POTENTIAL OF SOME YEAST STRAINS IN THE STEREOSELECTIVE BIOSYNTHESIS OF ACYLOINS

Mihai Lăcătuş[1], Viktória Bódai[2] ✉, László Nagy-Győr[2], László-Csaba Bencze[1], Csaba Paizs[1], László Poppe[3], Florin-Dan Irimie[1] ✉

[1] *Biocatalysis and Biotransformation Research Group, Babeş-Bolyai University, Cluj-Napoca, Romania*

[2] *Fermentia Ltd, Budapest, Hungary*

[3] *Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Budapest, Hungary*

E-mail: *vbodai@gmail.com, irimie@chem.ubbcluj.ro*

Introduction

The biocatalytic synthesis of asymmetric chiral acyloins as intermediates in the production of active pharmaceutical ingredients is an attractive goal. For example, *R*-(-)-phenylacetylcarbinol (*R*-PAC), is the key chiral precursor for pseudoephedrine production[1]. The use of pyruvate decarboxilase (PDC, E.C.4.1.1.1), a Mg(II) and thiamine diphosphate-dependent enzyme from yeasts as a biocatalyst, has been extensively studied and is now a well-recognized method for condensing aldehydes and pyruvic acid to form acyloins(α-hydroxy ketones)[2]. In whole cells mediated biotransformation, by-product formation can not be avoided consequently, screening for new microorganisms with high pyruvate decarboxilase and low oxido-reductase activity is still a challenging task.[3] Moreover, by-product formation can be minimised by optimizing reaction conditions. In this work, the screening of several newly isolated yeasts in lyophilized whole cells form for acyloin production in aqueous media was investigated.

Experimental part

Dry baker's yeast (*Saccharomyces cerevisiae*) was purchased from local market. Newly isolated yeasts strains were received as lyophilized cells. The aromatic aldehydes **1a-e** were purchased from Merck and were freshly distilled before use. Authentic racemic acyloins *rac*-**6a-e** diols *rac*-**7a-e** and diketones **8a-e** were chemically synthesized starting from the aromatic propanones **3a-e** (**Figure 1**).

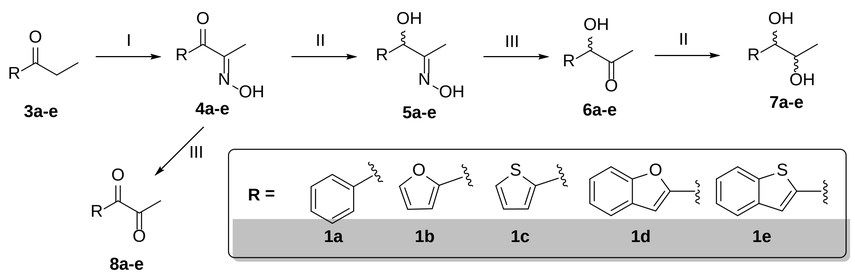

Figure 1. Chemical synthesis of *rac*-**6,7a-e** and **8a-e**.Reagents and conditions: **I.** *n*-BuONO,CH_3OH, 10M HCl, 60ºC; **II.** $NaBH_4$,CH_3OH,r.t.; **III.** CH_2O,HCl,reflux;

To investigate the selectivity and conversion of the newly isolated yeasts on acyloin condensation of different aldehydes, reactions were carried out as batch processes using lyophilized yeast cells suspended in aqueous buffer media (pH 7). Sodium pyruvate was used as acyl source. Reactions were incubated at 25ºC and shaked at 250 rpm. The progress of the bioconversions were monitored by TLC and GC analysis. The enantiomeric composition and conversion of the chiral acyloins were performed by GC analysis on a B-DM chiral column **(Figure 3).** The absolute configuration of the obtained acyloins was the same as stated in the literature[4].

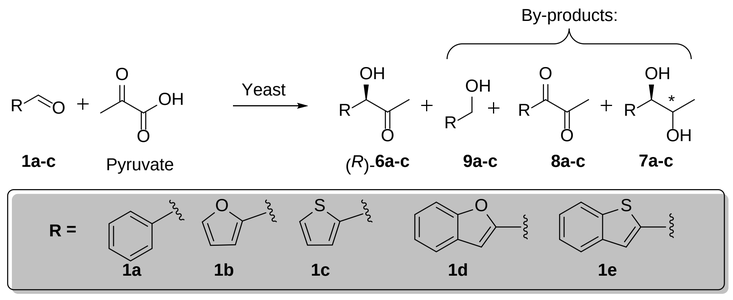

Figure 2. Yeast promoted biotransformation of aldehydes **1a-e** for the efficient stereoselective synthesis of acyloins **6a-e**.

Results and discussion

Preliminary experiments

Prior to the screening of yeast strains for condensation activity, initial experiments were conducted on aldehyde **1a** using fermenting and non-fermenting baker's yeast cells and in both cases, acyloin formation was observed but the major product was the alcohol **9a**. Results indicated that non-fermenting yeast produced significantly more acyloin than fermenting yeast.

Incubation time of the yeast cells was also studied and it was found that in the absence of externally added pyruvate, a longer incubation time prior to addition of the substrate favoured the production of acyloin and not the alcohol. When pyruvate was added to the reaction medium together with the substrate, similar results were obtained for different incubation periods, with 1 hour being the optimum.

As the results indicate, the presence of pyruvate has a great impact on the distribution of aldehyde **1a** into alcohol **9a** and acyloin **6a**. To determine the optimum concentration of pyruvate, a screening of pyruvate concentration was performed using baker's yeast and the results are shown in **Table 1**.

Screening of yeast strains for optimal acyloin production

Using the preliminary results, a screening of 9 yeast strains was attempted using aldehyde **2a** as substrate, with 10 equivalents of sodium pyruvate added to the reaction medium. Accordingly, almost no acyloin was detected (<5%) for all 9 yeast and the major product was the alcohol **9a**. Because high pyruvate concentrations inhibits the acyloin condensation, a further screening was attempted using only 2 equivalents of pyruvate and acyloin **6a** was detected in quantitative concentrations in all cases (**Table 2).**

For aldehydes **1b** and **1c**, the screening of yeast was performed in the same conditions as mentioned above, but acyloins were formed with very low conversions (<5% in most cases) while the major products were the alcohol **9b** and **9c**. In the case of aldehydes **1d** and **1e**, which were novel substrates for yeast mediated acyloin condensations, the production of acyloins **6d-e** was confirmed by NMR analysis, and will be followed by the determination of absolute configuration, conversion and enantiomeric excess values.

Table 1.Effect of pyruvate concentration on acyloin condensation with baker's yeast of benzaldehyde **1a**.

Pyruvate equiv.	0	0.1	1	2	5	10
Conv.(%) of 6a[1]	33	25	24	35	49	62
R^2	0.67	0.44	0.46	0.69	1.25	2.74

1.Enantiomeric exces of product in all cases was >98% and the major enatiomer was *R*-(-)-**6a**
2. **R** corresponds to molar *ratio* of acyloin **6a** and alcohol **9a**

Table 2. The results obtained by screening yeast strains for **1a**

Yeast strain[1]	ee	Conv.(%) of 6a	R^2
Pichia sp.	97	65	2.01
Lodderomyces sp.	97	77	3.71
Wickerhamonyces sp. 1A	96	19	8.64
Candida sp. 1	97	17	4.82
Cryptococcus sp.	92	9	4.52
Debaryomyces sp.	98	12	10.39
Rhodotorula sp.	95	17	0.22
Candida sp. 2	97	33	0.56
Wickerhamomyces sp. 1B	97	23	2.12
Saccharomyces cerevisiae	98	35	0.69

1.Conditions: 20mg of benzaldehyde(dissolved in 100 µl of isopropanol) and 2 equiv. of sodium pyruvate(414 mg) were added to a 50-mL Falcon tube containing 150 mg yeast in phospate buffer pH 7 (5mL).
2. **R** corresponds to molar ratio of acyloin **6a** and alcohol **9a**

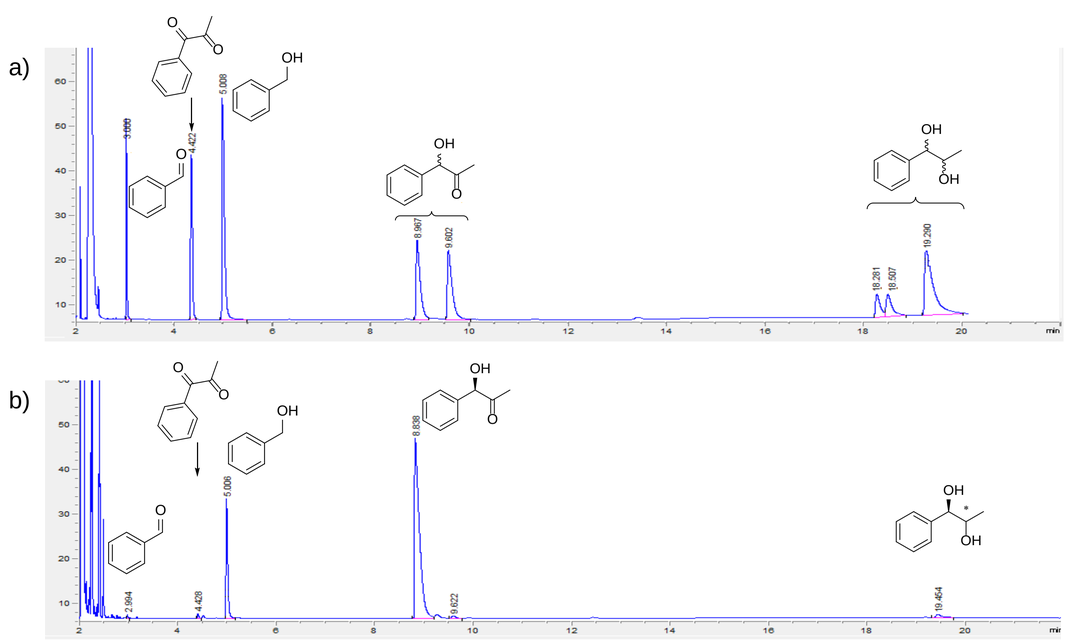

Figure 3. GC chromatograms of: a) *Separation of authentic substrates, products and byproducts for acyloin condensation of* ***1a***; b) *Acyloin condensation of* ***1a*** *with Lodderomyces sp. after 5 hour of reaction.*

Conclusions

- ❑ Preliminary results on the biotransformation of benzaldehyde with dry baker's yeast indicated that non-fermenting conditions and preincubation of dry yeast favours the acyloin formation.
- ❑ The used pyruvate concentration strongly influences acyloin formation, depending on the yeast strain. High levels of pyruvate can promote or inhibit acyloin biosynthesis.
- ❑ Screening studies with various yeasts showed that a *Pichia sp* and a *Lodderomyces sp* are potent candidates for the efficient and highly stereoselective acyloin synthesis.

Selected References

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Acknowledgements

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