Conversion of agro-industrial wastes into non-toxic protein-rich food additives by basidial fungi strains isolated from diverse ecosystems of Georgia

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Abstract Great interest to basidial fungi is determined by their potential to convert number of lignocellulosic wastes into nontoxic compounds, cheap food, pharmaceuticals, etc. The majority of basidial fungi strains are characterized by high activity of enzymes degrading plant biopolymers, including lignin, which attracts special attention because of their use in different industries and medicine. Among 53 tested basidial fungi strains best results were obtained by *Pleurotus drynus* IN11 and *Ganoderma* sp. GV02 strains while growing on orange wastes, where 20% and 27% of protein, respectively, and up to 50% of carbohydrates were accumulated. After 10 - 20 days of solid state fermentation (SSF) in the same biomass the content of lignin was detected only in trace amounts. The content of microelements was (mg/kg): calcium 1200 - 6200, potassium 2100 - 2800, and sodium 90 - 708. Further investigation of strains allowed selection of active producers of soluble polysaccharides among which *Pleurotus ostreotus* and the representatives of genus *Ganoderma* sp. (10 - 14 g/L) were the most active. During the submerged fermentation (SF) the highest potential to produce extracellular antioxidants were detected in several strains of *Ganoderma* sp. (up to 6 g/L). Some selected *Ganoderma* strains expressed very high laccase extracellular activity (about 125000 U/L).

Keywords: agro-industrial wastes, converstion, basidial fungi, protein-rich food additives.

1. Introduction

Basidiomycetes is a special group of organisms capable of degrading lignocellulose and consequently forming biologically active compounds (Huang et al., 2008). In recent years much attention has been paid to the production of bioactive compounds from white rot basidiomycetes. Many of their species are used as a source of food and pharmaceuticals (Roupas et al., 2012; Cohen et al., 2014). Basidiomycetes mushrooms are characterized by content of high protein, all essential amino acids, vitamins, micro- and macro elements, carbohydrates, low fat and no cholesterol (Liu et al., 2012). Even more important is the fact that it is possible to grow mushrooms at low prices on cheap lignocellulosic materials and obtain high quality dietary protein and biomass rich in bioactive compounds for direct human consumption (Panjabrao et al., 2007). In the mentioned processes, the role of white rot basidial fungi capable of synthesizing hydrolyzing and oxidizing enzymes, which play an important role in degradation of polysaccharides and lignin of plant biomass, should be especially noted (Huang et al., 2008). Effective enzymatic hydrolysis is one of the major prerequisites in the development of a successful lignocellulosic biorefinery (Dey et al., 2021).

2. Methods

Basidial fungi strains, collected from different ecological niches of Georgia and available on market lignocellulosic wastes were applied in experiments. All residues were dried at 50°C and milled to dust extent (<1 mm). Fungal inocula for SSF and SF of different lignocellulosic wastes were prepared on a rotary shaker at 180 rpm, at 27°C (Tsiklauri et al., 2014). Carboxymethyl cellulase (CMCase) activity was assayed according to IUPAC recommendations (Ghose, 1987). Xylanase activity was determined by the method of Bailey et al., 1992. Laccase activity was determined according to Bourbonnais, 1990. Protein concentration in the biomass was determined by the Kjeldahl method with Nessler's reagent. Antioxidant activity was determined spectrophotometrically (FRAP) (Benzie, 1996).

3. Results and discussion

3.1 Effect of the lignocellulosic substrates on fungi enzymes activity

Effect of various lignocellulosic substrates on the accumulation of enzymes by the basidiomycetes strains was studied. Preliminary selected 53 strains of basidiomycetes different genera and species were checked on their enzymes biosynthetic ability. While SF of these strains on orange peel substrates their various potentials were revealed in terms of accumulation both hydrolyzing (CMCase, xylanase) and oxidizing (laccase) enzymes (Table 1). *Pleurotus ostreatus* GK10 was found to be the best producer of hydrolyzing enzymes – CMCase and xylanase with activities 15.4 U/ml and 26.7 U/ml, respectively. As for laccase, the highest activity (108.5 U/) showed the strain *Ganoderma* sp. GV 02 (Table 1).

Basidiomycetes strains of the genus *Pleurotus* were the best producers of the hydrolyzing enzymes while growing on substrates: mandarin peels, orange peels, wheat bran and tea peels (Fig. 1, 2).

Ganoderma sp GV-02 proved to be the best laccase producer while cultivation on almost all tested lignocelluloses with, the highest activity 125.7 U/ ml when grown on mandarin peels.

Table 1. Basidiomycetes enzymes activities, (U/ml) during submerged fermentation on orange peels

Fungus	Laccase	CMCase	Xylanase
Ganoderma applanatum IN18	13.5	9.2	8.4
Ganoderma sp. GM 04	2.1	4.0	6.1
Ganoderma sp. GV01	98.7	5.1	8.2
Ganoderma sp. G02	108.5	3.4	7.5
Ganoderma lucidum IG74	1.6	3.5	5.3
Ganoderma sp. IN59	15.3	2.2	11.5
Pleurotus ostreatus IN22	0.5	8.2	12.5
Pleurotus ostreatus GV12	2.7	9.2	13.1
Pleurotus ostreatus GK10	1.3	15.4	26.7
Pleurotus ostreatus GK52	1.5	13.6	20.8
Pleurotus drynus IN 11	39.8	3.2	7.3
Pleurotus sp. GD41	0.9	5.4	9.6
Pleurotus sp. ID 20	1.9	4.0	10.1

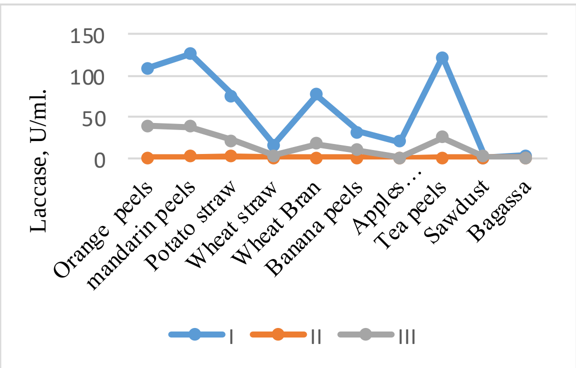

Fig. 1. Effect of lignocellulosic substrates on the laccase activity while SF of: I - *Ganoderma* sp. GV - 02; II - *P. ostreatus* GK10; III - *P. drynus* IN 11.

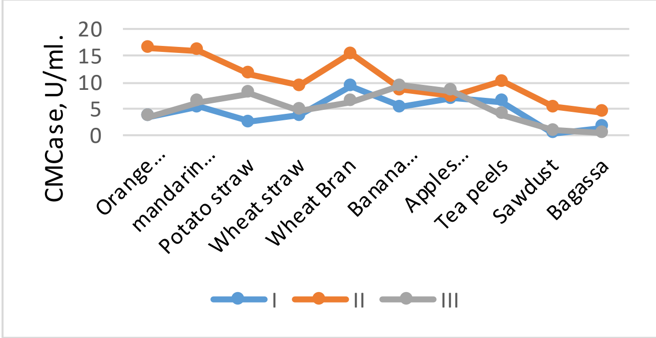

Fig. 2. Effect of lignocellulosic substrates on the CMCase activity while SF of: I - *Ganoderma* sp GV -02; II - *P. ostreatus* GK10; III - *P. drynus* IN 11.

3.2 Effect of carbon sources on fungi antioxidant activity

There is a great demand in new, natural antioxidants in pharmaceutical biotechnology. In the last years special attention is paid to the antioxidants of the fungi fruiting bodies or the fungal extracts. Total antioxidant activity of the selected three strains during the SF was studied in culture liquids and dried mycelia. Higher antioxidant activities were obtained in hot water extracts (s compared to alcohol extracts) of the dry micelia. The highest antioxidant activities were observed during the fungi SF with lignocellulosic substrates as a source of carbon in the media. Maximum total antioxidant activities were shown for *P. ostreatus* GK10 and *P. drynus* IN11 in case of wheat straw – 6mg/ml. For *Ganoderma* sp. GV02, mandarin peels and tea peels were the best substrates (Fig. 3).

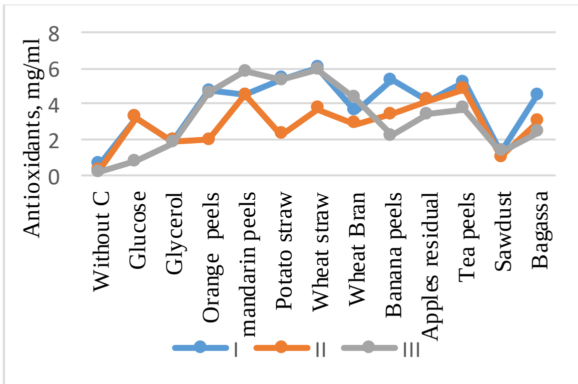

Fig. 3. Total antioxidant activity of hot-water extracts of dry mycelia during the SF on different lignocel-lulosic substrates, (mg/ml). I - *Ganoderma* sp. GV -02; II - *P. ostreatus* GK10; III - *P. drynus* IN 11.

3.3 Effect of the carbon source on fungi extracellular polysaccharides synthesis

Basidial fungi are known as producers of antitumor compounds, such as polysaccharides. The carbon is the major component of nutrient medium in a cultivation process, which must ensure the best growth of microorganisms and the highest yield of the desired product. In this study 12 different carbon sources were tested on the exopolysaccharides (EPS) synthesis by the selected basidiomycetes strains. *Ganoderma* sp. GV02 showed rather high EPS accumulation - 14.0 mg/ml on mandarin peels; *Pleurotus ostreatus* GK10 and *Pleurotus drynus* IN11 accumulated the highest amount of EPS on wheat straw: 10.0 mg/ml and 11.3 mg/ml, respectively (Fig. 4).

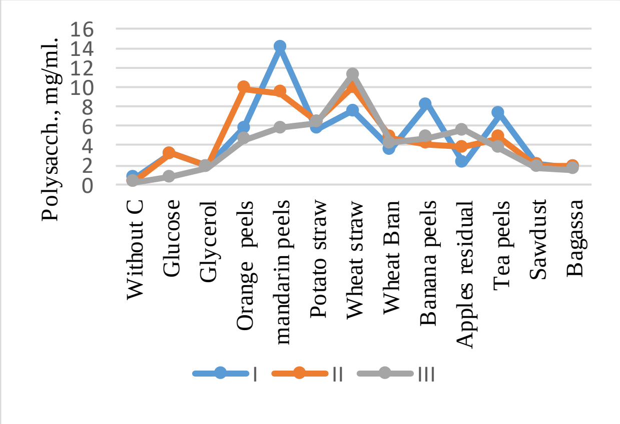

Fig. 4. Extracellular polysaccharides accumulation during the SF on different lignocellulosic substrates, mg/ml: I - *Ganoderma* sp GV 02; II - *P. ostreatus* GK10; III- *P. drynus* IN11.

3.4 Effect of carbon sources on the accumulation of elements by basidial fungi

Content of Ca, Na and K were studied in 13 above listed fungi strains during the SSF on various lignocellulosic substrates. The content of the microelements on the basis of dry weight, mg/kg was as follows: Ca: 1200 - 6200, K: 2100 - 2800, Na: 90 – 708.

3.5 Effect of different lignocellulosic substrates on the production of protein-rich biomass by basidiomycetes

Accumulation of protein in the studied basidiomycetes biomasses during the SSF on different lignocellulosic substrates varied significantly. The best results were obtained by *Ganoderma* sp. GV02 and *P. drynus* IN11 on orange peels, mandarin peels and banana peels (Fig.5). As seen, the amount of protein in the best case is increased 10 times.

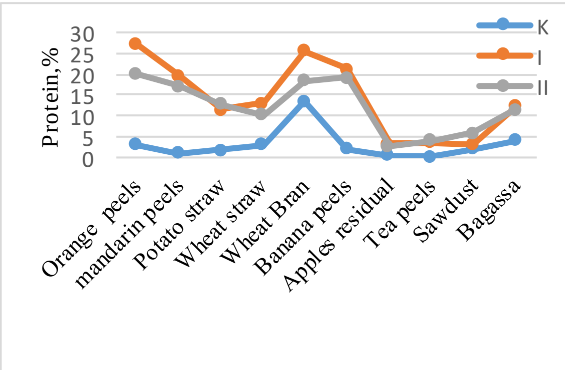

Fig. 5. Accumulation of protein by basidial fungi strains during the SSF on different lignocellulosic substrates, (%). I – Control (protein content in the lignocellulosic substrate), II - *Ganoderma* sp. GV -02; III - *P. drynus* IN11.

Conclusions

The studies revealed promising strains of the genera *Pleurotus* and *Ganoderma*, producers of lignocellulose deconstruction enzymes. The best producer of oxidative enzymes is *Ganoderma* sp. GV02; the best producer of hydrolizing enzymes is *Pleurotus ostreatus* GK10. Three selected basidiomycetes strains: *Ganoderma* sp. GV02, *P. ostreatus* GK10 and *P. drynus* N11 could be used for production of biomasses rich in polysaccharides, antioxidants, micro elements and protein from lignocellulosic residues, as cheap food additives.

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