA, USA). Restriction enzymes that generated unique TRFs were determined using Restriction Endonuclease Picker (REPK v1.3) (Collins and Rocap 2007). Preliminary experiments showed that *Hinf*I was the best endonuclease because it produced more TRFs than the other enzymes, and results presented here are for this endonuclease.

Enzyme reactions were quenched at 65 °C for 10 min to inactivate enzyme activity, purified and 0.5 µl aliquots were mixed with 1 µL of loading buffer (5 deionised formamide:1 loading dye) and 0.3 µl of DNA fragment length standard (Genescan 500 Rox; Perkin-Elmer). Reaction mixtures were denatured at 92 °C for 2 min and chilled on ice prior to electrophoresis. Samples (1.75 µL) were applied on 6% denaturing polyacrylamide gels and fluorescently labelled terminal restriction sizes were analysed using an ABI 3730x 1 genetic analyser automated DNA sequencer (University of Calgary, Calgary, AB, Canada). The TRFs were only scored as positive

when they had more than 50 fluorescence units. Fragments were sized using Peak Scanner software v1.0 (Applied Biosystems) and the resulting data was imported into T-REX software (Culman et al. 2009) for further processing. Data were subjected to quality control procedures including TRF alignment (clustering threshold = 2 bp), noise filtering (peak area, standard deviation multiplier = 1), and elimination of TRFs < 50 bp. The total fluorescence within each T-RFLP profile was calculated by summing the heights of peaks detected. Additive Main Effects and Multiplicative Interaction Model (AMMI) analysis was carried using T-REX software (Culman et al. 2009). The TRFs obtained from each electropherogram after running in T-REX software were used in PAT+ program from Microbial Community Analysis (MiCA3) software server application (Shyu et al. 2007) to assign putative taxonomic identities. Operational taxonomic units (OTUs) of each type of endophyte were derived from the number of TRFs obtained from each treatment.

The online server Chang BioScience (2004) was used to calculate indices of diversity: Shannon-Wiener index (H'), species richness (S) and evenness (E). Average TRFs were taken from three analyses for each sample, and the TRFs from all four field experimental replicates were included in the statistical analysis of H', S and E.

Construction of 16S rRNA Gene Library

Amplified PCR products of 16S rRNA genes from the isolates were purified using QuickA PCR purification kit (Qiagen, Gaithursburg, MD, USA) according to the manufacturer's instructions and were cloned into pGEM-T-easy vector (Promega, Madison, WI, USA), and then transformed into competent cells of *Escherichia coli* DH5α. Positive colonies were selected using the white-

185 blue-colony method on Luria–Bertani (LB) medium containing X-Gal (5-bromo-4-chloro-3-
186 indolyl-β-D-galactopyranoside), IPTG (isopropyl- β-D-thiogalactopyranoside), and 50 μg mL^{-1}
187 ampicillin and cultured in liquid LB culture medium. Cloned DNA was extracted from the
188 culture broth of positive colonies. Cloned products were sequenced at Génome Québec
189 Innovation Center (McGill University, Montreal, QC, Canada) using T7 and SP6 promoter's
190 primers. Purified PCR products of 16S rRNA gene were also sequenced using 8F and 1492R
191 primers. The 16S rRNA gene sequences were compared and identified by homology comparison
192 to entries in the GenBank nucleotide database using BLASTN (Altschul et al. 2004). All cultured
193 endophytes from various treatments are listed in Supplementary Table 1. Phylogenetic molecular
194 evolutionary analyses were conducted using ClustalW2 online software (Larkin et al. 2007) and
195 the aligned sequences were exported to MEGA 5.05 version (Tamura et al. 2011). All sequences
196 were screened for potential chimeric sequences using database-enabled code for ideal probe
197 hybridization employing R (DECIPHER) online software (Wright et al. 2012).
198
199 **Nucleotide Sequence Numbers**
200 All the nucleotide sequences of 16S rRNA gene from potato endophytes have been deposited in
201 the NCBI GenBank database under accession numbers JX912365 to JX912472.
202
203 **Statistical Analysis**
204 All data [cfu, Shannon-Wiener index (H'), species richness (S) and evenness (E)] were subjected
205 to analysis of variance (ANOVA) according the RCBD of the field experiment, using SAS
206 software (Ver. 9.1, SAS institute Inc., Cary, NC, USA). Unless otherwise specified, statistical
207 analyses were based on peak height used in T-REX software. Statistical significance was

established at P = 0.05 and means were separated by the least significant difference (LSD) test. Principal component analysis (PCA), a multivariate procedure, was used to classify the TRFs of the endophyte communities using PAST 2.02 software (Hammer et al. 2001).

RESULTS

Endophyte Populations

The lowest endophyte population, i.e., 2.83 x 10^3 cfu g^{-1} of potato root tissue, was found in 3-yr potato-bean-wheat rotation under CONV soil management, whereas the highest endophyte population, i.e., 7.65 x 10^3 cfu g^{-1} of root, was found in 5-yr potato-wheat-sugar beet-wheat-bean rotation under CONS soil management (Fig. 1). The endophytic populations in the 5-yr and 6-yr CONS rotations were significantly greater than those in the 3-yr and 4-yr CONV rotations. Between the two 3-yr rotations, the populations were significantly greater under CONS management than CONV management, and the same result was observed between the two 4-yr rotations.

Endophyte Diversity, Community Structures and Phylogenetic Identities: T-RFLP Analysis

Table 2 shows that differences in H' between treatments were similar to differences in endophyte populations described above (Fig. 1). Thus, between the two 3-yr or 4-yr rotations, the endophytes in rotations under CONS management were more diverse (greater H') than those under CONV management, and the 5- and 6-yr CONS rotations tended to have more diverse endophytes than 3- and 4-yr CONV rotations. Both S and E contributed to these differences in H' (H' is a composite of S and E).

231

232 Ordination of TRFs by PCA revealed differences in community structures of the endophytes in
233 the different treatments (Fig. 2). This analysis indicated that most (77%) of the variance in
234 endophyte community structures was explained by PC1 (x-axis, left to right in Fig. 2). Therefore
235 the endophyte communities in treatment T5 (5-yr CONS) and, to a lesser extent, T6 (6-yr CONS)
236 were very different from those in T1 (3-yr CONV) and, to a lesser extent, T3 (3-yr CONV) and
237 T2 (3-yr CONS). Fig. 2 also shows that α- and γ- *Proteobacteria* (see below) were more
238 associated with T5, and the *Firmicutes* with T6, than with other treatments.

239

240 The endophytes were identified and assigned to distinct putative phylogenetic groups. Many
241 TRFs shared more than one phylogenetic group and the identified TRFs revealed differences in
242 the relative population structures in various treatments. The TRFs were affiliated to
243 *Proteobacteria* (α, β, γ and δ), *Firmicutes*, *Cyanobacteria*, *Actinobacteria*, *Bacteriodetes*,
244 *Acidobacteria* and some unidentified bacteria (Fig. 3). Within 3-yr or 4-yr rotations, β-
245 *Proteobacteria* were more abundant in CONS management than in CONV management, but the
246 reverse was observed for *Acidobacteria*. A side-by-side comparison of the average distribution
247 for all CONV vs. CONS management shows that the sum of all *Proteobacteria* was greater
248 under CONS management than under CONV management, and so were *Bacteriodetes*, but the
249 reverse was true for *Acidobacteria* (Fig. 4).

250

251 **Taxonomic Identities: 16S rRNA Gene Sequencing**

252 A total of 108 endophytes from various treatments were selected for sequencing (Supplementary
253 Table 1). All of these 16S rRNA encoding gene sequences were identified by sequence

254 homology using NCBI BLAST followed by Ribosomal Database Project (RDP) (Larkin et al.
255 2007). Based on this sequencing, the dominant phyla in all treatments were *Proteobacteria*,
256 *Firmicutes*, *Cyanobacteria* and *Actinobacteria*, but their relative distribution in each treatment
257 was different (Fig. 5). Due to culturing difficulty (Jones et al. 2009), no *Acidobacteria* were
258 detected based on 16S rRNA gene sequencing. Within 3-yr or 4-yr rotations, *Proteobacteria*
259 were more abundant in CONS practices than CONV practices, whereas *Actinobacteria* were
260 more abundant in CONV practices than in CONS practices. Furthermore, *Bacteriodetes* were
261 only found in the longer rotations under CONS soil management. Relatively equal percentages of
262 *Firmicutes* were found in all treatments and the distribution of *Cyanobacteria* was variable.
263 Results of phylogenetic analysis of the 16S rRNA gene of endophytes from each treatment are
264 shown in Supplementary Figs. 1-6. The *Firmicutes* were mostly the genera *Bacillus*,
265 *Paenibacillus*, *Lysinibacillus*, *Solibacillus* and *Staphyloccocus*. Some of the genera belonging to
266 *Proteobacteria* were *Pseudomonas*, *Cedibacter*, *Agrobacterium*, *Rhizobium*, *Ralstonia* and
267 *Cupriavidus*. The *Cyanobacteria* were represented by *Halospirulina* and *Planktothricoides* spp.
268 Bacteria belonging to *Actinobacteria* included the genera *Kribbia*, *Brevibacterium*,
269 *Microbacterium* and *Arthrobacter*.
270

271 **DISCUSSION**

272 Bacterial endophytes in agricultural systems are subject to a wide range of management
273 practices. Characterizing these endophyte communities is important considering their potential
274 significance in plant growth promotion, nitrogen fixation, protection against disease, biotic and
275 abiotic stress tolerance, or sources of novel biomolecules for bioremediation (Trivedi et al.
276 2011). As endophytes occur in intercellular spaces of plant tissues, their role in supporting plant

277 growth is matter of discussion (Sessitsch et al. 2002). It is assumed that endophytic bacteria play
278 an important role in plant growth and its adaptation to the environment. Their presence indicates
279 that they are critical for plant health, growth and other ecologically relevant functions of plants.
280 The rhizosphere is an important source of root endophytes, and most of the root endophytes are
281 also present in the rhizopshere. Root endophytes enter the plant by local cellulose degradation or
282 fractures in the root system (Gough et al. 1997). In this study, conservation practices increased
283 soil microbial diversity and biomass (F. J. Larney, unpubl). We also found that changing the soil
284 management practices and lengthening crop rotations influenced bacterial endophyte populations
285 and altered their community structures.
286
287 Endophyte populations under CONS soil management were higher probably due to the improved
288 soil quality (Larkin et al. 2011), in this case resulting from direct seeding (zero or reduced
289 tillage), addition of composted manure, and cover-cropping. Many reports show that residue
290 retention with direct seeding increase the abundance of bacteria in no-till systems (Hammesfahr
291 et al. 2008; Ceja-Navarro et al. 2010). Agronomic practices that include reduced tillage and crop
292 residue retention can be adopted as sustainable agricultural practices that preserve and improve
293 the diversity of soil microbial communities (Ceja-Navarro et al. 2010). Tillage reduces microbial
294 populations because it accelerates the breakdown of soil organic C, a key substrate for
295 microorganisms. Tillage also reduces microbial diversity (Lupwayi et al. 1998), presumably
296 because it homogenizes the soil and the C substrates within it. Addition of organic amendments
297 like compost to agricultural soil increases the population of soil micro-organisms and recycling
298 of nutrients, resulting in improvement of both soil biological and chemical properties (Goyal et
299 al. 1999). Composting not only concentrates nutrients but also kills pathogens (Larney et al.,

2003) and weed seeds (Larney and Blackshaw 2003). . Composted manure is used to substitute for inorganic fertilizers and is widely used in organic farming systems (Lynch et al., 2011). Compost applied to agricultural fields improves soil structure and increases microbial activities due to the organic C inputs (Steger et al. 2007). Cover cropping, another practice employed in the CONS rotations, increases soil microbial populations because it increases soil organic C. Carrera et al. (2007) reported a significant effect of cover cropping on soil microbial community structure.

A consistent observation in both T-RFLP analysis for culture independent bacteria and 16S rRNA gene sequencing analysis for cultivated bacteria with regard to community structures of the endophytes was that CONS management systems contained more *Proteobacteria* than CONV management systems, and vice-versa for *Acidobacteria* (in T-RFLP). *Bacteriodetes* were found only in long rotations. The phylum *Proteobacteria* is the most heterogeneous of all bacterial phyla both morphologically and metabolically, and most bacteria of medical, industrial, and agricultural importance belong to this phylum (Oren 2010). In this study, the N_2-fixing *Rhizobium* was one of the genera found. Other endophytes found in rotations under CONS soil management also have potentially beneficial characteristics. For example, *Paenibacillus polymyxa* (in 5-yr rotation) is a nitrogen-fixing bacterium (Anand 2010) and *Kribbia dieselivorans* (in 6-yr rotation) has diesel-oil degradation activity (Jung et al. 2006). On the other hand, the phylum *Acidobacteria* is composed of a small group of acidophilic organotrophs and iron reducers (Oren 2010).

Sequencing of the 16S rRNA gene directly from potato root tissue (without culturing the endophytes) was not possible in constructing the gene library since the eubacterial primers used to amplify 16S rRNA are also homologous to chloroplast 16S and mitochondrial 18S rRNA of plants (Reiter et al. 2002, Sessitsch et al. 2002). The majority of clones contained mainly mitochondrial and, to a lesser extent, chloroplast small-subunit rRNA sequences. This limited our ability to compare the 16s rDNA clone library with a number of community members found by T-RFLP analysis. Hence, putative taxonomic analysis of TRFs was assigned based on PAT+ analysis from MiCA3 software (Shyu et al. 2007). Our phylogenetic and molecular evolutionary analyses (Supplementary Figs. 1-6) were based on cultured endophytes. The relative distribution of the most abundant phyla (from 16S rRNA gene sequencing) of the cultured endophytes in different treatments are shown in Figure 5. In previous studies of potato endophytes, α, β, and γ sub-divisions of *Proteobacteria*, *Actinobacteria*, *Flexibacter*, *Cytophaga*, and *Bacteriodetes* genera were identified (Reiter et al. 2002; Sessitsch et al. 2002). Pyrosequencing analysis of potato root endophytes also revealed *α-Proteobacteria*, *β-Proteobacteria*, *γ-Proteobacteria*, *δ-Proteobacteria*, *Cyanobacteria*, *Firmicutes*, *Acidobacteria*, *Actinobacteria*, *Bacteriodetes*, *Chloflexi*, *Plancomycetes*, *Fusobacteria*, *Verrucomicrobia*, *Gemmatimonadetes* and other unidentified bacteria families (Manter et al. 2010). More than 20 species of *Pseudomonas*, *Bacillus*, *Enterobacter* and *Agrobacterium* genera appear to be the most common culturable bacterial endophytes found in potato (Manter et al. 2010). Our results are in agreement with these observations.

In this study, we isolated, quantified and identified bacterial endophytes from potato grown in conventional and conservation soil management systems of different crop rotation lengths, and

demonstrated that the endophyte communities are complex. Conservation soil management practices and long crop rotations increased the populations and diversity on the endophytic bacteria compared with conventional soil management and short rotations. The community structures of the endophytes were also different in these treatments. This phylogenetic characterization of the endophytes needs to be followed by functional characterization to understand how these bacteria affect their host plants. Novel endophytes and their bioactive secondary metabolites could also be identified to develop technologies that increase crop growth and yields.

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Anand, R. 2010. Endophytic colonization and nitrogen fixation by *Paenibacillus polymyxa* in association with lodge pole pine and western red cedar. Ph.D. thesis, University of British Columbia, Vancouver, British Columbia, Canada.

Andreote, F. D., Arau, W. L. D., Azevedo, J. L. D., Elsas, J. D. V., Rocha, U. N. D. and Overbeek, L. S. V. 2009. Endophytic colonization of potato (*Solanum tuberosum* L.) by a novel competent bacterial endophyte, *Pseudomonas putida* Strain P9, and its effect on associated bacterial communities. Appl. Environ. Microbiol. **75**: 3396-3406.

Altschul, S. F., Madden, T. L., Schaeffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25**: 3389-3402.

Biederbeck, V. O., Zentner, R. P. and Campbell, C. A. 2005. Soil microbial populations and activities as influenced by legume green fallow in a semiarid climate. Soil Biol. Biochem. **37**: 1775-1784.

Carrera, L. M., Buyer, J. S., Vinyard, B, Abdul-Baki, A. A., Sikora, L. J. and Teasdale, J. R. 2007. Effects of cover crops, compost, and manure amendments on soil microbial community structure in tomato production systems. Appl. Soil Ecol. **37**: 247-255.

Ceja-Navarro, J. A., Rivera-Orduna, F. N., Patino-Zuniga, L., Vila-Sanjurjo, A., Crossa, J., Govaerts, B. and Dendooven, L. 2010. Phylogenetic and multivariate analyses to determine the effects of different tillage and residue management practices on soil bacterial communities. Appl. Environ. Microbiol. **76**: 3685–3691.

Chang BioScience, 2004. Shannon-Wiener diversity index/Shannon entropy calculator. http://www.changbioscience.com/genetics/shannon.html. 12 Oct. 2012.

Collins, R. E. and Rocap, G. 2007. REPK: an analytical web server to select restriction endonucleases for terminal restriction fragment length polymorphism analysis. Nucleic Acids Res. **35**: 58-62.

Conn, V. M. and Franco, C. M. M. 2004. Analysis of the endophytic actinobacterial population in the roots of wheat (*Triticum aestivum* L.) by terminal fragment length polymorphism and sequencing of 16S rRNA clones. Appl. Environ. Microbiol. **70**: 1787-1794.

390 **Culman, S. W., Bukowski, R., Gauch, H.G., Cadillo-Quiroz, H., and Buckley, D.H. 2009.** T-
391 REX: software for the processing and analysis of T-RFLP data. BMC Bioinformatics. **10**:
392 171.
393 **Doty, S. L., Dosher, M. R., Singleton, G. L., Moore, A. L., Van Aken, B., Stettler, R. F.,**
394 **Strand, S. E. and Gordon, M. P. 2005**. Identification of an endophytic *Rhizobium* in
395 stems of *Populus*. Symbiosis **39**: 27-36.
396 **Dunbar, J., Ticknor, L. O. and Kuske, C. R. 2000.** Assessment of microbial diversity in four
397 southwestern United States soils by 16S rRNA gene terminal restriction fragment
398 analysis. Appl. Environ. Microbiol. **66**: 2943-2950.
399 **Garbeva, P., Van Overbeek, L. S., Van Vurde, J. W. L. and Van Elsas, J. D. 2001.** Analysis
400 of endophytic bacterial communities of potato by plating and denaturing gradient gel
401 electrophoresis (DGGE) of 16S rDNA based fragments. Microb. Ecol. **41**: 369-383.
402 **Gough, C., Galera, C., Vasse, J., Webster, G., Cocking, E. C. and Denarie, J. 1997**. Specific
403 flavonoids promote intercellular root colonization of *Arabidopsis thaliana* by
404 *Azorhizobium caulinodans* ORS571. Mol. Plant-Microbe Interact. **10:** 560-570.
405 **Goyal, S., Chander, K., Mundra, M. C. and Kapoor, K. K. 1999.** Influence of inorganic
406 fertilizers and organic amendments on soil organic matter and soil microbial properties
407 under tropical conditions. Biol. Fertil. Soils. **29**: 196-200.
408 **Hammer, O., Harper, D. A. T. and Ryan, P. D. 2001.** PAST: PAleontological STatistics
409 software package for education and data analysis. Palaeontologia Electronica **4**: 1-9.
410 **Hammesfahr U., Heuer H., Manzke B., Smalla K. and Thiele-Bruhn, S. 2008.** Impact of the
411 antibiotic sulfadiazine and pig manure on the microbial community structure in
412 agricultural soils. Soil Biol. Biochem. **40**: 1583–1591.

Hung, P. Q., Kumar, S. M., Govindsamy, V. and Annapurna K. 2007. Isolation and characterization of endophytic bacteria from wild and cultivated soybean varieties. Biol. Fertil. Soils. **44**: 155-162.

Jones, R. T., Robeson, M. S., Lauber, C. L., Hamady, M., Knight, R. and Fierer, N. 2009. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. The ISME Journal. **3**: 442–453.

Jung, S. Y., Kim, H. S., Song, J. J., Lee, S. G., Oh, T. K. and Yoon, Y. H. 2006. *Kribbia dieselivorans* gen. nov., sp. nov., a novel member of the family *Intrasporangiaceae*. Int. J. Syst. Evol. Microbiol. **56**: 2427-32.

Khan, Z. and Doty, S. L. 2009. Characterization of bacterial endophytes of sweet potato. Plant Soil. **322**: 197-207.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H. et al. 2007. ClustalW and ClustalX version 2 Bioinformatics **23**: 2947-2948.

Larkin, R. P., Honeycut, C. W., Griffin, T. S., Olanya, O. M., Halloran, J. M. and He, Z. 2011. Effects of different potato cropping system approaches and water management on soil borne diseases and soil microbial communities. Phytopathol. **101**: 58-67.

Larney, F. J. and Blackshaw, R. E. 2003. Weed seed viability in composted beef cattle feedlot manure. J. Environ. Qual. **32**: 1105-1113.

Larney, F.J., Yanke, L.J., Miller, J.J., and McAllister, T.A. 2003. Fate of coliform bacteria in composted beef cattle feedlot manure. J. Environ. Qual. **32**: 1508–1515.

Liu, W. T., Marsh, T. L., Cheng, H. and, Forney, L. J. 1997. Characterization of microbial diversity by determining terminal fragment length polymorphisms of genes encoding 16S rRNA. Appl. Environ. Microbiol. **63**: 4516-4522.

436 **Luna, G. M., Anno, A. D. and Danovaro, R. 2006.** DNA extraction procedure: a critical issue
437 for bacterial diversity assessment in marine sediments. Environ. Microbiol. **8**: 308-320.
438 **Lupwayi, N. Z., Clayton, G. W., Hanson, K. G., Rice, W. A. and Biederbeck, V. O. 2004.**
439 Populations and functional diversity of bacteria associated with barley, wheat and canola
440 roots. Can. J. Soil Sci. **84**: 245-254.
441 **Lupwayi, N. Z., Rice, W. A. and Clayton, G.W. 1998.** Soil microbial diversity and community
442 structure under wheat as influenced by tillage and crop rotation. Soil Biol. Biochem. **30**:
443 1733-1741.
444 **Lynch, D.H., MacRae, R. and Martin, R.C.. 2011.** The carbon and global warming potential
445 impacts of organic farming: Does it have a significant role in an energy constrained
446 world? Sustainability, **3**: 322-362.
447 **Manter, D. K., Delgado, J. A., Holm, D. G. and Stong, R. A. 2010.** Pyrosequencing reveals a
448 highly diverse and cultivar-specific bacterial community in potato roots. Microb. Ecol.
449 **60**: 157-166.
450 **Marquez-Santacruz, H. A., Hernandez-Leon, R., Orozco-Mosqueda, M. C., Velazquez-**
451 **Sepulveda, I. and Santoyo, G. 2010.** Diversity of bacterial endophytes in roots of
452 Mexican husk tomato plants (*Physalis ixocarpa*) and their detection in the rhizosphere.
453 Genetics and Molecular Research **9**: 2372-2380.
454 **Oren, A. 2010.** The phyla of prokaryotes – cultured and uncultured. In Calster Academic Press,
455 Norfolk, UK.
456 **Osborn, A. M., Moore, E. R. B., and Timmis, K. N. 2000.** An evaluation of terminal-
457 restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial
458 community structure and dynamics. Environ. Microbiol. **2**: 39-50.

459 **Reiter, B., Pfeifer, U., Schwab, H. and Sessitsch, A. 2002.** Response of Endophytic Bacterial
460 Communities in Potato Plants to Infection with *Erwinia carotovora* subsp. *atroseptica*.
461 Appl. Environ. Microbiol. **68**: 2261-2268.
462 **Ryan, R. P., Germaine, K., Franks, A., Ryan, D. J. and Dowling, D. N. 2008.** Bacterial
463 endophytes: recent developments and applications. FEMS Microbiol. Lett. **278**: 1-9.
464 **Schulz, B. and Boyle, C. 2006.** Microbial Root Endophytes, Springer-Verlag, Berlin
465 Heidelberg, Soil Biol. **9**:1-13.
466 **Sessitsch, A., Reiter, B., Pfeifer, U. and Wilhelm, E. 2002.** Cultivation-independent population
467 analysis in bacterial endophytes in three potato varieties based on eubacterial and
468 *Actinomycetes*- specific PCR of 16S rRNA genes. FEMS Microbiol. Ecol. **39**: 23-32.
469 **Shyu, C., Soule, T., Bent, S. J., Foster, J. A. and Forney, L. J. 2007.** MiCA: A Web-based
470 tool for the analysis of microbial communities based on terminal-restriction fragment
471 length polymorphisms of 16S and 18S rRNA genes. J. Microb. Ecol. **53**: 562-570.
472 **Steger, K., Sjogren, A. M., Jarvis, A., Jansson, J. K. and Sundh, I. 2007.** Development of
473 compost maturity and *Actinobacteria* populations during full-scale composting of organic
474 household waste. J. Appl. Microbiol. **103**: 487–498.
475 **Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar S. 2011.** MEGA5:
476 Molecular evolutionary genetics analysis using maximum likelihood, evolutionary
477 distance, and maximum parsimony methods. Mol. Biol. Evol. **28**: 2731-2739.
478 **Trivedi, P., Spann, T. and Wang, N. 2011.** Isolation and characterization of beneficial bacteria
479 associated with citrus roots in Florida. Microb. Ecol. **62**: 324-336.
480 **West, E. R., Cother, E. J., Steel, C. C. and Ash, G. J. 2010.** The characterization and diversity
481 of bacterial endophytes of grapevine. Can. J. Microbiol. **56**: 209-16.

Wilson, D. 1995. Endophyte-the evolution of a term and clarification of its use and definition. Oikos, **73**: 274-276.

Wright, E. S., Yilmaz, L. S. and Noguera, D. R. 2012. DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences. Appl. Environ. Microbiol. **78**: 717-725.

Xin, G., Zhang, G., Kang, J. W., Staley, J. T. and Doty, S. L. 2009. A diazotropic, indole 3-acetic acid-producing endophyte from cottonwood. Biol. Fertil. Soils **45**: 669-674.

FIGURE CAPTIONS

Fig. 1. Effect of crop rotation length (3- to 6-yr) and soil management (CONV or CONS) on bacterial endophyte populations of potato roots. Each bar is an average of four field replications, and the standard error is indicated. Treatment means with the same letter are not significantly different at 5% significance level. W: wheat; B: beans; P: potato; SB: sugar beet; O (t): silage oats harvested July, timothy seeded August; T: timothy. CONV: Conventional soil management; CONS: Conservation soil management ; CONV: Conventional soil management; CONS: Conservation soil management.

Fig. 2. Ordination of endophyte TRFs (from T-RFLP analysis) of endophyte communities from different treatments (T1 to T6) by principal component analysis (PCA). Each treatment point is a mean of four replicates. The percentage of variance explained by each axis is indicated. T1: (Potato-Bean-Wheat) and T3: (Wheat-Sugar beet-Bean-Potato) under CONV soil management while T2: (Potato-Bean-Wheat), T4: (Wheat-Sugar beet-Bean-Potato), T5: (Potato-Wheat-Sugar beet) and T6: Oat-Timothy-Timothy-Sugar beet-Bean-Potato) under CONS soil management.

Fig. 3. Relative abundance of different bacterial phylogenetic groups (from T-RFLP analysis) in various treatments. Each data point was calculated from the average operational taxonomic units (OTUs) from four field replicates.

Fig. 4. Relative distribution of the different bacterial phylogenetic groups (from T-RFLP analysis) in conventional (CONV) and conservation (CONS) soil management treatments.

514 **Fig. 5.** Relative distribution of the most abundant phylogenetic groups (from 16S rRNA gene
515 sequencing) of cultured endophytes in various treatments. W: wheat; B: beans; P: potato; SB:
516 sugar beet; O (t): silage oats harvested July, timothy seeded August; T: timothy. CONV:
517 Conventional soil management; CONS: Conservation soil management ; CONV: Conventional
518 soil management; CONS: Conservation soil management.
519
520

SUPPLEMENTARY FIGURE CAPTIONS

Supplementary Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences of endophytes of potato from T1 treatment (3-yr Potato-Bean-Wheat rotation) under conventional soil management using neighbor-joining method in ClustalW2 program. EA1 to EA19, are the endophytes identified from treatment T1.

Supplementary Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences of endophytes of potato from T2 treatment (3-yr Potato-Bean-Wheat rotation) under conservation soil management using neighbor-joining method ClustalW2 program. EB1 to EB19 are the endophytes identified from treatment T2.

Supplementary Fig. 3. Phylogenetic tree based on 16S rRNA gene sequences of endophytes of potato from T3 treatment (4-yr Wheat-Sugar beet-Bean-Potato rotation) under conventional soil management using neighbor-joining method ClustalW2 program. EC1-EC16 are the endophytes identified from treatment T3.

Supplementary Fig. 4. Phylogenetic tree based on 16S rRNA gene sequences of endophytes of potato from T4 treatment (4-yr Wheat-Sugar beet-Bean-Potato rotation) under conservation soil management using neighbor-joining method ClustalW2 program. ED1 to ED17 are the endophytes identified from treatment T4.

Supplementary Fig. 5. Phylogenetic tree based on 16S rRNA gene sequences of endophytes of potato from T5 treatment (5-yr Potato-Wheat-Sugar beet-Wheat-Bean rotation) under conservation soil management using neighbor-joining method ClustalW2 program. EE1 to EE14 are the endophytes identified from treatment T5.

Supplementary Fig. 6. Phylogenetic tree based on 16S rRNA gene sequences of endophytes of potato from T6 treatment (6-yr Oat-Timothy-Timothy-Sugar beet-Bean-Potato rotation) under conservation soil management using neighbor-joining method ClustalW2 program. EF1 to EF23 are the endophytes identified from treatment T6.

1

Table 1. Irrigated rotation treatments at Vauxhall, Alberta. W: wheat; B: beans; P: potato; SB: sugar beet; O (t): silage oats harvested July, timothy seeded August; T: timothy. CONV: Conventional soil management; CONS: Conservation soil management.

Treatment	Length, yr	Rotation	Management
1	3	P-B-W	CONV
2	3	P-B-W	CONS
3	4	W-SB-B-P	CONV
4	4	W-SB-B-P	CONS
5	5	P-W-SB-W-B	CONS
6	6	O(t)-T-T-SB-B-P	CONS

2

3

Table 2. Diversity of bacterial endophytes isolated from potato in various treatments. Average values (± standard error) of shannon index, species richness and evenness are from four field replicates. Means with the same letter in a column are not significantly different 5% significance level. W: wheat; B: beans; P: potato; SB: sugar beet; O (t): silage oats harvested July, timothy seeded August; T: timothy. CONV: Conventional soil management; CONS: Conservation soil management.

Treatment	Management	Shannon Index (H')	Species Richness (S)	Evenness (E)
P-B-W	CONV	1.99 ± 0.20 bc	8.75 ± 1.5 ab	0.92 ± 0.03 ab
P-B-W	CONS	2.11 ± 0.09 a	9.75 ± 0.5 a	0.93 ± 0.02 ab
W-SB-B-P	CONV	1.92 ± 0.21 c	8.50 ± 1.9 b	0.91 ± 0.01 b
W-SB-B-P	CONS	2.11 ± 0.07 a	9.50 ± 0.6 ab	0.94 ± 0.02 a
P-W-SB-W-B	CONS	2.07 ± 0.16 ab	9.25 ± 1.5 ab	0.94 ± 0.01 a
O(t)-T-T-SB-B-P	CONS	2.09 ± 0.12 a	9.50 ± 1.0 ab	0.93 ± 0.01 ab

Fig. 1

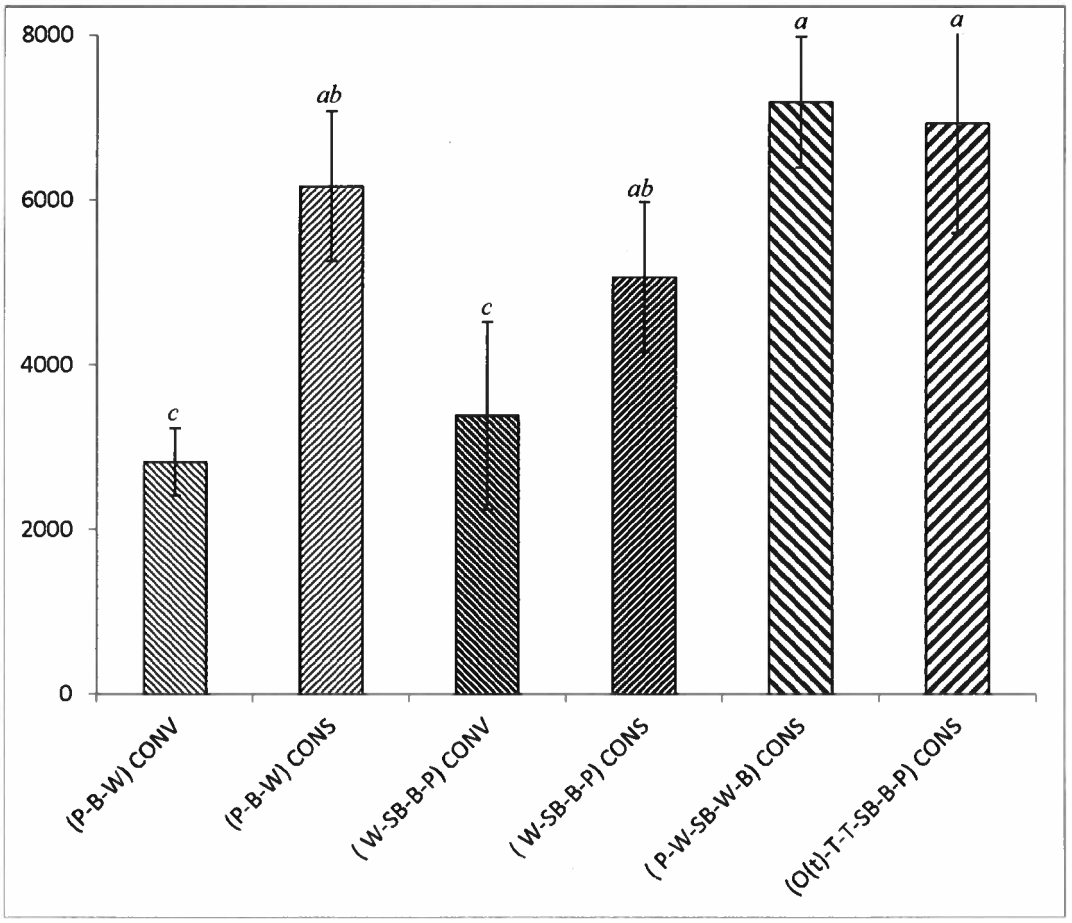
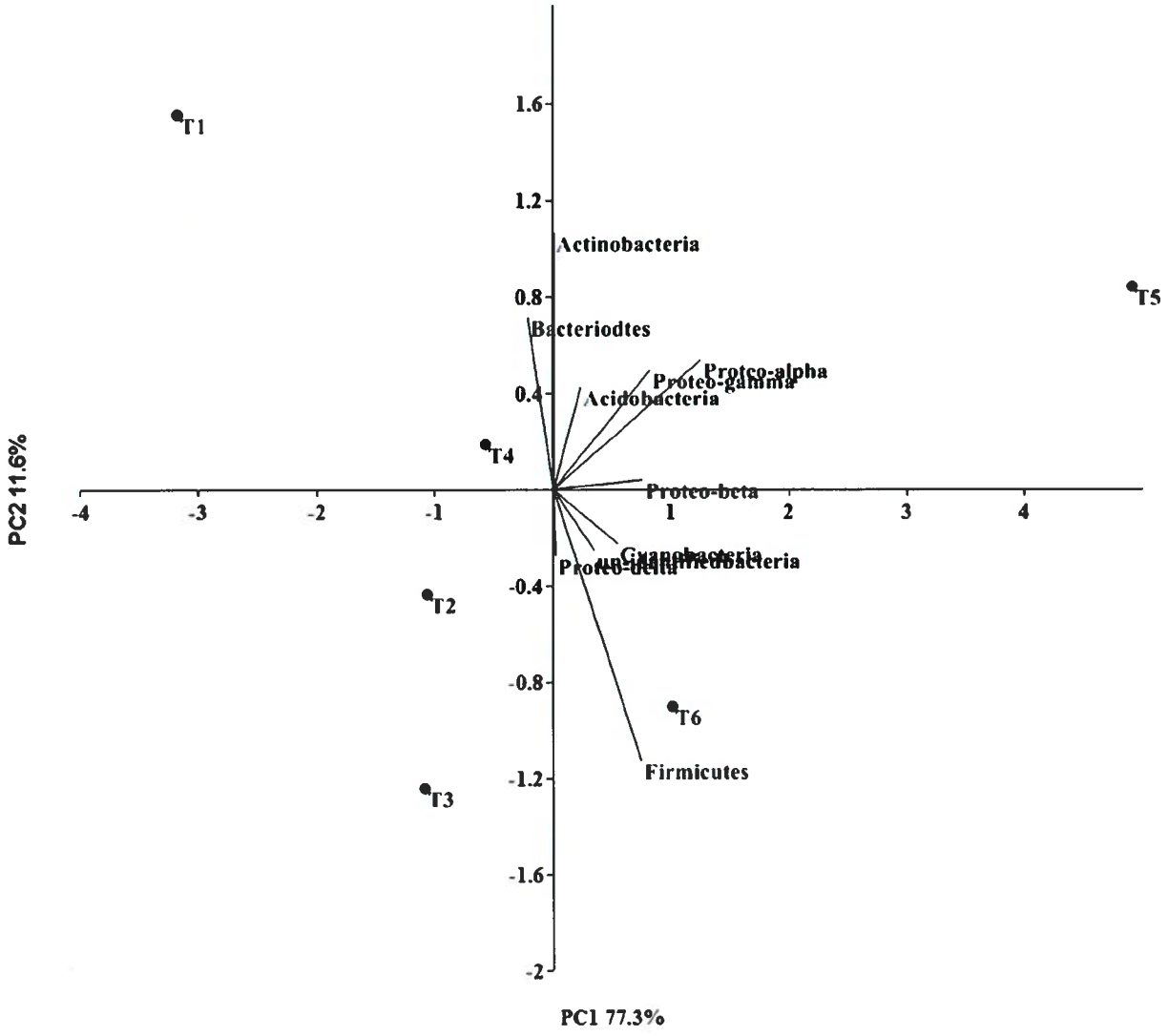


Fig. 2.



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14 Fig. 3

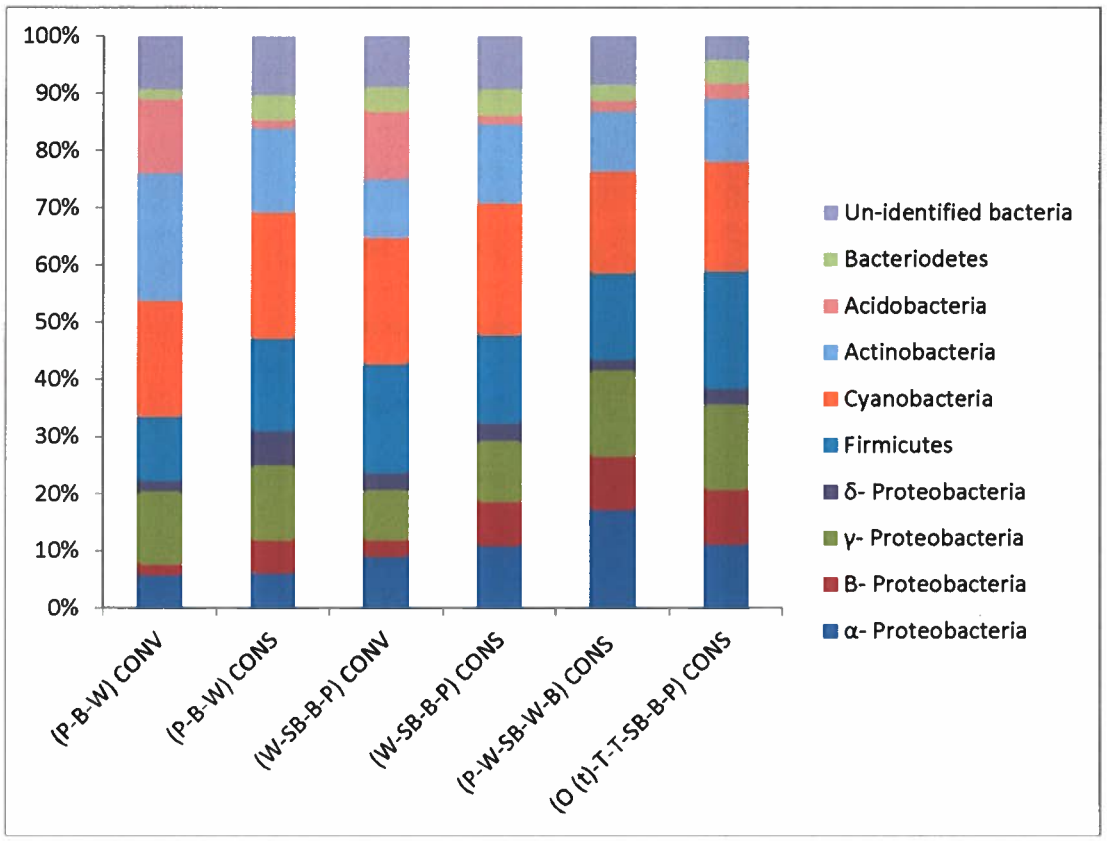

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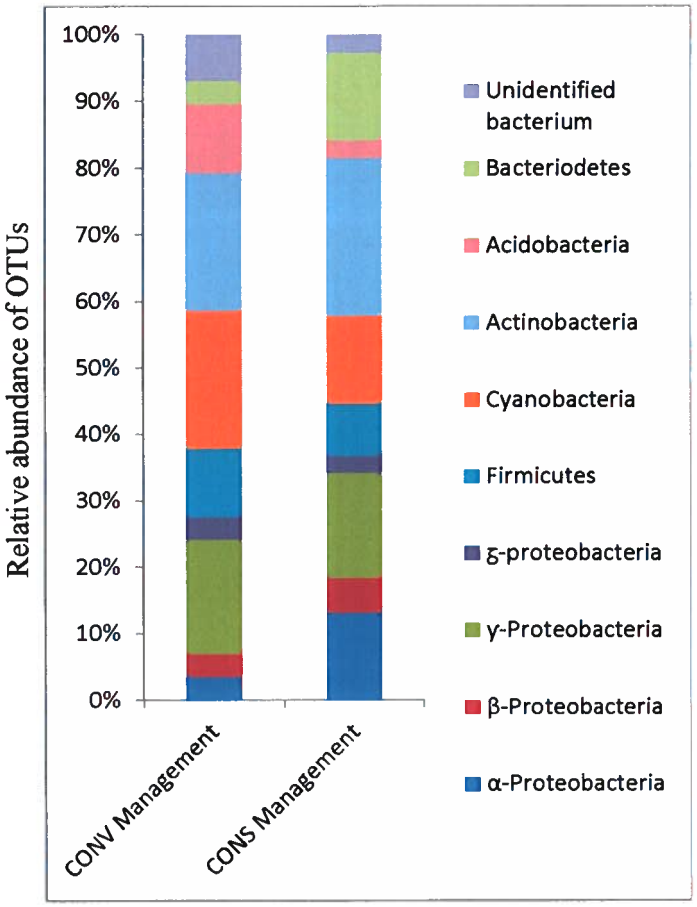
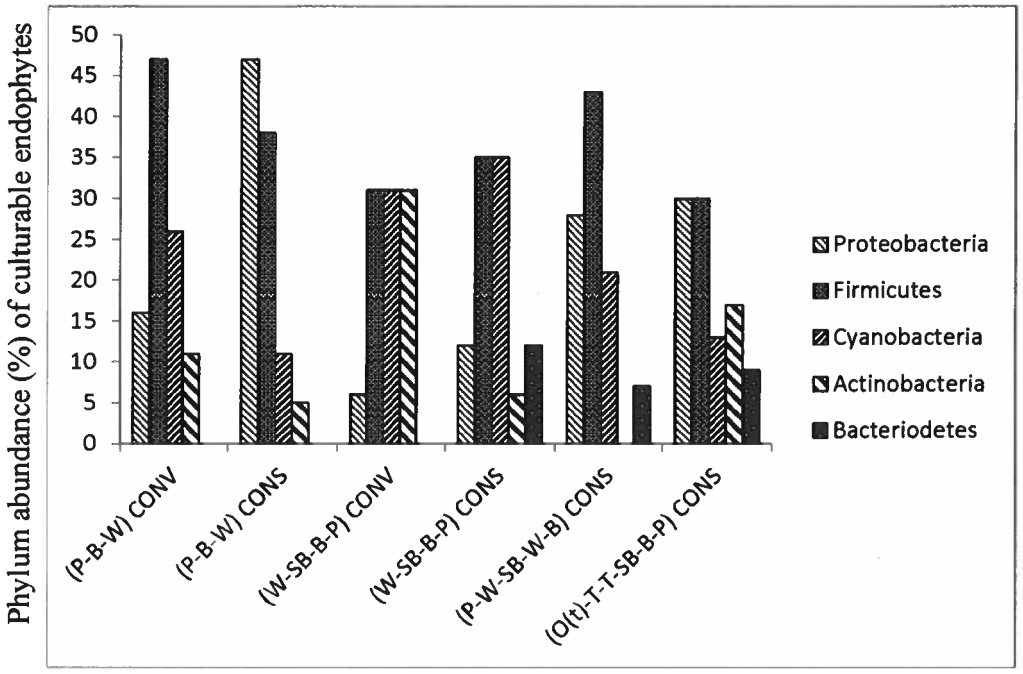
Fig. 4





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Supplementary Table 1. Endophytic bacteria cultured and identified based on 16S rRNA gene sequencing. TMT: Treatment; T1: (P-B-W) and T3: (W-SB-B-P) under CONV soil management while T2: (P-B-W), T4: (W-SB-B-P), T5: (P-W-SB-W-B) and T6: O(t)-T-T-SB-B-P under CONS soil management.

Endophyte	TMT.	Closest NCBI database match	% Similarity	NCBI accession Number
EA1	T1	*Staphylococcus warneri* strain AW 25(NR_025922)	99	JX912365
EA2	T1	*Paenibacillus tundrae* strain Ab10b (NR_044525)	99	JX912366
EA3	T1	*Pseudomonas brassicacearum* strain NFM421 (NR_074734)	99	JX912367
EA4	T1	*Bacillus marisflavi* strain TF-11 (NR_025240)	99	JX912368
EA5	T1	*Brevibacterium frigoritolerans* strain :DSM 8801 (NR_042639)	99	JX912369
EA6	T1	*Agrobacterium tumefaciens* strain IAM 12048 (NR_041396)	99	JX912370
EA7	T1	*Rhizobium selenitireducens* strain B1(NR_044216)	99	JX912371
EA8	T1	*Bacillus sp.* LMG 20238 (NR_042083)	98	JX912372
EA9	T1	*Halospirulina tapeticola* strain CCC Baja-95 Cl.2 (NR_026510)	84	JX912373
EA10	T1	*Planktothricoides raciborskii* NIES-207 strain (NR_040858)	84	JX912374
EA11	T1	*Halospirulina tapeticola* strain CCC Baja-95 Cl.2 (NR_026510)	85	JX912375
EA12	T1	*Planktothricoides raciborskii* NIES-207 strain (NR_040858)	84	JX912376
EA13	T1	*Halospirulina tapeticola* strain CCC Baja-95 Cl.2 (NR_026510)	84	JX912377
EA14	T1	*Paenibacillus tundrae* strain Ab10b (NR_044525)	99	JX912378
EA15	T1	*Bacillus megaterium* QM B1551 strain (NR_074290)	99	JX912379
EA16	T1	*Staphylococcus pasteuri* strain ATCC51129 (NR_024669)	99	JX912380
EA17	T1	*Bacillus thuringiensis* strain IAM 12077 (NR_043403)	99	JX912381
EA18	T1	*Bacillus thuringiensis* strain IAM 12077 (NR_043403)	99	JX912382
EA19	T1	*Staphylococcus warneri* strain AW 25(NR_025922)	99	JX912383

EB1	T2	*Cupriavidus metallidurans* strain CH34 (NR_074704)	99	JX912384
EB2	T2	*Bacillus weihenstephanensis* strain DSM11821 (NR_024697)	99	JX912385
EB3	T2	*Bacillus weihenstephanensis* strain DSM11821 (NR_024697)	99	JX912386
EB4	T2	*Bacillus megaterium* strain IAM 13418 (NR_043401)	99	JX912387
EB5	T2	*Paenibacillus tundrae* strain Ab10b (NR_044525)	98	JX912388
EB6	T2	*Bacillus megaterium* strain IAM 13418 (NR_043401)	99	JX912389
EB7	T2	*Brevibacterium frigoritolerans* strain :DSM 8801 (NR_042639)	99	JX912390
EB8	T2	*Pseudomonas brassicacearum* strain NFM421(NR_074834)	99	JX912391
EB9	T2	*Halospirulina tapeticola* strain CCC Baja-95 Cl.2 (NR_026510)	83	JX912392
EB10	T2	*Halospirulina tapeticola* strain CCC Baja-95 Cl.2 (NR_026510)	84	JX912393
EB11	T2	*Bacillus pumilus* SAFR-032 (NR_074977)	100	JX912394
EB12	T2	*Bacillus pumilus* SAFR-032 (NR_074977)	99	JX912395
EB13	T2	*Bacillus thuringiensis* strain IAM 12077 (NR_043403)	99	JX912396
EB14	T2	*Agrobacterium tumefaciens* strain IAM 12048 (NR_041396)	99	JX912397
EB15	T2	*Bacillus simplex* strain DSM 1321 (NR_042136)	99	JX912398
EB16	T2	*Bacillus megaterium* QM B1551 (NR_074290)	99	JX912399
EB17	T2	*Bacillus thuringiensis* strain IAM 12077 (NR_043403)	99	JX912400
EB18	T2	*Arhrobacter globiformis* strain DSM 20124 (NR_026187)	97	JX912401
EB19	T2	*Bacillus simplex* strain DSM 1321(NR_042136)	99	JX912402
EC1	T3	*Bacillus megaterium* QM B1551 (NR_074290)	99	JX912403
EC2	T3	*Brevibacterium frigoritolerans* strain :DSM 8801(NR_042639)	99	JX912404
EC3	T3	*Pseudomonas kilonensis* strain 520-20 (NR_028929)	100	JX912405
EC4	T3	*Halospirulina tapeticola* strain CCC Baja-95 Cl.2 (NR_026510)	83	JX912406
EC5	T3	*Halospirulina tapeticola* strain CCC Baja-95 Cl.2 (NR_026510)	83	JX912407
EC6	T3	*Serratia ficaria* strain DSM 4569 (NR_041979)	99	JX912408
EC7	T3	*Pseudomonas veronii* strain CIP 104663 (NR_028706)	99	JX912409
EC8	T3	*Arthrobacter sulfonivorans* strain ALL (NR_025084)	99	JX912410
EC9	T3	*Bacillus cereus* ATCC 14579 (NR_074540)	99	JX912411

EC10	T3	*Bacillus anthracis* str. Ames strain (NR_0074453)	99	JX912412
EC11	T3	*Bacillus marisflavi* strain TF-11 (NR_025240)	100	JX912413
EC12	T3	*Rhizobium huautlense* strain SO2 (NR_024863)	97	JX912414
EC13	T3	*Rhizobium selenitireducens* strain B1 (NR_044216)	99	JX912415
EC14	T3	*Rhizobium selenitireducens* strain B1 (NR_044216)	98	JX912416
EC15	T3	*Arthrobacter sulfureus* strain DSM 20167 (NR_026237)	99	JX912417
EC16	T3	*Bacillus megaterium* strain IAM 13418 (NR_04341)	99	JX912418
ED1	T4	*Bacillus clausii* strain DSM 8716 (NR_026140)	99	JX912419
ED2	T4	*Bacillus clausii* KSM-K16 (NR_074988)	99	JX912420
ED3	T4	*Bacillus licheniformis* DSM 13 (NR074923)	99	JX912421
ED4	T4	*Staphylococcus warneri* strain AW (NR_025922)	99	JX912422
ED5	T4	*Pseudomonas kilonensis* strain 520-20 (NR_028929)	100	JX912423
ED6	T4	*Bacillus pumilus* ATCC 7061 (NR_043242)	98	JX912424
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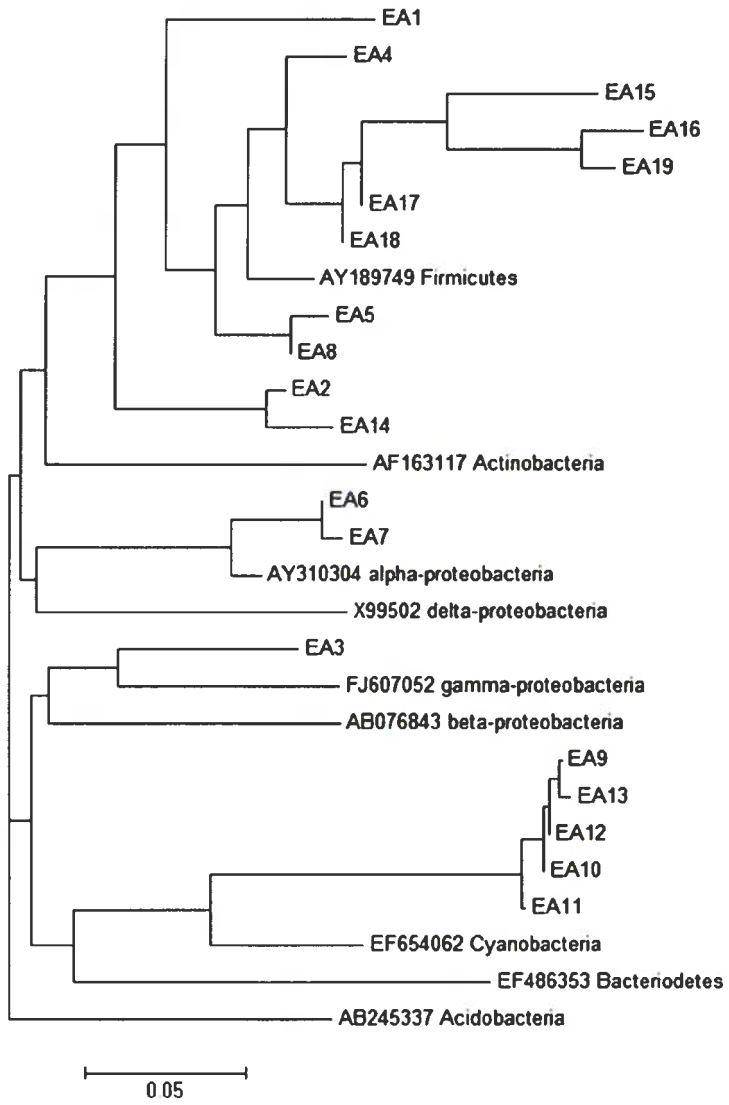
ED9	T4	*Brevibacterium frigoritolerans* strain :DSM 8801 (NR_042639)	99	JX912427
ED10	T4	*Pseudomonas lurida* strain : DSM 15835 (NR_042199)	99	JX912428
ED11	T4	*Halospirulina tapeticola* strain CCC Baja-95 Cl.2 (NR_026510)	83	JX912429
ED12	T4	*Brevibacterium frigoritolerans* strain :DSM 8801 (NR_042639)	100	JX912430
ED13	T4	*Lysinibacillus fusiformis* strain DSM 2898 (NR_042072)	99	JX912431
ED14	T4	*Bacillus vietnamensis* strain 15-1 (NR_024808)	97	JX912432
ED15	T4	*Agrobacterium tumefaciens* strain IAM 12048 (NR041396)	99	JX912433
ED16	T4	*Bacillus horikoshii* strain DSM8719 (NR_040852)	100	JX912434
ED17	T4	*Bacillus odysseyi* strain 34hs1 (NR_025258)	99	JX912435
EE1	T5	*Bacillus circulans* (NR_042726)	98	JX912436
EE2	T5	*Bacillus thuringiensis* strain IAM 12077 (NR_43403)	99	JX912437
EE3	T5	*Staphylococcus warneri* strain AW 25 (NR_025922)	99	JX912438

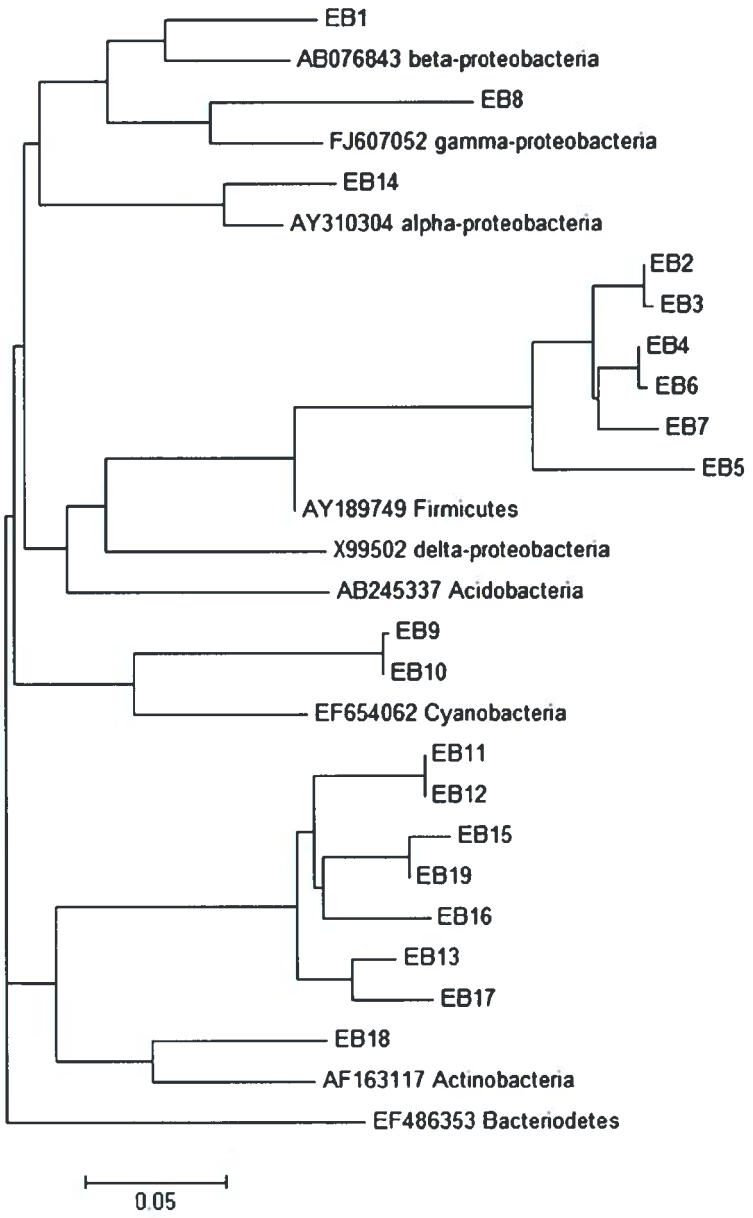
EE4	T5	*Pseudomonas brassicacearum* NFM421(NR_074834)	99	JX912439
EE5	T5	*Pseudomonas asplenii* strain ATCC 23835 (NR_040802)	98	JX912440
EE6	T5	*Microbacterium saperdae* strain IFO 15038 (NR_024637)	99	JX912441
EE7	T5	*Halospirulina tapeticola* strain CCC Baja-95 Cl.2 (NR_026510)	84	JX912442
EE8	T5	*Halospirulina tapeticola* strain CCC Baja-95 Cl.2 (NR_026510)	84	JX912443
EE9	T5	*Anabaena variabilis* ATCC 29413 (NR_074300)	86	JX912444
EE10	T5	*Bacillus marisflavi* strain TF-11 (NR_025240)	99	JX912445
EE11	T5	*Rhizobium daejeonense* strain L61 (NR_042851)	97	JX912446
EE12	T5	*Rhizobium selenitireducens* strain B1(NR_044216)	99	JX912447
EE13	T5	*Bacillus farraginis* strain R-6540 (NR_025785)	98	JX912448
EE14	T5	*Bacillus cereus* ATCC 14579 (NR_074540)	99	JX912449
EF1	T6	*Kribbia dieselivorans* strain N113 (NR_043763)	96	JX912450
EF2	T6	*Kribbia dieselivorans* strain N113 (NR_043763)	96	JX912451
EF3	T6	*Serinicoccus marinus* strain JC1078 (NR_025774)	96	JX912452
EF4	T6	*Bacillus atrophaeus* 1942 (NR_075016)	99	JX912453
EF5	T6	*Bacillus ginsengi* strain ge14 (NR_044193)	99	JX912454
EF6	T6	*Bacillus ginsengi* strain ge14 (NR_044193)	99	JX912455
EF7	T6	*Paenibacillus tundrae* strain Ab10b (NR_044525)	99	JX912456
EF8	T6	*Bacillus pumilus* SAFR-032 (NR_074977)	99	JX912457
EF9	T6	*Bacillus stratosphericus* strain :41KF2a (NR_042336)	99	JX912458
EF10	T6	*Ralstonia insidiosa* strain AU2944 (NR_025242)	100	JX912459
EF11	T6	*Cupriavidus metallidurans* CH34 (NR_074704)	99	JX912460
EF12	T6	*Solibacillus silvestris* strain HR3-23 (NR_028865)	99	JX912461
EF13	T6	*Arthrobacter polychromogenes* strain DSM 20136 (NR_026192)	99	JX912462
EF14	T6	*Agrobacterium tumefaciens* strain IAM 12048 (NR_041396)	99	JX912463
EF15	T6	*Rhizobium selenitireducens* strain B1(NR_044216)	98	JX912464
EF16	T6	*Anabaena variabilis* ATCC 29413 (NR_074300)	86	JX912465
EF17	T6	*Halospirulina tapeticola* strain CCC Baja-95 Cl.2 (NR_026510)	84	JX912466

EF18	T6	*Bacillus horikoshii* strain DSM8719 (NR_040852)	99	JX912467
EF19	T6	*Rhizobium daejeonense* strain L61(NR_042851)	98	JX912468
EF20	T6	*Bacillus foraminis* strain : CV53 (NR_042274)	98	JX912469
EF21	T6	*Cupriavidus metallidurans* CH34 (NR_074704)	99	JX912470
EF22	T6	*Ornithinimicrobium kibberense* strain K22-20 (NR_043056)	96	JX912471
EF23	T6	*Rhizobium selenitireducens* strain B1(NR_044216)	99	JX912472

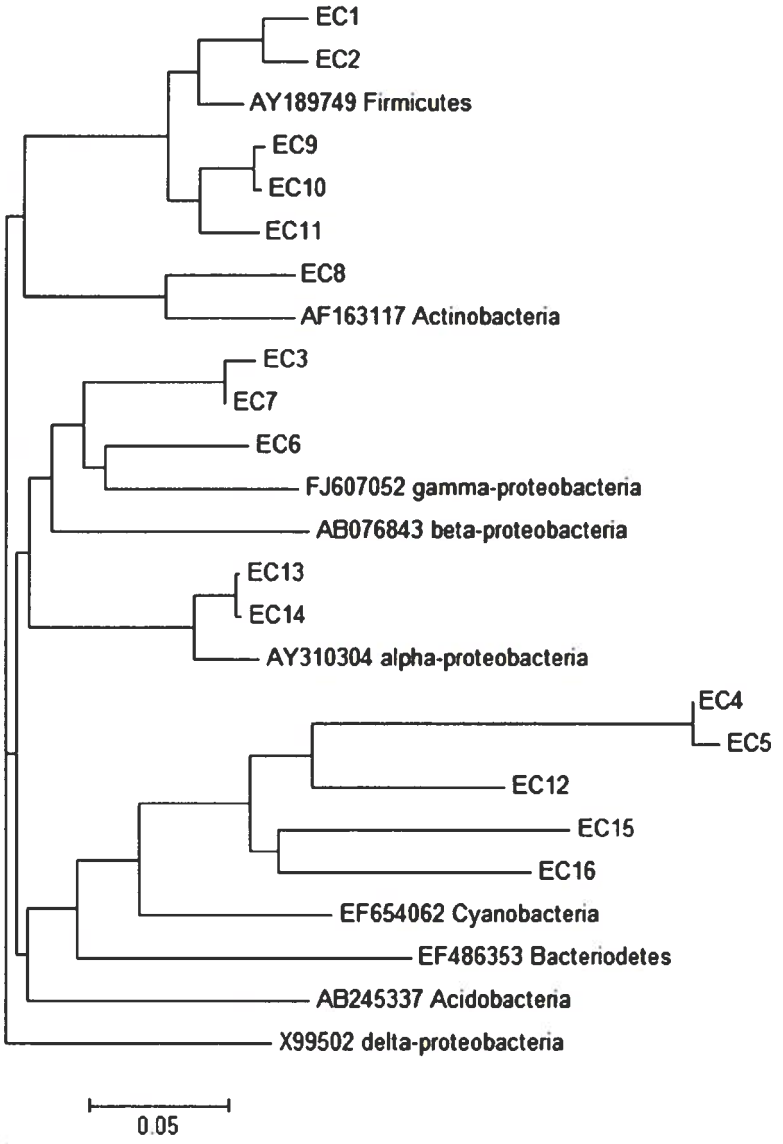
Supplementary Fig. 1



Supplementary Fig. 2



Supplementary Fig. 3



19 Supplementary Fig. 4

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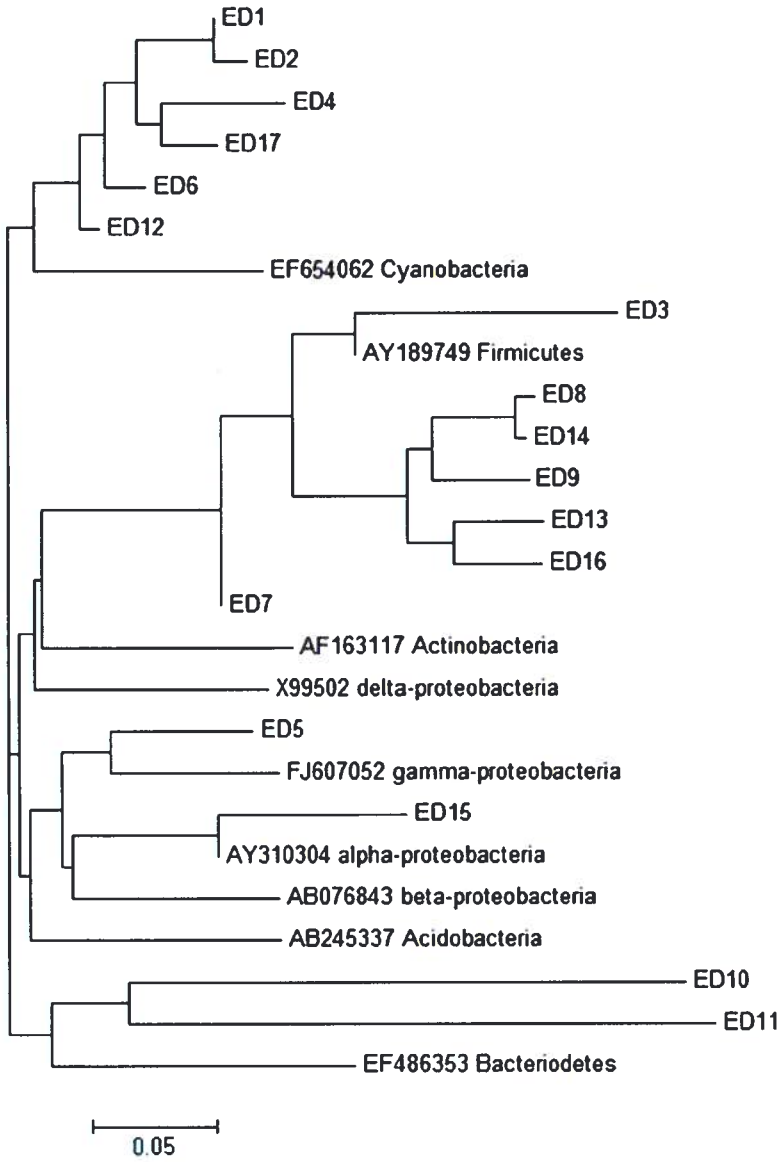

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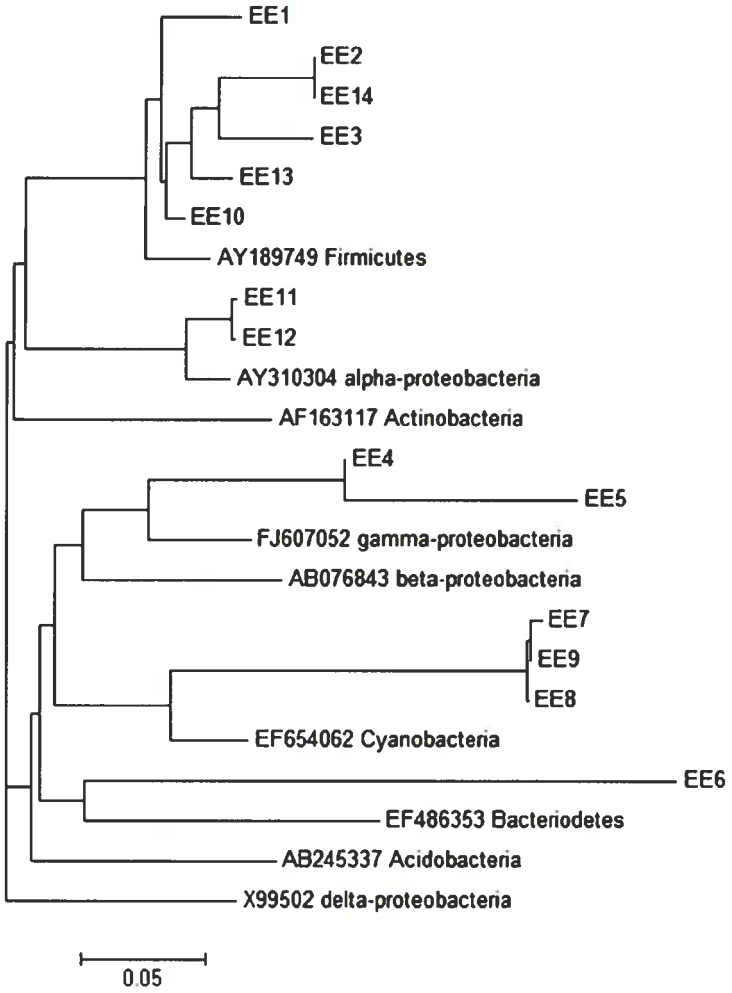
Supplementary Fig. 5



29 Supplementary Fig. 6

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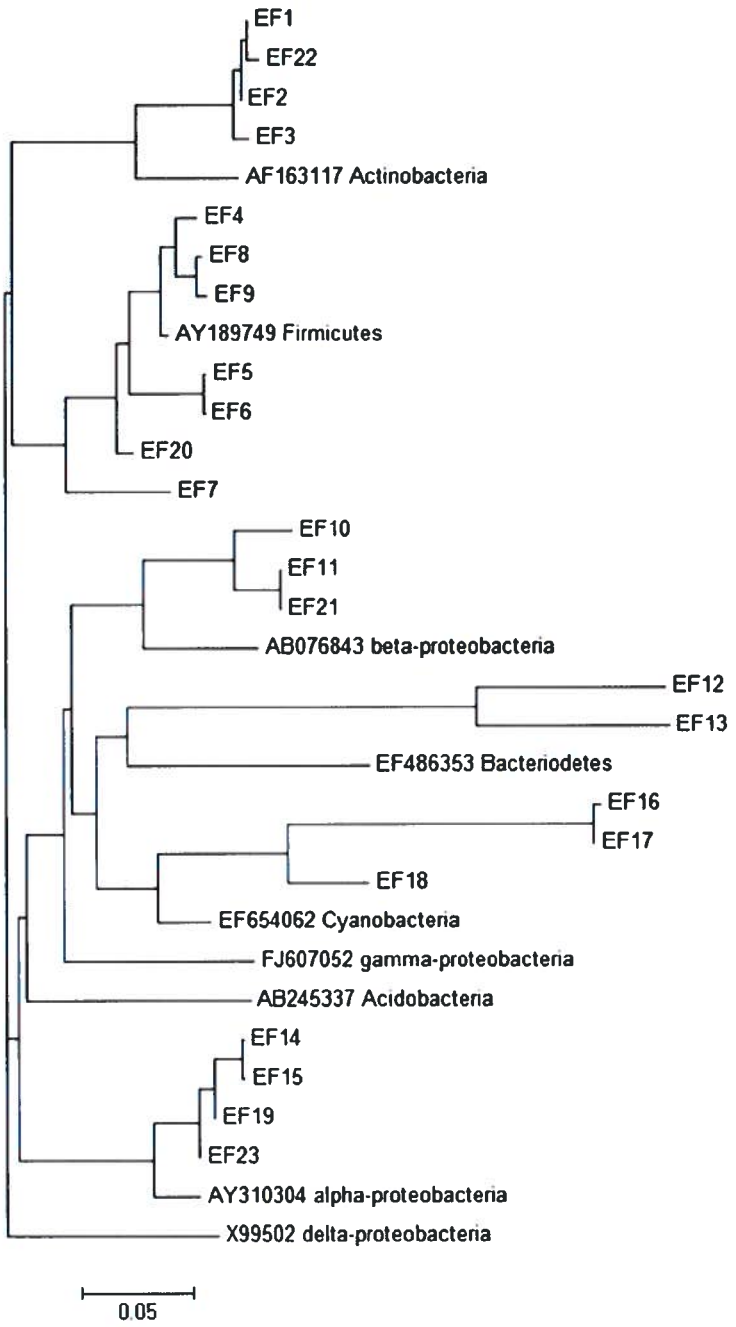

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