Research Article

Biodegradation Potential of StecchérinumOchraceum: Growth on Different Wood Types and Preliminary Evaluation of Enzymatic Activities

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Abstract. White-rot fungi isa source of a great variety of oxidative and hydrolytic enzymes suitable for biotechnological applications, e.g. in pulp and paper, textile and food industries, bioethanol production, degradation of recalcitrant environmental pollutants,and others. *Steccherinumochraceum* is a xylotrophicwhite-rot basidiomycetethat can be found in variousclimatic zones on different woody substrates (mostly well decayed). For this research, seventeenstrains of *S. ochraceum*were collected in different regions of Russia from various wood substrates (aspen, alder, oak, hazel, birch and willow). Phylogeneticanalyseswere performedbasedon the nucleotide sequences of ITS1, ITS2, 5.8S rRNA, 28S rRNA, β-tubulin and *tef1*.Oxidaseandcellulaseactivitieswereassessedbyplate-tests with ABTS and CMC. Forevaluation of biodegradation potential,solid state fermentation on alder and pine sawdust wasperformed. Weightanddensitylossaswellas the C:Nratioweremeasuredafter 90 days of cultivation.All *S. ochraceum* strains exhibited high oxidative activity towards ABTS, indicating secretion of oxidative enzymes (i.e. laccases and class II peroxidases). Cellulase activity was medium or low for most strains and in some strains – absent. Allstrainswereabletodegradealderandpinesawdust. There was no correlation between the enzymatic activity, biodegradation potential and geographic origin of *S. ochraceum* strains. However, *S. ochraceum* strains isolated from the same wood substrates exhibited similar characteristics in most cases. Strain LE-BIN 3398 was the most effective for degrading both alder and pine sawdust and could be regarded as a promising source of oxidative enzymes for biotechnology.

Keywords: basidiomycetes, biodegradation, solid state fermentation, oxidase activity, *Steccherinumochraceum*

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1. Introduction

White-rot fungi are the only organisms which can degrade all components of the plant cell wall including lignin. Plant cell wall decomposition is performed by a complex enzymatic system consisting of ligninases, cellulases and accessory enzymes[1]. Many of these enzymes are of a great interest for biotechnological applications, e.g. in pulp and paper, textile and food industries, in bioethanol production, for degradation of recalcitrant environmental pollutants and others[2–5].

The composition and properties of fungal enzymatic systems participating inlignocellulose degradation reflects not only the taxonomy of fungi but also the ecological niche which they occupy [6]. Therefore, when searching for producers of certain enzymes, not only systematic position of fungus should be taken into account, but also its life-style.

In addition, it should be noted that different strains of the same fungus may differ significantly in the level of secretion of the target enzyme. For example, various isolates of fungi from the genus Pycnoporusdemonstrated order of magnitude difference in the level of laccase activity in the culture liquid[7].

Steccherinumochraceum(Pers.) Gray is a white rot basidiomyceteoccurring in different regions of Russia. Being a secondary colonizer,thisfungususually occupies stumps, trunks, and fallen branches of various deciduous trees at the late decay stages. Previously, we reported *de novo* assembly and annotation of the genome of *S. ochraceum* strain LE-BIN 3174 and studied the features of its genome and enzymatic system reflecting the adaptation of *S. ochraceum*topre-degraded wood as a substrate [8]. Moreover, *S. ochraceum*and another two fungi from this genus *S. bourdotii*and *S. murashkinskyi*were reported as active producers of laccases with remarkably high thermostability and wide substrate specificity [9–12].

The aim of the present work was to study the biodegradation potential of different strains of *S. ochraceum* collected from various substrates in different regions of Russia and to evaluate their oxidase and cellulase activities.

TABLE 1: Theorigins and substrates of*S. ochraceum*strains.

Strain LE-BIN	Geographicalorigin	Substrate
1994	Russia, PrimorskyKrai	Deadfallenbranch
2134	Russia, RostovOblast	Dead fallen branch of deciduous tree
2203	Russia, PskovOblast	Deadfallenoakbranch
2286	Russia, AltaiKrai	Decayedpine
2296	Russia, AltaiKrai	Fallendeadwillow
3090	Russia, PrimorskyKrai	Decayedoakbranch
3120	Russia, OryolOblast	Fallendeadoak
3174	Russia, KalugaOblast	Dead fallen dry aspen branch
3398	Russia, VolgogradOblast	Dead fallen branch of black alder
3577	Russia, BryanskOblast	Fallendeadhazel
3611	Russia, KurskOblast	Fallendeadhazel
3617	Russia, KurskOblast	Fallendeadhazel
3622	Russia, KurskOblast	Fallendeadaspen
3824	Russia, LipetskOblast	Deadfallenbirchbranch
3827	Russia, LipetskOblast	Deadfallenhazeltrunk
3836	Russia, LipetskOblast	Dead fallen branch of deciduous tree
3870	Russia, Kazan	Deadfallenhazelbranch

2. Methods

2.1. Fungal strains

The fungal strains of *S. ochraceum*were obtained from KomarovBotanical Institute Basidiomycetes Culture Collection (LE-BIN; St. Petersburg, Russia)and kept on wort agar slants at 4 ^{o}C. The origins and the substrates from which *S. ochraceum* strains were collected are given in Table 1.

Theorigins and substrates of*S. ochraceum*strains.

2.2. DNA extraction, PCR amplification and phylogenetic analysis

For nucleic acid extraction, fungal mycelium was ground in liquid nitrogen. Total DNA extraction was performed using DNeasy Plant Mini Kit (Qiagen, USA).

The sequences of ITS1, ITS2, 5.8S rRNA, 28S rRNAribosomalloci were PCR amplified using standard oligonucleotide primers: ITS1F 5'–CTT GGT CAT TTA GAG GAA GTA A–3' and LR12 5'–GACTTAGAGGCGTTCAG –3'. The sequences of β-tubulinwere PCR amplified with F-btub1 [5'-CARGCYGGTCARTGYGGTAACCA-3'] and F-btub2r [5'-GGRATCCAYTCRACRAA-3'] primers; and the sequences *tef1*□ were PCR amplified with

EF1-983F [5'-GCYCCYGGHCAYCGTGAYTTYAT-3'] and
EF1-1567R [5'-ACHGTRCCRATACCACCRATCTT-3'] primers. PCR amplification of ribosomal loci was performed using the Taq PCR kit (Evrogen, Russia) under the following conditions: 1 cycle of 5 min at 95 °C; 25 cycles of (1 min at 95 °C, 1 min at 56 °C, and 3 min at 72 °C); 1 cycle of 5 min at 72 °C.For amplification of β-tubulin and *tef1* the extension time was 1 min with all the other parameters unaltered. PCR products were purified from agarose gel by a QIAquick Gel Extraction Kit (Qiagen, USA) and sequenced using the standard Sanger sequencing method.

The phylogenetic analysis was performed as described in [11].

2.3. Assessment of biodegradation potential

Forbiodegradationpotentialstudy solid state fermentation of *S.ochraceum*strains at wooden sawdust wasperformed in 750 mL flasks.Alder (15 g)and pine(10 g) sawdust 1-3 mm fractions with addition of 10 ml of distilled water were used as substrates. Flasks were inoculated with 20 days old cultures of *S.ochraceum*strains (25 mL). Cultivation was performed at 26°C under static conditions for 90 days. Every 30 days 15 mL of distilled water was added into each flask to maintain the moisture of the substrate. For weight anddensitydeterminationsawdustwasdriedtotheconstantweightat 70 °C. Carbon and nitrogen content was determined using Carlo Erba CHN 1500 elemental analyzer (Carlo-Erba Instruments, Italy) according to the manufacturer's protocol.

2.4. Plate tests

Plate tests for oxidase and cellulase activities of *S. ochraceum*strains were performed as described in [8]after 14 days of cultivation at MEA medium.

2.5. Principal component analysis

Principal component analysis was performed using R package factoextra, (http://www.sthda.com/english/rpkgs/factoextra).

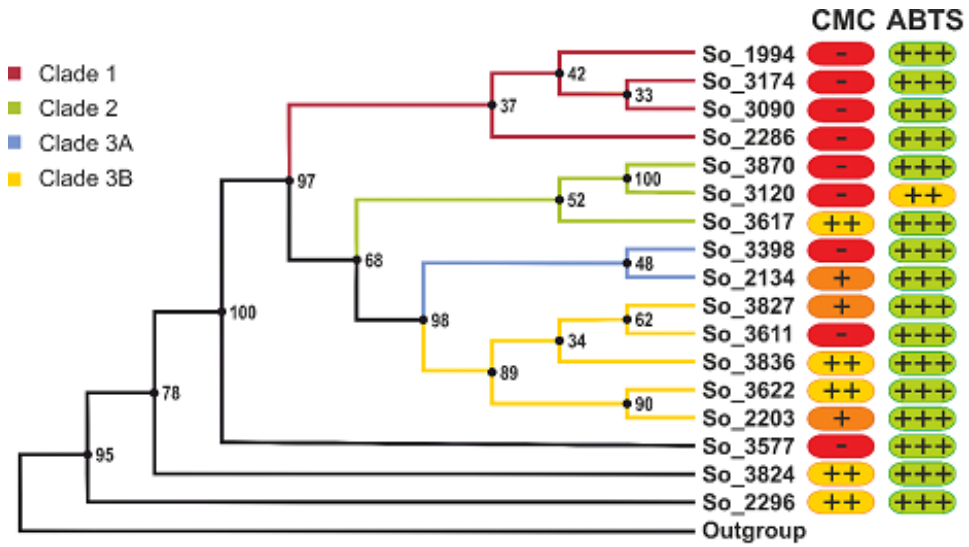

Figure 1: The phylogenetic analysis and plate test results..

3. Results

3.1. Phylogenetic analysis and assessment of overall oxidase and cellulase activities.

Toassesswhetherphylogenetic relationships among the strains of *S. ochraceum* correlate with their geographic origin and overall oxidase and cellulase activities, the multilocus phylogenetic analysis was performed, and mentioned activities were determined. For phylogenetic analysis the following 6 genetic loci were used: ITS1, ITS2, 5.8S rRNA, 28S rRNA, β-tubulin and *tef1*.The oxidase and cellulase activities were assessed by application method during solid-phase cultivation on MEA medium.

The multilocus phylogenetic analysis allowed determining several evolutionarily groups within the same species of *S. ochraceum* (Figure 1). Almost all *S. ochraceum* strains fell into one of three clades on the phylogenetic tree (i.e. 1, 2, 3A, and 3B). All the studied strains were characterized by the high oxidase activity. However, fungi from clade 1 and clade 2 completely lacked the cellulase activity, with an exception of strain So 3617 from clade 2 that demonstrate medium activity toward CMC. Almost all fungi included in the clade 3A and clade 3B exhibited low to medium cellulase activity.

As a result, no apparent relationshipswere found between the geographical origin of the strains, their phylogenyandenzymatic activities.

Table 2: Characteristics of alder and pine sawdust after 90 days of fungal growth.

Strain	Weight loss, %		Ash, %		C:N		Density, g/m^3	
	alder	pine	alder	pine	alder	pine	alder	pine
Control	0	0	0.42	0,23	64.4	338.1	209	120
S.o. 1994	13	12	0.98	0.75	117.5	250.4	138	102
S.o. 2203	12.6	10	0.87	0.76	110.7	183.3	139	107
S.o. 2296	22.6	0	1.02	0.81	122.8	150.5	124	120
S.o. 3090	12.6	10	0.8	0.7	132.3	236.7	140	106
S.o. 3174	16.6	10	0.92	0.68	155.6	199.8	133	106
S.o. 3398	21.3	15	0.94	0.76	127.2	173.6	124	100
S.o. 3617	16	12	0.84	0.73	132.4	172.4	134	103
S.o. 3824	16.6	10	0.94	0.74	125.1	250.79	132	107
S.o. 3827	16.6	10	0.94	0.76	126.8	192.1	131	106
S.o. 3870	16.6	14	0.9	0.8	122.4	206.8	132	105
S.o. 3622	22	12	1.05	0.86	137.5	199.7	124	102

3.2. Assessment of wood biodegradation potential

To assess the biodegradation potential, 11 strains isolated from different tree species and belonging to different phylogenetic clades (Figure 1) were selected. The biodegradation potential was assessed using sawdust from two types of wood – alder (deciduous) and pine (coniferous). The data regarding weight loss, ash content, carbon to nitrogen ratio and density of studied sawdust are present in Table 2.

For all the strains, the weight loss of alder sawdust varied from 12.6 to 22 %, while the weight loss of pine sawdust was, on average, lowerandcomprised10-15 %. The greatest weight loss of alder sawdust was observed for the So 2296, So3398 and So 3622 strains. In case of pine sawdust, strains So 3398 and So 3870 were the most effective, whileforSo 2296 strain no weight loss of sawdust was observed. For the alder sawdust the C:N ratio increased by 2-2.5 times, and for the pine sawdust – decreased by 1.5-2 times. Density of the dried sawdust decreases 1.5-1.7 times for the alder and 1.1-1.2 times for the pinesawdust, respectively.

Thus, coniferous sawdust was less efficiently degraded than deciduous.This is consistent with the fact that *S. ochraceum* usually colonizes deciduous wood types and is rare on conifers.

Table 2:

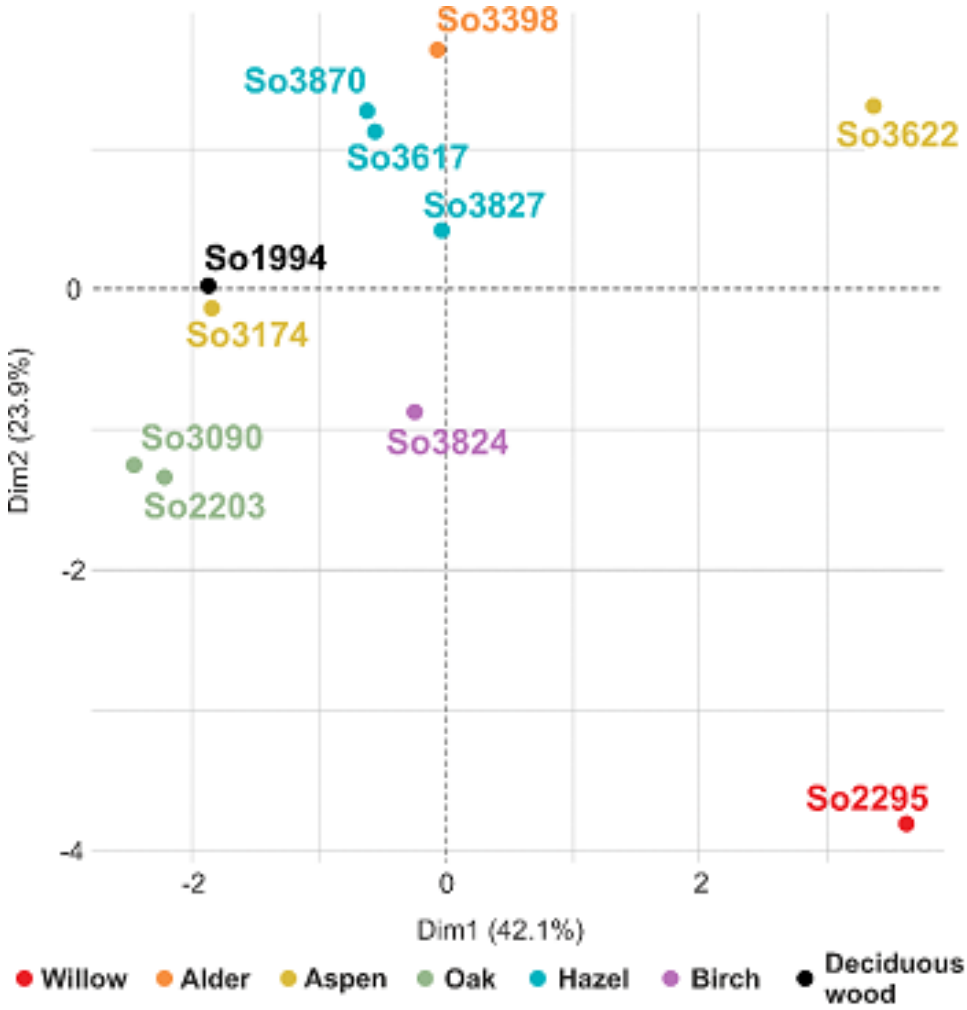

Figure 2: The principal component plot. Colors indicate the substrates from which the strains were collected.

3.3. The principal component analysis

Based on the data on the biodegradation of alder and pine sawdust, as well as activities in relation to ABTS and CMC, the principal component analysis (PCA) was carried out. On the PCA plot, the following *S. ochraceum*strains collected from the same substrate were grouped: 2203 and 3090 collected from oak and 3827, 3617, 3870 and 3398, growing naturally on hazel and alder. The exceptions were *S. ochraceum*3622 and 3174 collected from aspen which did not group with each other. Two strains *S. ochraceum* 3824 and *S. ochraceum* 2296 growing in nature on birch and willow were distant both from other groups and from each other.

4. Conclusions

Seventeenstrains of *S. ochraceum* collected in different regions of Russia from various wood substrates (aspen, alder, oak, hazel, birch and willow) were studied. All *S. ochraceum* strains exhibited high oxidative activity towards ABTS indicating secretion of oxidative enzymes (i.e. laccases and class II peroxidases). Cellulase activity of most *S. ochraceum* strains was medium or low and in some strains it was absent.

There was no correlation between the enzymatic activity, biodegradation potential and geographic origin of *S. ochraceum* strains. However, *S. ochraceum* strains isolated from the same wood substrates exhibited similar characteristics in most cases. *S. ochraceum* strain LE-BIN 3398 was the most effective for degradation both conifer and deciduous sawdust and could be regarded as a promising source of oxidative enzymes for biotechnology.

5. Funding

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