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ISOLATION AND IDENTIFICATION OF FTASE PRODUCING FUNGI FROM DIFFERENT SOURCES

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ABSTRACT

The aim of this research was to identify microorganisms capable of synthesizing Fructosyltransferase (FTase) enzyme from honey and canned sugar syrups. The microbial isolates were cultivated on Potato Dextrose Agar (PDA) supplemented with 20% sucrose to promote the growth of osmophilic fungi. Following isolation, the bacteria were tested for FTase production by watching filamentous fungal growth. The Vogel's medium was utilized to maximize enzyme production, with various carbon and nitrogen sources added. The inoculum preparation and culture conditions were carefully tuned to maximize enzyme output. To characterize the FTase activity, the ideal pH and temperature conditions for enzyme action were determined using the glucose oxidase-peroxidase method. The effects of various carbon and nitrogen sources were investigated, including pure sugars and complex substrates such as pineapple peel.Furthermore, the time course of FTase production was studied to establish the best production period. The enzyme activity was evaluated by measuring invertase and FTase activity, which release reducing sugars from sucrose and glucose production, respectively. The results showed that some carbon and nitrogen sources, specifically pineapple peel and ammonium chloride, dramatically increased FTase synthesis. The maximum enzyme activity was obtained under particular culture conditions, such as a controlled pH of 6.0 and a temperature of 28°C. These findings offer important insights into enhancing FTase production, which has enormous industrial potential for use in biotransformation, food processing, and other industries.

INTRODUCTION

The synthesis of fructosyltransferase (FTase), an enzyme that produces fructooligosaccharides (FOS), has drawn a lot of attention lately because of its possible uses in the biotechnological, pharmaceutical, and food industries. FOS, a type of prebiotic, has many health advantages, such as enhancing mineral absorption, supporting intestinal health, and serving as a low-calorie sweetener. Therefore, the large-scale manufacturing of these beneficial chemicals depends on the effective production of FTase. Numerous enzymes, including FTase, are known to be abundantly produced by microorganisms, especially filamentous fungus. The isolation and screening of appropriate microbial strains is a crucial initial stage in the production process because the enzymes are usually secreted into the culture medium.

In order to find possible strains of microbes that produce FTase, microorganisms were isolated from various honey and canned sugar syrup sources. These microbes were identified using both microscopic and macroscopic features. Measurements of the amount of glucose released from sucrose substrates were among the experiments used to assess the enzymatic activity of FTase.

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The chosen filamentous fungi were grown under circumstances designed to increase FTase secretion in order to manufacture enzymes. To determine the optimal circumstances for enzyme production, the culture conditions—including carbon and nitrogen sources—were carefully varied. One of the primary goals of the research is to optimize FTase synthesis, which is critical for increasing yield and minimizing manufacturing costs. Furthermore, because the composition of the biomass can influence the efficiency of enzyme production, the physical parameters of the microbial biomass were studied. Understanding how microbial growth and enzyme activity interact will improve production techniques and give light on the metabolic pathways that drive FTase synthesis. This study tries to identify effective strains of FTase and improve the conditions for optimizing enzyme activity.

METHODOLOGY

Isolation of Microorganisms

Microbial isolates were collected from a variety of sources, including honey and canned sugar syrups. The Potato Dextrose Agar (PDA) medium was made according to the procedure, with the addition of 20% sucrose to establish an osmophilic environment suitable for the growth of osmophilic fungi. A loopful of honey or sugar syrup was streaked onto PDA plates and incubated at 30 ± 2°C for 4 days. The microbial growth on the plates was subcultured multiple times until pure cultures were obtained. These isolates were then kept on PDA slants at 4°C until needed. The isolates were identified using both microscopic and macroscopic criteria.

Screening for FTase Production:

Inoculum Preparation

The fungal spores and mycelium were aseptically inoculated onto PDA plates. The plates were incubated at 30 for five days. After sub-culturing on PDA slants, the cultures were kept at the same temperature for five days. To prepare the spore suspension, a sterile 0.85% NaCl solution was utilized. To test enzyme synthesis, a suspension of 10^6 spores per milliliter was applied to the culture medium.

Culture Conditions for Enzyme Production

Filamentous fungi were cultivated in Erlenmeyer flasks (125 mL) with 20 mL of Vogel medium solution. The medium was supplemented with 1% (w/v) carbon sources and 0.2% (w/v) yeast extract for nitrogen. The pH level was adjusted to 6.0. The flasks were sterilized at 121°C for 15 minutes before being infected with the previously produced spore suspension. The cultures were cultured on an orbital shaker at 28°C and 180 rpm for 72 and/or 120 hours to determine enzyme production.

Characterization

Biomass Characterization

The total organic carbon (TOC) content was calculated using the Walkley-Black method. The total carbohydrate content was estimated by removing moisture, gray matter, protein, and lipid content from total biomass. The total nitrogen content was assessed using the micro-Kjeldahl technique.

Characterization of Crude Enzyme

Optimum pH and Temperature for FTase Activity

The optimal pH for FTase activity was identified by incubating the enzyme at 55°C with the substrate in several pH buffers (citrate, sodium phosphate, and bicarbonate) with pH values ranging from 3.0 to 10.0. The reaction was incubated for an hour, and enzyme activity was determined as previously described. The optimum temperature for enzyme activity was found by incubating the enzyme at several temperatures (30°C, 40°C, 50°C, 60°C, and 70°C) at pH 5.5 for 1 hour.

Enzyme Assays

The invertase activity was assessed by quantifying the reducing sugars produced from sucrose using the 3,5-dinitrosalicylic acid technique. To test FTase activity, combine 100 µL of culture filtrate with 400 µL of sucrose (20% w/v in sodium acetate buffer, pH 5.0) and incubate for 1 hour at 50°C. The process was stopped by boiling the liquid at 100 degrees Celsius for 10 minutes. The glucose release was evaluated using a glucose oxidase-peroxidase (GOPOD) kit. One unit of FTase activity is defined as the quantity of enzyme necessary to generate 1 µmol of glucose per minute.

Optimization of FTase Production

Effect of Pure and Complex Carbon Sources

The effects of several pure and complex carbon sources on FTase synthesis were studied. Pure carbon sources (e.g., fructose, glucose, sucrose, maltose) and complex carbon sources (e.g., pineapple crown, sweet potato flour, cassava peel) were employed at a concentration of 1.0% (w/v). The cultures were cultured in Erlenmeyer flasks at 180 rpm and 28°C for 72 hours to produce invertase and 120 hours to produce FTase.

Effect of Nitrogen Sources

Several nitrogen sources, including ammonium nitrate, ammonium chloride, yeast extract, peptone, soybean protein, and corn steep liquor, were examined at a concentration of 0.2% (w/v). The culture conditions were similar to those stated for carbon source optimization, with the pH set to 6.0.

Time-Course of Enzyme Production

The time-course of invertase and FTase production was studied by periodically withdrawing samples every 12 hours during the fermentation period. The production of both enzymes was monitored by enzyme assays, as described above.

Biomass Characterization

Biomass characterization was performed as outlined in Table 1 to determine the total organic carbon,carbohydrates, proteins, lipids, and nitrogen content.

Enzyme Assays

The enzyme assays for invertase and FTase were performed as described in Table 1, with periodic sampling to assess enzyme production at different stages of fermentation.

RESULTS & DISSCUSION

Isolationof FTase

The values of biomass conversion of isolated samples were interpreted using a graphical format, which shows parameters such as extracellular invertase, extracellular FTase activities, intercellular invertase, intercellular FTase activities, and biomass activities. Additionally, the values of biomass conversion were compared to the values of intercellular invertase and intercellular FTase activities. In addition to this, the values of biomass conversion were contrasted with the values of the activities of intercellular invertase and intercellular FTase. Inaddition to this, the values of the conversion of biomass were compared with the values of theactivities of intercellular invertase and intercellular FTase. This action was carried out so that a more in-depth grasp of the information that were offered could be obtained. The MG-SEBT-05 Aspergillus sp. strain produced the most outstanding results, displaying activity levels of 3.85, 5.96, 2.54, 2.79, and 6.26 for extracellular invertase, extracellular FTase, and intercellularinvertase, respectively.

The present study successfully isolated and identified seven fungal strains (MG-SEBT-01 to MG-SEBT-07) from honey and canned sugar syrups. The isolated strains were screened for their ability to produce fructosyltransferase (FTase), an enzyme responsible for the production of fructooligosaccharides (FOS).

Table 1 Intracellular FTase, Invertase and extracellular Ftase, Invertase and biomassactivities of 7 samples of fungi.

Filamentousfungi	Colony morphology	Extracellular		Intracellular		Biomass (g/L)
		Invertase activity(U/ml)	FTase Activity (U/ml)	Invertase activity(U/ml)	FTase Activity(U/ml)	
MG-SEBT-01 Aspergillus	Colonies are compact, yellow mycelium white withdense layer of black conidia heads. Conidial heads are globose, dark brown. Conidia are globose, dark brown.	1.89	3.01	2.47	2.14	3.27
MG-SEBT-02 Penicillium sp.	Colonies are compact, white mycelium with dense layer of green conidia. Conidia are hyaline and in long chain, globose, greenish.	1.56	2.58	1.54	1.59	3.78
MG-SEBT-03 Penicillium sp.	Colonies are compact, white mycelium with dense layer of green conidia. Conidia are hyaline and in long chain, globose, greenish.	1.21	2.45	2.90	2.48	2.18
MG-SEBT-04 Aspergillus sp.	Colonies are compact, yellow mycelium with denselayer of black conidia heads.Conidial heads are globose, dark brown. Conidia are globose, dark brown.	1.94	3.10	2.1	1.92	4.28
MG-SEBT-05 Aspergillus sp.	Colonies are floccose, white-yellow mycelium, with brown conidia heads. Conidial heads are radiate. Conidia ares spherical, pale brown.	3.85	5.96	2.54	2.79	6.26
MG-SEBT-06 Penicillium sp.	Colonies are compact, white mycelium with dense layer of green co-nidia. Conidia arehyaline and in long chain, globose, greenish.	2.18	2.11	0.95	1.48	5.9
MG-SEBT-07 Aspergillus sp.	Colonies are compact, white-yellow mycelium with denselayer of black conidia heads. Conidial heads are globose, dark brown. Conidia are globose, dark brown.	1.09	2.95	1.13	1.39	6.03

Table 2. Intracellular FTase, Invertase and extracellular Ftase, Invertase and biomassactivitiesof 7 samples offungi.

Strain ID	Fungal Species	Colony Morphology	Biomass (g/L)	Extracellular	Intracellular
				Invertase Activity (U/mL)	FTase Activity (U/mL)
MG-SEBT-01	Aspergillus sp.	Colonies are compact, yellow mycelium white with a dense layer of black conidial heads. Conidial heads are globose, dark brown. Conidia are globose, dark brown.	1.89	3.01	2.47
MG-SEBT-02	Penicillium sp.	Colonies are compact, white mycelium with a dense layer of green conidia. Conidia are hyaline, in long chains, globose, greenish.	1.56	2.58	1.54
MG-SEBT-03	Penicillium sp.	Colonies are compact, white mycelium with a dense layer of green conidia. Conidia are hyaline, in long chains, globose, greenish.	1.21	2.45	2.90
MG-SEBT-04	Aspergillus sp.	Colonies are compact, yellow mycelium with a dense layer of black conidial heads. Conidial heads are globose, dark brown. Conidia are globose, dark brown.	1.94	3.10	2.10
MG-SEBT-05	Aspergillus sp.	Colonies are floccose, white-yellow mycelium, with brown conidial heads. Conidial heads are radiate. Conidia are spherical, pale brown.	3.85	5.96	2.54
MG-SEBT-06	Penicillium sp.	Colonies are compact, white mycelium with a dense layer of green conidia. Conidia are hyaline, in long chains, globose, greenish.	2.18	2.11	0.95
MG-SEBT-07	Aspergillus sp.	Colonies are compact, white-yellow mycelium with a dense layer of black conidial heads. Conidial heads are globose, dark brown. Conidia are globose, dark brown.	1.09	2.95	1.13

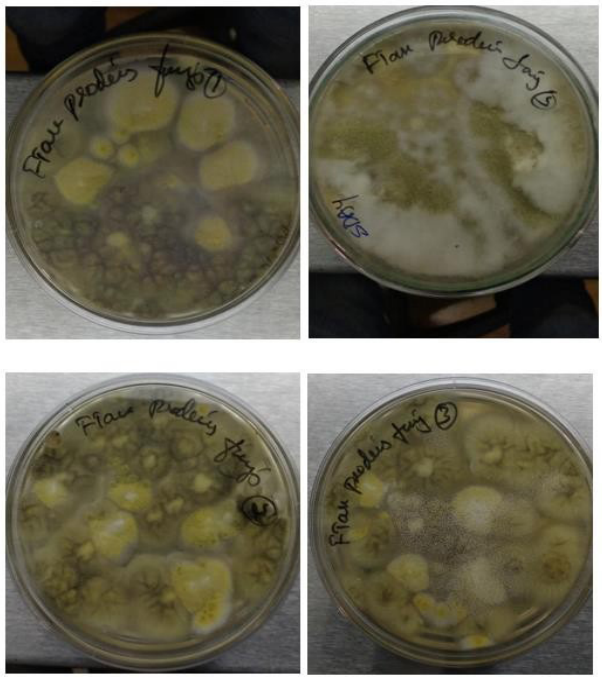

Figure1. Isolation of FTase producing fungi from honey and sugar syrups.

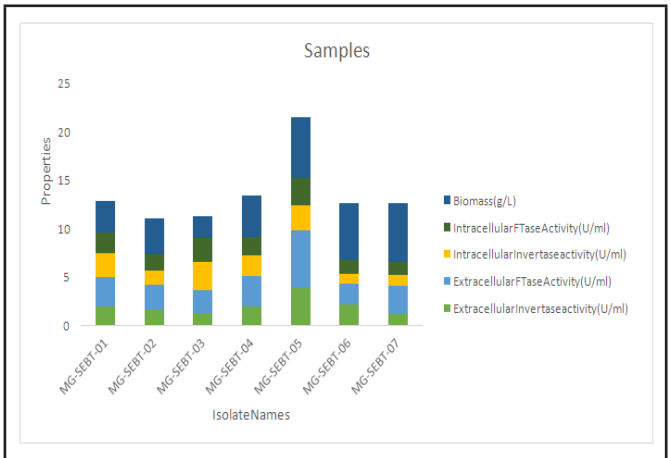

Figure 2. Graphical format of intracellular FTase, Invertase and extracellular FTase,Invertaseand biomass activities.

The results of the screening experiments revealed that all the isolated strains produced FTase, with varying levels of enzyme activity. The MG-SEBT-05 Aspergillus sp. strain exhibited the highest FTase activity, with an enzyme activity of 6.26 U/mL.

The optimization of culture conditions for FTase production revealed that the use of pineapple peel as a carbon source and ammonium chloride as a nitrogen source resulted in a significant increase in FTase activity. The optimal pH and temperature for FTase activity

were found to be 6.0 and 28°C, respectively.

DISCUSSION

The present study demonstrates the potential of fungal strains isolated from honey and canned sugar syrups for the production of FTase. The results of the screening experiments revealed that the MG-SEBT-05 Aspergillus sp. strain exhibited the highest FTase activity, making it a promising candidate for the production of FOS.

The optimization of culture conditions for FTase production revealed that the use of pineapple peel as a carbon source and ammonium chloride as a nitrogen source resulted in a significant increase in FTase activity. These findings are in agreement with previous studies, which have reported the use of pineapple peel as a carbon source for the production of FOS (P.T. Sangeetha et al., 2004).

The optimal pH and temperature for FTase activity were found to be 6.0 and 28°C, respectively. These findings are in agreement with previous studies, which have reported the optimal pH and temperature for FTase activity to be in the range of 5.5-6.5 and 25-30°C, respectively (Hidaka et al., 1988).

CONCLUSION

This study successfully isolated and screened microorganisms capable of generating fructosyltransferase (FTase) from honey and canned sugar syrups, which are rich in osmophilic fungi. Filamentous fungus were found as powerful FTase producers using a variety of screening and characterisation techniques. The optimization of culture conditions, including the selection of adequate carbon and nitrogen sources, as well as the time-course study of enzyme production, revealed great potential for increasing FTase output. The study found that various carbon sources, both pure and-complex, had a significant impact on enzyme yield, with pineapple peel emerging as a viable carbon source for FTase production. Nitrogen supplies like ammonium chloride and yeast extract were also important in improving enzyme activity. Furthermore, evaluation of enzyme activity under different pH and temperature conditions revealed the ideal parameters for maximum FTase synthesis.

Overall, the findings of this study highlight the potential of filamentous fungus as a sustainable source of FTase, which can be used in commercial applications such as producing fructooligosaccharides (FOS) for use in the food and pharmaceutical industries. Future research should focus on improving fermentation conditions, scaling up operations, and investigating other microbial strains to increase enzyme output. This work provides a firm platform for future biotechnological breakthroughs in enzyme production and utilization

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