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Resolution of racemic 1-arylethyl acetates by *Pseudomonas fluorescens* in the presence of a surfactant

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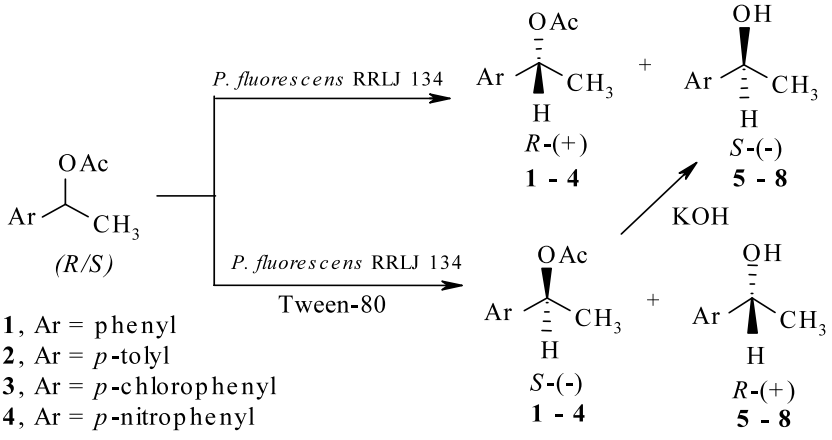
Abstract—The *Pseudomonas fluorescens* RRLJ 134 strain efficiently resolved (*R*/*S*)-1-arylethyl acetates into *S*-(−)-1-arylethyl acetates and *R*-(+)-1-arylethanol in the presence of the surfactant Tween-80. The surfactant reverses the enantioselectivity compared to the surfactant free medium.

The preparation of enantiomerically pure substrates using bio-catalysis has received considerable attention in recent years because of significant demand for optically pure compounds.[1] Indeed, the bio-catalytic resolution of racemates is now recognised as a valuable tool for the synthesis of optically active compounds.[2] Two approaches have been used for bio-catalytic resolutions—one involves the use of isolated enzymes and the other involves the use of microbial whole-cells. In comparison to enzymatic reactions, the whole-cell reactions usually afford poor enantioselectivity as several enzymes in the cell can participate in the reaction with one enzyme affording the desired enantiomer while another enzyme may produce its antipode.[3] However, because of the cost effectiveness, availability, scope for discovery of newer lipase activities and ease of handling, the whole-cell reactions continue to be preferred strategies in bio-organic transformations. To improve stereoselectivity, different methods have been developed by modification of the whole-cell reaction conditions such as treatment of microbial cells with additives,[4] addition of an inhibitor to specific enzymes[5] and the use of hydrophobic polymers.[6] Although these methods are effective, many reactions are unsuitable for these types of stereochemical control and consequently new strategies to increase the stereoselectivity of the microbial whole-cell reactions are still desirable.

There are several examples available for enzyme or whole-cell catalysed resolution of arylpropionic acids.[7] Further, the complete reversal of enantioselectivity of an enzyme catalysed reaction by directed evolution using error prone PCR and DNA shuffling has recently gained importance.[8] Also, the effect of the surfactant



1, Ar = phenyl
2, Ar = *p*-tolyl
3, Ar = *p*-chlorophenyl
4, Ar = *p*-nitrophenyl

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Table 1. The optical resolution of (*R*/*S*)-**1**–**4** by *P. fluorescens* RRLJ 134[a]

Entry	Acetate	Surfactant (%)	Conv. (%)[b]	Substrate			Product			E[d]
					Yield[c]	Ee (%)		Yield[c]	Ee (%)	
1	**1**	Nil	60	(*R*)-**1**	38	30	(*S*)-**5**	33	20	1.9
2	**1**	0.01	53	(*S*)-**1**	33	95	(*R*)-**5**	38	80	43
3	**2**	Nil	59	(*R*)-**2**	42	22	(*S*)-**6**	38	15	1.6
4	**2**	0.01	55	(*S*)-**2**	34	99	(*R*)-**6**	30	80	49
5	**3**	Nil	56	(*R*)-**3**	41	23	(*S*)-**7**	28	18	1.7
6	**3**	0.01	56	(*S*)-**3**	45	28	(*R*)-**7**	34	22	2
7	**4**	Nil	55	(*R*)-**4**	35	20	(*S*)-**8**	27	16	2.2
8	**4**	0.01	60	(*S*)-**4**	41	25	(*R*)-**8**	38	20	1.9

[a] Reaction time = 36 h.
[b] Conversions were deduced from the ees of the substrate (ee_s) and the product (ee_p): $c = ee_s/(ee_s+ee_p)$.
[c] Calculated taking into account the percentage of conversion.
[d] Enantiomeric ratio calculated according to Sih et al.[16]

Tween-80 on enantioselective hydrolysis of propionic esters by crude or purified lipases as well as whole-cell performance is documented.[9] To the best of our knowledge such a study on the kinetic resolution of an 1-arylethyl acetate is rare.

The fluorescent Pseudomonads are well known bacterial biocontrol agents[10] and are involved in various bio-transformation reactions.[11] Recently we have demonstrated that the whole-cell-catalysed reaction of *P. fluorescens* RRLJ 134 can be successfully applied to the hydrolysis of acetates into symmetrical alcohols.[12] Herein, we report the use of *P. fluorescens* RRLJ 134 in the hydrolytic kinetic resolution of (*R*/*S*)-1-arylethyl acetates with a slight preference for the *S*-alcohol. We further demonstrate that the enantioselectivity of the bio-catalyst can be completely reversed and significantly enhanced by using the surfactant Tween-80.

The kinetic resolution of (*R*/*S*)-1-phenylethyl acetate **1** with *P. fluorescens* RRLJ 134 in succinate media[13] at pH 7.0 afforded 1-phenylethyl acetate *R*-(+)-**1**, $[\alpha]_D^{25} = +25$ (*c* 1.5, $CHCl_3$) and 1-phenylethanol *S*-(−)-**5**, $[\alpha]_D^{25} = -14$ (*c* 1.4, $CHCl_3$) in poor enantioselectivity (Table 1, entry 1). The result suggested that the resolution of (*R*/*S*)-**1** by *P. fluorescens* was conducted by more than one enzyme, wherein one enzyme might hydrolyse the substrate to *S*-(−)-**5**, while another afforded the antipode on hydrolytic resolution.

The enhancing effect of the surfactant Tween-80 on lipase performance as well as whole-cell bacteria in the enantioselective hydrolysis of arylpropionic esters[9] prompted us to test the effect of the surfactant on the resolution reaction of (*R*/*S*)-**1** using whole-cell *P. fluorescens* RRLJ 134. We assumed that the relative activities of the enzymes that conduct hydrolytic resolution might be enhanced in the presence of the surfactant in comparison to the surfactant-free medium.

Thus, when the whole-cell *P. fluorescens* RRLJ 134 was incubated at 30°C with (*R*/*S*)-**1** in the presence of the surfactant Tween-80 (10 mg/100 ml of nutrient broth), the acetate *S*-(−)-**1**, $[\alpha]_D^{25} = -86$ (*c* 0.5, $CHCl_3$) and alcohol *R*-(+)-**5**, $[\alpha]_D^{25} = +52$ (*c* 1.4, $CHCl_3$) were obtained with reversal of enantioselectivity (Table 1, entry 2).[14] The enantiomeric ratio *E*, which reflects the relative reaction rate of the two enantiomers, turned out to be 43 in comparison to the surfactant-free medium ($E = 1.9$). Similarly (*R*/*S*)-**2** underwent enantioselective deracemization in the presence of Tween-80 to afford *S*-(−)-**2** and *R*-(+)-**6** in high enantiomeric excess. The enantiomeric ratio being $E = 49$ in comparison to the surfactant-free medium where $E = 1.6$ (Table 1, entries 3–4). However, the hydrolytic resolution of both (*R*/*S*)-**3** and (*R*/*S*)-**4** with surfactant or surfactant-free conditions afforded the products in poor enantioselectivities (Table 1, entries 5–8).

It was further observed that the enzyme activity was influenced by surfactant concentration. When the hydrolyses of (*R*/*S*)-(**1**–**2**) were carried at a concentration of 10 mg/100 ml (0.01%) of the nutrient broth, the

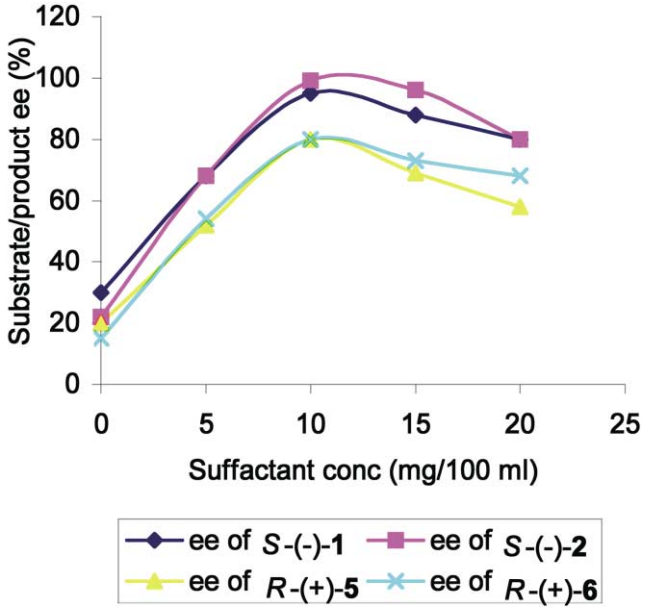

Figure 1. The effect of the surfactant Tween-80 on the enantioselective resolution of (*R*/*S*)-**1**–**2** by *P. fluorescens* RRLJ 134.

enantiomeric excesses (ee) of *S*-(−)-**1**–**2** and *R*-(+)-**5**–**6** were significantly enhanced (95–99%, and 80%).[15] However, changing the concentration of the surfactant to 5 mg/100 ml or 20 mg/100 ml (0.005 or 0.02%) led to a decrease in enantiomeric excesses (ee) of *S*-(−)-**1**–**2** and *R*-(+)-**5**–**6** (Fig. 1).

For the determination of the absolute configuration of the resolved products, products *S*-(−)-**1**–**4** were hydrolysed into *S*-(−)-**5**–**8** with methanolic KOH at room temperature. The *S*-configurations of the alcohols **5**–**8** were assigned by comparisons of their specific rotations with literature data.[17]

Thus we have found that the surfactant Tween-80 can reverse the enantioselectivity and enhance the reactivity of the *P. fluorescens* lipase that catalyses the resolution of (*R*/*S*)-**1**–**2** to *S*-(−)-**1**–**2** and *R*-(+)-**5**–**6**. The reversal of enantioselectivities could be due to an inhibitory influence of the surfactant Tween-80 on *S*-selective enzymes in preference to *R*-selective enzymes during hydrolysis. Further, the enantioselectivity is dependent on the surfactant concentration as well as the nature of the aryl substituents. An electron donating substituent on the aryl moiety favours enantioselectivity whereas an electron withdrawing group reduces the degree of resolution. The poor enantioselective resolution of substrates (*R*/*S*)-**3**–**4** could probably be due to the unfavourable electron deficient situation at the binding site of the enzyme-substrate due to the electron withdrawing group at the *p*-position of the aryl substrates.

In conclusion, we have reported the first example of the kinetic resolution of racemates with reversal of enantioselectivity using a microorganism in the presence of a surfactant in comparison with the surfactant-free medium. Furthermore, a *p*-substituent on the aryl group influences the enantioselectivities of the products thus providing an example for the electronic effect of the substituent on the aromatic ring[18] towards the binding site of the enzymes.

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14. **Typical experimental procedure**: To 100 ml of succinate media in a 250 ml Erlenmeyer flask were added (*R*/*S*)-1-phenylethyl acetate **1** (0.046 g, 0.278 mM) in methanol (2 ml), Tween-80 [polyoxyethylene(20) sorbitan monooleate, 10 mg] and *P. fluorescens* RRLJ 134 strain (0.5 ml) grown in King's B medium. The reaction was incubated (30°C) at 120 rpm rotation for 36 h with shaking. The resulting mixture was extracted with ethyl acetate, washed with water, dried over anhydrous sodium sulfate. Removal of the solvent and separation of the products by preparative silica gel TLC afforded *S*-(−)-1-phenylethyl acetate *S*-(−)-**1** and *R*-(+)-1-phenylethanol *R*-(+)-**5**. *S*-(−)-**1**, yield 33%, R_f = 0.6 (hexane/ethyl acetate = 90/10); IR (KBr) 1730 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.8–7.1 (m, 5H, aromatic protons), 5.58 (q, 1H, J = 7.0 Hz, methine proton), 1.75 (s, 3H, methyl protons), 1.28 (d, 3H, J = 7.0 Hz, methyl protons); MS (ESI) m/z 164 (M^+), 122 (M^+–42); ee 95%; $[\alpha]_D^{25} = -86$ (*c* 0.5, $CHCl_3$). *R*-(+)-**5**: yield 38%, R_f = 0.35 (hexane/ethyl acetate = 90/10); IR (KBr) 3490 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.70–7.00 (m, 5H, aromatic protons), 4.40 (q, 1H, J = 6.5 Hz, methine proton), 3.55 (bs, 1H, hydroxyl proton), 1.18 (d, 3H, J = 7.0 Hz, methyl protons); MS (ESI) m/z 122 (M^+); ee 80%; $[\alpha]_D^{25} = +52$ (*c* 1.4, $CHCl_3$) [lit.,[17a] $[\alpha]_D^{25} = +41.5$ (neat)].

Hydrolysis of *S*-(−)-1 to 1-phenylethanol *S*-(−)-5

To a solution of *S*-(−)-**1** (0.05 g, 0.3 mM) in methanol (2 ml) was added a methanolic solution of KOH (0.3 ml, 10%) dropwise and the reaction mixture was stirred for 2 h at room temperature. It was then poured into water (50 ml), extracted with dichloromethane (2×5 ml) and dried over sodium sulfate. Removal of the solvent afforded a liquid product, which was purified by silica gel column chromatography to afford *S*-(−)-**5**, yield 0.03 g (80%), R_f = 0.35 (hexane/ethyl acetate = 90/10); IR (KBr) 3490 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.70–7.00 (m, 5H, aromatic protons), 4.40 (q, 1H, J = 6.5 Hz, methine proton), 3.50 (bs, 1H, hydroxyl proton), 1.18 (d, 3H, J = 7.0 Hz, methyl protons); MS (ESI) m/z 122 (M^+); ee 94%; $[\alpha]_D^{25} = -60.2$ (*c* 1.4, $CHCl_3$); [lit.,[17b] $[\alpha]_D^{27} = -66.5$ (*c* 1.4, $CHCl_3$)].

S-(−)-**2**, yield 34%, R_f = 0.65 (hexane/ethyl acetate = 90/10); IR (KBr) 1730 cm^{-1}; MS (ESI) m/z 178 (M^+), 136 (M^+–42); ee 99%; $[\alpha]_D^{25} = -49$ (*c* 0.5, $CHCl_3$). *R*-(+)-**6**: yield 30%, R_f = 0.35 (hexane/ethyl acetate = 90/10); IR (KBr) 3495 cm^{-1}; MS (ESI) m/z 136 (M^+); ee 77%; $[\alpha]_D^{25} = +32$ (neat); [lit.,[17c] $[\alpha]_D^{27} = +56$ (neat)].

S-(−)-**3**, yield 45%, R_f = 0.70 (hexane/ethyl acetate = 90/10); IR (KBr) 1730 cm^{-1}; MS (ESI) m/z 198 (M^+); ee 28%; $[\alpha]_D^{25} = -15$ (*c* 0.5, $CHCl_3$). *R*-(+)-**7**: yield 34%, R_f = 0.30 (hexane/ethyl acetate = 90/10); IR (KBr) 3495 cm^{-1}; MS (ESI) m/z 156 (M^+); ee 22%; $[\alpha]_D^{25} = +12$ (*c* 0.5, $CHCl_3$).

S-(−)-**4**, yield 41%, R_f = 0.60 (hexane/ethyl acetate = 90/10); IR (KBr) 1730, 1535, 1348 cm^{-1}; MS (ESI) m/z 209 (M^+); ee 25%; $[\alpha]_D^{25} = -15$ (*c* 0.5, $CHCl_3$). *R*-(+)-**8**: yield 38%, R_f = 0.30 (hexane/ethyl acetate = 90/10); IR (KBr) 3495 cm^{-1}; MS (ESI) m/z 167 (M^+); ee 20%; $[\alpha]_D^{25} = +20$ (*c* 0.5, $CHCl_3$).

15. Enantiomeric excesses (ees) were determined by HPLC analysis with a chiral cell OD column.

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