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Research Article

Production of Single Cell Protein using *Kluveromyces marxianus* isolated from paneer whey

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Abstract

Objectives: This work emphasizes on yeast biomass production (SCP) from paneer whey using lactose fermenting yeast strain.
Methods: In the current study, 11 yeast strains were isolated from paneer whey sample collected from a dairy center in Chennai. The isolates were screened for their lactose fermenting ability and the strain that showed maximum growth in paneer whey was selected for biomass production. This strain was further tested for its ability to reduce BOD and to produce biomass.
Results: The isolate which produced maximum biomass and crude protein content was identified as *Kluveromyces marxianus*.
Conclusion: The results of the study show that the isolated strain *Kluveromyces marxianus* was capable of reducing the BOD and producing biomass of significant nutritional quality.
Keywords: SCP, paneer whey, *Kluveromyces marxianus*, BOD

1. Introduction

Whey is the liquid remaining after the production of cheese or the removal of fat and casein (80% of the proteins) from milk. Whey still contains about 50% of the nutrients present in milk, comprising milk sugar (lactose), serum proteins (whey proteins), minerals, a small amount of fat, and most of the water soluble minor nutrients from milk such as vitamins[1]. The low concentration of these components makes their recovery un-economical process[2]. Whey is treated as a waste and discharged without any treatment creating pollution problems. Lactose the main nutrient in whey can be economically utilized by its conversion to single cell protein[3]. Single cell protein production technologies arose as a promising way to solve the problem of worldwide protein shortage. It has evolved as a bioconversion process which turned low value by-products, often wastes, into products with added nutritional and market values and since SCP belongs to one of the economical protein products in the market, its production being profitable .

As compared with plants and animals for providing protein for food or feed, large scale industrial production of microbial biomass for the same has characteristic advantages such as, high rates of multiplication of microbes, high protein yield (30-80% protein in terms of dry weight etc. They can utilize a large number of low cost carbon sources including waste materials. Production installations also occupy limited areas and give high yields[4].

2. Materials and Method

2.1 Sample collection and biochemical analysis

Paneer whey was collected from a dairy center located in Chennai using sterile bottles and brought to the laboratory within a time period of 4 Hrs. Biochemical parameters such as pH, Total Dissolved Solids (TDS), Biological Oxygen Demand (BOD), fat and protein contents were analysed[5].

2.2 Isolation of yeast strain

The collected whey sample was allowed to ferment naturally at room temperature for 4 days. After four days, the sample was serially diluted and different dilutions were inoculated into Yeast Mannitol (YM) agar with chloramphenicol (0.1g/L) by pour plate and spread plate methods to isolate yeast strains. The plates were incubated at 25°C for 72 hrs. Colonies with distinct morphological features were selected and purified by sub-culturing on Potato Dextrose Agar (PDA). The purified isolates were stored on PDA slants at 4°C [2, 6].

2.3 Identification of the yeast strain

Primary identification was done by microscopic examination of culture stained with lactophenol cotton blue (LPCB). The strain, which showed maximum growth in paneer whey, was selected by checking cell concentrations at 620 nm[7]. For DNA extraction, the strain was routinely grown on PDA agar plate at 28°C for 24 to 48 hours. A single colony was then grown overnight on YM broth at 28°C with shaking at 150 rpm. DNA was extracted from the culture by adapting the method described by[8]. The 18s rRNA sequencing of the yeast was done for identification purpose.

2.4 Media preparation and Biomass Production

The pH of the whey was adjusted to 5 and boiled at 100ºC for 15 min. To prevent protein precipitation during heat sterilization, the proteins were eliminated from whey by cooling and filtering the sedimented proteins. One litre of the filtrate obtained (greenish yellow) was sterilized at 115ºC for 10 min and then inoculated with 5% of actively growing isolates. The medium was incubated at 28ºC for 5 days with constant shaking at 150 rpm[9]. After incubation, the biomass of the yeast cells was obtained by centrifugation at 4000 rpm and washed twice with distilled water. Dry weight was determined after drying overnight at 105 ºC. The protein percentage of biomass was estimated by micro-kjeldahl method with 6.25 as conversion factor.

2.5 Bioremediation by yeast culture

The pure culture of the selected isolate was studied for its ability to reduce BOD of whey. The whey sample was inoculated with actively growing cells of the isolate and incubated at room temperature with constant stirring. After 5 days of incubation, the biomass was removed by centrifugation and the supernatant was collected and subjected to BOD and TDS determination.

3. Results

The biochemical parameters, of the freshly collected whey samples, such as BOD, TDS, fat content, protein content and pH are depicted in Table 1.

Table .1 Biochemical analysis of paneer whey

Parameter	Amount
BOD	30000 mg/L
TDS	8300mg/L
Fat	0.5-0.6%
Protein	98.6mg/ml
pH	3.5

3.2 Isolation of yeast strain

A total of 11 yeast strains were isolated from the collected sample. No microbial contamination was observed during fermentation at room temperature for 4 days. Among the yeast strains isolated from paneer whey sample, one isolate showed maximum growth with a biomass yield of 45g/L with crude protein content of 48.1%.

Table 2: Result of biomass yield and protein percentage

Parameter	Amount
Biomass yield (g/L) Dry cell weight	4.5
Crude Protein	48.1(%)

3.3 Identification of the isolate

The isolate which showed maximum grow in whey sample was identified to be budding yeast cells which was confirmed by LPCB staining (Fig. 1). The rRNA sequencing analysis of the strain revealed that the isolate was Kluveromyces marxianus (Fig.2).

Figure 1: Budding cells of the isolate stained with LPCB

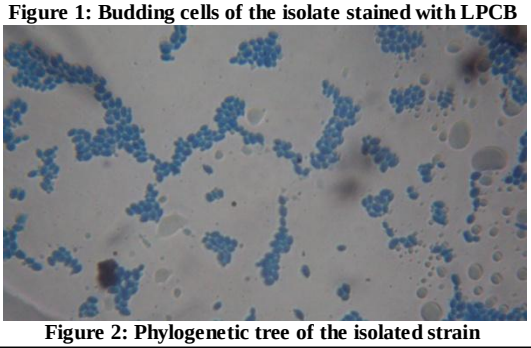
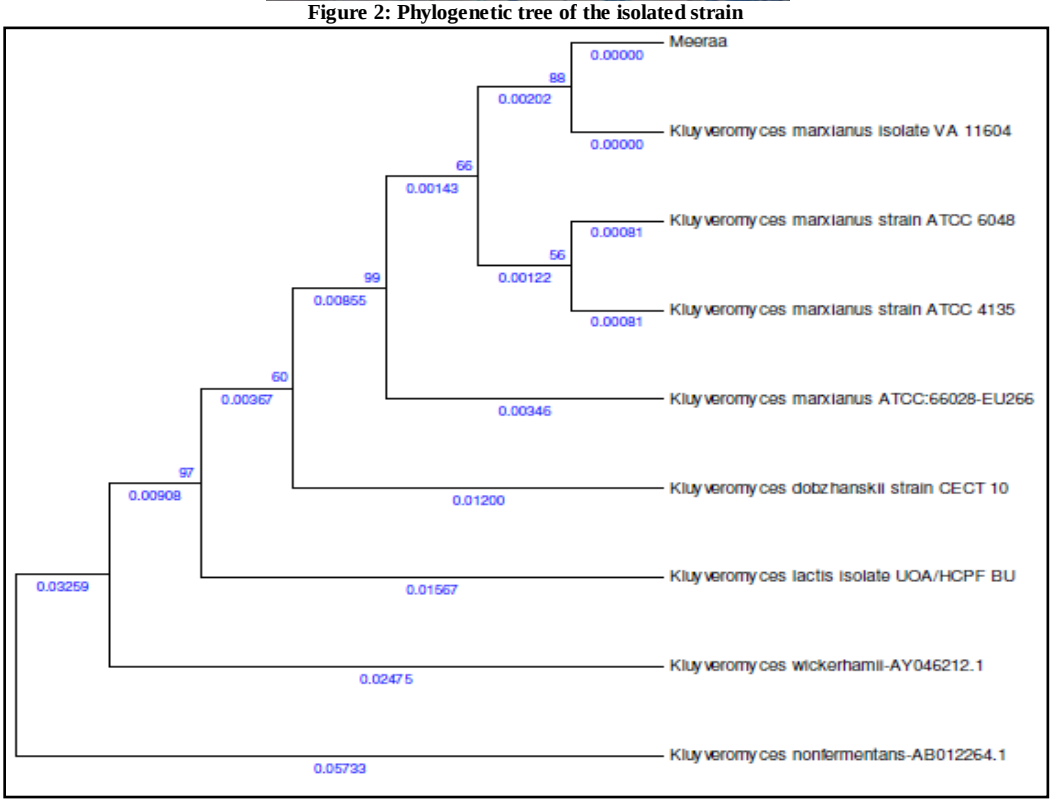


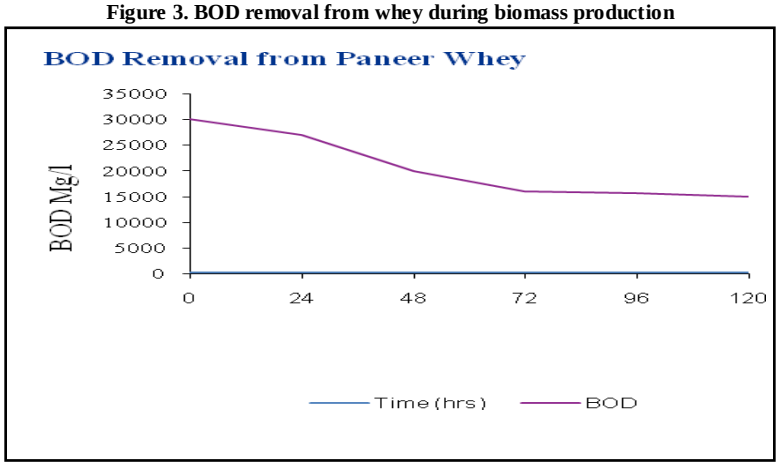
Figure 2: Phylogenetic tree of the isolated strain



3.4 Bioremediation studies

The results of the bioremediation studies reveal that the isolated strain *Kluveromyces marxianus* was effective in BOD removal from 30,000mg/L to around 15,000mg/L i.e., 50% reduction in BOD level was obtained.

Figure 3. BOD removal from whey during biomass production



4. Discussion

Paneer whey is considered as a waste and large quantities of it are discharged in to dairy effluent creating pollution problems. Paneer whey is acidic having a pH of 3.5 and also has a high biological oxygen demand[10]. Like other types of whey such as cheese whey, paneer whey also can be used as a cheaper source of carbon. Since it contains lactose and other milk proteins it dramatically enhances biomass production[11]. The isolated lactose fermenting yeast strain showed good biomass production in paneer whey which is significant when compared to that produced in an earlier study. Conversion of paneer whey in to SCP helped to reduce the BOD level.

5. Conclusion

The results of the current study suggest that the isolated strain *Kluveromyces marxianus* was found to be significant in biomass production, BOD reduction and protein production. Hence it can be used in bioremediation processes and a significant source of SCP.

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