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Review

Investigation of cytochromes P450 in myxobacteria: Excavation of cytochromes P450 from the genome of *Sorangium cellulosum* So ce56

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ABSTRACT

The exploitation of cytochromes P450 for novel biotechnological application and for the investigation of their physiological function is of great scientific interest in this post genomic era, where an extraordinary biodiversity of P450 genes has been derived from all forms of life. The study of P450s in the myxobacterium *Sorangium cellulosum* strain So ce56, the producer of novel secondary metabolites of pharmaceutical interest is the research topic, in which we were engaged since the beginning of its genome sequencing project. We herein disclosed the cytochrome P450 complements (CYPomes) of spore-forming myxobacterial species, *Stigmatella aurantiaca* DW4/3-1, *Haliangium ochraceum* DSM 14365 and *Myxococcus xanthus* DK1622, and their potential pharmaceutical significance has been discussed.

1. Introduction

The cytochromes P450 are a group of heme-mono-oxygenase enzymes and are ubiquitously distributed in all forms of life. They are involved in essential or crucial steps in metabolism such as bioactivation and detoxification of a wide variety of drugs and xenobiotics [1,2], and synthesis of important endogenous compounds and tailoring of secondary metabolite products [3–6]. The catalytic function of the P450 in most cases requires reducing equivalents derived from NAD(P)H (via different electron carriers) and molecular oxygen [7].

Myxobacteria are Gram-negative gliding δ-proteobacteria [8,9] with a high G + C content, which can perform a complex series of cellular differentiation processes that culminate in the formation of fruiting bodies during starvation. They are a phylogenetically coherent group and can be isolated from terrestrial [10] and marine environments [11]. Myxobacteria exhibit a multitude of possibilities for the production of bioactive substances of pharmaceutical importance. The approximately 7500 identified myxobacterial strains have yielded at least 100 distinct core structures and some 500 derivatives [12,13]. The genus *Sorangium* alone can produce nearly 50% of the metabolites isolated from myxobacteria [14]. Different strains of *Sorangium* produce several novel antimicrobial macrolides, the leupyrrins [15], the thuggacins [5,16], the sorangicin [17], phoxalone [18] and other components like a new sesquiterpene, sorangiodenosine [19], a free-radial scavenger, soraphinol C [20], as well as a novel class of antineoplastic agents, the epothilones and their analogs [21]. The selected strain *S. cellulosum* So ce56 produces the natural secondary metabolites chivosazol, etnangien and myxochelin [22–24]. Moreover, the complex life cycle of *S. cellulosum* So ce56 is mediated by an extensive regulatory network, including enhancer binding proteins, two component regulatory systems, extra cytoplasmic function family protein sigma factors, and serine/threonine/tyrosine protein kinases [25].

The genomic sequence information of organisms, which is being determined at an accelerating rate, has provided a novel tool for the investigation of gene and corresponding protein functions. The genomic sequencing projects continue to disclose the ever increasing number of orphan cytochrome P450 genes and have revealed the extraordinary biodiversity of this superfamily. To date, 12,456 P450s are named, with about 6000 more that are known, but not yet annotated [26]. The large pool of P450s identified in the genome has attracted much attention as a resource for new oxidation biocatalyst having endogenous function and biotechnological applications.

The genome sequence of the pharmaceutically and physiologically important myxobacterium *S. cellulosum* So ce56 revealed 21

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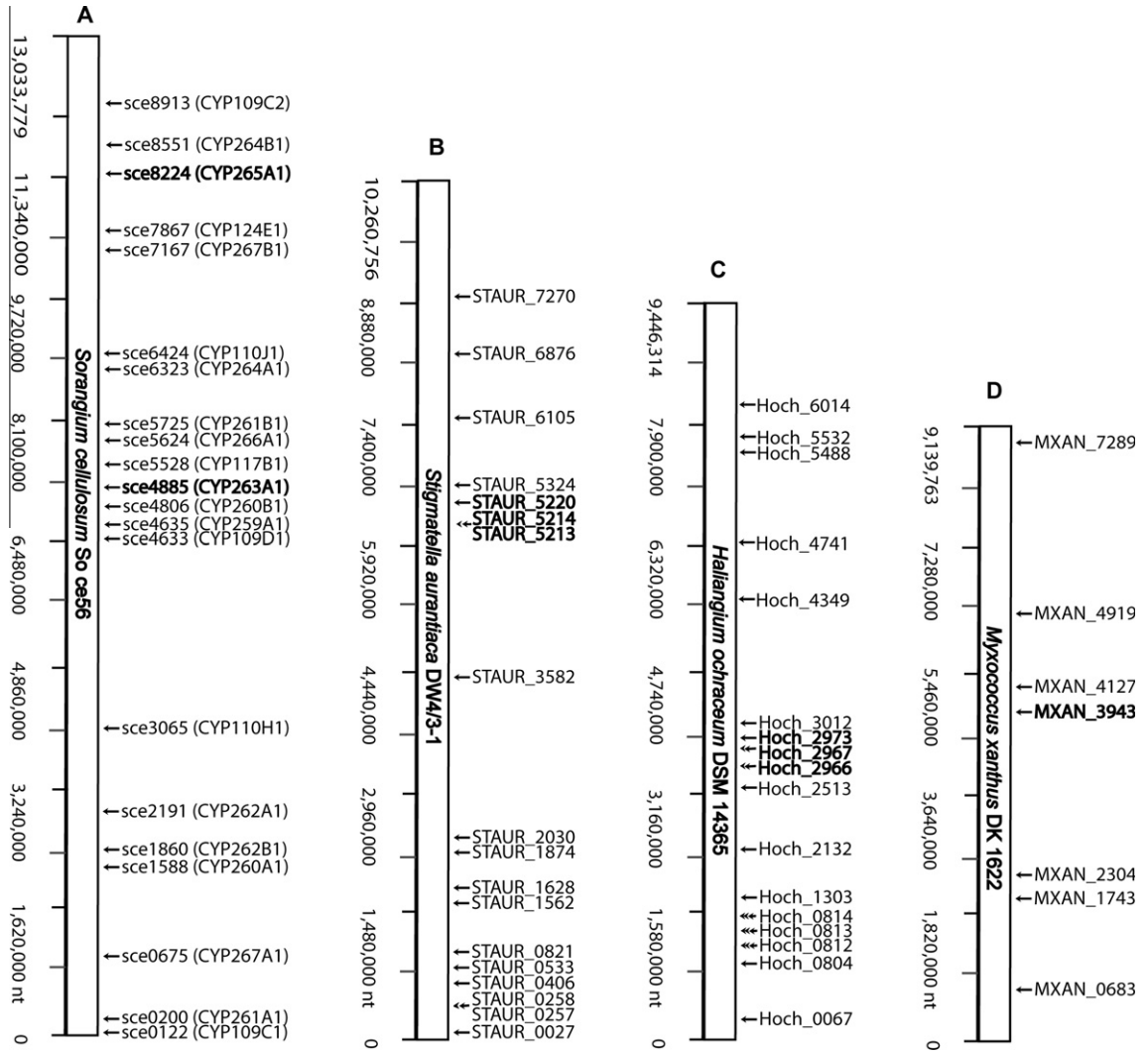

Fig. 1. Physical map showing the distribution of cytochromes P450 in the genome of spore forming myxobacterial species. The cytochrome P450 complements (CYPomes) are assigned in the genomic location of the respective genome of *Sorangium cellulosum* So ce56 (refseq: NC_010162) (A), *Stigmatella aurantiaca* DW4/3-1 (refseq: NC_014623) (B), *Haliangium ochraceum* DSM 14365 (refseq: NC_013440) (C) and *Myxococcus xanthus* DK1622 (refseq: NC_008095) (D) with their corresponding gene locus showing 21, 18, 17 and 7 P450s, respectively. The P450s with distinct PKS/NRPS are shown in bold. The double or triple arrows represent the location of two or more P450s in adjacent position.

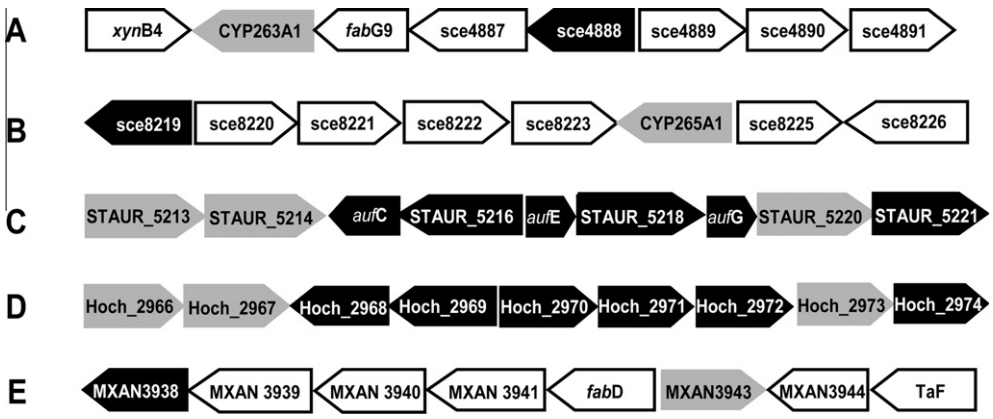

Fig. 2. The genomic organization of P450s potentially involved in secondary metabolite biosynthesis. The genetic organization of the cluster of CYP263A1 (A) and CYP265A1 (B) of *Sorangium cellulosum* So ce56; three P450s (STAUR_5213, STAUR_5214 and STAUR_5220) of *Stigmatella aurantiaca* DW4/3-1 (C); three P450s (Hoch_2966, Hoch_2967 and Hoch_2973) of *Haliangium ochraceum* DSM 14365 (D); and a P450 (MXAN3943) of *Myxococcus xanthus* DK 1622 (E) are illustrated. The genomic foot prints in accordance with the genome databases showing the direction of transcription (arrow head) are depicted. The P450 genes and the PKS/NRKS genes are shown in grey and black, respectively. The putative gene products encoded by the analyzed clusters are listed in the Supplemental Table S2.

P450s [22,27]. Since our laboratory has a particular interest in the enzymology of the P450 systems of myxobacteria, we report here the characterization and comparison of the cytochrome P450 complements (CYPomes) of spore-forming myxobacteria *Sorangium cellulosum* strain So ce56 [22], *Myxococcus xanthus* strain DK1622 [28], *Stigmatella aurantiaca* DW4/3-1 [29], and *Haliangium ochraceum* strain DSM 14365 [30]. Our study particularly focused on the CYPome of *S. cellulosum* So ce56.

2. Comparison of the CYPomes of spore-forming myxobacteria

As myxobacteria have a complex life cycle and a multitude of possibilities for the production of bioactive compounds, the exploitation of the inherent P450s for the elucidation of their physiological function or pharmaceutical applications is of great interest. At the time of this study, the complete genome sequence information was available for nine myxobacterial strains, the soil isolates (*S. cellulosum* strain So ce56, *M. xanthus* strain DK1622, *S. aurantiaca* DW4/3-1, *Anaeromyxobacter dehalogenans* strains 2CP-1, 2CP-C, Fw109-5 and K) and the marine isolates (*Plesiocystis pacifica* SIR-1 and *H. ochraceum* DSM 14365) [31]. Among the nine sequenced myxobacterial genomes, *S. cellulosum* strain So ce56 has the largest bacterial genome (13,033,779 bp, refseq-NC_010162), followed by *Plesiocystis pacifica* SIR-1 (10,587,646 bp, refseq-NZ_ABCS00000000), *S. aurantiaca* strain DW4/3-1 (10,260,756 bp, refseq-NC_014623), *H. ochraceum* strain DSM 14365 (9,446,314 bp, refseq-NC_013440), *M. xanthus*

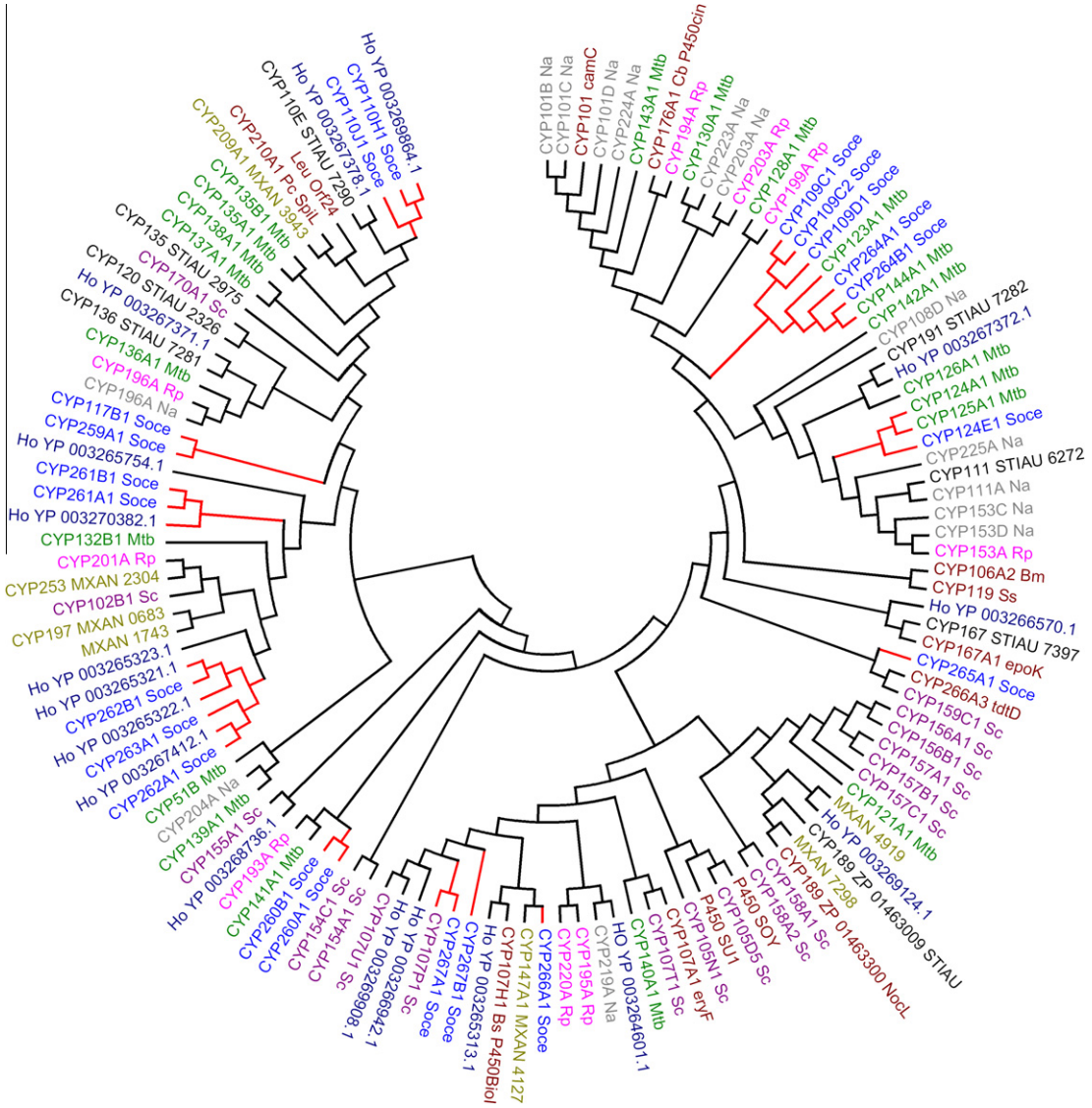

Fig. 3. The radial view of an unrooted phylogenetic tree obtained by MEGA4 (version 4.0) analysis for the determination of relatedness of the P450s from *Sorangium cellulosum* So ce56 with other myxobacterial species and well-characterized bacterial P450s. The clusters of *S. cellulosum* So ce56 are shown in red branches. The amino acid sequences of the P450s from the fruiting bodies forming strains of the myxobacteria, *S. cellulosum* So ce56 (21 P450s in blue with suffix 'Soce') [22], *Stigmatella aurantiaca* DW4/3-1 (eight P450s in black with 'STIAU') [29], *Haliangium ochraceum* strain DSM 14365 (17 P450s in dark blue with prefix 'Ho' and gene identification number) [30], and *Myxococcus xanthus* (seven P450s in light green with 'MXAN') [28] are selected. The tree also comprises the CYPome of *Mycobacterium tuberculosis* (20 P450s in green with prefix 'Mtb') [36], *Streptomyces coelicolor* A(3)2 (18 P450s in violet with prefix 'Sc') [37], *Rhodopedomonas palustris* (nine P450s in pink with prefix 'Rp') and *Novosphingobium aromaticivorans* DSM 12444 (14 P450s in grey with prefix 'Na') (http://www.cyped.uni-stuttgart.de). The well characterized bacterial P450s, CYP176A1_P450cin from *Citrobacter braakii* (AF456128_1) [38], CYP107H1_P450BioI from *Bacillus subtilis* (NP_390897.1) [39], CYP106A2_Bm from *Bacillus megaterium* (CAA79985) [40], CYP119_Ss from *Sulfolobus acidocaldarius* DSM 639 (1F4T_A) [41], CYP107A1_eryF from *Saccharopolyspora erythraea* NRRL 2338 (1Z8O_A) [42], CYP101_camC from *Pseudomonas putida* (1RF9_A) [43], CYP266A3_tdtD from *Pseudomonas diterpeniphila* (AF274704_2) [44], CYP167A1_epoK from *Sorangium cellulosum* (AF217189_9) [45], P-450soy (CAA45146) [46] and P450-SU1 (CYP105A1) [47] from *Streptomyces griseolus* (AAA26823), CYP210A1_SpiL from *Polyangium cellulosum* (CAD43453) [48] and Leu_Orf24 from *Chondromyces crocatus* (CAQ18837) [49] (all shown in brown), are also included. The relatedness of the *S. cellulosum* So ce56 clustered with the other P450s are shown.

(9,139,763 bp, refseq-NC_008095), and *A. dehalogenans* strains Fw109-5 (5,277,990 bp, refseq-NC_009675), K (5,061,623 bp, refseq-NC_011145), 2CP-1 (5,029,329 bp, refseq-NC_011891) and 2CP-C (5,013,479 bp, refseq-NC_007760) (www.ncbi.nlm.nih.gov/genome). All the five myxobacterial species, except *A. dehalogenans* strains, have been demonstrated to initiate fruiting body formation and sporulation in response to starvation [31,32]. In this manuscript, we focused only on the four spore-forming strains, the most investigated myxobacteria, *S. cellulosum* So ce56, *M. xanthus* DK1622, *S. aurantiaca* DW4/3-1 and *H. ochraceum* DSM 14365.

The genome sequencing project of the *S. cellulosum* strain So ce56 revealed 21 P450s, where nine P450s (~66.66%) (CYP259, CYP260, CYP261, CYP262, CYP263, CYP264, CYP265, CYP266 and CYP267) belong to novel families [27]. The bioinformatic analysis of the *S. cellulosum* So ce56 genome also revealed the presence of eight ferredoxin (Fdx) and two reductase genes (FdR) [33], but none of them is organized in a cluster with any of the P450 genes. Thus, no conclusion about the possible autologous electron transfer pathways could be drawn from the genomic context. Beside this, the CYPomes from the above mentioned other myxobacterial strains have not been studied to date. Therefore, we investigated the cytochrome P450 encoding genes in the myxobacteria based on their corresponding genome-sequence data. We revealed 18 P450s genes in *S. aurantiaca* followed by *H. ochraceum* and *M. xanthus* with 17 and 7 P450s, respectively. The comparison of the conserved P450 signatures and the variants in the I-helix, the K-helix and the heme-domain of the four spore-forming myxobacteria is illustrated in Supplemental Table S1. The physical map of these myxobacterial strains showing the distribution of P450s is depicted in Fig. 1.

Analyzing the neighboring genes of the P450s on the genomes, we disclosed P450s clustered with genes coding for polyketide synthase (PKS) and/or non ribosomal polyketide synthase (NRPS) proteins. In the genomic sequence of S. *cellulosum* So ce56, two P450s (CYP263A1 and CYP265A1) are clustered with PKS and/or NRPS proteins encoding genes, whose products were not isolated to date (Fig. 2A and B, Supplemental Table S2). In the genome of *S. aurantiaca* DW4/3-1 (refseq: NC_014623), three of the P450s (STAUR_5213, STAUR_5214 and STAUR_5220) are found embedded in the PKS gene-cluster (Fig. 2C, Supplemental Table S2), which synthesize the myxobacterial polyketide secondary metabolites aurafuron A and B [34]. However, a detailed characterization of those P450s has not yet been performed. In *H. ochraceum* DSM 14365 (refseq: NC_013440) three of the P450s (Hoch_2966, Hoch_2967 and Hoch_2973) are inside a putative PKS gene cluster (Fig. 2D, Supplemental Table S2), whose product has not been isolated so far. Similarly, the genome of *M. xanthus* DK 1622 (Refseq: NC_008095) also contains a P450 (MXAN_3943) in a putative PKS gene cluster (Fig. 2E, Supplemental Table S2), but the product has not been studied to date.

3. The relationship of P450s of *S. cellulosum* So ce56 with other bacterial P450s

Since so far experimental data are unavailable for *S. cellulosum* So ce56 P450s besides those published [27,33,35], the bioinformatics approach seemed to be a sensible route to derive some hints for potential P450 functions. Therefore, the protein sequences of individual P450s of *S. cellulosum* So ce56 were compared with other bacterial P450s and their relatedness was analyzed (Fig. 3 and Supplemental Table S3). Among the 21 P450s of *S. cellulosum* So ce56, only four families (CYP109, CYP110, CYP117 and CYP124) belong to previously assigned P450 families. The CYP109 family of this bacterium has three members (CYP109C1, CYP109C2 and CYP109D1) and they share a sequence identity between 30% and 39% with CYP107H1 and CYP109B1 of *Bacillus subtilis* and 30% and 31% sequence identity with CYP105D5 of *Streptomyces coelicolor* A3(2) (Supplemental Table S3). Since these P450 family members are shown to be fatty acid hydroxylases [50–52], the relatedness of the CYP109 family of *S. cellulosum* So ce56 with them indicate a similar potential function. Indeed, our experimental results demonstrated that CYP109D1 was able to hydroxylate saturated fatty acids [27].

CYP110H1 and CYP110J1 of *S. cellulosum* So ce56 are members of a larger family having almost 40% sequence identity with CYP110E1 of *Nostoc* sp PCC7120, which was shown to have an affinity for alkanes and fatty acids [53]. In addition, CYP110H1 and CYP110J1 of *S. cellulosum* So ce56 show 34–37% identity with members of the CYP135 family of *Mycobacterium tuberculosis,* with CYP210A1 of *Polyangium cellulosum* and with CYP209A1 of *M. xanthus* (Supplemental Table S3). However, experimental data on the function of these P450s are not available. CYP117B1 of *S. cellulosum* So ce56 showed almost 41% and 31% sequence identity with CYP117A1 of *Bradyrhizobium japonicum* and CYP196A3 of *Novosphingobium aromaticivorans,* respectively (Supplemental Table S3), the first of which was predicted to have an affinity towards terpenoid compounds [54]. However, there are no experimental data available confirming the substrate specificity of either CYP117A1 or CYP196A3.

CYP124E1 of *S. cellulosum* So ce56 showed 40–44% sequence identity with CYP125A1, CYP142A1 and CYP124A1 of *M. tuberculosis* (Supplemental Table S3), which were shown to bind and convert branched fatty acids, and to hydroxylate steroids [55,56]. So, it is assumed that CYP124A1 of *S. cellulosum* So ce56 might be a potential candidate for the hydroxylation of branched fatty acids, which are

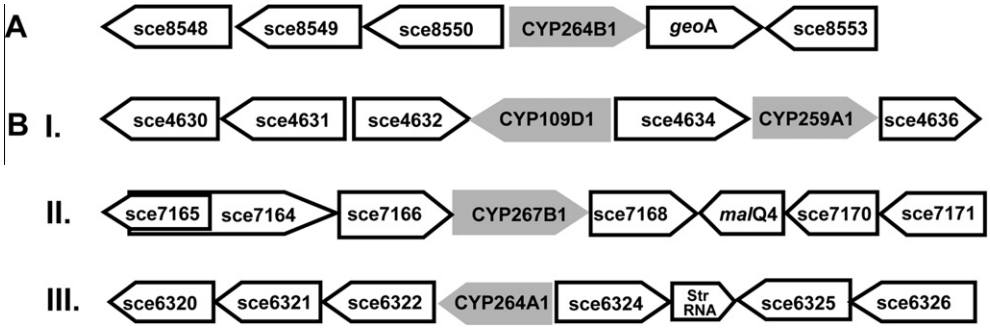

Fig. 4. The genomic organization of P450s clustered with a terpene cyclase and other unique P450 clusters of *S. cellulosum* So ce56. CYP264B1 (A) adjacent downstream of a putative terpene cyclase, and the unique cluster of CYP109D1 and CYP259A1 (B. I), CYP267B1 (B. II) and CYP264A1 (B. III) are shown. The genomic footprints in accordance with the genome databases showing the direction of transcription (arrow head) are illustrated. The P450 genes are shown in grey. The downstream genes (sce7165 and sce7164) of CYP267B1 are shown overlapped. The structural RNA at the upstream of CYP264A1 is shown as Str-RNA. The putative gene products encoded by the analyzed clusters are listed in the Supplemental Table S4.

Table 1
Analysis of the genomic context of cytochrome P450 genes of *Sorangium cellulosum* So ce56. The genomic information of the P450s and the surrounding genes were analyzed from the complete genomic information of *S. cellulosum* So ce56 (13,033,779 nt) available at http://www.ncbi.nlm.nih.gov/genome (the reference genome is NC_010162).

CYP[a]/Gene[b]	Genomic information and other key facts
1. P450s potentially involved in secondary metabolite formation	
CYP263A1 (sce4885)	Member of a novel bacterial P450 family, which lies three genes upstream of a polyketide synthase (PKS) module (sce4888) (Fig. 2A and Supplemental Table S2).
CYP265A1 (sce8224)	Member of a novel bacterial P450 family, which lies five genes downstream of a nonribosomal polyketide synthase (NRPS) module (sce8219) (Fig. 2B and Supplemental Table S2). A similar PKS/NRPS module is present in the genome of several *Streptomyces* and *Mycobacterium* sp. [57–59]. In *Streptomyces* sp., the P450s are involved in many polyketide biosynthetic pathways and catalyze the stereo- and regio-specific oxidative tailoring of antibiotics e.g. novobiocin from *S. spheroides* with CYP163A1 [60], doxorubicin from *S. peucitius* with CYP129A2 [61], amphotericin B from *S. nodosus* with CYP105H4 and CYP161A3 [62], complestatin from *S. lavendulae* with CYP165E1 and CYP165B5 [63], avermectin from *S. avermitilis* with CYP171A1 [64], PKSIII of *S. coelicolor* with CYP158A2 [65].
2. P450 clustered with a terpene cyclase	
CYP264B1 (sce8551)	Member of a novel bacterial P450 family. This is the only P450 clustered with a terpene cyclase gene (*geo*A, sce8552) adjacent downstream and separated by 63 bp (Fig. 4A and Supplemental Table S4), which could possibly be arranged in an operon. The genomic organization of this ORF resembles that of CYP170A1 of *S. coelicolor* A3(2), in which a coupled action of this CYP170A1 with epi-isozizaene synthetase for the biosynthesis of albaflavenone was described [4]. A similar cluster was also reported in *S. avermitilis* where the gene SAV2999 encoding CYP183A1 has been described to catalyze the conversion of pentalenene(3) to pentalen-13-al(7) by stepwise allylic oxidation via pentalen-13-ol(6) [66].
3. P450s with unique clusters	
CYP109D1 (sce4633) and CYP259A1 (sce4635)	CYP109D1 is a member of the pre-assigned CYP109 family, which contains saturated fatty acid and isoprenoid hydroxylases [27,35]. CYP259A1 represents a member of a novel bacterial P450 family. CYP109D1 and CYP259A1 are positioned in an anti-directional orientation in the genome and separated by a single gene, sce4634, encoding an anti-anti sigma regulatory factor (Fig. 4BI and Supplemental Table S4). The adjacent upstream gene (sce4632) of CYP109D1 belongs to the beta-CA superfamily protein and encodes a carbonate hydratase (CA), which catalyzes the reversible hydration of carbon dioxide, and is also found as a conserved gene in *Myxococcus xanthus* DK1622 (73% identity), *Streptococcus* sp N1 (52% identity) and in several *Acinetobacter* sp ADP1 (52% identity). The putative product of the adjacent downstream gene (sce4636) of CYP259A1 (sce4635) shows in parts sequence identity with a squalene cyclase (i.e. 343-416 amino acids out of 511 amino acid sequence), which is also conserved in *Rhizobium* sp (40% identity), *Mesorhizobium* sp (39% identity), and *Mycobacterium tuberculosis* CDC1551 (30% identity) as a putative cyclase.
CYP267B1 (sce7167)	Member of a novel bacterial P450 family, which is clustered with a DNA repair exonuclease family protein encoding gene (sce7166) upstream and a hypothetical protein encoding gene (sce7168) downstream (Fig. 4. B.II and Supplemental Table S4). A unique cluster of a tymovirus analogue gene (sce7164), coding for a tymovirus 45/70 Kd protein, is present in the upstream region, where a hypothetical protein encoding gene (sce7165) is found overlapping sce7164. Downstream of CYP267B1, genes encoding an α-gluconotransferase (sce7169), an α-amylase family protein (sce7170), and an ABC transporter ATP family protein (sce7171) are available.
CYP264A1 (sce6323)	Member of a novel bacterial P450 family, which is next to a sigma-54 dependent transcriptional regulator (sce6324) showing a very unique XylR-N/v4r signal domain. This domain is present as an activator in several proteobacteria, including activators of phenol degradation such as XylR [67]. A putative function of this P450 can not be hypothesized as all other ORFs upstream and downstream encode hypothetical proteins (Fig. 4B.III and Supplemental Table S4).
4. P450s clustered with other genes having distinct physiological roles	
CYP124E1 (sce7867)	The gene is located directly upstream of sce7866, which has a putative function in protocatechuate catabolism and four genes upstream of a putative phenylacetic acid (PA) degradation protein encoding gene (sce7863) (Supplemental Fig. S1(A)). PA genes are found in pathogenic and nonpathogenic mycobacterial species, actinomycetes, and some proteobacteria [68,69]. Moreover, CYP124E1 in Mycobacterium tuberculosis performed ω-hydroxylation of methyl-branched lipids and degradation of the cholesterol [70,71].
CYP262A1 (sce2191)	Member of a novel bacterial P450 family, which lies 3 genes downstream of a putative hydrolase encoding gene (*xys*A, sce2188) (Supplemental Fig. S1(B)).
CYP266A1 (sce5624)	CYP266A1 represents a member of a novel bacterial P450 family and lies adjacent upstream of *ubi*E4 (sce5625), the gene encoding a putative ubiquinone/menaquinone biosynthesis protein. CYP266A1 is the only P450 in *S. cellulosum* So ce56, which is located in proximity to a NADH-flavin oxidoreductase (ferredoxin reductase, sce5629) within the distance of 4 genes (Supplemental Fig. S1(C)).
5. P450s clustered with carbohydrate-metabolism related genes	
CYP109C1 (sce0122)	The P450 encoding gene is clustered with a predicted universal stress protein encoding gene *(uspA,* sce0123) adjacent downstream and a beta-xylosidase (*yag*H, sce0125), 3 genes downstream (Supplemental Fig. S2(A)).
CYP267A1 (sce0675)	Member of a novel bacterial P450 family, which is clustered with a polyphosphate-glucose phospho transferase encoding gene (*ppg*K, sce0674) adjacent upstream and a succinyl glutamic semialdehyde dehydrogenase encoding gene (sce0676) adjacent downstream (Supplemental Fig. S2(B)).
6. P450 clustered with regulatory elements	
CYP109C2 (sce8913)	This gene is clustered with the transcriptional regulator AraC family protein encoding gene (sce8912) adjacent upstream and a protein kinase gene (sce8916) downstream (Supplemental Fig. S3(A)). Another member of the CYP109 family, CYP109B1 from *Bacillus subtilis*, is known to perform hydroxylation of valencene, n-alcohols, terpenoids and fatty acids [51,72].
CYP110H1 (sce3065)	This gene is clustered with a two components hybrid system kinase encoding gene (sce3063) as a regulator, 2 genes upstream, and a conserved hypothetical protein encoding gene (sce3066), having identity with the DUF899 thioredoxin family protein adjacent downstream (Supplemental Fig. S3(B)). CYP110 from *Anabaena* 7120 showed the binding of long-chain saturated and unsaturated fatty acids [53].
CYP110J1 (sce6424)	This P450 gene is clustered with regulator encoding genes, ArsR (sce6422) and MaR (sce6423) family transcriptional regulator gene upstream and a protein kinase encoding gene (sce6427), 3 genes downstream (Supplemental Fig. S3(C)).
CYP260A1 (sce1588)	Member of a novel bacterial P450 family, which is clustered with a protein kinase regulator (sce1587) adjacent upstream. The rest of the neighboring genes of CYP260A1 are encoding for hypothetical protein (Supplemental Fig. S3(D)).
CYP261A1 (sce0200)	Member of a novel bacterial P450 family, which is clustered with a two components sensor histidine kinase regulator gene (sce0195) upstream. All the neighboring genes both upstream and downstream code for hypothetical proteins (Supplemental Fig. S3(E)).
CYP262B1 (sce1860)	Member of a novel bacterial P450 family, which is clustered with a regulatory gene (sce1859) adjacent upstream (Supplemental Fig. S3(F)). The presence of regulatory elements encoding genes for two components regulators and serine/threonine/tyrosine

Table 1 (*continued*)

CYP[a]/Gene[b]	Genomic information and other key facts
	kinases are predominant in the genome *S. cellulosum* So ce56. These kinds of regulators are generally the eukaryotic-like-regulators (ELR), which were studied in this bacterium [25]. The real significance of these regulators with respect to P450s has not been studied so far.

[a] P450 names as annotated at the website: http://drnelson.utmem.edu/CytochromeP450.html and described [27].
[b] Gene accession deposition in NCBI.

essential physiological derivatives required during the life cycle of this bacterium.

Interestingly, two novel P450 families, CYP266 and CYP267, of *S. cellulosum* So ce56 were clustered in the phylogenetic tree (Fig. 3) with the fatty acid hydroxylase CYP107H1 (P450Biol) of *B. subtilis* [39] and members of the CYP107 family from *Streptomyces coelicolor* A3(2), respectively, which suggests that these P450s might have a similar substrate spectrum (fatty acids and several xenobiotic compounds as well as terpenoids). The other members of new P450 families (CYP259, CYP260, CYP261, CYP262, CYP263, CYP264 and CYP265) of *S. cellulosum* So ce56 were only homologous to uncharacterized bacterial P450s (Supplemental Table S3), implying that a putative function of those P450s is not possible to disclose with the published data presently available (see Fig. 4).

4. Genome organization of cytochromes P450 of *S. cellulosum* So ce56

Sequence alignment, homology modeling and spin-shift analysis are the most commonly used approaches for the prediction of potential substrates for orphan cytochromes P450. These approaches generally do not consider the existing information present on neighboring genes of the P450 in a genome. The investigation of proximal and neighboring genes is more beneficial for those P450s, which are in a cluster or in an operon with redox partners or other known genes. Studying the genomic organization of corresponding P450s may help to hypothesize some potential natural redox partners and/or potential natural/analogue substrates. Although the endogenous role of P450s present in several polyketide synthetase (PKS) gene clusters during secondary metabolite formation has already been verified in some other strains of *S. cellulosum* [5,45], the genome of *S. cellulosum* So ce56 has not been investigated so far in this respect. As this is the very first detailed study on the genomic clusters of P450s in *S. cellulosum* So ce56, the detailed genomic organization for each of the P450s with respect to the neighboring genes was analyzed using the genome database of *S. cellulosum* So ce56 (http://www.ncbi.nlm.nih.gov/genome; refseq: NC_010162). The P450s were categorized into six groups, as summarized in Table 1.

5. Conclusions and perspectives

The genome sequencing projects of microbes continue to draw the attention of scientists for the investigation of novel P450 enzymes and to the diversity of their redox partners. The multitude of possibilities for the production of bioactive substances of pharmaceutical importance and the complex life cycle of the myxobacteria attracted us to study their cytochromes P450. Because of the limited availability of information regarding the function of these P450s, we have extensively studied the genomic information on the neighboring genes of each individual P450, which disclosed several characteristic features on the genomic level and also provided some information for putative substrates (at least for some novel P450s).

Genome mining of the P450s also disclosed the presence of several complex regulator genes and other hypothetical protein encoding genes, which have not been studied with regard to their influence on the function of P450s. As we were able to find some P450s embedded in the cluster of PKS/NRPS proteins encoding genes of myxobacteria (two P450s in *S. cellulosum* So ce56, three P450s in *S. aurantiaca*, three P450s in *H. ochraceum* and one in *M. xanthus*), the investigation of their potential to produce important pharmaceutical derivatives could be a promising research topics for the elucidation of the functional role of the P450s. However, sequence information alone is often not sufficient to determine the catalytic functions or actual substrates of the orphan P450s. Thus, other strategies like screening of substance libraries and knock-outs of the genes with subsequent metabolomic analysis might help to disclose the functions of the identified P450s. Interestingly, none of the P450s of *S. cellulosum* So ce56 has been found close to a putative redox partner such as Fdx or FdR. However, we were able to identify two autologous electron transport chains for CYP260A1 [33] and CYP109D1 [27,35]. Using these systems, regio- and stereo-selective derivatives of terpenes and terpenoids could be obtained supporting the idea of a potential biotechnological impact of myxobacterial P450s.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2011.04.035.

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