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A N I N T E R N A T I O N A L J O U R N A L

Biotransformation of Benzaldehyde to L-Phenylacetylcarbinol (L-PAC) by Free Cells of *Torulaspora delbrueckii* in presence of Beta-Cyclodextrin

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ABSTRACT

Studies were carried out to explore the possibility of decreasing the toxic and inhibitory effects of the substrate benzaldehyde during its biotransformation to L-PAC by free cells of Torulaspora delbrueckii using β-cyclodextrin (β-CD). Use of various levels of benzaldehyde and acetaldehyde in presence of 2% of β-CD showed that, in presence of β-CD, the organism could tolerate higher levels of benzaldehyde and acetaldehyde. Semi-continuous feeding of benzaldehyde and acetaldehyde was found to increase the yield of L-PAC in comparison with one time feeding.

Key words: Benzaldehyde, Biotransformation, Cyclodextrin, L-phenylacetycarbinol, *Torulaspora delbrueckii*

INTRODUCTION

Most of the literature concerning the synthesis of L-Phenylacetylcarbinol (L-PAC) and benzyl alcohol by fermenting yeast, deals with yield optimization by free cells (Agrawal et al., 1987; Cardillo et al., 1991; Zeeman et al., 1992). Studies on L-PAC formation from benzaldehyde have shown that under normal fermentative conditions using yeast, quantitative conversion of benzaldehyde into L-PAC has never been achieved because of formation of by-products like benzyl alcohol, PAC-diol (Smith and Hendlin, 1953; Gupta et al., 1979; Netraval and Vojtisek, 1982; Agrawal and Basu, 1989; Tripathi et al.,1988, Long et al., 1989). The yeast can not be used for multiple batches because of the toxic and inhibitory effects of substrate and products (Long et al., 1989; Coughlin et al., 1991). Use of cyclodextrin to decrease the toxicity of benzaldehyde for bioconversion using immobilized cells has been reported (Coughlin et al., 1991; Mahmoud et al., 1990). Considering these facts, it was attempted to study the effect of addition of β-cyclodextrin on biotransformation of benzaldehyde to L-PAC by the cells of *Torulaspora delbrueckii* and results are reported here.

MATERIALS AND METHODS

Materials - Microbial media components (Hi-Media Ltd. Mumbai), AR grade solvents and chemicals (S.D. Fine Chemicals Ltd. Mumbai and Merck India Ltd, Mumbai) and β-cyclodextrin

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(a gift sample from S.A. Chemicals Ltd, Mumbai) were used. An yeast isolate from molasses identified as Torulaspora delbrueckii was used. The composition of the maintenance medium [Long et al., 1989] used was: glucose 2%, peptone 1%, yeast extract, 1%, agar 1% and had pH 5.5. The growth medium [Long et al., 1989] contained glucose 2%, peptone 2%, yeast extract 1% and had pH 5.5. The biotransformation medium [Nikolova and Ward, 1991] having composition glucose 5%, peptone 0.6% and pH 4.5 was used.

Culture growth - 1 ml suspension of cells of the isolate *Torulaspora delbrueckii* containing 10^6 cells was inoculated into 9ml of growth medium and incubated on a rotary shaker at 30±2°C at 240 rpm for 24 h. The culture so obtained was inoculated into 100ml of the same medium and allowed to grow for 24 h. under same conditions and cells were harvested by centrifuging at 10, 000 rpm for 15 min at 15°C. The biomass obtained was washed with water, centrifuged and used for biotransformation studies.

Biotransformation of benzaldehyde to L-PAC - The biotransformation medium (100 ml) was inoculated with 3.0 g (wet wt.) of cell mass obtained as above. The flask was incubated on a shaker at 30±2°C and 240 rpm for1h for adaptation of cells to the medium. Benzaldehyde and acetaldehyde were added at concentrations ranging from 700 to 1200mg/100ml and 700 to 1200µl/100ml respectively and flasks were again incubated for the biotransformation on a shaker at 30±2°C and 240 rpm.

Effect of β-cyclodextrin addition on biotransformation of benzaldehyde - Earlier studies had established that *Torulaspora delbrueckii* cells could tolerate benzaldehyde up to 600mg/100ml of biotransformation medium and 600µl of 30-35% aqueous solution of acetaldehyde. Therefore, effect of 2% β-cyclodextrin (β-CD) was studied at benzaldehyde and acetaldehyde levels ranging from 700mg to 1200mg/100ml and 700µl to 1200µl/100ml respectively. The reaction was allowed to take place for 3h at 30±2°C and 240 rpm. From these studies 900mg of benzaldehyde and 1000µl acetaldehyde were the levels found to be optimal. To study the effect of β-CD level, concentration of β-CD was varied in the range of 0.5 to 2.5%. Semi-continuous feeding of different levels of benzaldehyde and acetaldehyde was also carried out 4 times at intervals of 30 min in presence of 2% β-CD.

Analysis of biotransformation products - After biotransformation, the medium was centrifuged at 10, 000rpm for 15min. The supernatant was extracted three times with equal volumes of diethyl ether. The combined extract was dried over anhydrous sodium sulphate and concentrated over a controlled temperature water bath. The residue obtained was dissolved in methanol and submitted to GC analysis. The conditions used for GC were as follows- GC model used was Chemito-8510 with Oracle -1 computing integrator. A 4 meter long column of 5%OV-17 was used. The injector temperature was 250°C and detector temperature (FID) was 250°C. Column programming was as follows, 75°C for 3 min then 10°C/min up to 250°C and holding for 5min. Retention times of benzaldehyde, benzyl alcohol, L-PAC, PAC-diol were 11min, 13min, 17min and 18.5 min respectively. The concentrations of these compounds were determined using peak area method (Shukla and Kulkarni, 1999). Each experiment was repeated 3 times and was found to be reproducible within ± 5 percent limits.

RESULTS AND DISCUSSION

Biotransformation of benzaldehyde by immobilized cell mass of *Saccharomyces cerevisiae* ATCC 834 at higher levels of benzaldehyde has been reported using β-cyclodextrin (Mahmoud et al., 1990; Coughlin et al., 1991).

Cyclodextrins are known to offer protective effect by forming inclusion complexes (Szetji, 1998; Uekama et al., 1998).

In the present work, study of effect of level of benzaldehyde in presence of 2 % β-CD showed that free cells of Torulaspora delbrueckii could easily tolerate up to 900mg of benzaldehyde (Fig.1) confirming the protective effect of β-CD.

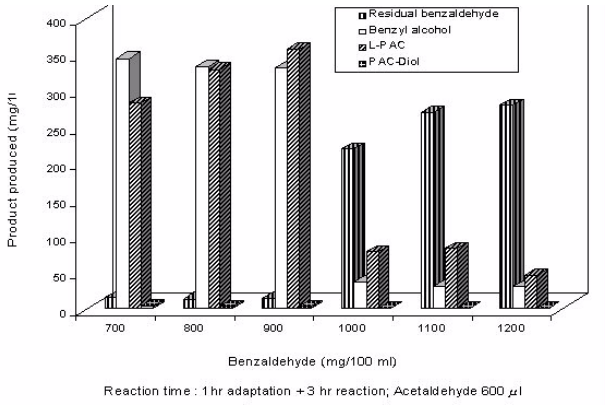

Figure 1 - Effect of concentration of benzaldehyde on product profile in presence of 2% Beta - Cyclodextrin.

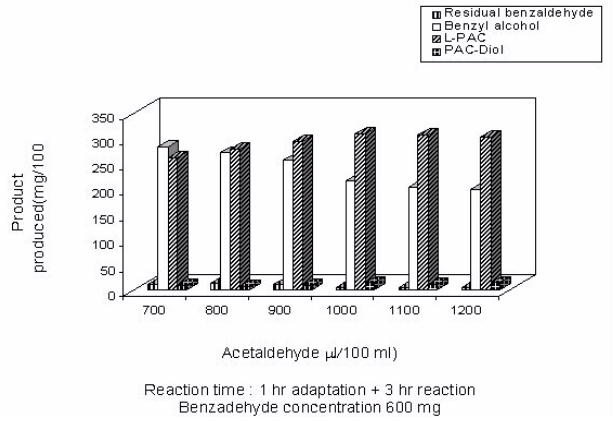

Figure 2 - Effect of concentration of acetaldehyde on biotransformation product profile in presence of 2% Beta - CD.

The same organism has been reported to tolerate only 600mg of benzaldehyde in absence of β-CD (Shukla, 1999). Addition of acetaldehyde is also known to increase L-PAC production (Groeger et al., 1966; Subramanian et al., 1987). Earlier reports from our laboratory have proved that acetaldehyde addition up to 600 µl proved beneficial (Shukla, 1999). In the present work effect of addition of acetaldehyde in the presence of 2% β-CD was studied at benzaldehyde concentration of 600mg. As shown in Fig. 2 with addition of acetaldehyde up to 1000µl, L-PAC production increased and thereafter remained constant till 1200 µl. A simultaneous, decrease in benzyl alcohol was observed as the acetaldehyde level increased from 700 to 1200 µl level.

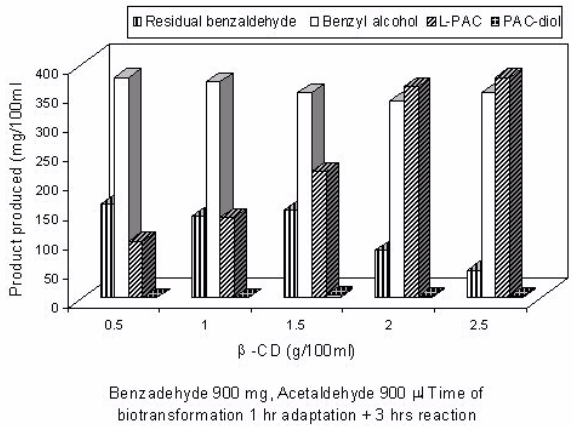

Figure 3 - Effect of concentration of β-CD on product profile.

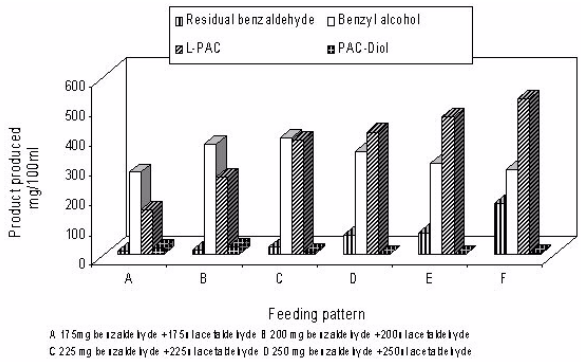

Figure 4 - Effect of semi-continuous feeding of benzaldehyde & acetaldehyde in the presence of 2% β-CD on the biotransformation product profile.

To study the effect of level of β-CD on biotransformation product profile different levels of β-CD were used. As shown in Fig. 3 at lower levels i.e. 0.5 to 1.5% β-CD, less L-PAC was formed giving more residual benzaldehyde. On increasing β-CD level to 2.5 g, production of L-PAC increased, whereas benzyl alcohol production was almost constant at all levels from 0.5 to 2.0 %. This suggested that probably a higher level of β-CD may be preventing oxidation of benzaldehyde molecule and diverting it to production of L-PAC and benzyl alcohol.

To confirm the beneficial effect of semi-continuous addition of benzaldehyde and acetaldehyde on production of L-PAC as well as to take advantage of tolerance of organism to high level of benzaldehyde and acetaldehyde in the presence of β-CD, semi-continuous feeding of

both of these at various levels was studied in presence of β-CD. Semi-continuous feeding for four times at the interval of 30 min resulted in a product profile as in Fig.4shown. With increase in total cumulative dose of benzaldehyde and acetaldehyde, the yield of L-PAC increased steadily. The organism could produce a maximum of 527mg/100ml of L-PAC at a total level of 1200mg of benzaldehyde and 1200 µl of acetaldehyde. Netraval and Vojtisek (1982) have reported a yield of 6.3g/L L-PAC with *S. carlsbergensis* 'Budvar' with semi-continuous feeding of benzaldehyde, acetaldehyde and sucrose, where as Cardillo et al. (1991) have reported 60 % conversion of benzaldehyde to L-PAC when *S. delbrueckii* was used.

Thus in presence of 2% β-CD, *Torulaspora delbrueckii* cells could tolerate 900mg of benzaldehyde and 1000 µl of acetaldehyde as against 600mg benzaldehyde and 600 µl of acetaldehyde in its absence and in presence of 2% β-CD, *Torulaspora delbrueckii* cells could produce 527mg/100ml of L-PAC when benzaldehyde and acetaldehyde were fed semi-continuously at 300mg and 300 µl respectively for four times.

REFERENCES

Agrawal, S. C. and Basu, S. K. (1989), Biotransformation of benzaldehyde to L-acetylphenylcarbinol by fed batch culture system. *J. Microbiol Biotechnol.*, **4** : (2), 84-86.

Agrawal, S. C.; Basu, S. K.; Vora, V. C.; Mason, J. R. and Pirt, S. J. (1987), Studies on the production of L-acetyl phenyl carbinol by yeast employing benzaldehyde as precursor. *Biotechol. Bioeng.*, **29** : (6), 783-785.

Cardillo R., Servi S. and Tinti. C. (1991), Biotransformation of unsaturated aldehydes by micro organisms with pyruvate decarboxylase activity. *Applied Microbiol. Biotechnol.*, **36 :** (3), 300-303.

Coughlin, R.W.; Mahmoud, W. M. and El-sayed, A. H. (1991), Enhanced bioconversion of toxic substances *US Patent 5173-413. (28.02 .91).*

Gupta, K. G.; Singh, J.; Sahani, G. and Dhavan, S. (1979), Production of phenyl acetyl carbinol by yeasts. *Biotechnol. Bioeng.*, **21** : (6), 1085-1089.

Long, A.; James, P. and Ward, O. P. (1989), Aromatic aldehydes as substrate for yeast and yeast alcohol dehydrogenase. *Biotechnol. Bioeng.*, **33** : (5), 657-60.

Mahmoud, W. M.; El Sayed, A. H. M. M. and Coughlin, R. W. (1990), Effect of β-Cyclodextrin on production of L-phenyl acetyl carbinol by immobilized cells of *Saccharomyces* cerevisae. Biotechnol. *Bioeng.*, **36** : (3), 256-262.

Netraval, J. and Vojtisek, V. (1982), Production of phenylacetylcarbinol in various yeast species. *Eur. J. Appl. Microbiol. Biotechnol*., **16**, 35-38.

Nikolova, P. and Ward, O. P. (1991), Production of L-phenyl acetyl carbinol by biotransformation : product and by-product formation and activities of the key enzymes in wild type and ADH - isoenzyme mutants of *Saccharomyces cerevisiae. Biotechnol. Bioeng.*, **20**, 493-498.

Shukla, V. B. (1999), Studies in Microbial Biotransformations. Ph.D. thesis University of Mumbai.

Shukla, V. B. and Kulkarni, P. R. (1999), Downstream processing of biotransformation broth for recovery and purification of L-phenyl acetyl carbinol (L-PAC). *J. Sci. Indus. Res.*, **58 :** (8), 591-593.

Smith, P. F. and Hendlin, D. (1953), Mechanism of phenyl acetyl carbinol synthesis by yeast. *J. Bacteriol*,. **65**, 440-445.

Subramanian, P. M.; Chatterjee, S. K. and Bhatia, M. C. (1987), Synthesis of (1RS, 2SR) - (±) - 2- amino - 1 - phenyl - 1 - propanol from (R) - (-) - 1 - hydroxy - 1 - phenyl - 2 – propanone. *J. Chemical Technol. Biotechnol.,* **39** : (4), 215-218.

Szejtli, J. (1998), Introduction and general overview of cyclodextrin chemistry. *Chem. Rev*., **98**, 1743-1753.

Tripathi, C. K. M.; Basu, S. K.; Vora, V. C., Mason, J. R. and Pirt, S. J. (1988), Continuous cultivation of yeast strain for biotransformation of L-acetyl phenyl carbinol (L-PAC) from benzaldehyde. *Biotechnol. Lett.*, **10** : (9), 635-36.

Uekama, K.; Hirayama, F. and Irie, T. (1998), Cyclodextrin drug carrier systems. *Chem. Rev*., **98**, 2045-2076.

Zeeman, R.; Netrval, J.; Bulantova, H. and Vodnasky, M. (1992), Biosynthesis of phenyl acetyl carbinol in yeast *Saccharomyces cerevisiae* fermentation. *Pharmazie*, **47** : (4), 291-94.

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