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Lactoserum as a moistening medium and crude inducer for fungal cellulase and hemicellulase induction through solid-state fermentation of apple pomace

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ABSTRACT

Objectives: The potential of lactoserum as a crude inducer and moistening medium for fungal cellulase and hemicellulase induction was tested through solid-state fermentation.
Methodology: Solid-state fermentation was conducted in Erlenmeyer flasks and plastic trays using apple pomace (AP) as solid substrate by *Aspergillus niger* NRRL 567 and *A. niger* NRRL 2001.
Results: Solid-state tray fermentation resulted in cellulase activities (IU g^{-1} dry weight basis) of 383.7 ± 17.9, 425.3 ± 21.3, 336.1 ± 16.2 and 4868 ± 39.8, respectively for FPase (filter paper cellulase), CMCase (carboxymethyl cellulase), BGL (β-glucosidase) and xylanase using *A. niger* NRRL 567. Similarly, *A. niger* NRRL 2001 resulted in enzyme activities (IU g^{-1}) of 401 ± 23.8, 544.7 ± 24.5, 285.4 ± 11.7 and 4580.7 ± 34.5, respectively for FPase, CMCase, BGL and xylanase. The enzyme loading of FPase 50 IU g^{-1} substrate in the hydrolysis of AP resulted in (g kg^{-1}): 509.3 ± 22.9 total sugar (48 h), 109.3 ± 5.7 reducing sugar (48 h), 16.1 ± 0.8 glucose (60 h) and 10.4 ± 2.0 xylose, respectively whereas hydrolysis of BSG resulted in (g kg^{-1}): 375.7 ± 16.2 total sugars (36 h), 114.6 ± 4.0 reducing sugar (60 h), 18.1 ± 0.9 glucose (60 h) and 19.8 ± 2.0 xylose (48 h), respectively.
Conclusions: This study demonstrated the potential of SSF as a simple and cheap technology for higher cellulase and hemicellulase production using negative cost AP waste which could eventually help to utilize abundant lignocellulosic biomass for production of biofuels and other value-added products.

1. Introduction

Currently, cellulases are the third largest industrial enzymes being marketed worldwide [1]. However, due to their widespread applications in fast growing biofuel industry, the market of cellulases is expected to expand to become the largest volume industrial enzyme [2]. Other than biofuels, they are widely used in chemical industry, textile, paper and

Abbreviations: ANOVA, analysis of variance; AP, apple pomace; BGL, β-glucosidase; BSG, brewery spent grain; CMC, carboxymethyl cellulose; CMCase, carboxymethyl cellulase; DNS, 3,5-Dinitrosalicylic acid; FPase, FP cellulase; LAC, lactoserum; RS, reducing sugar; SD, standard deviation; SSF, solid-state fermentation; TS, total sugar.

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pulp, detergent, beverage, juice extraction, agriculture and animal feed among others [3–5].

Lignocellulosic biomass is present in abundant quantity with production rate of 200 billion tons biomass per year (200 Gt y^{-1}) [6]. Owing to large quantities of carbohydrates present in biomass, worldwide research has been focused on the efficient saccharification of biomass for fermentable sugars. The resulting biomass syrup that have five and six carbon sugars can be used as carbon sources in biotechnological fermentations to produce bulk chemicals including bioethanol, antibiotics and industrial enzymes, among others.

However, for biotechnological valorization of lignocellulosic biomass, the polysaccharides present must be first hydrolyzed by acidic or enzymatic methods. Acidic hydrolysis is a mature technology but laden with disadvantages of generation of hazardous acidic waste and further recovery of fermentable sugars from the acid is technically difficult. Enzymatic hydrolysis is the preferred and more efficient method due to requirement of ambient and non-corrosive physico-chemical operating conditions. The enzymatic hydrolysis process is under rapid development and has immense potential for improvement in cost and efficiency of biomass utilization.

Conventionally, the production of enzymes is very expensive and raw material translates into 40–60% of the production cost. According to an estimate, cellulases procured from commercial sources contribute 22.5–43.4% to the total cost of cellulosic ethanol bioproduction [7]. The cost economics of cellulases can be brought down by multifaceted strategies, such as by using low cost lignocellulosic substrates, cost effective fermentation, such as solid-state fermentation (SSF) (koji fermentation) and use of crude inducers for higher cellulase production. Since, arrays of enzyme activities are required for the complete hydrolysis of cellulose, crude cellulase preparations are considered superior over the purified enzymes for industrial use [8]. Indeed, the efficiency of lignocellulosic hydrolysis can be dramatically increased if the crude cellulase from the fermentor is directly used for industrial purpose. Various researchers have used the crude enzyme preparations to validate their efficiencies [3,9,10].

As discussed earlier, inducers can enhance cellulases production. However, the cost of inducers also adds to the process economics. The use of crude inducers can helps to achieve higher cellulase production by fungi. Solid-state fermentation has recently gained attention for the production of microbial enzymes due to various advantages over traditional submerged fermentations. Among various microorganisms, filamentous fungi are widely employed in SSF due to their ability to grow efficiently on complex solid substrates and production of wide range of extracellular hydrolytic enzymes. Among the filamentous fungi, *Aspergillus niger* is widely used for cellulases production due to higher levels than other fungi, bacteria and yeasts.

The present study was conducted to evaluate the potential of lactoserum (LAC) as a crude inducer and moistening medium for obtaining higher cellulase production using apple pomace (AP) by *A. niger* NRRL 567 and *A. niger* NRRL 2001 through SSF. AP contains high carbohydrate content and other vital nutrients essential for the fungal growth (Table 1). LAC contains disaccharide, lactose which acts as an inducer for cellulase production [11]. The enzyme extraction was carried out using different extraction solutions, such as 50 mol m^{-3} citrate buffer (pH 4.8), distilled H_2O, milliQ H_2O, 1 kg m^{-3} Tween 80 solution and 10.0 kg m^{-3} of NaCl solution. Saccharification potential of crude cellulase and hemicellulase was evaluated by using 100 mol m^{-3} HCl and 100 mol m^{-3} NaOH pretreated AP and brewers' spent grain (BSG). So far to the best of our knowledge, there is no study which describes the use of LAC as a crude inducer for cellulase bioproduction through SSF.

Table 1 – Proximate composition of feed stocks.

Biomass components	Composition (DW basis)	
	AP	BSG
Total carbon (g kg^{-1})	127.9	106.9
Cellulose (g kg^{-1})	7.2	13.8–25.4
Hemicellulose (g kg^{-1})	–	28.4–30.0
Lignin (g kg^{-1})	23.5	11.9–27.8
Total carbohydrates (g kg^{-1})	480–838	345–433
Protein (g kg^{-1})	29–57	103–239
Reducing sugars (g kg^{-1})	108–150	–
Glucose (% of reducing sugar)	22.7	21.5[a]
Fructose (% of reducing sugar)	23.6	–
Sucrose (% of reducing sugar)	1.8	–
Arabinose (% of reducing sugar)	–	8.5[a]
Galactose (% of reducing sugar)	–	1.3[a]
Xylose (% of reducing sugar)	0.1	17.6[a]

Abbreviations: AP - apple pomace; BSG - brewer's spent grain; DW - dry weight.
a % of monosaccharide's on dry weight basis.

2. Material and methods

2.1. *Microorganism propagation and inoculums preparation*

A. niger NRRL 567 and *A. niger* NRRL 2001 was procured from Agricultural Research Services (ARS) culture collection, IL-USA. The culture conditions, maintenance and inoculum preparation have been described in Dhillon et al. [12]. The counting of spores was performed by direct microscopy using Neubauer cell counter chamber.

2.2. *Substrate preparation*

The AP sample was obtained from Lassonde Inc., Rougemont, Montreal, Canada (45.438333, −73.037778). The AP was collected after the pressing and meshing stage in storage conditions. The apples (*Malus domestica)* used for processing was of same quality. The pomace sample was homogeneous and it was stored at −20 °C for only 2 weeks to avoid any microbial contamination. Generally, the apple industries supplement apples with rice husk = 1% during processing of apples for better hold and efficient meshing. Hence, the pomace obtained from the industry was already amended with rice husk. The BSG is the by-product of brewery industry. The brewer's spent grain of barley (*Hordeum vulgare*), variety 2 Tau