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Determination of Desirable Attributes of an Indigenous *Burkholderia* Isolate Towards Biological Control of Plant Pathogenic Fungi and Its Microbial Enzyme Production

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ABSTRACT

Purpose: *Microorganisms are proven bio-resources for the environmentally-friendly and sustainable biological control of plant diseases and microbial enzyme production. The present study confirms the identity of an indigenous Burkholderia isolate, determines its desirable features as a biological control agent of plant pathogenic fungi and an enzyme producer and analyzes molecular relationships with selected environmental isolates of Burkholderia.*

Research Method: *In vitro antagonism of Burkholderia isolate on colony growth and spore germination of five fungal pathogens causing field and postharvest diseases were tested. Antifungal ability of cell-free filtrate and effect of temperature on antifungal ability were determined. Extracellular enzyme production of Burkholderia isolate was screened and phylogenetic relationships were elucidated.*

Findings: *Burkholderia isolate inhibited colony growth of the five fungal pathogens by a range of 45 - 59 %, reduced the spore germination ability forming spores of a typical morphology. Antifungal ability was lost beyond 57 oC and cell-free filtrates did not show antagonism against the tested fungi. The bacterial isolate gave the best match with Burkholderia A45 strain and it was; catalase, gelatinase, lipase and casein hydrolysis positive. The indigenous bacterial isolate grouped together with Burkholderia strains, having biodegradation ability of environmental pollutants.*

Research Limitations: *Absence of a comprehensive image on extracellular enzyme producing ability and information on biodegradation ability of the bacterial isolate.*

Originality/value: *Findings will be useful to exploit the potential of the bacterium in integrated management of pre- and postharvest fungal diseases and in microbial enzyme production.*

Keywords: *Bio-Control, Burkholderia,Extracellular enzymes, In vitro antagonism, Pre- and Post-harvest fungal pathogens*

INTRODUCTION

Pre and Post-harvest fungal diseases cause a significant loss of the agricultural production, both quantitatively and qualitatively, worldwide. Application of synthetic fungicides is the most commonly used method to manage the fungal diseases of crop plants. However, health and environmental hazards caused by synthetic fungicides have become a major concern among the consuming communities both local and international (Wisniewski and Wilson, 1992, Babychan *et al.*, 2017), hence environmentally-friendly alternatives are a timely need.

Colletotrichum gloeosporioides, *Colletotrichum capsici*, *Fusarium* spp., *Botryodiplodia* spp.

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and *Pestalotia* spp. are common fungal plant pathogens causing significant economic losses in fresh agricultural produce due to pre- and post-harvest diseases. *C. gloeosporioides* causes anthracnose diseases on a variety of agricultural crops such as papaya, apple, guava, citrus, grapes and strawberry (Sharma and Kulshrestha, 2015). *Colletotrichum capsici* causes anthracnose in chilli (Yun *et al.*, 2009) where *Fusarium* spp. are responsible for dry rots and vascular wilts in many economically important crop plants such as banana, potato and melon, to name a few (Saremi *et al*., 2011). Moreover, several types of leaf spots are caused by *Pestalotia* spp. (Luan *et al*., 2008) whereas stem-end rots in many fruit crops such as avocado, mango are caused by *Botryodiplodia* spp. Therefore, successful management of these fungal pathogens has become a crucial factor in order to achieve good quality agricultural produce with high economic returns.

Microorganisms naturally inhabiting the plant surfaces such as phyllosphere and fructosphere and surroundings of the plants such as rhizosphere have shown promising effects in controlling plant diseases caused by fungal pathogens (Heydari and Pessarakli, 2010). The genus *Burkholderia* comprised of several genomovars that are naturally present in the environment and have been reported to act as microbial antagonists of several plant pathogenic fungi through different modes of action (Coenye *et al.*, 2001). For example, siderophores of *Burkholderia cepacia* XXVI has the biocontrol capacity against *C. gloeosporioides* (Villalobos *et al*., 2012). According to De Costa *et al.,* (2008) *B. spinosa* has been proved for its antagonistic ability against *C. musae* under *in-vitro* conditions. *B. spinosa* has also been reported for its ability to reduce anthracnose disease in different banana varieties when applied as a post-harvest dip treatment. Silva and De Costa (2014) have reported the antagonistic ability of the *B. spinosa* in controlling pathogenic microorganisms such as *Aspergillus* spp. and *Fusarium* spp. present in banana phyllosphere when applied as pre-harvest soil drenches and foliar applications.

Burkholderia species produce a variety of enzymes which are biotechnologically-important, promote plant growth and health and degrade recalcitrant pollutants (Eberl and Vandamme, 2016). Despite the agriculturally and environmentally beneficial *Burkholderia* species, some of the members of the genus have been identified as threats to human, animal and plant health (Eberl and Vandamme, 2016). When an indigenous microorganism is isolated from environmental samples, in-depth investigations are essential on its desirable attributes before its introduction as a biological control agent.

With the above background, the present study was conducted to confirm the identity of the *Burkholderia* isolate (*Burkholderia* BSL isolate) which has been isolated and characterized from peel tissues of banana in a previous study and to determine desirable characteristics possessed by the *Burkholderia* BSL isolate for its use as a potential biological control agent against fungal pathogens. Further, it was aimed to determine the extracellular enzyme production potential of the test bacterium (*Burkholderia* BSL isolate) and to analyze its phylogenetic relationship with several environmental isolates belonging to the genus *Burkholderia*.

MATERIALS AND METHODS

Microbial cultures

A bacterial culture, which was originally-isolated from peel tissues of banana and identified as a species of *Burkholderia* by BIOLOG method (BIOLOG, USA) (De Costa *et al*., 2008) and designated as *Burkholderia* BSL isolate, was used in this study as the nucleus. Bacterium was retrieved from a preserved culture in 15 % glycerol, which had been stored at -80 ^{o}C ultralow temperature lab freezer (Sanyo, Japan). Purity was confirmed by streaking the bacterium on Nutrient Agar (NA) medium and based on the unique yellow coloured circular colonies formed on NA medium.

Three fungal pathogens causing postharvest

diseases of fruits, namely *C. gloeosporioides*, *C. capsici* and *Botryodiplodia* sp. and two fungi causing field diseases of plants, namely *Fusarium* sp. and *Pestalotia* sp. were isolated from respective specimens showing typical symptoms. Pure cultures were maintained on Potato Dextrose Agar (PDA) medium and identity of the fungal cultures was confirmed by colony and spore morphology.

Confirmation of the Identity of the Burkholderia BSL Isolate

To confirm the identity of the *Burkholderia* BSL isolate by molecular methods, genomic DNA was extracted by Chen and Kuo (1993) method and subjected to PCR using universal primers, namely 27F and 1492R primers (27F-5'AGAGTTTGATCMTGGCTCAG3'and 1492R-5'TACGGYTACCTTGTTACGACTT 3') to amplify 16s rDNA region of the bacterium. PCR cycle conditions included 35 cycles of denaturing step at 94 ^{o}C for 45 sec, annealing step at 50.2 ^{o}C for 45 sec and an extension step at 72 ^{o}C for 1 min. Expected size of the PCR product was approximately 1500 bp (Awad *et al*., 2015). PCR products were analyzed on 1.5 % agarose gel and amplicons having expected molecular size were sequenced at Asiri Diagnostic Services (Pvt) Ltd and subjected to DNA homology search using BLAST, NCBI.

Colony Growth Inhibition of Fungal Pathogens by Burkholderia BSL Isolate

Each purified fungal culture (treatment) was separately co-cultured with the *Burkholderia* BSL isolate in PDA medium by dual culture plate technique (Haidara *et al.*, 2016) while placing the one-day old bacterium as four lines on four sides of the Petri dish using an inoculation loop. A control treatment was maintained for each fungal isolate without the *Burkholderia* BSL isolate. Each treatment was replicated three times and arranged in a complete randomized design. Plates were incubated at 28 ^{o}C for six days and radial growth of each fungal isolate was measured. Percentage colony growth inhibition by *Burkholderia* isolate was calculated according to Haidara *et al.,* (2016) using the following equation;

Percentage colony growth inhibition = $[(R_2 - R_1)/R_2] \times 100$

Where,
R_1 = Minimal distance between the center of the mycelial disc and the fungal colony margin towards the direction of *Burkholderia*-BSL bacterium

R_2 = Fungal colony radius of the control plate (distance between the center of mycelial disc and the fungal colony margin)

Effect of Burkholderia BSL Isolate on Spore Germination of Fungal Pathogens

Spore suspensions of *C. gloeosporioides*, *C. capsici, Botryodiplodia* sp. *and Fusarium* sp. having a spore concentration of 1×10^4 spores/ml were prepared in sterile distilled water. Aliquots of 2 ml of the spore suspensions were mixed separately with equal volumes of a one-day old *Burkholderia* BSL culture grown in PD broth, in a test tube having a diameter of 1.5 cm. The mixtures were incubated in a shaking water bath (BW 200, Shaking water bath, Yamato, Japan) with 80 RPM shaking speed at 28 ^{o}C. Control treatments were maintained for each fungal culture without mixing with *Burkholderia* BSL isolate. Each fungal culture under the influence of *Burkholderia* BSL isolate was replicated 3 times. Seventeen hours after incubation, spores were observed under x 400 magnification for the presence of the germ tubes (De Cota and Chandima, 2014)

Antagonistic Ability of Burkholderia BSL Isolate Exposed to Different Temperatures

Eppendorf tubes each having 1.5 ml of one-day old broth culture of *Burkholderia* BSL isolate were incubated for 15 min at six selected temperatures, namely 27, 37, 47, 57, 67 and 77 ^{o}C using a heating block (Grant-BTD digital dry block heater for microtubes). After the heat treatment, each culture was plated on PDA plates along with *Botryodiplodia* culture according to the standard dual culture plate technique. For each temperature, control

treatment (without*Burkholderia* BSL isolate) was maintained. Each treatment was replicated three times. Three days after incubation at 28 ^{o}C, percentage colony growth inhibition of *Botryodiplodia* sp. was quantified as described above.

Antagonistic Ability of the Cell-Free Filtrate of Burkholderia BSL Isolate

Eight-day old nutrient broth culture of *Burkholderia* BSL isolate was centrifuged at 4000 rpm at 4 ^{o}C for 15 minutes to pellet out the bacterial cells. Supernatant was filtered through a Whatman No. 1 filter paper and the resultant filtrate was centrifuged again at 4000 rpm at 4 ^{o}C for 15 min. The supernatant was sterilized by filtering through a 0.2 µm syringe filter (Whatman 0.2 µm syringe filter). Cell free filtrate of the *Burkholderia* BSL isolate was subjected to dual culture plate technique against the five fungal pathogens separately. Each fungal culture (treatment) was triplicated with control treatments and incubated at room temperature (28 ^{o}C). Six days after incubation, percentage colony growth inhibition was calculated as described above.

Ability of Burkholderia BSL Isolate to Secrete Extracellular Enzymes

To confirm the gelatinase producing ability, Gelatin liquefaction test was done by stab inoculating the *Burkholderia* BSL isolate into test tubes containing semisolid gelatin agar (7.5 g of agar per L) and incubated at room temperature for 48 hr. Liquefaction was confirmed by placing at 4 ^{o}C as described by Balan *et al.,* (2012).

Catalase production ability was determined by mixing a loopful of a freshly grown *Burkholderia* BSL culture with 3 % H_2O_2 drop on a clean glass slide and observing for O_2 bubble formation (Reiner, 2010).

Starch hydrolysis test was done for the *Burkholderia* BSL isolate to determine its ability to produce α-amylase enzyme. Bacterium was streaked on a starch agar plate and incubated for 48 hr at 30 ^{o}C. Lugol's Iodine solution was added on to the medium and observed the formation of a clear zone around the bacterial colony (positive result for starch hydrolysis) (Hankin and Anagnostakis, 1975).

Lypolytic activity of the *Burkholderia* BSL isolate was determined by Hankin and Angnostakis (1975) method, in which the bacterium was heavily inoculated on a basal medium containing Difico peptone 10g/L, NaCl 5 g/L, $CaCl_2.2H_2O$ 0.1g/L, Agar 20 g/L, pH 6 and 1% (v/v) Tween 20. Basal medium inoculated with the *Burkholderia* BSL isolate was observed for the presence of clear halosor precipitates around the line of bacterial growth 48 hr after incubation at room temperature.

Casein hydrolysis ability of the *Burkholderia* BSL isolate was determined according to Powthong *et al.,* (2017). Briefly, the bacterium was heavily inoculated on a Basal Mineral Salts Medium (BMSM) containing skim milk (10 g/L). The casein hydrolysis ability of the isolate was detected by the presence of a clear halo around the bacterial colony after 48 hrs of incubation. The results were confirmed by adding 2 ml of 0.1 mol/L hydrochloric acid (HCl) to the plate in order to observe clear halos.

To determine the cellulolytic activity, the *Burkholderia* BSL isolate was heavily inoculated on the BMSM-containing carboxymethyl cellulose (10 g/L). The cellulose hydrolysis ability of the isolate was noted by checking for the presence or absence of clear halos around the colonies after 7 days of incubation and by flooding the culture plate with 0.2% Congo red and de-staining with 1 M NaCl for 15 min (Powthong *et al.*,2017).

Burkholderia BSL isolate was heavily inoculated on the BMSM medium containing 0.08% chitin azure. The detection of chitinase activity of the isolate was done by checking the presence or absence of clear halos around the bacterial colonies after incubation at room temperature for 7 days (depending on the growth rate of the bacterium) (Powthong *et al.*, 2017).

Phylogenetic Analysis

Genetic divergence of the *Burkholderia* BSL isolate used in the present study was determined by comparing with 18 other *Burkholderia* isolates previously reportedly having beneficial attributes based on sequence information available in DNA databases. In addition, *B. cepacia* B9 strain reported as a banana pathogen (Lee *et al*., 2003) was also included in the analysis (AY207313.1). Sequence data retrieved from NCBI and EMBL-EBI databases were used to construct a phylogenetic tree using MEGA6 software by Maximum Likelihood method based on the Jukes-Cantor model with 1000 number of bootstrap replications.

RESULTS

Molecular Identification of the Burkholderia BSL isolate

According to BLAST analysis, *Burkholderia* BSL isolate of the present study gave the best homology with *Burkholderia* sp. A45 strain (GenBank accession number KF788025) with a 92 % query cover, 0.00 E-value and 95 % identity. Sequence information of *Burkholderia*-BSL isolate was deposited in GenBank, NCBI under the accession number MK838493.

Colony Growth Inhibition of Fungal Pathogens by Burkholderia BSL Isolate

Burkholderia BSL isolate showed a percentage colony growth inhibition of 45 - 59% towards the five fungal pathogens by the 6th day of incubation (Table 01). Antibiosis as a mode of antagonism of the *Burkholderia* BSL isolate was identified by the inhibition zones produced on dual culture plates (Figure 01).

Effect on Spore Germination of fungal Pathogens by Burkholderia BSL Isolate

None of the spores of any of the fungi germinated after 17 hours of incubation. However, reduction of the number of spores present and changes in the spore morphology was observed in the samples treated with *Burkholderia* BSL isolate. These treated samples showed atypical spore morphology and shrunk cellular content compared to the control maintained.

Antagonistic Ability of Burkholderia BSL Isolate Exposed to Different Temperatures

With increasing temperature at which the *Burkholderia* BSL isolate was exposed to, a decreasing trend in colony growth inhibition of the fungal pathogen *Botryodiplodia* sp. was observed (Table 03). *Burkholderia* BSL cultures subjected to 57 ^{o}C and above had no ability to inhibit the colony growth of the fungal pathogen. Further studies are needed to determine the antagonistic ability of the *Burkholderia* BSL isolate against other fungi used in the present study at different temperatures.

Antagonistic Ability of the Cell-Free Filtrate of Burkholderia BSL Isolate

No colony growth inhibition of any fungal pathogens was observed when dual plate culture technique was performed using cell free filtrate of *Burkholderia* BSL isolate.

Table 01: Percentage colony growth inhibition of fungal pathogens by *Burkholderia* BSL isolate, 6 days after incubation.

Fungal pathogen	Mean* colony growth inhibition (%)
C. gloeosporioides	50± 3.14
C. capsici	54±1.54
Fusarium sp.	45± 0.59
Botryodiplodia sp.	58± 0.66
Pestalotia sp.	59± 4.21

**Values are means of 12 replicates*

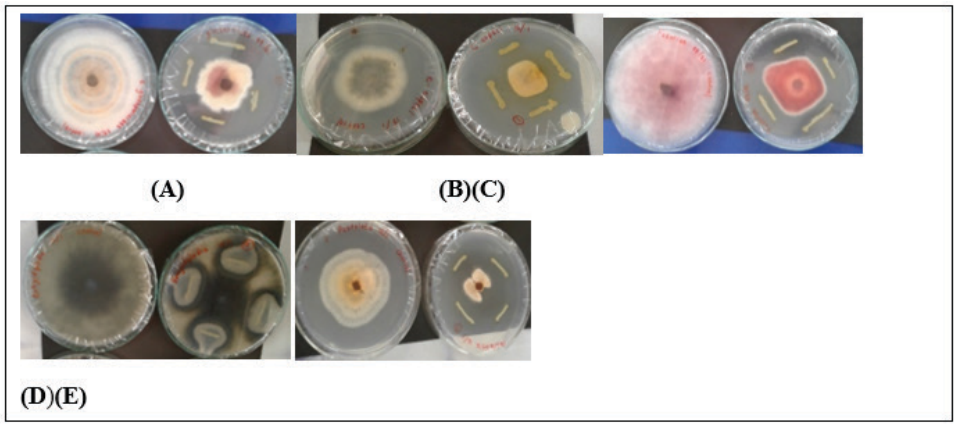

Figure 01: **Colony growth inhibition of five fungal pathogens (A-*C. gloeosporioides*, B- *C. capsici*, C- *Fusarium* sp., D- *Botryodiplodia* sp., E- *Pestalotia* sp.) by the *Burkholderia* BSL isolate, in comparison to the colony growth without the influence of *Burkholderia*BSL isolate.**

Table 02: **Total number of spores observed after 17 hours of incubation.**

Fungal culture	Total number of spores observed per ocular area	
	In Control Sample	In Treated Sample
C. gloeosporioides	92	35
C. capsici	25	6
Botryodiplodia sp.	5	1
Fusarium sp.	12	8

Table 03: **Percentage colony growth inhibition of *Botryodiplodia* sp. by *Burkholderia* BSL isolate exposed to different temperatures.**

Temperature (^{0}C)	Mean* percentage colony growth inhibition
27	65± 0.67
37	62±0.49
47	58± 1.47
57	0
67	0
77	0

**Values are means of 12 replicate*

Ability to Secrete Extracellular Enzymes by the Burkholderia BSL Isolate

In vitro assays confirmed that *Burkholderia* BSL isolate is capable of secreting catalase, gelatinase, lipase and casein hydrolyzing enzyme but not chitinase and starch hydrolyzing enzymes such as α-amylase and cellulose (Table 04).

Phylogenetic Analysis

Burkholderia BSL isolate is grouped in a separate clade with three other *Burkholderia* spp. (Figure 02). *Burkholderia* sp. A45 (KF88025.1) was the nearest match to the *Burkholderia* BSL isolate in the DNA homology search. Table 05 describes the details of members having a closer taxonomic relationship with BSL isolate. It is clear that the *B. cepacia* B9 strain which is responsible for finger rot of banana (Lee *et al.*, 2004), has no closer genetic relationship with the BSL isolate. No detailed information is available about the desirable features of *Burkholderia* sp. A45 (KF88025.1).

Table 04: Results given by the *Burkholderia*BSL isolate for different enzymatic assays.

Test	Results given by the *Burkholderia* isolate
Catalase Test	Positive (Formed bubbles with H_2O_2)
Gelatin Liquefaction Test	Positive (Gelatin Liquefaction was observed in treated test tubes)
Starch Hydrolysis Test	Negative (Clear zone was not observed around the line of bacterial growth)
Lipase Test	Positive (Clear visible precipitate was observed around the line of bacterial growth)
Casein Hydrolysis Test	Positive (Clear zone was observed around the line of bacterial growth)
Cellulose Hydrolysis Test	Negative (Clear zone was not observed around the line of bacterial growth)
Chitinase Test	Not yet confirmed. (One day after incubation- Negative / Clear zone was not observed around the line of bacterial growth, but need to keep for several days for a clear observation)

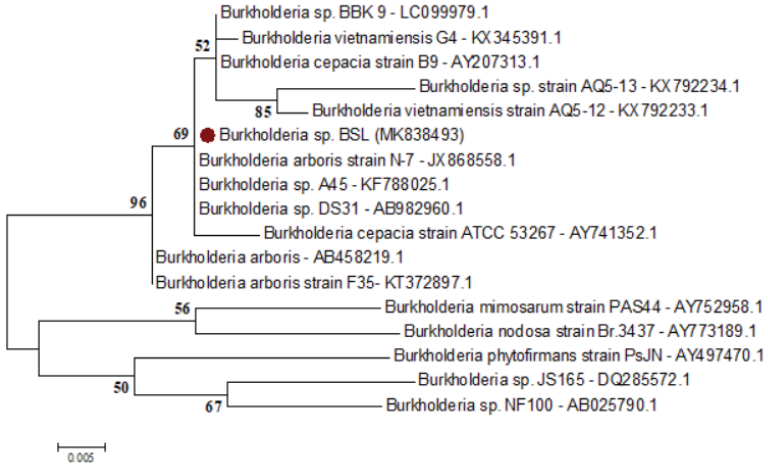

Figure 02: Phylogenetic tree showing the taxonomic location of *Burkholderia* BSL isolate built on Maximum Likelihood method based on the Jukes-Cantor model with 1000 replication of bootstrap.

DISCUSSION

Broad spectrum antifungal potential of the *Burkholderia* BSL isolate was clearly evident, as it inhibited the colony growth and percent spore germination of the five test fungal pathogens. It was evident that the fungal colony growth was inhibited by antibiosis through the formation of inhibition zones by the *Burkholderia* BSL isolate. In par with our observation, antifungal activity by *B. cepacia*, a soil borne bacterium has been reported against a wide range of plant pathogens, namely *Pythium* (Parke *et al.,* 1991), *Aphanomyces* (King and Parke 1993), *Rhizoctonia* (Cartwright and Benson 1994), *Alternaria alternata* Keiss, *Fusariumoxysporum* f. sp. *spinaciae* O-27, *Bipolaris sorokiniana* (Sacc. ex Scrok.) Shoem and *Colletotrichumlindemuthianum* (Sacc. and Magn. Bri. and Cav) (Li *et al*., 2007). In addition, several *Burkholderia* spp., namely, *Burkholderia phenazinium*, *Burkholderia megapolitana* and *Burkholderia bryophila* isolated from mosses, have been identified as potential biological control agents of a wide range of phytopathogens (Vandamme *et al.*, 2007, Eberl and Vandamme, 2016). Inhibitory activity of *B. cepacia* is correlated with the production of various secondary metabolites, such as altericidins, cepacin and other unidentified volatile or nonvolatile compounds (Parker *et al.,* 1984; Kirinuki *et al.*, 1997, EI-Banna and Winkelmann 1998). Our observations on the reduction of spore germination ability and typical morphological features and damage to the spores, by *Burkholderia* BSL isolate are compatible with the results of Li *et al.,* (2007) on fungal phytopathogens, *Curvularia lunata, F. graminearum* and *F. oxysporum* under the influence of an antifungal compound extracted from *B. cepacia.*

Table 05: Details on desirable features of *Burkholderia* isolates.

Name of the isolate/accession number	Desirable feature	Reference
Burkholderia sp. Strain BBK 9/ (LC099979)	Degradation of propiconazole	Satapute and Kaliwal (2016)
Burkholderia sp. DS31/(AB982960)	Degradation of insecticide	Tago *et al.,* (Unpublished)
Burkholderia arboris strain N-7/ (JX868558)	Degrading of petroleum	Liu *et al.,* (Unpublished)
Burkholderia vietnamiensis strain AU4i/ (KF114029)	Phosphate solubilization, indole-3-acetic acid production, N_2 fixation, ammonia production, siderophore production, HCN production, and inhibits growth of pathogenic fungi	Devi *et al.,* (2015)

The antifungal ability of *Burkholderia* BSL isolate was active until 47 ^{o}C and gave a 58 % colony growth inhibition of *Botryodiplodia* sp. and the activity was totally lost at 57 ^{o}C. Generally, antifungal activity and the stability of chemical compounds are usually influenced by the temperature. A *B. cepacia* isolate having the antifungal ability even at 160 ^{o}C has been reported (Li *et al*., 2007), hence possibility of using them for biotechnological applications, which involve higher temperatures may be possible. However, further investigations between 47-57 ^{o}C are needed to determine the exact thermal inactivation point of the antifungal substances produced by *Burkholderia* BSL isolate. Even with the available information on temperature effect, *Burkholderia* BSL isolate could be recommended to be used for integrated management in combination with hot water treatments for the management of fungal infections.

Some *Burkholderia* strains, namely, *B. gladioli* pv. *agaricicola* (*Bga*), Burkholderia 2.2 N and *B. cepacia* B23 have shown antimicrobial ability of the cell-free extract against several plant pathogenic fungi (Cain *et al*., 2000; Kadir *et al*., 2008; Elshafie *et al*., 2012). However, results of the present study have revealed the lack of antifungal ability of the cell-free extract of the *Burkholderia* BSL isolate. Cell bound nature of some extracellular enzymes or the essential need to have specific number of live bacterial cells and the fungal inducers to activate the quorum sensing like mechanisms and thereby to secrete the antifungal compounds might be the possible reasons to have such observations (Burns and Wallenstein, 2010).

In addition to the biological control ability of *Burkholderia* BSL isolate, it was also revealed to be a potent producer of several extracellular hydrolytic enzymes, namely catalase, gelatinase, lipase and caseinase. These enzymes and the microbial producers of them play significant roles in various biotechnological applications. Gelatinase producing microbes have a biotechnological significance as they are useful for the pharmaceutical industry to develop drugs because of their potential role in connective tissue degradation associated with tumor metastasis (Balan *et al*., 2012).

According to Kumar *et al.,* (2016), enzymes having the ability to hydrolyze caseins in camel milk have a significant importance in pharmaceutical and food industries since that hydrolysis derives some peptides having antioxidant, anti-cancer, mineral binding, growth stimulating and antimicrobial activities.

Lipase producing ability of bacterial genera such *as Burkholderia, Pseudomonas* and *Bacillus* has previously been reported (Gurung *et al*., 2013) and wild or recombinant forms of such bacteria are used to produce commercial products, especially in food, cosmetic, detergent and textile industry (Gurung *et al*., 2013). Further, ability of lipase in oil biodegradation has been reported (Gurung *et al*., 2013). Bacterial lipases are more stable than plant and animal lipases and they are considered as a major group of

biotechnologically valuable enzymes, mainly due to the versatility of their applied properties and easy of mass production (Gurung *et al*., 2013). As lipase production has been reportedly associated with virulence of bacteria (Gurung *et al*., 2013), these microbial enzyme producers could be safely used in detergent and textile industries and also for biodegradation of environmental pollutants.

Based on the information gathered through the phylogenetic analysis of the present study, genetic potential of *Burkholderia* BSL isolate for biodegradation of hazardous chemicals is evident and therefore, it should be further investigated. Phylogenetic tree constructed and the enzyme assays of the present study along with the information of Eberl and Vandamme (2016), the *Burkholderia* BSL isolate could be confirmed as a member of Bcc (*B. cepacia* Complex) group of *Burkholderia* having antifungal and hydrolytic enzyme production ability.

CONCLUSIONS

Identity of the *Burkholderia* BSL isolate was confirmed as *Burkholderia* sp. which belonged to Bcc group having antifungal ability against five different fungal pathogens causing field and postharvest diseases. Antifungal activity of the bacterium was lost in cell free extracts and at temperatures above 57 ^{o}C. The test bacterial isolate was catalase, gelatinase, lipase and casein hydrolytic enzyme positive but α-amylase negative. Based on these results, *Burkholderia* BSL isolate used in the present study can be recognized as a potential biological control agent of fungal plant pathogens and as an industrial enzyme producer.

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