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Amidation of polyaromatic carboxylic acids in aqueous medium

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Abstract

The amidation of polyaromatic carboxylic acids, such as 4-biphenylcarboxylic acid, 4-biphenylacetic acid, 4-carboxy-4′-hydroxybiphenyl, and *N*-phenylanthranilic acid, was satisfactorily carried out to give the corresponding amides using a wild-type bacterium, *Bacillus cereus* (w), in an aqueous medium. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: biotransformation; amidation; *Bacillus cereus*; polyaromatic carboxylic acid.

Amide compounds of polyaromatic carboxylic acids (PAA) are useful in fine chemical industries as materials of liquid crystals[1,2] and as intermediates for pharmaceuticals. Such amide compounds are generally obtained by the ammonolysis of acid chlorides under anhydrous conditions or by heating carboxylic acids in the presence of ammonia in an autoclave. Since undesired by-products are often generated during these amidation reactions, a new method to prepare amide compounds under milder reaction conditions is required.

Biotransformation technology has been developed as a new synthetic method that gives the desired compounds with high enantioselectivity and high substrate specificity under clean conditions without the use of organic solvents. This technology has been applied to various transformations, such as hydroxylation, oxidation and reduction, but there have been few reports on application to amidation of PAA.[3] Biotin has been transformed into biotinamide using *Rhodotorula flava* in 30% yield by a five day reaction at 30°C.[4] Cultivation of *Bacillus cereus* 50 with 12-hydroxy-octadecanoic acid at 30°C for five days afforded the corresponding 12-hydroxy-octadecanamide in 24% yield.[5] It has been found that oleic acid decomposes to give a mixture of 9(z)-octadecenamide, hexadecenamide, tetradecenamide, and tetradecanamide (5–7% yields) in the presence of *Bacillus megaterium* B-3437.[6] Thus, water-soluble natural products were used as the substrates in all of these reactions because aqueous media are required for such biotransformation. Therefore, although there have been a few reports on successful amidation by biotransformation, there have been no reports on amidation of water-insoluble substrates such as PAA.

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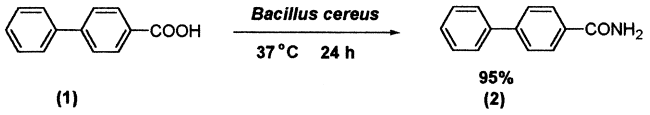
To prepare amide compounds corresponding to PPA, we focused on the biotransformation ability of *Bacillus cereus*.[7] Using *Bacillus cereus* (w) from soil, 4-biphenylcarboxylic acid (**1**) was satisfactorily transformed to 4-phenylbenzamide (**2**) in excellent yield (95%) by 24 h reaction at 37°C (Scheme 1).[8]



Scheme 1.

Through monitoring of the reaction by HPLC, several optimal reaction conditions, (substrate concentration, temperature, reaction time, and atmosphere) were determined, as shown in Table 1. Since the amount of **2** transformed from **1** increased with the growth of bacteria, the effect of cell density in the reaction mixture was also investigated. As a result of these examinations, the highest yield (95%) was obtained under the following conditions: cell density, 20 g/l; substrate concentration, 1 mM; temperature, 37°C; and reaction time, 24 h.

The supply of oxygen was found to be indispensable, since the reaction stopped under N_2 or CO_2 atmosphere. Other *Bacillus cereus* strain IFO 3001 obtainable from the list of cultures[9] also gave **2** in 92% yield. On the other hand, no amidation of PAA occurred using either *E. coli* or baker's yeast.

Table 1
Amidation of 4-biphenylcarboxylic acid in nutrient broth

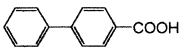
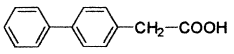
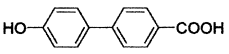
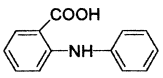
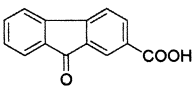
Substrate Concentration (mM)	Temp. (°C)	Time (h)	Atmosphere	Yield (%)
1.0	25	24	Air	84
1.0	30	24	Air	88
1.0	37	12	Air	73
1.0	37	24	Air	95
1.0	40	24	Air	86
1.0	37	24	N_2	1
1.0	37	24	CO_2	0
2.0	37	24	Air	70
4.0	37	24	Air	38

Reaction was carried out in 10 ml of nutrient in the presence of 0.2 wet-g of bacterial cells.

Our amidation system was also applied to substrates other than **1**. Interestingly, a simple aromatic carboxylic acid having one benzene ring, such as benzoic acid, could not be amidated by our system. Several PAAs, such as 4-carboxy-4-hydroxybiphenyl, *N*-phenylanthranilic acid, 9-fluorenone-2-carboxylic acid, and 4-biphenylacetic acid, were satisfactorily transformed to the corresponding amide compounds using *Bacillus cereus* (w), as shown in Table 2.[10]

The difference between conversion rates and yields shows a loss of each product during extraction with ethyl acetate. In the case of 9-fluorenone-2-carboxylic acid, although monitoring

Table 2
Amidation of polyaromatic carboxylic acids

Substrate	Conversion (%)	Yield (%)
	98	95
	69	67
	83	69
	70	57
	75	41

Ten ml of nutrient broth and 0.2 wet-g of cells were used, and the reaction was carried out at 37°C for 24 h. The substrate concentration was 1 mM.

by HPLC showed that the conversion of the carboxyl group to amide occurred in 75% yield, the amide product was obtained in only 41% yield. The rates of these losses are thought to depend on the solubility in ethyl acetate. Thus, our system is applicable to the amidation of PAA.

In summary, we have demonstrated that polyaromatic carboxylic acids can be specifically transformed to the corresponding amide compounds using *Bacillus cereus* (w) in an aqueous medium at 37°C. On the other hand, biotransformation of several aliphatic[11] and aromatic[12] nitrile compounds with nitrile hydratases also affords the corresponding amide compounds and this technology has been applied to acrylamide production.[13] Therefore, our biocatalytic amidation system can be used as a simple, direct and clean alternative to organochemical amidation methods.

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8. Into an L-shaped test tube were added PAA, seed bacteria and a sterilized culture solution composed of peptone (1%), fish meat (bonito) extracts (1%), and NaCl (0.2%). The reaction was started by shaking the tube in a 37°C water bath. The decrease in the substrate and the increase in amide were analyzed for the extracts of ethyl acetate by HPLC (Gilson Co. model 102, column YMC pac, ODS-A, 4 mm i.d.×150 mm in length) with a gradient system (MeOH:H_2O = 3:7 to 7:3) monitored by a UV detector at 270 nm. The evolution of ammonia from nutrients increased the pH from 7.0 to 8.4 during the reaction.

9. List of cultures; Inst. Fermentation: Osaka (IFO), **1996**; p. 131.
10. The substrate (0.2 mmol), seed bacteria (4 wet-g) and medium (200 ml) in a 500 ml flask was shaken reciprocally (120/min) at 37°C for 24 h. After the reaction the products were extracted with ethyl acetate and dried on anhydrous sodium sulfate. Evaporation of the solvent left a white powder of amide. Further purification was carried out on a separate column (YMC-pac, ODS, 20 mm i.d.×150 mm in length).
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