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Effect Of β-Cyclodextrin In The Production Of L-PAC

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ABSTRACT

Novel strains of yeasts from natural sources like fruits and sugarcane juice were isolated and screened for biotransformation studies. Production of L-phenyl acetyl carbinol (L-PAC) through biotransformation of benzaldehyde by immobilized cells of the yeast of different strains has been attempted. In our experiments the effect of β-cyclodextrin on L-PAC production was studied by adding 1 % β-cyclodextrin in all experiments.

KEYWORDS

Benzaldehyde;
Novel strains;
β-Cyclodextrin;
Immobilization;
Biotransformation;
L-PAC.

INTRODUCTION

The role of novel strains and their active principles with the aim of achieving desirable conversions of various substrates[1] in biotechnology is an important aspect. L-phenyl acetyl carbinol is the starting material for chemical synthesis of L-ephedrine hydro chloride and pseudo ephedrine pharmaceutical compounds used as decongestant, antiasthmatics[2] and recently reported, used in obesity control[3,4]. Aromatic substrate benzaldehyde will give L-PAC by biotransformation method. Certain yeast strains possess pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) enzymes that produce L-PAC and benzyl alcohol, a by product, respectively from benzaldehyde[5]. Biotransformation potentials of the growing cells free harvested cells immobilized cells and isolated crude as well as purified enzyme have been extensively studied[6-8].

The importance of novel strains in the bioconversion cannot be ignored. L-PAC production was studied[1] by free and immobilized cells of *saccharomyces cerevisiae* under various growth and biotransformation conditions. But we have studied L-PAC production from benzaldehyde by using various novel strains and the effect of β-cyclodextrin under vari-

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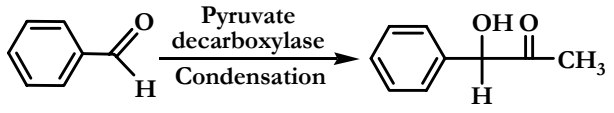

Benzaldehyde (Co-substrate)

L-Phenylacetylcarbinol (L-PAC)

SCHEME 1

ous growth and biotransformation modalities. L-PAC production is given in SCHEME 1.

MATERIALS AND METHODS

Isolation and screening of yeasts

BY strain: 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 resultant solution was aseptically transferred onto sterile YEMA slants with sterile transferring loop. The inoculated YEMA slants were incubated at room temperture (28°C) for 2-3 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 *Saccharomyces cerevislae* were observed. This organism was designated as BY and used as standard strain for comparison of biotransformation with other yeast strains isolated from different sources for their bioconversion potential.

Totally four yeast strains were used to study their biotransformation potential to produce L-PAC from benzaldehyde. Three yeast strains were isolated from three different sources like black grapes, date fruit and sugarcane juice.

Novel strains: Cleanly washed black grapes were added into 100mL conical flasks containing sterile water and incubated at room temperature for two to three days. After incubation one loop fill from each flask was added aseptically to separate petriplates containing YEMA medium. Antibiotics like strepto mcycin 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 pertriplates 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 100mL 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 separate petriplates containing YEMA medium. Antibiotics like streptomycin and griseofilvin 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 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 100mL 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 petriplates containing YEMA medium. Antibiotics like streptomcycin 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 pertriplates 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.

Immobilization

A measured volume of the resuspended inoculum prepared as explained was added to a 4% (W/

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V) solution of sodium alginate to obtain a final concentration of 3% (W/V) sodium alginate. The resulting cell suspension was extruded as drops into 2% calcium chloride solution to form beads, which were held in the solution for gelation period of 1hr. After filtering and washing in a bouchner funnel, the alginate beads containing the yeast were used to inoculate the fermentations. Weighed portions (25g) of alginate beads (containing measured mass of yeast) were inoculated into 100 ml volumes of fermentation medium in 250 ml flasks. Equal amount of free cells was used for inoculation of the same volume of fermentation medium. The flasks were incubated at 28-30°C on a rotary shaker for 24h. At the end of the incubation time, the beads were washed with sterile water then reintroduced into fresh 100 ml medium free cells were centrifuged (3000 x g for 10 min at 4°C), washed and then resuspended in fresh medium. After 1hr of incubation in the flasks, addition of the benzaldehyde (co-substrate) was begun simultaneously.

After 24hr shaking on rotary shaker the broth was filtered off from the beads and the resulting broth was subjected for extraction with equal volume of benzene. L-PAC was concentrated by simple distillation at 70 °C and subjected for analysis.

β-Cyclodextrin : The effect of β-cyclodextrin on L-PAC production was studied by adding 1.0% β-cyclodextrin in all experiments. The study was conducted by following the earlier mentioned immo bilisation method for the production of L-PAC by different yeasts. Our attempts to isolate L-PAC producing yeast from different sources has resulted in the isolation of some novel yeast strains. The L-PAC producing capacity of these was compared to that of bakers yeast.

RESULTS AND DISCUSSIONS

The central objective of the present investigation was to study the bioconversion of benzaldehyde to L-phenyl acetyl carbinol by using yeasts isolated from different natural sources like fruits and plants juice etc.. The investigation concentrated on the isolation and selection of improved yeast strains and their mutants and the use of batch process to carry out the biotransformation process. The investigation was carried out in two steps. First, isolation and screening of microorganisms capable of transforming benzaldehyde to L-PAC and secondly, the study of the bioconversion of benzaldehyde to L-PAC using isolated organisms and comparing bioconversion potential of new isolates with standard strain of *Saccharomyces cerevisiae* (BY). A comparative study of the percentage biotransformation obtained by various yeast strains and their mutants and effect of different additives on biotransformation was also carried out. In most of the studies molasses was used as production medium whereas in comparative studies sugarcane juice was used as production medium in biotransformation reactions. Basal broth medium was used for the biotransformation of immobilized cells of different isolates.

Grape garden (vineyard) soil, orchards soil and the soil collected from sugar factory premises etc., were considered to be rich in yeasts and these soil samples were used for screening. Due to heavy bacterial and fungal contamination despite of using antibacterial and anti-fungal agents we could not isolate any pure yeast strains from any soil samples. Fruits like black grapes, dates, pineapple and plant juice like sugarcane juice were also used for the iso-

TABLE 1: Effect of β-cyclodextrin in the production of L-PAC by immobilized cells

Type of organism	Additive	Description	L-PAC Concentration g/L	% Bioconversion
Bakers Yeast	β-cyclodextrin	Treated	1.71	27.29
		control	1.54	24.54
Candida pseudointermedia MTCC No. 6225	β-cyclodextrin	Treated	1.46	23.42
		control	1.36	21.68
Candida pseudointermedia MTCC No. 6352	β-cyclodextrin	Treated	1.85	29.54
		control	1.66	26.46
Issatchenkia orientalis MTCC No. 6351	β-cyclodextrin	Treated	2.11	33.73
		control	1.88	30.10

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lation of yeasts. Effect of β-cyclodextrin in the production of L-PAC by immobilized cells of baker yeast, *Candida pseudointermedia* MTCC No. 6225, *Candida pseudointermedia* MTCC No. 6352, *Issatchenkia orientalis* MTCC No. 6351 were presented in TABLE 1.

CONCLUSION

The effect of β-cyclodextrin in the production of L-PAC by immobilized cells of baker yeast, *candida pseudointermedia* MTCC No. 6225, *candida pseudointermedia* MTCC No. 6352, *Issatchenkia orientalis* MTCC No. 6351 is resulted in the increase of bioconversion. These bioconversion for immobilized cells in presence of β-cyclodextrin is greater than bioconversion values for free cells in absence of β-cyclodextrin. Finally the presence of β-cyclodextrin to the cells increases the bioconversion rate.

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