Endophytic bacteria improve plant growth through induction of phytohormones related gene expression in jute

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Abstract

Plants live in association with complex populations of microorganisms, including Plant Growth-Promoting Rhizobacteria (PGPR) which confers improved growth and enhanced stress tolerance to plants. This large and diverse group includes endophytic bacteria that can colonize the internal tissues of plants. This study aimed to identify the molecular and physiological characteristics of a non-rhizobial bacterial species from the surface-sterilized root of healthy and nematode-infested jute (*Corchorus capsularis*; *Corchorus olitorius* and an advanced variety of *Corchorus olitorius*, Robi-1), an annual fiber bearing plant species. Using universal primers to amplify bacterial 16S rDNA, we identified 59 culturable gram-positive bacterial isolates from healthy and nematode-infected jute plants. All the selected isolates were gram-positive of *Bacillus* and *Staphylococcus* genus. The endophytes were positive for pectinase, xylanase, cellulase, and phosphatase, all of which may influence jute physiology. Selected bacterial species increased the root length, shoot length, and germination rate. *B. cereus* significantly increased the growth phenotype and biomass of *C. capsularis* (CVL-1) whereas *S. hominis* showed significant growth increment in *C. olitorius* (O-9897) and the combination of all bacteria produced the same effect in the advanced line of *C. olitorius* (Robi-1). Higher expression of growth-promoting hormones GA-20 and GA-03 oxidase consolidate that plant growth was increased through induction of increased production of growth hormone gibberellin. Altogether, our results demonstrate that *B. cereus* (MCN3) and *S. hominis* (MON1) display plant growth-promoting traits of potential interest for agricultural applications.

1. Introduction

Symbiosis is one of the driving forces shaping inter-species interaction in an ecosystem. Plants and microbes appear to be invariably connected, with all plants harboring a diverse community of microbes (Bodenhausen et al. 2014). Endophytes are of great importance to plants, and they are abundantly found in all examined plants with great diversity (Shah et al.1992). The term endophyte was first coined for solely endophytic fungi; it nowadays also encompasses endosphere bacteria (Hardoim et al. 2008). Bacteria that complete their entire life cycle or part of it inside the plant; colonizing both intracellular and inter-cellular spaces without causing any disadvantageous side-effects to their host are referred to as endorhizosphere microbiota (Hardoim et al. 2008; Charles et al. 2006). In general, endophytic bacteria tend to have a favorable relationship for both themselves and their host plant, through plant growth promotion, plant defense induction, or the production of antimicrobial metabolites. The diversity of microbes associated with plant roots is enormous and the complex plant-associated microbial community, also referred to as the second genome of the plant, is crucial for plant health (Charles et al. 2006). The dynamic community structure of endophytes and rhizosphere is influenced by abiotic and biotic factors (soil conditions, biogeography, plant species, microbe-microbe, and plant-microbe interactions (Jonathan et al. 2013). Root exudates are often divided into two classes of compounds. Low-molecular-weight compounds such as amino acids, organic acids, sugars, phenolics, and other secondary metabolites account for much of the diversity of root exudates, whereas high-molecular weight exudates, such as mucilage (polysaccharides) and proteins, are less diverse but often compose a larger proportion of the root exudates by mass and the magnitude of photosynthates secreted as root exudates vary with the type of soil, age, and physiological state of the plant, and nutrient availability (Brady et al. 1999; Brimecombe et al. 2001). Positive interactions mediated by root exudates also include growth facilitators or growth regulator mimics that support the growth of other plants and also perform cross-species signaling with rhizospheric invertebrates. Contrastingly, negative interactions mediated by root exudates involve the secretion of antimicrobials, phytotoxins, nematicidal, and insecticidal compounds (Harsh et al. 2006). The most common mode of entry of endophytic bacteria into plant tissues is through primary and lateral root cracks, and diverse tissue wounds occur as a result of plant growth (Sprent et al. 1998; Agarwhal et al. 1987; Sørensen et al. 2015). Root wounds allow the leakage of plant metabolites, they become sites that attract bacteria (Egamberdieva et al. 2008). Plants can shape their rhizosphere microbiome and upon pathogen attack recruit protective microorganisms and enhance microbial activity to suppress pathogens in the rhizosphere (Roeland et al. 2012; Doornbos et al. 2012; Lugtenberg et al. 2009; Ryan et al. 2008). Plant polymer degrading enzymes such as cellulases and pectinases have also been suspected to play a role in internal colonization. In grapevine, it has been shown that polygalacturonidase and endoglucanase of *Xylella fastidosa* enlarge the pore sizes of pit membranes, polysaccharide structures that separate adjacent xylem vessels, and limit bacterial passage, thereby presumably facilitating the systemic spread of these bacteria in the plant. Thus, successful endophytes can be expected to be equipped with a set of cell wall degrading enzymes with confined, localized activity (Donoso et al. 2010). On the other hand, the bacterial species H*erbaspirillum seropedicae*, which lacks genes for degrading plant cell walls, is also a successful endophyte, confirming the existence of other strategies to penetrate plant tissues (Pedrosa et al. 2011; Wisniewski-Dyé et al. 2011). It is reported that bacteria can Endophytes can pass through the plant cell wall and enter the root cell either directly by the secretion of plant cell-wall-degrading enzymes and passage through the plant plasma membrane or by rhizophagy by which plants get microbes from the soil into their cells and digest them as a source of essential nutrients (White et al. 2014).

Jute dicotyledonous fibre-yielding plant of the Corchorus genus, the golden fibre of Bangladesh (Topdar et al. 2013; Choudhary et al. 2013; Heywood et al. 2007; Kubitzki et al. 2003) is an abundant source of biodegradable and renewable lignocellulose fibre (Niu et al. 2015). Jute fibre is produced mainly from two commercially important species, namely White Jute (*Corchorus capsularis*), and Tossa Jute (*Corchorus olitorius*) (Tanmoy et al. 2015). Both the species of jute, *C. capsularis* and *C. olitorius*, suffer from several diseases at all stages of their development from seed germination to the harvested fruits. Root-knot is caused by the nematodes *Meloidogyne incognit*a and *M. javanica* (Ahmed et al. 1961; Isaiah et al. 2016; Khan et al. 2004) and forms gall through feeding end parasitically root cortex, resulting in necrosis and stunting of severely infected root systems (Lin et al. 1992).

Here, we have identified endophytic bacteria from both *Corchorus* species (*C. capsularis and C. olitorius*) before and after nematode infection through 16s rRNA analysis and observed their hydrolytic enzyme production capability, efficiency in seed germination, and their efficiency in plant growth through expression analysis of growth-promoting genes. Among the identified bacteria *B. megaterium, Bacillus sp., B. licheniformis, B. subtilis, B. velezensis, B. pumilus, B. albus, B. altitudinis, B. pseudomycoides*, is well known as plant growth promoting bacteria through fixing N_2, contributing

auxin and ethylene signaling pathway, by making various nutrient available and some suppressing numerous pathogen. *B. megaterium* increases the biomass and positively modifies the root architecture of seedlings of the model plant species Arabidopsis thaliana both in physical contact with its roots and via the production of volatile organic compounds and induces different phytohormone-responsive marker genes (Dahmani et al. 2020). It is also proved to be nematostatic against citrus nematode *Tylenchulus semipenetrans* (López-Bucio et al. 2007; Ding et al. 2005; El-Zawahry et al. 2015). *Bacillus licheniformis* exhibit multiple plant growth-promoting characteristics/traits such as ammonia production, indole acetic acid production, phosphate solubilization, catalase production, heavy metal tolerance, and ACC deaminase activity (Saharan et al. 2014). *B. licheniformis* is responsible for the stimulation of root nodulation (Kefela et al. 2015; Jung et al. 2007), siderophore and cellulose production (Gutierrez-Manero et al. 2001), and phytohormone production such as gibberellin (Gutierrez-Manero et al. 2001). *S*ome strains of *B. cereus* can produce IAA and promote the growth of wheat (Hassan et al. 2008). *Bacillus cereus* promotes plant growth. Some strains induce resistance in tomato foliage against different pathogens namely *Pseudomonas syringae*, *Xanthomonas vesicatoria*, *Alternaria solani*, and *Corynespora cassicola* (Reginaldo et al. 2010). Some strains reduce larvae hatching of *Meloidogyne javanica* and increase the root, shoot length, and biomass of the plant (Dawar et al. 2008). Some strains induce systematic resistance and promote plant growth in *Arabidopsis thaliana* by simultaneously activating salicylate, jasmonate/ethylene-dependent signaling pathways (Niu et al. 2011). Some strains control *M. incognita* by producing C16 Sphingosine and Phytosphingosine which makes them potential for bio-control agents for *M. incognita* and *Caenorhabditis elegans* (Gao et al. 2016). *B. amyloliquefaciens*, *B. velezensis*, and *B. siamensis* form an "Operational Group *B. amyloliquefaciens*" within the *B. subtilis* Species Complex (Fan et al. 2017). *B. amyloliquefaciens* can colonize the plant rhizosphere, stimulate plant growth, and suppress by competing with phytopathogenic bacteria and fungi (Qiao et al. 2017). *B. velezensis* (previously known as *Bacillus amyloliquefaciens subsp. plantarum*) is reported to enhance the growth of several plants, including maize, soybean, oil-seed rape *(Brassica napus)*, and *Arabidopsis thaliana* [Hassan et al. 2019; Idriss et al. 2002; Buensanteai et al. 2008; Danielsson et al. 2007]. The root-colonizing *B. subtilis* PTS-394 supported the growth of tomato plants and suppressed soil-borne diseases (Danielsson et al. 2017). *B. subtilis* can also solubilize soil P, enhance nitrogen fixation, and produce siderophores that promote growth and suppresses pathogens. *B. subtilis* enhances stress tolerance in their plant hosts by inducing the expression of stress-response genes, phytohormones, and stress-related metabolites (Danielsson et al. 2019). NemOut™ (*B. subtilis* + *B. licheniformis* + *Trichoderma longibrachiatum*) reduced the *M. incognita* population density (Silva et al. 2017). Application of *Bacillus spp.*, significantly reduced hatching of larvae of *M. javanica* root knot whereas mortality of larvae was significantly increased with the increase in time (Dawar et al. 2014). *B. pseudomycoides*, is reported to produce pseudomycoisin, novel lantibiotics possessing antimicrobial activity. However, it is not reported to be a plant growth-promoting bacteria (Basi-Chipalu et al. 2015). *B. pseudomycoides* increase the availability of potassium in soil and uptake in tea plants when inoculated with Mica waste (Pramanik et al. 2019). Whereas, *B. mycoides* strain R2 isolated from rhizosphere soil of tomato plants exhibited high nematicidal activity against the free-living nematode *Caenorhabditis elegans* and the root-knot nematode *M. incognita* (Luo et al. 2018). The endophytic *B. altitudinis* had a notable influence on plant growth and was also a potential biocontrol agent of Grape Downy Mildew (Zhang et al. 2021; Zeng at al. 2021). *Staphylococcus hominis* was found in a different part of the plant tissue of hybrid maize (*Zea mays*) (Marag et al. 2018). *Staphylococcus hominis subsp. novobiosepticus* strain KE6 increased the total amount of chlorophyll, sugars and protein content, biomass yield, and vegetative growth parameters (Karuppiah et al. 2022).

It is reported that endophytic bacteria are responsible for the stimulation of root nodulation, siderophore, cellulose, and phytohormone production such as gibberellin and auxin (Kefela et al. 2015; Jung et al. 2007; Gutierrez-Manero et al. 2001). GA 20-oxidase catalyzes all the reactions involving the successive oxidation steps of carbon 20 between GA 53 and GA 20, including the removal of C-20 as CO_2. GA-03-oxidase functions as a 3β-hydroxylase, adding a hydroxyl group to C-3 to form the active gibberellin, GA 1. GA 2-oxidase inactivates GA 1 by catalyzing the addition of a hydroxyl group to C-2 (Lodewyckx et al. 2002). Here, we have identified endophytic bacteria from both *Corchorus* species (*C. capsularis and C. olitorius*) before and after nematode infection through 16s rRNA analysis, and observed their different lytic enzyme production capability, efficiency in seed germination, observed their efficiency in plant growth through expression analysis of growth-promoting genes.

2. Methods

2.1 Sample Collection and Isolation of Endophytes

Roots (Healthy and Nematode infected) of 90 days aged *Corchorus capsularis* and *C. olitorius* were collected from Bangladesh Jute Research Institute, Manikganj Regional Station (23°52' N, 90°1.4'E), Bangladesh.

2.2 Isolation and Preservation of Jute Endophytes

The roots were thoroughly washed to remove soil and organic matter, and surface sterilization was performed with a 60-second wash with 70% ethanol followed by a 20-minute wash in 0.1% Mercuric chloride and a 30-second wash with 70% ethanol. Then the residual Mercuric chloride was removed by a ten times wash with autoclaved distilled followed by a final rinse with sterile reverse osmosis-treated (RO) water (Sameer et al. 2016). Then the root (Healthy jute) and root galls (Nematode Infected plant) were carefully collected and soaked for 4 hours at room temperature in a pectinase solution and gently mashed with a pestle. The resultant slurry was transferred to a 250 mL glass flask with 50 mL distilled 0.2 M PBS buffer (pH 7.0) and then shaken for 10 minutes to disperse the root materials. The homogenized solution was filtered through a series of stacked sieves: 250-µm, a 75-µm, and a 25-µm pore sieve to remove the host plant and nematode material (Jenora et al. 2006). Then the 1000 µl (1 ml) of the filtrate was serially diluted up to 10^{-5} and spread onto Nutrient Agar (Peptone 5g/L, Yeast extract 2g/L, Meat extract 1g/L, NaCl 5g/L, Agar 15g/L; pH 7.2±0.2) and incubated both aerobic and anaerobic condition for 48 hours at 37°C. A triplicate set of plates were used for all the treatments throughout the study. Bacterial isolates were preserved in 30% glycerol at -80°C.

2.3 Phosphate and lignin hydrolysis test

The phosphatase and lignase activity were tested on Pikovskaya's agar (PA) containing Tricalcium phosphate as an insoluble phosphate source (Pikovskaya et al. 1948) and lignin agar respectively. Pikovskaya's agar plates were prepared as per following composition (g/l): $C_6H_{12}O_6$ 10.0g, $CaHPO_4$ 5.0g, $(NH_4)_2$ SO_4 0.5g, NaCl 0.2g, $MgSO_4.7H_2O$ 0.1g, KCl 0.2g, yeast extract 0.5g, $MnSO_4.H_2O$ 0.002g, $FeSO_4.7H_2O$ 0.002g containing Bromo phenol blue 0.24g, pH 7.2±0.2. Freshly grown cultures of bacteria were spot inoculated on PA and lignin agar plates with the help of a sterile loop. Inoculated plates were incubated at 37°C for 72h. The formation of a halo zone around the colonies is indicative of positive enzyme activity. Solubilizing index (SI) of the isolates was determined by using the formula as follows:

SI = (Halo zone + Colony diameter)/Colony diameter.

2.4 Extra-cellular enzyme assay

For the screening of extra-cellular enzyme producing the isolates, the procedure described by Dingle et. al. (Dingle et al. 1953) was followed where bacteria were cultured in 100 ml Nutrient Broth (Peptone 5g/L, Yeast extract 2g/L, Meat extract 1g/L, NaCl 5g/L; pH7.2±0.2) and incubated at 37°C in shaking incubator with 200 rpm for 24 hours. After incubation, a suitable volume of bacterial cultures was centrifuged at 22,000x g rpm for 20 min and filtered the supernatant through a Millipore filter (0.22 µm). The filtrate was subjected to the screening of Pectinase, Xylanase, and Cellulase enzyme (Dingle et al. 1953). The 6 mm diameter wells were prepared in agar plates containing pectin, xylan, and CMC substrate with sterile cork borer to inoculate 50 µl of filtrate. After 24 hours of incubation at 37°C, the plates were flooded with iodine solution and the presence of a clear zone around the well indicates the enzymatic activity of the isolates. The average diameter (mm) of clear zones of 3 replications for each isolate was recorded.

2.5 Identification of bacteria by sequencing the 16S rRNA gene

Comparison of the bacterial 16S rRNA gene sequence allows differentiation among organisms at the genus level across all major phyla of bacteria along with classifying strains at the species and subspecies level (Jill et al. 2004; Woese et al. 1985; Woese et al. 2000).
The bacterial communities were analyzed by genomic DNA isolation, PCR amplification, and sequencing of the 16S rDNA fragments.

DNA Isolation

Total genomic DNA was isolated according to the "Bacterial genomic DNA isolation using CTAB" protocol described by JGI (William et al. 2004) briefly bacterial isolates were grown in Nutrient broth at 37°C for 16 hours at 180 rpm shaking. Then the culture was centrifuged at 10,000 rpm for 5 minutes and the pellet was re-suspended in TE (10mM Tris; 1 mM EDTA, pH 8.0) to adjust $OD_{600} \cong 1.0$ concentration. Then added lysozyme at 100 mg/ml concentration and incubated at 37°C for 30 minutes. Then 10% SDS was added and mixed thoroughly followed by the addition of Proteinase K (10mg/ml) and incubation for 1 hour at 56°C. Finally Incubated at 65°C for 10 min after the addition of 5 M NaCl and CTAB/NaCl (heated to 65°C) and mixing. Afterward phenol: chloroform: isoamyl alcohol (25:24:1) was added and mixed well and centrifuged at max speed for 10 min at room temperature and the aqueous phase was transferred to a clean microcentrifuge tube followed by another wash with chloroform: isoamyl alcohol (24:1) and finally 0.6 volume isopropanol (-20°C) was added to the collected aqueous phase and incubated at -20°C for overnight. After centrifuged at max speed for 15 min at 4°C the pellet was washed with ice-cold 70% ethanol (directly from -20°C freezers) and spun at max speed for 5 min and the pellet was air dried at room temperature and was re-suspended in ~170 µl of DNase-free water and RNAse treated (with a final concentration of 0.5 U/µl) and incubated at 37°C for 1 hr. The purity and concentration of DNA were estimated by visual examination on ethidium bromide-stained agarose gel as well as using the Thermo Scientific NanoDrop 2000.

Amplification of 16S rDNA and PCR product purification

PCR amplification of 16s rDNA gene was carried out in GeneAmp PCR System 9700 (Applied Biosystems, Life Technologies, USA) by using forward primer 533F (5'-GAGTTTGATCCTGGCTCAG-3') and the reverse primer 1492RI (5'-ACCTTGTTACGACTT-3') (Idriss et al. 2002). The final volume of PCR mixture was 50 µl containing 5µl of 50ng/µl genomic DNA, 0.25µl of Platinum Taq DNA Polymerase (Invitrogen, Life Technologies, USA), 5µl of 10x PCR buffer with 2µl of 20mM MgSO4, 1µl of 10mM dNTP (Invitrogen, Life Technologies, USA), 2µl Dimethyl sulfoxide (DMSO), 2µl of 10µM each primer and 30.75µl of Nano pure distilled water. The cycling parameter consisted of 35 cycles: denaturation at 94°C, 30s; primer annealing at 55°C, 45s; extension at 72°C, 1min. Before the amplification cycle, DNA was denatured for 5 min at 94°C and after amplification, an extension step for 7 min at 72°C was performed. All the amplified PCR products were eluted from agarose gel using Qiagen QuickSpin PCR purification columns (Qiagen, Catalog No. 28706).

Sequencing, assembly, and BLAST

The amplified and purified PCR fragments were sequenced in ABI 3730XL DNA Analyzer (Applied Biosystems, Life Technologies, USA) with the 533F (5'-TTA CCGCGGCTGCTGGCAC-3'), 1492RI (5'-ACCTTGTTACGACTT-3') primer. The
sequencing reaction was performed by using Big Dye V3.1 sequencing reagents (Applied Biosystems,
Life Technologies, USA) following the manufacturer's protocol. The two sequences for each sample were assembled using CAP3 (Buensanteai et al.

2008) and the contig of each isolate was compared to public databases available in NCBI and species level identification was determined as a 16S rDNA sequence similarity of >99% with that of the prototype strain sequences in the GenBank.

Phylogenetic Analysis

Phylogenetic analysis was conducted using a multiple sequence alignment program Clustal omega. Then Newick file of the tree was given to MEGAX (Kumar et al. 2018) to construct a phylogenetic tree by the neighbor-joining method program where significant levels of interior branch points obtained were determined by bootstrap analysis (1000 data re-sampling).

Effects of Endophytes on Seed Germination

The selected endophytic bacterial strain was grown in Nutrient Broth for 16 h (OD_{600}=1) at 34°C with 180 rpm shaking followed by the collection of bacterial cells by centrifugation (13,000 rpm for 10 min at 4°C). Cells were re-suspended with sterile 0.05 mM PBS (pH 7.0) to prepare a final concentration of 10^8 CFU/ml for the uniform population of bacteria for seedlings inoculation (Taghavi et al. 2009). 1 gm of healthy seeds were treated with endophytic bacteria ($1x10^8$ CFU/ml) for 2h with 180 rpm shaking, placed on moist germination paper, and incubated at room temperature. Seeds treated with sterile distilled water served as control. Vigor index (VI = Mean Root Length + Mean Shoot Length x % Germination) was calculated at the end of 7 days (Baki et al. 1973). Three replicates of 10 seeds were used per treatment.

Effect of Endophytic Bacteria on Jute Growth

Seeds treated with endophytic bacterial isolates as well as sterile distilled water were sown to clay pots containing a sterilized mixture of Coconut husk: Soil: Manure (1:1:1) in a greenhouse (30°C, 70% humidity). Two ml of the endophytic bacteria cells were added around the roots of 5-day-old aseptic seedlings of *C. olitorius*, *C. capsularis,* and Robi-1, and 2 ml of phosphate buffer of heat-killed endophytic bacteria cells were added as a negative control. Vegetative growth parameters such as Shoot length, Shoot diameter, root length, fresh weight, number of leaves, and leaf area were recorded after 30 days of growth. The fresh weights of the plants were determined by weighing the individual plants immediately after harvesting.

Primer design, RNA extraction, and expression profile

The primers of GA-20, GA-3, and GA-2 oxidase were designed using a web-based tool from IDT (http://sg.idtdna.com) and GenScript (www.genscript.com) followed by verification with Multiple Primer Analyzer (https://www.thermofisher.com). Primer specificity was evaluated by 1% agarose gel electrophoresis and melting curve analysis during qRT-PCR.

One gram of sample tissues was disrupted into a fine powder with mortar-pestle, and an in-house modified CTAB (Hossain et al. 2019) protocol was used to extract total RNA. Genomic DNA contamination was removed by amplification grade DNase I (Sigma Aldrich, Germany). NanoDrop 2000 spectrophotometer (NanoDrop, Thermo Scientific) determined the concentration of RNA, while the integrity was evaluated by 1% agarose gel electrophoresis. Complementary DNA (cDNA) was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) and random primers from 1 µg of total RNA according to the manufacturer's protocol. Then the samples were treated with RNaseH (Thermo Fisher Scientific, USA) to remove residual RNA. The quantitative RT-PCR was performed with a Quantstudio Real-time PCR system (Applied Biosystems, USA) using 96 well-plate with a final volume of 20 µL containing 5 ng cDNA, 10 ul of 2X PowerUpTMSYBRTM Green Master Mix (Applied Biosystems, USA) and 300 nM of each primer. The qRT-PCR cycling for all genes was carried out according to the following conditions: an initial activation step of 2 min at 50°C, then a denaturation step for 10 min at 95°C followed by 40 cycles of 95°C for 15 s, the primer specific annealing temperatures (Supplementary table 1) for 20 s, and 72°C for 20 s. To check the specificity of the primers, a melting curve was generated at end of qRT-PCR cycles by a constant increment of temperature from 60°C to 95°C. The qRT-PCR reactions were performed with three biological replicates and three technical replicates for each biological replicate along with no template control. The expression level of the selected Gibberellin genes was analyzed with the comparative CT method (2-ΔΔCt) [Donoso et al. 2010] and two housekeeping genes such as HK-9 [Pedrosa et al. 2011] were used as internal control genes for normalization of qRT-PCR analysis.

3. Results

3.1 Sample Collection and Isolation of bacteria

Based on different colony morphology (**Supplementary Figure 1**), a total of 59 isolates were isolated from healthy and nematode-infected jute plants (**Table 1**).

Table 1: Different bacteria isolated from healthy and nematode-infected jute plants.

Sample	Healthy	Nematode Infected
C. capsularis	13	17
C. olitorius	13	16

3.2 Identification of Endophytic bacteria in Jute

We have found only gram-positive bacteria in both species of jute in healthy and nematode-infected conditions. The 16s rRNA gene sequencing and BLAST search revealed that most of the isolates were different *Bacillus* and *Staphylococcus* strains with 97%-100% sequence similarity (**Supplementary Table 1**). The Maximum Likelihood (ML) tree was built by combining the sequence data of endophytic bacteria from jute endophytes (**Figure 1**). Healthy and diseased *C. capsularis* harbored similar bacterial species. The diseased plant harbored *B. mycoides* and *B. pseudomycoides*. Likely healthy and diseased *C. olitorius harbored* almost similar bacterial species, however, diseased plant harbored *P. megaterium, B. siamensis*, *B. amyloliquefaciens,* and healthy plant harbored *S. hominis* (**Figure 2**).

3.3 Hydrolytic Enzymatic activity

Most of the isolates from jute endophytes possess plant polymer degrading enzymes with some exceptions (**Figure 3)**. Isolates with the highest EI from each bacterial species were selected for growth regulation tests.

3.4 Selection of bacterial strain: seed bacterization and root colonization

Certain bacterial species increased the root length, shoot length, and germination rate compared to the control. *B. aryabhattai*, *B. proteolyticus*, *B. velezensis,* and *Staphylococcus hominis* increased plant growth in the CVL-1 variety. Whereas almost all isolates improved the growth of Robi-1 and O-9897 variety including *B. megaterium*, *B. aryabhattai*, *B. proteolyticus*, *B. flexus,* and *B. haynesii*. Vigor Index [Vigor Index= (Mean Root Length + Mean Shoot Length x % Germination)] was higher in plants inoculated with *S. hominis* (**Figure 4**).

3.5 Effects of Endophytes on Jute

The endophytic bacteria showed significant growth-promoting effects on the jute. *B. cereus* significantly increased the growth phenotype and biomass of *C. capsularis* (CVL-1) whereas *S. hominis* showed significant growth increment in *C. olitorius* (O-9897) and likewise, the combination of all bacteria increased the growth of Robi-1 (**Figure 5A; 5B; 5C**).

The phenotypic growth (root, shoot length) (**Figure 6A, B**), biomass (root mass, shoot mass) (**Figure 6C, D**), and Leaf number and leaf area (**Figure 6E, F**) greatly increased with bacteria inoculation.

3.6 Expression profile analysis

Increased stem length ensures increased fibre yield. We have selected the best phenotypic growth showing plant from each jute variety for expression profile of different growth-promoting hormone gibberellin such as GA-20, GA-03, and GA-02 oxidase. GA-20 oxidase was highly expressed in CVL-1(7.5x) and O-9897 (5x) while the expression was only 2-fold in Robi-1. The expression of GA-03 oxidase was highest in O-9897 (>5x) while the expression was only 2-fold in CVL-1 and Robi-1 (**Figure 7A, B, C**). Higher expression of GA-20 and GA-03 oxidase consolidate that plant growth was increased through induction of increased production of growth hormone gibberellin. Higher expression of GA-20, GA-03 along with increased plant height indicates that bacteria stimulates the GA-20 and GA-03 production. However, GA-02 was highly expressed in O-9897. As it is reported that plant gibberellin production is regulated by feedback regulation and increased GA activity also increases GA-02 production through the feedback regulation plant has reached the homeostasis condition and increased plant growth.

4. Discussion

Sustainable agricultural practices are fundamental to meet the future world's agricultural demands i.e. increased production and agroecosystem protection. Plant growth-promoting rhizobacteria (PGPR) promote plant growth without environmental contamination (Khandelval et al. 2021). They stimulate plant growth through mobilizing nutrients in soils, producing numerous plant growth regulators, protecting plants from phytopathogens by controlling or inhibiting them, improving soil structure, and bioremediating the polluted soils by sequestering toxic heavy metals and degrading xenobiotic compounds like pesticides (García-Fraile et al. 2012). Commercial production of synthetic P fertilizers requires is highly expensive and ~ 75–90% of the applied P fertilizers are precipitated in the soil system (Jill et al. 2004). Phosphate solubilizing bacteria can reduce the cost of fertilizer by making unavailable P-sources available form and consequently enhancing the yield of crops.

In this study, we have isolated and identified endophytic bacteria from healthy and nematode-infected jute roots (*C. capsularis, C. olitorius*, and Robi-1) through pure culture and 16S rDNA genomic sequencing. We have found different species of *Bacillus* and *Staphylococcus*. Physiological characterization included pectin, xylan, cellulose, lignin, and phosphate hydrolysis. Such enzymatic activities may assist the seeds through germination (Li et al. 2019) and the transmission into the plant tissues. Xylanolytic and cellulolytic enzymes are particularly important for the degradation of tissues surrounding the bast fibre, an essential process for harvesting jute fibres (Munshi et al. 2008; Ali 1958). Most of the endophytes were positive for Pectin, xylan, cellulose, and phosphate hydrolysis. Phosphatase activity of endophytes may help in nutrient uptake and enhance plant growth. Here, we explored an environment-friendly option for boosting jute plant growth through the application of plant growth-promoting probiotics. Significant improvement in plant growth was observed through the application of both *B. cereus, S. hominis*, and a combination of endophytic bacteria in *C. capsularis, C olitorius*, and Robi-1 respectively. Although plant growth promotion by the application of various species of *Bacillus* and *Staphylococcus* has been reported (Tamoor et al. 2018; Karuppiah et al. 2022), this study demonstrated that *B. cereus* significantly increased root length (44%) and weight (32%), Shoot length (14%) and mass (23%), leaf area 2% and leaf number (19%) in *C. capsularis*. Whereas, *S. hominis* increased root length (22%) and mass (9%), while shoot length (4%) and mass (10%), leaf area (13%), and leaf number (11%) in *C. olitorius*. The bacterial combination

increased the growth of Robi-1. Root length (35%), root mass (36%), shoot length (12%), shoot mass (27%), leaf area (11%), and leaf number (18%) were increased in Robi-1. *B. cereus* significantly increased GA-20, and GA-03 by 7.5 and 2-fold, and a decreased expression of GA-03 (Fig. 7A, B, C) corroborates that the growth promotion was due to the increased production of active growth-promoting gibberellin hormone. Higher expression of GA-20 (3 fold), GA-03 (2 fold), and GA-02 (2 fold) was observed in the *S. hominis* inoculated O-9897 (Fig. 7A, B, C). Likely in Robi-1, the higher expression of GA-20 (5-fold) and GA-03 (5.2 fold) indicates plant growth promotion by active gibberellin production compared to the control plant proves that endophytic bacteria induce gene expression in the host (Fig. 7A, B, C). Higher GA-02 (4-fold in O-9897 and 2-fold in Robi-1) expression was also observed. GA production is regulated by feedback regulation and increased GA activity increases GA-02 production to deactivate the active GA. Through the feedback regulation of GA, the plant has reached the homeostasis condition and increased plant growth (Hedden et al. 2012). It is evident from our findings that plant is able to shape their endophytic microbiome and before and after a pathogen attack they primarily harbored growth-promoting bacteria. The application of *B. cereus* and *S. hominis* significantly increased the growth of *C. capsularis* and *C. olitorius* respectively. Also, a combination of eleven bacterial species promoted significant plant growth in an advanced line of *C. olitorius* Robi-1. These findings suggest plant endophytes isolated from the native environment could be used as natural agents for increased production of jute.

Declarations

Data availability

All 16S rRNA partial gene sequences are publicly available from the GenBank under the accession number OP492092 to OP492153 (Release Date: October 16, 2023), with specific numbers listed in Supplementary Table 1.

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Author Contribution

N.A., and M.S.I. conceived the study, and N.A, M.S.H., M.R.A., and R.A., performed the investigations. N.A., B.A., M.W.U., and Q.M.H. contributed to collect samples and physico-chemical property analysis of jute endophyte. N.A isolated genomic DNA. M.S.H., R.A., and N.A. performed genomic sequencing and assembly of 16s rDNA. N.A. identified the bacteria and performed phylogenetic analysis. N.A prepared the figures. N.A. wrote the paper. N.A., M.W.U., and M.S.H reviewed paper. All authors read and approved the final paper.

Ethics declarations

Competing Interests

The authors declare no competing interests.

Supplementary Material

Supplementary Table 1: 16s rRNA sequence analysis of jute endophytes.

Supplementary Table 2: Lytic enzyme Index of the isolates.

Supplementary Table 3: Vigor-Index of the selected isolates.

Supplementary Figure 1: Colony Picture of the selected isolates.

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Figures

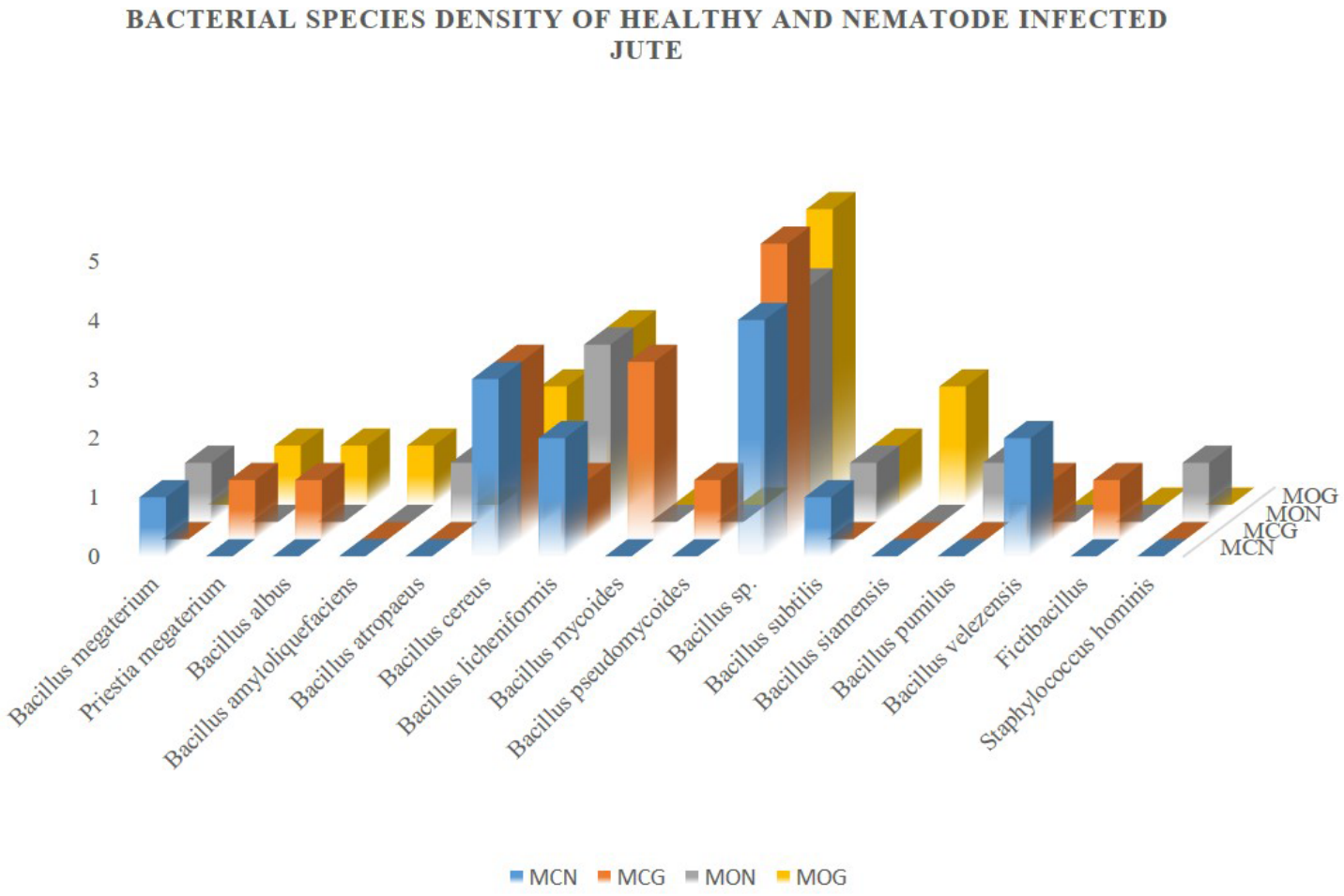

Figure 1

Species diversity of healthy and diseased jute endophyte.

Figure 2

Maximum likelihood (ML) tree of culturable endophytic bacteria. The tree was constructed using Mega 6.0 software and the ML method with a bootstrap value of 1,000. Two major clades represent two different genera. And different species are shown in different colors.

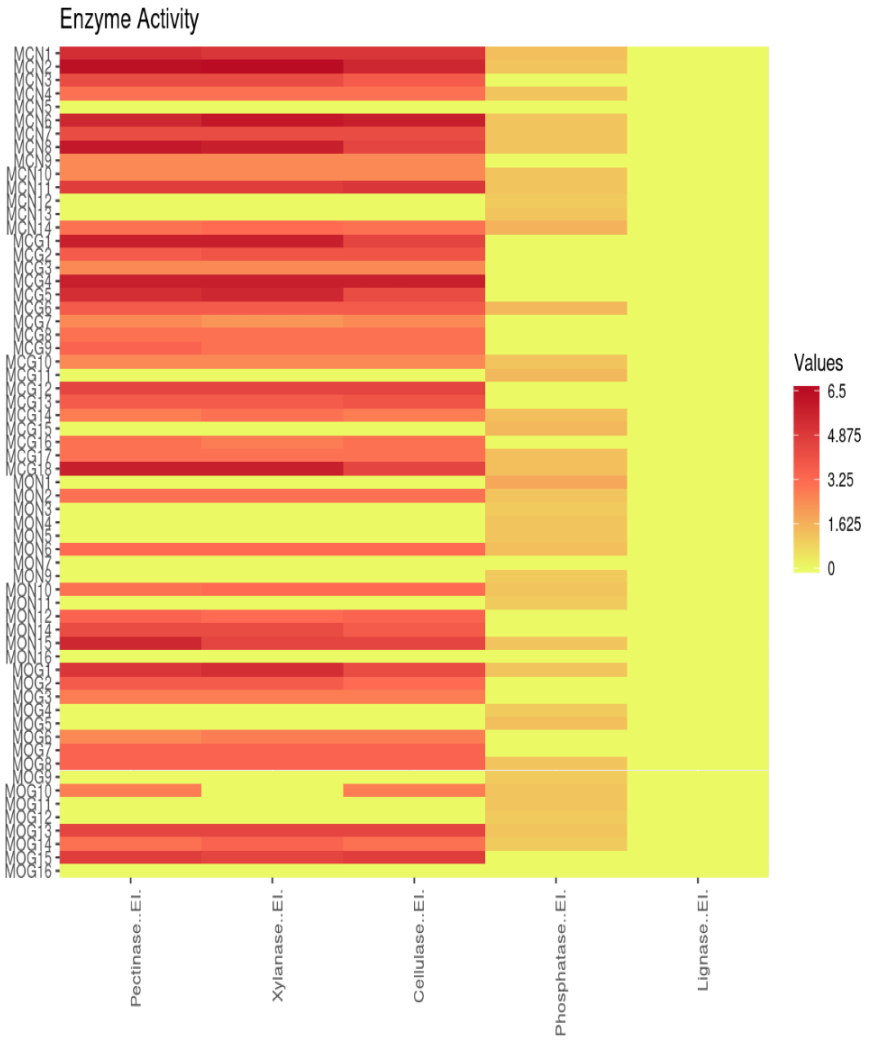

Figure 3

Lytic Enzyme activity of Jute Endophytes.

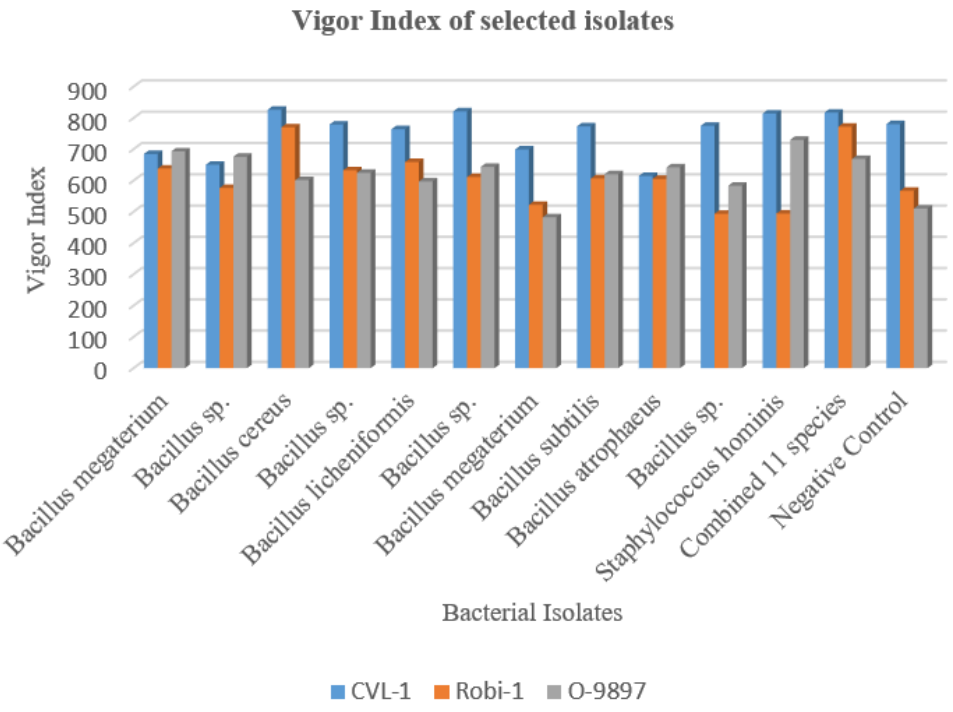

Figure 4

Effect of seed treatment with promising endophytic bacteria on seed germination and seedling vigor of jute.

Figure 5

Effect of *B. proteolyticus, Staphylococcus hominis,* and a combination of endophytic bacteria on vegetative growth of (A) *C. capsularis* cv CVL-1 (B) *C. olitorius* cv. O-9897 (C) *C. olitorius* cv. Robi-1. Photos were taken on the 30th day of inoculation.

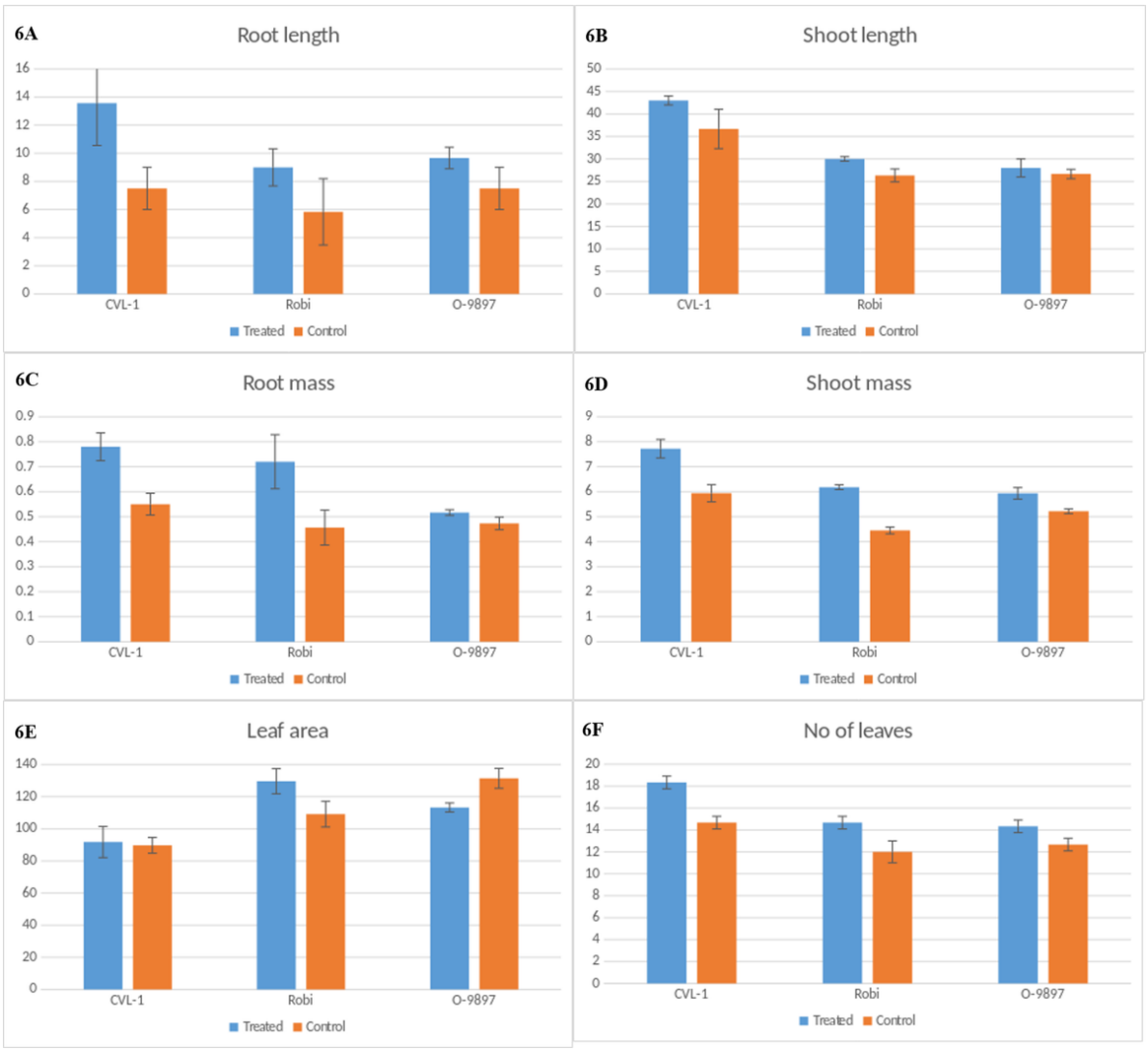

Figure 6

Different growth parameters increased by growth-promoting endophytic bacteria.

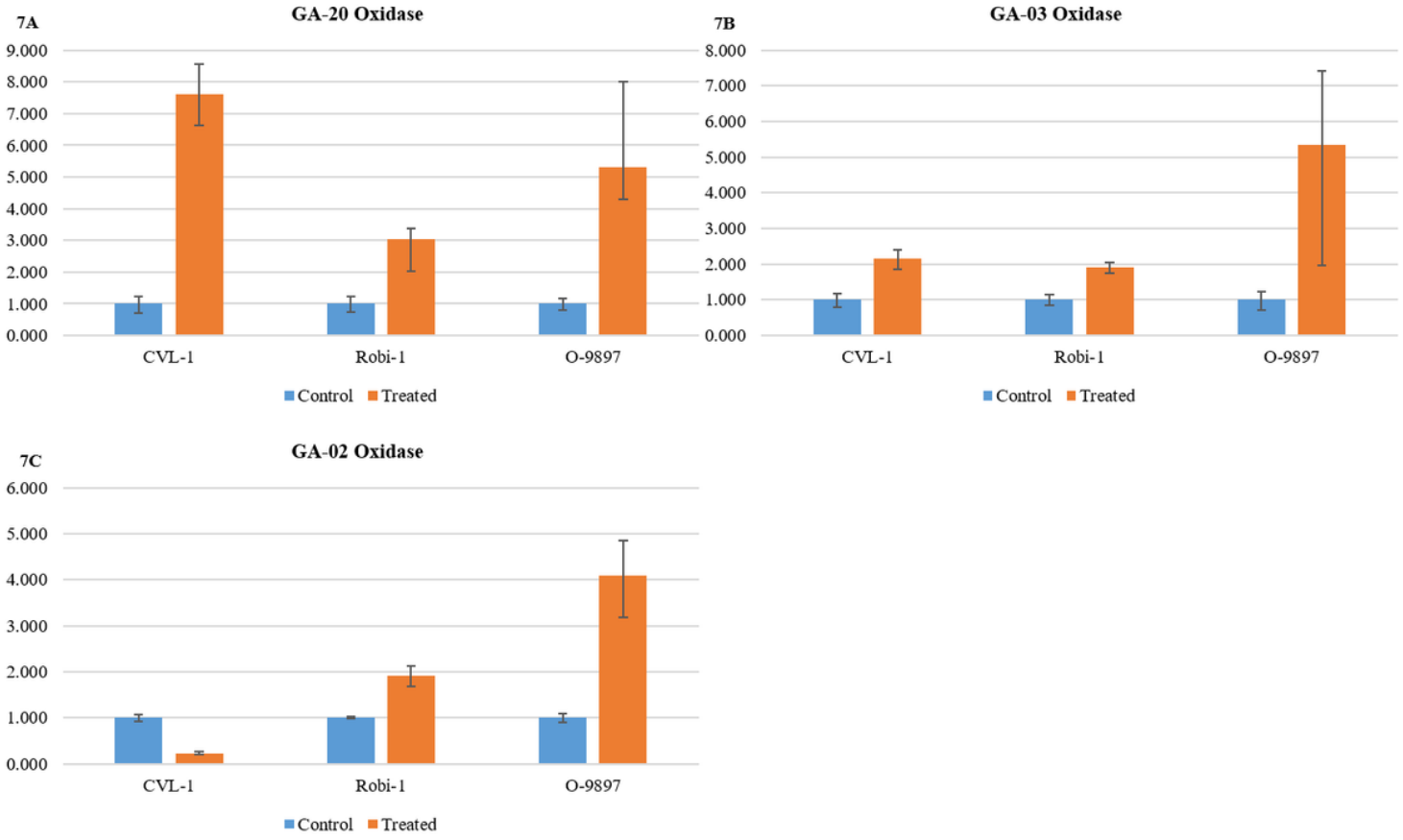

Figure 7

Expression profile of (A) GA-20, (B) GA-03, and (C) GA-02 in CVL-1, Robi-1, and O-9897.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableandfigure.docx