Potential of Cometabolic Transformation of Polysaccharides and Lignin in Lignocellulose by Soil Actinobacteria

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Abstract

While it is known that several Actinobacteria produce enzymes that decompose polysaccharides or phenolic compounds in dead plant biomass, the occurrence of these traits in the environment remains largely unclear. The aim of this work was to screen isolated actinobacterial strains to explore their ability to produce extracellular enzymes that participate in the degradation of polysaccharides and their ability to cometabolically transform phenolic compounds of various complexities. Actinobacterial strains were isolated from meadow and forest soils and screened for their ability to grow on lignocellulose. The potential to transform ¹⁴C-labelled phenolic substrates (dehydrogenation polymer (DHP), lignin and catechol) and to produce a range of extracellular, hydrolytic enzymes was investigated in three strains of Streptomyces spp. that possessed high lignocellulose degrading activity. Isolated strains showed high variation in their ability to produce cellulose- and hemicellulose-degrading enzymes and were able to mineralise up to 1.1% and to solubilise up to 4% of poplar lignin and to mineralise up to 11.4% and to solubilise up to 64% of catechol, while only minimal mineralisation of DHP was observed. The results confirm the potential importance of Actinobacteria in lignocellulose degradation, although it is likely that the decomposition of biopolymers is limited to strains that represent only a minor portion of the entire community, while the range of simple, carbon-containing compounds that serve as sources for actinobacterial growth is relatively wide.

Introduction

Lignocellulose represents the dominant portion of plant biomass and is thus a key pool of carbon in terrestrial ecosystems. The decomposition of lignocellulose in soil environments, where it originates as aboveground or belowground litter, is thus an essential process of the carbon cycle. Microorganisms represent the key decomposers of lignocellulose in soils and especially fungi are often regarded as major lignocellulose decomposers [1], most likely because their larger, multicellular and often filamentous bodies are better suited for the exploitation of bulky substrates [2]. This potential has led to the evolution of efficient enzymatic systems responsible for the decomposition of biopolymers in several fungi [1,3–5].

The process of lignocellulose decomposition is mediated by extracellular enzymes that target its main components: the polysaccharides cellulose and hemicelluloses and polyphenolic lignin [6]. A wide array of enzymes is necessary for the complete decomposition of lignocellulose. The system for cellulose decomposition typically consists of endoglucanases, cellulobiohydrolases (exocellulases) and β-glucosidases. The hemicellulolytic system is composed of multiple glycosyl hydrolases that are specific for xylose-, mannose-, arabinose- and galactose-containing polysaccharides; and lignin degradation is mediated by oxidative enzymes, such as oxidases (laccases), peroxidases and auxiliary enzymes, that produce hydrogen peroxide [1,7,8].

Although fungi vary largely in their production of extracellular enzymes, several groups, including saprotrophic wood decomposers and cord-forming fungi, that inhabit litter and soil were shown to produce complete arrays of extracellular enzymes that decompose all of the components of lignocellulose [4,9]. Current advances in genome sequencing indicate that the theoretical potential of bacteria to degrade certain components of lignocellulose, e.g., cellulose, is relatively widespread [10]; and, for certain taxa, enzymes involved in decomposition were characterised [11]. Moreover, recent reports also show that bacteria may play a significant role in cellulose decomposition in soil environments [12]. However, the composition of bacterial enzymatic systems has not been systematically addressed, and it is difficult to estimate their potential to transform individual lignocellulose components.

Actinobacteria seem to be good candidates for efficient lignocellulose decomposition, and their filamentous growth may help them access and utilise polymeric substrates [13]. Therefore, the involvement of certain Actinobacteria in the degradation of polysaccharides or phenolic compounds in dead plant biomass is generally accepted [14,15]. This is based on previous reports that suggest the presence of decomposer traits in several actinobacterial taxa. In the case of cellulose, the production of endocellulase by
the genera Streptomyces, Cellulomonas and Acidothiernas was reported [16–18], while efficient exocellulases often combined with xylanase activity were found in Thermobifida, Cellulomonas and Cellulosimicrobium [19–21]. β-Glucosidases have been characterised in the above genera as well as in Gluconobacter, Terrabacter, Micrococcus, Microbacterium and Bjelfbacterium [22–26]. Recently, many putative cellulose-degrading enzymes were found in the sequenced genomes of several Actinobacteria [27], and this phylum showed the highest percentage of genomes that harboured putative cellolytic enzymes. Approximately 1/5 of the 514 characterised genomes harbour at least one putative cellulase [10]. Although information on hemicellulose-degrading enzymes is scarce, individual enzymes were reported in several genera, including Streptomyces, Cellulomonas, Cellulosimicrobium and Kocuria [28–31].

Although the major lignin degraders are white-rot fungi, there are also many reports about bacterial strains that are able to degrade lignin. In addition to the Proteobacteria and Firmicutes [32–34], these reports also mention actinobacterial taxa. Studies on the decomposition of natural and synthetic lignins, for example, isolated lignin, prepared 14C-synthetic lignins or model compounds, indicated that the genera Arthrobacter, Nocardioides and Streptomyces were capable of lignin utilisation, although their efficiencies varied widely and did not reach the level that was observed in ligninolytic fungi [35–38].

Despite the relative abundance of reports on the decomposer abilities of Actinobacteria, the current information remains rather fragmented. Different strains have been studied for the production of individual enzymes, and the abilities to use plant lignocellulose as a growth substrate were not studied in much detail. The aim of this study was to explore the ability of soil Actinobacteria to act as decomposers of dead plant biomass. To achieve this goal, a set of natural isolates was screened for their production of cellulosic enzymes, and active strains were tested for their ability to use lignocellulose in the form of wheat straw as a growth source. The potential decomposers were further screened for the production of multiple lignocellulose-degrading enzymes and for their ability to cometabolically transform 14C-labelled phenolic substances (lignin, DHP and catechol) during their growth on wheat straw. The Actinobacteria in this study were isolated from soil with mixed-metal pollution. Due to their heavy metal resistance, Actinobacteria are frequently found in such soils, and this group may replace the more sensitive fungi as the main decomposers in such soils [39,40]. We thus expected that the decomposition of lignocellulose would be a common trait of strains isolated from this environment.

**Materials and Methods**

**Isolation of Bacterial Strains**

The study was carried out on private land. No specific permission was required for the activities covered by this study. However, for more intensive research activities, individual private owners have to grant the permit to conduct such research. The field studies did not involve endangered or protected species. Bacterial strains were isolated from the organic horizons of the grassland and forest soils near Príbram, Czech Republic (49°42′22.207″N, 13°38′27.296″E). The soil is a cambisol with pH of approximately 5.5 and has a clay/silt/sand ratio of approx. 40:30:30% and an elevated heavy metal content (namely, Cu, Zn, Cd, Zn, Pb and As) due to its location near a polymetallic smelter [41]. The study was performed on private land; no specific permit was required for the activities performed as part of this study.

Physical and chemical treatments were used for the selective isolation of soil Actinobacteria. Soil samples were pre-treated by dry heating (120°C) and phenol treatment (1.5%), and water extracts

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**Table 1. Screening of actinobacterial strains for their ability to produce celllobiohydrolase and 1,4-β-glucosidase.**

| Strain | Celllobiohydrolase | 1,4-β-Glucosidase |
|--------|-------------------|------------------|
| pl18   | ++                | +++              |
| pl21   | –                 | +                |
| pl23   | +                 | ++               |
| pl28   | +                 | ++               |
| pl36   | +++               | +++              |
| pl41   | +++               | +++              |
| pl67   | ++                | –                |
| pl70   | –                 | +                |
| pl73   | –                 | +                |
| pl75   | –                 | +                |
| pl77   | –                 | +++              |
| pl80   | –                 | +                |
| pl81   | –                 | +                |
| pl84   | +                 | ++               |
| pl86   | +                 | –                |
| pl88   | +++               | +++              |
| pl95   | +++               | +++              |
| pl98   | –                 | +                |
| pl100  | –                 | +                |
| pl107  | –                 | +                |
| pl112  | –                 | +                |
| pl116  | +++               | +++              |
| pl118  | +                 | +++              |
| pl123  | +++               | +++              |
| pl124  | ++                | +                |
| pl129  | +                 | +                |
| pl131  | –                 | +                |
| pl134  | –                 | +                |
| pl136  | –                 | +                |
| pl138  | –                 | +                |
| pl149  | +                 | +                |
| pl150  | –                 | +++              |
| pl153  | +++               | +++              |
| pl154  | ++                | +++              |
| pr10   | +                 | –                |
| pr22   | –                 | +++              |
| pr24   | +                 | –                |
| pr3    | +                 | +                |
| pr30   | +                 | +++              |
| pr4    | –                 | +                |
| pr40   | +                 | –                |
| pr41   | +++               | ++               |
| pr45   | –                 | +++              |
| pr48   | –                 | ++               |
| pr49   | +                 | +                |
| pr52   | +                 | –                |
| pr55   | +++               | +                |
| pr57   | +                 | ++               |
were used to inoculate plates containing selective media, either humic acid-vitamin agar (1 g L\(^{-1}\) humic acid, 0.5 g L\(^{-1}\) Na\(_2\)HPO\(_4\), 7.7 g L\(^{-1}\) KCl, 0.05 g L\(^{-1}\) MgSO\(_4\) \(\cdot\) 7H\(_2\)O, 0.01 g L\(^{-1}\) thiamine-HCl, riboflavin, niacin, pyridoxine, inositol, Ca-pantothenate, p-aminobenzoic acid and 0.25 mg L\(^{-1}\) biotin, 18 g L\(^{-1}\) agar, pH 7.2) or lignin-soy bean flour-vitamin agar containing soil extract (1 g L\(^{-1}\) lignin, 0.2 g L\(^{-1}\) soy bean flour, 0.5 g L\(^{-1}\) Na\(_2\)HPO\(_4\), 7.7 g L\(^{-1}\) KCl, 0.05 g L\(^{-1}\) MgSO\(_4\) \(\cdot\) 7H\(_2\)O, 0.01 g L\(^{-1}\) FeSO\(_4\) \(\cdot\) 7H\(_2\)O, 0.02 g L\(^{-1}\) CaCO\(_3\), B-vitamins: 0.5 mg L\(^{-1}\) thiamine-HCl, riboflavin, niacin, pyridoxine, inositol, Ca-pantothenate, p-aminobenzoic acid and 0.25 mg L\(^{-1}\) biotin, 18 g L\(^{-1}\) agar, pH 7.5) that was supplemented with the antibiotics kanamycin (20 mg L\(^{-1}\)) and nalidixic acid (10 mg L\(^{-1}\)) [42,43]. Pure cultures of bacteria were obtained from agar plates, and those strains that were identified as *Actinobacteria* were retained. Strains were stored in a sterile, 50% glycerol solution in 25 mM Tris at −20°C and subcultured on GYM agar (4 g L\(^{-1}\) glucose, 4 g L\(^{-1}\) yeast extract, 10 g L\(^{-1}\) malt extract, 2 g L\(^{-1}\) CaCO\(_3\), 12 g L\(^{-1}\) agar, pH 7.2) at 25°C.

### Screening for Lignocellulose-degrading Strains

Efficient decomposition of cellulose, which is the major component of dead plant biomass, depends on the production of cellbiohydrolase and β-glucosidase. To screen for the production of these two enzymes, isolated actinobacterial strains were cultivated in liquid GYM medium for 14 days at 25°C without agitation (three replicates). The cultivation liquid was collected, and the activities of the extracellular enzymes were measured spectrophotometrically as described previously [44]. Cellbiohydrolase (exocellulase, EC 3.2.1.91) and 1,4-β-glucosidase (EC 3.2.1.21) were assayed using 4-methylumbelliferyl-β-D-glucopyranoside and 4-methylumbelliferyl-β-D-glucopyranoside, respectively, in 50 mM sodium acetate buffer (pH 5.0). The reaction mixtures were incubated at 40°C for 120 min and terminated by sodium carbonate addition [44].

The activity of each strain was ranked on a scale of negative, low, medium or high. Cellbiohydrolase: negative = 0 to 0.5 μmol min\(^{-1}\) mL\(^{-1}\), low = from >1 to 15 μmol min\(^{-1}\) mL\(^{-1}\), medium from >15 to 50 μmol min\(^{-1}\) mL\(^{-1}\), and high >50 μmol min\(^{-1}\) mL\(^{-1}\). 1,4-β-glucosidase: negative = 0 to 1 μmol min\(^{-1}\) mL\(^{-1}\), low = from >1 to 10, medium from >10 to 20 μmol min\(^{-1}\) mL\(^{-1}\), and high >20 μmol min\(^{-1}\) mL\(^{-1}\). Strains that produced cellbiohydrolase and 1,4-β-glucosidase and exhibited high activity of at least one of those enzymes were identified, and their ability to grow on lignocellulose as a carbon source was examined. One gram of air-dried, milled wheat straw was added into 100-mL thick-walled flasks to form a uniform layer. Each flask was supplemented with 5 mL of distilled water and sterilised by autoclaving (2 x 30 min at 121°C with cooling to room temperature between the two cycles). The flasks were inoculated with 1 mL of cell suspension that had been pre-grown for three days on liquid GYM media. Triplicate flasks for each strain were incubated for 21 days at 25°C. After incubation, the enzymes were extracted in 15 mL of distilled water, and the extracts were filtered and used for enzyme activity measurements.

The activities of 1,4-β-glucosidase, cellbiohydrolase and 1,4-β-xylisolase in the extracts were assessed using 4-methylumbelliferyl-β-D-glucopyranoside, MUF-β-D-cellulobiose and MUF-β-D-xylopyranoside, respectively, in 50 mM sodium acetate buffer, pH 5.0, as previously described [44]. Substrates (100 μL in DMSO) at a final concentration of 500 μM were combined with the three technical replicates of the 100-μL extracts in a 96-well multiwell plate. For the background fluorescence measurement, 100 μL of sodium acetate buffer was combined with 100 μL of the 4-methylumbelliferol standards to correct for fluorescence quenching. The multiwell plates were incubated at 40°C, and fluorescence was recorded from 5 min to 125 min using the Infinite microplate reader (TECAN, Austria) at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. The quantitative enzymatic activities after blank subtraction were calculated based on standard curves of 4-methylumbelliferone, and enzyme activity was expressed per g of straw dry mass.

Of the 14 strains, only six exhibited visual growth and produced extracellular enzymes in straw. Among these, three strains, pl88, pr6 and pr55, that highly produced cellbiohydrolase were selected for the detailed characterisation of glycosyl hydrodrolase production and the decomposition of phenolic compounds.

To analyse the spectra of the extracellular enzymes that were produced by the bacterial strains, pl88, pr6 and pr55, that highly produced cellbiohydrolase were cultivated on diluted GYM media with either cellulose as a specific inducer or with finely milled wheat straw (mesh size 0.2 mm) as a complex inducer. In 50-mL flasks, 10 mL of 10× diluted GYM media was combined with 50 mg of cellulose or wheat straw and sterilised by autoclaving. The flasks were inoculated with 100 μL of cell suspension that had been pre-grown for three days in liquid GYM media. Triplicate flasks for each strain were incubated for 21 days at 25°C. After incubation, the 1,4-β-glucosidase, cellbiohydrolase and 1,4-β-xylisolase activities were measured, as described above. The activities of 1,4-α-glucosidase, 1,4-α-arabinosidase, 1,4-β-galactosidase, 1,4-β-mannosidase and 1,4-β-gluconidase in the extracts were assessed using 4-methylumbelliferyl-α-D-glucopyranoside, 4-methylumbelliferyl-α-D-arabinopyranoside, 4-methylumbelliferyl-β-D-galactopyranoside, 4-methylumbelliferyl-β-D-galactosidase, 4-methylumbelliferyl-β-D-glucuronidase, and the same method. The activities of endo-1,4-β-glucanase (endo-cellulase) and endo-1,4-β-xylanase (endo-xylanase) were assayed using azo-dyed carboxymethyl cellulose and birchwood xylan, respectively, according to the manufacturer’s instructions (Megazyme, Ireland). Reaction mixture containing 0.2 mL of 2% dyed substrate in 200 mM sodium acetate buffer, pH 5.0, and 0.2 mL of sample was incubated at 40°C for 60 min, and the reaction was ended by adding 1 mL of ethanol followed by 10 s of vortexing and 10 min of centrifugation [10,000 × g] [45]. The amount of released dye was measured at 595 nm, and the enzyme activity was calculated according to standard curves that correlated dye release with the release of reducing sugars.

### Transformation of \(^{14}C\)-labelled Phenolic Compounds

The transformation of phenolic compounds of various complexities was studied using \(^{14}C\)-labelled compounds. \(^{14}C\)-labeled dehydrogenation polymer (\(^{14}C\)-DHP) was synthesised according to Brunow [46] and dissolved in a N,N-dimethylformamide-water suspension [1:20 v/v] [47]. \(^{14}C\)-labeled lignin was extracted from labelled poplar trees that were prepared according to Odier [48] and used as a solid material. \(^{14}C\)-labeled catechol in an ethanol

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**Table 1. Cont.**

| Strain | Cellbiohydrolase | 1,4-β-Glucosidase |
|--------|------------------|------------------|
| pr6    | ++               | +++              |
| pr7    | +                | ++               |
| pr9    | +                | ++               |

Twenty-five isolates produced neither of the enzymes. Legend: “++” high production of enzyme (activity >100 mM/mL), “+” average production (100 > activity > 10), small production (activity <10) and “−” no enzyme production.

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flask. In the lignin flasks, 10 mg of fine, 14C-poplar lignin powder was added, which resulted in a final radioactivity of 550,500 dpm per flask, and CO2 was trapped by bubbling the released air through sterile water was added. The flasks were sealed with rubber septa and milled wheat straw, which was added to form a uniform layer. The suspension was then incubated flasks were shaken for 2 h (280 rpm) at room temperature and then, 750 μL of 14C-DHP in a N,N-dimethylformamide-water suspension was added drop-wise onto the surface of the straw layer, which resulted in a final radioactivity of 127,500 dpm per flask. In the catechol flasks, 750 μL of 14C-catechol in an ethanol-water solution was used directly.

The cometabolic transformation of phenolic compounds was studied in 100-mL, thick-walled flasks containing 1 g of air-dried and milled wheat straw, which was added to form a uniform layer. Each flask was supplemented with 5 mL of distilled water and sterilised by autoclaving (2 x 30 min at 121°C with cooling to room temperature between the two cycles). The flasks were inoculated with 1 mL of cell suspension that had been pre-grown for three days in liquid GYM media (five flasks per strain). Control flasks were left uninoculated. The following day, 14C-labelled DHP, catechol and poplar lignin were added. In the DHP flasks, 750 μL of 14C-DHP in a N,N-dimethylformamide-water suspension was added drop-wise onto the surface of the straw layer, which resulted in a final radioactivity of 127,500 dpm per flask. In the catechol flasks, 750 μL of 14C-catechol in an ethanol-water solution was added, which resulted in a final radioactivity of 550,500 dpm per flask. In the lignin flasks, 10 mg of fine, 14C-poplar lignin powder was added onto the surface of the straw layer, which resulted in a final radioactivity of 230,000 dpm per flask, and then, 750 μL of sterile water was added. The flasks were sealed with rubber septa and aluminium caps.

Incubation proceeded for 76 days at 24°C in the dark. Volatile compounds were flushed out of the flasks every week using sterile air, and CO2 was trapped by bubbling the released air through two sequential flasks containing Opti-Fluor and Carbosorb/Opti-Fluor (Packard Instruments) every week. A liquid scintillation counter (Model 1411, WallacOy, Finland) was used to quantify the trapped 14CO2 during the experiment. After incubation, the flasks supplemented with the 14C-labelled substances were stored at −18°C until mass-balance extraction.

The flasks containing the residual 14C material were extracted twice with 6 mL of distilled water. After the addition of water, the incubation flasks were shaken for 2 h (280 rpm) at room temperature on a table rotary shaker. The suspension was then poured into a 60-mL syringe containing a pre-weighed cotton plug, and the aqueous extract was pushed through the syringe. For measurements, 1 mL of water extract was diluted with 7 mL of distilled water, and the dilutions were mixed with 10 mL of LUMAGEL. The radioactivity of each extract was measured using a liquid scintillation counter (Model 1411, WallacOy, Finland). The cotton plugs that were used in the filtrations and the residual solid material were air dried and then burned in a combustion chamber (Junitek, Finland). The radioactivity was then counted using a liquid scintillation counter as reported previously [49]. The efficiency of combustion was verified using 14C-labelled standards.

A one-way analysis of variance with the Fisher’s least significant difference post hoc test was used to analyse the statistical significance of differences among treatments. Differences with a P<0.05 were regarded as statistically significant.

Identification of Actinobacterial Strains

DNA was isolated from the actinobacterial biomass that was obtained by cultivation in liquid GYM medium using the modified Miller-SK method [50]. Isolated genomic DNA was used as a template in PCR reactions using universal primers for the bacterial

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**Table 2.** Identification of selected actinobacterial strains and the accession numbers of their partial 16S rRNA gene sequences.

| Strain | Accession No | Closest hit                          | Accession No | Similarity (%) | Coverage (%) |
|-------|--------------|--------------------------------------|--------------|----------------|--------------|
| pl18  | KC789721     | Micromonospora saelcensis strain L6   | JN862845     | 99.6           | 99.3         |
| pl36  | KC789723     | Streptomyces ciscaucicus strain HBUMB3169 | EU841585     | 99.9           | 99.8         |
| pl41  | KC789724     | Curtobacterium flaccumfaciens strain LMG 3645 | NR025467     | 100.0          | 99.3         |
| pl88  | KC789730     | Streptomyces atratus strain HBUM173340 | FJ486302     | 100.0          | 94.2         |
| pl95  | KC789731     | Streptomyces aureus strain HBUM174596 | EU841581     | 99.4           | 100.0        |
| pl116 | KC789734     | Kribella antibiotica strain YIM 31530 | NR029048     | 98.8           | 99.8         |
| pl118 | KC789719     | Streptomyces sangleri strain IHB B 6004 | KF475877     | 96.6           | 99.9         |
| pl123 | KC789735     | Nocardia exalbida W9709               | GQ376167     | 99.2           | 100.0        |
| pl153 | KC789738     | Streptomyces hygroscopicus subsp. gelidanus strain NBRC14620 | AB184606     | 99.8           | 99.9         |
| pl154 | KC789739     | Microbyspora rosea subsp. rosea strain AO11 | AB369120     | 99.8           | 95.6         |
| pr6   | KC789740     | Streptomyces mauvicolor strain 7534   | JN180187     | 99.2           | 99.3         |
| pr30  | KC789741     | Amycolatopsis saalfeldensis strain HK 0474 | DQ792502     | 99.2           | 100.0        |
| pr41  | KC789742     | Streptomyces setonensis strain 17-1   | EU367980     | 99.4           | 99.1         |
| pr55  | KC789744     | Streptomyces sannanensis strain 126195 | JN180213     | 98.9           | 99.4         |

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and archaeal 16S rRNA gene, pH-T7 (5’-TAATACGACTCACTATAGGAGGTGTGATCAG-3’) and pA (5’-AAGGAGGTGATCCAGCCGCA-3’). Each 50-μl reaction mixture contained 5 μl of 10× buffer for DyNAzyme DNA Polymerase (Finnzymes), 3 μl of purified BSA (10 mg mL⁻¹), 2 μl of each primer (0.01 mM), 1 μl of PCR Nucleotide Mix (10 mM each), 1.5 μl of DyNAZyme II DNA Polymerase (2 U μl⁻¹, Finnzymes) and 1 μl of isolated genomic DNA. The cycling conditions were as follows: 16 94°C 5 min, 35 (94°C 1 min, 57°C for 45 s min and 72°C for 90 s) followed by 72°C for 10 min. The PCR products were directly sequenced by Macrogen (Seoul, Korea), and the sequences were manually edited using the BioEdit program (http://www.mbio.ncsu.edu/bioedit/bioedit.html) and corrected prior to a BLASTn search against the nucleotide database at the NCBI (http://www.ncbi.nlm.nih.gov/blast).

Results and Discussion

Seventy-six strains of soil Actinobacteria were isolated from the soils of the study area and screened for their ability to produce enzymes involved in cellulose decomposition, including cellobiohydrolase and 1,4-β-glucosidase. Of these strains, 32% did not produce any of the tested enzymes, while 31% produced both of them. The production of 1,4-β-glucosidase was more common (57% of strains) than that of cellobiohydrolase (41% of strains; Table 1). The percentage of strains that produced cellobiohydrolase roughly corresponded to the percentage of actinobacterial strains that harboured a gene for endocellulase or cellobiohydrolase (i.e., the glycosyl hydrolase family GH5, 6, 8, 9, 12, 44, 45 or 48), which was one-third of all of the sequenced actinobacterial genomes that were analysed in a recent study [10]. The percentage of strains that did not produce detectable amounts of any enzyme (32%) was higher than what was inferred from the analysis of the genomes, which was less than 20% of the genomes [10]. It is thus possible that some of the strains that harbour 1,4-β-glucosidase do not express the gene or show only low expression levels.

Fourteen strains that produced both enzymes, and highly produced at least one, were selected for further studies and were identified by 16S rRNA sequencing. Of these, eight strains showed highest similarity with members of the genus Streptomyces, while the best hits for the others were from the genera Amycolatopsis, Curtobacterium, Kribbella, Microbispora, Micromonospora and Nocardia (Table 2). The activity of cellobiohydrolase and 1,4-β-glucosidase in the genera Amycolatopsis, Kribbella, Micromonospora, Nocardia and Streptomyces corresponded well with the presence of the corresponding genes in their genomes [10]. The currently analysed genomes of Nocardia did not contain a cellobiohydrolase gene, and the genomes of Curtobacterium and Microbispora have not been analysed. Despite their high cellulolytic activity, only six of the fourteen analysed isolates (pl88, pl95, pl118, pr6, pr30 and pr55) showed visually detectable growth on milled wheat straw after 21 days of culturing. All of these strains produced extracellular glycosyl hydrolases: cellobiohydrolase, 1,4-β-glucosidase and 1,4-
b-xylosidase, although their activities differed (Figure 1). The strains that highly produced cellobiohydrolase (>2 μmol min⁻¹ g⁻¹ straw dry mass) were selected for further experiments. Cellobiohydrolase represents the rate-limiting enzyme in the decomposition of cellulose, the most abundant and rapidly decomposable polysaccharide in plant litter [12,51].

All studied strains produced a complete set of cellulolytic enzymes: endocellulase, cellobiohydrolase and 1,4-b-glucosidase, and all strains produced the xylanolytic enzymes endoxylanase and 1,4-b-xylosidase as well as the hemicellulases 1,4-b-galactosidase and 1,4-b-mannosidase. 1,4-α-Arabinosidase was only produced by pr6 and pr55, while amylase was produced by pr6

Figure 3. Mineralization of phenolic compounds by selected Actinobacteria. Time course of 14CO₂ production during the transformation of 14C-catechol, 14C-poplar lignin and 14C-DHP in wheat straw microcosms by the selected actinobacterial strains. Control treatments contained sterile straw. The data represent the means and standard errors.
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and pl88. The bacteria produced the same enzymes, regardless of whether cellulose or milled wheat straw was used as the carbon source (Figure 2). However, wheat straw, which is a complex substrate that contains various polysaccharides, increased the production of endocellulase was studied. Transformation of a substrate that contains various polysaccharides, increased the relative share of water-soluble phenolics, and this was most apparent for catechol, where insoluble 14C compounds represented 47% of the total in the control, while they were 19–26% of the total after incubation with bacteria. The amounts of soluble 14C in the DHP- and lignin-containing flasks also increased after bacterial treatment, but only slightly, by 2–4% and 0.7–2.0%, respectively (Table 3).

The mineralisation of lignin by Streptomyces was first reported by Crawford, who found that mineralisation rates of 14C-lignin-labelled fir varied from 1.5 to 3% after nearly 42 days [57]. Similar rates were also reported by Pasti et al. [58]. Occasionally, higher lignin mineralisation rates were reported, such as 2.9% after 10 days by Arthrobacter sp. [59] or up to 5% after 15 days by Nocardi a sp. [38]. In comparison, the mineralisation rates that were observed in our isolates seemed to be relatively low, but care must be taken in these comparisons because the modes of lignin labelling and preparation greatly affect mineralisation rates. The mineralisation rates of the recalcitrant lignin model compound 14C-DHP that were reported in this study indicates that the ability of the studied Actinobacteria to catalyse the complete decomposition of lignin is low and that cometabolic lignin degradation during the growth of active decomposers on lignocellulose is rather negligible.

Although solubilisation is the first important step in lignin decomposition because it makes the polar lignin residues available to other microorganisms, only a few studies have reported the amount of solubilised lignin. Our results indicate only a limited solubilisation of lignin, and these data are comparable to those published for several Streptomyces (6–10%) [58].

Catechol is the first intermediate product of phenol degradation, and its cleavage is a critical step in the aerobic degradation of aromatic compounds in microorganisms [60]. Several bacterial strains that are capable of catechol degradation belong to various phyla, such as Actinobacteria, Alphaproteobacteria, Betaproteobacteria or Gammaproteobacteria. Catechol degradation was investigated mainly in studies that focused on the treatment of phenolic wastes, and efficient degraders were found in the genera Pseudomonas, Actinobacter and Klebsiella [61]. All of the isolates in our study were able to efficiently degrade or solubilise catechol. Although the importance of phenolics as a growth substrate for bacteria is not clear, low molecular mass phenolic compounds are relatively common in litter [62] and their transformation might be of importance.

The results confirm the potential importance of Actinobacteria in lignocellulose degradation, although it is likely that the decomposition of biopolymers is limited to strains that represent only a minor portion of the entire community. Our results indicate that strains that are capable of growth on a complex lignocellulose substrate exist; these taxa are able to decompose cellulose and hemicelluloses, and at least some of them may prefer the latter. The importance of cometabolic degradation of lignin seems to be limited when compared with the abilities of saprotrophic fungi, but Actinobacteria may still contribute to the solubilisation of phenolics, especially low-molecular-mass compounds. Future studies using genomic, transcriptomic or proteomic approaches are needed to

Table 3. Mass balance of 14C-catechol, 14C-popolar lignin and 14C-DHP lignin after a 76-day incubation in wheat straw microcosms with the selected actinobacterial strains.

| Substrate      | Strain  | Respired | Soluble        | Bound         |
|----------------|---------|----------|----------------|---------------|
| catechol       | pl88    | 11.64±0.85 a | 65.2±0.7 c | 23.2±0.6 c |
|                | pr6     | 3.33±0.29 b  | 70.6±1.1 b   | 26.1±0.8 b  |
| DHP            | pr55    | 6.36±0.60 c  | 74.4±0.5 a   | 19.2±1.1 d  |
| poplar lignin  | pr6     | 1.12±0.13 a  | 40.0±0.2 a   | 94.9±0.2 c  |
|                | pl88    | 1.04±0.03 a  | 2.7±0.1 c    | 96.2±0.1 b  |
|                | pr55    | 0.81±0.07 b  | 3.4±0.2 b    | 95.8±0.2 b  |
|                | control | 0.94±0.02 c  | 2.0±0.2 d    | 97.9±0.3 a  |
| DHP            | pl88    | 0.67±0.06 a  | 21.7±0.4 a   | 77.7±0.4 b  |
|                | pr6     | 0.64±0.11 a  | 22.4±0.5 a   | 76.9±0.6 b  |
|                | pr55    | 0.45±0.02 b  | 23.1±0.5 a   | 76.4±0.6 b  |
|                | control | 0.11±0.03 c  | 18.9±1.1 b   | 81.0±1.1 a  |

The data (% of the total) represent the means and standard errors. Different letters indicate statistically significant differences at P≤0.05. doi:10.1371/journal.pone.0089108.t003
explore the link between the genetic potential of Actinobacteria and their actual activities as decomposers of organic matter.

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Author Contributions

Conceived and designed the experiments: TV PB. Performed the experiments: TV KS. Analyzed the data: TV KS PB. Wrote the paper: TV PB.
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