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Isolation of Novel Strains from Natural Sources for Biotransformation Studies

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Abstract: Isolation and screening of novel strains of yeasts from natural sources for biotransformation studies were investigated. Three strains were isolated from different natural sources like blackgrapes, date fruit and sugarcane juice. These strains were identified at the Institute of Microbial Technology, Chandigarh, basing on sequencing of D₁/D₂ domain of 26S r RNA gene and assigned MTCC numbers. These three strains were designated as *Candida pseudointermedia* MTCC No.6225 (BGY), *Issatchenkia orientalis* MTCC No.6351(DY), *Candida pseudointermedia* MTCC No.6352(SCY), respectively. These strains were used for biotransformation studies.

Key words: Isolation, screening, novel strains, blackgrapes, date fruit, sugarcane juice, biotransformation

INTRODUCTION

Biotechnology employs microorganism as well as higher cells and their active principles with the aim of achieving desirable conversions of various substrates (Tripathi *et al.*, 1997). The importance of novel strains in the bioconversion is a great task. Biotransformation potentials of the growing cells free harvested cells immobilized cells and isolated crude as well as purified enzyme have been extensively studied (Liew *et al.*, 1995; Shin and Rogars, 1996a, b). L-Phenyl acetyl carbinol is routinely produced via biotransformation of aromatic substrate benzaldehyde. L-PAC is starting material for chemical synthesis of L-ephedrine hydrochloride and pseudo ephedrine, pharmaceutical compounds used as decongestant, antiasthmatics (Shin and Rogars, 1995) and recently reported, used in obesity control (Astrup *et al.*, 1992). Certain yeast strains possess enzymes that produce L-PAC and benzyl alcohol, a by product, respectively from benzaldehyde (Nikolova and Ward, 1991).

We have studied L-PAC production from benzaldehyde by producing various novel strains under various growth and biotransformation modalities with a view to monitor the ideal conditions permitting maximum product yield at constant substrate concentration and cell density.

MATERIALS AND METHODS

Isolation and Screening of Yeasts

Fresh commercial grade Bakers yeast pills were added aseptically into 5 mL sterile water present in a test tube. After addition of yeast pills the cotton plug was replaced and the test tube was rolled between two palms for complete dissolution of mass in the water. One loop full of resulted solution was aseptically transferred onto sterile YEMA slants with sterile transferring loop. The inoculated YEMA slants were incubated at room temperature (28°C) for two three days. After incubation the pure yeast growth was observed on medium. A small amount of cell mass was smeared on clean glass

slide and allowed to dry. The smear was stained with crystal violet and focused under microscope. Large oval shaped pure cells of *S. cerevisiae* were observed. This organism was designated as BY and used as standard strain for comparison of biotransformation with other isolates through this study.

Totally four yeast strains were used to study their biotransformation potential to produce L-PAC from benzaldehyde. One of the strains is *Saccharomyces cerevisiae* which was isolated from commercial fresh bakers yeast mentioned as BY and was used as a standard strain to compare with other yeast strains isolated from different sources for their bioconversion potential.

Three yeast strains were isolated from three different sources like black grapes, date fruit and sugarcane juice.

Cleanly washed black grapes were added into 100 mL conical flasks containing sterile water and incubated at room temperature for two to three days. After incubation one loop full from each flask was added aseptically to separate petri plates containing YEMA medium. Antibiotics like streptomycin and griseofulvin were added to YEMA medium to prevent the growth of bacteria and fungi respectively. After inoculation plates were incubated for two to three days at room temperature and yeast growth was observed. The obtained yeast colonies were further purified by streaking on petriplates containing the same medium. The strain was mentioned as BGY.

Paste of date fruit prepared by grinding in sterile mortar and pestle, was added into 100 mL conical flask containing sterile water and incubated at room temperature for two to three days. After incubation one loop full from each flask was added aseptically to separate petri plates containing YEMA medium. Antibiotics like streptomycin and griseofulvin were incubated for two to three days at room temperature and yeast growth was observed. The obtained yeast colonies were further purified by streaking on petri plates containing the same medium. The strain was mentioned as DY.

Medium

Yeast Extract Malt Extract Agar (YEMA) Medium was used for isolating and maintaining cultures.

The Compositions of YEMA is as follows:

Yeast Extract 0.4%
Dextrose 0.4%
Agar 1.75%
Malt Extract 1%
Water To make up to 100 mL
pH 5.5

Sugar cane juice was added into 100 mL conical flasks containing sterile water and incubated at room temperature for two to three days. After incubation one loop full from each flask was added aseptically to separate petri plates containing YEMA medium. Antibiotics like streptomycin and griseofulvin were added to YEMA medium to prevent the growth of bacteria and fungi respectively. After inoculation, the plates were incubated for two to three days at room temperature and yeast growth was observed. The obtained yeast colonies were further purified by streaking on petriplates containing the same medium. The strain was mentioned as SCY.

The strains were mentioned as BGY, DY and SCY throughout the work until their identification and were used for different studies. These strains were identified at the Institute of Microbial Technology, Chandigarh, basing on sequencing of D1/D2 domain of 26 S rRNA gene and assigned MTCC numbers. These three strains were identified as *Candida pseudointermedia* MTCC No.6225 (BGY), *Issatchenkia orientalis* MTCC No. 6351 (DY), *Candida pseudointermedia* MTCC No. 6352 (SCY) respectively.

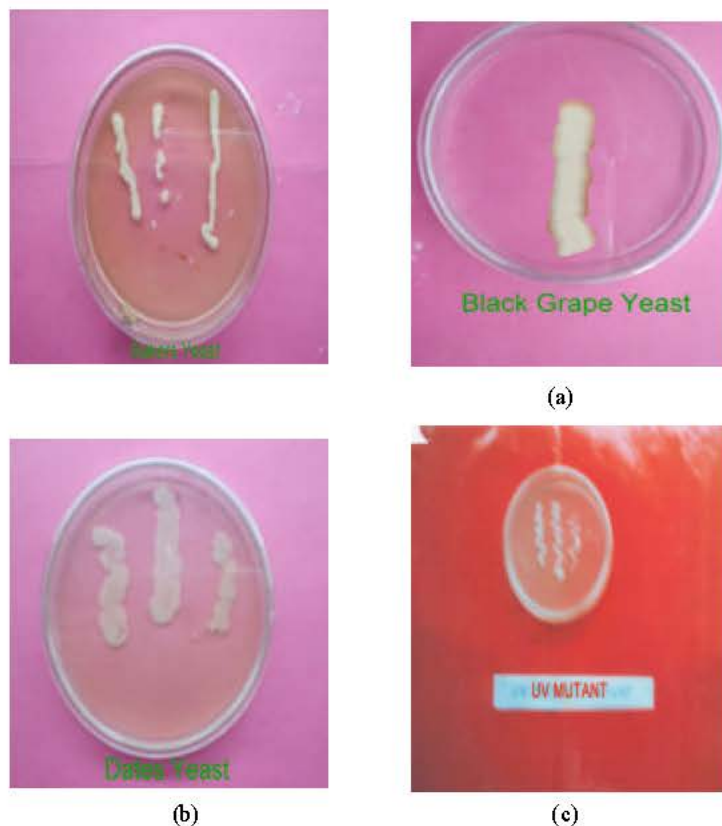


Fig. 1: Different yeast cultures (a) MTCC No. 6225 (BGY), (b) MTCC No. 6351 (DY) and (c) MTCC No. 6352 (SCY)

RESULTS AND DISCUSSION

Different fruits were taken, washed with sterile distilled water and transferred aseptically into sterile water taken in sterilized 100 mL conical flasks. The flasks were incubated at room temperature for two to three days. Later, by using an inoculation loop, a loopfull of suspension was streaked on sterile YEMA medium (previously added with streptomycin and griseofilvin) containing petri plates. The petri plates were incubated at room temperature for three to four days. The colonies obtained on incubation were subjected to morphological studies and purity studies. Pure yeast colonies were used for the study. From pure yeast growth sub-culturing was done on YEMA slants. Those cultures were maintained in the laboratory and used in the entire study.

We could isolate different yeast cultures which have been identified as *Candida pseudointermedia* MTCC No. 6225, *Candida pseudointermedia* MTCC No. 6352, *Issatchenkia orientalis* MTCC No. 6351. These cultures are shown in Fig. 1a-c. Since bioconversions using these yeasts have not been reported earlier, attempts have been made to use these yeasts for production of L-PAC.

Since L-PAC is optically active compound. The concentration of L-PAC was estimated by both polarimetric and calorimetric methods (Grotger and Erge, 1965). L-PAC concentration values obtained by polarimeter were consistent with the concentration values obtained by colorimeter.

In 1954 Paul F. Smith and David Hendlin conducted fermentations with whole cells of the fresh pressed baker yeast in cane molasses at pH 5.5.

CONCLUSIONS

In conclusion the present procedure for the Isolation and screening of novel strains of yeasts from natural sources for biotransformation studies were investigated. Three strains were isolated from different natural sources like blackgrapes, date fruit and sugarcane juice. These strains were identified at the Institute of Microbial Technology, Chandigarh, basing on sequencing of D₁/D₂ domain of 26S rRNA gene and assigned MTCC numbers. These three strains were designated as *Candida pseudointermedia* MTCC No.6225 (BGY), *Issatchenkia orientalis* MTCC No.6351 (DY), *Candida pseudointermedia* MTCC No.6352 (SCY). Which will be an important addition to the present existing procedures. The novel strains we can explore for the different chemical reactions. Further studies in this direction are in progress.

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