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Identification and analysis of the chivosazol biosynthetic gene cluster from the myxobacterial model strain *Sorangium cellulosum* So ce56

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Abstract

Myxobacteria belonging to the genus *Sorangium* are known to produce a variety of biologically active secondary metabolites. Chivosazol is a macrocyclic antibiotic active against yeast, filamentous fungi and especially against mammalian cells. The compound specifically destroys the actin skeleton of eucaryotic cells and does not show activity against bacteria. Chivosazol contains an oxazole ring and a glycosidically bound 6-deoxyglucose (except for chivosazol F). In this paper we describe the biosynthetic gene cluster that directs chivosazol biosynthesis in the model strain *Sorangium cellulosum* So ce56. This biosynthetic gene cluster spans 92 kbp on the chromosome and contains four polyketide synthase genes and one hybrid polyketide synthase/nonribosomal peptide synthetase gene. An additional gene encoding a protein with similarity to different methyltransferases and presumably involved in post-polyketide modification was identified downstream of the core biosynthetic gene cluster. The chivosazol biosynthetic gene locus belongs to the recently identified and rapidly growing class of *trans*-acyltransferase polyketide synthases, which do not contain acyltransferase domains integrated into the multimodular megasynthetases.

Keywords: Myxobacteria; *Sorangium cellulosum*; Secondary metabolism; Chivosazol; PKS; NRPS; Biosynthetic gene cluster

1. Introduction

Sorangium cellulosum is a Gram-negative soil bacterium with several unusual features. The myxobacterium is able to glide in swarms over solid surfaces, to exhibit "social behaviour", to form fruiting bodies upon starvation and to produce secondary metabolites of high biotechnological and economic importance (Reichenbach and Höfle, 1993). The group of secondary metabolites known to be produced from *S. cellulosum* strains contains the antifungal ambruticins (Höfle et al., 1991), the ratjadons (Gerth et al., 1995)

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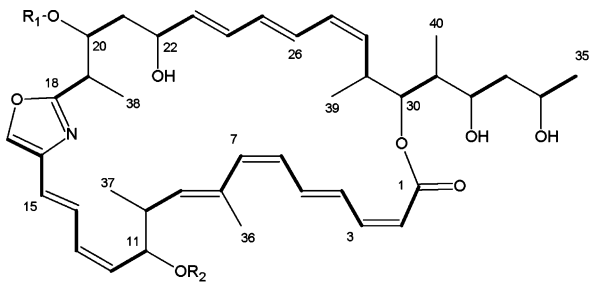

Chivosazol	R_1	R_2	R_3	R_4
A	Me		Me	Me
A_1	Me		Me	Me
B	H		Me	Me
C	Me		H	H
D	H		H	Me
E	H		H	H
F	Me	H	-	-

Fig. 1. The chemical structure of chivosazoles A–F (Irschik et al., 1995a; Jansen et al., 1997). A and A_1 differ in the configuration of the 6, 7 double bond (A:Z; A_1:E)

and the tubulin interacting and therefore highly cytotoxic disorazols (Irschik et al., 1995b) and epothilones (Bollag et al., 1995; Gerth et al., 1996). Jerangolid, sorangicin, soraphen and a variety of other substances exhibit a wide range of biological activities (Gerth et al., 2003). Epothilone acts as a microtubule stabilizing agent and is of clinical interest as an anticancer agent. *Sorangium* strains often do not produce a single metabolite but several different natural compounds. *S. cellulosum* strain So ce12, for example, produces several different biologically active compounds: sorangiolides, which are active against Gram-positive bacteria, sorangicin A and variants, disorazoles and chivosazoles (Irschik et al., 1987, 1995a,c; Jansen et al., 1994).

S. cellulosum strain So ce56 was chosen as model strain for a functional genomic project not only because of the biotechnological importance of the genus *Sorangium* (Gerth et al., 2003) but also because of its advantageous features, such as homogenous growth in liquid culture and a (relatively) short generation time in comparison to most other *Sorangium* strains. This strain is being sequenced within the Bielefeld GenoMik network of the German Ministry of Education and Research (http://www.genetik.uni-bielefeld.de/GenoMik/cluster6.html) and provides insight into the biology of this group of secondary metabolite producers.

So ce56 is known as a producer of chivosazol, etnangien, myxochelin and some other yet uncharacterized natural products (Gaitatzis et al., 2005 and K.G., unpublished). The aim of our work is to better understand the biochemical basis of secondary metabolite formation and its regulation in myxobacteria. Thus, the identification of the corresponding biosynthetic gene clusters is a prerequisite for genetic manipulation to improve the production of biotechnologically interesting secondary metabolites.

The model strain *S. cellulosum* So ce56 as well as many other *Sorangium* strains produce chivosazol (Irschik et al., 1995a; Jansen et al., 1997) (Fig. 1). This compound is active against filamentous fungi and yeast and is especially active against mammalian cells (cytotoxic activity of chivosazoles in concentration of as little as 9 ng ml^{-1}) (Jansen et al., 1997). Chivosazol belongs to the group of natural products that act on the cytoskeleton of higher cells and are thus of potential use for pharmaceutical applications (Fig. 2). The compound does not show activity against bacteria. The structure represents a macrocyclic polyketide incorporating one oxazole ring decorated with a glycosidically