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Full Paper

The Thioesterase Bhp is Involved in the Formation of β -Hydroxytyrosine during Balhimycin Biosynthesis in *Amycolatopsis balhimycina*

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Article first published online: 8 DEC 2009

DOI: 10.1002/cbic.200900600

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Issue



ChemBioChem

Volume 11, Issue 2, ([/doi/10.1002/cbic.v11:2/issuetoc](https://doi.org/10.1002/cbic.v11:2/issuetoc)) pages 266–271, January 25, 2010

Additional Information

How to Cite

Mulyani, S., Egel, E., Kittel, C., Turkanovic, S., Wohlleben, W., Süssmuth, R. D. and van Pée, K.-H. (2010), The Thioesterase Bhp is Involved in the Formation of β -Hydroxytyrosine during Balhimycin Biosynthesis in *Amycolatopsis balhimycina*. *ChemBioChem*, 11: 266–271. doi: 10.1002/cbic.200900600

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Publication History

1. Issue published online: 20 JAN 2010
2. Article first published online: 8 DEC 2009
3. Manuscript Received: 29 SEP 2009

Funded by

- The World Bank
- Deutsche Forschungsgemeinschaft. Grant Numbers: DFG Pe 438/16–1 and 16–2, DFG Su 239/3–3
- EU. Grant Number: COMBIG-TOP–LSHB-CT-2003-503491

- Abstract
- [Article \(/doi/10.1002/cbic.200900600/full\)](/doi/10.1002/cbic.200900600/full)
- [References \(/doi/10.1002/cbic.200900600/references\)](/doi/10.1002/cbic.200900600/references)
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Keywords:

antibiotics; balhimycin; beta-hydroxytyrosine; glycopeptides; thioesterases; vancomycin

Abstract

The putative hydrolase gene bhp from the balhimycin biosynthetic gene cluster has been cloned and overexpressed in Escherichia coli. The corresponding enzyme Bhp was purified to homogeneity by nickel-chelating chromatography and characterized. Although Bhp has sequence similarities to hydrolases with “haloperoxidase”/perhydrolase activity, it did not show any enzymatic activity with standard “haloperoxidase”/perhydrolase substrates (e.g., monochlorodimedone and phenol red), nonspecific esterase substrates (such as p-nitrophenyl acetate, p-nitrophenyl phosphate and S-thiophenyl acetate) or the model lactonase substrate dihydrocoumarin. However, Bhp could be shown to catalyse the hydrolysis of S-β-hydroxytyrosyl-N-acetyl cysteamine thioester (β-OH-Tyr-SNAC) with 15 times the efficiency of S-L-tyrosyl-N-acetyl cysteamine thioester (L-Tyr-SNAC). This is in agreement with the suggestion that Bhp is involved in balhimycin biosynthesis, during which it was supposed to catalyse the hydrolysis of β-OH-Tyr-S-PCP (PCP=peptidyl carrier protein) to free β-hydroxytyrosine (β-OH-Tyr) and strongly suggests that Bhp is a thioesterase with high substrate specificity for PCP-bound β-OH-Tyr and not a “haloperoxidase”/perhydrolase or nonspecific esterase.

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