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RESEARCH ARTICLE

Microbial biodiesel production: novel method of utilizing sago wastewater for lipid production using oleaginous yeast, *Candida tropicalis* ASY1

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ABSTRACT

An investigation was aimed at identification and screening of oleaginous yeast which accumulate high level lipid in its biomass by using sago processing waste water as substrate. A total of three isolates obtained from sago waste water sample were screened for their ability to utilize starch by amylase secretion. Of which, the isolate ASY1 found to be positive for amylase activity and this yeast was identified as *Candida tropicalis* by 18srRNA sequencing. Cultivation of *C. tropicalis* ASY1 for ten days and kinetic analyses of various parameters like pH, biomass, amylase, starch, protein and cellular lipid revealed that the amount of lipid obtained was **1.37 g.L^{-1}**, registering **48.96% of** lipid and amylase activity reaching maximum on 7th day (**10.88 IU.ml^{-1}**). Therefore, *Candida tropicalis* ASY1 could be a potential candidate effectively used for next generation biodiesel production.

Key words: oleaginous yeast, Microbial lipid, Biodiesel, sago processing wastewater

Introduction:

Tapioca (cassava) is one of the major staple food around the globe and it is industrially used to get starch, sago (Sabudana), chips and wafers. Sago handling industry is one of the imperative agro based industry. There are around 500 sago preparing ven- tures, of which 470 units are in Salem area of Tamil Nadu, India. Salem area is one of driving makers of cassava in India. Around 90% of cassava produced is consumed by sago preparing industry. The waste water from such starch industry is utilized for irriga- tion due to water Scarcity. Starch waste water is rich in organic content, COD and BOD hence eutrophica- tion is one of the major environmental problem. So the waste water can be viably utilized for microbial lipid production and lessen the earth contamination brought on by the water. In this investigation we prin- cipally focus on oleaginous yeast on the grounds that different strains of yeast have appeared to use differ- ent carbohydrate substrate to aggregate microbial oil particularly lipid under stress conditions.

Materials and Methods:

Sago Processing waste water sample was col- lected from T.A Perumal sago industries Salem, Tamil Nadu, (India) for oleaginous yeast isolation. The sam- ple was serially diluted and plated in Yeast extract agar medium and incubated at 30 °C for about 48 hr. Based on colony morphology, three isolates were picked, sub cultured and purified. Later on these iso- lates namely ASY1, ASY2, ASY3 were distinguished as yeast by microscopic examination. Then these three yeast species were subjected to amylase activity by plate screening. Out of these three isolates ASY1 showed positive result for amylase activity and the DNA from the isolate was extracted by Bust n' Grab method and its concentration and purity was ob- served by Nano drop spectrophotometer and agarose gel electrophoresis. PCR amplification of 18srRNA of isolate was performed using NL1 and NL4 primers. The PCR products were sequenced and upon analyses of retrieved sequences, resulted in identification of yeast isolates as ***Candida tropicalis.***

Kinetic study of *Candida tropicalis* was done with sago waste water as substrate for its growth. Starch content of the wastewater was measured by Phenol sulfuric acid method (Dubois *et al*., 1965) and the total ammonia content by Kjeldahl method (Kjeldahl, 1883). Finally the C:N ratio was adjusted to 30:1. Fifty ml of this prepared medium was added in 250ml conical flask in three replicates and sterilized at 121 °c at 15psi for 15 min. Then 72 hr grown yeast culture was inoculated into the culture medium. From the flasks culture media was withdrawn at customary interims and subjected to parametric investigations. Spent culture medium was centrifuged at 10,000 rpm for 10 min. The supernatant was utilized for analyzing pH, dissolvable starch, amylase (DNS method), pro- tein (Bradford method) and cellular Lipid estimation of yeast was done by using Folch method.

Results and Discussion:

Using sago processed wastewater as a sample, 3 yeast isolates were made namely ASY1, ASY2, ASY3 and of which ASY1 showed positive for amylase ac- tivity (Fig 1). PCR amplification using NL1 and NL4 primers yielded 600bp product (Fig: 2) which was then sequenced. After sequencing, it was identified as *Candida tropicalis* for which a phylogenetic tree was constructed as shown below (fig 3)

Sago wastewater having C: N ratio 30:1 was used as growth medium for which kinetic parameters (pH, biomass, starch, lipid, and protein) were measured at periodical intervals for 10 days of yeast growth. It was noticed that a maximum cellular lipid of **1.37 g.L^{-1}**, registering **48.96 %** lipid was recorded on 7th day of culture. Interestingly, maximum amylase activity of

10.88 IU.ml^{-1} was observed for this isolate which sug- gest that the *C. tropicalis* ASY1 utilized the starch for its growth and produced a maximum amount of lipid. Hence, this isolate could be a potential candidate for high level lipid production leading to biodiesel gener- ation using cheaply available wastewater.

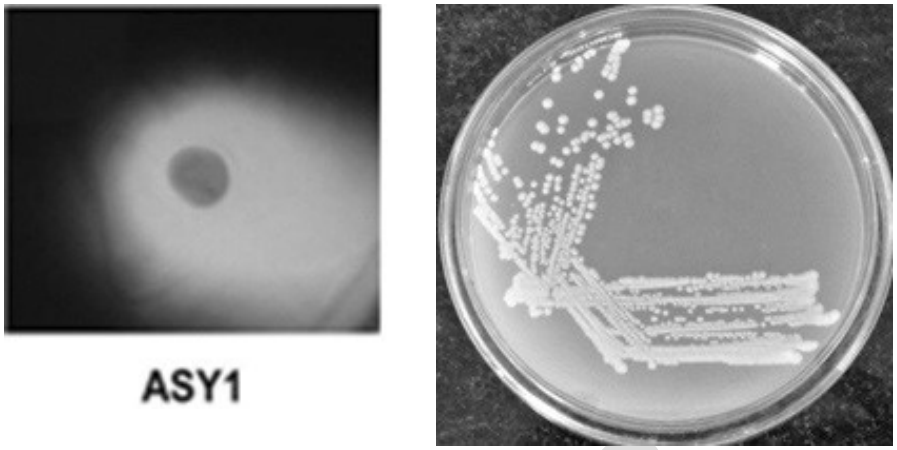

Fig 1: ASY1 showing positive amylase activity

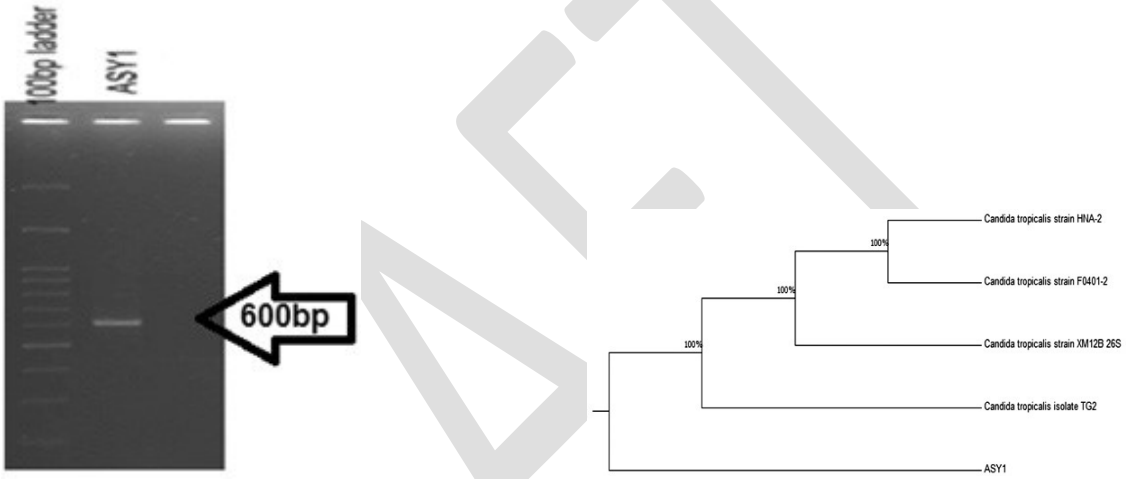

Fig 2: gel picture of DNA extracted from yeast isolate using NL1 and NL5 primers

Conclusion:

Considering the suitability of sago wastewater as a substrate for cultivation of *Candida tropicalis* ASY1, its starch hydrolyzing ability and simultaneous lipid production make this as potential candidate for biodiesel generation.

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