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Production Of L-Phenyl Acetyl Carbinol Through Biotransformation Of Benzaldehyde By Free And Immobilized Yeast Cells

Corresponding Author

M.Lakshmi Narasu
Centre for Biotechnology,
Institute of Science and Technology,
J.N.T.University, Kukatpally,
Hyderabad-500072 (INDIA)

Co-Authors

M.Ravi Kumar, M.Adharvana Chari
Centre for Biotechnology,
Institute of Science and Technology,
J.N.T.University, Kukatpally,
Hyderabad-500072 (INDIA)

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ABSTRACT

Novel strains of yeast from natural sources like fruits for biotransformation studies were investigated. In our experiments the L-PAC product obtained from benzaldehyde through biotransformation of benzaldehyde by free and immobilized cells of the yeast of different strains has been attempted and results were compared. Free and immobilized cells of all isolates were prepared and studied for their bioconversion potential in molasses medium. Free cells of all isolates showed slight increase in percentage bioconversion than immobilised cells.

KEYWORDS

Benzaldehyde;
Biotransformation;
Novel strains;
Free cells;
Immobilised cells;
L-PAC.

INTRODUCTION

Microorganisms as well as higher cells are governing an important role in the desirable conversions of various substrates in chemo enzymatic synthesis[1]. L-phenyl acetyl carbinol is the starting material for chemical synthesis of L-ephedrine hydrochloride and pseudoephedrine 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 decaroxylase (PDC) and alchol 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

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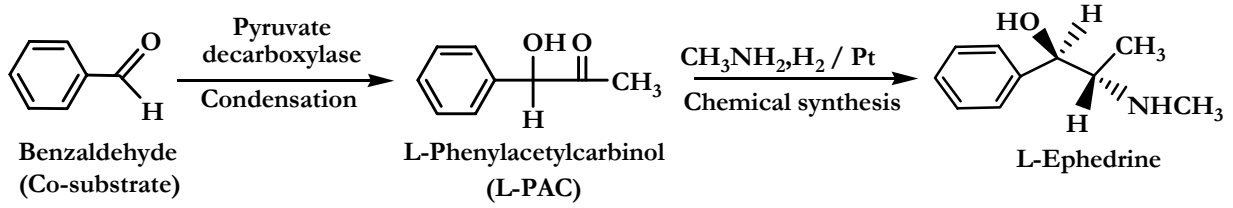

SCHEME 1

extensively studied[6-8].

The role of novel strains in the bioconversion is an important aspect. 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 free and immobilized 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. L-PAC production is given in SCHEME 1.

EXPERIMENTAL

Free and immobilized new isolated cells and one *Saccharomyces cerevisiae* (BY) were used in this study. The cultures were maintained on YEMA medium and were used for their capacity to produce L-phenyl acetyl carbinol (L-PAC).

The composition of basal broth medium used for growth of microorganisms and production of L-PAC was as composed (per liter) of: yeast extract, 6.0 g $(NH_4)_2SO_4$, 4.0g $MgSO_4$, 0.6 g KH_2PO_4 1.0 g , dextrose 100.0 g. The pH was adjusted to 6.2 at the beginning of the fermentation. Dextrose solution was autoclaved separately and then added aseptically to the previously sterilized components.

Yeast inoculum was a 24-hr-old culture, grown on the basal broth medium, then centrifuged (3000 x g for 10 min at 4°C), resuspended in sterile water and recentrifuged. The cell mass so obtained was weighed while wet and resuspended in a desired volume of sterile water for inoculation of free cell fermentations. All biomass amounts mentioned herein are given in terms of wet weight.

Immobilization method

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

RESULTS AND DISCUSSIONS

Biotransformation potential of immobilized cells

In 1996 Shin and Rogers reported[9] that cell immobilization of yeast is able to reduce the toxic substrate gradient. The immobiliazation of C.Utilis in calcium alginate beads increased the yield in shake flasks but reduced the yield in continuous bioreactor.

In 1989 Wafa M. Mahmad Abdul Halim M.M. E. Sayed et al., reported[10] that immobilization of

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TABLE 1: Biotransformation potential of immobilized cells of different yeast isolates in molasses medium

Sr. no.	Name of the organism	L-PAC concentration obtained in immobilized cells g/L	% Bioconversion for immobilized cells	% Bioconversion value for free cells
1.	*Saccharomyces cerevisiae*	1.54	24.49	25
2.	*Candida pseudointermedia* MTCC No. 6225	1.36	21.62	23.43
3.	*Candida pseudointermedia* MTCC No. 6352	1.66	26.40	33.47
4.	*Issatchenkia orientalis* MTCC No. 6351	1.88	30.00	37.16

Saccharomyces cerevisae enhanced the yield of L-PAC in shake flasks.

In 1997 Tripathi, Agarwal and Vinod Bihari et al., reported[1] that free cells were found to be more efficient biocatalysts for L-PAC production, as compared with the immobilized cells. On the same lines we attempted to immobilze different yeasts in calcium alginate beads to study biotransforamtion potentials. Immobilized cells gave less yield than free cells on shake flasks.

A measured volume of the resuspended inoculum prepared was added to a 4% (w/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 a geletion period of 1h. After filtering and washing beads containing the yeast were used to inoculate the fermentations.

Immobilized cells of all isolates were prepared and studied for their bioconversion potential in molasses medium.

Immobilized cells of *S.Cerevisiae* showed slight decrease in percentage bioconversion than free cells, immobilized cells are *Candida pseudointermedia* MTCC No. 6225 showed 1.75% decrease in percentage bioconversion.

Immobilized cells are *Candida pseudointermedia* MTCC No. 6352 and *Issatchenkia orientalis* MTCC No. 6351 showed almost same decrease that is 7% in percentage bioconversion than free cells. Several workers[11] conducted fermentation in molasses as production medium, in addition to that in industry use molasses medium for bioconversion reaction in the L-PAC production.

From the experimental results we can say that the free cells are better biocatalysts than the immobilized one in molasses medium. On the contrary Mahmoud et al. have reported[12] immobilized cells to be superior biotransformants, though at higher concentration of benzaldehyde. The results are presented in TABLE 1.

CONCLUSION

In conclusion the present procedure for the usage of free and immobilized novel strains of yeasts from natural sources for biotransformation studies were investigated and used for the bioconversion of benzaldehyde to L-PAC. Three strains were isolated from different natural sources like black grapes, date fruit and sugarcane juice and were identified at the Institute of Microbial Technology, Chandigarh. These three strains were designated as *Candida pseudointermedia* MTCC No.6225 (BGY), *Issatchenkia orientials* MTCC No.6351 (DY), *Candida pseudointermedia* MTCC No.6352 (SCY). Which will be an important addition to the present existing procedures. This is the first report on bioconversion of benzaldehyde to L-PAC by *Candida pseudointermedia* and *Issatchenkia orientalis*. The novel free and immobilized cells, we can explore for the different chemical reactions. Further studies in this direction are in progress.

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