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Pretreatment of Agro-Industrial Wastes with Basidial Fungi Strains for Effective Delignification of Lignocellulosic Wastes

GIORGI KVESITADZE[1,2,*], NINO TSIKLAURI[1], LALI KUTATELADZE[1], TAMAR URUSHADZE[1], IZOLDA KHOKHASHVILI[1], TINATIN SADUNISHVILI[1]

[1]Sergi Durmishidze Institute of Biochemistry and Biotechnology, Agricultural University of Georgia, 0159 Tbilisi, Georgia
[2]Georgian National Academy of Sciences, 0108 Tbilisi, Georgia
*corresponding author: kvesitadze@hotmail.com

Abstract Lignocellulosic biomass, including agro-industrial wastes, is an ideal cheap and abundant source of glucose for ethanol production. The complex structure of lignin makes it difficult to hydrolyze the biomass cellulose to sugars and other organic compounds. In recent years, it has become more and more important to develop an environmentally friendly pretreatment method for delignification of plant residues. The aim of the present work was to study the delignification of agro-industrial residues by higher basidial fungi. Solid state fermentation (SSF) of 35 tested strains on wheat, rice and corn straw, potato above-ground mass (AMP) and sunflower lignocellulosic waste (SLW) showed different levels of oxidase enzyme production. In particular, laccase activity varied from 0.5 to 91.8 U/ml, and manganese peroxidase (MnP) activity from 0 to 2.1 U/ml, depending on the substrates and the type of the fungi. The best lignin destroyers were: *Trametes maxima* GK-02, *Ganoderma* sp. GV 51, *Pleurotus* sp. IN-93. The best delignification result was achieved with the strain *Pleurotus* sp. IN-93 in corn straw and wheat straw, from 17.8% to 7.7% and from 19.1% to 10.6%, respectively. In the case of delignification of the AMP, the amount of lignin decreased by 4.0-6.9%. *Trametes maxima* GK-02 was the most effective in case of SLW - lignin degradation from 19.7% to 10.6%.
Keywords: Agro-industrial wastes, Basidial fungi, Pre-treatment, Fermentation.

1. Introduction

Conversion of abundant lignocellulosic biomass to biofuels as transportation fuels presents a viable option for improving energy security and reducing greenhouse emissions (Kumar et al., 2009). Lignocellulose has been recognized as a potential and major source for biofuels and other value-added products (Elumalai et al., 2018). Agricultural wastes could be of valuable use in biofuel production as a sizeable quantity of simple sugars for the onward production of biofuel may be obtainable from them. Thus, rather than being allowed to constitute environmental pollutants, they can be used as an alternative source of biofuel (Fadeyi & Akiode, 2022). Pretreatment of lignocellulosic feedstock is an important method for biomass conversion processes. The goal of the pretreatment process is to break down the lignin structure and disrupt the crystalline structure of cellulose, so that the enzymes can easily access and hydrolyze the cellulose (Mosier et al., 2005). In recent years, the interest in application of mycelial fungi potential for biotechnological processes is significantly increased. Biological treatment using various types of rot fungi, a safe and environmentally friendly method, is increasingly being advocated as a process that does not require high energy for lignin removal from a lignocellulosic biomass, despite extensive lignin degradation (Okano et al., 2005). Lignin degradation by white-rot fungi occurs through the action of lignin-degrading enzymes such as peroxidases and laccase (Lee et al., 2007). Selection of proper plant raw materials, and of the basidial fungi strains, fermentation of which results in their best delignification is the aim of current research.

2. Methods

Higher Basidiomycetes strains available in the culture collection of the Institute of Biochemistry and Biotechnology, Agricultural University of Georgia, were used in this study. The production of lignocellulolytic enzymes by white-rot basidiomycetes species was investigated under the solid-state fermentation (SSF) on lignocellulosic materials. Available at the local and international market lignocellulosic wastes were dried at 60°C and milled to dust extent (<1 mm). Fungal inoculums were prepared on a rotary shaker at 180 rpm, at 27°C (Tsiklauri et al., 2014) and then were inoculated for SSF on different lignocellulosic residues.

Laccase activity was determined by monitoring the changes in A420 related to the rate of oxidation of 1 mM 2,2-azino-bis-[3-ethylthiazoline-6-sulfonate] (ABTS) in 50 mM Na-acetate buffer (pH 3.8) (Bourbonnais & Paice, 1990). MnP activity was measured at 270 nm by following the formation of Mn3+-malonate-complexes (Wariishi et al. 1992). One unit of laccase or MnP activity was defined as the amount of the enzyme that leads to the oxidation of 1 μmol of the substrate per minute. Chemical Composition of Agro-industrial wastes was determined by weight method (Kumar et al., 2015).

3.

4. Results and discussion

3.1. Fungi enzymes activity

The ligninase enzyme production by the selected basidial fungi was studied while grown on wheat straw under SSF. Their various potentials were revealed in terms of accumulation both manganese peroxidase and laccase enzymes (Table 1). Activities of synthesized enzymes were tested on 6th, 15th and 20th days of cultivation; maximum amount was obtained at 15th day of cultivation. The indices of the enzymes production varied in rather wide range: laccase - 0.3-71.5 U/ml, manganese peroxidase - 0-1.77 U/ml. The best producers of manganese peroxidase were *Pleurotus* sp. IN-93 (1.77 U/ml) and *Ganoderma* sp. GV 51 (1.45 U/ml), while *Ganoderma* sp. GV 51 (71.5 U/ml), *Pleurotus drynus* IN 11 (38.3 U/ml) and *Trametes maxima* GK-02 (28. 4 U/ml) showed the best results for laccase. The strain *Ganoderma* sp. GV 51 showed a good productivity to both enzymes studied.

Table 1. ~~Title of the table~~ Accumulation of manganese peroxidase and laccase enzymes during SSF of wheat straw by basidial fungi

Fungus	**Laccase, U/ml**	**MnPase, U/ml**
Fomes fomentarius GK33	9.2	0
Fomitopsis pinicola IK45	**0.5**	0
Ganoderma applanatum IN08	13.5	0.33
Ganoderma lucidum IG74	15.3	0.18
Ganoderma sp. GV 51	**71.5**	1.45
Pseudotrametes gibbosa IK-78	12.6	0.29
Pleurotus ostreatus IN22	**0.5**	0.17
Pleurotus ostreatus GV12	2.7	0
Pleurotus sp. IN-93	38.3	**1.77**
Piptoporus betulinus IK-26	1.8	0
Trametes maxima GK-02	28. 4	**1.15**
Trametes maxima GK-15	21. 8	0.2

The fungus *Ganoderma* sp. GV 51 show best laccase producing ability on all tested substrates, while rice straw, corn straw and SLM were the best (Fig. 1). The higher Mn-peroxidase was synthesized by *Ganoderma* sp. GV 51and *Pleurotus* sp. IN-93. The best substrate for the *Pleurotus* sp. IN-93 was SLM, with enzyme activity 2.11 U/ml. For the *Ganoderma* sp. GV 51 the best substrate was AMP - 1.85 U/ml.

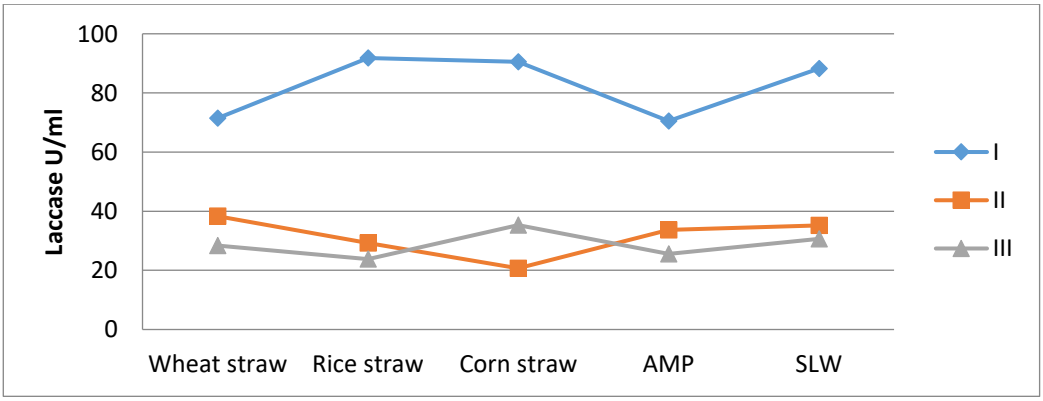

Figure1. Laccase activity of *Ganoderma* sp. GV 51 (I)*; Pleurotus* sp. IN-93 (II)*; Trametes maxima* GK-02 (III) while SSF on lignocellulosic wastes.

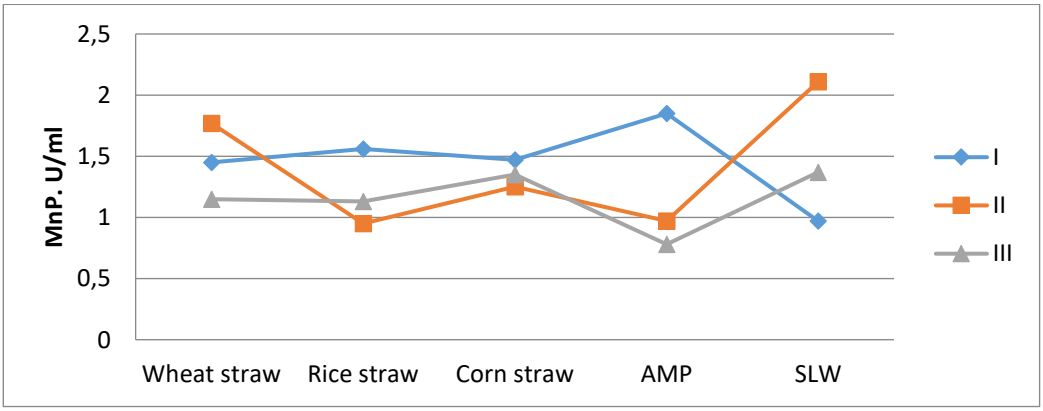

Figure 2. MnP activity of *Ganoderma* sp. GV 51 (I)*; Pleurotus* sp. IN-93 (II)*;*

Trametes maxima GK-02 (III) while SSF on lignocellulosic wastes.

3.2 Evaluation of delignification effect in biomasses treated with basidial fungi

Pleurotus sp. IN-93 was found to be a better destructor of lignin in wheat straw; its amount decreased from 19.1% to 10.6% (Fig. 3). In the case of rice straw, a 40% reduction of lignin was obtained in biomass fermented with *Ganoderma* sp. GV 51 (Fig. 4). The best delignification results were found in the biomasses while cultivation of both fungi strains on corn straw (Fig. 3, 4). In the case of *Pleurotus* sp. IN-93, lignin decreased from 17.8% to 7.7%, while in case of *Ganoderma* sp. GV 51, lignin decreased to 6.5%. A significant delignification ability of SLM was revealed in biomass fermented with *Pleurotus* sp. IN-93, from 19.7% to 8.6% (Fig. 3). *T. maxima* GK-02, which was also a good producer of laccase, showed a comparatively low delignification ability; it was the most effective in case of SLW - lignin degradation from 19.7% to 10.6% (Fig. 5).

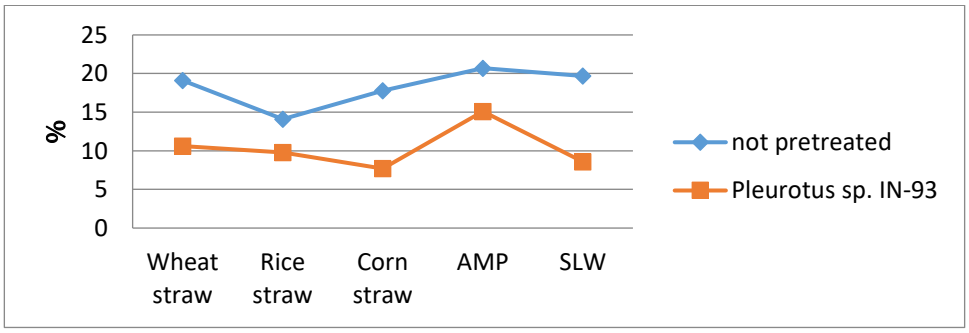

Figure 3. Delignification of lignocellulosic wastes pretreated with *Pleurotus* sp. IN-93 by means of SSF during 20 days.

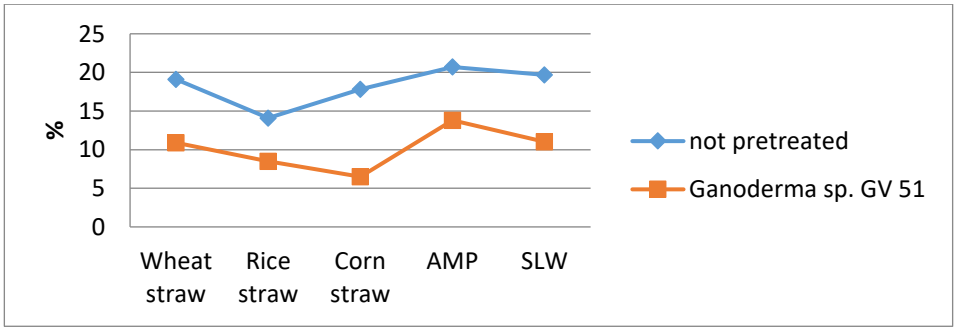

Figure 4. Delignification of lignocellulosic wastes pretreated with *Ganoderma* sp. GV 51 by means of SSF during 20 days.

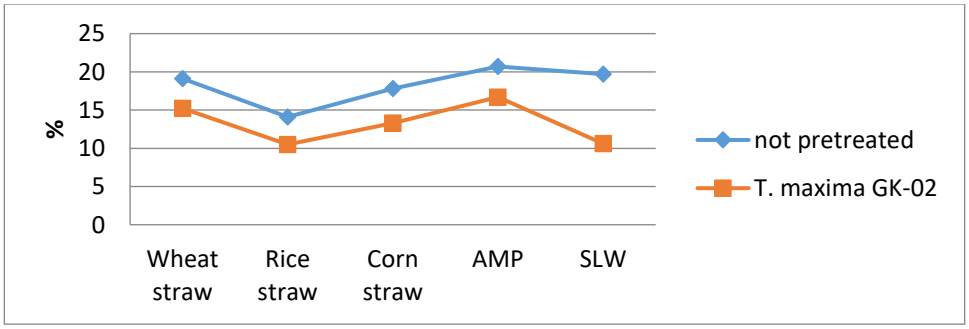

Figure 5. Delignification of lignocellulosic wastes pretreated with *T. maxima* GK-02 by means of SSF during 20 days.

Conclusion

The bio-conversion of lignocellulosic biomass by industrial enzymes is a potential sustainable approach to develop value-added bio-products. An important step in this process is pretreatment for the substrates delignification. ~~Out of tested basidial fungi strains, *Pleurotus* sp. IN 93 and *Ganoderma* sp. GV 51 have been revealed as effective delignifiers of wheat, rice and corn straw, AMP and SLW.~~ Technologically important basidial fungi strains *Pleurotus* sp. IN-93 and *Ganoderma* sp. GV 51 have been selected, which reduced the lignin content in wheat, rice and corn straw, AMP and SLW by 2.0-2.5 times after 20 days SSF. Further improvement of the process is possible by means of optimization of the fermentation conditions.

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