Notes

OBAFLUORIN, A NOVEL β-LACTONE PRODUCED BY *PSEUDOMONAS FLUORESCENS*. TAXONOMY, FERMENTATION AND BIOLOGICAL PROPERTIES

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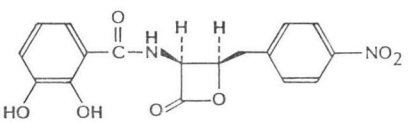
In the past we have reported on the isolation of β-lactones from bacteria; namely *Bacillus* sp., *Pseudomonas* sp. and a soil coryneform[1]. These molecules in addition to carrying an acylamino group at the 3-position have a 4-β methyl group. Here we report on obafluorin, a β-lactone having a *p*-nitrobenzyl group as the 4-substituent (Fig. 1). This paper describes the taxonomy of the obafluorin-producing organism, fermentation conditions and biological properties of obafluorin.

Fig. 1. Structure of obafluorin.



Pseudomonas fluorescens SC 12,936 (ATCC 39502) was isolated from a plant specimen obtained in Princeton, New Jersey. Subsequent screening of bacteria isolated from a wide variety of samples and from many different environments has revealed the widespread occurrence of this molecule among *Pseudomonas* strains (Table 1).

The producing strain of *P. fluorescens* SC 12,936 is an aerobic, Gram-negative, motile rod, occurring singly or as short plump diplobacilli. Motility is achieved by means of one or more

Table 1. Collection sites and types of samples yielding *Pseudomonas* strains producing obafluorin.

Sample	Site
Plant litter	Montgomery Township, New Jersey
Salt marsh mud	Cheesequake, New Jersey
Forest litter and bracket fungi	Great Swamp, New Jersey
Ferns	West Windsor, New Jersey
Rotting oak bark	Lawrenceville, New Jersey
Elm leaf litter plant material and hay compost	Princeton, New Jersey
Sewage aeration tank waters	Kingston, New Jersey
Soil and plant material	Hopewell, New Jersey
Moss, algae and mineral spring water	Monessen, Pennsylvania
Rotting gladiolas	Fairless Hills, Pennsylvania
Leaf litter and mulch	Waterbury, Connecticut
Moss and soil	Surbridge Forest, Massachusetts
Silty clay	Hawaii
Decaying plants	St. Thomas
Red clay soil	Brazil
Soil	Hungary
Plant materials	Regensburg, Germany
Brewery sewage and forest soil	Germany
Riverbank soil	Hamburg, Germany
Soil	Saint Cergue, Switzerland
Soil	Johannesburg, South Africa
Soil, moss, leaves and debris	North Wales
Soil and plants	Barbados
Muds, soils, lichens, fresh and rotting plant material	England

polar flagella. The organism is oxidative both on triple sugar - iron agar and HUGH - LEIFSON's O (oxidative)/F (fermentative) glucose test medium. It is cytochrome oxidase positive and is fluorescent on KING's B medium. The following test responses were positive: catalase, arginine dihydrolase and gelatinase. It produces lipase and lecithinase and forms levan from sucrose. The organism does not hydrolyze starch, form indole or accumulate poly-β-hydroxybutyrate. The organism can utilize the following as sole carbon sources: D,L-arginine, betaine, trehalose, *m*-inositol, L-arabinose, sucrose, propionate, and adonitol but not butyrate or ethanol. These characteristics serve to identify the obafluorin producer as *P. fluorescens*.

Fermentation was initiated by transferring a loopful of surface growth from an agar slant of *P. fluorescens* SC 12,936 into 500-ml Erlenmeyer flasks, each containing 100 ml of the following sterilized medium: yeast extract 0.5%, glucose 0.5%, $MgSO_4 \cdot 7H_2O$ 0.01% and $FeSO_4 \cdot 7H_2O$ 0.01% in a soil extract filtrate* and tap H_2O (mixed in a ratio of 1 part filtrate to 4 parts tap water). After inoculation, the flasks were incubated at 25°C on a rotary shaker (300 rpm; 5-cm stroke) for approximately 24 hours. A 1% (vol/vol) transfer of this culture growth was made to fifty 500-ml Erlenmeyer flasks each containing 100 ml of the sterilized medium described above. The fermentation was continued for approximately 17~18 hours using the same temperature and rotary shaker conditions described above. Antibiotic production was monitored by a paper disc agar diffusion assay using *Bacillus licheniformis* (SC 9262) as test organism. The antibiotic was isolated and its structure determination will be published elsewhere.

Table 2. Antibacterial activity of obafluorin.

Organism	SC	Zone of inhibition (mm)[a]	
		Obafluorin (10 μg)[b]	Ampicillin (10 μg)
Staphylococcus aureus	1276	8.7	33.4
	2399	7.4	42.2
	2400	7.9	19.7
Escherichia coli	8294	—	21.5
	10857	10.5	36.8
	10896	12.8	21.6
	10909	13.4	30.9
Enterobacter cloacae	8236	9.3	28.3
Pseudomonas rettgeri	8479	7.7	36.5
P. aeruginosa	9545	9.1	25.8

[a] Inoculum adjusted to Mc Farland 0.5 turbidity standard and tested on K10 agar (pH 6.7) consisting of: beef extract 0.15%, yeast extract 0.3%, peptone 0.6% and glucose 0.1% in distilled water.

[b] Obafluorin, 2 mg/ml in acetonitrile, 5 μl applied to 6.3 mm Whatman #4 filter paper disks.

* Soil extract filtrate: 1 vol. soil plus 2 vols. H_2O extracted at 100°C for one hour and filtered.

Table 3. Action of β-lactamases on obafluorin[a].

Compound	Relative rate of hydrolysis with β-lactamase type		
	P99	TEM-2	K1
Cephaloridine	100	100	100
Obafluorin	4	110	140

[a] Assays were performed in 10 mM phosphate buffer (pH 6.0) - EtOH - acetonitrile (320: 80: 3), using lactone at 35 μg/ml. Quantitation of hydrolysis was accomplished using HPLC to identify hydrolysis product. Cephaloridine at 35 μg/ml was used as a standard under the same conditions, but reaction was followed spectrophotometrically.

Obafluorin showed weak antibacterial activity against a range of bacteria by disk diffusion (Table 2) but on MIC testing all bacteria tested exhibited values greater than 100 μg/ml. Interaction studies of obafluorin with β-lactamases are shown in Table 3. As can be seen, obafluorin was efficiently hydrolyzed by TEM and K1 β-lactamases and showed some susceptibility to hydrolysis by P99. To our knowledge, this is the first report of a non-β-lactam antibiotic showing such a high degree of susceptibility to hydrolysis by β-lactamases.

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Reference

1) WELLS, J. S.; J. C. HUNTER, G. L. ASTLE, J. C. SHERWOOD, C. M. RICCA, W. H. TREJO, D. P. BONNER & R. B. SYKES: Distribution of β-lactam and β-lactone producing bacteria in nature. J. Antibiotics 35: 814~821, 1982