Novel autochthonous fungi for the treatment of lignocellulosic biomass

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Lignocellulosic biomass has become one of the most explored renewable substrates for the production of many valuable chemicals, biofuels, and food or feed ingredients or products. However, a complex structure blocs its direct utilization and directs to utilization of particular pretreatments in order to remove lignin and facilitate the biomass hydrolysis prior to its further processing. The pretreatments could be done by chemical, physical, physicochemical means, or using biological methods. Biological methods involving the use of fungi or their enzymes in the pretreatment and hydrolysis of the lignocellulosic substrate are ecologically friendly, rather time consuming, but with no formation of harmless components that can inhibit the production microorganism (Nair and Sivakumar, 2020). The limitations, such as process longevity and loss of valuable sugars, can be alleviated or overcome by utilization of novel efficient microorganisms, e.g. fungi which are selective biomass decomposers, as well as by optimizing conditions of the pretreatment.

In this study, a potential use of novel isolated Serbian autochthonous fungi in the pretreatment of lignocellulosic biomass was studied. Isolates were collected in the southern Serbia near the City of Leskovac. The strains were isolated from stumps, fallen trees and branches, fallen leaves, or living trees found in the oak forest and orchards around the forest. Among 12 isolated fungi, the isolates identified as *Trametes hirsuta* F13 and *Stereum gausapatum* F28 stood out as ligninolytic enzyme producers and were selected for potential use in the pretreatment of waste lignocellulosic biomass (beechwood sawdust). Table 1. shows biomass and lignin reduction and selectivity coefficients relative to the total lignin reduction (selectivity coefficient 1), and relative to the Klason's lignin reduction (selectivity coefficient 2) after 35 days of incubation under non optimized conditions. More lignin was degraded by *T. hirsuta* F13 than *S. gausapatum* F28: its selectivity coefficient, relative to the total lignin, was 1.7, while the selectivity coefficient obtained for *S. gausapatum* was 1.1. The values of the selectivity coefficient, relative to Klason's lignin, were 1.47 for the isolate F13 and 0.8 for the isolate F28, which confirmed that *T. hirsuta* F13 was the best candidate among the isolated fungi for use for the biomass pretreatment.

Table. 1. Reduction of biomass and lignin by the selected fungi.

Monitored parameter	Fungal isolate	
	T. hirshuta F13	*S.gauspatum* F28
Biomass reduction (%)	19	24
Total lignin reduction (%)	33.8	28
Selectivity coefficient 1	1.7	1.1
Klason's lignin reduction (%)	28	19
Selectivity coefficient 2	1.47	0.8

An isolate identified as *Myrmaecium fulvopruinatum* F14 showed high hydrolytic activity, but negligible ligninolytic activity, and was selected as a potential producer of important industrial hydrolytic enzymes. The three novel fungal isolates with pronounced lignocellulolytic activities were identified using ITS sequences, and the sequences were deposited in the NCBI GenBank database. Their accession numbers are KY264754.1 (*Trametes hirsuta* F13), KY264753.1 (*Stereum gausapatum* F28), and MF521930.1 (*Myrmaecium fulvopruinatum* F28) (Jović *et al*., 2018).

The isolate *T. hirsuta* F13 which was selected as the best candidate for the pretreatment of lignocelulosic biomass was further studied in order to enhance its biomass degrading efficiency by optimizing the pretreatment conditions. It was found that the supplementation with sugar beet molasses stillage (MS) could significantly improve the biological pretreatment. The initial results showed that the addition of MS could improve ligninolytic activity, but also that the type of a dominant enzyme activity differed depending on the stillage concentration. Besides, other pretreatment parameters such as substrate moisture and temperature of the treatment were found important. The results have shown that the use of MS at the concentration of 13% as a supplement at the temperature of 25 ^{0}C and substrate moisture of 63% can shorten the time of the biological

pretreatment from 35 to 18 days, while the selectivity of biomass degradation was also improved of (Jović *et al*., 2020).

Finally, this study examined and selected three novel autochthones fungal isolates, *T. hirsuta* F13, *M. fulvopruinatum* F14, and *S. gausapatum* F28, which can be used in the production of industrially important lignocellulolytic enzymes and/or in biomass pretreatment under determined conditions.

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