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Rhizobacteria Influence Potato Tuberization Through Enhancing Lipoxygenase Activity

Akula Nookaraju · Sajeesh Kappachery · Jae Woong Yu · Se Won Park

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Abstract Many rhizobacteria are known to influence several plant growth parameters in many crop species. The influence of rhizobacteria on potato tuber production was reported in few earlier studies but none of the studies explored the molecular mechanism involved in such effects. In the present study, we isolated and identified 13 bacteria from potato rhizosphere and these isolates were tested for their influence on potato tuberization under *in vitro* and *ex vitro* conditions. Under *in vitro* conditions, mini-tubers formed as a result of sub-apical swelling of stolons in control while mostly sessile tubers formed in the axils of nodal segments in few of the bacterial treatments. Among all the isolated strains, bacterial isolate 6 induced highest average tuber yield followed by isolate 4 and 5 under both *in vitro* and *ex vitro* conditions. As lipoxygenase (LOX) expression is associated with plant-microbe interactions and suggested involvement of LOX in potato tuberization, we tested the hypothesis on LOX activity related role of these rhizobacteria on tuber induction and tuber yield in potato through studying the *LOX1* gene expression and LOX activity. Significant increase in *LOX1* mRNA expression and LOX enzyme activities were detected in stolons and tubers from the rhizobacterial treatments as compared to untreated control. Also a strong positive correlation between rhizobacteria-induced *LOX1* gene expression and enhanced tuber induction and tuber yields were observed.

Resumen Se sabe que muchas rhizobacterias influencian varios parámetros del crecimiento de las plantas en muchas especies de cultivos. Se ha reportado la influencia de rhizobacteria en la producción de tubérculos de papa en algunos estudios previos, pero ninguno exploró el mecanismo molecular involucrado en tales efectos. En el presente estudio, aislamos e identificamos 13 bacterias de la rhizosfera de la papa y se probaron estos aislamientos para ver su influencia en la tuberización de papa bajo condiciones *in vitro* y *ex vitro*. Bajo las condiciones *in vitro*, se formaron minitubérculos como resultado de hinchamientos subapicales de estolones en el testigo, mientras que en algunos de los tratamientos con bacterias se formaron tubérculos mayormente sésiles en las axilas de los segmentos nodales. De entre todas las variantes aisladas, el aislamiento 6 indujo el promedio más alto de rendimiento de tubérculo seguido por los aislamientos 4 y 5, tanto en condiciones *in vitro* como *ex vitro*. Considerando que la expresión de la lipoxigenasa (LOX) se asocia con interacciones planta-microbio, y que se sugiere el involucramiento de LOX en la tuberización de la papa, probamos la hipótesis del papel relacionado con la actividad de LOX de estas rhizobacterias en la inducción y rendimiento de tubérculo en papa mediante el estudio de la expresión del gen LOX1 y de la actividad de LOX. Se detectaron aumentos significativos en la expresión del ARNm de LOX y en sus actividades enzimáticas en estolones y tubérculos de los tratamientos con rhizobacterias en comparación con los testigos sin tratamientos. También se observó una fuerte correlación positiva entre la expresión del gen LOX inducida por rhizobacteria y el incremento de la inducción y rendimiento de tubérculo.

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Introduction

Potato tuberization is a complex phenomenon influenced by a variety of intrinsic and extrinsic factors such as temperature (Ewing and Struik 1992), light, photoperiod (Jackson 1999), Ca^{2+} level in soil (Balamani et al. 1986) and N availability (Krauss 1978). In addition to these factors, some strains of rhizobacteria were reported to influence tuber number and tuber yield in potato (Burr et al. 1978; Vrany and Fiker 1984; Sturz 1995; Oswald et al. 2010). Burr et al. (1978) reported a significant increase in growth and yield of potato plants after the treatment of seed tubers with *Pseudomonas* spp., while Vrany and Fiker (1984) reported a 4 to 30% increase in plant growth and tuber yield in potato. Similarly, Sturz (1995) found an increased tuber number and average tuber weight with the application of plant growth promoting rhizobacteria (PGPR). Apart from tuberization, they were reported to have an effect on plant growth, root number and lignin content in potato (Frommel et al. 1991). Recently, Oswald et al. (2010) screened PGPRs in potato and attributed the increased tuber yields in PGPR treated plants to early tuber induction, fast leaf area development and greater photosynthetic rates.

Many genes exhibit differential expression during potato tuberization, although their direct involvement in tuber development remains ambiguous (Sarkar 2008). Among those, up-regulation of lipoxygenases (LOXs) has been directly linked to both tuber induction and tuber development (Kolomiets et al. 2001). It is also documented that lipoxygenases, iron containing dioxygenases are induced in plants in response to microbial attack to produce 9-hydroperoxides enhancing plant defenses against pathogens (Reddy et al. 2000). Hydroperoxides are precursors of jasmonic acid (JA), methyl jasmonate (Me-JA), and tuberonic acid (TA) that function as plant stress hormones and exhibit tuber-inducing activity in potato under *in vitro* conditions (Koda et al. 1988; Pelacho and Mingo-Castel 1991). *In-situ* hybridization studies showed the accumulation of *LOX1* class transcripts in the apical and sub-apical regions of the newly formed tubers (Kolomiets et al. 2001). Further, the increased LOX activity showed a positive correlation with tuber induction and tuber growth in potato (Nam et al. 2005). These reports show that the activity of LOX is coincided with tuberization and its activity in turn can be influenced by interaction with microbes. Hence, present study was taken to investigate whether the bacterial isolates from potato rhizosphere can directly induce LOX activity to modulate the potato tuberization.

Materials and Methods

Isolation of Bacteria from Potato Rhizosphere

Soil samples were collected from potato fields of Highland Agriculture Research Centre (HARC), Pyeongchang, Republic of Korea, located at 37° 26′ N latitude and 128° 32′ E longitude. Rhizobacteria were isolated and pure cultures were maintained on TSA (Trypt Soy Agar with Casein, pH 7.3) as described by Jafra et al. (2006) with minor modifications. Ten gram of soil adhering to the tubers and roots of potato was suspended in 100 mL of sterile triple de-ionized water by shaking on rotary shaker for 25 min at 250 rpm. One milliliter of soil suspension was used for preparing serial ten-fold dilutions in 0.8% (*w*/*v*) NaCl. For isolation of individual bacteria, 100 μL of 10^{-4} fraction was spread on Petri dishes (90 mm Diameter x 15 mm Height) containing TSA. The Petri dishes were then incubated in dark at 26°C for 48 h. Individual bacterial colonies were picked, sub-cultured and streaked on TSA medium for establishing pure cultures (Jafra et al. 2006). Gram staining was performed using a Gram Stain Kit (BD Diagnostics, New Jersey, USA).

PCR Amplification and Sequencing of PCR Products

Genomic DNA from bacterial isolates was isolated using modified CTAB method (Maloy 1990). PCR amplification targeting bacterial 16S rRNA region was performed in a thermal cycler (Takara, Shiga, Japan) using universal primers: CAH16S 1F (5′-AAT ACA TGC AAG TCG AAC GA-3′) and CAH16S 1R (5′-TTA ACC CAA CAT CTC ACG AC-3′) (Marshal et al. 1999). PCR reactions were performed in a 25 μL reaction mixture containing 0.3 U of *Taq* DNA polymerase (Takara, Shiga, Japan) in 1X buffer, 50 ng of template DNA, 400 μM of each dNTPs and 7 pmol of each primer. PCR was performed using the following program: Initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 45°C for 45 s and extension at 72°C for 1 min with a final extension at 72°C for 5 min. The PCR products were purified using PCR purification kit (RBC, Chung Ho, Taiwan) and sequenced. The sequences of PCR products from the bacterial 16S rRNA genome were compared with reported 16S rRNA gene sequences in the NCBI gene bank for identification of the bacteria.

Screening of Bacterial Isolates for their Effect on Potato Tuberization

During *in vitro* studies, addition of live bacteria to the tuber induction medium resulted in severe bacterial growth on the medium and nodal explants were killed subsequently. Due to

this limitation, we used heat-treated broths of rhizobacteria in the tuber induction medium. For this, single colony of individual bacterial isolates was grown overnight in 5 mL of MS (Murashige and Skoog's 1962) liquid medium at 26°C and 250 rpm. The pH of MS medium was adjusted to 7.3 to optimize the bacterial growth. Bacterial broths (<2% *v/v* of tuber induction medium) were heat-treated (80°C for 20 min) and supplemented at a concentration of 10^7 cfu mL^{-1} in the sterile MS medium containing 7% (*w/v*) sucrose used for tuberization. Though the heat-treatment procedure did not completely kill the *Bacillus* sp, it could minimize the spread of the bacterium on MS medium and explants were healthy. Single node segments from 30 day old *in vitro* grown shoots of potato cv. Desiree were used as explants for *in vitro* tuberization studies (Fig. 1a). Desiree was selected as it responded well to bacterial treatments among three cultivars screened during preliminary studies in our laboratory (data not shown). The nodal explants were inoculated in Petri dishes (120 mm Diameter × 20 mm Height) containing MS medium supplemented with heat-treated bacterial broths. The MS medium without the bacterial supplements served as control. Cultures were incubated in a growth chamber maintained at 20±1°C temperature with 65% relative humidity under continuous dark. Each treatment contained 16 explants and the experiment was repeated three times. Observations on stolon initiation, tuber number and tuber yield were recorded for a period of 60 days.

For *ex vitro* studies, *in vitro* rooted shoots (>5 cm height) of potato cv. Desiree were used. These shoots were produced from *in vitro* cultures initiated from seed potato tubers and maintained on MS medium in culture boxes (90 mm Diameter × 120 mm Height). The plants were dipped in broths of live rhizobacteria (10^7 cfu mL^{-1}) for 10 min and planted in plastic pots (7 cm diameter) containing autoclaved soil-sand (1:1) mixture. The plants were irrigated with Hoagland's solution (Hoagland and Snyder 1933) and covered with polythene bags. The plants were initially kept in growth chamber maintained at 23±2°C for hardening. After 4 weeks, the hardened plants were transferred to bigger pots (20 cm Diameter and 30 cm Deep) containing sterile soil-sand (1:1) mixture and plants were moved to greenhouse. The plants were irrigated with tap water at regular interval. When the plants withered completely, tubers were harvested and tuber yields were determined.

LOX1 Gene Expression by RT-PCR and Quantitative Real-Time PCR

We tested the hypothesis that rhizobacteria and bacterial components will affect *lipoxygenase* gene expression and

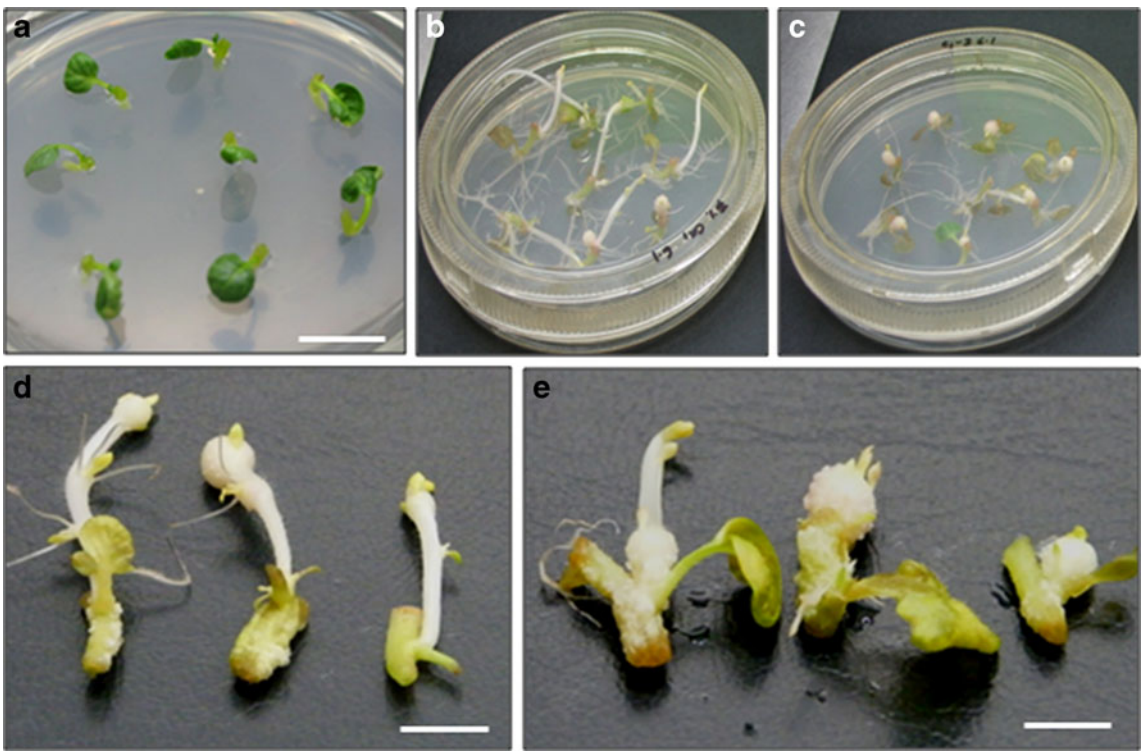

Fig. 1 Influence of heat-treated broths of rhizobacteria isolates on *in vitro* tuberization in potato cv. Desiree. Nodal segments used for *in vitro* tuberization studies (**a**), bar = 2.5 cm; Tuberization pattern in control (**b**) and treatment containing bacterial isolate 6 (**c**), bar = 2 cm; Tubers formed on stolons in untreated control (**d**), bar = 3 mm; Sessile tubers formed directly in the axils of nodal segments in MS medium supplemented with heat-treated broth of bacterial isolate 6, bar = 3 mm (**e**)

influence tuber induction and tuber yield. Total RNA was isolated from stolons (S1), tuber initials (<2 mm, S2), developing tubers (2–4 mm, S3) and large tubers (>4 mm) (matured tubers in case of *ex vitro*, S4) from *in vitro* and *ex vitro* experiments under the influence of rhizobacteria and untreated control using TRI reagent (Sigma-Aldrich, St. Louis, USA). First strand cDNA was synthesized using SuperScript™ First Strand cDNA Synthesis kit following manufacturer's protocol (Invitrogen, California, USA). One microliter of cDNA was used as template for RT-PCR using the *lipoxygenase* (*LOX1*) gene specific primers (F: 5′-TTG CGG TGA ATG ACG TTG GTG TTC-3′ and R: 5′-TTC AGG AGT TCC TGG TTC AGG CAT-3′). PCR was performed in a thermal cycler (Takara, Shiga, Japan) using the following program: Initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 45 s and extension at 72°C for 1 min with a final extension at 72°C for 5 min. Actin served as loading control. PCR products were separated on 1% agarose gel stained with ethidium bromide and the gels were photographed using gel documentation system (Bio-Rad, Hertfordshire, UK). For quantitative real time PCR, cDNA samples were amplified using an ABI Prism 7700 sequence detector (Applied Biosystems, California, USA). The real-time PCR amplification of *LOX1* gene was carried out using gene specific primers as mentioned above. The PCR was performed using a SYBR green PCR kit (Bio-Rad, Hertfordshire, UK) with actin serving as internal control. Comparative threshold (Ct) values were normalized to actin control and compared to obtain relative expression levels.

Estimation of Lipoxygenase (LOX) Enzyme Activity

Stolons (S1), tuber initials (S2), developing (S3) and large tubers (S4) collected from bacterial treatments as well as control were used for crude protein extraction. About 100 mg of frozen tissue was homogenized in 500 μL of triple de-ionized water at 4°C using a mortar and pestle. The homogenate was centrifuged at 15,000*g* for 30 min at 4°C. The resulting supernatant was used as the crude enzyme extract. The protein determinations were carried out using the dye-binding method of Bradford (1976). LOX enzyme activity in tuber samples was determined according to the procedure described by Gökmen et al. (2002) with minor modifications. The substrate solution for the LOX enzyme was prepared by mixing 157.2 μL of pure linoleic acid, 157.2 μL of Tween-20 and 10 mL of triple de-ionized water. The solution was clarified by adding 1 mL of 1 N NaOH and the mixture was diluted to 200 mL with 0.1 M potassium phosphate buffer (pH 6.3), giving a 2.5 mM final concentration of linoleic acid. The enzyme reaction was initiated by adding 25 μL of enzyme extract to the reaction mixture. The formation of hydroperoxides was monitored spectrophotometrically at 234 nm. One unit of LOX activity was defined as an increase in absorbance of 0.001 at 234 nm per min per mg of protein under assay conditions. A double beam spectrophotometer (Model No. UV-1700, Shimadzu, Kyoto, Japan) and 1 cm path-length cuvettes were used for the study.

Statistical Analysis

The data on various parameters were analyzed using analysis of variance (ANOVA) using SPSS.10 (SPSS, Chicago, IL, USA). The treatment means were compared using Duncan's multiple range test (Duncan 1955) and the mean differences were considered significant at $^{**}p<0.01$ or $^{*}p<0.05$.

Results

Thirteen morphologically distinct bacterial types were isolated from representative soil samples collected from experimental fields of HARC, Korea. Soil dilution fraction at 10^{-4} was found optimum for isolation of individual bacterial colonies. Using the universal primers CAH16S 1F and CAH16S 1R we were able to amplify approximately 1.0 kb in 16S rRNA region of all the bacterial isolates. The bacterial isolates were identified to genus level by nucleotide sequence comparison with the reported 16S rRNA sequences in the NCBI database (Table 1). *Bacillus* was found to be the dominant species in the potato rhizosphere. The other isolates include genera *Variovorax*, *Proteobacterium*, *Chrysobacterium, Staphylococcus, Agrobacterium* and *Plantibacter*. The isolates represented both Gram-negative (*Chysobacterium, Agrobacterium, Proteobacterium* and *Variovorax*) and Gram-positive (*Bacillus, Staphylococcus* and *Plantibacter*) bacteria.

All isolates (Table 1) were tested individually for their effect on *in vitro* potato tuberization but data was shown only for the isolates which were found to produce significant positive effect. Tuber initials were observed early in rhizobacteria treatments as compared to untreated control (Table 2). First tuber initials were observed at 4 to 6 days after culture of explants in bacterial treatments, whereas tuber initiation was delayed by 2 to 3 days in untreated control. In case of untreated control, the *in vitro* tubers were formed as a result of sub-apical swelling of stolons (Fig. 1b and d), whereas several sessile tubers were formed directly in the axils of nodal explants cultured on MS medium supplemented with heat-treated broths of bacterial isolates (Fig. 1c and e). Substantial increase in average tuber size was also observed in the explants cultured on tuber induction medium supplemented with

Table 1 Identification of bacteria form potato rhizosphere by 16S rRNA gene sequencing

PGPR	Gram reaction	Primer pair used	Close match NCBI accession number	Match with organism 16S rRNA sequences	% Homology based on nucleotide
Isolate-1	−	CAH16S 1F/R	AF375840	*Chryseobacterium* sp. U3	99
2	−	CAH16S 1F/R	FJ785222	*Agrobacterium tumefaciens* strain ISSDS-425	99
3	+	CAH16S 1F/R	FJ790330	*Bacillus cereus* strain HZB	98
4	+	CAH16S 1F/R	FJ774005	*Bacillus pumilus* strain DH-11	99
5	+	CAH16S 1F/R	FJ380964	*Staphylococcus epidermidis* strain BQN1N-02d	99
6	+	CAH16S 1F/R	FJ613615	*Bacillus firmus* strain 40	98
7	+	CAH16S 1F/R	FJ613603	*Bacillus firmus* strain 17	98
8	−	CAH16S 1F/R	EF702968	Uncultured beta *proteobacterium* strain MS092A1 C05	98
9	−	CAH16S 1F/R	FJ719347	*Variovorax* sp. T71	100
10	+	CAH16S 1F/R	FJ613622	*Bacillus* sp. 77	99
11	+	CAH16S 1F/R	FJ755919	*Bacillus thuringiensis* strain IS2	100
12	+	CAH16S 1F/R	FJ006927	*Plantibacter* sp. WPCB192	100
13	−	CAH16S 1F/R	EU441166	*Variovorax paradoxus* strain SFWT	100

Genomic DNA from bacterial isolates was amplified with universal primers flanking 16S rRNA. Based on the nucleotide sequence comparison with reported 16S rRNA sequences in NCBI gene bank, the bacteria were identified to their genus level

heat-treated broths of bacterial isolates. The tuber yield was highest (Ca. 12.56 g per treatment; 220% higher than untreated control) when MS medium was supplemented with the heat-treated broth of bacterial isolate 6 (*Bacillus firmus* strain 40) (Table 2).

Based on their best performance under *in vitro* studies, isolates 4, 5 and 6 were selected for the greenhouse experiments. Similar results were recorded from greenhouse studies too with positive effect of the three selected isolates (4, 5 and 6) on potato tuber number, average tuber size and total yield. The inoculations had no significant influence on average plant height (Table 3). Among these isolates, plants inoculated with isolate 6 produced highest tuber yield (183.5 g per plant; 120% increase over control) followed by 94.5 g by potato plants inoculated with isolate 4. The control treatment yielded 83.2 g tubers per plant. These results clearly showed the strong positive influence of bacterial isolates 6 and 4 on potato tuber yield both under *in vitro* and *ex vitro* conditions.

The RT-PCR results showed that the *LOX1* mRNA expression in stolons and tuber samples collected from *in vitro* and greenhouse experiments was found to be higher in

Table 2 Effect of heat-treated broths of rhizobacterial isolates on *in vitro* tuberization of potato cv. Desiree

PGPR treatment	No. of stolons induced	Av. stolon length (cm) (mean ± SE)	[a]Days to tuber initiation	Total tubers induced	Av. tuber size (mm) (mean ± SE)	[b]Av. tuber yield (g) (mean ± SE)
Control	19	7.4 ± 0.8^{a}	7–8	4	3.5 ± 0.3^{d}	3.91 ± 0.26^{d}
Isolate-1	10	6.8 ± 1.3^{ab}	5	8	4.2 ± 0.1^{d}	4.03 ± 0.29^{d}
Isolate-2	8	4.8 ± 0.7^{d}	3–6	6	4.2 ± 0.2^{d}	2.96 ± 0.37^{e}
Isolate-3	9	6.2 ± 0.3^{b}	6–7	6	4.3 ± 0.3^{d}	3.63 ± 0.70^{de}
Isolate-4	8	5.9 ± 0.4^{bc}	4–5	8	6.6 ± 0.7^{c}	10.03 ± 0.13^{c}
Isolate-5	7	4.7 ± 2.6^{d}	4–6	8	7.7 ± 0.2^{b}	11.36 ± 0.04^{b}
Isolate-6	6	4.9 ± 2.1^{cd}	4–6	8	9.8 ± 0.1^{a}	12.56 ± 0.24^{a}
Isolate-7	18	5.5 ± 1.8^{c}	9	7	4.3 ± 0.1^{d}	4.18 ± 0.15^{d}
Isolate-8	15	5.9 ± 0.9^{bc}	7	9	4.6 ± 0.6^{d}	4.28 ± 0.49^{d}
ANOVA	**	**	*	*	**	**

Basal medium: MS + sucrose 7% (*w*/*v*), pH 5.7

Each treatment contained 16 explants and the experiment was repeated three times (total explants per treatment = 48). Any two mean values with no letter in common are significantly different at $*p\leq0.05$, $**p\leq0.01$

[a] Indicates the first appearance of tuber from the explants given the treatment

[b] Av. tuber yield was calculated per treatment consisting 16 explants

Table 3 Effect of rhizobacteria isolates on tuber yield of potato cv. Desiree under greenhouse conditions

PGPR treatment	Days to harvest	Plant height at maturity	No. of tubers produced per plant	Av. tuber size (mm) (mean ± SE)	Av. tuber yield (g) (mean ± SE)
Control	80–85	55.3	8	20.9 ± 1.2^{b}	83.20 ± 1.11^{bc}
Isolate-4	85	59.0	12	24.3 ± 1.3^{ab}	94.50 ± 1.73^{b}
Isolate-5	85–95	62.5	6	26.0 ± 1.3^{ab}	77.20 ± 1.27^{c}
Isolate-6	90–95	65.2	16	29.2 ± 1.3^{a}	183.52 ± 4.62^{a}
ANOVA	ns	ns	*	*	*

Each treatment contained two plants and the experiment was repeated three times (total plants per treatment = 6). Any two mean values with no letter in common are significantly different at $*p\leq0.05$. *ns* not significant

bacterial treatments than in untreated controls (Fig. 2). The *LOX1* gene expression as assessed by real time-PCR showed a 1.2 to 2.1 fold increase in stolon tissues in bacterial treatments over that of untreated control (Fig. 3a). The highest expression of *LOX1* (2.1 fold over control) was displayed by stolons formed from explants cultured on tuber induction medium supplemented with heat-treated broth of bacterial isolate 6. There was a gradual decrease in *LOX1* mRNA expression from tuber initials to large tubers (Fig. 3b–d). Similar results were observed in case of explants obtained from greenhouse studies. The *LOX1* expression in stolons from plants treated with the isolate 6 showed a 2 fold increase over that of control. At tuber maturity there was no significant difference in *LOX1* expression among the treatments (Fig. 3d). LOX enzyme activity also displayed similar trend showing highest activity in stolons formed in bacterial treatments as compared to the control (Fig. 4). Among the isolates, the LOX enzyme activity was highest in stolons (S_1) derived from MS medium supplemented with heat-treated broth of bacterial isolate 6 (*Bacillus firmus* strain 40) which was 1.3 fold increase over untreated control (Fig. 4a). Similar results were observed in case of explants derived from greenhouse plants treated with the live isolates showing superiority of isolate 6 over other isolates.

Discussion

Thirteen morphologically distinct bacterial types were isolated from the soil samples collected from potato fields of HARC and the isolated bacteria were identified using

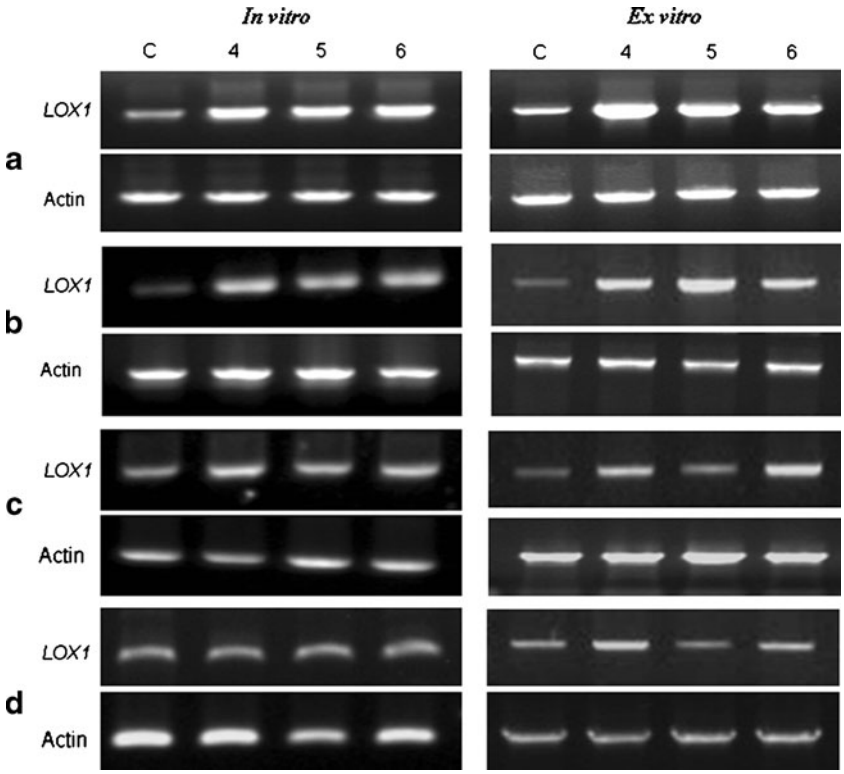

Fig. 2 RT-PCR analyses for *LOX1* mRNA expression using *LOX1* gene specific primers. *LOX1* mRNA expression in stolons (S_1) (**a**), tuber initials (S_2) (**b**), developing tubers (S_3) (**c**) and large tubers (S_4) (**d**). Actin served as loading control. *C*: stolons and tubers from control; *4*, *5* and *6* are from treatments supplemented with PGPR isolates *4*, *5* and *6*

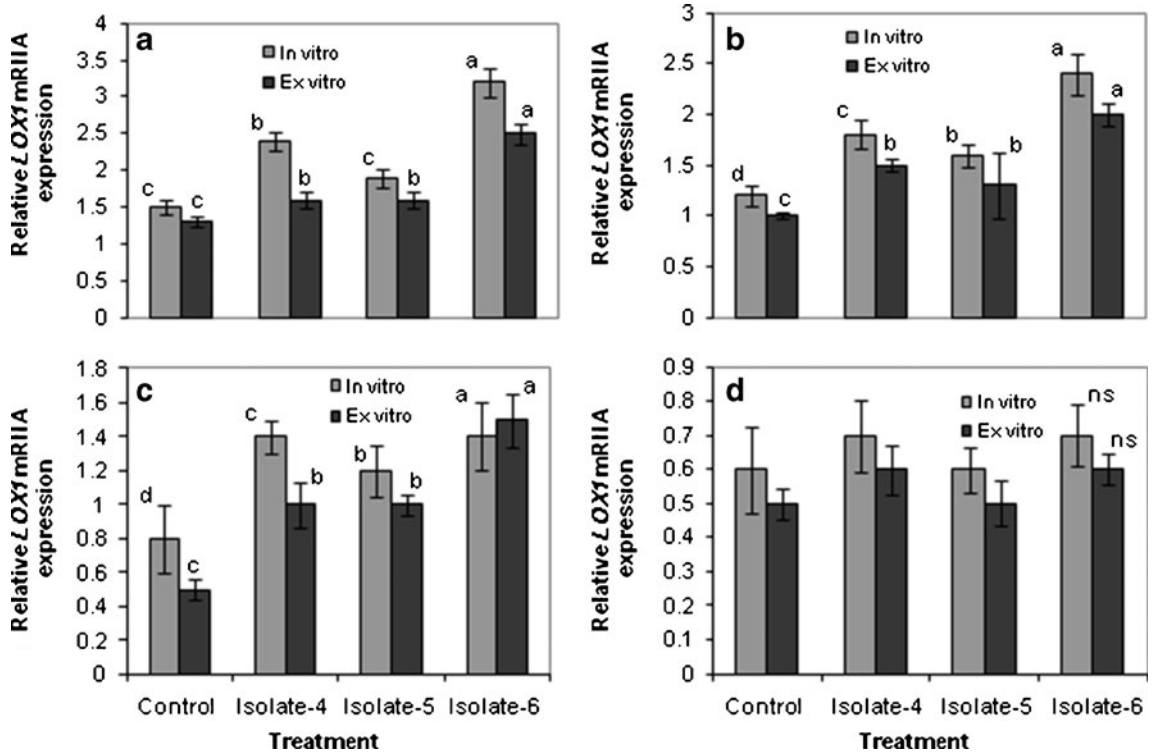

Fig. 3 Quantitative real time PCR analysis of *LOX1* mRNA expression using *LOX1* gene specific primers. The cDNA was normalized in dependence of the transcript level of actin mRNA. *LOX1* mRNA expression in stolons (**a**), tuber initials (**b**), developing tubers (**c**) and large tubers (**d**). The values are presented as the mean ± SE (n=3). Bars represented by different letters are significantly different at $p<0.01$. *ns* non significant

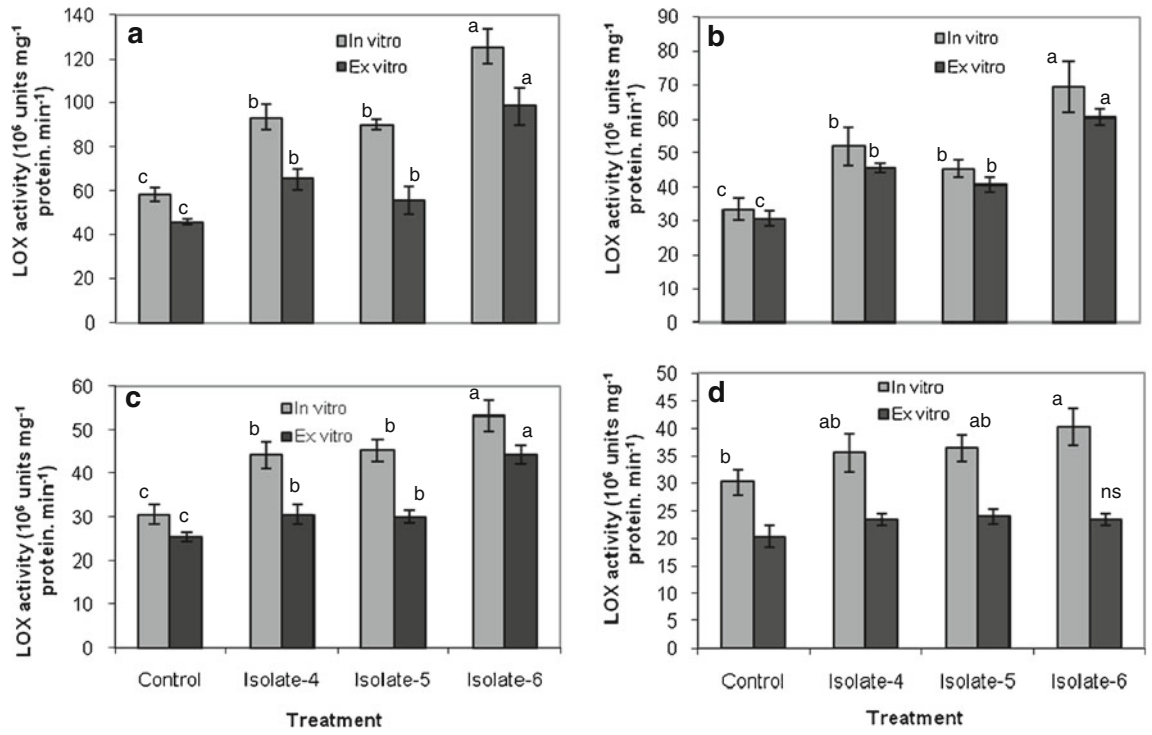

Fig. 4 LOX enzyme activity in tuber samples of potato. LOX activity in stolons (**a**), tuber initials (**b**), developing tubers (**c**) and large tubers (**d**) from control and bacterial treatments. The values are presented as the mean ± SE (n=3). Bars represented by different letters are significantly different at $p<0.01$. *ns* not significant

16S rRNA sequencing. Identification based on 16S rRNA is considered to be reliable method due to its high conservation within a species and among the species of the same genus. The identified bacteria belong to different genera among which, *Bacillus* was found to be the major genus. Few bacteria belong to these genera were reported to be beneficial for the crop plants in several ways (Bashan and de-Bashan 2005).

In our study, few of our bacterial isolates showed significant positive influence on potato tuber yield under *in vitro* studies (Table 2). Tuber induction was early by 2 or 3 days in bacterial treatments as compared to untreated control. In untreated control, tubers formed as a result of sub-apical swelling of stolons while several sessile tubers formed directly in the axils of nodal cuttings cultured on MS medium supplemented with few heat-treated broths of rhizobacterial isolates. Formation of sessile tubers directly in the axils of the nodal segments is an indication of strong tuber induction (Ewing 1978). Further, the early tuber induction in bacterial treatment lots might have lead to early tuber growth and higher tuber yield. The *in vitro* results were comparable with the results from greenhouse studies in terms of tuber number, tuber size and tuber yield. Beneficial effects of live or heat-killed form of different PGPR on various plant processes are documented in many plant species by various workers (Tan et al. 1999; Siddiqui 2000; Smith et al. 2003; Jaizme-Vega et al. 2004). In our studies few heat-treated bacterial isolates when supplemented in tuber induction medium could lead to a strong tuber induction and higher tuber yield. The results from *in vitro* studies were supported by comparable results obtained from greenhouse experiments, where live bacteria were used. Also, the results from *in vitro* studies strongly suggest the retention of beneficial properties of bacteria even after heat-treatment corroborating earlier reports (Siddiqui 2000; Baker et al. 2001). These reports also suggested that similar to live cells, heat-killed cells of bacteria can produce hypersensitive reactions and modify various morphogenic processes in plants through specific cell wall compounds like glycoproteins and terpenoid alexins.

The induction of *LOX1* gene expression by heat-treated form of bacterial isolates in potato tissues could generate LOX derived metabolites (such as JA, TA and TAG) that might have promoted potato tuberization in the present study (Koda et al. 1988) by antagonizing the effects of gibberelic acid (GA) (Jackson and Willmitzer 1994; Jackson 1999). The enhanced activity of LOX might have generated reactive oxygen species (ROS) (Zimmermann and Zentgraf 2005) and the generated ROS, especially superoxide ions could regulate tuber development in potato through modulating GA biosynthesis (Kim et al. 2007). Thus enhanced tuber induction and tuber yield in bacterial treatments can be partly attributed to the increased LOX enzyme activity (Kolomiets et al. 2001) with subsequent production of LOX-derived metabolites and increased ROS generation due to high LOX activity. Lipoxygenases are also known to regulate the reorientation of microtubules and allow radial cell expansion leading to tuber enlargement (Matsuki et al. 1992). Apart from LOX signaling, few strains of Gram positive bacteria like *Bacillus* spp. produce plant growth hormones such as indole-3-acetic acid (IAA) and were reported to promote plant growth and yield in several crop species (Ramos et al. 2003; Yan et al. 2003). The inductive role of IAA on stolon initiation and tuber induction in potato was documented in earlier studies (Dragicevic et al. 2008). A similar influence of *Bacillus* strains in promoting potato tuberization under *in vitro* and *ex vitro* conditions in our study could be expected.

In conclusion, the PCR based method allowed for rapid identification of bacteria from potato rhizosphere. Few of the tested bacteria isolates showed significant positive influence on potato tuberization under *in vitro* and *ex vitro* conditions supporting the important role of bacteria inhabiting in the rhizosphere in potato tuberization. In the present study, increased *LOX1* mRNA expression and LOX enzyme activity was correlated with the increased tuber induction, growth and tuber yield in the bacterial treatments. A detailed biochemical and molecular studies on identification and functional characterization of signal molecules of bacterial origin can bring further clues for deducing PGPR-induced signaling mechanism involved in potato tuberization.

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