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Rapid Isolation/Selection of Best Yeast Culture and Its Metabolic Control for the Biotransformation of Benzaldehyde to 1-Hydroxy-1-phenyl-2-propanone

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Abstract.- Present work describes the rapid isolation and selection of thirteen yeast culture having high resistance to acetaldehyde and benzaldehyde, directly added to the medium at isolation stage. All isolates had 100% similarity with type strain *Saccharomyces cerevisiae* during molecular characterization. *S. cerevisiae* APL-2 showed better kinetic parameters and ultimately lead to better production of 1-hydroxy-1-phenyl-2-propanone. This fermentation involved two phases, first phase to attain the suitable level of cell density while second phase is the biotransformation phase. Effect of temperature (15-30°C) was studied during second phase (at the time of acetaldehyde and benzaldehyde dozing). The maximum level of biotransformation was achieved when temperature was maintained at 15-18°C. Addition of benzyl alcohol also enhanced the biotransformation level.

Keywords: *Saccharomyces cerevisiae,* biotransformation, L-PAC, benzaldehyde

INTRODUCTION

1-hydroxy-1-phenyl-2-propanone or Phenylacetylcarbinol (L-PAC) or α-hydroxy-benzyl methyl ketone is an important intermediate for the production of L-ephedrine, norephedrine, pseudoephedrine, nor-pseudoephedrine, amphetamine, adrenaline, phenylpropanolamine methamphetamine and phenyl-amine (Abourashed *et al*., 2003). L-ephedrine is an ingredient of pharmaceutical preparations used as antiasthmatics and decongestants (Rogers *et al.,* 1998).

1-hydroxy-1-phenyl-2-propanone can be prepared by chemical synthesis from cyanohydrins (Brusse *et al.,* 1988; Jackson *et al.,* 1990) but it is prepared industrially by the biotransformation of benzaldehyde (Netrval and Vojtisek, 1982). The production of the 1-hydroxy-1-phenyl-2-propanone is carried out by pyruvate decarboxylase (PDC) and is accompanied with the formation of by-products, *i.e*. benzyl alcohol, due to the action of an alcohol dehydrogenase (ADH) and oxidoreductases (Fig. 1). Benzoic acid in trace amounts as a by-product has also been reported (Khan and Daugulis, 2011).

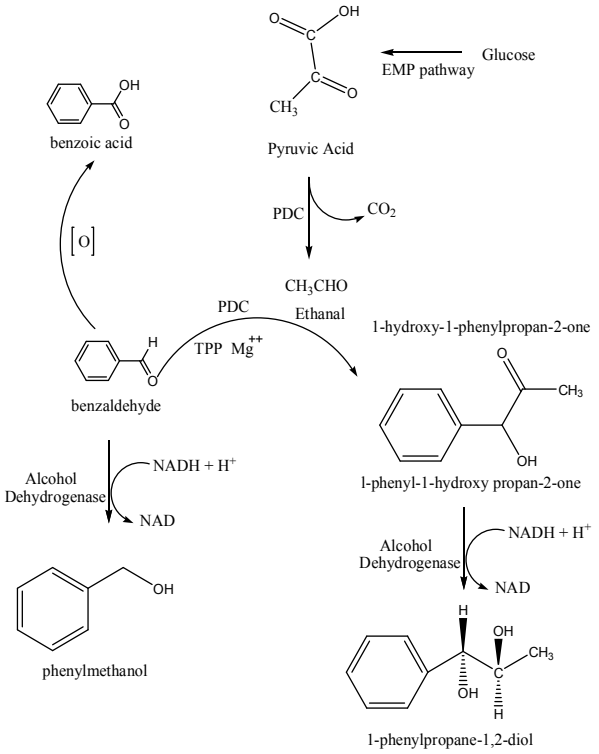

Fig.1. Pathway for production of 1-hydroxy-1-phenyl-2-propanone and associated products (Adopted from Shin and Rogers, 1996)

Various microorganisms such as bacteria like *Zymomonas mobilis* (Bringer-Meyer and Sahm 1988; Cardillo *et al*., 1991), yeast like *Hansenula*

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anomala, Saccharomyces carlsbergensis, Saccharomyces cerevisiae, *Saccharomyces ellipsoideus, Candida utilis* and *Torula utilis* (Gupta *et al*., 1979; Agarwal *et al*., 1987) and filamentous fungi like *Polyporus eucalyptorum*, *Aspergillus niger*, *Fusarium* sp., *Neurospora* sp., *Rhizopus javanicus, Rhizopus oryzae* etc. transform benzyldehyde into phenylacetylcarbinol (Cardillo *et al*., 1991).

Several attempts have been made by different workers in order to produce economical and cost effective 1-hydroxy-1-phenyl-2-propanone by various cultures and cultural techniques such as optimization of growth and biotransformation conditions, control of cell metabolism and method of benzaldehyde addition but still there is a room for the search of potent culture (Becvarova *et al.,* 1963; Netrval and Vojtisek, 1982, Miguez *et al.,* 2012). This work described the rapid isolation/ selection of a good 1-hydroxy-1-phenyl-2-propanone producer and to increase its production by metabolic control.

METHODOLOGY

Yeast cultures producing 1-hydroxy-1-phenyl-2-propanone

Yeast cultures were isolated from different rotten fruit samples collected from local market. One gram sample was added to 100 mL sterile saline water (0.5%) and vortexed. An aliquot of 1.0 mL were transferred to 50 mL of Yeast extract Peptone Dextrose liquid medium (20 g peptone,20 g glucose and 10 g yeast extract dissolved per liter of distilled water) supplemented with 400 µL benzaldehyde, one milliliter acetaldehyde and 10 mg/L ampicillin, then incubated at 30^{o}C in a shaking incubator until the growth was visible. One hundred microliter from this culture was streaked on malt extract agar/YPD medium in the petri plates. These plates were incubated at 30^{o}C for 3-4 days. Independent colonies were picked up and maintained on potato dextrose agar and YPD slants (Modified method reported by Shakoori *et al.,* 2005; Rehman *et al.,* 2007). Pure cultures were identified with morphological and biochemical characterization, then verified by molecular methods by amplification of the internal transcribed spacer region of the 18S rDNA. Following primer sequences were used for the amplification of ITS1 region of the 18S rDNA after White *et al*. (1990).

NS1 5'-GTAGTCATATGCTTGTCTC-3'
ITS2 5'-GCTGCGTTCTTCATCGATGC-3'

PCR products were treated with two restriction endonucleases, *Msp*I and *Hae*III, and then subjected to electrophoresis and compared the results with type strain of *Saccharomyces cerevisiae* (Redzepovic *et al.,* 2002).

Culture conditions and biotransformation

The acetaldehyde and benzaldehyde resistant cultures were screened through submerged fermentation after Shukla *et al*. (2001). Fifty milliliters of molasses medium containing (g/L); over-limed molasses (20 brix), urea (10), KH_2PO_4 (1.0), $MgSO_4$ (10), yeast extract (4.0), peptone (4.0), K_2HPO_4 (1.0) transferred to 250 mL Erlenmeyer flask and was inoculated with 15.0% (v/v) inoculum ($2.40x10^8$ cells/mL or OD_{595}=0.4) of age 14 h. The flask was incubated at 30^{o}C to achieve a cell density of $1.20x10^8$cells/mL. At this stage, dosing of acetaldehyde (420 µL, 342 µL, 285 µL, 228 µL and 142 µL) and benzaldehyde (210 µL, 171 µL, 142 µL, 114 µL and 71 µL) was completed in five intervals of 40 min. All the experiments were run parallel in triplicates. Fermented broth was utilized for the estimation of L-PAC. 1-hydroxy-1-phenyl-2-propanone formed was extracted from the fermentation broth using toluene (sample to volume ratio of 1:2) in a separating funnel. The sample was then used for the estimation of 1-hydroxy-1-phenyl-2-propanone. 1-hydroxy-1-phenyl-2-propanone was estimated with the help of polarimeter (Netraval and Vojtisek, 1982; Becvarova *et al*., 1963). Optical rotation (OR) was recorded and 1-hydroxy-1-phenyl-2-propanone (g/L) was calculated using the following formula.

g/L= 0R x 2 (dilution factor) x 1.11 (density of 1-hydroxy-1-phenyl-2-propanone**)**

RESULTS AND DISCUSSION

Thirteen yeast strains were isolated capable of growing in YPD medium supplemented with 400

µL benzaldehyde, one milliliter acetaldehyde and 10 mg/L of ampicillin. These cultures were further screened for their biotransformation potential in shake flask. All of them had 100% similarity with type strain of *Saccharomyces cerevisiae*. Table I shows the natural habitat from where they were isolated and their potential of benzaldehyde biotransformation to 1-hydroxy-1-phenyl-2-propanone. Thirteen *S. cerevisiae* strains were isolated from eight different sources such as apple, apricot, banana, guava, mango, peach, pomegranate and white grapes. Out of these, *S. cerevisiae* APL-2 isolated from apple gave the maximum production of 1-hydroxy-1-phenyl-2-propanone produced (3.07 g/L).

Effect of different temperature (15-35°C) was studied on the production of 1-hydroxy-1-phenyl-2-propanone at the time acetaldehyde and benzaldehyde dosing (Fig. 2). Maximum production was obtained at 15-18°C. Further increase in temperature decreased the production of 1-hydroxy-1-phenyl-2-propanone. It may be due to the fact that the boiling point of acetaldehyde is 20°C. So at temperature higher than 18°C, the acetaldehyde might have converted in to vapors resulting in low yield of 1-hydroxy-1-phenyl-2-propanone. Shin and Rogers (1995) checked the effect of temperature on the formation of L-PAC and concluded that predominant production of L-PAC was obtained at 4°C.

Effect of benzyl alcohol supplementation (0-2.5 g/L) in the fermentation medium was studied on the production of 1-hydroxy-1-phenyl-2-propanone (Fig. 3). Production of 1-hydroxy-1-phenyl-2-propanone increased with an increase in the concentration of benzyl alcohol supplementation up to 2.0%. Many enzymes of the de novo biosynthetic cascades exhibit the phenomenon of feedback inhibition, where a metabolite that is the final or penultimate product of the cascade functions as a heterotropic inhibitor of one of the enzymes that is present early in the biosynthetic cascade. The increase in production may be due to feedback inhibition of alcohol dehydrogenase by benzyl alcohol. However, the production of 1-hydroxy-1-phenyl-2-propanone decreased when the concentration of benzyl alcohol supplementation exceeded above 2.0%. It may be due to an equilibrium effect or inhibition (reversible or non-reversible) of key enzymes by substrate and/or products, progressive reduction in cell viability, overall inhibition of cellular metabolism due to toxicity effects of substrate and/or products which may denature enzymes or permeabilise membranes leading to disruption of membrane-bound enzymes or the release of essential cofactors is also a possibility (Shin and Rogers, 1995; Zhang *et al.,* 2008).

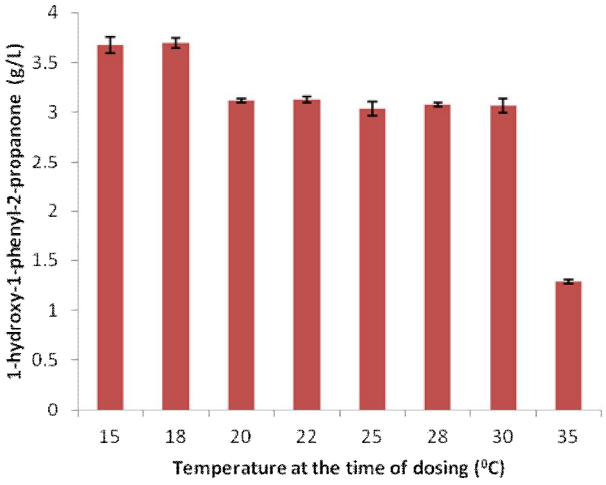

Fig. 2. Effect of temperature on the production of 1-hydroxy-1-phenyl-2-propanone at the time dosing

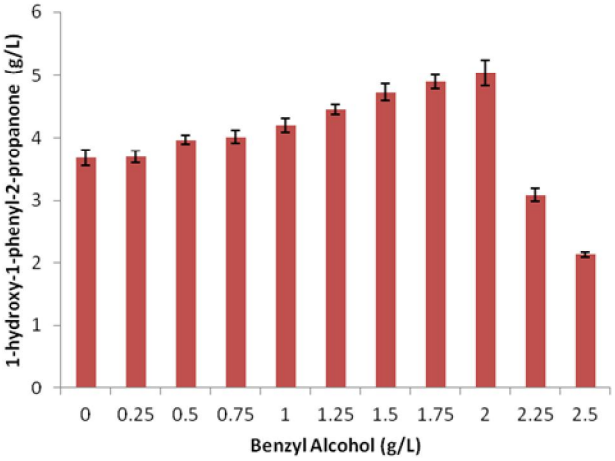

Fig. 3. Effect of benzyl alcohol supplementation in the fermentation medium on the production of 1-hydroxy-1-phenyl-2-propanone.

Table I.- Screening of *Saccharomyces cerevisiae* strains for the production of 1-hydroxy-1-phenyl-2-propanone produced.

Sr. #	Source/Habitat	IRN[a]	FSC[b]	μ[c]	Δt[d]	Δp[e]
1	Apple	APL-1	*S. cerevisiae* APL-1	0.11	9.0	1.87
2	Apple	APL-2	*S. cerevisiae* APL-2	0.23	6.5	3.07
3	Apricot	APT-3	*S. cerevisiae* APT-3	0.08	12	0.57
4	Apricot	APT-4	*S. cerevisiae* APT-4	0.06	14	1.16
5	Apricot	APT-5	*S. cerevisiae* APT-5	0.16	7	2.21
6	Banana	BNN-6	*S. cerevisiae* BNN-6	0.11	9	2.08
7	Banana	BNN-7	*S. cerevisiae* BNN-7	0.12	8.5	1.65
8	Guava	GUA-8	*S. cerevisiae* GUA-8	0.06	14	1.12
9	Mango	MGO-9	*S. cerevisiae* MGO-9	0.02	16	0.50
10	Mango	MGO-10	*S. cerevisiae* MGO-10	0.09	11	0.60
11	Peach	PCH-11	*S. cerevisiae* PCH-11	0.10	10	1.12
12	Pomegranate	PGT-12	*S. cerevisiae* PGT-12	0.13	8	2.31
13	White Grapes	WGP-13	*S. cerevisiae* WGP-13	0.19	8	2.35

[a]IRN: Isolation reference Number
[b]FSC: Final specific Code
[c]μ: Specific growth rate at exponential phase of growth(g/L/h)
[d]Δt:Time to attain the cell Density of $1.20x10^8$ cells/mL (h)
[e]Δp: Amount of 1-hydroxy-1-phenyl-2-propanone produced (g/L)

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