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Amylolytic Potential of Oleaginous Yeast in Sago Processing Wastewater (SWW) under Submerged Fermentation

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Authors' contributions

This work was carried out in collaboration among all authors. Author SU has received research grants from DBT and revised the manuscript. *Authors KT and NS carried out the experiments and wrote the manuscript. Author PS supervised the works. All authors read and approved the final manuscript.*

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Original Research Article

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ABSTRACT

Sago processing wastewater was assessed for their suitability as growth substrates using oleaginous yeasts, for the production of a useful enzyme (amylase) under submerged fermentation (SmF). Sago wastewater (pH was adjusted to 6) containing starch concentration (10% w/v) were inoculated with yeast strain and incubated at 30ºC for 10 d in an incubator shaker (150 rpm). The results of the amylase activity of oleaginous yeast and in its substratum SWW were compared with the different processing wastes (potato peel, banana peel, cassava peel, corn residue, rice husk, wheat bran, yam peel and barley husk) and oleaginous yeasts (R*hodotorula mucilaginosa*, *Saccharomyces pastorianus*, *Lipomyces starkeyi* and *Rhodotorula glutinis*). Compared to other oleaginous yeast, our yeast strain found to produce higher amylase

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activity of 1.51 IU mL^{-1}. Furthermore, SWW produced more amylase activity than the other compared wastes. This research finding illustrates the environmental friendly and alternate use of sago processing wastewater, towards their valorization as substrates for valuable enzymes and chemical production.

Keywords: Oleaginous yeast; amylase activity; starch; sago wastewater.

1. INTRODUCTION

Amylase is one of the three most abundant classes of the industrial enzyme. This class accounts for around 30% of the world's enzymes production [1]. All amylases act as glycoside hydrolyzers and hydrolyze α-1,4 glycoside bonds [2]. Amylolytic enzymes are used in the production of syrups, sweeteners, saccharification and liquefaction of starch, cheese ripening and flavoring, flour, infant cereal, breweries, silage and food, animal feed, ethanol, detergents, pharmaceuticals and cosmetics. It is also used in the paper industry and textile industries [3 -9].

Starch is a complex molecule requiring multiple hydrolytic enzymes such as α-amylases and glucoamylases to generate simple sugars. To date, the price of amylases has influenced the feasibility of transforming this polysaccharide into fuels and other chemicals significantly to date [10,11]. Since the carbon source is one of the major factors influencing the cost of enzyme production considerable attention has been paid to the use of agro-industrial residues in the fermentation process [12].

Sago processing wastewater is one of the prominent wastewater released from the sago processing units during the processing of tapioca tubers. Approximately 20,000 – 30,000 liter of water was used for the production of one ton of sago [13]. This water directly emanated from the industries as an effluent. This effluent is rich in starch, fermentable sugars, and other nutrients to justify its use as a carbon source in the fermentation process. Despite the great potential, this wastewater has not yet been exploited as a possible carbon source for microbial enzymes production, such as amylases. To explore the potential of SWW for enzyme production, the present study was undertaken using oleaginous yeast under submerged fermentation.

2. MATERIALS AND METHODS

2.1 Strain and Culture Conditions

The oleaginous yeast strain *Candida tropicalis* ASY2 (Accession number: MH011502) was isolated, identified from sago processing wastewater (SWW) by biotrap enrichment at Sree Selliamman sago factory was used in the present study (Fig. 1). The culture is maintained in YEME slants at 4ºC.

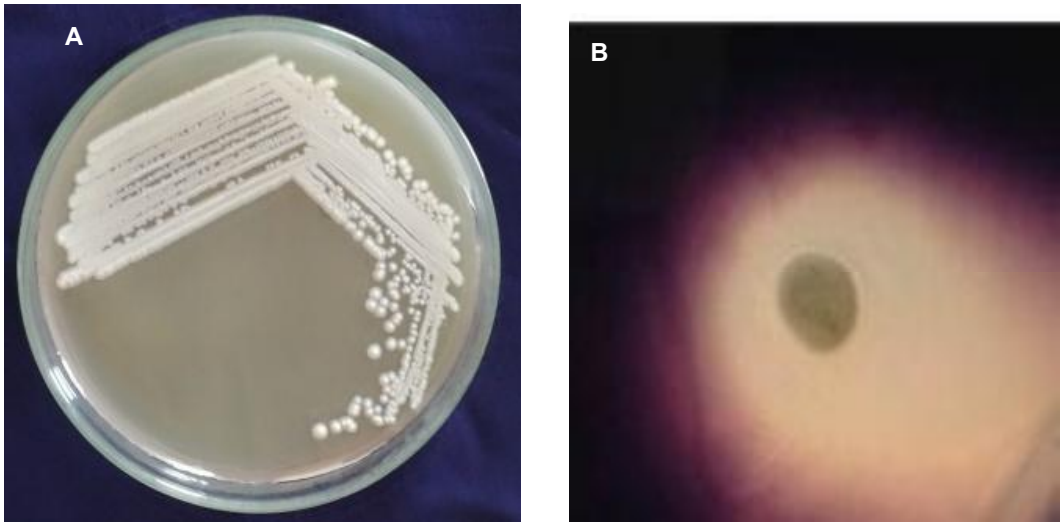

Fig. 1 A. Streak plate of *C. tropicalis* ASY2 in yeast extract mannitol agar medium
B. Clearance zone formed by *C. tropicalis* ASY2 in starch agar medium

2.2 Submerged Fermentation

The yeast culture was prepared 24 h before the experiment with a concentration of 10^6 cells mL^{-1}. The active seed inoculum was inoculated into a 250 ml conical flask containing 50 ml of SWW (pH was adjusted to 6 for the growth of yeast cells). It was incubated in a mechanical shaker (Orbitek, Scigensis Biotech, India) at 150 rpm for 10 d (30±2°C). Periodically, the SWW samples along with yeast biomass were withdrawn and harvested by centrifugation at 3000 rpm for 10 min and the supernatant was stored at 4ºC for further analysis.

2.3 Kinetic of Amylase in SWW

The residual glucose in the SWW was analyzed using the DNS method [14]. About 1 mL of the supernatant sample was added into 15 mL glass tubes. The tubes were then added with 3 mL of DNS reagent and placed in a water bath at 90°C for 5 min. Then, 1 mL of 40% Rochelle salt solution (potassium sodium tartrate) was added to the tubes when the contents were still warm. After the tubes were cooled to ambient temperature, the dark red colored intensity was read at a wavelength of 510 nm in a spectrophotometer (SpectraMax i3X, China). One mL of distilled water was added in a 15 mL glass tube and used as a blank sample; the procedure mentioned above was repeated for all SWW samples. A series of glucose solutions with known glucose mass (0 – 500 µg mL^{-1}) were run in parallel for calibration purposes.

2.4 Amylase Activity at Different Starch Concentration

To study the effect of different starch concentration on amylase secretion by *C. tropicalis* ASY2 in SWW, soluble starch was added to the SWW separately with different concentration ranged from 10 to 80 g L^{-1} and sterilized. The pH of SWW was adjusted to 6 and inoculated with 5% active incoulum of *C. tropicalis* ASY2 (10^6 cfu mL^{-1}). The amylase activity was determined through the DNS method [15] at on 5^{th} day of fermentation.

2.5 Statistical Analysis

All the experiments were run in triplicates. Mean values with the standard error of deviation are presented. The experiments were subjected to one way analysis of variance (ANOVA) and the significance of differences between samples means were calculated by XLSTAT 2010 software using Duncan's multiple range tests. P values ≤ 0.05 were regarded as significant.

3. RESULTS AND DISCUSSION

The present work explored the production of amylase, by utilizing sago processing wastewater as a substrate using *C. tropicalis* ASY2 yeast strain under submerged fermentation. The amylase produced from SWW was comparatively evaluated with various starchy wastes and oleaginous yeast. The results of the present study are discussed below.

3.1 Kinetics of Amylase Activity in SWW

In SWW, the amylase activity was monitored for an incubation period of 10 d. The amylase activity was commenced from the first day by consuming 35% (3.45 g L^{-1}) of starch in SWW by secreting 0.93 IU ml^{-1} (Fig. 2) of amylase. The activity was gradually increased up to 204 h of fermentation. Afterwards, there was no much difference observed in amylase activity. The maximum amylase activity was recorded at unit 240 h of fermentation with the enzyme activity of 1.79 IU mL^{-1} at 240 h of fermentation (Fig. 2). While comparing our findings with Paludo et al [15], it was observed that the optimized simple starch-based medium was as a substratum for growing of *Coprinus comatus* for amylase production and reported a maximum amylase activity of 5.84 $U.mL^{-1}$ at 48 h fermentation. Even though, yeast strain in the present study recorded lower activity than Paludo et al. [15] there is a possibility for increasing the activity by providing additional starch, optimization conditions etc.

3.2 Amylase Activity at Different Starch Concentrations in SWW

In SWW, additional starch was added to evaluate its influence on amylase secretion. Among the different starch concentrations (10 to 80 g L^{-1}) evaluated, maximum amylase activity of 1.51 IU mL^{-1} is observed in SWW with 20 g L^{-1} of starch concentration (Fig. 3). After this concentration, the amylase activity was decreased with an increase in starch concentration (1.09 IU mL^{-1} at 80 g/L). This might be due to the presence of excess starch content. It is very clear from Fig. 3, that the maximum response for enzyme production occurred when transitional substrate concentrations were used. According to Amim et al. [16] as the substrate concentration increases,

enzyme production increases proportionally until the medium is saturated with the substrate. When this saturation point is reached, the addition of extra substrate does not influence the levels of enzyme produced. In the present study also, it was observed that as the concentration of starch increases, the enzyme becomes saturated and thereafter recorded lesser enzyme units. Only after the empty of the catalytic site of amylase, the additional starch substrates will undergo the reaction. The rate of glucose formation now depends on the enzyme's activity itself, and adding more starch will not affect the reaction rate to any significant effect. Bothast and Schlicher reported earlier on the role of amylase in converting raw starch into glucose within 48–72 h to produce the desired product [17]. Wild et al. [18], reported higher development of *Lipomyces starkeyi* lipids on the starch substrate (rice residue) than glucose, since the strain is capable of directly converting starch using amylase enzyme.

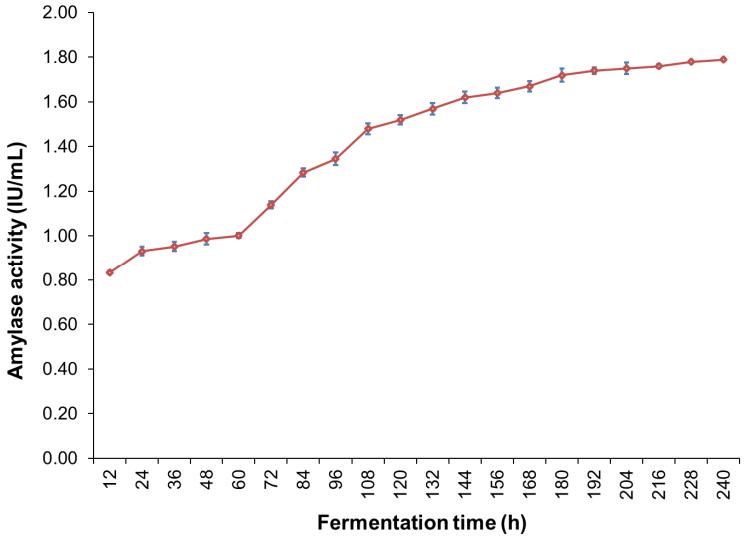

Fig. 2. Time course study of amylase activity of *C. tropicalis* ASY2 in SWW

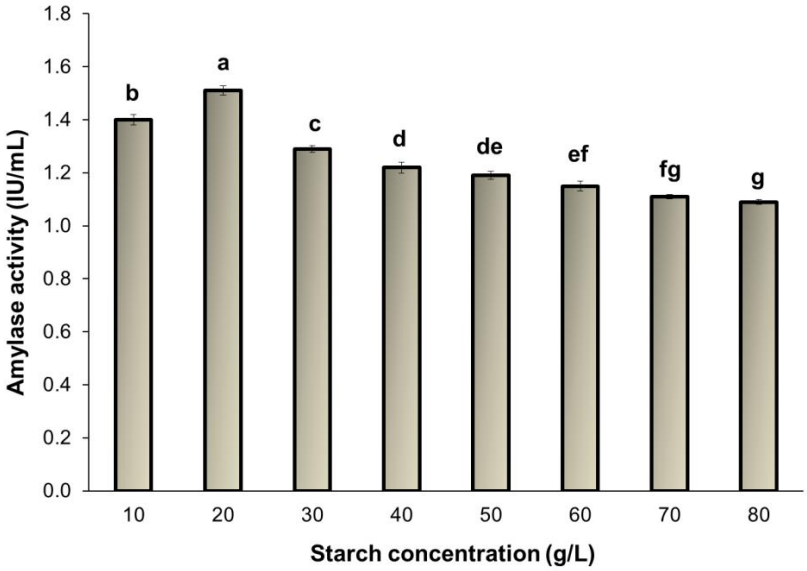

Fig. 3. Amylase activity of *C. tropicalis* ASY2 in SWW at different starch concentrations

Table 1. Comparative analysis of amylase activity of *C.tropicalis* ASY2, using different starchy wastes (adopted from Chaturvedi et al., [19])

Substrate	RG	RM	SP	LS	GW	CT
Potato peel	0.73 ± 0.03	0.07 ± 0.05	0.35 ± 0.07	0.20 ± 0.05	0.23 ± 0.07	-
Banana peel	0.72 ± 0.01	0.23 ± 0.20	0.39 ± 0.14	1.12 ± 0.06	0.33 ± 0.18	-
Cassava peel	0.69 ± 0.04	0.05 ± 0.01	0.40 ± 0.06	0.92 ± 0.05	0.33 ± 0.20	-
Corn residue	0.12 ± 0.01	0.14 ± 0.01	0.63 ± 0.01	0.94 ± 0.01	0.33 ± 0.21	-
Rice residue	1.38 ± 0.11	0.02 ± 0.01	0.39 ± 0.04	0.26 ± 0.09	050 ± 0.07	-
Wheat bran	0.79 ± 0.04	0.05 ± 0.00	0.56 ± 0.02	0.59 ± 0.21	0.35 ± 0.00	-
Yam peel	0.53 ± 0.01	0.08 ± 0.05	0.63 ± 0.00	0.34 ± 0.01	0.39 ± 0.02	-
Barley residue	0.47 ± 0.08	0.16 ± 0.13	0.31 ± 0.05	0.32 ± 0.02	0.39 ± 0.03	-
Sago processing wastewater	-	-	-	-	-	1.48 ± 0.02 (5^{th} day)

Note: R. glutinis RG, R. mucilaginosa RM, S. pastorianus SP, L. starkeyi LS, G. wiirorense GW, Candida tropicalis ASY2 CT, 10% of substrate used

3.3 Comparative Analysis of Amylase Activity of *C. tropicalis ASY2* in SWW's with Various Starchy Wastes using Different Oleaginous Yeasts

In this present study, our results of the amylase activity of yeast strain in SWW was compared with various starchy wastes and oleaginous yeast and presented the results in Table 1. Highest amylase activity of 1.48 and 1.51 IU mL^{-1} is observed at starch concentration of 10 and 20 g L^{-1}, respectively at 120 h of fermentation compared to other agro-starchy wastes (Table 1) such as potato peel (0.73 IU mL^{-1}), banana peel (0.72 IU mL^{-1}), cassava peel (0.69 IU mL^{-1}), corn residue (0.12 IU mL^{-1}), rice residue (1.38 IU mL^{-1}), wheat bran (0.79 IU mL^{-1}), yam peel (0.53 IU mL^{-1}) and barley husk (0.47 IU mL^{-1}) by *R. glutinis* [19] on 8^{th} day of fermentation(Table 1). The yeast strain ASY2 produced higher amylase activity of 1.51 IU mL^{-1} as compared to *Rhodotorula glutinis, Lipomyces starkeyi* and other agricultural wastes [19]. Based on the statistical analysis, the starch concentration at 20 g L^{-1} is the best concentration compared to other concentrations.

4. CONCLUSION

The remarkable ability of the yeast strain *Candida tropicalis* ASY2 to produce on amylase activity in SWW shows economic viability of the enzyme production technology using starchy Waste streams.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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