Subsystem: Inorganic sulfur (sulfate) assimilation

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Introduction

Sulfur is required for the biosynthesis of several essential compounds like amino acids (cysteine and methionine), vitamins (biotin, thiamin), and prosthetic groups (Fe-S clusters) in all organisms. In order to synthesize these compounds, the sulfur has usually to be in a reduced state, most commonly as (hydrogen) sulfide. In the absence of an environmental supply of reduced sulfur moieties (e.g. a black smoker or another organism), organisms have to reduce the needed sulfur themselves. In many microorganisms this function is performed by a very common pathway for assimilatory sulfate reduction, leading from sulfate to sulfide, which is then incorporated in various sulfur containing metabolites (Fig. 1).

Subsystem overview

For each of the reaction steps there seem to exist at least two functional variants (Fig. 2) that appear to be only weakly correlated with each other, so that almost any combination can be found (Fig. 3).

The first step in the pathway is the uptake of oxidized inorganic sulfur compounds, usually sulfate or thiosulfate. Several transporters are known to be involved in this, like the ABC-type transporter Sbp CysAWPT in *Escherichia coli* [1] or the Pit-type permease (SulP) in *Bacillus subtilis* [2]. Following uptake, intracellular sulfate is activated by adenylylation, yielding adenosine phosphosulfate (APS). Two different enzyme families of sulfate adenylyltransferase are commonly involved in this reaction: a heteromeric form (SAT1+2, usually called CysDN) known from *E. coli;* and the homomeric form (DSAT) described for example in *B. subtilis* [2], which is usually used in dissimilatory sulfate reduction [3]. The next step is the reduction of the activated sulfate. APS is either phosphorylated to phosphoadenosine phosphosulfate (PAPS) by APS kinase (ASK, CysC) and subsequently reduced to sulfite by PAPS reductase (PAPSR, CysH) or converted directly by APS reductase (APSR, also called CysH). Which way is used in particular is hard to determine by sequence similarity alone as APS and PAPS reductases belong to the same protein family and APS reductase has been shown to also act on PAPS [4,5,6]. If not verified experimentally, it should therefore be assumed that APS reductase is bifunctional in an organism if APS kinase is also present. The last step of the pathway is the conversion of sulfite to sulfide. In *E. coli* and *B. subtilis* this step is catalyzed by a heteromeric form of sulfite reductase (SIR FP+HP, CysIJ), using NADPH directly as an electron donor [1]. Interestingly, there are a lot of organisms where only the hemoprotein subunit (SIR HP) or a protein more similar to the ferredoxin-dependent sulfite and nitrite reductases known from plants is present. In the latter case, electrons for sulfite/nitrite reductase are derived either from the photosystem I or, in non-photosynthetic tissues, from NADPH [7]. These electrons are then transferred via an ferredoxin—NADPH reductase onto a ferredoxin that in turn delivers them to the homomeric form of sulfite reductase.

At least one functional variant for each of the four steps leading from extracellular sulfate to intracellular sulfide was identified in about 80 bacterial strains and species using SEED analytical tools. More importantly, two novel hypothetical variants were predicted for each sulfate uptake and reduction of sulfite to sulfide (see next page), delivering new testable targets for functional genomics (Fig. 2).

Using SEED to identify "missing genes" and to predict novel functional variants in Sulfur assimilation pathway

Sulfate uptake

Several organisms lack a clear homologue of the ABC-type sulfate/thiosulfate transporter known from *E.coli* or the Pit-type sulfate permease found, e.g., in *B. subtilis*. Based on co-occurrence two possible alternatives could be found:

1. A gene encoding a putative permease (CysZ, 7) is clustered with genes involved in sulfate reduction in two corynebacterial species (Fig. 3, boxed in dark green, and Fig. 5; identifiable by a consecutive numbering in the species row). This permease might therefore be involved in sulfate uptake. The experimental verification/falsification of this assumption is currently under way in our group.
2. Genes encoding an ABC-type transporter of unknown specificity are clustered with genes involved in sulfate reduction in at least four bacterial species (Fig. 3, light green box).

Reduction of sulfite to sulfide

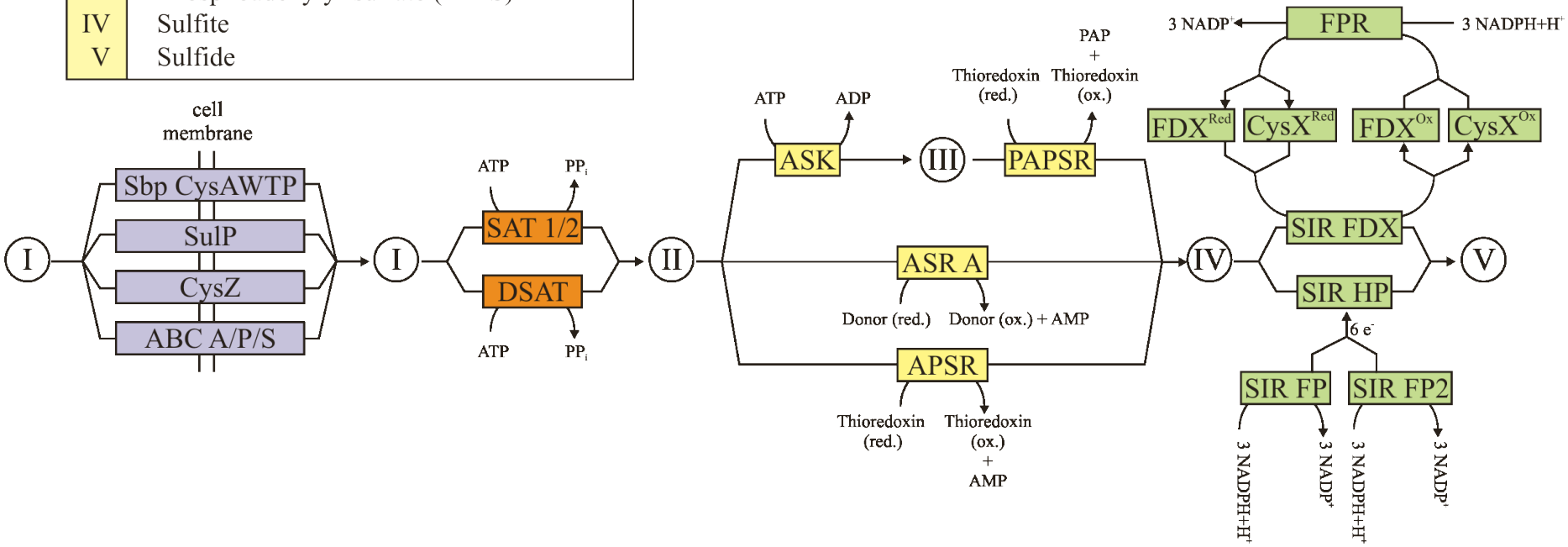
A significant number (about 40 out of 80 in total) of bacterial species currently present in the subsystem lack the flavoprotein subunit of sulfite reductase (SIR FP, 18) known from *E.coli* and *B. subtilis*. Using SEED two possible alternatives were identified in this case as well:

1. In the genomes of more than 40 organisms (25 of which are currently present in the subsystem), SIR FP seems to be replaced by a yet uncharacterized oxidoreductase (SIR FP2, 19; see Fig. 3, highlighted in bold red) which is clustered with the hemoprotein subunit of sulfite reductase (SIR HP, 20) (Fig. 4, boxed in red). This variant can be found, e.g., in *Sinorhizobium meliloti* and *Bordetella parapertussis*.
2. A second variant resembling the system found in plants can be found in the bacterial order of the *Actinomycetales* (Fig. 3, boxed in orange, and Fig. 5) but cannot be found in other bacteria with exception of the *Deinococcales*. It consists of a ferredoxin-dependent sulfite reductase (SIR FDX, 22), a ferredoxin—NADP(+) reductase (FPR, 23) and either a ferredoxin (FDX, 24) or a small, ferredoxin-like protein (CysX, 25).
   An interesting observation is the possible functional coupling and even fusions of FPR, FDX, and CysX detected in SEED: In some organisms (e.g., *M. tuberculosis* and *N. farcinica*) fusion proteins of FDX and FPR are found, identifiable by the same number in both columns (Fig. 5, highlighted in bold red). In all *Actinomyetales* present in the subsystem that lack this fusion (e.g. in *C. efficiens*), a small protein distantly related to ferredoxins (CysX) is clustered with other genes involved in sulfate reduction. Apparent deviations in *C. glutamicum* and *T. fusca* (Fig. 5, marked with arrows) are due to miscalling of this small ORF by an automatic software - in both cases a clear CysX homologue exists.

The novel genes for both novel functional variants for the reduction of sulfite to sulfide are currently under study in our group to elucidate whether our predictions are correct.

Fig. 1: Subsystem diagram illustrating functional variants in assimilatory sulfate reduction pathway

Intermediates	
I	Sulfate
II	Adenylyl-sulfate (APS)
III	Phosphoadenylyl-sulfate (PAPS)
IV	Sulfite
V	Sulfide



Abbreviated functional roles			
Sbp/CysAWTP	Sulfate and thiosulfate ABC transporter	APSR	Adenylyl-sulfate reductase
SulP	Sulfate permease, Pit-type	PAPSR	Phosphoadenylyl-sulfate reductase
CysZ	Sulfate permease, hypothetical	SIR FP	Sulfite reductase, flavoprotein subunit
CysZ	Sulfate ABC transporter, hypothetical	SIR FP2	Oxidoreductase, probable SIR flavoprotein analogue
SAT 1	Sulfate adenylyltransferase, subunit 1 (ATP-binding)	SIR HP	Sulfite reductase, hemoprotein subunit
SAT 2	Sulfate adenylyltransferase, subunit 2	SIR FDX	Sulfite reductase, ferredoxin-dependent
DSAT	Sulfate adenylyltransferase, dissimilatory type	FPR	Ferredoxin--NADPH reductase
ASK	Adenylyl-sulfate kinase	FDX	Ferredoxin
ASR A	Adenylyl-sulfate reductase, α-subunit	CysX	Ferredoxin-like protein

Fig. 2: Functional roles and their assignment to functional variants

The list of functional roles included in the subsystem Inorganic sulfur assimilation in SEED. Potential novel functional variants identified with SEED are highlighted.

Found in Variant	Column	Abbrev	Functional Role
1***	1	CysA	Sulfate and thiosulfate import ATP -binding protein cysA (EC 3.6.3.25)
	2	CysW	Sulfate transport system permease protein cysW
	3	CysT	Sulfate transport system permease protein cysT
	4	CysP	Sulfate and thiosulfate binding protein cysP
	5	SBP	Sulfate-binding protein sbp
2***	6	SulP	Sulfate permease, Pit-type
3***	7	CysZ	Sulfate permease, CysZ-type
4***	8	ABC P	ABC-type probable sulfate transporter, permease protein
	9	ABC A	ABC-type probable sulfate transporter, ATPase component
	10	ABC S	ABC-type probable sulfate transporter, periplasmic binding protein
*1**	11	SAT1	Sulfate adenylyltransferase subunit 1 (EC 2.7.7.4)
	12	SAT2	Sulfate adenylyltransferase subunit 2 (EC 2.7.7.4)
*2**	13	DSAT	Sulfate adenylyltransferase, dissimilatory-type (EC 2.7.7.4)
**1*	14	APSR	Adenylyl sulfate reductase [thioredoxin] (EC 1.8.4.10)
**2*	15	ASK	Adenylylsulfate kinase (EC 2.7.1.25)
	16	PAPSR	Phosphoadenylyl-sulfate reductase [thioredoxin] (EC 1.8.4.8)
-	17	ASR A	Adenylylsulphate reductase alpha-subunit (EC 1.8.99.2)
***1	18	SIR FP	Sulfite reductase [NADPH] flavoprotein alpha-component (EC 1.8.1.2)
***2	19	SIR FP2	Oxidoreductase probably involved in sulfite reduction
***1 ***2	20	SIR HP	Sulfite reductase [NADPH] hemoprotein beta-component (EC 1.8.1.2)
***4	21	SIR FDX	Ferredoxin--sulfite reductase (EC 1.8.7.1)
	22	FPR	Ferredoxin--NADP(+) reductase (EC 1.18.1.2)
	23	FDX	Ferredoxin
	24	CysX	Ferredoxin-like protein involved in electron transfer

Novel (proposed) functional variants

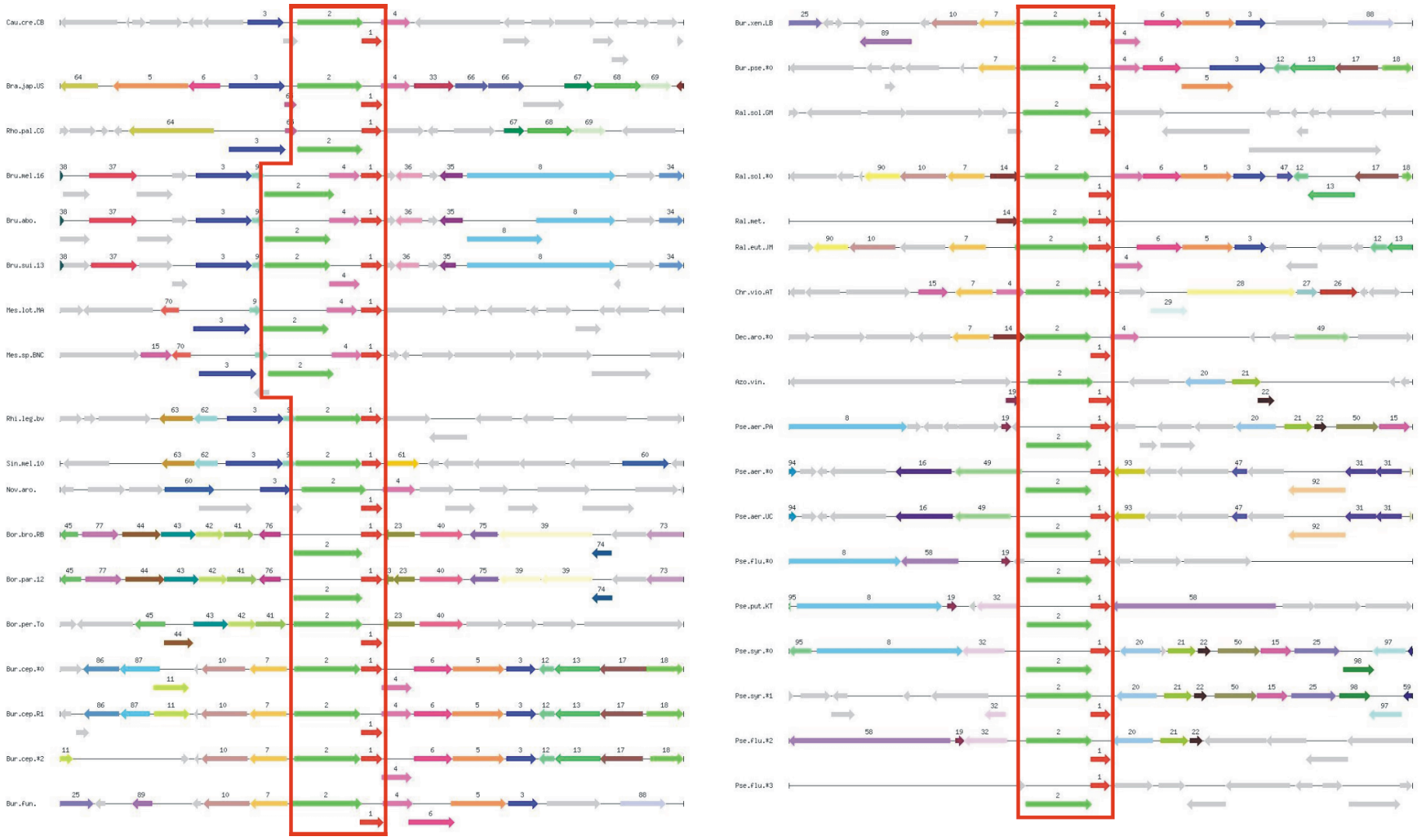
Sulfate uptake
Var. 1: A putative permease (CysZ, 7) is clustered with genes involved in sulfate reduction in two corynebacterial species.
Var. 2: An ABC-type transporter (ABC A|P|S, 8-10) is clustered with genes involved in sulfate reduction in several bacteria.

Reduction of sulfite to sulfide
Var. 1: A small putative oxidoreductase (SIR FP2, 19) might act as functional replacement of the flavoprotein subunit of sulfite reductase (SIR FP, 18).
Var. 2: A ferredoxin-dependent sulfite reductase (SIR FDX, 21) replaces the heteromeric form. Electrons are transferred from NADPH via ferredoxin--NADPH reductase (FPR, 22) to either ferredoxin (FDX, 23) or CysX (24).

Fig. 3: A snapshot of the subsystem spreadsheet illustrating various combinations of functional variants in different reaction steps.

		Sulfate Uptake				Sulfate activation		Reduction Sulfate to Sulfite			Reduction Sulfite to Sulfide			
Organism	Variant Code	CysA-1 CysW-2 CysT-3 CysP-4 Sbp-5	SulP-6	CysZ-7	ABC P-8 ABC A-9 ABC S-10	CysD-11 CysN-12	DSat-13	APS-14	ASK-15 PAPS-16	ASR A-17	SIR FP-18 SIR HP-20	SIR FP2-19 SIR HP-20	SIR FDX-21 FPR-22 CysX-24	FDX-23
Thermobifida fusca [B]	0134					998-11 997-12		2715-14	1003-15 2715-16				2716-21 2693-22	2001 518
Oceanobacillus iheyensis HTE831 [B]	0231						1662-13	1655-14	1664-15 1655-16		**1656-18** 1657-20			1732 2579
Deinococcus radiodurans R1 [B]	0234						2830-13	2829-14	2828-15 2829-16				2827-21 678-22	2257 2510
Nitrosomonas europaea ATCC 19718 [B]	1111	559-1 560-2 561-3 565-5				830-11 829-12		828-14			**826-18** 825-20			6
Bordetella parapertussis 12822 [B]	1112	1569-1 1568-2 1567-3 1566-4				1572-11 1571-12		1570-14				**380-19** 381-20		2521 3130
Escherichia coli K12 [B]	1121	2390-1 2391-2 2392-3 2393-4 3836-5				2707-11 2708-12			2706-15 2718-16		**2720-18** 2719-20			2209 2532
Vibrio vulnificus CMCP6 [B]	1131	3598-1 3597-2 3596-3 3595-4				687-11 688-12			685-15 1293-16		**1291-18** 1292-20			4194 448 878
Sinorhizobium meliloti 1021 [B]	1132	5344-1 5345-2 5346-3 5347-4				2236-11 469-11 5363-11 2237-12 468-12 5362-12		2238-14	469-15 5363-15 2238-16			**2766-19** 2765-20		2433 3269 4552
Mycobacterium tuberculosis H37Rv [B]	1134	2399-1 2400-2 2401-3 2402-4				1288-11 1287-12		2394-14	1288-15 2394-16				2393-21 3108-22 887-22	1179 2009 887
Bacillus subtilis subsp. subtilis str. 168 [B]	2231		1560-6				1092-13 1561-13	1093-14 1559-14	1091-15 1562-15 1093-16 1559-16		**3350-18** 3349-20			
Corynebacterium efficiens YS-314 [B]	3114			2638-7		2640-11 2641-12		2642-14					2644-21 2592-22 2645-22 2643-24	1160
Streptomyces avermitilis MA-4680 [B]	4134				2138-8 2137-9 2136-10	2134-11 2316-11 2133-12 2315-12		2131-14	2132-15 2314-15 2131-16				2129-21 1508-22 2130-24	1269 2298 3131 6957
Clostridium acetobutylicum ATCC 824 [B]	4100				282-8 281-9 280-10	284-11 283-12			277-15	278-17				249 279 3642 3733 473

Fig. 4: Positional coupling of the hemoprotein subunit of sulfite reductase (SIR HP) with an uncharacterized oxidoreductase (SIR FP2)



1	SIR FP2:	Uncharacterized involved probably oxidoreductase in sulfite reduction
2	SIR HP:	Sulfite reductase [NADPH] hemoprotein beta-component (EC 1.8.1.2)

Fig. 5: Conservation of the ferredoxin-dependent sulfite reduction in different *Actinomycetales*

		Sulfate Uptake				Sulfate activation		Reduction Sulfate to Sulfite			Reduction Sulfite to Sulfide			
Organism	**Variant Code**	**CysA-1 CysW-2 CysT-3 CysP-4 Sbp-5**	**SulP-6**	**CysZ-7**	**ABC P-8 ABC A-9 ABC S-10**	**CysD-11 CysN-12**	**DSat-13**	**APS-14**	**ASK-15 PAPS-16**	**ASR A-17**	**SIR FP-18 SIR HP-20**	**SIR FP2-19 SIR HP-20**	**SIR FDX-21 FPR-22 CysX-24**	**FDX-23**
Corynebacterium efficiens YS-314 [B]	3114			2638-7		2640-11 2641-12		2642-14					2644-21 2592-22 **2645-22** **2643-24**	1160
Corynebacterium glutamicum ATCC 13032 [B]	3114			2713-7		2715-11 2716-12		2717-14					2718-21 2658-22 **2719-22**	1057 2856
Mycobacterium tuberculosis H37Rv [B]	1134	2399-1 2400-2 2401-3 2402-4				1288-11 1287-12		2394-14	1288-15 2394-16				2393-21 3108-22 **887-22**	1179 2009 **887**
Mycobacterium avium ssp. paratuberculosis str. k10 [B]	1134	2210-1 2211-2 2212-3 2213-4				2484-11 2485-12		2036-14 2209-14	2484-15 2036-16 2209-16				2035-21 2208-21 3176-22 **825-22**	2039 2607 2726 **825**
Streptomyces avermitilis MA-4680 [B]	4134				2138-8 2137-9 2136-10	2134-11 2316-11 2133-12 2315-12		2131-14	2132-15 2314-15 2131-16				2129-21 **1508-22** **2130-24**	1269 2298 3131 6957
Streptomyces coelicolor A3(2) [B]	4134				6039-8 6040-9 6041-10	6042-11 6043-12		6045-14	6044-15 6045-16				6047-21 **651-22** **6046-24**	3241 5090 7044 7172
Nocardia farcinica IFM 10152 [B]	1134	1437-1 1436-2 1435-3 1434-4				1446-11 2421-11 3315-11 1147-12 2422-12 3316-12		1448-14	2421-11 3315-15 1448-16				1449-21 **1439-22** **2548-22** **4888-22** 5155-22	1245 **1439** **2548** 4186 4570 **4888** 5478 5539
Thermobifida fusca [B]	0134					998-11 997-12		2715-14	1003-15 2715-16				2716-21 **2693-22**	2001 518

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