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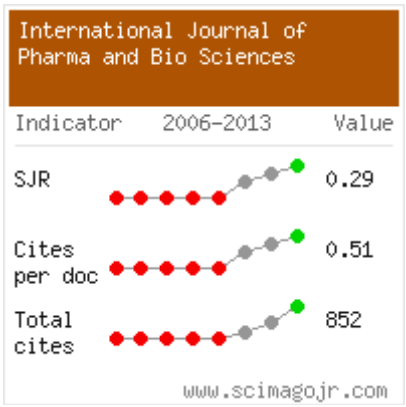

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STUDIES ON THE OPTIMIIZATION OF BIOMASS PRODUCTION USING *KLUYVEROMYCES MARXIANUS* ISOLATED FROM PANEER WHEY

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ABSTRACT

In the present study *Kluyveromyces marxianus* isolated from paneer whey was employed for biomass production. Various parameters like pH, temperature and nitrogen source were optimized for maximum biomass yield. The biomass yield was studied to vary with different nitrogen sources, pH and temperature. Ammonium sulphate, ammonium nitrate, beef extract and yeast extract were used as nitrogen sources. In addition, the concentration of the nitrogen source at which the biomass yield proved to be high was also optimized. Among the nitrogen sources tested, ammonium sulphate showed maximum yield at a concentration of 0.4mg/ml. The results of the study conclude that *Kluyveromyces marxianus* could be effectively produced from paneer whey at optimal conditions and used for various industrial applications.

KEYWORDS: ***Kluyveromyces marxianus*, Paneer whey, biomass, nitrogen sources.**

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INTRODUCTION

Whey is the major by-product of dairy industry and its disposal without expensive sewage treatments represents a major source of water pollution due to the bulk quantities and its high organic matter content[1]. The main solute in cheese whey is lactose present at a concentration of about 3-8.Other components are protein salts and vitamins that are present in minor amounts. The low concentration of these components makes their recovery uneconomical[2]. Lactose the main nutrient in whey can be economically utilized by its conversion to single cell protein[3].Comparative analysis of cheese whey and paneer whey indicated significantly higher concentration of sodium, potassium, calcium and chloride contents in paneer whey than cheese whey. The analysis is important as paneer whey can be utilized more efficiently otherwise creating environmental pollution especially in India. In India there has been a substantial increase in the production of paneer, resulting in an increased accessibility of whey. India's annual production is estimated at150000 tones of paneer and concerning 2 million tones of whey containing about 130000 tones of valuable milk nutrients are produced per annum[1]. As compared with plants and animals for providing protein for food or feed, large scale industrial production of microbial biomass for the same as has great characteristic advantages such as; micro organisms in general have a high rate of multiplication and a high protein content(30-80 protein in terms of dry weight; they can utilize a large number of low cost carbon sources including waste materials; Production installations occupy limited areas and give high yields except for algae microbial production is independent of seasonal and climatic variations and there for more easy to plan[4].

MATERIALS AND METHODS

Collection of paneer whey and isolation of Kluyveromyces marxianus

Paneer whey was collected from a dairy centre located in Chennai; kept in insulated ice packs and brought to the laboratory in sterile bottles. The collected whey was boiled and the precipitated protein was removed by centrifugation. The paneer whey was poured in 15ml screw cap tubes for further use. The fungal strains were isolated, screened and identified by the authors in their previously reported work[5]. The strain *Kluyveromyces marxianus,* maintained on Potato Dextrose Agar (PDA) plates, obtained in their earlier work was taken for the current study. A portion of the whey sample was stored under refrigerated conditions for further use.

Optimization of pH for the growth of K. marxianus

The pH of the production medium (paneer whey) was adjusted to 3, 4, 5, 6, 7 and 8, autoclaved at 121°C for 15min. The sterile medium was cooled to room temperature and was inoculated with 10% of seed culture (*Kluyveromyces marxianus*) and incubated at 28°C for 24 hr in a shaking incubator (180rpm). The biomass yield was determined by measuring the OD at 620nm.

Optimization of temperature for the growth of K. marxianus

Paneer whey medium prepared according to the optimized pH was taken in 15 ml screw cap tubes and autoclaved. 10% of inoculum was added aseptically to the sterile medium and incubated at various temperatures ranging from 20 to 45°C under shaking condition (180rpm) for 24hrs. After incubation, OD was determined at 620nm to find the optimal temperature that shows maximum growth of the fungal strain.

Effect of different nitrogen sources on biomass yield

The effect of various nitrogen sources on biomass yield was studied by amending the whey medium with ammonium sulphate, ammonium nitrate, yeast extract and beef extract. In addition, the effect of varying concentrations of these nitrogen sources (0.2, 0.4, 0.6, 0.8, 1, and 1.2%) was also studied. The inoculated whey medium was incubated for 5 days under shaking condition at the optimized temperature. After incubation the cells were collected by centrifugation at 4000rpm and washed twice with distilled water[6]. Dry weight was determined after

drying overnight at 105°C. All experiments were done in duplicates.

RESULTS

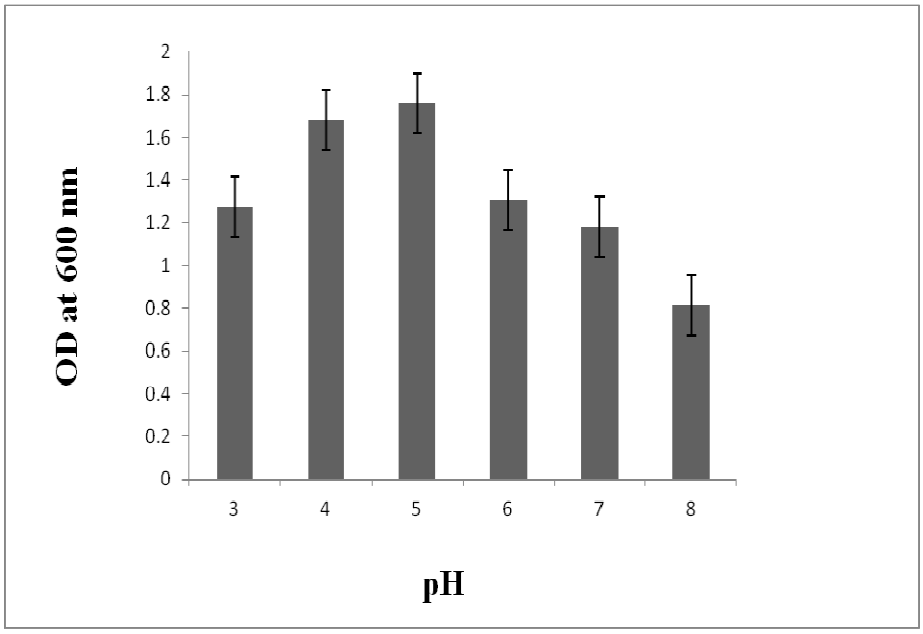
The *Kluyveromyces marxianus* strain thus obtained was stored on PDA at 4°C for further use.

Figure 1
Pure culture of Kluyveromyces marxianus

Optimization of pH for the growth of Kluyveromyces marxianus

The result of the pH optimization suggests that among the varying pH tested, maximum growth of *K. marxianus* was observed at pH5 at which the absorbance was recorded as 1.759. Also, it was observed that growth was significantly reduced when the pH of the production medium was increased above 5.

Figure 2
Effect of different pH on Kluyveromyces marxianus growth in paneer whey

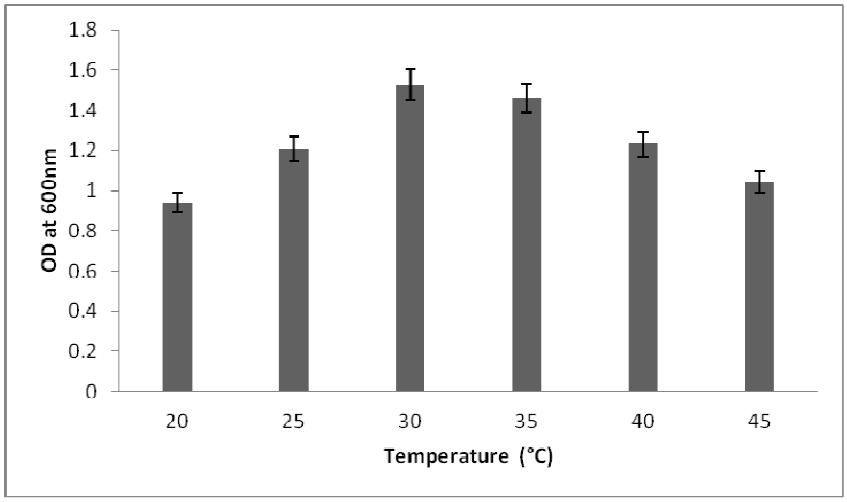


Optimization of temperature for the growth of Kluyveromyces marxianus

The optimal temperature for the maximum growth of *K. marxianus* in paneer whey was found to be 30°C, which is evident from the highest absorbance value recorded (1.528). In the varying temperatures tested, the fungal growth was found to vary in the range of 0.94-1.5 and the growth gradually reduced at temperatures higher than 30°C.

Figure 3

Effect of temperature on the growth of Kluyveromyces marxianus in paneer whey



Effect of different nitrogen sources on biomass yield

The results of nitrogen source optimization depict that, almost all the tested nitrogen sources such as Ammonium sulphate, ammonium nitrate, yeast extract and beef extract enhanced the yield of fungal biomass (Fig 4-7). The biomass yield using these nitrogen sources was studied to be in the range of 3.2-6g/L. However, ammonium sulphate showed the maximum biomass yield of 6g/L. Also, the concentration of ammonium sulphate that produced maximum biomass yield was recorded as 0.4%.

Figure 4

Effect of different concentration of ammonium sulphate on biomass production

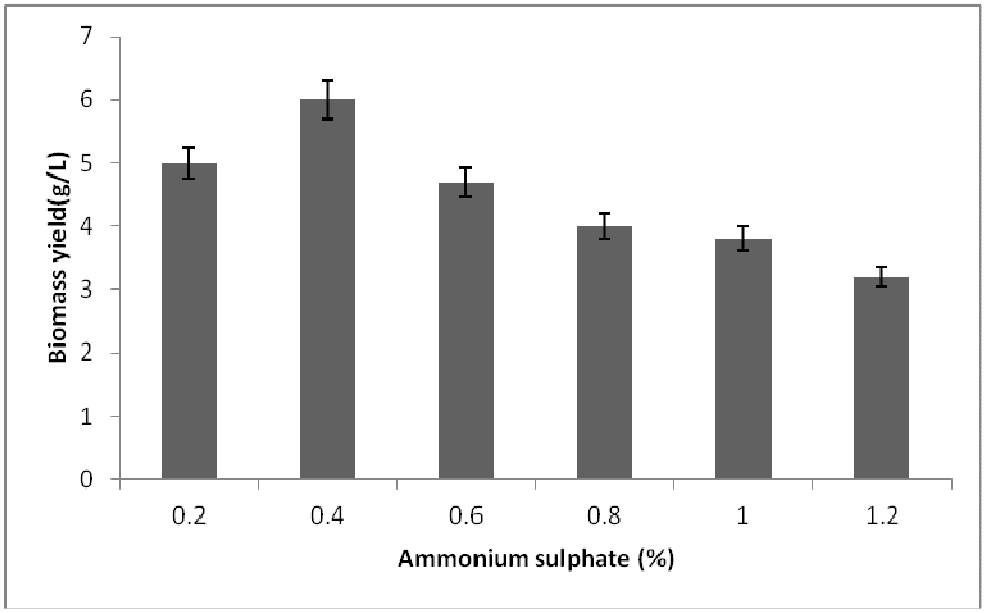


Figure 5
Effect of different concentration of ammonium nitrate on biomass yield

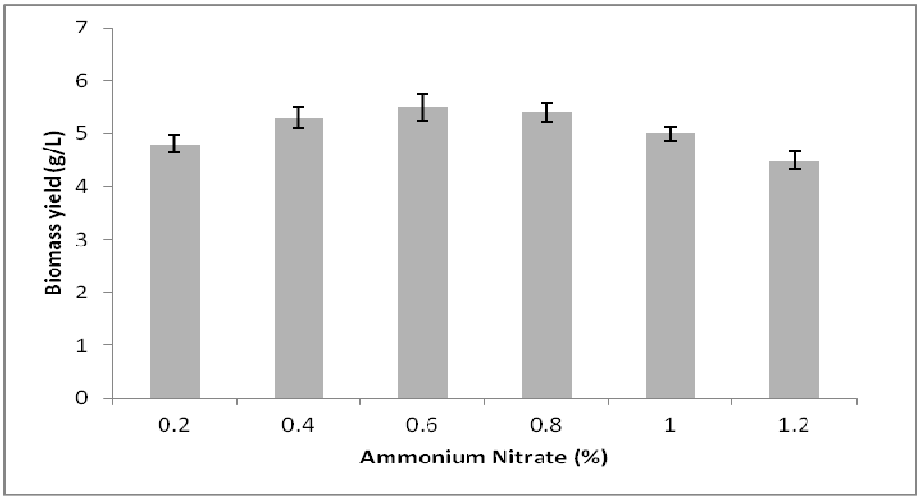


Figure 6
Effect of different concentrations of yeast extract on biomass yield

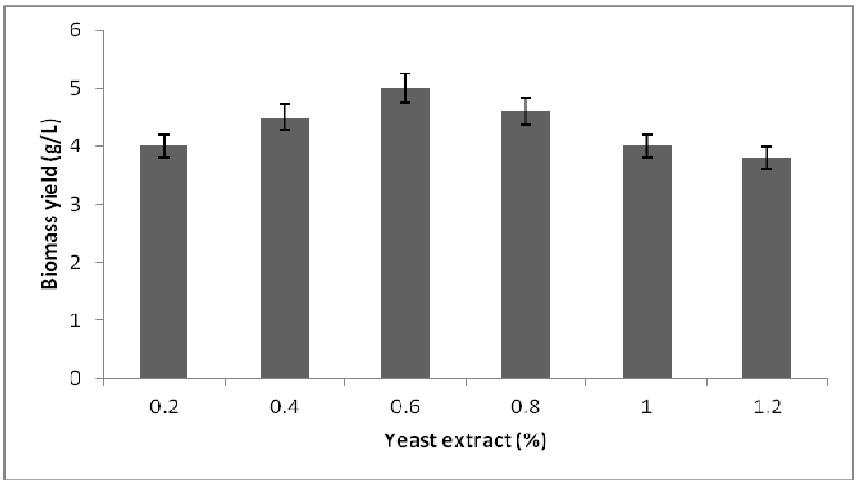
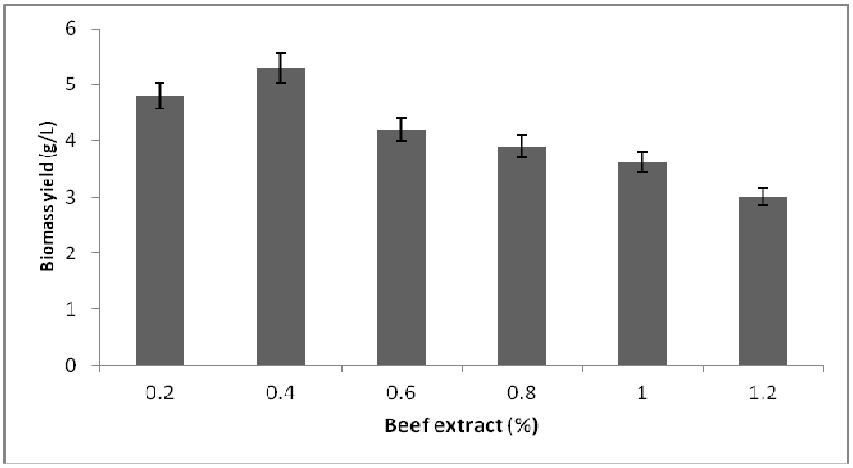


Figure 7
Effect of different concentrations of beef extract on biomass yield



DISCUSSION

From the study it was observed that the yeast strain *Kluyveromyces marxianus* showed maximum growth at conditions as the following, temperature 30°C, pH 5, 0.4% ammonium sulphate as a nitrogen source. The pH of culture medium is considered as one of the pivotal factors for microbial metabolism and secondary metabolites biosynthesis. pH is said to be related to cell wall and membrane permeability characteristics and thus has got an effect on either ion uptake or loss to the nutrient medium[7]. As various literature exists on the growth of fungus in acidic conditions, our study also found that *K. marxianus* grows in between pH 5-7. Different studies have proved that incubation temperature is one of the major conditions affecting the growth rate of microorganisms[8]. Incubation temperature ranging between 20 and 25°C was detected to be optimum for mycelial growth of *Rhizoctonia solani*[9]. The results of temperature optimization of the current study are in coherence with a similar study which suggests that the increase of incubation temperature from 25 to 30ºC enhances the growth of the cells and production of bioactive metabolite from *Aspergillus* strain TSF 146[10]. A similar study has proved that *Neolentinus kauffmanii* was grown in a different temperature range among which best mycelia yield (301mg/100cm^3) was attained at 25°C after 17 days of incubation[11]. Various nitrogen sources showed better biomass yield when compared to yield in paneer whey without supplimentation. Among nitrogen sources like ammonium nitrate ,ammonium sulphate, beef extact and yeast extract, ammonium sulphate showed much biomass yield at0.4gm/l. In conclusion the growth rate of *Kluyveromyces* strain can be increased by the supplementation of nitrogen source.

CONCLUSION

Thus the current study concludes that *Kluyveromyces marxianus* could be effectively produced from paneer whey at optimal conditions and used for various industrial applications.

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