Screening of microorganisms for ω-transaminase activity

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INTRODUCTION

Transaminases (TA) are pyridoxal phosphate dependent enzymes which are capable of transferring amino groups to achiral ketones. TA´s can be used for asymmetric synthesis of amines and also for kinetic resolution of racemic amines.[1]

The aim of this work is to screen microorganisms for their ω-TA activity for the kinetic resolution of 1-*rac*-phenylethylamine (Figure 1).

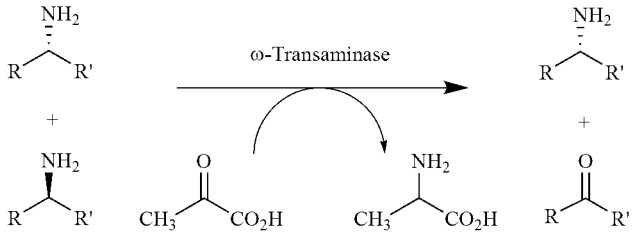

Figure 1. Kinetic resolution of *rac*-amines using microorganisms containing ω-TA.

RESULTS AND DISCUSSION

We tested six *Pseudomonas* sp. strains (*P. alcaligens, P. putida, P. stutzeri, P. aeruginosa, P. fluorescens* and *P. oleovorans* DSM 1045) and *Janibacter terrae* DSM 13953. The strains *P. oleovorans* and *Janibacter terrae* gave the most promising results. The kinetic resolution of *rac*-phenylehtylamine was performed using lyophilized cells previously cultivated in standard medium (glucose/bacteriological peptone/yeast extract). The cells were rehydrated with phosphate buffer containing PLP (pH 7.5) and then, were added of 1-*rac*-phenylethylamine (1 eq) and sodium pyruvate (1 eq). The reaction mixture was shaken at 30 ºC and 120 rpm for 24 or 48 hours. Afterwards, the reaction was stopped by extraction with ethyl acetate.[2] The organic phase was dried with anhydrous sodium sulphate prior GC analysis.

Conversions for all reactions were determined on a GC-MS cromatograph using a Rtx-5MS column (thickness: 0.25 µm, diameter: 0.25 mm, length: 30 m). The enantiomeric excess was accessed using GC-FID cromatograph using a chiral column BetaDex 225 (thickness: 0.25 µm, diameter: 0.25 mm, length: 30 m).

The results for the most promising strains evaluated (*P. oleovorans* and *Janibacter terrae*) are summarized in Table 1.

Table 1. Kinetic resolution of 1-phenylethylamine using *P. oleovorans* and *Janibacter terrae*.

Microorganism	Time (hours)	Phenyl-ethylamine (%)	Acetophenone (%)	*ee*
P. oleovorans	24	13	87	*80 R*
	48	18	82	*90 R*
J. terrae	24	54	46	*21 R*
	48	27	73	*37 R*

According to Table 1, good conversions and enantiomeric excess were obtained for kinetic resolutions using both strains. Furthermore, similar results were achieved with 24 or 48 hours of reaction. To reach better enantiomeric excess we are currently carrying on the reaction with a shorter incubation period.

CONCLUSION

Amongst all tested microorganisms, *P. oleovorans* and *Janibacter terrae* showed interesting results for the kinetic resolution of 1-*rac*-phenylehylamine. Further studies to improve the enantiometic excesses of these reactions and the screening of other strains are in progress in our laboratory.

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