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Production of L-phenyl Acetyl Carbinol (L-PAC) by Different Novel Strains of Yeasts in Molasses and Sugar Cane Juice as Production Medium

M. Ravi Kumar, M. Adharvana Chari and M. Lakshmi Narasu
Centre for Biotechnology, Institute of Science and Technology,
J.N.T. University, Kukatpally, Hyderabad-500072, India

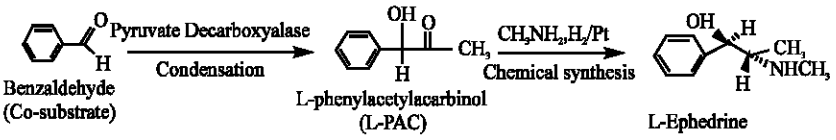
Abstract: Isolation and screening of novel strains of yeasts from natural sources like fruits and sugarcane juice for biotransformation studies was investigated. These strains were identified at the Institute of Microbial Technology, Chandigarh, basing on sequencing of D_1/D_2 domain of 26S rRNA gene and assigned MTCC numbers. These three strains were designated as *Candida pseudointermedia* MTCC No. 6225 (BGY), *Issatchenkia orientials* MTCC No. 6351 (DY), *Candida pseudointermedia* MTCC No. 6352 (SCY), respectively. Production of L-phenyl acetyl carbinol (L-PAC) through biotransformation of benzaldehyde by free cells of the yeast of different strains has been attempted. In our experiments the L-PAC product obtained from benzaldehyde through biotransformation by four different yeasts like *baker's yeast*, *Candida pseudointermedia* (6225), *Candida pseudointermedia* (6352) and *Issatchenkia orientials* (6351).

Key words: Benzaldehyde, novel strains, molasses, sugarcane juice, biotransformation, L-PAC

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). 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 (Shin and Rogers, 1995) and recently reported, used in obesity control (Astrup *et al*., 1992). 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 (Nikolova and Ward, 1991). Biotransformation potentials of the growing cells free harvested cells immobilized cells and isolated crude as well as purified enzyme have bee n extensively studied (Liew *et al*., 1995; Shin and Rogers, 1996a, b).

The role of novel strains in the bioconversion is an important aspect. L-PAC production was studied 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 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.



Scheme 1:

Corresponding Author: M. Adharvana Chari, Centre for Biotechnology, Institute of Science and Technology, J.N.T. University, Kukatpally, Hyderabad-500072, India

Materials and Methods

Production of L-phenyl Acetyl Carbinol with Normal Cells of Sccharomuces Cerevisiae (BY)

Production of L-Pac (a key intermediate for many drugs) from benzaldehyde by yeasts is the potential route in the Fermentation Industry for the production of Ephedrine and other drugs. The present study was conducted by the author in Sultan-Ul -Uloom college of Pharmacy, located in Hyderabad, India in the year 2004.The stock culture of baker's yeast was freshly subcultured (Ellaiah and Krishna, 1987) on fresh sterile YEMA medium slants and incubated at room temperature (about 28°C) for 36 h. Thus the 36 h culture was used for harvesting. The culture of Bakers yeast was harvested by shaking in 5 mL sterile water. Harvested microbial suspension was transferred into Inoculum medium-I. The composition of inoculum medium is as follows:

Molasses	15%
Urea	0.2%
In Sterile water	To make up to 100 mL
pH	5.5

The flask was incubated at 28°C on a rotary shaker (180 RPM) for 24 h.

The microbial count was made by using Neubauer counting chamber. The Microbial suspension was diluted so that each mL of suspension contained 200×10^6 cells. Ten milliliter of inoculum from IM-I was transferred to 100 mL of inoculation medium-II who's composition is indicated below:

Molasses	10%
Urea	0.25%
In Sterile water	To make up to 100 mL
pH	5.5

The flasks were incubated on a rotary shaker (180 RPM) for 16 h.

One hundred milliliter of production media were prepared. In most of the study, molasses medium used as a production medium and for comparison study sugarcane juice medium with urea was used as a production medium. Ten milliliter of inoculum from IM-II was transferred to production media whose composition is same as IM-II and incubated for 9 h on rotary shaker. At 9 h, nutrient (20 mL of 50% molasses) was added to molasses production medium and nutrient (20 mL of 50% sugar cane juice) was added to sugar cane production medium respectively and incubated on rotary shaker. From 10 h onwards 0.6% of distilled benzaldehyde was added in 6 divided doses of half an hour intervals to production medium.

Then flasks were incubated on the rotary shaker for 24 h. the flasks containing 130 mL broth (i.e.,100 mL production medium+10 mL inoculum+20 mL nutrient medium) was treated with 130 mL benzene (solvent) and shaken for 15 min in separating funnels. Then the organic layer was separated and filtered through absorbent cotton. Finally the solvent benzene was distilled of to get L-PAC product.

Production of L-phenyl Acetyl Carbinol with New Isolates of Yeasts:
Procedures for Bioconversion:

In comparative studies the novel cultures were used to estimate and compare their biotransformation potential with bakers yeast. The stock cultures of *Candida pseudointermedia* MTCC No. 6225, *Candida pseudountermedia* MTCC No. 6352 and *Issatchenkia orientalis* MTCC No. 6351 were subcultured aseptically on Strile YEMA slants with sterilized transferring loop in the sterile area (Laminar air flow).

Previously mentioned procedure (Ellaiah and Krishna, 1987) was repeated for the novel strains also. In most of the study molasses medium was used as a production medium and for comparative study sugarcane juice medium with urea (Kaur and Kocher, 2002) was used as a production medium.

Results and Discussion

L-Phenyl acetyl carbinol is a yellow colour liquid. Its specific gravity reported is 0.93 at room temperature. L-PAC produced by isolates including Bakers yeasts (*S.cerevisia*) showed the same specific gravity value. pH value of L-PAC (1: 1 Ratio of L-PAC sample and water) is reported as 3.84. In this experiments the L-PAC product obtained through biotransformation by four different yeasts like baker's yeast, *Candida pseudointermedia* (6225), *Candida pseudointermedia* (6352) and *Issatchenkia orientials* (6351) showed the same pH values. R_f value of L-PAC was reported (Groger and Erge, 1965) in chloroform as mobile phase on silica gel Iodine vapors were used for the detection of spots.

In developing chromatograms chloroform as a front solvent was compared with solvent mixture (30% ethyl acetate 70% hexane) as mobile phase. The later solvent front showed better separation than chloroform. So we used 30% ethyl acetate and 70% hexane as solvent front in all our experiments.

L-PAC is reactive in UV light, iodine vapours and β-Methoxynaphthalene charring also. The Rf value of standard L-PAC is around 0.33. L-PAC product of different isolates showed the same Rf value.

The methyl ketone present in L-PAC undergoes the Idoform reaction (Smith and Hendlin, 1954). It initially undergoes halogenation and the cleavage in the presence of alkali like NaOH to give rise to Idoform. This reaction is very specific for L-PAC and does not occur with by products. L-PAC produced by different isolates gave rise to Idoform.

By polarimetric and colorimetric estimations, the percentage bioconversion was estimated in different yeast isolates. Other fermentation products (e.g., benzyl alcohol, benzoic acid as well as unconverted benzaldehyde were assayed by High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC). The chemical structure of L-PAC was identified and conformed to ^{1}H NMR and UV spectral data.

Several workers (Ellaiah and Krishna, 1987) conducted fermentation in molasses as production medium, in addition to that in industry use molasses medium for bioconversion reaction in the L-PAC production.

In the present investigation we tried the sugar cane juice as production medium in L-PAC production. Sugar cane juice with 0.25% urea was used as production medium which yielded more product of L-PAC than molasses. In addition to that product extraction from sugarcane juice medium was very convenient than from the molasses medium. The% bioconversion obtained with different yeast isolates is shown in Table 1.

Biotransformation potential of free cells of *Bakers yeast* (*S.cerevisiae*) was studied in both molasses and sugarcane juice as production medium. In molasses medium 25% bioconversion was observed where as in sugarcane juice medium 28% bioconversion was observed.

Table 1. Conversion of molasses and sugarcane juice as production media on production of L-PAC

Name of the organism	Medium used	L-PAC concentration (g L^{-1})	Bioconversion (%)
Saccharomyces cerevisiae	Molasses	1.58	25.00
Candida pseudointermedia MTCC No. 6225	Molasses	1.47	23.43
Candida pseudointermedia MTCC No. 6352	Molasses	2.10	33.47
Issatchankia orientalis MTCC No. 6351	Molasses	2.33	37.16
Saccharomyces cerevisiae	Sugarcane Juice	1.841	28.00
Candida pseudointermedia MTCC No. 6225	Sugarcane Juice	1.49	23.75
Candida pseudointermedia MTCC No. 6352	Sugarcane Juice	2.97	47.48
Issatchankia orientalis MTCC No. 6351	Sugarcane Juice	3.80	60.61

Biotransformation potential of free cells of *Candida pseudointermedia* MTCC No. 6225 was studied in both molasses and sugarcane juice as production medium. In molasses 23.43% bioconversion was observed where as in sugarcane juice medium 23.75% bioconversion was observed.

Biotransformation potential of free cells of *Candida pseudointermedia* MTCC No. 6352 was studied in both molasses and sugarcane juice as production medium. In molasses 33.47% bioconversion was observed where as in sugarcane juice medium 48.76% bioconversion was observed.

Biotransformation potential of free cells of *Issatchenkia orientalis* MTCC No. 6351 was studied in both molasses and sugarcane juice as production medium. In molasses 37.16% bioconversion was observed where as in sugarcane juice medium 60.61% bioconversion was observed.

Conclusion

In conclusion the present procedure for the usage of 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 blackgrapes, 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. The most significant findings of the present research are use of 3 new strains of yeasts for the production of L-PAC and the use of Sugarcane juice as production medium for the production of L-PAC. We are successful in both attempt and there is considerable increase in the percentage yield of L-L-PAC when sugarcane juice was used as production medium. Though sugarcane juice is expensive compared to Molasses, the extraction procedure was proved to be much easier with sugarcane juice compared to Molasses. Further research modalities like different factors, immobilization studies, mutation studies etc., could help in establishing the cost effective methods for the production of L-PAC by using sugarcane juice as a production medium. This is the first report on using *Candida pseudointermedia* and *Issatchenkia orientalis* for bioconversion studies of benzaldehyde to L-PAC. Use of sugarcane juice as production medium in biotransformation studies of benzaldehyde to L-PAC is also the first report of its kind. Moreover sugarcane juice showed increased bioconversion potential than molasses medium. The novel strains we can explore for the different chemical reactions. Further studies in this direction are in progress.

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