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MICROWAVE-ASSISTED EXTRACTION OF SULFATED FUCANS FROM BROWN SEAWEED AND EVALUATION OF FUNGAL STRAINS FOR ENZYMES ACTIVE PRODUCTION TOWARD THIS CLASS OF POLYSACCHARIDE

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ABSTRACT

Sulphated polysaccharides from brown seaweedss comprise a complex group of macromolecules with a wide range of important biological properties such as anticoagulant, antioxidant, antiproliferative, antitumoral, anticomplementary, anti-inflammatory, antiviral, antipeptic and antiadhesive activities. Fucoidan is one of the main sulphated fucan, mostly interesting for their biological activities specially the potential to inhibit HIV reverse transcriptase and the possible application as active compound in antiretroviral drugs. However, algae remain largely unexploited and seaweeds can be found in sufficient amount for the commercial exploitation. Usually, most of the processes to recover sulfated polysaccharides from natural sources consist in acid extractions during long reaction times. Specific enzymes able to degrade fucoidan matrix (fucoidanases) are important tools to establish structural characteristics and biological functions of this polysaccharide. Such enzymes, have been only isolated from marine organisms. Reports of fungal microorganisms with enzymatic activity over this sulfated-polysaccharide are scarce. The aims of the present work were: 1) to recovery of sulphated polysaccharides (fucoidan) by microwave-assisted extraction under different operational conditions and 2) the identification of fungal strains able to growth over fucoidan-based media and to produce active fucoidanases.

MATERIALS AND METHODS

Microwave-assisted extraction

Fucus vesiculosus brown seaweed from North Portugal was used in the experiments. The extraction reactions were performed in a microwave digestion oven MDS-2000 (CEM Corporation), under different operational conditions: time (1, 11 and 31 min), pressure (30, 75 and 120 psi) and alga/water ratio (1/25, 3/25 and 5/25 g/ml), according to a 2^3 full factorial design. Total sugars content, monosaccharide composition and sulphated content were quantified

Screening of fungal strains

Aspergillus niger PSH, *Penicillium purpurogenum* GH2 and *Mucor sp.* 3P were the screened strains.. Fucoidan of *Laminaria japonica* and urea were used as carbon and nitrogen source. Radial growth rate (U_r) was kinetically monitored measuring colony diameters. Hyphal length (L_{av}) and diameter (D_h) were quantified by image analyses measurements.

Fermentation for culture media selection

Fermentation assays were performed with *Aspergillus niger* PSH. The tested media were Czapek Dox and Pontecorvo, supplemented with two carbon sources: a primary source composed by fucoidan *Laminaria japonica* (10 gL^{-1}) and a secondary source composed by glucose, sucrose, lactose, fructose or sodium acetate, (5 gL^{-1}); urea (5 gL^{-1}) was used as nitrogen supply. The experiments were carried out in 100 mL Erlenmeyer flasks at 140 rpm, 30 °C, and $1x10^6$ spores·ml^{-1} of conidial concentration. Biomass production, substrate consumption, extracellular and intracellular activity and protein content were evaluated. Kinetic parameters adjusted to Velhurst-Pearl and Luedeking y Piret models were estimated.

RESULTS AND DISCUSSION

Sulfated polysaccharide production

The percentage of fucoidan recovered and the total sugar yield were highest when using 120 psi, 1 min, and 1 g alga/25 ml water (18.22% and 27.62%, respectively). However, similar values were found when using 30 psi, 31 min, and 1 g alga/25 ml water (15.61% and 24.52%, respectively), which can be associated to hydrolysis effect measured by the severity parameter (Ro=2.14 and 2.12, respectively). Sulfate content in the hydrolysates was not affected by the operational conditions, maintaining values higher than 22%. Monosaccharide composition analyses showed mainly the presence of fucose, galactose and xylose.

Strains selection and hyphal growth measurements

Mycelial growth and morphology parameters of the selected molds are showed in Table 1. Strains showed a direct relation between the kinetic (Ur) and micrometrical parameters (Lav and Dh). Highest parameter values were obtained by cultivation of the Mucor 3P strain. Mineral salts addition to the media (CZ) enhanced germination and growth of germ tube. In CZ media, *Mucor* 3P, *A. niger* PSH, and P. *purpurogenum* GH2 presented elongation rates 33%, 12% and 29% higher than those obtained in MM media. Larger spores provided fast growing of thick hyphae (Mucor sp.), whereas the small spores of species produced slow growing of thin hyphae (*A*. PSH and *P*. GH2).

Table 1: Growth Parameters of Fungal Strains Cultivated on Fucoidan-Urea Petri Plates

Strain/ Media	U_r (µm h^{-1})	L_{av} (µm)	D_h (µm)	μ (h^{-1})
Mucor 3P/ MM	579.90 ± 0.01	251.63 ± 89.2	4.44 ± 0.89	0.40
Aspergillus PSH/ MM	350.43 ± 0.03	184.47 ± 48.1	4.41 ± 0.77	0.37
Penicillium GH2/ MM	136.93 ± 0.09	158.99 ± 66.07	3.40 ± 1.17	0.16
Mucor 3P/ CZ	755.07 ± 0.01	336.68 ± 107.69	7.05 ± 1.72	0.40
Aspergillus PSH/ CZ	390.67 ± 0.01	208.33 ± 61.77	5.37 ± 1.06	0.36
Penicillium GH2/ CZ	232.80 ± 0.16	206.29 ± 53.09	3.51 ± 0.80	0.19

Ur: Radial growth rate of hyphae; *Lav*: Length of hyphae; *Dh*: Diameter of hyphal tubules; µ: Specific growth rate. MM: minimal media, CZ: Czapek media

Effect of Combined Media on Induction of Fucoidan-Degrading Enzymes

Maximum biomass production (5.79 g L-1) was reached on Czapek medium supplemented with sucrose. Low mycelia production was using fucoidan as sole carbon source or supplemented with lactose or sodium acetate. Fucoidan hydrolytic enzymes were only expressed as extracellular metabolites. Enzyme activity was highest in the sucrose supplemented medium (2.77 U L-1) and in the medium containing fucoidan as sole carbon source (1.88 U L-1). On the contrary, metabolic parameters showed that the highest enzyme productivity were with sucrose-fucoidan and fucoidan as sole carbon source (Figure 1)

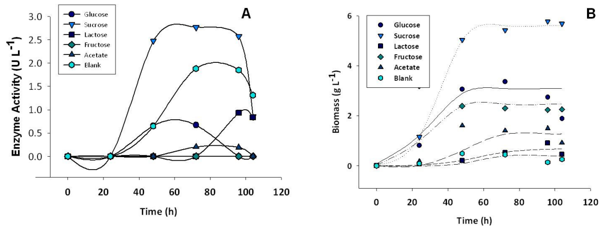

Figure 1: Biomass (A) and Enzyme Activity (B) with *A. niger* PSH over Combined Media.

CONCLUSIONS

Hydrothermal-microwave extraction, as a green technology, showed to be an effective method for sulfated polysaccharides recovery from brown seaweeds with shorter times than those reported in the literature. *Aspergillus niger PSH* has the capacity for synthesize fucoidan hydrolytic enzymes, being potential microbiology tools for used in fermentation process and microbial growth induction. These are the first results describing the production of enzymes from terrestrial fungus with ability to degrade fucoidan

REFERENCES

Bakunina, I. Y., Nedashkovskaya, O. I., Alekseeva, S. A., Ivanova, E. P., Romanenko, L. A., Gorshkova, N. M. 2002. Microbiology, 71, 41–47

Furukawa, S., Fijikawa, T., Koga, D., Ide, A. 1992. Bioscience, Biotechnology, and Biochemistry, 56, 1829–1834

Sakai, T., Kawai, T., & Kato, I. 2004. Marine Biotechnology, 6, 335–346

Urvantseva, A. M., Bakunina, I. Y., Nedashkovskaya, O. I., Kim, S. B., Zvyagintseva, T. N. 2006. Applied Biochemistry and Microbiology, 42, 484–491

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