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Isolation of Glucosidase Enzyme Producing Bacteria

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Abstract: *Beta-Glucosidase producing microorganisms are major sources which can be employed for bioconversion of cellulose. In the current study, 9 morphologically different bacterial isolates were isolated from dairy effluent and 7 were isolated from fermented barley. 4 of the bacteria from the dairy effluent and 5 from barley source were found to possess beta-glucosidase activity. The above activity was tested by growth in medium supplemented with esculin and ferric ammonium citrate.*

Keywords: B-glucosidase, microorganisms, ferric ammonium citrate barley

I. INTRODUCTION

B-Glucosidases are the focus of much research recently because of their important roles in a variety of fundamental biological and biotechnological processes (1). B -Glucosidase [B-glucoside glucohydrolase, EC 3.2.1.21] is one among the members of cellulase enzyme system, together with endoglucanase and cellobiohydrolase.B -glucosidase catalyzes the hydrolysis of the B-glucosidic linkages of aryl and alkyl B-glucosides,linked oligosaccharides and the number of all other oligosaccharides with the release of glucose.That is involved in the last step of cellulose saccharification cleaving cellobiose to glucose (2)

B-Glucosidase is beneficial in increasing isoflavone aglycones during fermentation of soymilk (3) within the bioconversion of phenolic anti-oxidants from defatted soybean powder (4).Micro-organisms producing this enzyme are employed directly for the bioconversion of majorginsenoside Rb1 from Panax ginseng to minor ginsenosides of more therapeutic interest. These are isolated from soil in a ginseng field (5) or performed by employing food grade microorganisms (6). B-Glucosidases of intestinal microflora in lower bowel can hydrolyze the glucoside isoflavones to aglycones and promote their absorption (2).Therefore, bacteria with B-glucosidase activity are potentially important within the production of compounds with higher estrogenecity and better absorption, facilitating the biological availability of isoflavones (5)

II. MATERIALS AND METHODS

Selection of source for isolation of microorganisms:

The dairy effluent sample (source A) was collected from Prabha Dairy industry, Mumbai. The sample was collected in a clean sterile container and stored at 3 degree celsius until the analysis was carried out.The barley grains (source B) were resourced from local market.

Isolation of microorganisms from selected sources:

1.0 g of soil sample from sample A was weighed and the microorganisms were isolated by employing standard serial dilution plating technique (Jensen, 1968). 0.1 ml of aliquot from eachdilution was plated on R2A agar medium for the isolation of bacterial colonies and in the further step the plates were incubated at 37oC for 2-3 days.

Barley sample (10 g) was soaked in water (100 ml) and allowed for fermentation for a period of 4 days. For the isolation of microorganisms from this source, varying volumes (10, 100, 200 and 1000 µl) of the fermented supernatant was plated on R2A agar. Theplates were incubated at 37°C for a period of 7 days.

Identification of bacteria with B-glucosidase activity:

The morphologically different colonies obtained from source B were identified on MRS agar and from source A on R2A agar, both supplemented with esculin (3 g/l) and ferric ammonium citrate (0.2 g/l). The plates were incubated at

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37°C for about 48 hoursand colonies that were producing browning or blackening of the medium were noted as esculin hydrolyzing bacteria. The esculin positive bacterial colonies were identified and single colonies were obtained on MRSAgar.

III. RESULTS AND DISCUSSION

Isolation of microorganisms from selected sources

9 morphologically different bacteria were identified from source A as a result of serial dilution. They were identified on the basis of shape of the colour, shape of colony, and their characteristics on their growth medium. Barley grains were allowed to ferment and were screened for microorganisms. The Barley grains were soaked in water for a period of 3-4 days and when the supernatant was used for isolation, morphologically different bacteria were identified on R2A agar. Neither the paste of soakedbarley nor raw powdered barley, when plated resulted in any colony growth suggesting the absence of anyendophytic bacteria in barley. After identification of morphologically different colonies from sample A (barley water) each colony was identified to verify its growth on MRS (de Mann Rogosa Sharpe) agar. Previously, food grade bacterial cultures have been maintained on this medium and used for bioconversion experiments. MR Sagar is specific for the growth of Lactobacillus and related species which are predominant in fermented food (2,7). For further studies of source B, MRS medium was used.

Identification of bacteria with B-glucosidase activity

Morphologically different bacteria from source A were screened for the production of -glucosidase enzyme by using esculin R2A agar. 4 reddish brown colonies onthe esculin R2A agar indicated that of the 9 isolated bacteria 4 of them possess glucosidase activity. In order to check for the β-glucosidase activity of the isolated bacteria from source B, each individual bacterial colony was spotted on MRS agar supplemented with esculin and ferric ammonium citrate. 5 bacteria from barley source were found to be esculin positive cultures as they resulted in the blackening of the medium around the colony B(8).

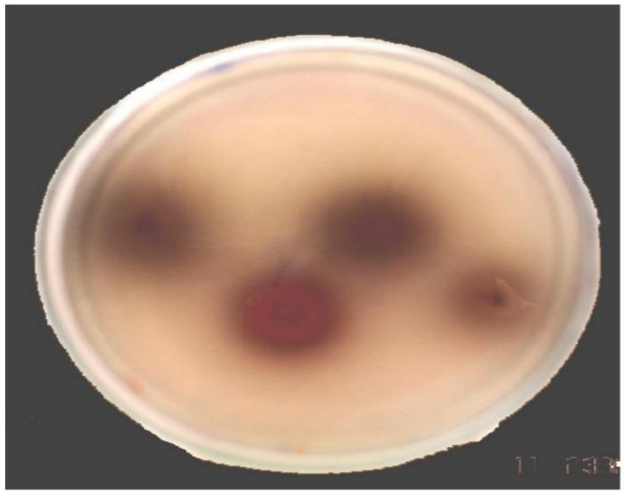

Figure 1. Esculin positive cultures on R2A agar isolated from dairy effluent source (Source A).

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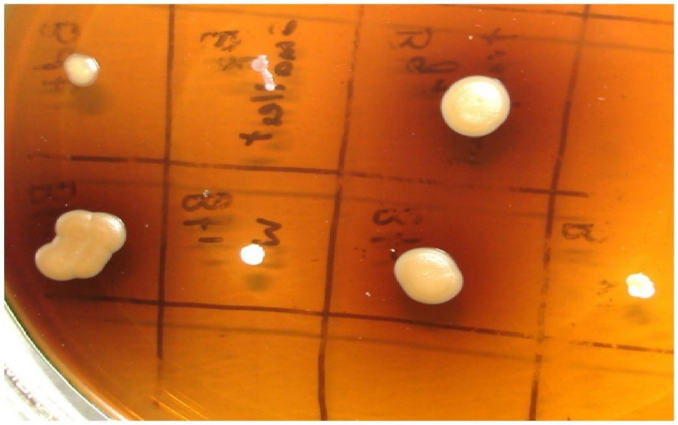

Figure 2. Esculin positive cultures on MRS agar isolated from fermented barley source (source B).

REFERENCES

[1]. Chi H, Ji GE (2005). Transformation of ginsenosides Rb1 and Re fromPanax ginseng by food microorganisms, Biotechnol. Lett. 27: 765-771.

[2]. Czjzek M, Cicek M, Zamboni V, Bevan DR, Hennrissat B, Esen A(2000). The mechanism of substrate (aglycone) specificity in B-glucosidases is revealed by crystal structures of mutant maize B-glucosidase DIMBOA, DIMBOAGlc, and dhurrin complexes, PNAS,97(25): 13555-13560.

[3]. Hendrich S (2002). Bioavailability of isoflavones, J. Chromatography B 777: 203-210.

[4]. Hu YB, Luan HB, Haoa DB, Xiao HC, Yang SA, Yang L (2007),Purification and characterization of a novel ginsenoside-hydrolyzingB-glucosidase from the China white jade snail (Achatina fulica),Enzyme Microbial. Technol. 40: 1358-1366.

[5]. Kim MK, Lee JW, Lee KY, Yang DC (2005). Microbial Conversion ofMajor ginsenoside Rb1 to pharmaceutically active minor ginsenosideRd J. Microbiol. 43(5): 456-462.

[6]. McCue P, Shetty K (2003). Role of carbohydrate-cleaving enzymes inphenolic antioxidant mobilization from whole soybean fermented withRhizopus oligosporus, Food Biotechnol. 27(l): 27-37.

[7]. Otieno DO, Shah NP (2007). Endogenous -glucosidase and B-galactosidase activities from selected probiotic micro-organisms andtheir role in isoflavone biotransformation in soymilk, J. Appl. Microbiol.103: 910-912.

[8]. Yun SI, Jeong CS, Chung DK, Choi HS (2001). Purification and some properties of a -glucosidase from Trichoderma harzanium Type C-4, Biosci. Biotechnol. Biochem. 65(9): 2028-2032.