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Pullulan production from cane molasses by *Aureobasidium mausonii* strain NCIM 1226

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ABSTRACT

A less studied strain *Aureobasidium mausonii* NCIM 1226 was used for pullulan production employing cane molasses as growth medium. The maximum yield of pullulan (70 g l^{-1}) was obtained with 10% molasses in absence of nitrogen source after 120 h of fermentation. Molasses supported growth of Aureobasidium mausonii and also showed adequate pullulan production as compared to other media. Molasses is a rich feedstock along with adequate micronutrients and available in plenty in India. Pullulan produced by Aureobasidium mausonii was compared with standard pullulan by I.R. Spectroscopy. The results indicated that molasses can be an expedient medium for production of pullulan.

KEYWORDS

Aureobasidium mausonii;
Molasses;
Pullulan;
I.R.spectroscopy;
Nitrogen sources.

INTRODUCTION

Pullulan is a neutral, water soluble, fungal exopolysacchride produced by *Aureobasidium* spp.[10,15]. It is an extracellular, unbranched homopolysaccharide which consists of maltotriose and maltotetraose units with both α-(1-6) and α-(1-4) linkages[12]. Pullulan has many attractive properties like- non-toxic, non-immunogenic, non-mutagenic and non-carcinogenic. It is transparent, colorless, viscous and tasteless[10,6]. Because of all these properties pullulan is gaining an eminence in exploring various biomedical applications which include targeted drug and gene delivery, surface modification, tissue engineering, etc.[18]. It also has wide applications in food and cosmetic industries[10,18]. Esterified and etherified pullulan derivatives can be used in manufacture of pastes and adhesives, with application galore'. Pullulan can also be used in textile industry, paints, foundry, printing, plywood, etc. 1994[10].

Pullulans are produced by yeast like fungi-*Aureobasidia* species. These are ubiquitous saprophytes found world widely, commonly in phyllosphere of many crops, plants[6] and in air. The structure and fermentation of pullulan has been studied in greater details. Various substrates like beet molasses[25], agro- industrial waste[5], sucrose supplemented with olive oil, peat hydrolysate[3], straw hydrolysate[26], low cost substrates[8], coco-

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nut by-products[9], jagerry, brewery waste, hydrolysed potato starch waste[4], fuel ethanol by-products, carob pod[19], deproteinized whey [20,21],cassava starch residue[17], lactose, various industrial by-products and agricultural wastes[1], various carbon sources[2], starch hydrolysate[23], sucrose supplemented with soyabean oil[22], spent wash[14] have been used to grow *Aureobasidia* and to produce pullulan.

Amongst the above substrates some investigations involved non-conventional practices like-adaptation technique and mixed culture system [20,21], high cell density inoculation (HCDI) - two stage batch fermentation[1], etc. Pretreatment was required for some substrates like beet molasses[25], peat hydrolysate[3], starch hydrolysate[23], hydrolysed potato starch waste[4], fuel ethanol by-products[11] etc. In some instances, nutrient supplements were incorporated into these substrates in the form of corn steep liquor, $(NH_4)_2PO_4$, and K_2HPO_4[3]. Many of these publications revolved on various parameters like pH[7,9,19,20,21] sugar concentrations[2,20,21,25], biomass production[20,21,5,9,19], fermentation time[7,9], etc. to opimize conditions for pullulan production.

Of the known 15 species of *Aureobasidia*, *Aureobasidium pullulans* is the most studied while *Aureobasidium mausonii* is the least. Known literature indicates paucity on the use of molasses. Therefore, we attempted to explore *Aureobasidium mausonii*-molasses combination.

EXPERIMENTAL

Organism

Aureobasidium mausonii (NCIM 1226*)* was obtained from National Collection of Industrial Microorganisms, (NCIM), National Chemical Laboratory, Pune 411008. Organism was maintained on Potato Dextrose Agar slants and grown on sterilized Potato Dextrose Broth; distributed in 250 ml flasks, sterilized at 121^0C for 15 min.Subsequent to inoculation, flasks were incubated at (30 ± 2^0C) for 48 hours on a rotary shaker (150 rpm).

Feed stock

Cane molasses was obtained from Yashwantrao Sahakari Sakhar Karkhana (YSSK) Ltd., Theur. District-Pune. Whenever required it was supplemented with various nitrogen sources (ammonium chloride, ammonium sulfate, and potassium nitrate) at a concentration of 0.25g/100ml and appropriate controls were kept. Supplemented molasses was distributed into flasks (50ml per 250 ml flask) and sterilized at 121^0C for 15 min. Inocula of *Aureobasidium mausonii* were prepared as above and inoculated into molasses containing flasks at 5%v/v.

Typical pre-and post-fermentation analyses were done for necessary comparisons. Total carbohydrate was estimated using Anthrone method. Estimation of nitrogen content was done by Nessler's method[13]. After fermentation contents were centrifuged at 6000 rpm using cooling centrifuge at 4-6^0C for 20min. Residues were washed and dried at 105^0C for 18-20 hours to a constant dry weight. Ethyl acetate was added to supernatant and kept at room temperature overnight. Ethyl acetate was removed and ethanol was added (1:1proportion) and kept overnight. The mixture was centrifuged at 6000 rpm for 20 min. The residue was dried to a constant dry weight at 60^0C and considered as pullulan. Supernatant was dried for estimation of residual sugar by Anthrone's method. Pullulan present in residue was analyzed for confirmation and comparison with standard pullulan (Hayashibara Co.Ltd.Okayama.Japan.) using I.R. Spectroscopy (FTIR Schimatzu).

ABBEREVIATIONS

ml : milliliter;
0C : degree centigrade;
Rpm : rotation per minutes
I.R. : Spectroscopy – Infra red spectroscopy.
v/v : volume by volume.
w/v : weight by volume
g l^{-1} : gram per liter.

RESULTS & DISCUSSION

In the present study it was observed that initial carbohydrate and nitrogen contents were 50 g l^{-1} and 1 g l^{-1} respectively. After complete fermentation (120 hours); good growth of *A. mausonii* was observed on molasses. *A. mausonii* produced pul-

TABLE 1 : Biomass and pullulan production characteristic of *Aureobasidium mausonii* using molasses – based medium.

Nitrogen source(0.25%w/v)	Cell biomass ($g\ l^{-1}$, dry weight)	Pullulan ($g\ l^{-1}$, dry weight)	Total residual sugar($g\ l^{-1}$)
Ammonium chloride	10	51	2.4*
Ammonium sulphate	35	65	2.8
Potassium nitrate	20	47	2.8
control	10	70*	3

lulan on molasses with concomitant appreciable fall in carbohydrate and nitrogen content of molasses (85% and traces, respectively). Pullulan and biomass produced using variables at the end of fermentation are presented in TABLE 1.

We observed that in molasses- based media, *A. mausonii* showed maximum production of pullulan in absence of nitrogen support (70g l^{-1}). Residual sugar was the least (2.4mg l^{-1}) when ammonium chloride was added. I.R. Spectroscopy data confirmed that exopolysacchride produced by *A. mausonii* was indeed pullulan as confirmed by comparison of wave numbers of standard and sample.

Our work confirmed that on molasses based medium pullulan production was to the tune of 70 g l^{-1} using *A. mausonii* as a novel candidate organism. As mentioned earlier, a variety of substrates have been used previously. Pullulan yield data on these substrates varied e.g., beet molasses- 19 g l^{-1} [25], olive oil and sucrose- 51.1 g l^{-1}[7] peat hydrolysate- 12-14 g l^{-1}[3], low cost substrate- 80 g l^{-1}[8], coconut by-products- 58 g l^{-1}[9], jagerry- 51.1 g l^{-1} [24], brewery waste- 6 g l^{-1}[20,21], hydrolysed potato starch waste- 87gl^{-1}[4], carob pod- 6.5 g l^{-1}[19], deproteinized whey 11.0 ± 0.5g l^{-1}[20,21], cassava starch residue- 27.5 g l^{-1}[17], various industrial by-products and agricultural wastes- 65.3 g l^{-1}[1] and starch hydrolysate 15 g l^{-1}[23].

Some of these substrates did require supplementation, e.g. clarified cane molasses (10% sugars), potato starch waste (3%), enzyme hydrolyzed sweet whey (5% lactose) + 0.05% glutamic + 0.298% KH_2PO_4 and hydrolyzed rice straw (4%) + 1% sucrose[1]. The present study using 10% cane molasses indicates that in the absence of any nitrogen source growth of *A. mausonii* and a maximum of 70 g l^{-1} of pullulan can be produced. Nitrogen supplements did not enhance yields. On the contrary, nitrogen additions shift the process in favor of biomass rather than pullulan. Literature indicates that the highest concentration of pullulan obtained so far is with using cashew fruit juice (90.5. g l^{-1})[5].

Availability of approximately 1% nitrogen and 48 % of sugars along with other essential minerals like ash, potassium, chloride, magnesium, sulfur, sodium, copper, iron etc. in molasses appears as wholesome as compared to other feed stocks. However, it does contain some impurities. All the same, pretreatments do help substantially. We conclude that molasses- based media were economical and expedient.

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