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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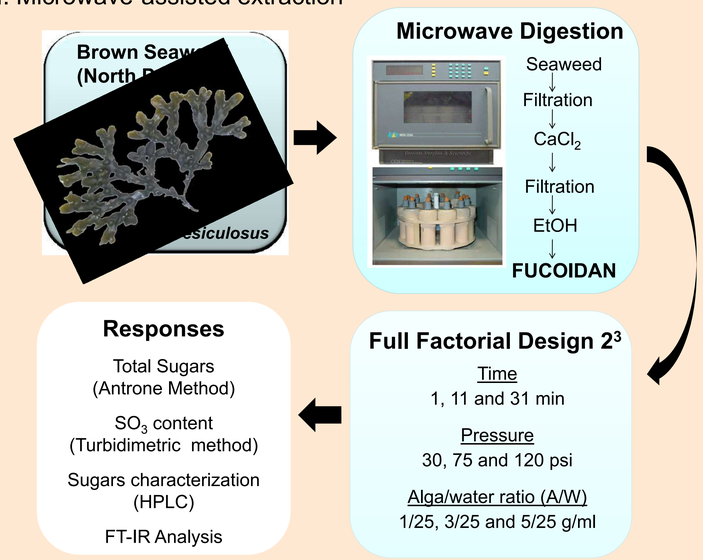
Introduction

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.

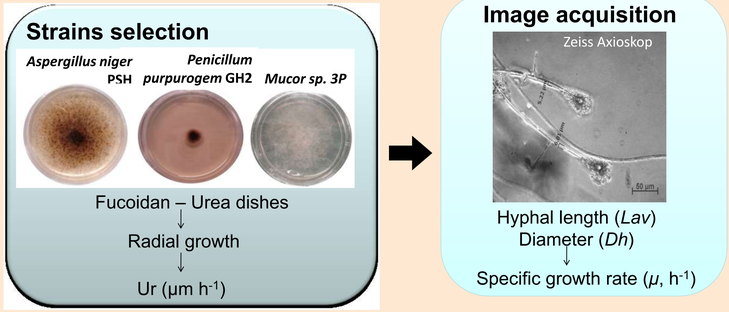
The aims of the present work were: 1) to recovery of sulphated polysaccharides (fucoidan) by microwave-assisted extraction under different operational conditions and 2) the identification of fungal strains able to growth over fucoidan-based media and to produce active fucoidanases.

Materials and Methods

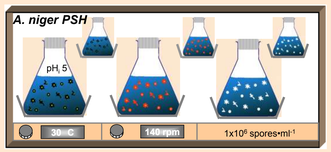
I. Microwave-assisted extraction



II. Screening of fungal strains



III. Fermentation for culture media selection



Media
- Czapek
- Pontecorvo

1st C Source
- Fucoidan (10 gL-1)

N2 Source
- Urea (5 gL-1)

2nd C Source
- Glucose,
- Sucrose
- Lactose
- Fructose
- Sodium Acetate (5 gL-1)

Variables
- Biomass
- Substrate consumption
- Extracellular activity
- Intracellular activity
- Protein content

Mathematical models
- Logistic equation
- Kinetic parameters

Results and Discussion

I. Sulfated polysaccharide production

Under the conditions evaluated significant differences ($p<0.05$) were found varying the pressure, time and alga/water relation levels (Table 1). The percentage of fucoidan recovered and the total sugar yield were highest when using 120 psi, 1 min, and 1 g seaweed/25 ml water.

Table 1. Recovery yields of *Fucus vesiculosus* fucoidan extraction by microwave

P (psi)	t (min)	A/W (g/ml)	Yield %TS-AM	%AH	%Fuc	%TS/alga	%SO3	% Fucose
30	1	1/25	9.42	28.82	6.25	1.25	20.08	13.34
30	1	5/25	1.39	27.92	1.08	0.20	16.87	18.70
30	31	1/25	24.52	48.99	15.61	3.95	22.76	15.35
30	31	5/25	3.59	42.57	8.60	2.20	27.63	23.57
120	1	1/25	27.62	51.36	18.22	4.97	21.09	20.47
120	1	5/25	4.39	46.33	10.93	2.81	24.88	18.62
120	31	1/25	25.54	67.98	6.93	1.14	30.31	4.83
120	31	5/25	3.68	42.59	5.74	0.96	35.55	6.09
75	16	3/25	9.83	48.42	12.15	3.01	23.74	15.55

%TS-AM: Total Sugars liberated after microwave ; %AH: Alga hydrolysis; %Fuc: Fucoidan yield; %TS-Fuc: Total Sugars of precipitated fucoidan

II. Strains selection and hyphal growth measurements

Mycelial growth and morphology parameters of the selected molds are showed in Table 2. 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.

Table 2. 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

III. Effect of Combined Media on Induction of Fucoidan-Degrading Enzymes

Maximum biomass production (5.79 gL-1) was reached on Czapek medium supplemented with sucrose. Fucoidan hydrolytic enzymes were only expressed as extracellular metabolites. Enzyme activity was highest in the sucrose supplemented medium (2.77 UL-1) and in the medium containing fucoidan as sole carbon source (1.88 UL-1). metabolic parameters showed the highest enzyme productivity were with sucrose-fucoidan and fucoidan as sole carbon source (Table 3)

Table 3. Metabolic parameters of liquid fermentations for fucoidanase production.

Media	$Y_{X/S}$	$Y_{E/X}$	P_R
Glu/Fuc	0.32	0.17	0.09
Suc/Fuc	0.87	0.48	0.40
Fru/Fuc	0.39	0.00	0.00
Lac/Fuc	0.21	1.02	0.08
Ac/Fuc	0.54	0.13	0.04
Fuc	0.62	3.55	0.50

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. *A. niger PSH* has the capacity for synthesize fucoidan hydrolytic enzymes, being potential microbiology tools for used in fermentation process and microbial growth induction.