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ORIGINAL RESEARCH

Screening of Wild-Type Fungal Isolates for Cellulolytic Activity

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Abstract:
Background: In this study, wild-type fungal isolates, producing highly effective cellulolytic enzymes were selected for bioconversion of residues and waste from agriculture and rational utilization of energy resources for food production.
Methods: We screened wild-type fungal isolates of *Aspergillus*, *Penicillium*, and *Trichoderma* with an enhanced ability to produce extracellular cellulase. We carried out solid-state fermentation on a medium of agricultural waste products, including wheat bran, beet peels, and cotton oil cake, as well as additional sources of nitrogen and mineral elements. Enzyme production by the fungal isolates was detected within 14 days of cultivation.
Results: Of 17 strains of *Trichoderma*, *Aspergillus*, and *Penicillium* tested, we identified *Penicillium* strain K-2-25 and *Trichoderma lignorium* strain T-22 to have high cellulolytic activity. K-2-25 demonstrated the highest activity after 48 hours of cultivation. T-22 also showed significant cellulolytic activity. *Penicillium* strain K-2-25 showed cellulolytic activity for 98–270 hours during cultivation, and the amount of reduced glucose was 945 mg. *T. lignorium* T-22 was the second most active strain, with glucose reduction of 835 mg.
Conclusion: The strains K-2-25 and T-22 will are be recommended for biotechnological applications, especially for bioconversion of poor hardly decomposable vegetable waste products, such as like straw, into useful biomass.

Keywords: screening, wild-type fungal isolates, solid–state fermentation, cellulolytic activity

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Introduction

In nature, lignocellulose is derived from wood, grass, agricultural residues, forestry wastes, and solid municipal wastes. Lignocellulose consists of three types of polymers, ie, cellulose, hemicellulose, and lignin. Each polymer is degraded by a variety of microorganisms which produce a battery of enzymes working synergistically. Recycling of these natural polymers is important in the carbon cycle.[1,2] The major component of lignocellulose is cellulose, which is the most abundant renewable organic resource, and comprises approximately 45% of dry wood weight. Cellulose is also the major component of solid municipal waste. Vast quantities of this material are produced from food processing, the timber industry, papermaking, cereal grain harvesting, and sugar cane processing.[3] This linear polymer is composed of D-glucose subunits linked by b-1,4 glycosidic bonds, forming cellobiose molecules. The number of D-glucose subunits vibrates significantly from 15–10,000 in one molecule.[4] Cellulose can appear in crystalline form, and is known as crystalline cellulose. In addition, there is a small proportion of nonorganized cellulose chains which form amorphous cellulose. In this conformation, cellulose is more susceptible to enzymatic degradation.[5] Biological degradation of cellulose has attracted the interest of microbiologists and biotechnologists for many years.[1,6–12]

Most cellulolytic microorganisms are eubacteria and fungi, although some anaerobic protozoa and slime molds are able to degrade cellulose. Cellulolytic micro-organisms can establish synergistic relationships with noncellulolytic species in cellulose wastes. Interactions between these populations lead to complete degradation of cellulose, with release of carbon dioxide and water under aerobic conditions, and carbon dioxide, methane, and water under anaerobic conditions.[5,12,13]

In nature, hydrolysis of cellulose occurs as a result of the action of a cellulase complex produced by cellulolytic microorganisms. This complex consists with different actions, ie, endoglucanases (endo-1,4-b-glucanases), cellobiohydrolases (exo-1,4-b-glucanases), and b-glucosidases. Endoglucanases (endo-1,4-b-glucanases) can hydrolyze internal bonds (preferentiallyably in cellulose amorphous cellulose regions), and release new terminal ends, whereas cellobiohydrolases act on the existing or endoglucanase-generated chain ends. Both enzymes can degrade amorphous cellulose but, with some exceptions, cellobiohydrolases are the only enzymes that efficiently degrade crystalline cellulose. Cellobiohydrolases and endoglucanases release cellobiose molecules. Effective hydrolysis of cellulose also requires b -glucosidases, which break down cellobiose, releasing two glucose molecules.[1,5,14,15]

Bacterial and fungal degradation of cellulose has been extensively studied and reviewed.[9,11,16–19] Among prokaryotes, the *Corynebacterium* spp, *Cellulomonas fimi*, and the anaerobe *Clostridium thermocellum* are the best known examples.[8] Of the aerobic cellulolytic bacteria, *Cellulomonas, Pseudomonas*, and *Streptomyces* spp. are well investigated.[5] Among the bacteria, much work has been done on lignocellulose degradation by *Streptomyces* spp,[16] mainly with *Streptomyces viridosporus* T7A, which degrades both lignin and the carbohydrate portions of lignocelluloses.[17,20] Cellulolytic fungi play an important role in natural ecosystems, participating in transformation of cellulose from various vegetable complexes. Fungi capable of decomposing cellulose are well represented in various laboratory collections. They are easily isolated in culture, and the strains are easily kept and supported in subculture. This opens up a broad range of opportunities for using active strains in industry.

The soft-rot fungi, *Trichoderma viride* and *Trichoderma reesei,* are by far, the most extensively studied ones. During the past few years, investigations of these fungi have made significant progress towards elucidating the enzymology of cellulose degradation.[21]

Two *P. chrysosporium* enzymes, one cell-bound and one extracellular, have been identified.[15] Relative to endoglucanases and cellobiohydrolases, low levels of the *T. reesei* glucosidase are secreted in submerged culture.

Molecular research of the genome sites responsible for biosynthesis of oxidizing enzymes is being actively conducted. For example, sequences have been reported for both genomic and cDNA clones encoding cbh1,[22–24] cbh2,[12,25] egl,[26] eg3,[27] and fl-gh, tcosidase from *T. reesei*. [28]

Extracellular enzymes may be produced in liquid or solid media. The use of solid media permits fast screening of large populations of fungi, allowing detection of specific enzymes.[29–31] Enzymes capable of transforming cellulose are of great importance for economic

activities, eg, in wood processing, paper production, and textiles. Nowadays, bioconversion of straw into fuel, fodder, foodstuffs, and semiproducts is considered to be one of the key problems of biotechnology in the chemical and microbiological industries.[1,6,10,32–36]

Every year, significant amounts of unused straw biomass are produced as waste products from agricultural production on the fields of Kyrgyzstan, and could be transformed into useful biomass for feeding animals. Enzymes are usually derived from mesophile and thermophile micro-organisms which are more suitable for fermentation. Mesophiles are obtained found easily from natural sources. Enzymes obtained from thermophiles have suitable properties for the industry because they are thermostable. Industrial companies are now interested in finding useful extremophiles, including cold-adapted micro-organisms. In addition, from the viewpoint of energy-saving and environmental protection, cold-active enzymes are preferable to energy-consuming mesophilic and thermophilic enzymes.[18,37]

In this study, we have screened the ability of fungal strains isolated from alpine and mountainous soils to produce cellulolytic enzymes for use in the bioconversion of waste products into valuable biomass products.

Materials and methods

Microorganisms and culture maintenance

Wild-type fungal strains of *Aspergillus*, *Penicillium*, and *Trichoderma* were isolated from different biotopes in Kyrgysztan and stored in the microbiology laboratory collection at Kyrgyz-Turkish Manas University (see Table 1). Stock cultures were maintained at 4 °C on potato-dextrose agar for 2–12 weeks. In all experiments, spores from stock slants were used as the initial inoculum.

Screening of wild-type fungal strains for cellulolytic activity

The endoglucanases are commonly assayed by viscosity reductions in carboxymethyl cellulose solution.[19] The cellulolytic activity of fungal strains was determined by their ability to grow and form cleared zones around colonies on selective medium with 0.1% sodium carboxymethyl cellulose. The surface of the media with the developed fungi colonies was mixed with 0.1% Congo Red and incubated for 15 minutes at room temperature. The dye was then removed and NaCl 1 M solution was added, followed by incubation for a further 10 minutes at room temperature. We then measured the ratio of the diameter of the clear zone to the diameter of the colony.

For primary screening of the cellulolytic activity of the fungi, we also used the following method: a sterile ashless filter was put on the surfaces of the fungi colonies grown on Hutchinson agar (K_2HPO_4, 1.0; $CaCl_2$ 0.1; $MgSO_4$ 0.3; NaCl 0.1; $FeCL_3$ 0.01;and $NaNO_3$ 2.5) at pH 5.6–6.0 pressed onto the agar plate. To avoid desiccation, the plates were kept in a moist incubation chamber at 26–27 °C for 1–2 weeks. Cellulolytic activity was estimated by the extent of destruction of the strips of filter paper.

Table 1. Natural sites and soil types for sample selection and isolation of fungi.

Place of sample selection	Collection number of strain	Soil type	Taxonomy
At Bashy valley, a foothill zone, 2020 m	KP-4	Brightly chestnut	*Aspergillus* sp
At Bashy valley, subalpine zone, 3020 m	BS-4	Dark chestnut	*Aspergillus* sp
Kadjisay town, quartz mineral processing mine (400 m from the center), 2000 m	K-2-25	Gray-brown stony deserted soils	*Penicillium* sp
Shor Bulak, Ton region, 1700 m	Sh-4	Meadow salty, dark gray soils	*Penicillium* sp
Granary, flour-grinding	Asp-1	Grain wheat	*Aspergillus flavus*
Chuy valley, arable ground	T-22	Gray soils, arable layer under crops	*Trichoderma lignorium*
Bodies of plants	T-20	Wild plants	*Trichoderma* sp
1 km from Ak-Tuz ore factory, 1800 m	5H-3	Dark chestnut	*Aspergillus* sp
2 km from Ak-Tuz ore factory, 1780 m	P-2	Dark chestnut	*Cladosporium* sp

Solid-state fermentation

The fungal cultures were grown by solid-state fermentation consisting of 99.0% agricultural residues, ie, wheat bran, beet peels, and cotton oil cake, with nitrogen 1.0%, NH_4NO_3 0.1%, $NH_4H_2PO_4$ 0.1%, and $MgSO_4 \cdot 7H_2O$ 0.1%, at pH 5.3. Five gram samples of this medium were put into 500 mL bottles, autoclaved dry (121 °C, one hour), and inoculated with 50 mL of active cell suspension from 48-hour cultures grown in shake flasks containing Capek's medium at 30 °C with shaking at 150 rpm (Lab Companion® digital incubator/refrigerated shaker, Korea). The inoculated lignocellulose substrate was then distributed on the inside surface of the bottle by gentle rolling on a bench top. Inoculated cultures in replicates of three and noninoculated controls dampened with sterile medium were incubated at 30 °C and 67% humidity for 14 days, and harvested after prespecified periods of time.

Preparation of crude enzyme extract

After each 48 hours of solid-state fermentation, the growing mycelium fungus was harvested by adding 100 mL of acetate buffer (pH 5.0), mixing, and filtering under vacuum and centrifugation. The supernatant was used as a crude enzyme extract to assay for extracellular cellulase.

Enzyme assays

Endoglucanase with carboxymethyl cellulose

Endoglucanase was assayed using low-viscosity carboxymethyl cellulose (Sigma-Aldrich Chemie GmbH, Munich) as the substrate. A total of 3 mL of sodium carboxymethyl cellulose 1.667% (wt/vol) in 0.067 M phosphate buffer (pH 6.0) was added to a test tube containing 2.0 mL of unconcentrated culture supernatant. The tubes were incubated at 45 °C for 0, two, four, and six minutes. The reaction was stopped by adding 3.0 mL of 3,5-dinitrosalicylate reagent (Sigma-Aldrich Chemie GmbH). The tubes containing 3,5-dinitrosalicylate were then boiled for 20 minutes, and 1.0 mL of (wt/vol) sodium potassium tartrate 40% was added to each tube. The tubes were then cooled, and the A575 was recorded. The amount of sugar released was calculated based on a standard glucose curve. One unit of enzyme activity represents the amount of enzyme which produced 1 μmol of reduced sugar per minute.

Endoglucanase with cellulose substrate

This method was based on hydrolysis of straw polysaccharides by the enzyme, with subsequent determination of reducing sugar. Cellulase activity describes the ability of the enzyme to catalyze the splitting of straw polysaccharides to glucose under the experimental conditions. One unit of cellulase activity is the quantity of enzyme which, for one hour at 50 °C, catalyzes the splitting of 1 mg of straw polysaccharides under the experimental conditions.

We added 50 mg of finely crushed wheat straw to 5 mL of buffer enzyme extract. The mixture obtained was kept in a water bath at 50 °C for one hour, and the incubated mix was then filtered through a No 3 or No 4 filter. The amount of reducing sugar released was detected using a modification of the method described by Somogyi-Nelson.[38] One milliliter of the incubated mixture obtained as a result of straw hydrolysis was added to 1 mL of Somogyi reactant and heated in a boiling water bath for 20 minutes. We quickly cooled the tubes and added 1 mL of Nelson reactant. The A660 was recorded. The buffer enzyme extract was measured for light absorption, and the amount of reducing sugar was calculated from the standard glucose curve formula:

$$X = \frac{a * b}{y}$$

where X = unit of activity, a = amount of reducing sugar on light absorption, b = dilution of the aqueous extract of the enzyme, and y = number of enzymes. One unit of enzyme activity was represented by the amount of enzyme which produced 1 μmol of reduced sugar. The biomass of hydrolysis of lignocellulose substrate was calculated by a weight method. The aqueous extract of raw biomass diluted in acetate buffer (pH 5.0) was used as the negative control.

All results were obtained after three-fold repetition of experiments. The probability of obtaining the observed data under the null hypothesis was calculated by the method of McDonald.[39]

Results and discussion

Cultures of *Aspergillus*, *Penicillium*, and *Trichoderma* (see Table 1) isolated from various natural sites in Kyrgyzstan were used in this study.

Screening of wild-type fungal strains for cellulolytic activity

The cellulolytic activity of the fungal strains was determined according to their ability to grow and form clear zones around fungal colonies in a sodium carboxymethyl cellulose 0.1% medium. Some fungal isolates (K-25, KP-4, Sh-4) were able to grow on selective medium with sodium carboxymethyl cellulose 0.1% and to show a clear zone around the colonies, but the ratio of the diameter of the clear zone to the diameter of the colony was not significant (Fig. 1). Therefore, we used a simple screening method to select the active strains for their ability to destroy the sterile filter paper used as the cellulose substrate. A sterile ashless filter was put on the surface of the fungal colonies grown on Hutchinson agar.

Of all the strains tested, the fungal isolates T-22, KP-4, and K-2-25 showed the highest cellulolytic activity (Fig. 2). These strains were able to destroy 70%–90% of the filter paper in 25 days, and the KP-4 strain caused 100% decomposition of the filter paper, as shown in Figures 3 and 4.

Growth of fungal cultures on waste product surfaces

All fungal strains showed good growth, covering their nutrient substrates with mycelium. For example:

- T-22 (*Trichoderma*) formed a friable mycelium of dark green color (Fig. 5)
- Kp-4 (*Aspergillus*) formed a dense mycelium of bright bluish-turquoise color (Fig. 5)
- K-2-25 (*Penicillium*) formed a dense mycelium of blue color with a greenish shade substrate (Fig. 6)
- Asp-1 (*Aspergillus flavus*) formed a friable dense mycelium of grayish-red color (Fig. 6)
- T-20 (*Trichoderma*) formed a friable powder mycelium of dark-green color (Fig. 6)
- Sh-4 (*Penicillium*) formed a dense air mycelium of green-blue color (Fig. 6).
- The water extract obtained during this period was light-brown colored, passed quite quickly through a No 4 filter, and the mycelium in all variants was dense.

Activity of fungi on extracellular cellulase

Strains T-22, K-2-25, and Kp-4 synthesized extracellular cellulase after 48 hours of growth on the nutrient mix consisting of waste products. The enzymatic activity was variable. Production of extracellular cellulase over the 48-hour period by *Penicillium* strain K-2-25 was so high that the amount of glucose reduced to 1.6 µmol, but the other strains did not show such results, although the *T. lignorium* strain T-22 showed significant activity compared with the other strains, with glucose reduction of 1.4 µmol, and Kp-4-1 had the third highest activity at 0.9 µmol.

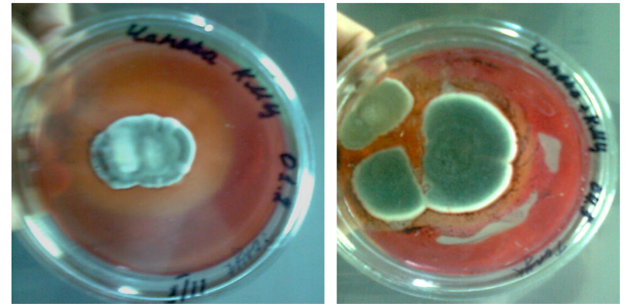

Figure 1. Clear zone around the fungal colonies on selective medium with 0.1% sodium carboxymethyl cellulose.

After 96 hours, the extracellular cellulolytic activity of the three strains, ie, T-22, Kp-4, and K-2-25, was decreased by almost two-fold compared with the activity after 48 hours of growth. At this time, the Asp-1 and Sh-4 strains showed the highest activity in comparison with the other cultures, and their cellulolytic activity was 0.78 µmol and 0.77 µmol of glucose reduction, respectively. After 144 hours, strain Sh-4 showed the highest activity, achieving a glucose reduction of 0.68 µmol. The other strains showed low activity. After 192 hours, the culture of Asp-1 had the highest activity at 0.7 µmol of glucose

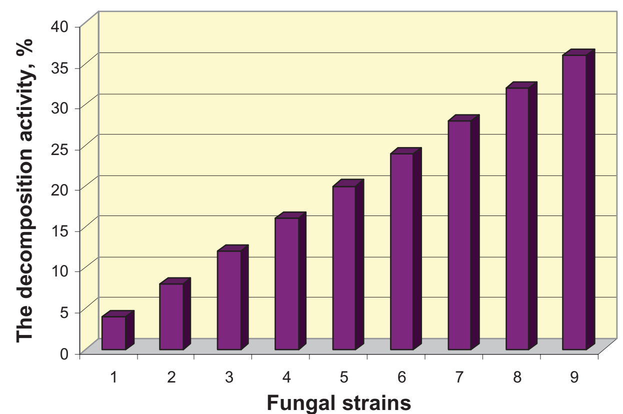

Figure 2. Decomposition activity of filter paper according to fungal strains 1-T-20 (*Trichoderma*), 2-Asp-1 (*Aspergillus*), 3-Sh-4 (*Penicillium*), 4–5H-3 (*Aspergillus*), 5-P-2 (*Cladosporium*); 6-BS-4 (*Aspergillus*), 7-T-22 (*Trichoderma*), 8-KP-4 (*Aspergillus*), and 9-K-2-25 (*Penicillium*).

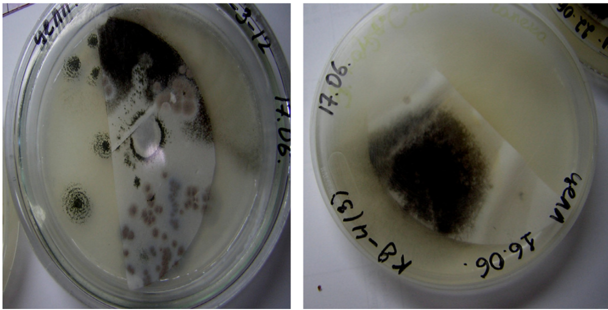

Figure 3. Colonization of Whatman filter paper by fungal mycelium two days after inoculation.

reduction. Strains Kp-4-1 and T-20 had cellulolytic activity of 0.6 and 0.7 µmol. The other cultures had low activity.

After 240 hours, it was observed that the biomass was diluted for all variants. It was especially appreciable in flasks containing strains Sh-4, Asp-1, and K-2-25. The biomass in flasks with strains T-22, Kp-4-1, and T-20 appeared to be increased on direct vision. For this incubation time, the *Penicillium* strain K-2-25 showed the highest results, at 1.4 µmol of reduced glucose, which is similar to the activity seen after 48 hours of growth. The *T. lignorium* strain T-22 and Kp-4-1 had identical glucose reduction activity at 0.800 µmol.

After 288 hours, full dilution was observed in flasks containing cultures of Asp-1, Sh-4, and 132.20, and the *Penicillium* strain K-2-25 showed higher activity than the other isolates, with a glucose reduction activity of 1.15 µmol.

After 336 hours, the Kp-4-1 culture showed higher activity than the others, with a glucose reduction activity of 1.0 µmol.

After 739 hours, all substrata with mycelium were washed off into porcelain cups and placed in an incubator at 55 °C, after which their cellulolytic activity

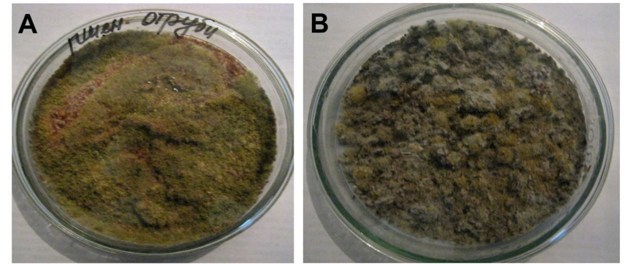

Figure 5. A) Growth of *Trichoderma* strain T-22 and **B**) growth of *Aspergillus* strain Kp-4 on waste products of agricultural production.

was defined. The Kp-4-1 and T-22 strains showed good results, and the activity of the *Penicillium* strain was 1.0 µmol, and was 0.9 µmol for the *Trichoderma* strain (Fig. 7).

The average cellulolytic activity of the fungi was calculated by summarizing the data obtained after 14 days of cultivation. The *Penicillium* strain K-2-25 had, on average, the most active cellulolytic activity. The amount of reduced glucose was 945 mg, corresponding to the unit of activity determined by the formula. The *T. lignorium* strain T-22 took second position, with 835 mg of reduced glucose (Fig. 8).

Thus, our data show that the strains investigated produced different amounts of extracellular cellulase, with varying activity within 14 days of cultivation on agricultural waste products. *Penicillium* strain K-2-25 was the most active producer of extracellular cellulase, the maximum output of which was observed after 48 hours of cultivation. However, after 96, 144, and 192 hours of cultivation, the output of the enzyme was low, which reflects features of the synthesis process and fungal metabolism. After 240 hours of growth, high activity was observed again for K-2-25, with production of a significant amount of the enzyme. After 288 hours, the cellulolytic activity of this enzyme in culture remained at a higher level

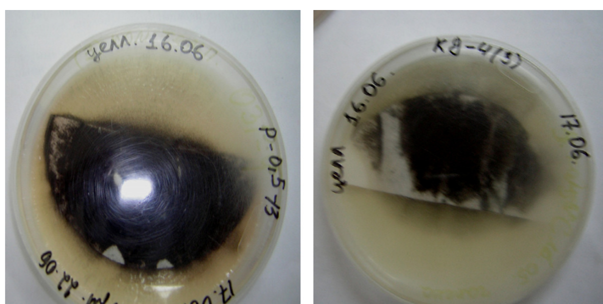

Figure 4. Decomposition of Whatman filter paper 25 days after inoculation with *Penicillium* sp isolate K-2-25 and *Aspergillus* sp isolate K P-4.

Figure 6. Growth of Asper-1 (*Aspergillus*), K-2-25 (*Aspergillus*), Sh-4 (*Penicillium*), and T-20 (*Trichoderma*) on waste products of agricultural production.

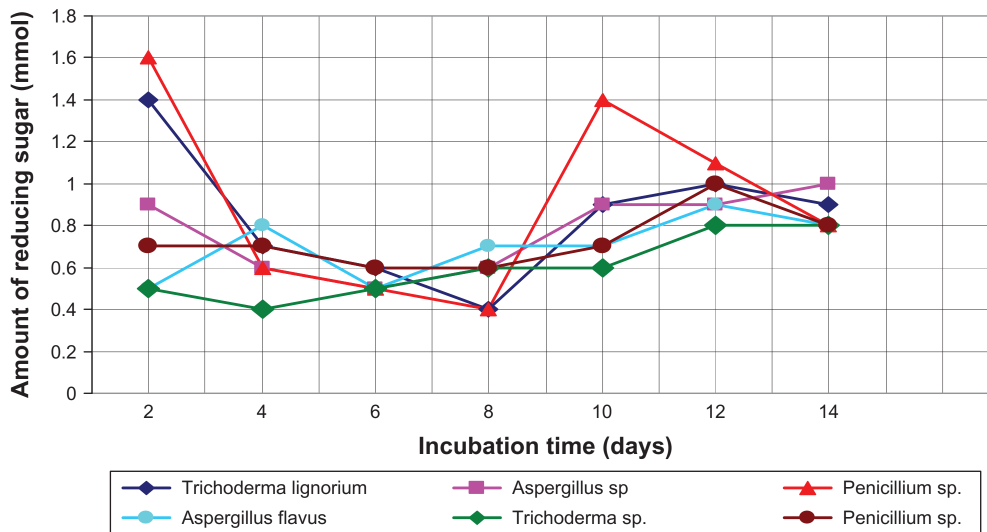

Figure 7. Cellulolytic activity of fungi according to the amount of glucose reduced in each 48 hours over 14 days.

in comparison with the other strains. A gradual recession of enzyme activity was seen on the last experimental day for this culture.

T. lignorium strain T-22 also showed significant cellulolytic activity, taking second position after K-2-25. This strain also produced extracellular cellulase, with variable activity according to duration of cultivation. After 48 hours, the activity of T-22 was quite high after K-2-25 but, on other days, its activity increased or decreased. The average sum of the data obtained showed that the T-22 strain took second position to K-2-25 for cellulolytic activity.

We compared the cellulolytic activity of our two active fungal strains with the cellulolytic activity of already well-studied strains (Table 2). The highest activity was shown by a mutant strain of *Trichoderma viride* QM9123, which reduced 3.65 mg glucose for 24 days,[37,40] while unidentified strains of *Trichoderma*, T6 and T1, reduced 1.96 and 0.69 mg glucose for 24 days.[41] According to other authors,[42] *T. viride* reduced 1.52 mg of glucose for 35 days. The same authors also studied the cellulolytic activity of fungi from *Aspergillus and Penicillium* genera. *Aspergillus terreus* reduced 1.60 mg of glucose, while *Aspergillus flavus* reduced 0.33 mg of glucose for 35 days. Our selected natural strains, ie, *Penicillium* sp K-2-25 and *T. lignorium* T-22 reduced 945 mg and 835 mg of glucose, respectively, for 14 days .The time taken for fermentation to occur was almost half than that reported by other authors.[41,42]

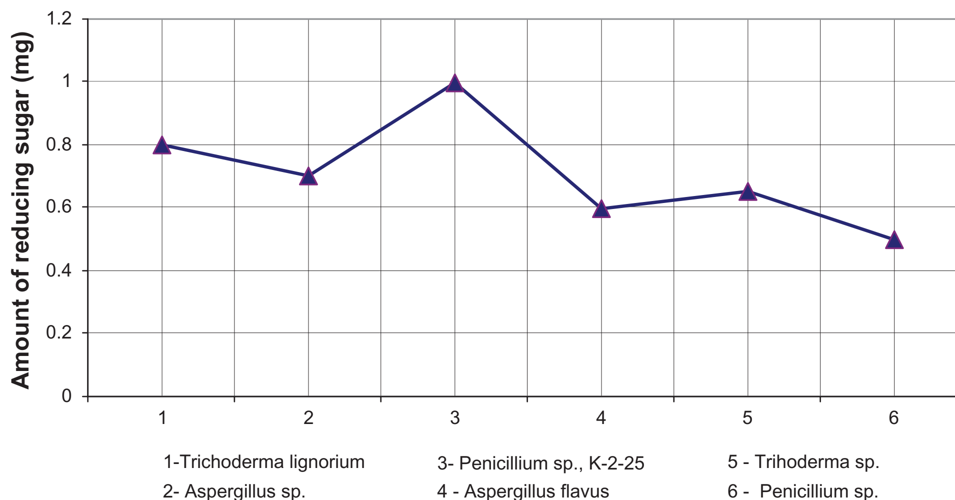

Figure 8. An average cellulolytic activity of fungi on the amount of glucose reduced for 14 days.

Table 2. A comparison of cellulolytic activity of our tested strains with well-studied strains of *Trichoderma*, *Penicillium*, and *Aspergillus* genera.

Strains of *Trichoderma, Penicillium,* and *Aspergillus* genera	Cellulolytıc activity as mg of glucose using the procedure of Mandels and Weber[39]	Cellulolytıc activity as mg of glucose using the procedure of Poincelot and Day[31]	Cellulolytıc activity as mg of glucose using the procedure of Rautela and Cowling[36]	Cellulolytıc activity as mg of glucose in the present study
Trichoderma viride QM9123	3.65 for 24 days			
Unidentified strains of *Trichoderma,* *T*6 and T1		1.96 for 24 days 0.69 for 24 days		
Trichoderma viride QM 6a			1.52 for 35 days	
Penicillium capsulatum QM 26			0.00 for 35 days	
Aspergillus flavus link QMIOe			0.33 for 35 days	
Aspergillus terreus QM 82j			1.60 for 35 days	
Trichoderma lignorium strain T-22				945 mg for 14 days
Penicillium sp strain K-2-25				835 mg for 14 days

On the basis of these observations, our results confirm that the cellulolytic activity of the selected strains was not less than that of the well-known natural strains, and certainly less than the activity of mutant strains. Thus, the activity of our strain T-22 *(T. lignorium)* was almost equal to the activity of *Trichoderma* T6, which showed a 1.96 mg glucose reduction over 24 days.[41] According to the authors,[42] their *Penicillium* strains did not show any cellulolytic activity, whereas our studies have identified a natural isolate of *Penicillium* showing high activity even in comparison with *Trichoderma* strains. Our isolate of *Penicillium* sp, K-2-25, had the highest potential (945 mg of reduced glucose) for cellulolytic activity among the *Penicillium* genera. Our results identified two fungal strains, ie, K-2-25 and T-22, which are suitable for biotechnological use, especially in bioconversion of poorly decomposable vegetable waste products, for example, straw.

Discussion

Our planet is facing an energy crisis as a result of using nonrenewable energy resources, such as coal, oil, and gas. Their supplies are not unlimited, and will be consumed in a finite period of time. Various branches of science are conducting research on renewable energy resources. One of the most important resources in this regard is biomass, a renewable organic substance generated from photosynthesis by plants and algae. Thus, exploitation of biomass as an energy resource allows us to not only conserve nonrenewable sources of energy, but also to preserve biodiversity in general.

In turn, microbiological science brings knowledge that can dramatically enhance the efficiency of the biomass trough towards a vegetable organism on the one hand, and can transform plant waste into productive biomass on the other. Vegetable biomass is a renewable and available source of raw material.

The grain industry in Kyrgyzstan produces a valuable biomass of straw as a waste product every year. However, this potentially valuable biomass is lost due to lack of adequate technology for processing into productive bioresources. Straw is mainly composed of cellulose, a high molecular weight insoluble polymer of glucose, which is not decomposable by animal enzymes. However, it is a perfect substrate for microbiological fermentation processes, that allows homogeneous, high-quality, and inexpensive raw fodder to be obtained from straw. The action of

cellulolytic enzymes is key to the biological degradation of cellulose.

The basic sources of enzymes that hydrolyze polysaccharides are the various fungi which secrete enzymes into the environment. *Trichoderma*, *Aspergillus*, and *Penicillium* constitute a group of micro-organisms that produce the cellulase which hydrolyzes cellulose. Extracellular enzymes can be produced in liquid or solid media, although the use of solid media enables rapid screening of large populations of fungi, which is the best way to detect specific enzymes.

We isolated fungi from a number of different types of material, and screened a large number of fungi to determine their ability to degrade the polysaccharides present in biomass and to produce protein or higher value products. Strains K-2-25 and T-22 were identified to have high cellulolytic activity from 17 tested strains of *Trichoderma*, *Aspergillus*, and *Penicillium*, most of which were isolated from cold and extreme habitats. We isolated enzymes expressed by cold-adapted micro-organisms living in high alpine regions and glaciers which had never been screened before. Industrial companies are now very interested in useful extremophiles, including cold-adapted micro-organisms. In addition, from the viewpoint of energy conservation and environmental protection, cold-active enzymes are more attractive than energy-consuming mesophilic and thermophilic enzymes.

Major obstacles to the exploitation of enzymes for various industrial needs are their yield, stability, specificity, and production costs. New enzymes are required to have desirable biochemical and physicochemical characteristics and a low cost of production

Solid-state fermentation was carried out on a medium composed of agricultural waste products, including wheat bran, beet peels, cotton oilcake, and additional nitrogen and mineral elements. The activity of enzyme production by the fungal isolates was determined after 14 days of cultivation. *Penicillium* strain K-2-25 demonstrated maximum activity after 48 hours of cultivation. *Trichoderma lignorium* strain T-22 also showed significant cellulolytic activity, taking second position after the K-2-25 strain.

The results of this study have enabled us to select two fungal strains suitable for biotechnological applications, especially for bioconversion of poorly decomposable vegetable waste products like straw. There are thousands of tons of straw left as waste products after a wheat crop in the many fields of our country, which can be transformed into useful biomass.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

References

1. Perez J, Munoz-Dorado T, De la Rubia J, Martınez J. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: An overview. *Int Microbiol*. 2002;5:53–63.
2. Vallander L, Erikson KE. Enzymatic recirculation in saccharification of lignocellulosic materials. *Enzym Microb Technol*. 1987;9:714–20.
3. Shoemaker S, Schweickart V, Ladner M, et al. Molecular cloning of exocellobiohydrolase I derived from *Trichoderma reesei* strain L27. *Bio/Technol*. 1983;1:691–6.
4. Jeffries TW. Biodegradation of lignin and hemicelluloses. In: Ratledge C, editor. *Biochemistry of Microbial Degradation*. Dordrecht: Kluwer; 1994.
5. Beguin P, Aubert JP. The biological degradation of cellulose. *FEMS Microbiol Rev*. 1994;13:25–58.
6. Chandrakant P, Bisaria VS. Simultaneous bioconversion of cellulose and hemicellulose to ethanol. *Crit Rev Biotechnol*. 1998;18:295–331.
7. Cullen D, Kersten P. Fungal enzymes for lignocellulose degradation. In: Kinghorn JR, Turner G, editoris. *Applied Molecular Genetics of Filamentous Fungi*. New York, NY: Chapman & Hall; .Chapter 4. 1992.
8. Kirk TK, Higuchi T, Chang H, editors. *Lignin Biodegradation and Microbiology, Chemistry, and Potential Applications*. *Volume 1*. Boca Raton, FL: CRC Press Inc; 1980.
9. Kirk K, Cullen D. Enzymology and molecular genetics of wood degradation by white rot fungi. In: Young RA, Akhtar M, editors. *Environmentally Friendly Technologies for the Pulp And Paper Industry*. New York, NY: Wiley & Sons; 1998.
10. Lee J. Biological conversion of lignocellulosic biomass to ethanol. *J Biotechnol*. 1997;56:1–24.
11. Raudonene B, Varnayte R. Bioconversion of waste rye crops by micromycetes. *Bıologıja*. 1997;4:43–7.
12. Whyman CE, Goodman BJ. Biotechnology for production of fuels, chemicals and materials from biomass. *Appl Biochem Biotechnol*. 1993;39:41–59.
13. Leschine SB. Cellulose degradation in anaerobic environments. *Annu Rev Microbiol*. 1995;49:399–426.
14. Deshpande V, Eriksson KE, Pettersson B. Production, purification and partial characterisation of 1,4-β-glucosidase enzymes from Sporotrichum pulverulentum. (1978). *Eur J Biochemistry*. 1978;90:191–8.
15. Gong CS, Chen LF, Trao GT. Affinity chromatography of endoglucanase of *Trichoderma viride* by concanavalin A-agarose. *Biotechnol Bioeng*. 1979; 21:167–71.
16. Crawford DL. Lignocellulose degradation by selected *Streptomyces* strains. *Appl Environ Microbiol*. 1978;35:1041–5.
17. Deobald LA, Crawford DL. Activities of cellulase and other extracellular enzymes during lignin solubilization by *Streptomyces viridosporus*. *Appl Microbiol Biotechnol*. 1987;26:158–63.
18. Romanelli A, Houston CW, Barnett SM. Studies on thermophilic cellulolytic fungi. *Appl Microbiol*. 1975;30:276–81.

19. Thompson DP, Eribo BE. Extracellular enzyme production by *Rhizopus and Mucor* species on solid media. *Can J Microbiol*. 1984;30:126–8.
20. Crawford DL, Barder MJ, Pometto AL IIIrd, Crawford RL. Chemistry of softwood lignin degradation by a Streptomyces. *Arch Microbiol*. 1982; 131:140–5.
21. Saloheimo M, Lehtovaara P, Penttila M, et al. EGIII, a new endoglucanase from *Trichoderma reesei*: The characterization of both gene and enzyme. *Gene*. 1988;63:11–21.
22. Teeri T, Salovuori I, Knowles JK. The molecular cloning of the major cellulase gene from Trichoderma reesei. *Bio/Technology*. 1983;1:696–9.
23. Teeri T, Lehtovaara P, Kauppinen S, Salovuori I, Knowles JK. Homologous domains in *Trichoderma reesei* cellulolytic enzymes: Gene sequence and expression of cellobiohydrolase II. *Gene*. 1987;51:43–52.
24. Wynne ES, Pemberton JM. Cloning of a gene cluster from *Cellvibrio mixtus* which codes for cellulase, chitinase, amylase, and pectinase. *Appl Environ Microbiol*. 1986;52:1362–7.
25. Chen CM, Gritzali M, Stafford DW. Nucleotide sequence and deduced primary structure of cellobiohydrolase II from *Trichoderma reesei*. *Biotechnology (N Y)*. 1987;5:274–8.
26. Penttila M, Lehtovaara P, Nevalainen H, Bhikabhai R, Knowles J. Homology between cellulase genes of *Trichoderma reesei*: Complete nucleotide sequence of the endoglucanase I gene. *Gene*. 1986;45:253–63.
27. Sternberg D. Beta-glucosidase of Trichoderma: Its biosynthesis and role in saccharification of cellulose. *Appl Envir Microbiology*. 1976;31:648–54.
28. Barnett CC, Berka RM, Fowler T. Cloning and amplification of the gene encoding an extracellular glucosidase from *Trichoderma reesei*: Evidence for improved rates of saccharification of cellulosic substrates. *Biotechnology*. 1991;9:562–7.
29. Hankin L, Anagnostakis SL. The use of solid media for detection of enzyme production by fungi. *Mycology*. 1975;67:597–606.
30. Wood TM, McCrae SL. The cellulase of *Trichoderma koningii*. Purification and properties of some endoglucanase components with special reference to their action on cellulose when acting alone and in synergism with the cellobiohydrolase. *Bıochem J*. 1978;171:61–72.
31. Zare-Maivan H, Shearer CA. Extracellular enzyme production and cell wall degradation by freshwater lignicolous fungi. *Mycology*. 1988;80:365–75.
32. Adler E. Lignin chemistry—past, present and future. *Wood Sci Technol*. 1977;11:169–218.
33. Aleksandrov GP, Pertov AN, Medvedova SA, Babkin BA. The selection of lignin degradation fungi for biotechnology process. *J Appl Biochem Microbiol*. 1998;34:270–5.
34. Mikalidze GG, Keshelashvili NG, Sharashenidze NN. The development of food protein from symbiosis cultivation of yeasts and mycelium fungi on plant wastes of agriculture production. In: *Use the Biomass of Microorganisms for Food Aims* Pushino: Scientific Center on Biological Research of Academy Science of the USSR; 1985.
35. Ohgiya S, Hoshino T, Okuyama H, Tanaka S, Ishizaki K. Biotechnology of enzymes from cold adapted microorganisms. In: Margesin R, Franz Schinner F, editors. *Biotechnological Applications of Cold-Adapted Organisms*. Berlin: Springer-Verlag; 1999.
36. Reshetnikov IA. *Fungi Mycelium as a Source of Feed and Food Protein*. Moscow: Publishing House of Moscow State University; 1989.
37. Mandels M, Weber J. Cellulases and their applications. In: Gould RF, editor. *Advances in Chemistry Series 95*. Washington, DC: American Chemical Society; 1969.
38. Somogyi M. A new reagent for the determination of sugars. *J Biol Chem*. 1952;195:19–23.
39. McDonald JH. *Handbook of Biological Statistics*. 2nd ed. Baltimore, MD: Sparky House Publishing; 2009.
40. Mandels M, Weber J, Parizek R. Enhanced cellulase production by a mutant of *Trichoderma viride*. *Appl Microbiol*. 1971;21:152–4.
41. Poincelot RP, Day PR. Simple dye release assay for determining cellulolytic activity of fungi. *Appl Microbiol*. 1972;23:875–9.
42. Rautela GS, Cowling EB. Simple cultural test for relative cellulolytic activity of fungi. *Appl Microbiol*. 1966;14:892–8.

Klesov AA, Rabinovich ML, Sinizin AP, Griroposh SU, Churila IB. *Bioorganic Chemistry*. 1980;6:1225–42.

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