Biocatalytic potential of *Streptomyces* spp. isolates from rhizosphere of plants and mycorrhizosphere of fungi

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Abstract

Biocatalytic potential of *Streptomyces* strains isolated from the rhizosphere of plants and from mycorrhizosphere of fungi has been investigated. A total of 118 *Streptomyces* isolates were selected and functionally screened for 10 different biotechnologically important enzymatic activities: hydrolase (cellulase, cutinase, gelatinase, lipase, protease, polyhydroxyalkanoate (PHA) depolymerase), phenol oxidase and peroxidase (laccase, tyrosinase and lignin peroxidase) and aminotransferase. Out of 118 tested *Streptomyces* spp. 90% showed at least one enzymatic activity. The most abundant were enzymes involved in the biomass degradation, as the production of cutinase, cellulase and lignin-peroxidase were detected in 31, 40 and 48% of the isolates, respectively. The improved specific activity of lipase (isolates BV315 and BV100) and tyrosinase (isolates BV87 and BV88) were shown in comparison to industrially relevant activities of *Pseudomonas* strains. Plant rhizosphere soils were more prolific source of *Streptomyces* strains with biocatalytic potential in comparison to mycorrhizosphere soils. Overall, 284 enzyme activities among 118 *Streptomyces* isolates have been detected. This is the first comprehensive screening of *Streptomyces* isolates from rhizosphere and mycorrhizosphere soils for novel biocatalysts, showing that specific environmental habitats, such as rhizosphere soils, are “treasure troves” of *Streptomyces* with biocatalytic potential.

1. Introduction

Growing interest for the development of more environmentally acceptable processes in chemical and biotechnological industries placed biocatalysis before organocatalysis [1]. Characteristics of microbial enzymes like stability, catalytic activity, and ease of production and optimization in comparison to plant and animal enzymes are the reasons for their widespread uses as biocatalysts [2, 3]. Of more than a hundred enzymes being used industrially, over one third are from bacteria [4]. Microbial cells and enzymes have been successfully employed as biocatalysts in the production of fuels, chemicals, polymers and other materials [5, 6]. The obstacle in the even wider application of biocatalysis is often the availability of the suitable enzymes. Given the remarkable biodiversity of microorganisms, it is highly probable that many valuable activities and properties still have to be found among wild-type enzymes [7].

Soil microbial diversity is relatively unexplored with respect to enzymes with synthetically useful properties [8]. The rhizosphere represents a unique environment, very crowded in comparison to the bulk soil which results in high competition for nutrient sources [9]. Members of the rhizosphere microbial communities both bacteria and fungi have adopted distinct strategies for utilizing organic matter released from the plant roots by producing different types of specific extracellular enzymes [10].

Streptomyces species, renowned for their ability to synthesize bioactive molecules, are one of the dominant component of the microbial population in most soils [11]. They are aerobic, filamentous Gram-positive, GC rich bacteria that can also secrete a wide range of extracellular enzymes for degradation of natural polymers [12]. By recycling nutrients associated with polymers such as keratin, lignocelluloses, starch, mannan, xylan and chitin they are important for biodegradation and humus formation in the soil [13, 14]. The enzymes

originating from *Streptomyces* are more attractive for industrial application than enzymes from other sources because of high stability and unusual substrate specificity [11]. In the past few years, bioprospecting *Streptomyces* species for different enzymatic activities has received more attention [15-17].

Streptomyces originating from the rhizosphere of plants or mycorrhizosphere of fungi have previously been explored for the production of bioactive molecules [18-20], however they have not been exploited for biocatalysis. Considering potential uses of hydrolases, phenol oxidases and peroxidases, as well as aminotransferases, we functionally screened 118 *Streptomyces* isolates from rhizosphere of plants or mycorrhizosphere of fungi for 10 different enzymes (hydrolase (cellulase EC 3.2.1.4, cutinase EC 3.1.1.74, gelatinase EC 3.4.24.35, lipase EC 3.1.1.3, protease EC 3.4.21, PHA depolymerase EC 3.1.1.75), phenol oxidase and peroxidase (laccase EC 1.10.3.2, tyrosinase EC 1.14.18.1 and lignin peroxidase EC 1.11.1.14) and aminotransferase EC 2.6.1).

In this study we screened *Streptomyces* species isolated from rhizosphere soils of plants including two Balkan endemorelict plants (*Ramonda serbica* and *Ramonda nathaliae*), 11 plants used in traditional medicine (*Papaver rhoeas*, *Salvia pratensis*, *Matricaria chamomilla*, *Urtica dioica*, *Glechoma hederacea*, *Taraxacum officinale*, *Verbascum*, *Viola* sp., *Cirsium arvense*, *Quercus cerris* and *Bryum* sp.) and mycorrhizosphere soils of 6 different fungi (*Lactarius deterrimus*, *Entoloma* sp., *Suillus* sp, *Russula* sp., *Clitophilus* sp., *Tuber brumale*) also utilized in traditional medicine [21, 22].

2. Materials and Methods

2.1. Reagents

Substrates for enzymatic activity screens such as carboxymethyl cellulose (CMC), L-tyrosine, DL-alpha-methylbenyzlamine (mixture of (S)- and (R)- enantiomers), lignin, guaiacol, gelatine, Coomassie blue, Congo red and Rhodamine B and all other chemicals were purchased from Sigma–Aldrich (Munich, Germany) and Acros Organics (Thermo Fisher Scientific, Waltham, USA). Glucose, mannitol, tryptone, peptone, yeast extract and other media components were purchased either from Oxoid (Cambridge, UK) or Becton–Dickinson (Sparks, USA). All kits used for PCR, PCR purification and sequencing were either from QIAGEN (Hilden, Germany) or Applied Biosystems (Thermo Fisher Scientific, Waltham, USA).

2.2 Media for growth of *Streptomyces* and *Pseudomonas* species

Mannitol soy flour medium (MSF) [23]: 20 g/L of mannitol, 20 g/L of soy flour, 20 g/L agar and 10 mmol/L $CaCl_2$. Tryptone Soy Broth (TSB) [23]: 30 g/L TSB powder. LB medium: 10 g/L NaCl, 5 g/L yeast extract, 10 g/L tryptone. CMC medium: 2.5 g/L glucose, 5 g/L lactose, 5 g/L CMC, 20 g/L peptone, 0.29 g/L $CaCl_2x2H_2O$, 1.45 g/L NH_4Cl and 15 g/L agar. Yeast Peptone Agar (YPA): 5 g/L NaCl, 15 g/L agar, 5 g/L peptone, 3 g/L yeast extract. R2 minimal medium [23]: 0.25 g/L K_2SO_4, 10 g/L $MgCl_2$ x $6H_2O$, 2 g/L glucose, 15 g/L agar supplemented with 10 ml/L 0.5% KH_2PO_4 (w/v), 80 ml/L 3.68% $CaCl_2$ x $2H_2O$ (w/v), 100 ml/L TES buffer, 2 ml/L Trace elements solution, 5 ml/L 1 mol/L NaOH and 15 ml/L 20% L-proline (w/v).

2.3 Sampling and isolation of *Streptomyces* species

Rhizosphere and mycorrhizosphere soils samples of medicinal plants (*Papaver rhoeas*, *Salvia pratensis*, *Matricaria chamomilla*, *Urtica dioica*, *Glechoma hederacea*, *Taraxacum officinale*, *Verbascum*, *Viola* sp., *Cirsium arvense*, *Bryum* sp.) and fungi (*Lactarius deterrimus*, *Entoloma* sp., *Suillus* sp, *Russula* sp., *Clitophilus* sp.) were collected in Serbia during the year 2015 and 2016 under sterile conditions. Soil samples were stored at +4°C and transferred to the laboratory. *Streptomyces* were isolated after dilution of the samples and plating on MSF medium [23]. Plates were incubated at 30°C for 7 days, and colonies of distinct morphological appearance were cultured in MSF to obtain pure isolates. Spore suspensions were stored in glycerol (20%, v/v), maintained at -80°C and used for the inoculation of cultures for further experiments. At the time of usage, spore suspensions (20 µl) were inoculated onto solid MSF medium and grown for 7 days at 30°C. A total of 118 isolates (available upon request) were selected from *Streptomyces* sp. laboratory collection for further tests. For all enzymatic tests colonies were cultivated for 72 h at 30°C in TSB and 10µl of liquid culture was inoculated on solid media with the tested substrate.

2.4 DNA isolation and sequencing of the 16S rRNA gene

Genomic DNA was isolated by the method of Nikodinovic *et al.*[24]. Gene for 16S rRNA was amplified from genomic DNA using universal bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). PCR amplification was performed in 2720 Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, USA) using KAPA Taq PCR kit (KAPA Biosystems, USA) following manufacturer's protocol. PCR product was purified using PCR purification kit (Qiagen, Germany). Sequencing was done with a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Thermo Fisher Scientific, Waltham, USA) on an

Applied Biosystems 3130 Genetic Analyzer. 16S rRNA gene sequences were identified using BLASTN programme (http://www.ncbi.nlm.nih.gov) [25].

2.5 16S rRNA gene phylogeny

Sequences of 29 selected isolates were analyzed and forward and reverse sequence assembled by SeqMan Pro software (DNASTAR Inc., Madison, USA). Alignment of sequences and homologous sequences taken from GeneBank was performed with Clustal W 2.0 algorithm [26]. The phylogenetic tree was constructed by the maximum-likelihood algorithm using Jukes-Cantor distance correction and Bootstrap resampling method, all included in the MEGA7 package [27]. The tree was rooted using 16S rRNA gene sequence of *Bacillus subtilis* 168 (AL009126) as an outgroup. Sequences of nearest type strains, as well as, outgroup strain were taken from GenBank. 16S rRNA gene sequences (at least 1300 nt) were deposited in GeneBank under accession numbers MF11780-MF11807.

2.6 Preparation of cell-free extracts

Streptomyces isolates were inoculated in liquid TSB medium and grown for 96 h at 30°C with shaking at 200 rpm while *Pseudomonas aeruginosa* PAO1 and *Pseudomonas putida* F6 were grown in LB medium overnight at 37°C and 180 rpm. Pellets were harvested by centrifugation for 20 minutes at 5000 rpm and resuspended in 10 ml of 100 mmol/L potassium phosphate buffer pH 7.0. Cells were disrupted by sonication with 6 short burst of 15 s followed by an interval of 30 s of cooling. Cell pellets were removed by centrifugation for 30 min at 14000 rpm at 4°C.

2.7 Extracellular enzymatic activities

Activities of all tested enzymes, except for cutinase and aminotransferase, were observed in enzyme-specific tests in solid media after inoculation of 20 µl of 96 h old bacterial liquid cultures and incubation at 30°C for 7 days.

For the detection of cutinase and aminotransferase activity, cell-free extracts (CFE) and supernatants were used in enzyme assay for the preparation of enzyme specific reaction mixtures, which were incubated for 24h at 30°C with shaking at 180 rpm.

2.7.1 Screening for cellulase producing bacteria

Screening for cellulase enzymes in isolated bacteria was performed on cellulose agar containing 2 g/L, 5 g lactose, 5 g/l CMC, 20 g/l peptone, 0.3 g/l $CaCl_2xH_2O$, 0.8 g/L NH_4Cl, 15 g/L agar. Cellulase producing bacteria were selected based on discoloration zones after 15 minutes staining in 0.1 % Congo red (w/v) and destaining in 1 mol/L NaCl and 1 % acetic acid (v/v).

2.7.2 Screening for cutinase producing bacteria

Screening of cutinase producing bacteria was done as previously reported by Gupta and co-workers [28]. Cutinase activity was determined by a spectrophotometric assay using *p*-nitrophenyl butyrate (*p*-NPB) as the substrate in a reaction mixture comprising of 70 µl 100 mmol/L TRIS-HCl buffer pH 8.2, 80 µl substrate mixture (0.158 g/L p-NPB, 0.17 g/L SDS, 10 g/L TRITON X-100) and 50 µl CFE or culture supernatant. The reaction was followed by measuring absorbance at $A_{410.}$

2.7.3 Screening for lipase, protease and gelatinase producing bacteria

The screening was done on YPA medium supplemented with 25 ml/L olive oil and 10 mg/L of rhodamine B for lipase [29], 1% skimmed milk (w/v) for protease [10] and 10 g/L of gelatine for gelatinase [10]. Lipase producing bacteria were selected based on the formation of an orange-colored fluorescent halo around colonies detected under UV light. Protease producing bacteria were selected based on the appearance of clear zones around colonies. Gelatinase producing bacteria were selected based on the formation of clear zones around the colony after staining with Coomassie blue (0.25%, w/v) in methanol and acetic acid and water (5:1:4) and destaining by methanol and acetic acid (5:1) [10].

2.7.4 Screening for PHA depolymerase producing bacteria

Screening polyhydroxyalkanoate (PHA) depolymerase producing bacteria was done on R2 minimal medium [23] supplemented with polyhydroxybutyrate (PHB). Preparation of PHA was done as previously reported [30] by dissolving 2 g PHB in 5 ml of methylene-chloride and 20 ml H_2O, followed by sonification with 8 bursts of 1 min followed by an interval of 30 s for cooling. PHA depolymerase activity was detected as the presence of clear zones around colonies.

2.7.5 Screening for laccase and tyrosinase producing bacteria

Screening was done on YPA medium supplemented with 0.01% guaiacol (v/v) and 0.35 mmol/L $CuSO_4$ x $5H_20$ (w/v) for laccase [31] and 0.5% L-tyrosine (w/v, pH 7) for tyrosinase [32]. Laccase positive isolates were those with brown color around colonies, while tyrosinase positive colonies were pigmented brown to black.

2.7.6 Screening for lignin peroxidase producing bacteria

For testing lignin peroxidase activity R2 [23] minimal medium was supplemented with 4.3 g/L lignin and 0.1 g/L Azure Blue [33] and activity was detected after incubation in dark, as a presence discoloration zones around colonies.

2.7.7 Screening for aminotransferase producing bacteria

Screening of aminotransferase producing bacteria was done according to previously reported method [34, 35]. Isolates were grown on R2 medium supplemented with *ortho*-xylylenediamine as substrate and positives isolates were those which developed dark brown pigmentation during incubation. Aminotransferase activity was confirmed by HLPC analysis using MBA as the substrate in the reaction mixture comprising of 180 µl substrate mixture (50 mmol/L MBA (equimolar mixture of (S)- and (R)-MBA) and 1 mmol/L PLP), 30 µl 50 mmol/L pyruvate and 90 µl CFE. The reaction was followed by measuring the formation of acetophenone by HPLC [36].

2.8 Specific enzyme activity

2.8.1 Characterization of lipase activity with p*-nitrophenyl palmitate as substrate*

The activity of lipases from *Streptomyces* sp. BV100, *Streptomyces* sp. BV315 and *Pseudomonas aeruginosa* PAO1 were measured spectrophotometrically with the long chain fatty acyl ester *p*-nitrophenyl palmitate (C_{16}) as substrate at λmax 410 nm following the production of *p*-nitrophenol ($1.83x10^4$ $M^{-1} \times cm^{-1}$) [37]. Reactions were performed in triplicate with continuous monitoring of *p*-nitrophenol liberation over a period of 6 h at 30°C and 42°C. Reaction mixture contained 70 µl 100 mmol/L TRIS-HCl buffer pH 8.2, 80 µl substrate mixture (0.158 g/L *p*-NPP, 0.17 g/L SDS, 10 g/L TRITON X-100) and 0,2 mg/mL of total proteins in culture supernatant (determined by Bradford method). One unit of lipase

activity was defined as the amount of enzyme releasing 1 μmol of *p*-nitrophenol per min at 30°C or 42°C under standard reaction conditions. Statistically significant differences between the activity of lipase from *P. aeruginosa* PAO1 and lipases from *Streptomyces* sp. BV100 and *Streptomyces* sp. BV315 were evaluated by Student's *t*-test (ANOVA).

2.8.2 Characterization of tyrosinase activity with L-tyrosine as substrate

The activity of tyrosinases from *Streptomyces* sp. BV87, *Streptomyces* sp. BV88 and *Pseudomonas putida* F6 were measured spectrophotometrically with L-tyrosine as substrate at λmax 475 nm, following the production of dopachrome ($0.37x10^4$ $M^{-1} \times cm^{-1}$) [38]. Reactions were performed in triplicate with continuous monitoring of dopachrome formation over 24h at 30°C and 42°C in a microplate spectrophotometer. Reaction mixture contained 790 μl 50 mmol/L K-phosphate buffer pH 6.5, 10 μl of 100 mmol/L L-tyrosine (final 1 mmol/L substrate) and 0.2 mg/L of total proteins in culture supernatant (determined by Bradford method). One unit of tyrosinase activity was defined as the amount of enzyme releasing 1 μmol of dopachrome per min at 30°C or 42°C under standard reaction conditions. Statistically significant differences between the activity of tyrosinase from *P. putida* F6 and tyrosinases from *Streptomyces* sp. BV87 and *Streptomyces* sp. BV88 were evaluated by Student's *t*-test (ANOVA).

2.9 Statistical and correlation analysis

The heat map was constructed using R (R Development Core Team 2014, Vienna, Austria) [39] in order to summarize biocatalytic potential of 118 tested *Streptomyces* strains. Clustering algorithm that groups related rows together by similarity was used for computing a dendrogram. Results were approximated on the relative scale ranging from 0 (red) as no detected enzyme activity, progressing to yellow as moderate enzyme activity, then to 100 (green) as the positive enzyme activity.

3. Results

3.1 Isolation, selection and identification of *Streptomyces* strains

Approximately 700 *Streptomyces* spp. were isolated from rhizosphere soil of 13 different plants (*Papaver rhoeas*, *Salvia pratensis*, *Matricaria chamomilla*, *Urtica dioica*, *Glechoma hederacea*, *Taraxacum officinale*, *Verbascum*, *Viola*, *Ramonda nathaliae*, *Ramonda serbica*, *Cirsium arvense, Quercus cerris, Bryum* sp.) and mycorrhizosphere soil of 6 different fungi (*Lactarius deterrimus*, *Entoloma* sp., *Suillus* sp., *Russula* sp., *Clitophilus* sp., *Tuber brumale*) on the selective media over 6-years period (2010-2015). A total of 118 isolates were selected based on different morphology and partial 16S rRNA gene sequences for the assessment of 10 different enzymatic activities (Table 1).

3.2 Assessment of the enzymatic activities of 118 *Streptomyces* isolates

Streptomyces isolates were functionally screened for the production of enzymes belonging to three wide groups, namely hydrolases, phenol oxidases and peroxidases and transaminases. Out of 118 isolates 90% (106/118) showed at least one of tested enzymatic activities (Fig 1.). On the other hand, 12 isolates (10%), of which 8 were associated with fungi, showed negative in all enzyme tests (Fig 2.).

Cellulases were detected in 47 isolates, cutinases in 39, gelatinases in 17, lipases in 25, PHA depolymerases in 5 and caseinase in 23 isolates out of 118 isolates (Table S1). Therefore, the most abundant enzymes were those involved in the biomass degradation, as the production of cutinase, cellulase and lignin peroxidase activities were detected in 31, 40 and 48% of the isolates, respectively. On the other hand, PHA depolymerase with 4% was the least detected enzyme activity amongst 118 *Streptomyces* isolates. Laccases, tyrosinases and lignin

peroxidases were detected in 32, 26 and 57 isolates, respectively, out of 118 isolates (Table S1), while transaminase activity has been detected in 11 out of 118 isolates (Table S1).

Grouping of soil isolates according to positive enzyme activity is shown as a heat map (Fig 2.). Clustering of enzymes could be observed, as four major clusters could be seen on the heat map. Enzymes for biomass degradation including cellulases, laccases and tyrosinases grouped together, and cellulases grouped with cutinases. Interestingly, strains which exhibited laccase activity, which is lignin degradation activity, rarely showed lignin peroxidase activity (Fig 2.). Great number of strains, 34 isolates (29%), showed positive in 3 enzyme tests and only 2 strains, namely *Streptomyces* sp. BV286 (*Papaver rhoeas*) and *Streptomyces* sp. BV333 (*Salvia pratensis*), showed positive in 6 enzyme tests. Gelatinase and aminotransferase were two enzymatic activities common for both isolates (Table 2).

3.3 Phylogenetic relatedness of selected *Streptomyces* strains

From 118 tested isolates, 29 isolates (28 newly sequenced and one already sequenced and deposited at GenBank, *Streptomyces* sp. NP10 (JQ288108)) were selected for phylogenetic analysis (Fig 3.) based on the different morphology and the number of enzyme tests in which they showed good activity (Table 2).

3.4 Biocatalytic potential of *Streptomyces* spp. according to source of isolation

The most abundant enzymes in both groups of isolates, from the rhizosphere of plants (n=87) and mycorrhizosphere of fungi (n=31), were those involved in the biomass degradation. Out of 87 isolates from rhizospheres of plants 45% and 36% showed cellulase and laccase activities, respectively (Fig 4.a). Among 31 isolates from fungi, most detected enzymes were cutinase with 45% and lignin peroxidase with 55% (Fig 4.a).

Aminotransferases were mostly detected in strains isolated from the rhizospheres of *Verbascum thapsus* and *Papaver rhoeas*, both with around 27% (Fig 4.b). Cellulase, gelatinases, laccases and PHA depolymerases are the most abundant in strains isolated from *Papaver rhoeas* with 21, 29, 28 and 60%, respectively. Cutinases and lignin peroxidases with 36% and 30%, respectively, were predominantly detected among isolates from fungi, while lipases with 28% and proteases with 35% were detected in strains from the rhizosphere of *Salvia pratensis*. Tyrosinases with 21% were mostly detected among strains originating from the rhizosphere of *Taraxacum officinale,* the common dandelion.

3.5 Specific activities of lipase and tyrosinase

Lipase and tyrosinase activities of selected *Streptomyces* strains were compared with the activities of lipase producing *Pseudomonas aeruginosa* PAO1 [40] and tyrosinase producing *Pseudomonas putida* F6 [41] on agar plates inoculated with liquid cultures and in enzyme specific spectrophotometric assays in culture supernatants (Fig 5.). *Streptomyces* strains BV315 and BV100 were used for lipase activity comparison, while BV87 and BV88 were used for tyrosinase activity comparison. Selected *Streptomyces* strains showed larger positive zones faster than *Pseudomonas* species on agar plates supplemented with enzyme specific substrate (Fig 5.). Lipase and tyrosinase enzyme assays were carried out at 30°C and 42°C, and lipases from both tested strains performed better at 30°C while tyrosinases performed better at 42°C (Fig 5.). Specific lipase activity in culture supernatants of *Streptomyces* sp. BV315 and BV100 were 1.5 and 1.2 times higher in comparison to specific lipase activity in culture supernatant of *P. aeruginosa* PAO1 (Fig 5.a). The specific activity of tyrosinase from culture supernatants of *Streptomyces* sp. BV87 and BV88 were 2.1 and 2.7 times higher in comparison to the specific activity tyrosinase from culture supernatant of *P. putida* F6 tyrosinase (Fig 5.b).

4. Discussion

Microorganisms are very important in the biogeochemical cycle as their enzymes play an essential role in the decomposition of natural compounds [42]. Among them, actinomycetes, predominantly genus *Streptomyces*, are especially important for degradation of recalcitrant compounds, like lignin and cellulose. Therefore, our aim was to explore the wide biocatalytic potential of *Streptomyces* strains from rhizosphere and mycorrhizosphere soil of plants and fungi, anticipating high incidence and activity of enzymes able to act upon lignocelluloses.

Streptomyces isolates were functionally screened for the activity of enzymes belonging to three wide groups, namely hydrolases, phenol oxidases and peroxidases and transaminases, due to their prospective applications. Hydrolases (i.e. cellulase, cutinase, gelatinase, lipase, PHA depolymerase, protease/caseinase) are a group of enzymes that catalyze bond cleavage using water as a nucleophile. They have been extensively used in the industrial production of agrochemicals, pharmaceuticals and high-value added compounds [43]. The most abundant enzymes among tested isolates were those involved in the biomass degradation (cellulase, cutinase and lignin peroxidase). Cellulases convert cellulose to fermentable sugars and the largest known producers of cellulases with alkaline pH optimum and high thermostability are *Streptomyces* species [44]. Cutinases are mostly isolated from fungi and are widely used for PET-polymer degradation [45] and the potential industrial application of cutinases from *Streptomyces* has also been assessed [46]. On the other hand, PHA depolymerase was the least detected enzyme activity amongst 118 *Streptomyces* isolates. PHA depolymerase degrades extracellular PHA in the environment, so the released oligomers and monomers can be used as a source of carbon and energy [47].

In the previous studies on enzymes from soil microorganisms, based on culture dependent and culture-independent methods, most frequently detected enzymes were protease

(caseinase) and cellulase [48] and protease and lipase [49-51]. In the soil metagenomics study, clones included lipase, amylase and nuclease activities, with no functional cellulases activity detected [52].

Laccases and tyrosinases were detected in a quarter of isolates while lignin peroxidases were detected in almost half of the tested isolates (Table S1). Phenol oxidases (laccases and tyrosinases) and peroxidases (lignin peroxidases) have various biological functions including ontogeny, defense and the acquisition of carbon and nitrogen [53]. In the environment these enzymes play a role in the degradation of complex polymers (lignin, humic acid), lignification, detoxification, pathogenicity, morphogenesis, sporulation, polymerization of melanin and spore coat, while they have found application in the field of biotechnology, bioremediation, food processing, medicine, pharmacy and the textile, pulp and paper industry [54]. Previously isolated laccases from *Streptomyces* strains [55-57] together with lignin peroxidases [58] were shown to be involved in lignin degradation.

Transaminase activity has been detected in small number of tested isolates (11 out of 118) (Table S1). Transaminases are ubiquitous in nature and play a key role in nitrogen metabolism as they are transferring amino group between an amino donor and a prochiral ketone substrate [59]. The use of transaminase for biocatalytic application has been largely investigated in the last years to create new sustainable productive process for the preparation of drugs and chemical intermediates, such as chiral amines [60]. Although *Streptomyces* strains have many biosynthetic clusters and aminotransferases are usually included in them [61], very low number of aminotransferases was detected in 118 selected isolates.

In line with our study, Alves and coworkers [48], during the survey of hydrolytic microbial enzymes in soil, water, and plant microenvironments reported at least one enzymatic activity in 89% of screened microbial isolates (n=346) and activity of 5 enzymes in the single isolate

from soil including gelatinase, protease and cellulase which were also detected in our isolates producing multiple enzymes. The production of multiple and different enzymes is important since organisms that share the same microhabitat may contribute to the availability of nutrients. Microorganisms with combined hydrolytic activity might have application in processes involving multiple enzymatic steps like converting biomass to bioethanol, bioremediation and wastewater treatment [62].

The most abundant enzymes in both groups of isolates, from the rhizosphere of plants (n=87) and mycorrhizosphere of fungi (n=31), were those involved in the biomass degradation. Most detected enzymes among the rhizosphere isolates were cellulases and laccases. This was expected considering the source of isolation, and the fact that these enzymes are important for the symbiotic life of these microorganisms and for biomass degradation [63]. Among the isolates from myccorhizosphere of fungi, most detected enzymes were cutinase and lignin peroxidase. Cutinase and lignin peroxidase enzymes might play role in establishing contact of fungi with plant roots. Also, lignin, cellulose and cutin probably have a key role in providing microorganisms from these habitats with nutrition. There was no obvious predominance of certain enzymatic activities on the plant or fungi species level.

Grouping of isolates either according to enzyme activity or to the same habitat could not be observed on the phylogenetic basis (Fig 3.). Only isolates *Streptomyces* sp. BV302 and *Streptomyces* sp. BV307 which were isolated from the rhizosphere of *Papaver rhoeas*, which showed 4 and 5 enzyme activities respectively, were phylogenetically close sharing also laccase and lignin peroxidase activity. From 4 selected isolates in which no enzyme activity was detected three, namely *Streptomyces* sp. BV9, *Streptomyces* sp. BV19 and *Streptomyces* sp. BV52 grouped together, and they were all associated with fungi. Strain *Streptomyces* sp. BV333 with 6 enzyme activities was closely related to *Streptomyces* sp. BV 295 showing 4

enzymes activities in common (lipase, aminotransferase, lignin peroxidase and protease). Strain *Streptomyces* sp. BV286 which showed 6 enzyme activities grouped with *S. aurantiacus*. This strain is the producer of aurantimycin [64] and we could find only one report of bacteria identified by 16S rRNA gene sequencing as *S. aurantiacus* that could produce lipase and protease [65] which were not detected in our strain BV286. Commercially available and widely used enzyme from *Streptomyces* strain is protease Pronase P from *S. griseus* (Sigma Aldrich, P5147, Merck, Darmstadt, Germany) consisting of two metalloendopeptidase, designated as *Streptomyces griseus* metalloendopeptidases I and II [66]. Although we tested our isolates for proteolytic activity, none of the strains that were used for phylogenetic analysis showed close relateness to commercial protease (Pronase P) producer *S. griseus.* Similarly, *Streptomyces thermoviolaceus* secretes highly stable cellulase [67], but no phylogenetic similarity between this strain and cellulase producers from our study has been detected.

Activities of lipases from *Streptomyces* strains BV315 and BV100 were compared with the lipase from *Pseudomonas aeruginosa* PAO1 while the tyrosinases from *Streptomyces* sp. BV87 and BV88 were compared with the tyrosinase from *P. putida* F6. Although enzymes, especially lipases [68] originating from *Pseudomonas* strains, are widely used industrial biocatalysts, *Streptomyces* strains showed greater specific activity in culture supernatants under given conditions indicating the need for further characterisation and development towards biotechnological applications.

Detection of enzymes in *Streptomyces* isolates from rhizosphere and mycorrhizosphere soils is important to understand their biochemical activities and to verify the biotechnological potential of these microorganisms. In this study, 118 isolates showed 284 enzyme activities, with 90% of isolates showing at least one tested activity. All tested enzymes (cellulase,

cutinase, gelatinase, lipase, protease, polyhydroxyalkanoate (PHA) depolymerase, laccase, tyrosinase, lignin peroxidase and aminotransferase) have great biocatalytic potential and all of them found use in many industrial processes. This study revealed the potential of *Streptomyces* species to produce important enzymes, particularly lipases and tyrosinases, for further biotechnological exploration. As anticipated, predominance of enzymes involved in biomass degradation was found.

Results of this comprehensive functional screen showed that specific environmental habitats, such as rhizosphere soils, are "treasure troves" of *Streptomyces* with biocatalytic potential and that *Streptomyces* strains are still an untapped reservoir of biodiversity for bioprospecting, not only for antibiotics but for useful biocatalysts as well.

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Conflict of Interest

The authors declare no conflict of interest.

Supporting Information

Table S1. Total number of *Streptomyces* isolates scored positive per enzyme activity screened in this study.

References

[1] Hernaiz, M. J., Alcantara, A. R., Garcia, J. I., Sinisterra, J. V. (2010) *Chemistry (Weinheim an der Bergstrasse, Germany)* **16**, 9422-9437.

[2] Bornscheuer, U. T., Huisman, G. W., Kazlauskas, R. J., Lutz, S., Moore, J. C., Robins, K. (2012) *Nature* **485**, 185-194.

[3] Singh R., Kumar M., Mittal A., K., M. P. (2016) *3 Biotech* **6**, 174.

[4] Sanchez, S., Demain, A. L. (2011) *Org. Process Res. Dev.* **15**, 224-230.

[5] Illanes, A., Cauerhff, A., Wilson, L., Castro, G. R. (2012) *Bioresour. technol* **115**, 48-57.

[6] Nestl, B. M., Hammer, S. C., Nebel, B. A., Hauer, B. (2014) *Angewandte Chemie (International ed. in English)* **53**, 3070-3095.

[7] Guérard-Hélaine, C., de Berardinis, V., Besnard-Gonnet, M., Darii, E., Debacker, M., Debard, A., Fernandes, C., Hélaine, V., Mariage, A., Pellouin, V., Perret, A., Petit, J.-L., Sancelme, M., Lemaire, M., Salanoubat, M. (2015) *ChemCatChem* **7**, 1871-1879.

[8] Powthong, P., Sripean, A., Suntornthiticharoen, P. (2017) *AJPCR* **10**, 1-6.

[9] Perotto, S., Bonfante, P. (1997) *Trends. Microbiol.* **5**, 496-501.

[10] De Santi, C., Altermark, B., de Pascale, D., Willassen, N. P. (2016) *J. Basic. Microbiol.* **56**, 238-253.

[11] Prakash, D., Nawani, N., Prakash, M., Bodas, M., Mandal, A., Khetmalas, M., Kapadnis, B. (2013) *BioMed Res. Inter.* **2013**, 1-8.

[12] Sevillano, L., Vijgenboom, E., van Wezel, G. P., Díaz, M., Santamaría, R. I. (2016) *Microb. Cell Factor.***15**, 28.

[13] Lamilla, C., Pavez, M., Santos, A., Hermosilla, A., Llanquinao, V., Barrientos, L. (2017) *Polar Biol.* **40**, 719-726.

[14] Stach, J. E., Bull, A. T. (2005) *Antonie van Leeuwenhoek* **87**, 3-9.

[15] Sathya R., T., U. (2014) *Int. J. Pharm. Pharm. Sci.* **6**, 1-5.

[16] Das, P., Solanki, R., Khanna, M. (2015) *Int. J. Biotech Trends Technol.* **10**, 20-32.

[17] Lekshmi, M., Ayona Jayadev, Navami, S. S. (2014) *IJSER* **5**, 199-203.

[18] Lima, S. M., Melo, J. G., Militao, G. C., Lima, G. M., do Carmo, A. L. M., Aguiar, J. S., Araujo, R. M., Braz-Filho, R., Marchand, P., Araujo, J. M., Silva, T. G. (2017) *Appl. Microbiol. Biotechnol.* **101**, 711-723.

[19] Boudjeko, T., Tchinda, R. A., Zitouni, M., Nana, J. A., Lerat, S., Beaulieu, C. (2017) *Microbes Environ.* **32**, 24-31.

[20] Schulz, D., Nachtigall, J., Riedlinger, J., Schneider, K., Poralla, K., Imhoff, J. F., Beil, W., Nicholson, G., Fiedler, H. P., Sussmuth, R. D. (2009) *J. Antibiotics* **62**, 513-518.

[21] Feyzabadi, Z., Ghorbani, F. (2017), 10.1002/ptr.5909.

[22] Hobby, G. H., Quave, C. L., Nelson, K., Compadre, C. M., Beenken, K. E., Smeltzer, M. S. (2012) *J. Ethnopharmacol.* **144**, 812-815.

[23] Tobias Kieser, Mervyn J. Bibb, Mark J. Buttner, Keith F. Chater, Hopwood, D. A. (2000) Practical Streptomyces Genetics, John Innes Foundation.

[24] Nikodinovic, J., Barrow, K. D., Chuck, J. A. (2003) *BioTechniques* **35**, 932-934, 936.

[25] Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., Lipman, D. J. (1997) *Nucleic Acids Res.* **25**, 3389-3402.

[26] Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., Higgins, D. G. (2007) *Bioinformatics (Oxford, England)* **23**, 2947-2948.

[27] Kumar, S., Stecher, G., Tamura, K. (2016) *Molecular Biol. and evolution* **33**, 1870-1874.

[28] Winkler, U. K., Stuckmann, M. (1979) *J. Bacteriol.* **138**, 663-670.

[29] Ugur, A., Sarac, N., Boran, R., Ayaz, B., Ceylan, O., Okmen, G. (2014) *ISRN Biochem.* **2014**, 1-7.

[30] Teeraphatpornchai, T., Nakajima-Kambe, T., Shigeno-Akutsu, Y., Nakayama, M., Nomura, N., Nakahara, T., Uchiyama, H. (2003) *Biotechnol. Lett.* **25**, 23-28.

[31] Priyam Vandana, Peter, J. K. (2014) *Int. J. Engineer. Sci. Res. Terchnol.* **3**, 45-50.
[32] Roy, S., Das, I., Munjal, M., Karthik, L., Kumar, G., Kumar, S., Rao, K. V. B. (2014) *Front. Biol.* **9**, 306-316.
[33] Kameshwar, A. K. S., Qin, W. (2017) *BioEnergy Res.* **10**, 248-266.
[34] Shin, J. S., Yun, H., Jang, J. W., Park, I., Kim, B. G. (2003) *App. Microbiol. Biotech.* **61**, 463-471.
[35] Guo, F., Berglund, P. (2017) *Green Chem.* **19**, 333-360.
[36] Schatzle, S., Hohne, M., Redestad, E., Robins, K., Bornscheuer, U. T. (2009) *Analytical Chem.* **81**, 8244-8248.
[37] Gupta, R., Rathi, P., Gupta, N., Bradoo, S. (2003) *Biotechnol. Appl. Biochem.* **37**, 63-71.
[38] Ito, M., Oda, K. (2000) *Biosci. Biotechnol. Biochem.* **64**, 261-267.
[39] Team, R. C., (2014) R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria.
[40] Wohlfart, S., Hoesche, C., Strunk, C., Winkler, U. K. (1992) *Microbiology* **138**, 1325-1335.
[41] McMahon, A. M., Doyle, E. M., Brooks, S., O'Connor, K. E. (2007) *Enz. Microb. Technol.* **40**, 1435-1441.
[42] Ranjard, L., Poly, F., Combrisson, J., Richaume, A., Gourbiere, F., Thioulouse, J., Nazaret, S. (2000) *Microb. Ecol.* **39**, 263-272.
[43] Busto, E., Gotor-Fernandez, V., Gotor, V. (2010) *Chem. Soc. Rev.* **39**, 4504-4523.
[44] Jang, H. D., Chang, K. S. (2005) *Biotechnol. Lett.* **27**, 239-242.
[45] Ribitsch, D., Herrero Acero, E., Przylucka, A., Zitzenbacher, S., Marold, A., Gamerith, C., Tscheliessnig, R., Jungbauer, A., Rennhofer, H., Lichtenegger, H., Amenitsch, H., Bonazza, K., Kubicek, C. P., Druzhinina, I. S., Guebitz, G. M. (2015) *Appl. Environ. Microbiol.* **81**, 3586-3592.
[46] Fett, W. F., Gérard, H. C., Moreau, R. A., Osman, S. F., Jones, L. E. (1992) *Curr. Microbiol.* **25**, 165-171.
[47] Martinez, V., de Santos, P. G., Garcia-Hidalgo, J., Hormigo, D., Prieto, M. A., Arroyo, M., de la Mata, I. (2015) *Appl. Microbiol. Biotechnol*. **99**, 9605-9615.
[48] Alves, P. D. D., Siqueira, F. d. F., Facchin, S., Horta, C. C. R., Victória, J. M. N., Kalapothakis, E. (2014) *Open Microbiol. J.* **8**, 25-31.
[49] García, C., Hernández, T., Costa, F. (1994) *Soil Biol. Biochem.* **26**, 1185-1191.
[50] Lee, S. W., Won, K., Lim, H. K., Kim, J. C., Choi, G. J., Cho, K. Y. (2004) *Appl. Microbiol. Biotechnol* **65**, 720-726.
[51] Fujii, K., Oosugi, A., Sekiuchi, S. (2012) *Biosci. Biotechnol. Biochem.* **76**, 906-911.
[52] Rondon, M. R., August, P. R., Bettermann, A. D., Brady, S. F., Grossman, T. H., Liles, M. R., Loiacono, K. A., Lynch, B. A., MacNeil, I. A., Minor, C., Tiong, C. L., Gilman, M., Osburne, M. S., Clardy, J., Handelsman, J., Goodman, R. M. (2000) *Appl. Environ. Microbiol.* **66**, 2541-2547.
[53] Sinsabaugh, R. L. (2010) *Soil Biol. Biochem.* **42**, 391-404.
[54] Margot, J., Bennati-Granier, C., Maillard, J., Blanquez, P., Barry, D. A., Holliger, C. (2013) *AMB Express* **3**, 63.
[55] Davis, J. R., Goodwin, L., Teshima, H., Detter, C., Tapia, R., Han, C., Huntemann, M., Wei, C. L., Han, J., Chen, A., Kyrpides, N., Mavrommatis, K., Szeto, E., Markowitz, V., Ivanova, N., Mikhailova, N., Ovchinnikova, G., Pagani, I., Pati, A., Woyke, T., Pitluck, S., Peters, L., Nolan, M., Land, M., Sello, J. K. (2013) *Genome announcements* **1**.
[56] Majumdar, S., Lukk, T., Solbiati, J. O., Bauer, S., Nair, S. K., Cronan, J. E., Gerlt, J. A. (2014) *Biochemistry* **53**, 4047-4058.
[57] Prins, A., Kleinsmidt, L., Khan, N., Kirby, B., Kudanga, T., Vollmer, J., Pleiss, J., Burton, S., Le Roes-Hill, M. (2015) *Enzyme Microb. Technol*. **68**, 23-32.
[58] Saini, A., Aggarwal, N. K., Sharma, A., Yadav, A. (2015) *Enzyme Res.* **2015**, 15.
[59] Koszelewski, D., Tauber, K., Faber, K., Kroutil, W. (2010) *Trends Biotechnol.* **28**, 324-332.
[60] Nugent, T. C., El-Shazly, M. (2010) *Adv. Synth. Catal.* **352**, 753-819.
[61] Watve, M. G., Tickoo, R., Jog, M. M., Bhole, B. D. (2001) *Arch. Microbiol.* **176**, 386-390.

[62] Facchin, S., Alves, P., Siqueira, F., Barroca, T., Victória, J., Kalapothakis, E. (2013) *Open J. Ecol.* **3**, 34-37.
[63] Hütsch, B. W., Augustin, J., Merbach, W. (2002) *J. Plant Nutri. Soil Sci.* **165**, 397-407.
[64] Grafe, U., Schlegel, R., Ritzau, M., Ihn, W., Dornberger, K., Stengel, C., Fleck, W. F., Gutsche, W., Hartl, A., Paulus, E. F. (1995) *J. Anthibiotcs* **48**, 119-125.
[65] Gupta, N., Mishra, S., Basak, U. C. (2007) *Malays. J. Microbiol.* **3**, 1-8.
[66] Tsuyuki, H., Kajiwara, K., Fujita, A., Kumazaki, T., Ishii, S. (1991) *J. Biochem.* **110**, 339-344.
[67] Jones, B. E., Van Der Kleij, W. A., Van Solingen, P., Weyler, W., Goedegebuur, F., (2003) Cellulase producing actinomycetes, cellulase produced therefrom and method of producing same US, pp. 39.
[68] Hasan, F., Shah, A. A., Hameed, A. (2006) *Enzyme Microb. Technol.* **39**, 235-251.

Table 1. Origin of the 118 selected *Streptomyces* isolates

Rhizosphere/mycorrhizosphere soil	Number of isolates	*Streptomyces* sp isolates
Papaver rhoeas	20	BV285, BV286, BV288, BV289, BV290, BV291, BV292, BV293, BV294, BV295, BV296, BV298, BV300, BV302, BV305, BV307, BV308, BV309, BV312, BV313
Salvia pratensis	12	BV315, BV316, BV317, BV319, BV323, BV327, BV328, BV330, BV333, BV334, BV335, BV336
Matricaria chamomilla	9	BV339, BV341, BV346, BV358, BV359, BV407, BV409, BV413
Urtica dioica	4	BV213.2, BV221, BV222, BV230
Glechoma hederacea	3	BV134, BV135, BV140
Taraxacum officinale	9	BV77, BV77.1, BV81, BV82, BV83, BV86, BV87, BV88, BV113
Verbascum	11	BV92, BV96, BV98, BV99, BV100, BV101, BV103, BV105, BV110, BV152.2, BV364
Viola sp.	2	BV195.2, BV198.5
Ramonda nathaliae	3	RN26, RN27, RN32
Ramonda serbica	4	RS2, RS6, RS24, RS61
Cirsium arvense	1	BV245.2
Quercus cerris	1	NP10
Bryum sp.	8	BV121, BV121.1, BV121.2, BV122, BV124, BV125, BV128.1, BV129
Lactarius deterrimus	7	BV1, BV2, BV3, BV4, BV6, BV8, BV9
Entoloma sp.	4	BV10, BV13, BV19, BV20
Suillus sp	6	BV24, BV27, BV29, BV42, BV43, BV44
Russula sp.	5	BV45, BV46, BV47, BV50.2, BV52
Clitophilus sp.	6	BV57, BV61, BV64, BV66, BV69, BV76
Tuber brumale	3	BV282, BV365, BV366

Table 2. Selected *Streptomyces* isolates (n=29) for phylogenetic analysis with the detected enzymatic activities

Number of detected activities	Isolate	CEL[1]	CUT[2]	GEL[3]	LIP[4]	PRT[5]	PHA[6]	LAC[7]	TYR[8]	LIG[9]	AMT[10]
0	BV9	-	-	-	-	-	-	-	-	-	-
	BV52	-	-	-	-	-	-	-	-	-	-
	BV19	-	-	-	-	-	-	-	-	-	-
	BV140	-	-	-	-	-	-	-	-	-	-
1	BV290	+	-	-	-	-	-	-	-	-	-
	BV98	-	-	-	-	-	-	+	-	-	-
	BV121	-	-	-	-	-	-	-	+	-	-
2	BV103	+	+	-	-	-	-	-	-	-	-
	BV330	-	-	-	+	+	-	-	-	-	-
	BV294	+	+	-	-	-	-	-	-	-	-
	BV346	-	-	-	-	-	-	+	+	-	-
	NP10	+	+	-	-	-	-	-	-	-	-
3	BV96	+	+	-	-	-	-	-	-	+	-
	BV291	+		-	-	-	-	+	-	+	-
	BV305	+	+	-	-	-	-	-	-	+	-
	BV334	+	-	-	-	-	-	+	-	+	-
	BV221	-	-	-	+	+	-	-	-	+	-
4	BV302	+	-	-	+	-	-	+	-	+	-
	BV316	+	+	-	-	-	-	+	-	+	-
	BV129	+	-	-	+	-	-	-	-	+	+
	BV295	-	-	-	-	+	-	+	-	+	+
	BV293	-	+	-	-	+	-	+	-	+	-
	BV100	+	-	-	+	-	-	-	-	+	+
	BV364	-	-	+	-	-	-	+	+	-	+
5	BV101	+	-	-	+	+	-	-		+	+
	BV292	-	-	-	+		+	+	+	+	-
	BV307	-	-	+	-	-	+	-	+	+	+
6	BV286	+	+	+	-	-	-	+	+	-	-
	BV333	-	-	+	+	+	-	-	+	+	+

[1] CEL - Cellulase, [2]CUT - Cutinase, [3]GEL - Gelatinase, [4]LIP – Lipase, [5]PRT – Proteinase, [6]PHA – PHA, [7]LAC – Laccase, [8]TYR – Tyrosinase,[9]LIG – Lignin peroxidase, [10]AMT – Aminotransferase

Figure Legends

FIG 1. Representative functional screens of selected *Streptomyces* spp. isolates on solid media supplemented with enzyme specific substrates. Functional screen for (a) cellulase, (b) gelatinase, (c) lipase, (d) protease, (e) laccase, (f) tyrosinase, (g) lignin-peroxidase and (H) aminotransferase.

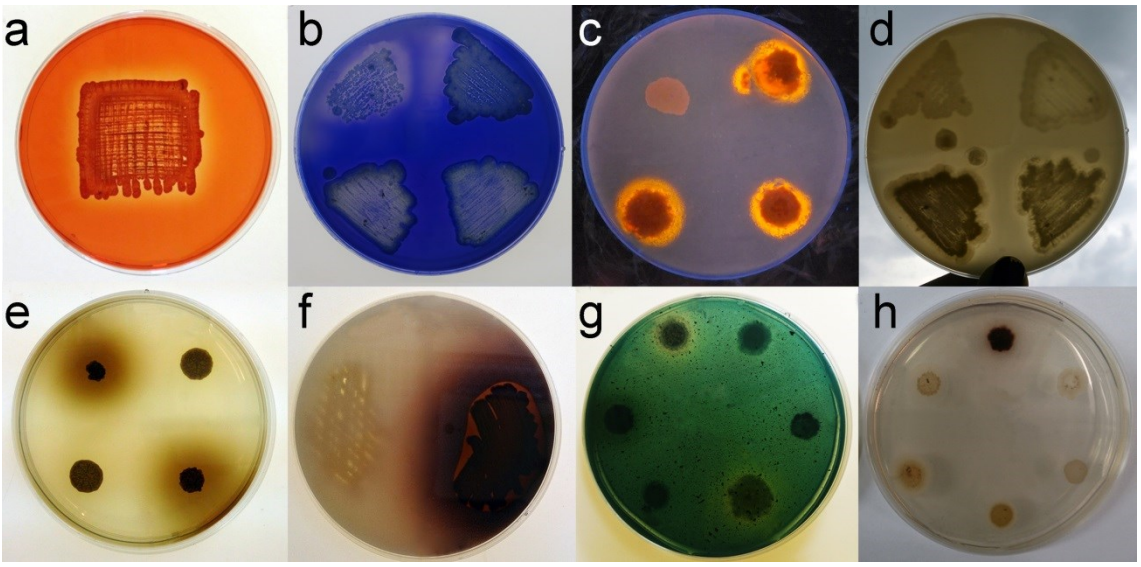

FIG 2. Heat map showing the enzyme activity of 118 isolated *Streptomyces* strains and clustering of enzyme groups. The columns of the heat map represent enzyme activity and the rows represent *Streptomyces* isolates. The color key scale is based on activity in enzyme assays where red is negative activity, yellow moderate and green positive enzyme activity.

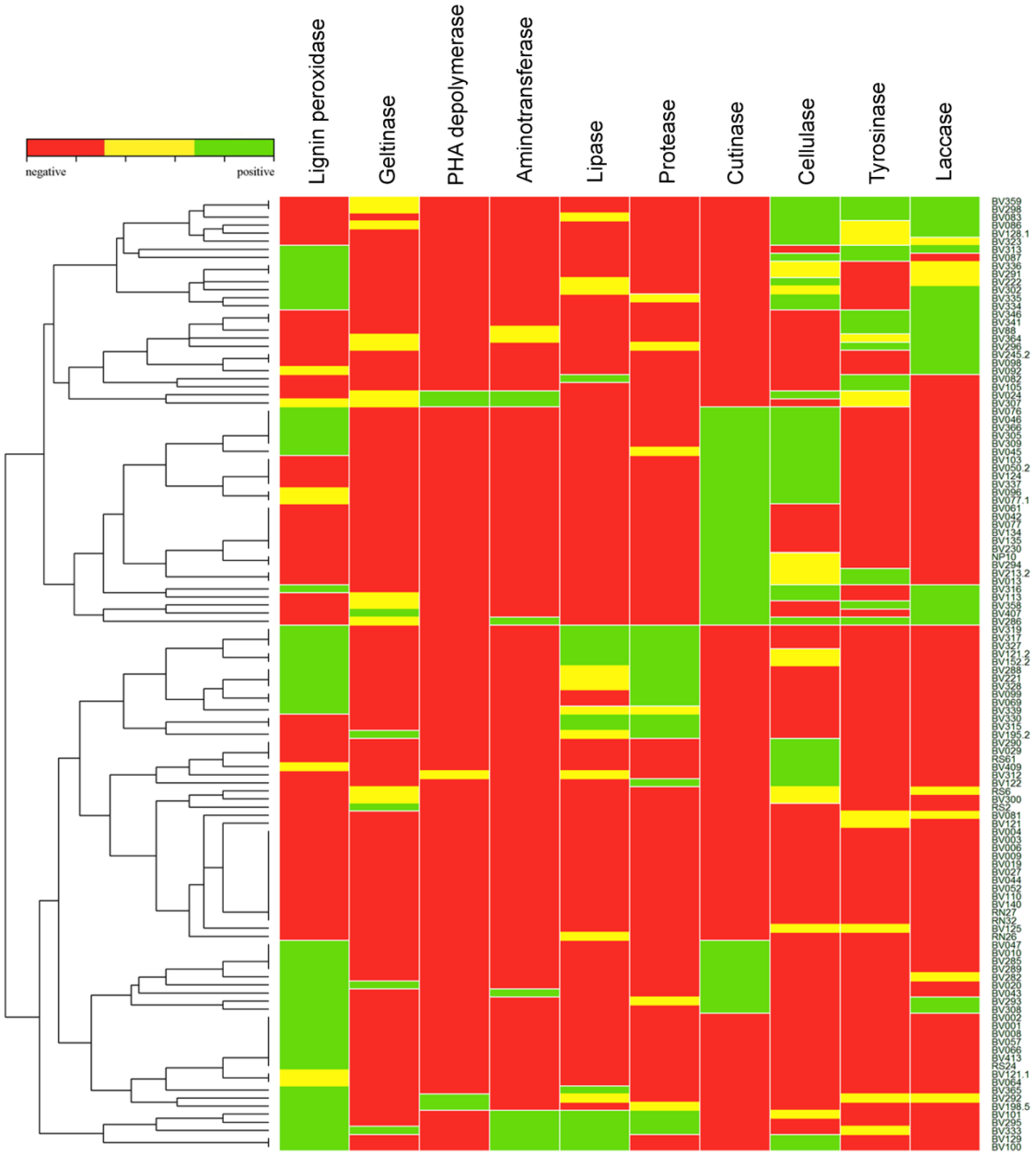

FIG 3. Phylogenetic tree showing the relationship among selected isolated *Streptomyces* strains based on the 16S rRNA gene homology. GeneBank accession number for 16S rRNA gene sequences are shown in parentheses. *Streptomyces* isolates are written in bold while type strains are marked with ^{T}. For the purpose of rooting the tree 16R rRNA gene sequence of *Bacillus subtilis* 168 (AL009129) was used as an outgroup.

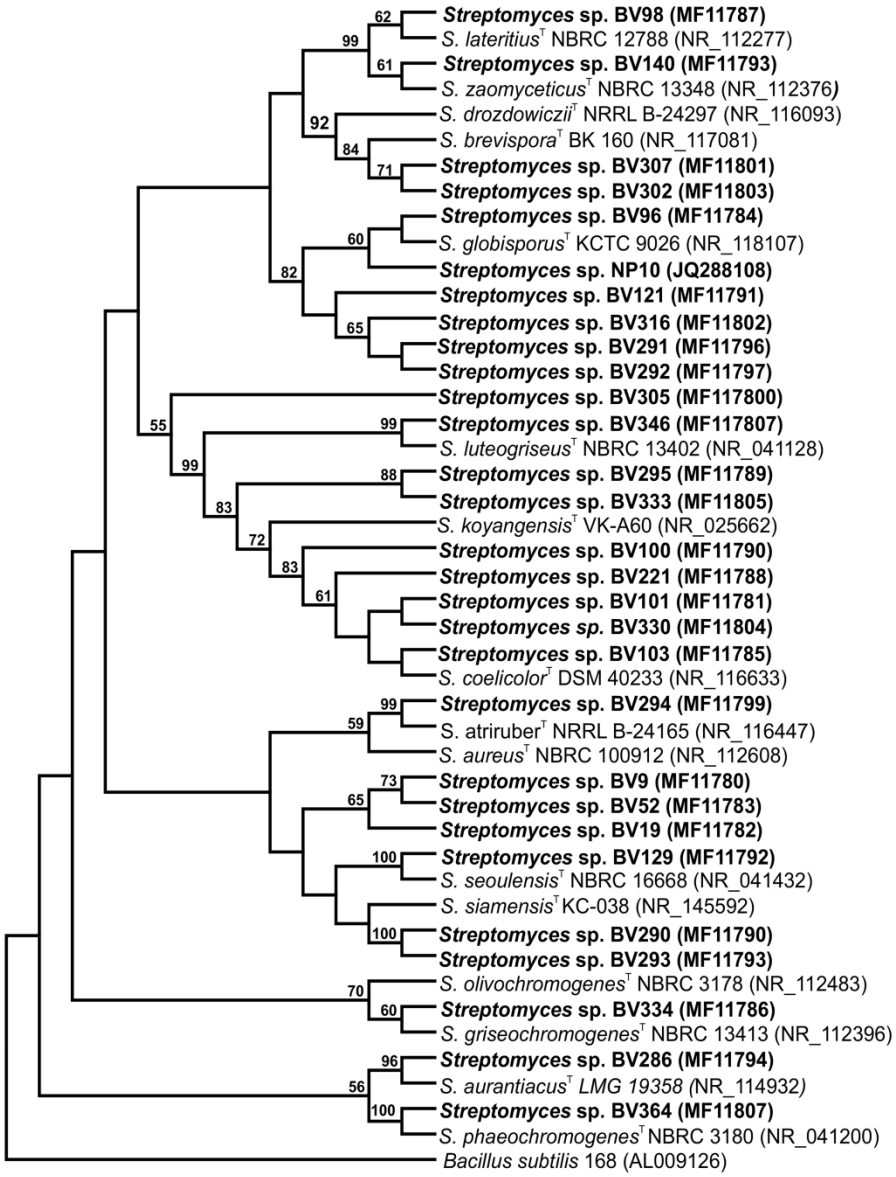

FIG 4. Distribution of different enzymatic activities of *Streptomyces* isolates. Distribution of enzymatic activities (a) between *Streptomyces* isolates from two soil sources (rhizospheres of plants and mycorrhizosphere of fungi) and (b) between *Streptomyces* associated with different species of plants and fungi.

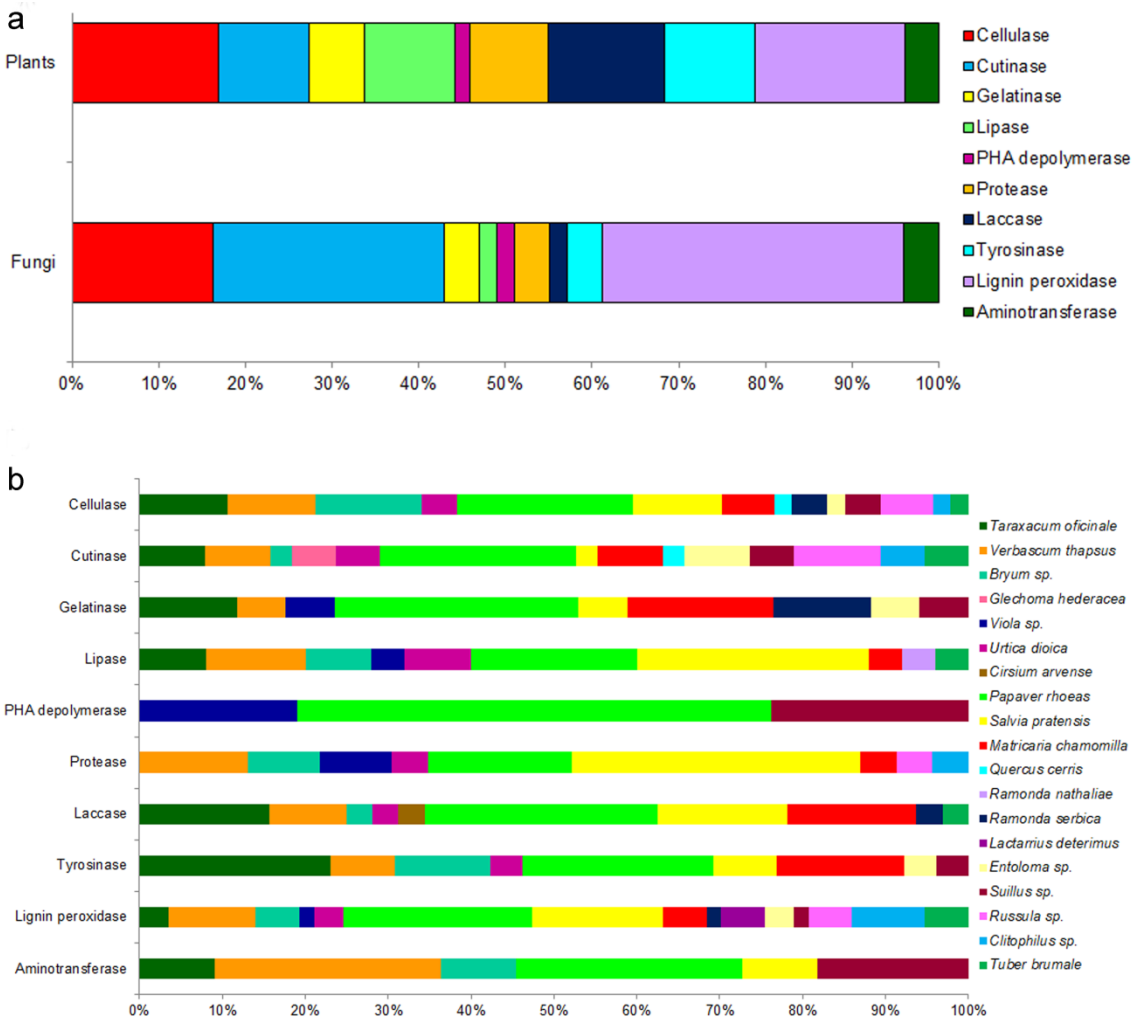

FIG 5. Comparison of A lipase and B tyrosinase activities from *Pseudomonas* and *Streptomyces* strains. Lipase activity of *Pseudomonas aeruginosa* PAO1, *Streptomyces* sp. BV315 and *Streptomyces* sp. BV100 on agar plates with olive oil as substrate (A-left) and in enzyme assay with p-NPP as substrate at 30°C represented as specific enzyme activity (A-right). Tyrosinase activity of *Pseudomonas putida* F6, *Streptomyces* sp. BV87 and *Streptomyces* sp. BV88 with tyrosine as a substrate on agar plates (B-left) and in enzyme assay at 42°C represented as specific enzyme activity (B-right). Statistically significant difference $P < 0.5$ is marked with *.