African Journal of Biotechnology Vol. 11(9), pp. 2154-2161, 31 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2876
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Genome analyses of *Nocardia farcinica* for the identification and comparison of cytochrome P450 complement with related actinomycetes

Ning Qiu[1], Meihu Ma[1]* and Gang Chen[2]

[1]College of Food Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, P. R. China.
[2]College of Life Science, South Central University for Nationalities, Wuhan, Hubei 430070, P. R. China.

Accepted 23 November, 2011

Nocardia **pathogenic strains are occasionally found to be resistant to many antibiotics but also have the ability to produce antibiotics, which involve a variety of cytochrome P450 (CYP) enzymes.** ***Nocardia farcinica*** **IFM 10152 contains 27 CYPs, most of which clustered with ferredoxin, ferredoxin reductase or regulatory genes. These CYPs were designated to subfamilies in this study and their genome contexts were detected to predict the possible metabolic functions. In addition, three new subfamilies as well as a conservon clustered with CYPs were revealed. Phylogenetic analysis of CYPs from** ***N. farcinica*** **IFM 10152, together with those from** ***Streptomyces coelicolor*** **A3(2) and** ***Mycobacterium tuberculosis*** **H37Rv were performed to demonstrate the evolution of** ***N. farcinica*** **CYPs. This comparative study will lead to a better understanding of various CYPs in** ***N. farcinica*** **and contribute to its future application in medicine.**

Key words: *Nocardia*, genome, cytochrome P450, actinomycetes, evolution.

INTRODUCTION

Nocardia are filamentous-growing gram-positive soil saprophytes that belong to the family *Actinomycetales*, which consists of clinically and industrially important genera such as *Mycobacterium*, *Streptomyces*, *Corynebacterium* and *Rhodococcus*. Many species of *Nocardia* cause the disease nocardiosis in human's and animals' lung, central nervous system, brain and cutaneous tissues (Ishikawa et al., 2004). Incidence of Nocardiosis was on the rise recently (Iannotti et al., 2009; Garbino et al., 2010), whereas few studies were performed on the mechanisms of nocardial virulence. *Nocardia* species are resistant to many front-line antibiotics including third-generation cephalosporins (Wallace et al., 1990). Since treatment for nocardiosis relies heavily on chemotherapy, their intrinsic multiple drug resistance is a serious problem (Ishikawa et al., 2004).

Nocardia farcinica, which was originally isolated by Nocard in 1888 from a case of bovine farcy, is the classical pathogen in bovine nocardiosis, while cases of human infection with *N. farcinica* are increasingly being diagnosed (Torres et al., 2000). In 2004, the genome of *N. farcinica* strain IFM10152 was completely sequenced. *N. farcinica* IFM 10152 is a gram positive, filamentous bacterium, and is considered as an opportunistic pathogen. On the other hand, some species of *Nocardia*, even clinical isolates, have the capability to produce antibiotics (Shigemori et al., 1998; Tanaka et al., 1997) and enzymes that are industrially important (Coco et al., 2001) which include aromatic compound-degrading or converting enzymes. This indicates the conceivable existence of a variety of cytochrome P450 (CYP) enzyme which may perform different functions in *N. farcinica* IFM 10152 genome.

CYP genes encode a superfamily of heme-thiolate-containing enzymes involved in the initial oxidation of xenobiotics. CYP enzymes are always involved in a vast array of biological processes and catalyze a variety of chemicals by different oxidative mechanisms including activation or inactivation of the substrates (Raucy and Allen, 2001). These substrate chemicals comprise of

*Corresponding author. E-mail: mameihuhn@yahoo.com.cn.

exogenous compounds (drugs, pollutants, pesticides, etc.) and endogenous chemicals (steroids, eicosanoids, etc). Thus, CYP genes play key roles in many areas. Genome sequencing makes it possible to uncover the remarkable biodiversity of CYP superfamily in and among related strains. Identification and distribution of cytochrome P450 complement (CYPome) in a lot of *Actinomycetales* stains were widely characterized (Lamb et al., 2002, 2003; Parajuli et al., 2004; Roberts et al., 2002; McLean et al., 2006). In this study, we annotated and characterized the CYPome of *N. farcinica* IFM 10152. Furthermore, CYP evolution among IFM 10152 and related genomes were discussed to reveal the diversity of IFM 10152 CYPs.

MATERIALS AND METHODS

The *N. farcinica* IFM 10152 genome database was established and searched using the CYP heme binding domain signature (unusually represented as FXXGXXXCXG, though there are exceptions at all three non-cysteine positions) as reported by Lamb et al. (2003). The open reading frames (ORFs) containing such motifs were further screened for the presence of a highly conserved threonine in the putative I-helix, which is proposed to be involved in CYP oxygen activation in most CYPs and the conserved EXXR motif present in the K-helix (Nelson et al., 1996). In order to assign the *N. farcinica* CYP proteins to subfamilies, the genes containing all three motifs were used as queries for BLAST searches to the GenBank non-redundant protein database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify their closest homologues. CYPs showing more than 40% amino acid sequence identity were placed in the same family, and CYPs having more than 55% identity were categorized in the same subfamily. New family was assigned for the CYP showing less than 40% similarity to other organisms.

The deduced amino acid sequences of the putative *N. farcinica* CYPs and the CYPs from *Streptomyces coelicolor* A3(2) as well as those from *Mycobacterium tuberculosis* H37Rv were aligned using ClustalX (Chenna et al., 2003) and subjected to phylogenetic analysis by both the maximum parsimony and distance with neighbour-joining methods using MEGA4 (Tamura et al., 2007).

RESULTS AND DISCUSSION

Detection of CYP genes in the *N. farcinica* genome data revealed 26 CYPs on the chromosome and one on the plasmid (Table 1). CYPs contributed to approximately 0.2, 0.5 and 0.4% of all the coding sequences in *S. coelicolor* A3(2), *M. tuberculosis* and *N. farcinica*, respectively. The relatively high diversity of CYPs probably reflects the enriched secondary metabolism pathways in these organisms. The genome search of *N. farcinica* revealed three new CYP families (nfa56380, nfa34990 and nfa33510) and 24 sequences fit into existing CYP families (Table 1). There were three main clusters in a single subfamily: CYP125, CYP154 and CYP156. Among these CYPs, six members (CYP51B1, CYP123A6, CYP125A15, CYP255A3, CYP255A4 and CYP256A2) show more than 70% identity to existing cytochrome P450s of other organisms (Table 1). The conserved I-helix, K-helix and heme-binding motif of these CYPs are demonstrated in Table 2.

The phylogenetic tree consisting of CYPs from *N. farcinica* IFM 10152, *S. coelicolor* A3(2) and *M. tuberculosis* H37Rv showed that there was no CYP homolog between *M. tuberculosis* H37Rv and *S. coelicolor* A3(2) genome and also indicated that *N. farcinica* CYPs are obviously divided into two clusters of *Mycobacterium*-like and *Streptomyces*-like ones (Figure 1). Nine CYPs belonging to four subfamilies (CYP154A, CYP154C, CYP156A and CYP157A) from *N. farcinica* are present in *S. coelicolor* A3 (2) branch. Simultaneously, CYPs of three subfamilies (CYP123A, CYP125A and CYP51B1), comprising five CYPs are present in *M. tuberculosis* H37Rv branch. CYP186, CYP193, CYP255 and CYP256, together with three CYPs presumed to be new CYP families (nfa56380, nfa34990, and nfa33510) were not found in either CYPome of these two genomes.

Cyp51 is considered as the most distributed P450 (Waterman and Lepesheva, 2004). CYP51B1, named as Cyp51s of bacteria is common in most mycobacteria and lies in a putative operon which consists of linear array of six ORFs, and comprises, in order, a regulator belonging to the TetR family, a CYP of CYP123A subfamily, (homologous to Rv0766c (CYP123) of *M. tuberculosis*), a probable oxidoreductase, CYP51, a ferredoxin (Fdx), and an ORF of unknown function (Kelly et al., 2003). This operon appears highly conserved within mycobacteria and was found in *M. tuberculosis*, *Mycobacterium bovis* BCG, *Mycobacterium paratuberculosis*, *Mycobacterium vanbaalenii* PYR-1, *Mycobacterium smegmatis* str. MC2 155, *Mycobacterium ulcerans* Agy99, *Mycobacterium* sp. MCS, *Mycobacterium avium* and even in *Rhodococcus* sp. RHA1 via genome search. CYP51B1 is also found in *N. farcinica* and shows 81 and 77% identity with the CYP51B1 in *Rhodococcus* sp. RHA1 and *M. tuberculosis* H37Rv, respectively (Table 1). Instead of locating in the operon mentioned above, *N. farcinica* CYP51B1 is directly followed by a ferredoxin reductase (Fdr) and surprisingly adjacent to *mce*7A in a divergent transcriptional direction. The *mce* genes were commonly thought to be related to the pathogenicity in *M. tuberculosis* (Brahmachari et al., 2003; Haile et al., 2002), however, *N. farcinica mce*7A coupled with CYP51B1 is an orphan gene comparative to the *mce* operon (*mce* 1-4 A, B, C, D, E, F) in *M. tuberculosis* H37Rv.

CYP125A15 from *N. farcinica* shares 72 and 67% identity with CYP125A14P and CYP125A1 of *Rhodococcus* sp. RHA1 and *M. tuberculosis* H37Rv, respectively. CYP125A15 is coupled with *fad*A3 which codes for an acetyl-CoA acetyltransferase related to the lipid metabolism. This couple of genes behaves as conserved DT-pairs classified as XX (Korbel et al., 2004), which is also found around CYP125A14P, CYP125A1, CYP125A7 and CYP125A2 of *Rhodococcus* sp. RHA1, *M. tuberculosis* H37Rv, *M. ulcerans* Agy99 and *Streptomyces avermitilis* MA-4680, respectively. Whether

Table 1. The CYPs of *N. farcinica* with their homologues.

CYP name[a]	Size[b]	Match in the database[c]				
		Species	CYP name	Protein identifier	Identity (%)	overlap[d]
CYP51B1	452	*Nocardia farcinica* IFM 10152	CYP51B1	YP_118800	100	452
		Rhodococcus sp. RHA1	CYP51B1	YP_704615	81	448
		Mycobacterium tuberculosis H37Rv	CYP51B1	P0A512	77	448
CYP125A15	422	*Mycobacterium tuberculosis* H37Rv	CYP125A1	P63709	67	421
		Mycobacterium tuberculosis H37Rv	CYP124A1	P0A516	40	391
CYP125A16	408	*Mycobacterium tuberculosis* H37Rv	CYP125A1	P63709	62	407
		Mycobacterium tuberculosis H37Rv	CYP124A1	P0A516	43	404
CYP125A17	408	*Mycobacterium tuberculosis* H37Rv	CYP125A1	P63709	63	407
		Mycobacterium tuberculosis H37Rv	CYP124A1	P0A516	42	395
CYP123A6	401	*Rhodococcus* sp. RHA1	CYP123A5	YP_704613	72	400
		Mycobacterium tuberculosis H37Rv	CYP123A1	P63707	60	400
CYP138A7	451	*Mycobacterium tuberculosis* H37Rv	CYP138A1	P63717	53	428
		Mycobacterium tuberculosis H37Rv	CYP137A1	O69653	39	418
CYP136B4	502	*Nocardia farcinica* IFM 10152	CYP136B4	YP_117347	100	502
		Mycobacterium flavescens PYR-GCK	CYP136B2	ZP_01192884	56	462
		Mycobacterium tuberculosis H37Rv	CYP136A1	P95099	41	448
CYP140B2	436	*Mycobacterium vanbaalenii* PYR-1	CYP140B1	YP_956297	52	426
		Mycobacterium tuberculosis H37Rv	CYP140A1	P63721	45	427
nfa56380	426	*Gloeobacter violaceus* PCC 7421	CYP110E5	NP_926010	37	423
		Gloeobacter violaceus PCC 7421	CYP110E4	NP_926009	36	404
CYP154A5	409	*Streptomyces coelicolor* A3(2)	CYP154A1	Q9KZR7	60	411
		Streptomyces coelicolor A3(2)	CYP154C1	Q9L142	47	408
CYP256A2	409	*Rhodococcus* sp. RHA1	CYP256A1	YP_708186	75	408
		Micromonospora griseorubida	CYP107E1	BAA03672	37	407
CYP154C3	410	*Streptomyces coelicolor* A3(2)	CYP154C1	Q9L142	52	401
		Streptomyces coelicolor A3(2)	CYP154A1	Q9KZR7	41	404
CYP154A6	414	*Streptomyces coelicolor* A3(2)	CYP154A1	Q9KZR7	54	403
		Streptomyces coelicolor A3(2)	CYP154C1	Q9L142	46	401
nfa34990	397	*Streptomyces coelicolor* A3(2)	CYP159A1	Q9RJQ7	38	361
		Mycobacterium vanbaalenii PYR-1	CYP187A6	YP_952830	36	349
CYP193C1	399	*Nocardia farcinica* IFM 10152		YP_117422	49	369
		Bradyrhizobium japonicum USDA 110	CYP193A1	NP_773652	35	397
CYP255A3	403	*Rhodococcus* sp. RHA1	CYP255A1	YP_702345	78	403
nfa33510	405	*Mycobacterium smegmatis* str. MC2 155	CYP279A5	YP_888490	33	352
		Mycobacterium vanbaalenii PYR-1	CYP279A3	YP_951227	29	378
CYP255A4	404	*Rhodococcus* sp. RHA1	CYP255A2	YP_703834	84	404
		Rhodococcus sp. RHA1	CYP255A1	YP_702345	56	404
CYP193B1	396	*Bradyrhizobium japonicum* USDA 110	CYP193A1	NP_773652	43	383
		Rhodococcus sp. RHA1	CYP255A2	YP_703834	32	397
CYP157A5	424	*Streptomyces coelicolor* A3(2)	CYP157A1	Q9L141	59	406
		Streptomyces coelicolor A3(2)	CYP157B1	Q9RJQ6	50	420
CYP156A2	421	*Streptomyces coelicolor* A3(2)	CYP156A1	Q9KZR8	59	402
		Streptomyces coelicolor A3(2)	CYP156B1	Q93RT1	41	414
CYP156A3	425	*Streptomyces coelicolor* A3(2)	CYP156A1	Q9KZR8	56	391
		Streptomyces coelicolor A3(2)	CYP156B1	Q93RT1	41	416
CYP156A4	434	*Streptomyces coelicolor* A3(2)	CYP156A1	Q9KZR8	59	388

Table 1. Contd.

CYP156A5	445	*Streptomyces coelicolor* A3(2)	CYP156B1	Q93RT1	42	401
		Streptomyces coelicolor A3(2)	CYP156A1	Q9KZR8	50	393
		Streptomyces coelicolor A3(2)	CYP156B1	Q93RT1	42	424
CYP156A6	417	*Streptomyces coelicolor* A3(2)	CYP156A1	Q9KZR8	55	393
		Streptomyces coelicolor A3(2)	CYP156B1	Q93RT1	40	417
CYP186A3	472	*Nocardia farcinica* IFM 10152	CYP186A3	YP_120573	100	472
		Mycobacterium smegmatis str. MC2 155	CYP186A1	YP_886147	65	440
CYP102H1	473	*Streptomyces coelicolor* A3(2)	CYP102B1	Q9RD76	46	466
		Bacillus cereus ATCC 14579	CYP102A5	NP_832952	40	458

[a]CYP names as annotated in the website: http://drnelson.utmem.edu/CytochromeP450.html; [b]number of amino acids; [c]Database search at NCBI; [d]number of amino acid overlap which exceeds the protein size, this is due to the introduction of gaps during BLAST comparison.

Table 2. Classification of putative cytochrome P450s in *N. farcinica*.

CYP name[a]	I-helix	K-helix	Heme-binding motif	Accession number
CYP51B1	^{256}AGHHTT^{261}	^{313}ETLR^{316}	^{387}FGAGRHRCVG^{396}	YP_118800
CYP125A15	^{255}AGNETT^{260}	^{292}EIIR^{295}	^{357}GGTGAHFCIG^{366}	YP_116727
CYP125A16	^{250}AGNETT^{255}	^{287}EIVR^{290}	^{352}GGTGTHYCVG^{361}	YP_118798
CYP125A17	^{250}AGNETT^{255}	^{287}EIVR^{290}	^{352}GGTGTHYCVG^{361}	YP_118643
CYP123A6	^{238}AGNETT^{243}	^{277}ETLR^{280}	^{342}FGAGVHFCLG^{351}	YP_118792
CYP138A7	^{256}AGHETT^{261}	^{300}EVQR^{303}	^{371}FGGGARRCIG^{380}	YP_118386
CYP136B4	^{312}AAHDTS^{317}	^{367}EALR^{370}	^{441}FGGGAHKCIG^{450}	YP_117347
CYP140B2	^{264}AGFETT^{270}	^{303}EILR^{306}	^{368}FSSGIHVCLG^{377}	YP_120856
nfa56380	^{254}AGHETT^{259}	^{293}ETLR^{296}	^{363}YGGGHRRCPG^{372}	YP_121854
CYP154A5	^{243}AGHETT^{248}	^{282}ESLR^{285}	^{348}FGYGAHHCLG^{357}	YP_118504
CYP256A2	^{241}GGFDNT^{246}	^{281}EILR^{284}	^{354}LSYGLHHCLG^{363}	YP_119599
CYP154C3	^{244}AGHETT^{249}	^{283}ETLR^{286}	^{350}FGHGPHICPG^{359}	YP_121527
CYP154A6	^{244}AGHETT^{249}	^{283}EALR^{286}	^{349}FGHGAHHCIG^{358}	YP_120733
nfa34990	^{230}AGADTT^{235}	^{270}EGLR^{273}	^{334}FGQGPHSCPG^{343}	YP_119711
CYP193C1	^{237}GGMNEP^{242}	^{276}ETVR^{279}	^{340}FGSGVHLCAG^{349}	YP_117425
CYP255A3	^{241}GAMQEP^{246}	^{280} ESLR^{283}	^{345}FGAGDHACAG^{354}	YP_118440
nfa33510	^{243}AGHDTT^{248}	^{281}ESLR^{284}	^{346}FGYGGHYCLG^{355}	YP_119562
CYP255A4	^{242}GALQEP^{247}	^{281}EGAR^{284}	^{346}FGAGNHACAG^{355}	YP_119270
CYP193B1	^{236}GGLNEP^{241}	^{274}ESVR^{277}	^{338}FGGGNHFCAG^{347}	YP_117422
CYP157A5	^{248}AGHLPT^{253}	^{287}EVLW^{290}	^{354}FSHGEYRCPF^{363}	YP_121526
CYP156A2	^{251}AGIEPE^{256}	^{290}EVLF^{293}	^{353}WSAGPHSCPA^{362}	YP_118503
CYP156A3	^{254}AGFEAQ^{259}	^{293}EVLF^{296}	^{358}WSTGPHACPA^{367}	YP_118344
CYP156A4	^{261}AGIEPQ^{266}	^{300}EVLF^{303}	^{360}WGAGPHACPA^{369}	YP_120732
CYP156A5	^{264}AGFETT^{286}	^{315}YVLF^{318}	^{375}FGAGPHRCPA^{384}	YP_116702
CYP156A6	^{276}AAGELL^{281}	^{291}EVLF^{294}	^{351}WGVGQHACPA^{360}	YP_117405
CYP186A3	^{284}FAFDAA^{289}	^{332}ESVR^{335}	^{407}FSAGPAECPG^{416}	YP_120573
CYP102H1	^{279}AGHETS^{284}	^{338}ETLR^{341}	^{415}FGTGLRACIG^{424}	YP_122047

[a]CYP names as annotated in the website: http://drnelson.utmem.edu/CytochromeP450.html.

these two proteins interact with each other as post-transcriptional regulator mentioned by Korbel et al. (2004) need more experimental evidence. It is noteworthy that CYP125A1 from *M. tuberculosis* H37Rv is induced during Mtb engulfment in macrophages and are required for Mtb survival in infected mice (Stoker et al., 2004; Schnappinger et al., 2003; Rubin and Sassetti, 2003), and thus supposed to have a pivotal role in bacterial viability and pathogenesis (McLean et al., 2006). CYP125A16 and CYP125A17, which exhibit 72% identity to each other and have 64 and 65% homology to CYP125A14P of *R.* sp. RHA1, do not share such a

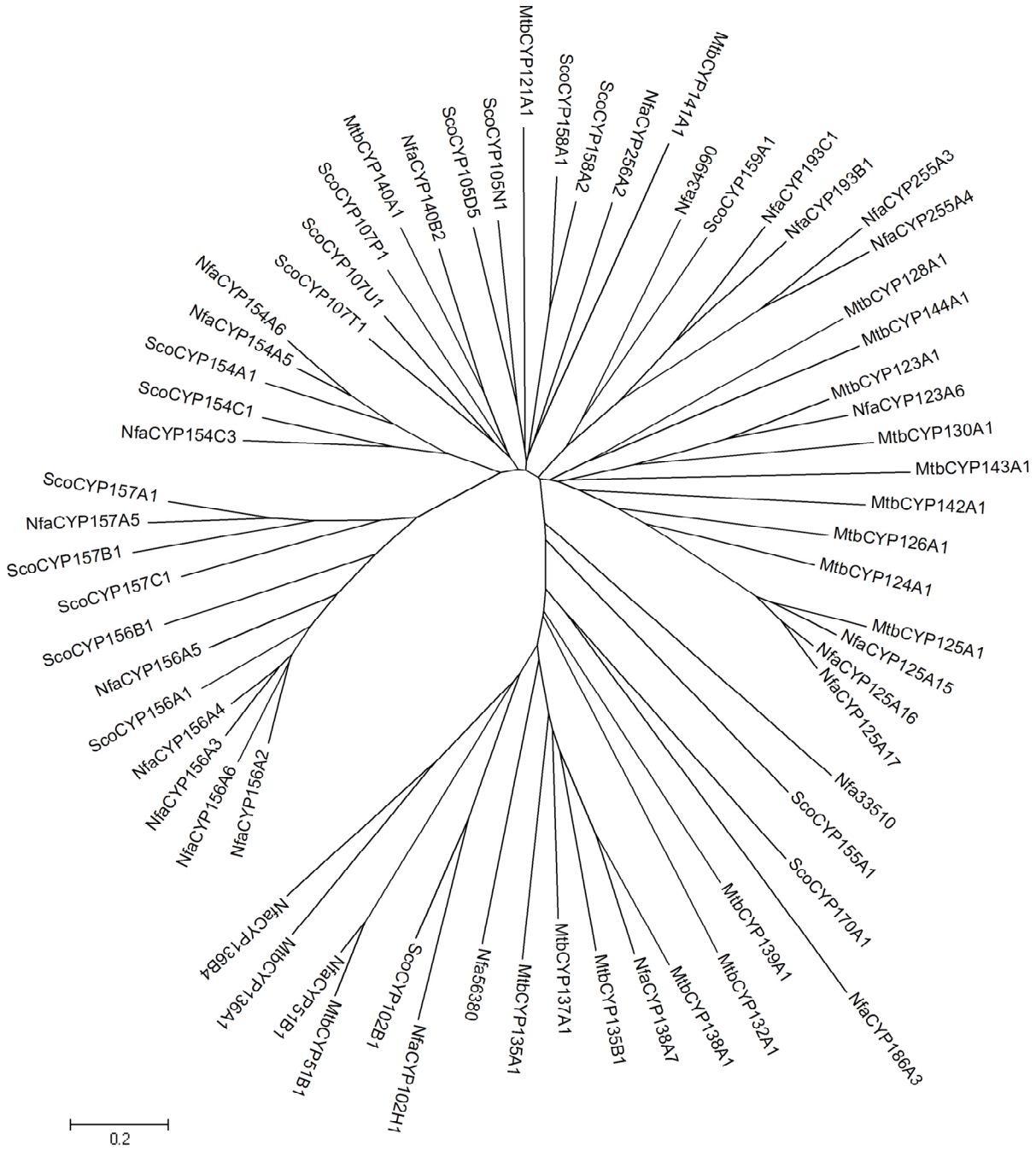

Figure 1. Phylogenetic tree of the cytochrome P450 superfamilies from *N. farcinica* IFM10152 as compared to those of *S. coelicolor* A3(2) and *M. tuberculosis* H37Rv.

conserved genome context with posttranscriptional regulator. CYP255A3 and CYP255A4 from *N. farcinica* show 78 and 84% identity to CYP255A1 and CYP255A2 of *Rhodococcus* sp. RHA1, respectively. CYP255A3 and CYP255A4 lie in the conserved divergently transcribed gene pairs (DT-pairs) (Korbel et al., 2004) with the transcriptional regulator of AraC family, and both adjacent to a putative phenol hydroxylase downstream. This module is also found encompassing CYP255A2 of *Rhodococcus* sp. RHA1. Upstream of the *ben*ABCDK gene cluster of *Rhodococcus* sp. RHA1 genome, such a region consists of AraC family transcriptional regulator and CYP255A1 DT-pairs is also found, however, a cytochrome P450 reductase instead of phenol hydroxylase follows CYP255A1. As the regulator in DT-pairs is implicated in controlling the divergently transcribed

gene (Korbel et al., 2004), CYP255A3 and CYP255A4 is supposed to be regulated by the AraC transcriptional regulator and involved in the phenol degradation. The striking example is that the conserved AraC transcriptional regulator and P450 DT-pairs which is found in *M. tuberculosis* H37Rv clustered with CYP132A1 is supposed to be involved in P450 regulation (Reyrat et al., 2003). The AraC transcriptional regulators, primarily transcriptional activators, are supposed to regulate diverse bacterial functions including sugar catabolism, responses to stress, and virulence (Martin and Rosner, 2001). Another P450 in *N. farcinica* appears to have the bidirectionally transcribed P450 and transcriptional regulator gene pairs is Nfa34990, which was suggested to belong to a new family. The transcriptional regulator was an IclR family member, which has the Helix-turn-helix motif at the N-terminus which is similar to that of AraC type. CYP256A2 from *N. farcinica* shows 75% identity to CYP256A1 from *Rhodococcus* sp. RHA1 (Table 1). This two P450s share very similar genome context. With a probable short chain dehydrogenase upstream, these two CYPs were followed by an Fdx and Fdr in tandem. CYP256A2 also share 36% identity with CYP107N1 (Orf3) involved in the biosynthesis of mitomycin C from *Streptomyces lavendulae*. *N. farcinica* CYP123A6 is clustered with an Fdx by the insertion of a short chain dehydrogenase between them and CYP125A16 downstream. CYP136B4, followed by an Fdx, shows 56, 48 and 41% identity to CYP136B2, CYP136C1 and CYP136A1 of *Mycobacterium gilvum* PYR-GCK, *Rhodococcus* sp. RHA1 and *M. tuberculosis* H37Rv, respectively. Such CYPs that clustered with ferredoxin and ferredoxin reductase were suggested to be probably enhanced by expression or activities. CYP136B4 is also coupled with a TetR family transcriptional regulator to form DT-pairs conserved in *M. gilvum*, *Mycobacterium smegmatis*, *M. tuberculosis* and *Rhodococcus* sp. RHA1. Another conserved DT-pairs is found in CYP138A1 coupled with a TetR family transcriptional regulator from *M. tuberculosis* H37Rv. The cognate in *N. farcinica*, CYP138A7, which shows 53% identity to CYP138A1, is also coupled with TetR family transcriptional regulator but in the same transcriptional direction. Other CYPs clustered with a transcriptional regulator in the same transcriptional direction from *N. farcinica* is CYP125A17, which is adjacent to a transcriptional regulator downstream and CYP140B2 clustered with a transcriptional regulator upstream. CYP140A1 from *M. tuberculosis* H37Rv shares 45% identity with *N. farcinica* CYP140B2, and is one of the important P450s that bind the azole antifungal drugs (McLean et al., 2002). CYP193B1 and CYP193C1, which show 43 and 35% identity to CYP193A1 from *Bradyrhizobium japonicum* USDA 110, belong to new subfamilies excluded in Actinomycetes. These two CYPs cluster together with the insertion of an Fdx and Fdr in tandem between them. As the cytochromes P450 found in *Bradyrhizobium japonicum* and other rhizobia were considered to be unique and hypothesized to be intracellular O_2 carriers involved in an efficient pathway of electron transport (Tully et al., 1998), the function of CYP193B1 and CYP193C1 in *N. farcinica* is open to much conjecture. Recent studies have revealed that *N. farcinica* CYP193B1 and CYP 256A2 were responsible for the hydroxylation of the A-ring of daidzein (Choi et al., 2009). CYP186A3 shows 65 and 56% identity to CYP186A1 and CYP186A2 from *M. smegmatis* str. MC2 155 and *Mycobacterium* sp. JLS. *N. farcinica* CYP186A3 resides at the end of a putative operon involved in phenol hydroxylation.

Three *N. farcinica* CYPs (nfa56380, nfa34990 and nfa33510) were not designated to the existing CYP families according to the criteria mentioned above, and thus suggested to form new families. Nfa34990 shows 38% identity to CYP159A1 from *S. coelicolor* A3(2); nfa33510 shows 33% identity to CYP279A5 from *M. smegmatis* str. MC2 155; nfa56380 shows 37% identity to CYP110E5 from *Gloeobacter violaceus* PCC 7421, and shows 48% identity to the deduced P450 (YP_640103.1) from *Mycobacterium* sp. MCS.

Sequence analysis of *N. farcinica* revealed that a large number of CYPs belongs to CYP156A subfamilies (5 copies). In addition, three CYPs are designated into CYP154 family as well as one in CYP157 family. CYPs belonging to these three families are frequently found downstream the unique conserved operons (conservon, *cvn*) among *Streptomyces* (Bentley et al., 2002; Lamb et al., 2003; Komatsu et al., 2006). Among the seven putative conservons in *N. farcinica*, only one conservon is clustered with CYP downstream named CYP157A5 and CYP154C3 (Figure 2). Such a conservon is quite similar to that of *cvn*10 clustered with CYP157A1 and CYP154C1 in *S. coelicolor* A3(2) and that clustered with CYP157A2 and CYP154C2 in *S. avermitilis*.

In conclusion, 27 cytochrome P450s were identified in *N. farcinica* IFM 10152 genome. Some CYPs clustered with ferredoxins or ferredoxin reductase while others clustered with regulatory genes. This indicates that CYPs may play various roles in *N. farcinica* such as involvement in the catabolism of secondary metabolites, and meanwhile, having the compound-degrading ability. Phylogenetic analysis among CYPs from the related stains (*N. farcinica* IFM 10152, *S. coelicolor* A3(2) and *M. tuberculosis* H37Rv) revealed the evolutionary history of these CYPs. These data will probably contribute towards further comparative analyses of CYPs in Actinomycetes, which will shed further light on the detection of the underlying CYP functions.

ACKNOWLEDGEMENTS

This work was financially supported by the Chinese National Natural Science Funds (Grant No. 31101366)

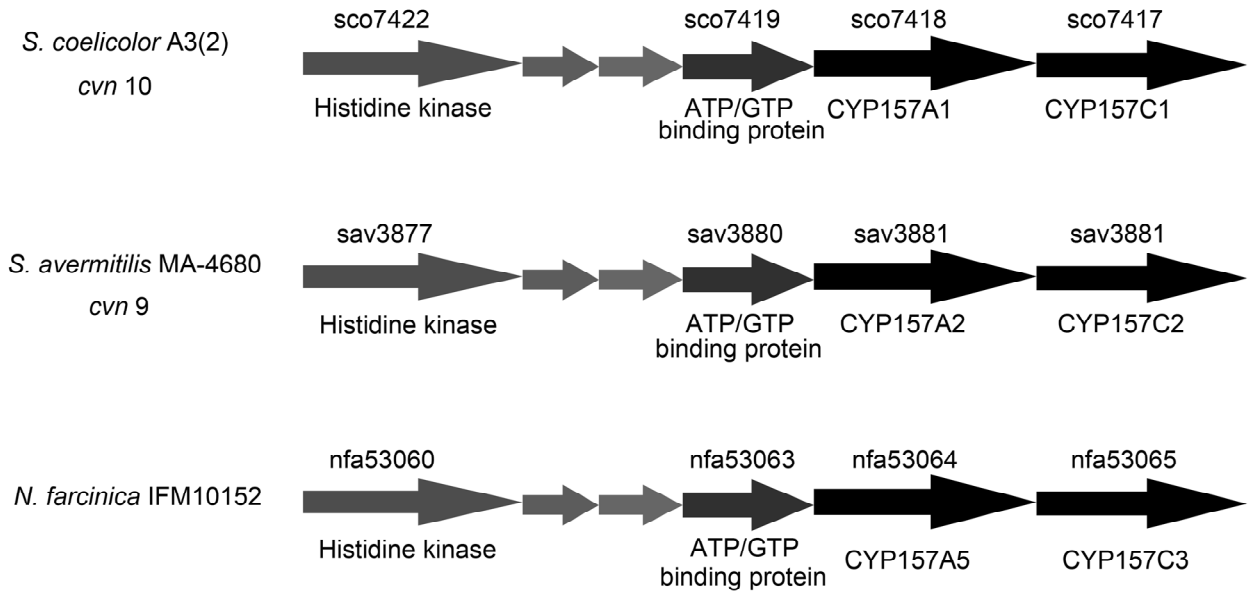

Figure 2. Analysis of conservons containing cytochromes P450 in the genome sequence of *N. farcinica* IFM10152.

and the earmarked fund for Modern Agro-industry Technology Research System (Project No. nycytx-41-g22).

REFERENCES

Bentley SD, Chater KF, Cerdeno-Tarraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D, Bateman A, Brown S, Chandra G, Chen CW, Collins M, Cronin A, Fraser A, Goble A, Hidalgo J, Hornsby T, Howarth S, Huang CH, Kieser T, Larke L, Murphy L, Oliver K, O'Neil S, Rabbinowitsch E, Rajandream MA, Rutherford K, Rutter S, Seeger K, Saunders D, Sharp S, Squares R, Squares S, Taylor K, Warren T, Wietzorrek A, Woodward J, Barrell BG, Parkhill J, Hopwood DA (2002). Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature, 417: 141-147.

Brahmachari V, Kumar A, Bose M (2003). Analysis of expression profile of mammalian cell entry(mce) operons of *Mycobacterium tuberculosis*. Infect. Immun. 71: 6083-6087.

Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG & Thompson JD (2003). Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res. 31: 3497-3500.

Choi KY, Kim TJ, Koh SK, Roh CH, Pandey BP, Lee N & Kim BG (2009). A-ring ortho-specific monohydroxylation of daidzein by cytochrome P450s of *Nocardia farcinica* IFM10152. Biotechnol. J. 4: 1586-1595.

Coco WM, Levinson WE, Crist MJ, Hektor HJ, Darzins A, Pienkos PT, Squires CH, Monticello DJ (2001). DNA shuffling method for generating highly recombined genes and evolved enzymes. Nat. Biotechnol. 19: 354-359.

Garbino J, Ambrosioni J, Lew D (2010). Nocardiosis: Updated Clinical Review and Experience at a Tertiary Center. Infection, 38: 89-97.

Haile Y, Caugant DA, Bjune G, Wiker HG (2002). *Mycobacterium tuberculosis* mammalian cell entry operon (mce) homologs in Mycobacterium other than tuberculosis (MOTT). FEMS Immunol. Med. Microbiol. 33: 125-132.

Iannotti CA, Hall GS, Procop GW, Tuohy MJ, Staugaitis SM, Weil RJ (2009). Solitary *Nocardia farcinica* brain abscess in an immunocompetent adult mimicking metastatic brain tumor: rapid diagnosis by pyrosequencing and successful treatment. Surg. Neurol. 72: 74-79.

Ishikawa J, Yamashita A, Mikami Y, Hoshino Y, Kurita H, Hotta K, Shiba T, Hattori M (2004). The complete genomic sequence of *Nocardia farcinica* IFM 10152. Proc. Natl. Acad. Sci. USA. 101: 14925-14930.

Kelly SL, Jackson CJ, Lamb DC, Marczylo TH, Parker JE, Manning NL, Kelly DE (2003). Conservation and cloning of CYP51: a sterol 14 alpha-demethylase from *Mycobacterium smegmatis*. Biochem. Biophys. Res. Commun. 301: 558-563.

Komatsu M, Takano H, Hiratsuka T, Ishigaki Y, Shimada K, Beppu T, Ueda K (2006). Proteins encoded by the conservon of *Streptomyces coelicolor* A3(2) comprise a membrane-associated heterocomplex that resembles eukaryotic G protein-coupled regulatory system. Mol. Microbiol. 62: 1534-1546.

Korbel JO, Jensen LJ, von Mering C, Bork P (2004). Analysis of genomic context: prediction of functional associations from conserved bidirectionally transcribed gene pairs. Nat. Biotechnol. 22: 911-917.

Lamb DC, Ikeda H, Nelson DR, Ishikawa J, Skaug T, Jackson C, Omura S, Waterman MR, Kelly SL (2003). Cytochrome p450 complement (CYPome) of the avermectin-producer *Streptomyces avermitilis* and comparison to that of *Streptomyces coelicolor* A3(2). Biochem. Biophys. Res. Commun. 307: 610-619.

Lamb DC, Skaug T, Song HL, Jackson CJ, Podust LM, Waterman MR, Kell DB, Kelly DE, Kelly SL (2002). The cytochrome P450 complement (CYPome) of *Streptomyces coelicolor* A3(2). J. Biol. Chem. 277: 24000-24005.

Martin RG, Rosner JL (2001). The AraC transcriptional activators. Curr. Opin. Microbiol. 4: 132-137.

McLean KJ, Clift D, Lewis DG, Sabri M, Balding PR, Sutcliffe MJ, Leys D, Munro AW (2006). The preponderance of P450s in the *Mycobacterium tuberculosis* genome. Trends Microbiol. 14: 220-228.

McLean KJ, Marshall KR, Richmond A, Hunter IS, Fowler K, Kieser T, Gurcha SS, Besra GS, Munro AW (2002). Azole antifungals are potent inhibitors of cytochrome P450 mono-oxygenases and bacterial growth in mycobacteria and streptomycetes. Microbiology, 148: 2937-2949.

Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus IC, Nebert DW (1996). P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics, 6: 1-42.

Parajuli N, Basnet DB, Chan Lee H, Sohng JK, Liou K (2004). Genome analyses of *Streptomyces peucetius* ATCC 27952 for the

identification and comparison of cytochrome P450 complement with other *Streptomyces*. Arch. Biochem. Biophys. 425: 233-241.
Raucy JL, Allen SW (2001). Recent advances in P450 research. Pharmacogenomics J. 1: 178-186.
Reyrat JM, Recchi C, Sclavi B, Rauzier J, Gicquel B (2003). *Mycobacterium tuberculosis* Rv1395 is a class III transcriptional regulator of the AraC family involved in cytochrome P450 regulation. J. Biol. Chem. 278: 33763-33773.
Roberts GA, Grogan G, Greter A, Flitsch SL, Turner NJ (2002). Identification of a new class of cytochrome P450 from a *Rhodococcus* sp. J. Bacteriol. 184: 3898-3908.
Rubin EJ, Sassetti CM (2003). Genetic requirements for mycobacterial survival during infection. Proc. Natl. Acad. Sci. USA. 100: 12989-12994.
Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, Dolganov G, Efron B, Butcher PD, Nathan C, Schoolnik GK (2003). Transcriptional adaptation of *Mycobacterium tuberculosis* within macrophages: Insights into the phagosomal environment. J. Exp. Med. 198: 693-704.
Shigemori H, Komaki H, Yazawa K, Mikami Y, Nemoto A, Tanaka Y, Sasaki T, In Y, Ishida T & Kobayashi J (1998). Brasilicardin A. A Novel Tricyclic Metabolite with Potent Immunosuppressive Activity from Actinomycete Nocardiabrasiliensis. J. Org. Chem. 63: 6900-6904.
Stoker NG, Kendall SL, Rison SCG, Movahedzadeh F, Frita R (2004). What do microarrays really tell us about M-tuberculosis? Trends Microbiol. 12: 537-544.
Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596-1599.
Tanaka Y, Komaki H, Yazawa K, Mikami Y, Nemoto A, Tojyo T, Kadowaki K, Shigemori H, Kobayashi J (1997). Brasilinolide A, a new macrolide antibiotic produced by Nocardia brasiliensis: producing strain, isolation and biological activity. J. Antibiot. (Tokyo). 50: 1036-1041.
Torres OH, Domingo P, Pericas R, Boiron P, Montiel JA, Vazquez G (2000). Infection caused by *Nocardia farcinica*: case report and review. Eur. J. Clin. Microbiol. Infect. Dis. 19: 205-212.
Tully RE, van Berkum P, Lovins KW, Keister DL (1998). Identification and sequencing of a cytochrome P450 gene cluster from *Bradyrhizobium japonicum*. Biochim. Biophys. Acta. 1398: 243-255.
Wallace Jr. RJ, Tsukamura M, Brown BA, Brown J, Steingrube VA, Zhang YS, Nash DR (1990). Cefotaxime-resistant Nocardia asteroides strains are isolates of the controversial species *Nocardia farcinica*. J. Clin. Microbiol. 28: 2726-2732.
Waterman MR, Lepesheva GI (2004). CYP51-the omnipotent P450. Mol. Cell. Endocrinol. 215: 165-170.