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REVIEW ARTICLE

Penicillin-binding proteins in *Actinobacteria*

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Because some *Actinobacteria*, especially *Streptomyces* species, are β-lactam-producing bacteria, they have to have some self-resistant mechanism. The β-lactam biosynthetic gene clusters include genes for β-lactamases and penicillin-binding proteins (PBPs), suggesting that these are involved in self-resistance. However, direct evidence for the involvement of β-lactamases does not exist at the present time. Instead, phylogenetic analysis revealed that PBPs in *Streptomyces* are distinct in that *Streptomyces* species have much more PBPs than other *Actinobacteria*, and that two to three pairs of similar PBPs are present in most *Streptomyces* species examined. Some of these PBPs bind benzylpenicillin with very low affinity and are highly similar in their amino-acid sequences. Furthermore, other low-affinity PBPs such as SCLAV_4179 in *Streptomyces clavuligerus*, a β-lactam-producing *Actinobacterium*, may strengthen further the self-resistance against β-lactams. This review discusses the role of PBPs in resistance to benzylpenicillin in *Streptomyces* belonging to *Actinobacteria*.
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INTRODUCTION

Drug resistance is a major problem in almost all the drug-related fields. Among bacterial resistance, β-lactam-antibiotic resistance is the most prevailing and threatening area in public health, because β-lactam antibiotics have been most widely used for chemotherapy of infectious diseases even in 80 years since penicillin's discovery.[1] β-Lactam antibiotic resistance is caused mainly by two mechanisms: antibiotic-degrading enzymes, β-lactamases[2] and modification of target sites, penicillin-binding proteins (PBPs).[3]

The phylum *Actinobacteria* constitute one of the largest phyla within the *Bacteria*.[4,5] *Streptomyces* species, which belong to *Actinobacteria,* are filamentous, soil-dwelling, high guanine+cytosine (G+C)-content Gram-positive bacteria and are characterized by their ability to undergo complex cellular differentiation like filamentous fungi.[6] In addition, *Streptomyces* species produce a wide variety of secondary metabolites including β-lactam antibiotics.[7–9] However, *Streptomyces* species are prokaryotic microorganisms and unlike penicillin- and cephalosporin-producing fungi they must protect themselves from the attack of antibiotics, thus they have to have self-resistant mechanisms.[10–13] In addition, *Streptomyces* species are known to be highly resistant to benzylpenicillin, although they are Gram-positive bacteria. This review discusses the role of PBPs in resistance to benzylpenicillin in *Streptomyces* belonging to *Actinobacteria*.

PBPS

The bacterial cell wall peptidoglycan is a three-dimensional, net-like mesh called sacculus in which glycan strands are cross-linked by peptide chains. It maintains cell shape and provides mechanical strength to resist osmotic pressure.[14,15] The peptidoglycan biosynthesis is catalyzed by glycosyltransferases to polymerize the glycan chains and by transpeptidases to catalyze peptide crosslinking between two adjacent glycan chains. The transpeptidases, also called PBPs, were initially identified as their ability to bind penicillins.[16,17] Depending on the structure and the catalytic activity of their N-terminal domain, they are classified into class A, B and C PBPs.[14,18–20] The C-terminal domains of both class A and class B PBPs have the transpeptidase activity. In class A PBPs, the N-terminal domain is responsible for their glycosyltransferase activity, whereas in class B PBPs, the glycosyltransferase domain is lacking. Class C PBPs are also called low-MW PBPs, having the carboxypeptidase activity, and are responsible for the maturation and recycling of the peptidoglycan.[19] They are not essential and are excluded from further study.

Table 1 summarizes the genome sizes, G+C contents, numbers of PBPs, types of PBPs, class A and class B PBPs and some characters of 113 Actinobacterial species including 30 *Streptomyces* species. Most species have both class A and class B PBPs. However, the phylogenetic distribution of PBPs among taxa is uneven; *Actinomyces odontolyticus* ATCC 17982 encodes two PBPs per genome, whereas *S. avermitilis* MA-4680 and *S. coelicolor* A3(2) have 13 PBPs. In general, *Streptomyces* species possess >10 PBPs including class A and class B PBPs.[21]

CLASS B PBPS

A phylogenetic tree constructed on the basis of their amino-acid sequences of 446 class B PBPs from 113 Actinobacterial species is classified into 10 clusters and is shown in Figure 1. In general, the PBPs from taxonomically related species go into the same clusters. PBPs of suborder *Propionibacterineae* belong to subcluster I. PBPs of the members of order *Actinomycetales*, such as *Thermobispora, Streptosporangium*, *Actinomyces* and *Mobiluncus*, form cluster II. PBPs of other members of *Actinomycetales*, such as *Kytococcus, Kineococcus, Isoptericola, Cellulomonas, Sanguibacter, Beutenbergia, Janibacter,*

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Table 1 The numbers and types of putative PBP genes

Bacteria	Prefix	Genome size (Mb)	G +C %	No. of PBP	Type of PBP	Class A PBP	Class B PBP	Comment
Acidimicrobium ferrooxidans DSM 10331	AFER	2.16	68.3	3	B3		0089, 0769, 1250	Ferrous-iron-oxidizing, moderately thermophilic, acidophilic bacteria, isolated from hot spring in Iceland, thermophile, nonsporulating
Acidothermus cellulolyticus 11B	ACEL	2.44	66.9	5	A2; B3	2004, 2135	0020, 0751, 1004	A moderately thermophilic, aerobic, cellulolytic bacterium originally recovered from the acidic hot springs
A. odontolyticus ATCC 17982	ACTODO	2.39	65.4	2	B2		00847, 01564	Isolated from human dental cavity, nonmotile, nonsporulating, mesophile, septicemia, oral abscesses, lung infection, eye infection
A. urogenitalis DORA_12	Q605	2.6	68.4	5	A3, B2	AUC00015G0001, AUC00927G0001, AUC01040G0002	AUC00266G0006 AUC00451G0003	Isolated from human urogenital tract
Actinoplanes missouriensis 431	AMIS	8.77	70.8	8	A5; B3	10850, 39800, 60090, 78300, 80960	00450, 15180, 71610	Aerobic, motile, filamentous, sporulating bacterium; produces actaplanin, azacytidine, D-xylose isomerase; degrades flavonoids and natural rubber;
Actinosynnema mirum DSM 43827	AMIR	8.25	73.7	6	A3; B3	0235, 5121, 7034	0023, 5772, 5886	A producer of nocardicin; Pseudonocardineae, isolated from blade of grass from Raritan River, aerobic, nonmotile, sporulating, mesophile, chemoorganotroph
Amycolicicoccus subflavus DQS3–9A1	AS9A	4.74	62.2	5	A2; B3	0253, 2577	0033, 4146, 4510	Isolated from a saline soil contaminated with crude oil; no mycolic acids and MK-8 was the major menaquinone
Arthrobacter aurescens TC1	AAUR	4.60	62.3	6	A2; B4	3369, 3416	0030, 1704, 3184, 4181	Reduce hexavalent chromium; found in extreme environments such as deep subsurface soils, arctic sea and radioactive waste tanks; catabolize a variety of xenobiotic compounds; used in the bioremediation
Atopobium parvulum DSM 20469	Apar	1.54	45.7	4	A1; B3	1010	0480, 0673, 1344	Isolated from gingival crevices of the human oral cavity, obligate anaerobic, coccus-shaped, nonmotile, nonsporulating, free living, mesophile, 37–45 °C
Beutenbergia cavernae DSM 12333	BCAV	4.67	73.1	5	A2; B3	0604, 4182	0028, 0389, 2416	Isolated from cave soil; no mycolic acid; not acid-fast, nonmotile, non-spore-forming and a rod-coccus growth cycle; peptidoglycan contains lysine in position 3 of the peptide subunit and an interpeptide bridge of L-LYS+ L-Glu.
Bifidobacterium adolescentis ATCC 15703	BAD	2.09	59.2	4	A2; B2	0157, 1336	0040, 1107	Normal inhabitants of healthy human and animal intestinal tracts, anaerobic, rod-shaped, nonsporulating, nonmotile, intestinal microflora
Bifidobacterium animalis subsp. *lactis* AD011	BLA	1.93	60.5	3	A1; B2	0208	0077, 0782	An anaerobic Gram-positive lactic acid bacterium commonly found in the guts of healthy humans; utilize nondigestible oligosaccharides, nonsporulating
Bifidobacterium animalis subsp. *lactis* BLC1	BLC1	1.94	60.5	4	A2; B2	0209, 1314	0079, 1166	
Bifidobacterium bifidum PRL2010	BBPR	2.21	62.7	4	A2; B2	0401, 1743	0104, 0550	Anaerobic, rod-shaped, nonmotile, nonsporulating, mesophile
Bifidobacterium bifidum S17	BBIF	2.19	62.8	4	A2; B2	0427, 1684	0137, 0574	
		2.33	58.7	4	A2; B2	0187, 1410	0067, 0606	

Table 1 (Continued)

Bacteria	Prefix	Genome size (Mb)	G +C %	No. of PBP	Type of PBP	Class A PBP	Class B PBP	Comment
Bifidobacterium breve ACS-071-V-Sch8b	HMPRE-F9228							Anaerobic, rod-haped, nonmotile, non-sporulating, mesophile, human vaginal microflora
Bifidobacterium breve UCC2003	BBR	2.42	58.7	4	A2; B2	0176, 0487	0069, 1263	Isolated from infant nursing stool, anaerobic,rod-shaped, nonsporulating, non-motile, intestinal microflora, probiotic, nonpathogenic
Bifidobacterium dentium Bd1	BDP	2.64	58.5	4	A2; B2	0244, 1801	0040, 1548	Isolated from dental caries, anaerobic, nonmotile, nonsporulating, mesophilic, probiotic, nonpathogenic
Bifidobacterium longum subsp. *infantis* 157F	BLIF	2.40	60.1	4	A2; B2	0139, 0411	0048, 1329	
Bifidobacterium longum subsp. *infantis* ATCC 15697	BLIJ	2.83	59.9	4	A2; B2	0174, 2144	0078, 0867	Uses milk oligosaccharides, produces fucosidases; anaerobic, non-halophilic, Gram-positive bacterium; hexose metabolism: phosphoketolase pathway (bifid shunt), probiotic, nonpathogenic
Bifidobacterium longum subsp. *longum* F8	BIL	2.38	59.9	2	A1; B1	14530	18970	Anaerobic, nonsporulating, mesophilic, human fecal, gastrointestinal tract, 37 °C
Bifidobacterium longum subsp. *longum* JCM 1217	BLLJ	2.39	60.3	4	A2; B2	0150, 0394	0060, 1278	Anaerobic, rod-shaped, nonsporulating, mesophile, commensal
Brevibacterium casei S18	C272	3.66	68.1	4	A3; B1	05329, 06024, 15065	05334	Isolated from human skin, aerobe, mesophile, symbiotic, commensal
Catenulispora acidiphila DSM 44928	CACI	10.47	69.8	8	A1; B7	1390	0037, 1307, 1448, 1656, 5826, 6659, 7282	Aerobic, free-living, nonmotile, acidophilic Gram-positive bacterium; resistant to lysozyme; peptidoglycan type is A3gamma ll-Dpm-Gly, filament shaped, sporulating
Cellulomonas flavigena DSM 20109	Cfla	4.12	74.3	3	B3		0027, 1590, 3460	Rod-shaped, nonsporulating, nonmotile, biomass degrader, cellulose degrader, nitrate reducer, xylan degrader
Clavibacter michiganensis subsp. *michiganensis* NCPPB 382	CMM	3.3	72.7	4	A2; B2	0915, 0919	0017, 1865	Aerobic, rod-shaped, nonmotile, nonsporulating, mesophile, plant pathogen, isolated from Lycopersicon esculentum
Conexibacter woesei DSM 14684	Cwoe	6.36	72.7	7	A1; B6	3542	0016, 1104, 1801, 2661, 3775, 5329	Isolated from forest soil, obligate aerobic, rod-shaped, motile, nonsporulating, mesophile, free living
C. diphtheriae NCTC 13129	DIP	2.49	53.5	5	A2; B3	0298, 2294	0055, 1497, 1604,	Gram-positive, nonmotile rods; produces the diphtheria toxin; isolated from soil, plant material, waste water and dairy products, diphtheria, respiratory infection, chemoorganotroph
C. glutamicum ATCC 13032	NCGL	3.31	53.8	5	A2; B3	0274, 2884	0042, 1933, 2084	Unusual outer membrane ~8 nm thick; mycolic acid-arabinoglactan-peptidoglycan polymer form the cell wall
C. pseudotuberculosis 1/06-A	CP106	2.28	52.2	5	A2; B3	0194, 1992	0032, 1278, 1380	A facultative intracellular pathogen; causes caseous lymphadenitis in animals; pleomorphic forms: coccoids and filamentous rods; nonsporulating, non-capsulated, nonmotile bacterium; it has fimbriae
C. pseudotuberculosis 1002	CP1002	2.34	52.2	5	A2; B3	0200, 2034	0035, 1298, 1397	

Table 1 (Continued)

Bacteria	Prefix	Genome size (Mb)	G +C %	No. of PBP	Type of PBP	Class A PBP	Class B PBP	Comment
C. pseudotuberculosis 42/02-A	CP4202	2.34	52.2	5	A2; B3	0198, 2027	0034, 1288, 1387	
C. resistens DSM 45100	CRES	2.6	57.1	6	A2; B4	2033, 2137	0042, 0263, 0788, 1250	Highly resistant to antimicrobial agents; anaerobic, rod-shaped, nonomitile, non-sporulating, mesophile
Cryptobacterium curtum DSM 15641	Ccur	1.62	50.9	3	A1; B2	02290	06100, 09550	Isolated from periodontal pocket, periodontal infection, caries, obligate anaerobic, rod-shaped, nonmotile, nonsporulating, mesophile, free living
Frankia alni ACN14a	FRAAL	7.50	72.8	7	A3; B4	1281, 6546, 6857	1919, 2190, 5852, 6753	Aerobic, filament-shpaed, nonmitile, sporulating, nitrogen-fixing; differentiate into sporangium and vesicles (specialized cell for nitrogen fixation), contain lipid components called hopanoids. Hopanoids
Frankia sp. CcI3	FRANCCI3	5.43	70.1	7	A3; B4	0754, 4277, 4526	1214, 1409, 3641, 4434	Isolated from root nodules of Casuarina cunninghamiana, aeroobic, filament shaped, sporulating, nonmotile,symbiotic, mutualistic, plant symbiont, soil, nitrogen fixation, nonpathogenic, chemoorganotroph
Frankia sp. EuI1c	FraEuI1c	8.82	72.3	8	A3; B5	0346, 6058, 7178	0085, 1014, 1303, 1960, 2368	Aerobic, sporulating, mesophile, filament shaped, symbiotic, mutualistic, nitrogen fixation, nonpathogenic, chemoorganotroph
Gardnerella vaginalis 409-05	HMPREF0424	1.62	42.0	4	A2; B2	0304, 1277	0032, 1107	Obligate anaerobic, rod-shaped, free-living Gram-variable bacterium; a risk factor for the acquisition of HIV, bacterial vaginosis, vagina microflora
G. vaginalis HMP9231	HMPREF9231	1.73	41.2	4	A2; B2	1162, 1288	0015, 1089	Anaerobic, rod-shaped, mesophile, human oral microflora, vagina microflora
Gordonia alkanivorans NBRC 16433	GOALK	5.07	67.4	7	A2; B5	00350, 01250	00210, 00240, 00270, 01580, 01900	Isolated from tar-contaminated soil/marine sediment; use alkanes as a carbon source
Gordonia polyisoprenivorans VH2	GPOL	5.67	67.0	6	A2; B4	07230, 49200	00240, 05610, 20210, 27800	Isolated from soil of a rubber tree plantation and from fouled water inside a decayed automobile tire; degrade natural and synthetic poly(cis-1,4-isoprene) rubber;
Isoptericola variabilis 225	ISOVA	3.31	73.9	5	A2; B3	2701, 3000	0021, 1284, 2892	A cellulolytic bacterium isolated from the termite hindgut; biofuels, biomass conversion; rod-shaped, motile
Janibacter sp. HTCC2649	JNB	4.23	68.4	4	A1; B3	13593	00615, 05649, 12079	Aquatic,marine, free living, mesophile, isolated from Sargasso Sea
Kineococcus radiotolerans SRS30216	KRAD	4.76	74.4	5	A2; B3	0429, 4341	0073, 0475, 3205	An aerobic, coccoid bacterium isolated from a high-level radioactive waste cell; resitant to ionizing γ-radiation and desiccation; marked change in colony morphology over prolonged incubation
Kitasatospora setae KM-6054	KSE	8.78	74.2	8	A4; B4	27750, 36430, 38960, 59840	26130, 39410, 46160, 46190,	An aerobic, soil-habiting mycelial Gram-positive bacterium isolated from oil; produce bafilomycin B1 and bafilomycin A1, 2, specific inhibitors of vacuolar ATPase; LL- and meso-DAP
Kocuria rhizophila DC2201	KRH	2.7	71.2	3	A1; B2	04490	14880, 20650	A coccoid, halotolerant, phenol-degrading Gram-positive bacterium isolated from the rhizosphere of narrowleaf cattail; amino-acid transporters and drug efflux pumps

Table 1 (Continued)

Bacteria	Prefix	Genome size (Mb)	G +C %	No. of PBP	Type of PBP	Class A PBP	Class B PBP	Comment
Kribbella flavida DSM 17836	Kfla	7.58	70.6	4	B4		0061, 2302, 2880, 6892	Obligate aerobe, nonmotile, sporulating, mesophile, free living
Kytococcus sedentarius DSM 20547	KSED	2.79	71.6	3	B3		00200, 09230, 16620	A free-living, nonmotile, human opportunistic pathogen; grows as spherical/coccoid; produces monesin A and B; isolated from varing environments such as human skin and groundwater
Leifsonia xyli subsp. *xyli* str. CTCB07	LXX	2.58	67.7	6	A4; B2	02090 03600, 05450, 23190	00230, 15320	An aerobic, rod-shaped, Gram-positive bacterium, causes ratoon stunting disease and affects sugarcane; a plant pathogen; no endospore or motility; an unusual cell wall peptidoglycan with 2,4-diaminobutyric acid
Micrococcus luteus NCTC 2665	Mlut	2.5	73.0	3	A1; B2	18460	00770, 13660	Aerobe, motile, nonsporulating,mesophile, metal resistant, free living, soil
Micromonospora aurantiaca ATCC 27029	MICAU	7.03	72.8	9	A6; B3	3350, 4230, 4961, 5144, 5927, 6271	0098, 4478, 5070	Metabolic versatility like nitrogen fixation, exhibit saphrophytic (living off dead tissues) and symbiotic lifestyles, ubiquitous in the environment, degrade plant cell walls and fibers; spore formation; bioremediation
Mobiluncus curtisii ATCC 43063	HMPRE-F0573	2.15	55.4	4	A2; B2	10708, 10751	10644, 11576	An anaerobic, rod shaped Gram-positive bacterium found in the human vagina
M. avium 104	MAV	5.48	69.0	5	A2; B3	0071, 0446	0020, 2330, 3723	*M. avium* complex causes a serious infection in people with advanced AIDS; mycolic acid-arabinoglactan-peptidoglycan polymer form the cell wall
M. bovis BCG str. Tokyo 172	JTY	4.37	65.6	5	A2; B3	0051, 3742	0016, 2174, 2881	A major cause of tuberculosis in animal species and man; an unusual outer membrane ~8 nm thick,the outer membrane and the mycolic acid-arabinoglactan-peptidoglycan polymer form the cell wall
M. leprae TN	ML	3.27	57.8	5	A2; B3	2308, 2688	0018, 0908, 1577	An unculturable very slow-growing, acid-fast, obligate intracellular bacterium; nonmotile and rod-shaped; responsible for leprosy.
M. tuberculosis H37Rv	RV	4.41	65.6	5	A2; B3	0050, 3682	0016c, 2163c, 2864c	Acid-fast, obligate aerobic, nonmotile, rod-shaped bacterium; the causative agent of tuberculosis; persists in a dormant or latent form for years
Nakamurella multipartita DSM 44233	NAMU	6.06	70.9	4	A1; B3	0707	0079, 2190, 3930	An obligately aerobic chemoorganotrophic, polysaccharide-accumulating Gram-positive bacterium isolated from active sludge
Nocardia farcinica IFM 10152	NFA	6.02	70.8	8	A3; B5	03390, 55490, 55570	820, 17600, 18430, 41160, 54970	A Gram-positive, filamentous-growing soil saprophyte, nocardiosis, mastitis, aerobic, filament shaped, sporulating
Nocardioides sp. JS614	NOCA	4.99	71.7	6	A2; B4	0326, 4676	0024, 3069, 3462, 4600	An aerobic mesophillic Gram-positive bacterium; grows on media containing vinyl chloride and ethane; monooxygenase system has a major role in the VC starvation response
Nocardiopsis dassonvillei subsp. *dassonvillei* DSM 43111	Ndas	5.77	72.8	5	B5		0890, 2552, 3385, 3720, 5248	Aerobic, mesophile, free living, pulmonary infection, actinomycetoma, lacks mycolic acid

Table 1 (Continued)

Bacteria	Prefix	Genome size (Mb)	G +C %	No. of PBP	Type of PBP	Class A PBP	Class B PBP	Comment
Propionibacterium acnes 266	PAZ	2.49	60.0	4	A2; B2	01380, 22310	01980, 08010	Anaerobic, rod-shaped, nonmotile, non-sporulating, mesophile, pathogen, human skin, acne
P. acnes KPA171202	PPA	2.56	60.0	4	A2; B2	0126, 2149	0185, 0752	Anaerobic, rod-shaped, nonmotile, non-sporulating, mesophile, pathogen, human skin, acne
P. acnes SK137	HMPRE-F0675	2.5	60.1	4	A2; B2	3139, 5213	3226, 3820	Anaerobic, nonspore forming, Gram-positive bacterium; produces propionic acid; non-toxigenic; a common resident of the pilosebaceous (hair follicle) glands of the human skin, acne vulgaris
Renibacterium salmoninarum ATCC 33209	RSA-L33209	3.16	56.3	4	A1; B3	2795	2241, 2500, 2891	A Gram-positive, rod-shaped bacterium that causes the bacterial kidney disease in salmonids
Rhodococcus erythropolis PR4	RER	6.52	62.3	8	A3; B5	04630, 58380, 58990	00300, 10660, 10670, 25560, 35580	Aerobic, Gram positive, capable of morphological differentiation; broad metabolic diversity; desulphurization of fossil fuels, production of acrylamide and extracellular polysaccarides; bioremediation
Rothia dentocariosa ATCC 17931	HMRE-F0733	2.51	53.7	3	A1; B2	10478	10948, 11665	Aerobic, pleomorphic, coccoid- to rod-shaped bacterium frequently isolated from the human oral cavity, periodontitis, opportunistic infection, meningitis, endocarditis
*Rubrobacter xylanophilu*s DSM 9941	Rxyl	3.23	70.5	5	A2; B3	1310, 2308	0022, 1138, 1498	Isolated from thermally polluted industrial runoff from a carpet factory, aerobic, rod-shaped, nonmotile, nonsporulating, thermophile, free living, radiation resistant, nonpathogen, heterotroph, soil
Saccharomonospora azurea NA-128	SACAZ	4.76	70.0	4	A2; B2	02388, 02721	01210, 02813	Aerobic, Gram-negative bacterium; aerial mycelium and single spores; degrades hemicellulose
Saccharomonospora viridis DSM 43017	SVIR	4.31	67.3	4	A2; B2	36250, 39340	24950, 33330	Aerobic, Gram-negative bacterium; typical mycelium morpphology of *Saccharomonospora*; found in hot compost and hay, its spores cause farmer's lung disease, bagassosis and humidifier fever; metabolize pentachlorophenol
Saccharomonospora marina XMU15	SACMA	5.97	68.9	5	A2; B3	5053, 5737	0205, 1393, 2176	Aerobic, Gram-positive bsacterium; forms substrate and aerial mycelia, nonmotile; isolated from ocean sediment; degrades hemicellulose; contains an unusually large number of glycosyltransferases
Saccharopolyspora erythraea NRRL 2338	SACE	8.21	71.1	7	A4; B3	0314, 0385, 6352, 7356	0046, 5864, 5990	Aerobic, filament-shpaed, nonmotile, sporulating, free-living, Gram-positive bacterium; produces erythromycin
Salinispora arenicola CNS-205	SARE	5.79	69.5	7	A5; B2	3240, 3923, 4021, 4796, 5078	0051, 3444	Isolated from tropical marine sediment; produces staurosporine and nifamycin; requires seawater for growth
Salinispora tropica CNB-440	STROP	5.18	69.5	7	A5; B2	3015, 3548, 3639, 4354, 4560	0046, 3218	Resides in ocean sediments; requires seawater for growth; produces salinosporamide A, a potent inhibitor of the 20S proteasome;
Sanguibacter keddieii DSM 10542	Sked	4.25	71.9	3	B3		00200, 22860, 36830	Isolated from blood of dairy cows, rod-shaped, nonsporulating, mesophile, free living

Table 1 (Continued)

Bacteria	Prefix	Genome size (Mb)	G +C %	No. of PBP	Type of PBP	Class A PBP	Class B PBP	Comment
Slackia heliotrinireducens DSM 20476	SHEL	3.17	60.2	3	A1; B2	08350	08800, 14210	A nonmotile, obligate anaerobe, pyrrolizidine alkaloids metabolizer Gram-positive bacterium, isolated from the rumen of a sheep
Stackebrandtia nassauensis DSM 44728	Snas	6.84	68.1	4	A2; B2	1056, 5713	2665, 6473	An aerobic, nonmotile, degrade or hydrolyze allantoin, casein, aesculin, gelatin, hypoxanthine, starch and tyrosine
S. albulus CCRC11814	K530	9.3	72.2	6	A1; B5	08126	01507, 21226, 23516, 34418, 52485	Production of poly-L-lysine
S. albus J1074	SSHG	6.84	73.3	10	A4; B6	01811, 02961, 03197, 04158	01149, 01599, 02906, 03834, 03835, 04427	Nonmotile, aerobic, sporulating, Gram-positive bacterium
S. avermitilis MA-4680	SAV	9.03	70.7	13	A6; B7	3225, 4294, 4423, 4583, 5179, 7219	2952, 3603, 3604, 4339, 5458, 6116, 6387	Soil and water Gram-positive filamentous bacteria, produces avermectin, a human and veterinary medicine
S. bingchenggensis BCW-1	SBI	11.94	70.8	12	A6; B6	03076, 04174, 05361, 05810, 06697, 09068	02283, 04376, 05407, 06233, 07119, 07873	A soil bacterium; the largest bacterial genome that has been sequenced to date; produces milbemycin, an anthelmintic macrolide
S.cattleya NRRL8057	SCAT	6.28	72.9	11	A4; B7	1929, 2889, 3140, 3906	0768, 1207, 1730, 1901, 3088, 4153, 5676	Produces thienamyci, cephamycin C, penicillin N and fluorinated metabolites
S. cattleya DSM46488	SCATT	6.28	72.9	11	A4; B7	19200, 28790, 31330, 38910	07700, 12070, 17240, 18950, 30790, 41420, 56770	
S. clavuligerus ATCC27064	SCLAV	6.76	72.5	12	A3; B9	2006, 2887, 3942	1087, 1301, 1774, 2276, 2947, 4154, 4179, 4180, 4198	Clavulanic acid, cephamycin C, deacetoxycephalosporin C and penicillin N; cannot use glucose as a carbon source, because it lacks a glucose transport system, has all the enzymes of the urea cycle
S. coelicoflavus ZG0656	SMCF	8.48	71.8	11	A3; B8	1708, 4389, 7595	3764, 4686, 7469, 7795, 7796, 8190, 8286, 8884	Produces novel acarviostatin family α-amylase inhibitors
S. coelicolor A3(2)	SCO	8.67	72.1	13	A4; B9	2897, 3580, 3901, 5039	1875, 2090, 2608, 3156, 3157, 3771, 3847, 4013, 5301	A filamentous, high G+C Gram-positive bacterium; degrades chitin, takes part in the nitrogen cycle
S. collinus Tu365	B446	8.27	72.6	9	A3; B6	15140, 19060, 23580	09755, 10960, 13820, 16355, 19320, 24955	Producer of kirromycin
S. davawensis JCM4913	BN159	9.47	70.6	11	A4; B7	3357, 4150, 4546, 5391	3075, 4478, 5121, 5122, 5684, 6352, 6632	Producer of roseoflavin
S. flavogriseus ATCC33331	SFLA	7.34	71.1	12	A4; B8	2228, 3158, 3398, 4003	0559, 1988, 3202, 3620, 3741, 4275, 4730, 4938	An aerobic, Gram-positive bacterium isolated from soil; produces cellulases and xyanases
S. ghanaensis ATCC 14672	SSFG	8.22	72.2	10	A4; B6	02387, 02608, 03635, 04479	02394, 03587, 04216, 04217, 04765, 05266	Aerobic, filamentous, nonmotile, high G +C Gram-positive bacterium, which produces moenomycin

Table 1 (Continued)

Bacteria	*Prefix*	*Genome size (Mb)*	*G +C %*	*No. of PBP*	*Type of PBP*	*Class A PBP*	*Class B PBP*	*Comment*
S. griseoaurantiacus M045	SGM	7.71	72.7	10	A5; B5	0550 1814, 3549, 4216, 6740	0325, 3502, 4547, 5988, 5989	Isolated from marine sediment, produces manumycin and chinikomycin
S. griseoflavus Tu4000	SSRG	7.36	71.7	12	A4; B8	02182, 02879, 03203, 03961	01957, 03076, 03158, 03705, 03706, 04177, 04634, 04850	Anaerobic, filamentous, nonmotile, free-living, gram-positive bacterium
S. griseus subsp. *griseus* NBRC 13350	SGR	8.55	72.2	10	A4; B6	2494, 3341, 3679, 4647	2203, 3726, 4232, 4340, 4934, 5621	Anaerobic, filamentous, nonmotile, free-living, Gram-positive bacterium; produces streptomycin
S. hygroscopicus subsp. *jinggangensis* 5008	SHJG	10.15	71.9	11	A5; B6	3853, 4373, 5171, 5432, 6136	3336, 4100, 4627, 4628, 5219, 6411	Produces validamycin;
S. lividans TK24	SSPG	8.19	72.2	11	A3; B8	02649, 03751, 04641	02381, 03670, 03808, 03892, 04382, 04383, 04919, 05673	A Gram-positive, filamentous, soil bacterium
S. pristinaespiralis ATCC 25486	SSDG	8.13	68.7	11	A3; B8	00591, 04208, 06322,	02572, 02766, 03053, 06246, 06247, 07051, 07138, 07139	Aerobic, filamentous, nonmotile, Gram-negative, sporulating bacterium; produces pristinamycin
S. rimosus subsp. *rimosus* ATCC10970	SRIM	9.5	71.9	11	A4; B7	00295, 08328, 13873, 22689	00065, 04191, 06646, 15770, 26297, 31850, 31885	Bioconversion of quercetin into a novel glycoside
S. roseosporus NRRL 11379	SROS-N1_01-01000	7.76	71	10	A4; B6	12023, 17747, 19666, 23998	05995, 09849, 13775, 14509, 17500, 25622	Aerobic, filamentous, nonmotile, Gram-positive bacterium
S. scabiei 87.22	SCAB	10.15	71.5	11	A4; B7	33601, 41401, 56801, 64431	10101, 29591, 45551, 53611, 53621, 60051, 70631	Aerobic, filamentous, nonmotile, sporulating bacterium; an important plant pathogen; produces phytotoxins called thaxtomins: pathogenicity island
Streptomyces sp. PAMC26508	F750	7.53	71.1	10	A4; B6	2719, 3337, 3596, 4580	1743, 2434, 2998, 3546, 4834, 6320	Production of clavaminate synthase 2
Streptomyces sp. SirexAA-E	SACTE	7.41	71.7	11	A4; B7	2371, 3027, 3329, 4291	1307, 1519, 2029, 2618, 2701, 3283, 4532	Aerobic, filamentous, nonmotile, sporulating free-living bacterium
S. sviceus ATCC 29083	SSEG	9.31	69.8	10	A4; B6	01073, 07525, 03439, 04164	00010, 00011, 00733, 01896, 09019, 09517,	Aerobic, filamentous, nonmotile, sporulating, free-living bacterium
S. tsukubaensis NRRL18488	STSU	7.67	71.5	10	A4; B6	11540, 15659, 19470, 23388	10421, 17409, 21621, 24378, 26744, 27686	Producer of tacrolimus (FK506)
S. venezuelae ATCC 10712	SVEN	8.23	72.4	10	A4; B6	2646, 3350, 3677, 4705	1522, 1745, 2386, 2985, 3631, 4995	Aerobic, filamentous, nonmotile, sporulating free-living bacterium; produces chloramphenicol,
S. violaceusniger Tu4113	STRVI	10.66	71.0	11	A5; B6	1350, 2314, 3845, 8252, 9005	0275, 1135, 3190, 7171, 7897, 7904	Aerobic, filamentous, motile, sporulating, mesophilic, bacterium; produces spirofungin, antifungal agent
S. viridochromogenes DSM 40736	SSQG	8.55	71.1	11	A5; B6	02328, 02941, 03901, 04279, 05113	01781, 02628, 03242, 03243, 03958, 05348	Aerobic, filamentous, nonmotile, sporulating bacterium
S. zinciresistens K42	SZN	8.22	72.5	10	A4; B6	06389, 16730, 18682, 28493	02952, 10458, 13352, 17932, 18819, 22026	Aerobic, filamentous, nonmotile, nonsporulating, halophilic bacterium; isolated from soil from a zinc and copper mine

Table 1 (Continued)

Bacteria	Prefix	Genome size (Mb)	G +C %	No. of PBP	Type of PBP	Class A PBP	Class B PBP	Comment
Streptosporangium roseum DSM 43021	SROS	10.34	70.9	11	A4; B7	2902, 3010, 8177, 9363	0113, 1441, 1456, 2864, 3583, 4062, 7683,	Aerobic, filamentous, nonmotile, sporulating, mesophilic bacterium; produces angucycline, an inhibitor of the endothelin-converting enzyme
Thermobifida fusca YX	TFU	3.64	67.5	4	A2; B2	0570, 3097	1416, 2475	Aerobic, rod-shaped, nonmotile, sporulating, moderate thermophilic soil bacterium; degrades organic material and dcllulose; farmer's lung, mushroom worker's disease, respiratory infection
Thermobispora bispora DSM 43833	TBIS	4.19	72.4	10	A4; B6	0195, 1426, 3106, 3566	0053, 0796, 1401, 1685, 1727, 2465	Aerobic, filamentous, nonmotile, sporulating, thermophilic bcterium
T. curvata DSM 43183	TCUR	5.64	71.6	8	A4; B4	1026, 1268, 4921, 4955	0065, 1542, 2932, 4002	An aerobic, cellulolytic, thermophilic Gram-positive bacterium; produces a number of industrially important compounds like cellulase, alpha-amylase and polygalacturonate lyase
Tropheryma whipplei str. Twist	TWT	0.93	46.3	3	A1; B2	0705	0222, 0776	Aerobic, rod-shaped, nonmotile, nonsporulating, mesophilic bacterium; deficient in amino acid metabolisms, the lack of clear thioredoxin and thioredoxin reductas; causes Whipple's disease
Tropheryma whipplei TW08/27	TW	0.93	46.3	3	A1; B2	0722	0548, 0787	A Gram-positive, filamentous, aerobic, soil-dwelling actinomycete. It is the causative agent of the Whipple's disease
Tsukamurella paurometabola DSM 20162	TPAU	4.38	68.4	7	A2; B5	3939, 4192	0029, 0349, 1690, 2652, 3973	Obligate aerobic, rod-shaped, nonomotile, nonsporulating, mesophilic bacterium; isolated from ovaries of Cimex lectularius; a human opportunistic pathogen

Abbreviations: G+C, guanine+cytosine; PBP, penicillin-binding protein.

Catenulispora, Renibacterium, Arthrobacter, Saccharomonospora and *Micromonospora*, constitute cluster IV. Some PBPs of *Actinomyces* and *Mobiluncus* fall into subcluster IV-2. PBP members that from suborder *Micrococcineae* are partly overlapped and distributed in the clusters IV and V. *Kytococcus, Isoptericola, Cellulomonas, Sanguibacter, Beutenbergia, Janibacter, Renibacterium* and *Arthrobacter* are members of suborder *Micrococcineae*. PBPs of order *Bifidobacteriales* form subcluster III. The cluster V includes PBPs of suborder *Micrococcineae* members such as *Clavibacter*, *Leifsonia*, *Tropheryma*, *Micrococcus*, *Rothia*, *Kocuria*, *Renibacterium* and *Arthorobacter*. However, PBPs of suborder *Micrococcineae* are also distributed in the cluster IV, as described above. No PBPs of *Micrococcineae* make a phylogenetically distinct, coherent cluster and are dispersed in clusters IV and V.[4] The subclusters VI-1, VI-2 and VI-3 consist of PBPs of suborder *Corynebacterineae* (*Corynebacterium*, *Tsukamurella*, *Gordonia*, *Mycobacterium*, *Rhodococcus*, *Nocardia* and *Amycolicicoccus*), suborder *Pseudonocardineae* (*Saccharopolyspora*, *Saccharomonospora* and *Actinosynnema*) and suborder *Frankineae* (*Nakamurella*). However, PBPs of *Corynebacterineae* and *Pseudonocardineae* compose different branches in the subclusters. The suborders *Corynebacterineae* and *Pseudonocardineae* are closely related taxonomically[4,22] and, in addition, *Nakamurella multipartite*, which is currently a member of suborder *Frankineae*, is proposed to be closely related to *Pseudonocardineae*.[4] The PBPs of most *Frankia* species belong to subclusters VII-1, VII-2, VII-4 and VII-5 which contain also PBPs of suborder *Frankineae* (*Frankia* and *Acidothermus*), suborder *Micromonosporineae* (*Salinispora*, *Micromonospora* and *Actinoplanes*) and suborder *Glycomycineae* (*Stackebrandtia*).

As described above, *Streptomyces* species carry more PBPs than other species. Reflecting this fact, PBPs of *Streptomyces* species form seven large subclusters of VIII-1, VIII-2, VIII-3, VIII-4, VIII-5, VIII-6 and VIII-7. SCAB_10101 possesses neither essential serine nor lysine residues which are involved in the enzymatic catalysis (the amino-acid sequence in this region is Thr-Thr-Phe-Ser), so that it is excluded from further analysis.

Intriguingly, more than half of *Streptomyces* species hold two successive class B PBPs (Figure 1 and Table 1). For example, SSHG_03834 and SSHG_03835 (a similarity value E is 1.2e−62, the same hereafter), SAV_3603 and SAV_3604 (3e−68), SCLAV_4179 and SCLAV_4180 (1.5e−11), SMCF_7795 and SMCF_7796 (1.1e−48), SCO3156 and SCO3157 (2.8e−47), BN159_5121 and BN159_5122 (3.4e−65), SSFG_04216 and SSFG_04217 (1.3e−56), SGM_5988 and SGM_5989 (1.3e−58), SSRG_03705 and SSRG_03706 (3.2e−62), SHJG_4627 and SHJG_4628 (8.6e−62), SSPG_04382 and SSPG_04383 (2.7e−59), SSDG_07138 (125aa) and SSDG_07139 (too short to compare), SCAB_53611 and SCAB_53621

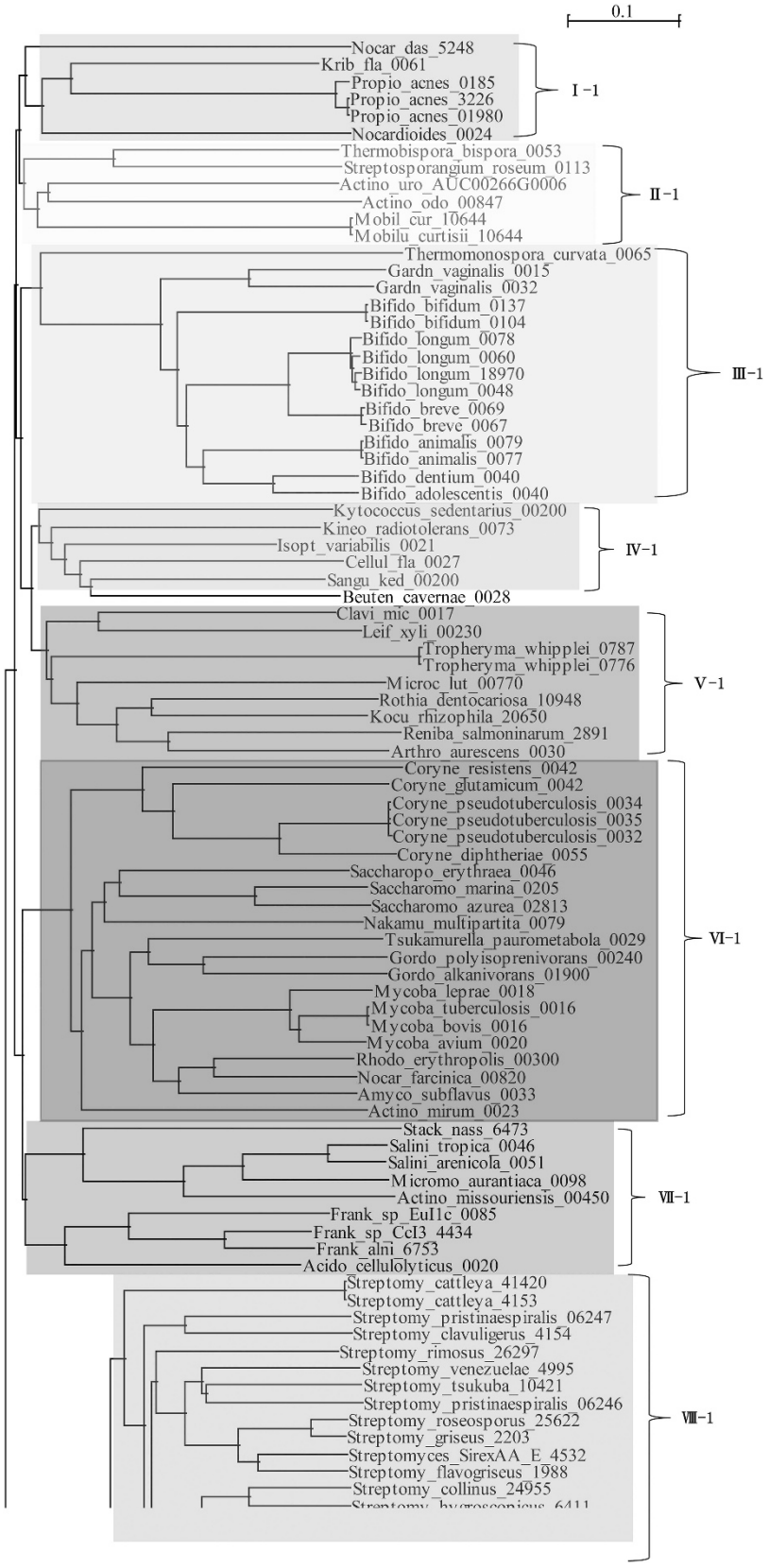
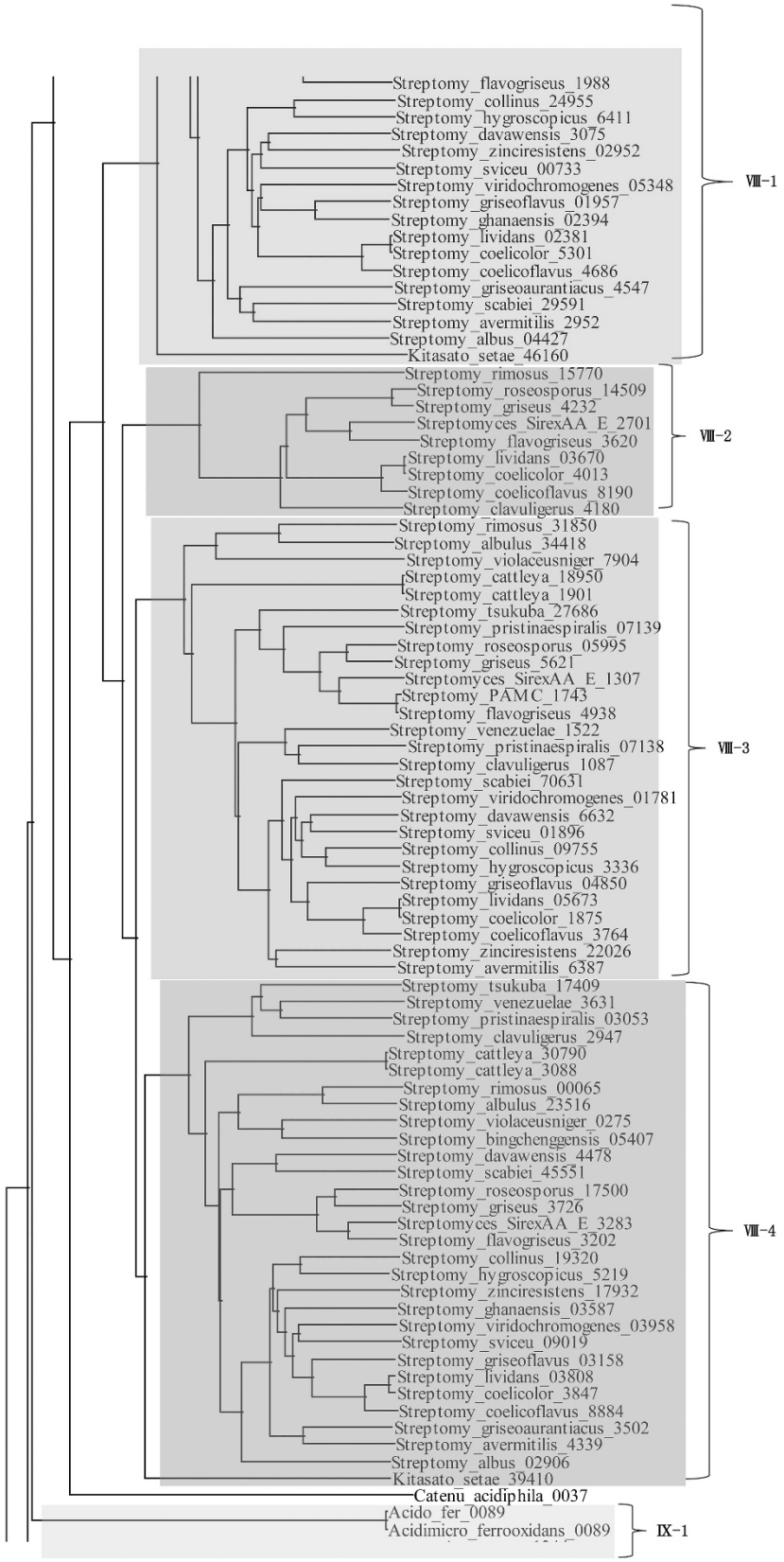


Figure 1 Phylogenetic tree of 446 class B PBPs listed in Table 1 from *Actinobacteria*. The tree was constructed by using ClustalX 2[40] as SCO4049 (penicillin acylase) as outgroup. A full color version of this figure is available at *The Journal of Antibiotics* journal online.



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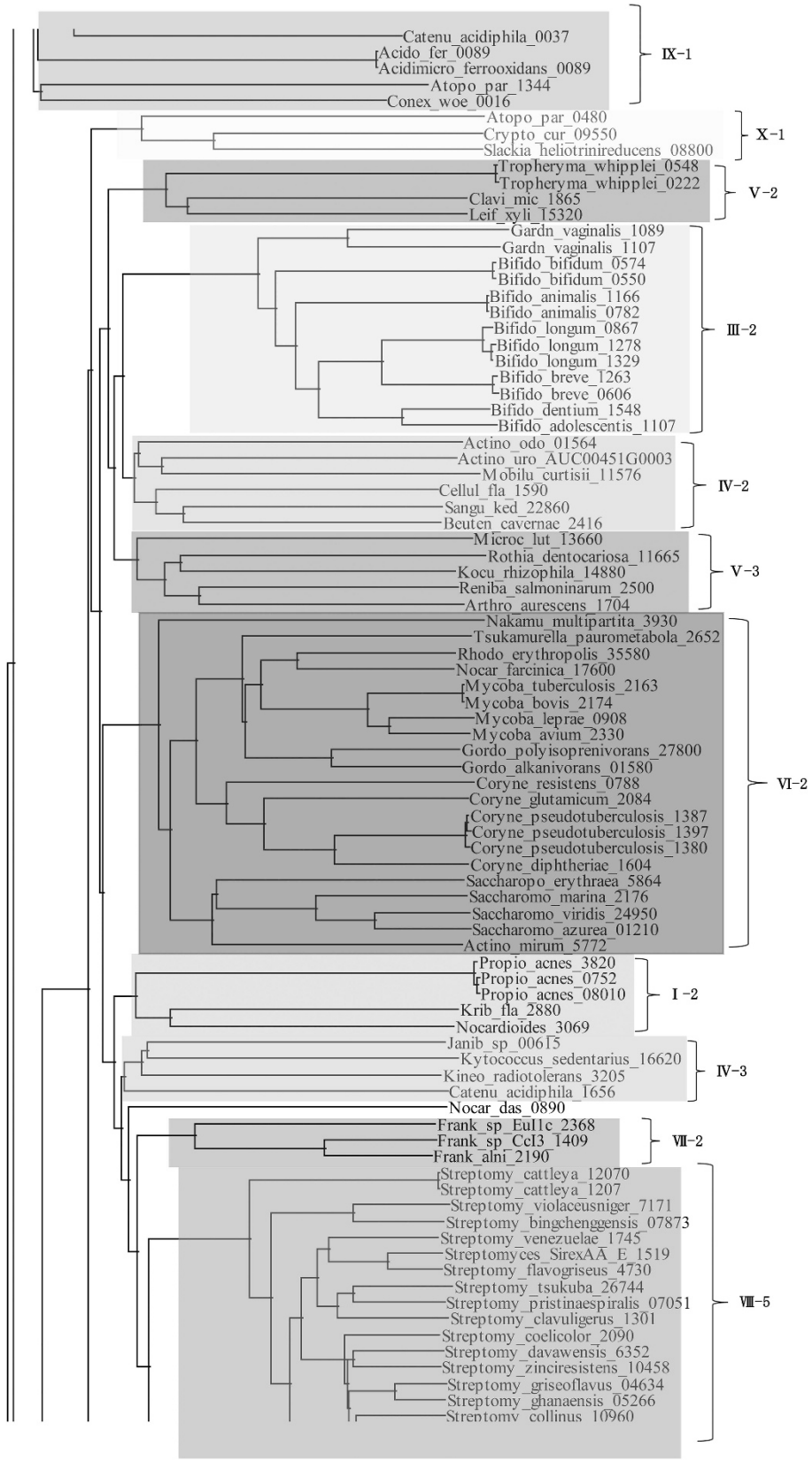

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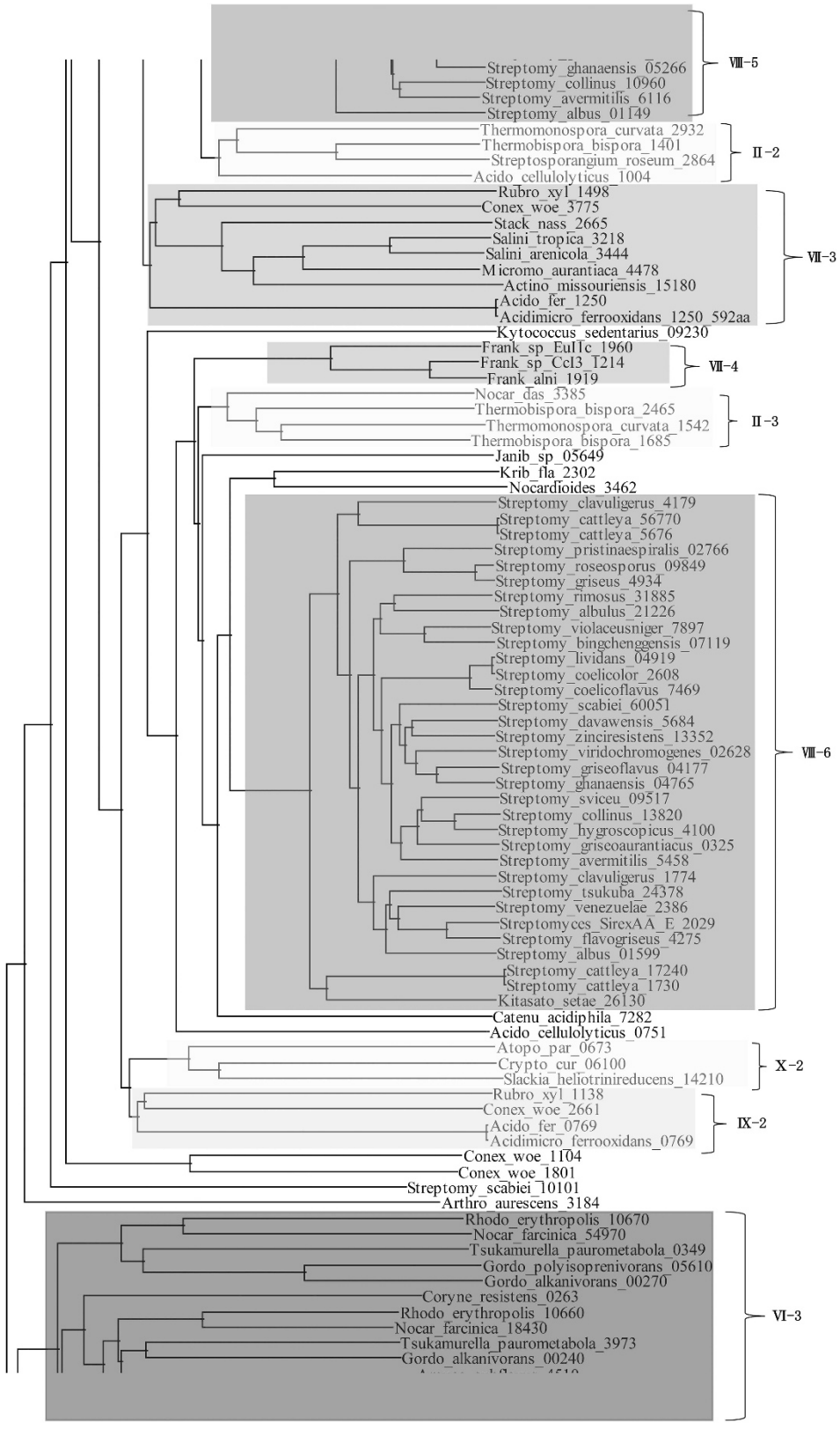

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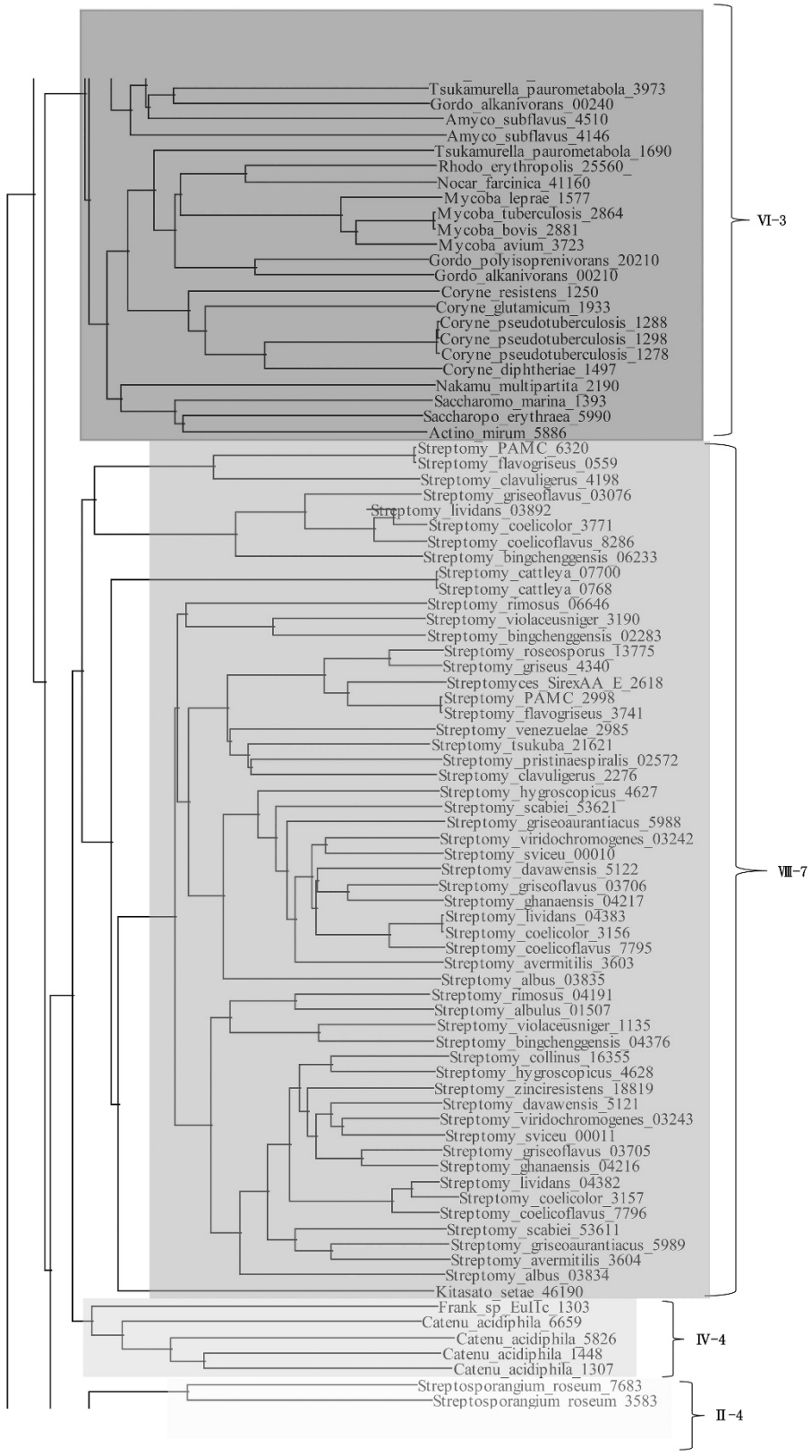

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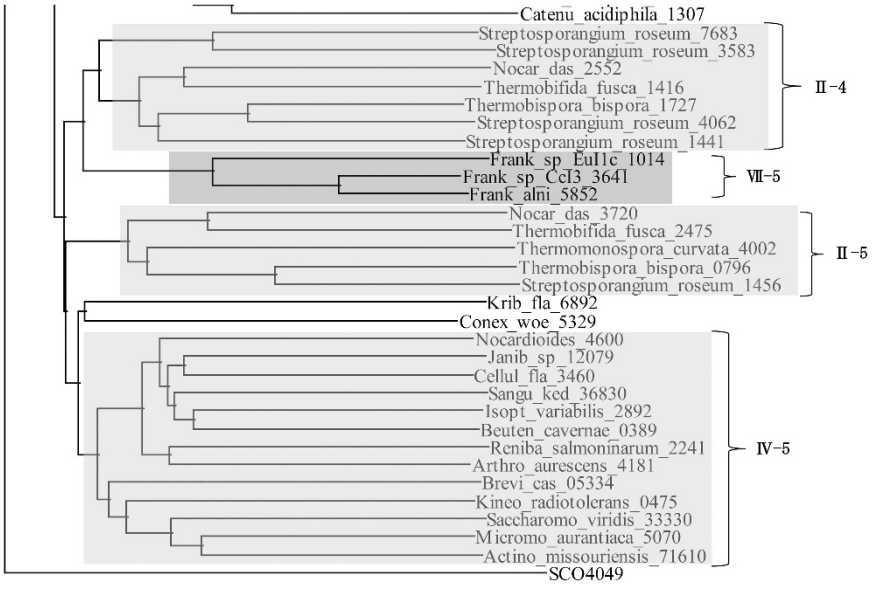

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Table 2 Comparison of genomic arrangements adjacent to PBPs in three Streptomyces species

S. clavuligerus (SCLAV)			*S. cattleya (SCAT)*			*S. coelicolor (SCO)*		
Gene no.	*No. of aa*	*Function*	*Gene no.*	*No. of aa*	*Function*	*Gene no.*	*No. of aa*	*Function*
1769	1456	Ribonuclease E	1725	136	Hypothetical protein	2603	518	Integrase
1770	251	Radical SAM protein	1726	148	Hypothetical protein	2604	311	Hypothetical protein
1771	651	Fe-S oxidoredactase	1727	61	Hypothetical protein	2605	233	Hypothetical protein
1772	520	Metal-binding protein	1728	651	Hypothetical protein	2606	661	Hypothetical protein
1773	401	RodA, FtsW	1729	401	Hypothetical protein	2607	398	Sfr protin
1774	773	Penicillin-binding protein	1730	738	Penicillin-binding protein	2608	769	Penicillin-binding protein
1775	219	MreD	1731	209	MreD	2609	223	MreD
1776	372	MreC	1732	302	MreC	2610	341	MreC
1777	343	MreB, Mbl	1733	343	MreB	2611	343	MreB
1778	144	Nucloside diphosphate kinase	1734	137	Nucloside diphosphate kinase	2612	137	Nucloside diphosphate kinase
1779	121	Membrane protein	1735	122	Hypothetical protein	2613	118	Hypothetical protein
1780	505	Dihydrofolate synthase	1736	510	Tetrahydrofolate synthase	2614	444	Tetrahydrofolate synthase
1781	417	Two component histidine kinase	1737	877	Valyl-tRNA synthetase	2615	874	Valyl-tRNA synthetase
1782	875	Valyl-tRNA synthetase	1738	284	Hypothetical protein	2616	335	Hypothetical protein
1783	386	PAT1 multi-domain protein	1739	427	ATP-dependent protease	2617	428	ATP-dependent protease
1784	464	ATP-dependent protease						

A full color version of this table is available at *The Journal of Antibiotics* journal online.

(3.2e−59), SSEG_00010 and SSEG_00011 (9.3e−61), and SSQG_03242 and SSQG_03243 (3.5e−61). The amino-acid sequences of these pairs of PBPs are not only very similar to each other, but also all the sequences are closely related and pertain to the subcluster VIII-7 in the phylogenetic tree (Figure 1). That is, the amino-acid sequence identity and similarity of PBPs in subcluster VIII-7 are in the range of 49.2–51.8% and 71.8–77.8%, respectively. Furthermore, the nucleotide sequences of each pair are arrayed in the same direction, indicating that they were duplicated and transferred to each other very recently. The pair of *S. clavuligerus* SCLAV_4179 and SCLAV_4180 is an exception. *S. clavuligerus* is a cephamycin and clavulanic acid producer. These PBPs of *S. clavuligerus* belong to the different subclusters (VIII-6 and VIII-2) and the similarity of the amino-acid sequences is very low (the E-value is 1.5e−11). Although *S. clavuligerus* possesses two PBPs in subcluster VIII-7, SCLAV_2276 and SCLAV_4198, their similarity value E is not so low (3.1e−28). In addition, *S. cattleya*, a cephamycin and thienamycin producer, carries only one PBP (SCAT_0768) in this subcluster. This peculiar behavior may be related to β-lactam production. The two PBPs of *S. clavuligerus* SCLAV_4179 and SCLAV_4180 are located at the end of cephamycin-clavulanic acid biosynthetic gene cluster, but arrayed in the reverse direction. Moreover, PBP SCLAV_4179 in *S. clavuligerus* is reported to have a low affinity to β-lactam antibiotics and is essential to the growth,[23] consequently it is presumed to be involved in the self-resistance. Interestingly, the amino-acid sequence of SCLAV_4179 is highly similar to that of SCAT_5676 (the similarity value E is 8.4e−186) of *S. cattleya*, indicating that the PBP genes were interchanged between the two species as a whole-cephamycin biosynthetic

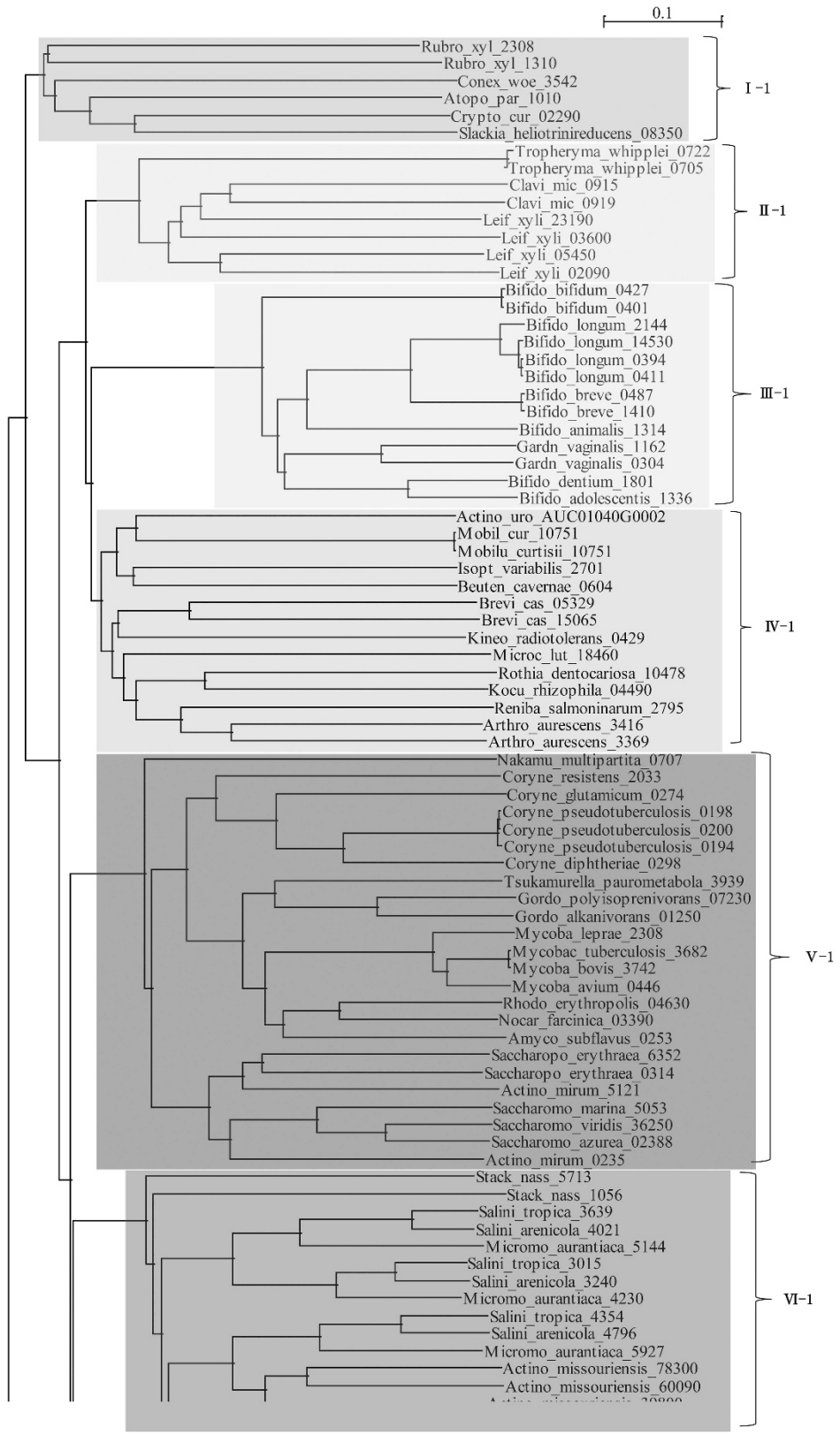


Figure 2 Phylogenetic tree of 292 class A PBPs listed in Table 1 from *Actinobacteria*. The tree was constructed by using ClustalX 2[40] as SCO4049 (penicillin acylase) as outgroup. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

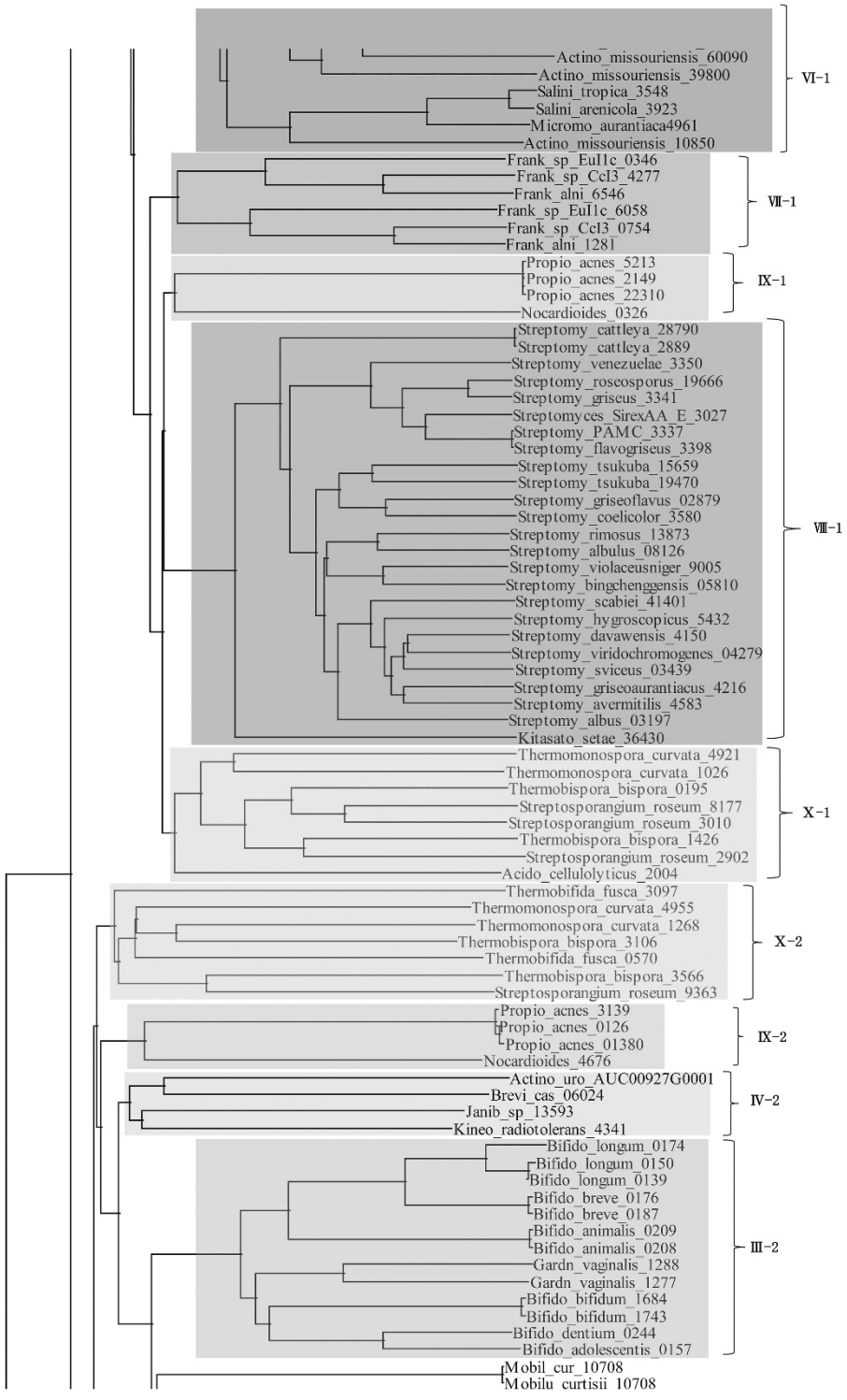
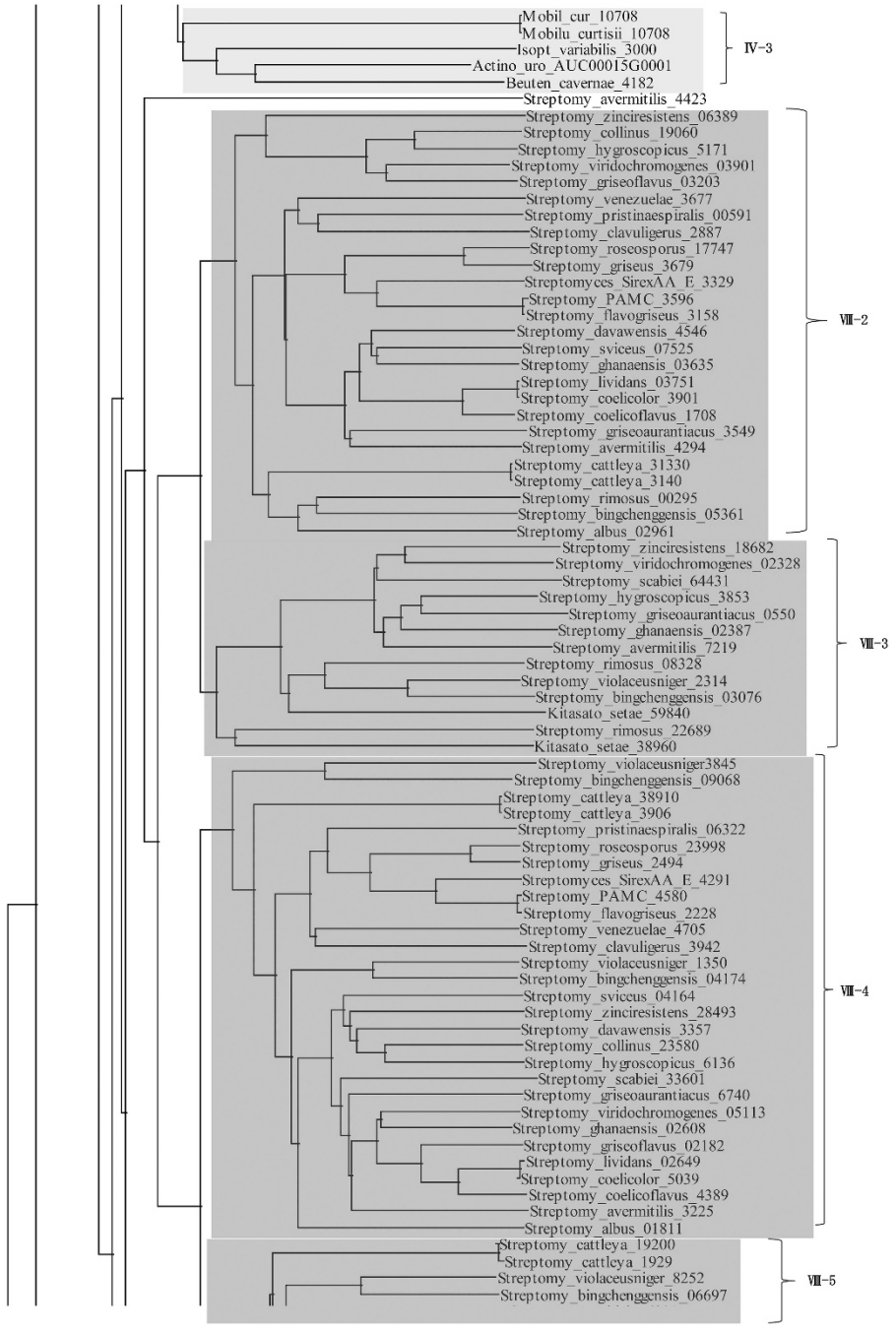


Figure 2 Continued.

gene cluster, and the clavulanic acid gene cluster was inserted in this region later. In *S. cattleya*, the protein of the similar amino-acid sequence to SCLAV_4180 is located not in the next to SCAT_5676 but in the completely different position as SCAT_3088, where no β-lactam biosynthetic gene is present. On the other hand, two proteins having highly similar amino-acid sequences to SCLAV_4179 (SCAT_5676 and SCAT_1730, the similarity E-values are 8.4e−186 and 1e−144, respectively) exist in *S. cattleya*. In *S. clavuligerus*, a similar PBP to SCLAV_4179 is present as SCLAV_1774 (E-value is 2.9e−159). Furthermore, a similar protein to SCLAV_4179 is also found in *S. coelicolor* (SCO2608, an E-value is 1.1e−160). Comparison of the genomic arrangements in these three species reveals similar arrangements of the genes, at least in the downstream of PBPs (Table 2). Moreover, similar proteins to SCLAV_4179 are present not only in



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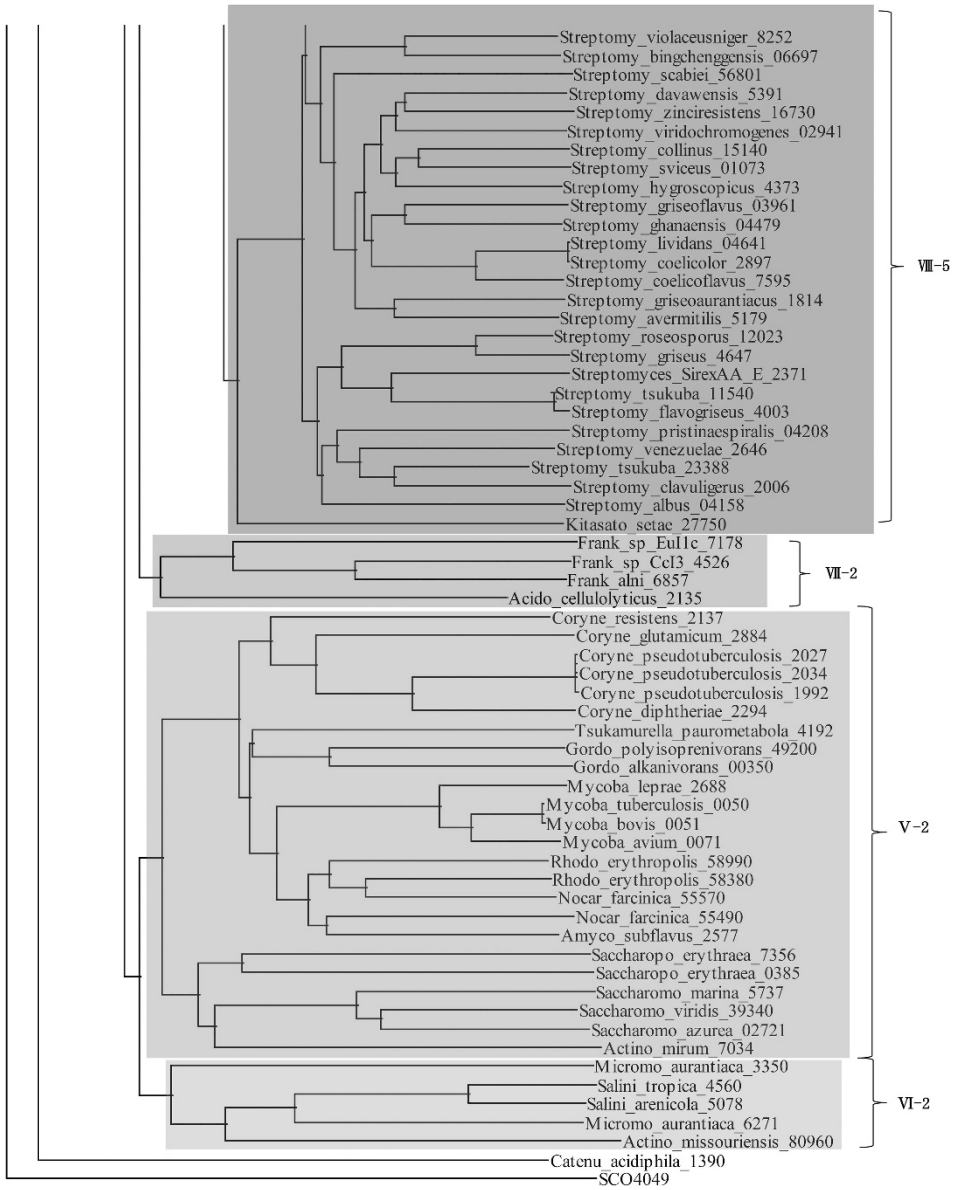
Streptomyces species such as *S. avermitilis* MA-4680 (SAV_5458, an E-value is 2.5e−158, the same hereafter), *S. lividans* TK24 (SSPG_04919, 3.7e−169), *S. viridochromogenes* DSM 40736 (SSQG_02628, 5.6e−161), *S. scabiei* 87.22 (SCAB_60051, 1.7e−174), *S. griseus* (SGR_4934, 4.5e−165) and *S. hygroscopicus* (SHJG_4100, 2.9e−163), but also in *Catenulispora acidiphila* DSM 44928 (Caci_7282, 1.2e−101), *Kitasatospora setae* KM-6054 (KSE_26130, 5.9e−137), *Kribbella flavida* DSM 17836 (Kfla_2302, 4.3e−105) and *Nocardioides* sp. JS614 (Noca_3462, 2.0e -108).[24] That is, the amino-acid sequence identity and similarity are in the range of 74.3–75.5% and 90.8–92.5% in *Streptomyces*, respectively, and 45.7–62.7% and 72.6–83.2% in other species, respectively. None of these species is reported to produce β-lactam antibiotics, suggesting that SCLAV_4179 and its analogs in *Streptomyces* at least are not related to β-lactam biosynthesis but associated only with β-lactam resistance. This is supported by the fact that similar proteins to SCLAV_4179 are also found in other *Actinobacteria*, such as *Thermomonospora curvata* DSM 43183 (Tcur_1542, 6.3e−99), *Frankia* sp. EuI1c (FraEuI1c_1960, 4.9e−76), *Nocardiopsis dassonvillei* (Ndas_3385, 5.2e−108) and *Janibacter* sp. HTCC2649 (JNB_05649, 4.3e−72), β-proteobacteria, such as *Methylobacillus flagellatus* KT (YP_546600, 5.2e−40) and *Janthinobacterium lividum* (WP_010393193, 3.9e−49), and γ-proteobacteria, such as *Plesiomonas shigelloides* (WP_010864271, 9.8e−36) and *Pseudomonas putida*



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(WP_009397921, 4.3e−38). The PBP of *S. clavuligerus*, SCLAV_4179, is reported to have a low affinity to β-lactam antibiotics.[23] Therefore, the low-affinity PBP gene of *S. clavuligerus* (SCLAV_4179) is supposed to overspread to most *Actinobacteria*, especially to *Streptomyces* species, to have a major role in β-lactam resistance and to reflect on the fact that most *Streptomyces* species, in particular, are highly resistance to benzylpenicillin, although they are Gram-positive bacteria.[25] In addition, two low-affinity type PBPs, SCLAV_1774 and SCLAV_4179, and SCAT_1730 and SCAT_5676, greedily present in *S. clavuligerus* and *S. cattleya*, β-lactam-antibiotic producers, reinforces their self-resistance to their own β-lactams.

Another PBP proposed to be involved in the self-resistance in *S. clavuligerus* is SCLAV_4198.[26,27] SCLAV_4198 belongs to subcluster VIII-7 together with SCLAV_2276. Most *Streptomyces* species occupy two or three PBPs in this subcluster. These PBPs are supposed to strengthen further the self-resistance against β-lactam antibiotics in these *Streptomyces* species. Furthermore, most of these PBPs are adjacent to each other, as described above. *S. clavuligerus* is again an exception.

Goffin and Ghuysen[18,26] showed that class B PBPs from Gram-positive bacteria were classified into three distinct subclasses, B1 (whose prototype is *Enterococcus faecium* PBP5, X84859), B4 (whose prototype is *S. pneumoniae* PBP2x, P14677) and B5 (whose prototype is *S. pneumoniae* PBP2b, P10524). Phylogenetic and similarity analyses indicate that all the PBPs from *Streptomyces* analyzed in this paper form disparate clusters from these subclass members, and SCLAV_4179 is only distantly related to class B1/B2 PBPs, low-affinity class PBPs, rather than class B4 or B5 PBPs, whether they are analyzed in whole sequence or penicillin-binding core sequences[26] (see Supplementary Figure 1).

Protein kinases are classified into two families based on their biochemical similarities and enzymatic specifications as following: the histidine kinase superfamily belonging to the two component systems[28] and the serine/threonine and tyrosine protein kinase

Table 3 Similarity of amino-acid sequences of class A PBPs in *Streptomyces* (E-values)

	SCAT_2889 ①	*SGR_3341* ①	*STSU_19470* ①	*SCAT_3140* ②	*SGR_3679* ②	*SRIM_00295* ②	*SAV_7219* ②	*SHJG_3853* ③	*SRIM_08328* ③	*SGR_2494* ④	*SAV_3225* ④	*SHJG_6136* ④	*SGR_4647* ⑤	*SHJG_4373* ⑤
SCAT_2889 ①														
SGR_3341 ①	1.2e−114													
STSU_19470 ①	6.3e−114	9.5e−121												
SRIM_13873 ①	3.6e−126	3.1e−127	5.2e−123	1.7e−26	1.2e−30	7.4e−29	4.7e−25	7.3e−22	1.1e−24	5.2e−29	7.6e−30	1.6e−28	6.0e−31	8.0e−26
SHJG_5432 ①	2.3e−121	1.7e−119	2.3e−135	7.4e−33	4.9e−33	1.3e−33	2.5e−27	4.8e−27	2.3e−27	6.1e−39	6.8e−31	2.1e−25	6.5e−32	8.7e−30
SCAT_3140 ②	2.0e−26	8.3e−29	1.1e−27											
SGR_3679 ②	8.7e−28	4.4e−28	7.8e−29	2.0e−93										
SRIM_00295 ②	3.6e−33	1.6e−31	6.0e−36	1.4e−98	9.1e−98									
SHJG_5171 ②	1.5e−30	8.5e−38	4.3e−40	4.5e−88	7.9e−92	3.5e−91	2.9e−64	3.5e−63	8.0e−70	9.8e−57	5.3e−52	6.9e−54	1.3e−54	1.9e−48
SAV_7219 ②	1.5e−24	3.6e−33	7.5e−31	5.6e−63	7.1e−70	2.5e−75								
SHJG_3853 ③	3.9e−28	1.5e−31	2.4e−30	3.4e−56	3.4e−80	2.6e−77	2.5e−135							
SRIM_08328 ③	2.8e−29	6.7e−38	1.9e−35	1.1e−87	3.8e−80	6.7e−79	2.5e−99	3.9e−91						
SGR_2494 ④	1.3e−31	3.7e−38	8.1e−30	2.5e−55	9.5e−60	1.7e−65	1.6e−40	1.2e−39	4.9e−49					
SAV_3225 ④	2.8e−34	6.0e−29	3.8e−30	5.0e−54	7.0e−50	2.1e−51	1.6e−44	2.2e−38	4.9e−45	1.2e−98				
SHJG_6136 ④	3.5e−31	3.9e−31	4.8e−31	1.1e−51	4.2e−57	1.2e−59	2.7e−46	2.2e−43	8.2e−52	7.6e−109	5.0e−120			
SCAT_3906 ④	2.2e−29	5.9e−39	1.4e−33	9.6e−47	1.7e−44	4.8e−56	1.7e−44	1.5e−40	3.4e−48	1.9e−81	2.8e−78	2.5e−79	1.8e−70	2.4e−59
SGR_4647 ⑤	1.9e−26	1.2e−34	4.0e−28	1.0e−57	8.6e−51	2.4e−56	1.9e−45	6.1e−43	2.9e−49	2.6e−66	2.0e−52	6.6e−70		
SHJG_4373 ⑤	1.3e−23	5.1e−29	1.1e−26	1.0e−54	1.7e−46	2.7e−50	1.2e−43	3.6e−43	1.9e−47	1.6e−62	1.7e−49	3.9e−63	2.4e−75	
SAV_5179 ⑤	1.4e−35	1.7e−33	1.7e−31	7.7e−49	1.0e−59	1.2e−52	1.9e−41	3.7e−41	3.7e−43	2.7e−67	1.5e−59	9.8e−71	5.4e−88	6.4e−89
SCAT_1929 ⑤	5.1e−26	5.2e−31	1.6e−32	2.5e−51	1.5e−55	6.6e−59	2.7e−43	1.0e−45	6.9e−48	3.5e−66	1.5e−60	9.2e−66	9.3e−86	2.4e−78
STSU_11540 ⑤	3.1e−29	1.9e−32	8.8e−29	1.3e−50	2.3e−47	7.5e−58	1.0e−46	2.7e−41	2.1e−47	7.0e−118	9.9e−99	4.0e−101	7.4e−71	2.3e−59
SFLA_4003 ⑤										1.9e−63	6.7e−60	6.4e−72		

Abbreviations: SCAT, *S. cattleya*; SGR, *S. griseus*; STSU, *S. tsukubaensis*; SRIM, *S. rimosus*; SAV, *S. avermitilis*; SHJG, *S. hygroscopicus*; SFLA, *S. flavogriseus*. Subcluster numbers are shown in the circle. A full color version of this table is available at *The Journal of Antibiotics* journal online.

superfamily.[29,30] Recently, these serine/threonine and tyrosine protein kinases were shown to be involved in the regulation of cell morphogenesis of *Streptococcus pneumonia*,[31] *Corynebacterium glutamicum*[32] and *Mycobacterium tuberculosis*,[33] germination of *Bacillus subtilis* spores[34] and polar growth and hyphal branching in *S. coelicolor*.[35] Although investigating the *S. coelicolor* homolog of PknB, a serine/threonine protein kinase of *M. tuberculosis*, Yeats *et al.*[36] identified a novel domain called PASTA domain that is found in the C-termini of eukaryotic-like serine/threonine kinases and PBPs. This domain binds β-lactam antibiotics and their peptidoglycan analogs. It is intriguing in this connection that serine/threonine protein kinases are present next to PBPs in *Streptomyces* species. Furthermore, these protein kinases carry four PASTA domains in tandem in these molecules. Such protein kinases are K530_23511, SSHG_02907, SAV_4338, SBI_05406, SCAT_3089, SCATT_30800, SCLAV_2946, SMCF_8885, SCO3848, B446_19315, BN159_4479, SFLA_3201, SSFG_03588, SGM_3503, SSRG_03159, SGR_3725, SHJG_5218, SSPG_03807, SSDG_03054, SRIM_00070, SrosN1_010100017505, SCAB_45561, F750_3547, SACTE_3284, SSEG_02705, STSU_17414, SVEN_3632, STRV1_0274, SSQG_03956 and SZN_17937. However, although these PBPs adjacent to the protein kinases belong to the same subcluster VIII-3, they have no PASTA domain in their molecules in contrast to PBPs in other bacteria. The PBPs in *Actinobacteria* such as *M. tuberculosis* class A PBP (accession number is YP_178005, the same hereafter), *Rhodococcus* sp. DK17 (WP_016884523) and *Nocardia* sp. BMG111209 (WP_019931711) possess one PASTA domain each in their C-terminal region. Intriguingly, the amino-acid sequences of the protein kinases in this group are almost the same with each other, especially in N-terminal regions containing the protein kinase domains. Protein kinases located adjacent to PBPs are also seen in other *Actinobacteria* such as Cfla_0025 and Cfla_0026, AMIR_0021 and AMIR_0022, TCUR_0063 and TCUR_0064, Snas_6471 and Snas_6472, and Afer_0087 and Afer_0088. Two protein kinases arrange in tandem, and then comes PBP. Although the function of these protein kinases and the relationship to PBPs are not known yet, they might involve in peptidoglycan biosynthesis in concert with PBPs.

CLASS A PBPS

A phylogenetic tree constructed on the basis of their amino-acid sequences of 292 class A PBPs from *Actinobacteria* is classified into 10 clusters and is shown in Figure 2. Like the class B PBPs, the PBPs from taxonomically related species form the same clusters. Accordingly, cluster I consists of PBPs of subclass *Rubrobacteridae* (*Rubrobacter* and *Conexibacter*) and *Coriobacteriae* (*Atopobium*, *Cryptobacterium* and *Slackia*). All the PBPs of *Trophyryma*, *Clavibacter* and *Leifsonia* compose cluster II. The similarity of the amino-acid sequences between TW_0722 and TWT_0705 is 100% except the C-terminal amino acid, where glutamic acid is replaced by aspartic acid. Amino-acid sequence similarity values (E-values) are 1.3e−68 between TW_0722 and CMM_0915, 1.2e−86 between TW_0722 and LXX_03600, and 2.5e−70 between TW_0722 and LXX_02090. Among *Micrococcineae* PBPs, PBPs of three genera (*Trophyryma*, *Clavibacter* and *Leifsonia*) behave as a group like the class B PBPs. Cluster III is made up of PBPs of order *Bifidobacteriales* (*Bi®dobacterium* and *Gardnerella*). These PBPs are divided into two subclusters, III-1 and III-2. PBPs of suborder *Actinomycineae* (*Actinomyces* and *Mobiluncus*) and *Micrococcineae* (*Isoptericola*, *Beutenbergia*, *Brevibacterium*, *Micrococcus*, *Rothia*, *Kocuria*, *Janibacter*, *Renibacterium* and *Arthrobacter*), together with two PBPs of genus *Kineococcus* (KRAD_0429 and KRAD_4341), form subclusters IV-1, IV-2 and

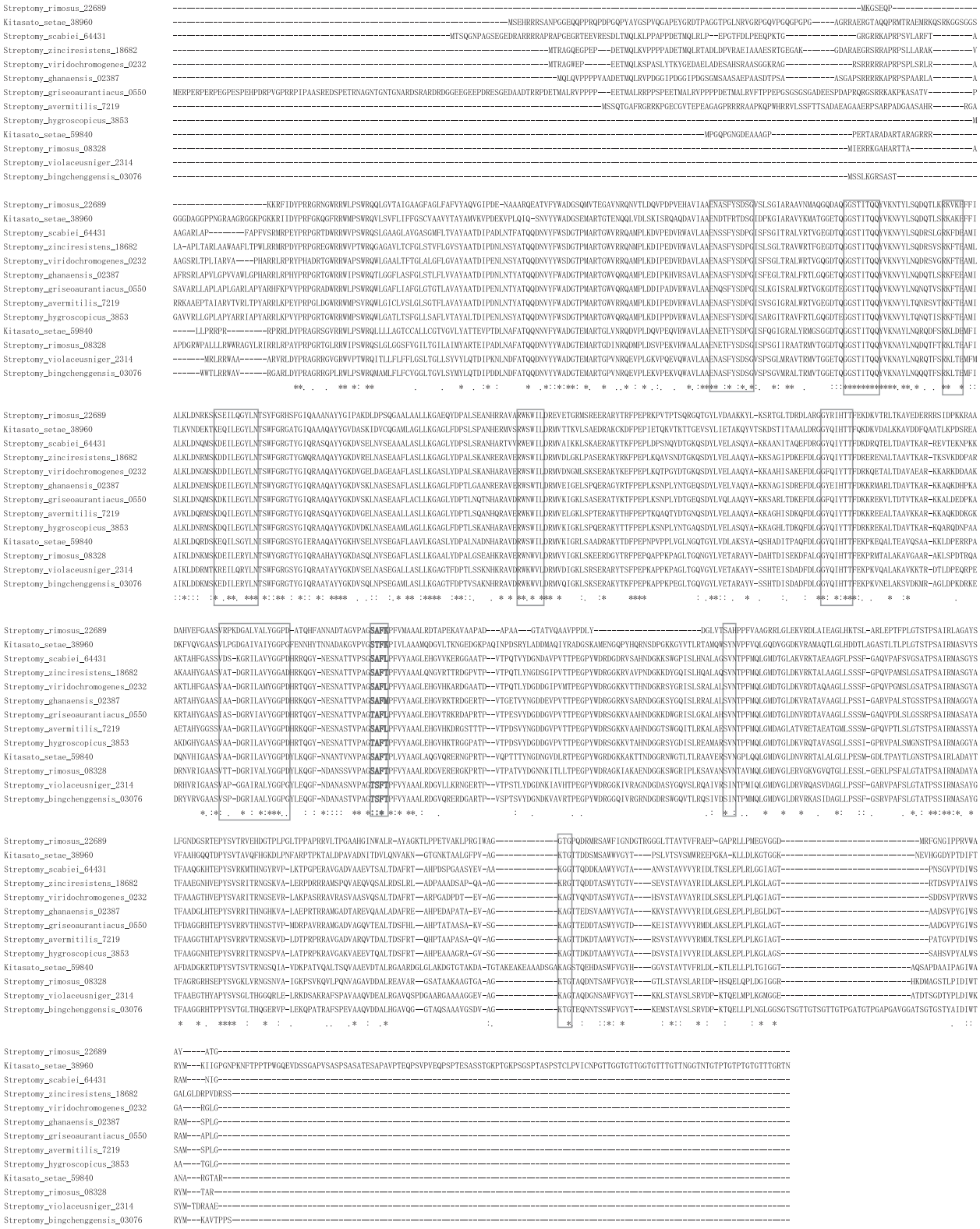

Figure 3 Amino-acid alignment of 13 PBPs in subcluster VIII-3 of the phylogenetic tree (Figure 2). The amino-acid sequences are aligned by using MUSCLE.[41] The conserved motifs[18] are boxed, and the essential SXXK sequences are marked with red, bold letters. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Table 4 Low-affinity and some closely related PBPs in Streptomyces

Penicillin-binding protein	*E-value (similarity value)*
Subcluster VIII-6	
Streptomy_clavuligerus_4179	7.2e−218 (100%)
Streptomy_clavuligerus_1774	1.4e−145
Streptomy_albulus_21226	3.8e−156
Streptomy_bingchenggensis_07119	4.1e−186
Streptomy_cattleya_5676	8.4e−186
Streptomy_coelicolor_2608	1.1e−160
Streptomy_davawensis_5684	1.1e−167
Streptomy_griseoflavus_04177	3.1e−167
Streptomy_griseus_4934	4.5e−165
Streptomy_hygroscopicus_4100	2.9e−163
Streptomy_scabiei_60051	1.7e−174
Streptomy_venezuelae_2386	3.7e−172
Subcluster VIII-7	
Streptomy_clavuligerus_4198	5.5e−144 (100%)
Streptomy_clavuligerus_2276	6.3e−34
Streptomy_albulus_01507	2.8e−28
Streptomy_bingchenggensis_02283	6.3e−30
Streptomy_cattleya_0768	6.7e−26
Streptomy_coelicolor_3156	2.5e−30
Streptomy_davawensis_5121	1.8e−30
Streptomy_griseoflavus_03076	1.9e−41
Streptomy_griseus_4340	1.1e−27
Streptomy_hygroscopicus_4628	8.5e−32
Streptomy_scabiei_53621	2.5e−25
Streptomy_venezuelae_2985	1.3e−24

IV-3. The class A PBPs of *Kineococcus radiotolerans* behave with those of suborder *Micrococcineae* ,such as *Brevibacterium*, but other PBPs of suborder *Micrococcineae*, such as *Isoptericola* and *Beutenbergia,* comport themselves with those of suborder *Actinomycineae*, such as *Actinomyces* and *Mobiluncus* (subclusters IV-1 and IV-3). PBPs of suborder *Corynebacterineae* (*Corynebacterium*, *Tsukamurella*, *Gordonia*, *Mycobacterium*, *Rhodococcus*, *Nocardia* and *Amycolicicoccus*) and *Pseudonocardineae* (*Saccharopolyspora*, *Saccharomonospora* and *Actinosynnema*) form subclusters V-1 and V-2 but different branches, although the amino acid similarity is not so different between PBPs of these branches. PBPs of genus *Nakamurella* move with those of suborder *Pseudonocardineae* like the class B PBPs, although genus *Nakamurella* is classified as suborder *Frankineae*. Subclusters VI-1 and VI-2 consist of PBPs of suborder *Micromonosporineae* (*Salinispora*, *Micromonospora* and *Actinoplanes*). PBPs of genus *Stackebrandtia* form outgroups in the phylogenetic tree as suggested by the taxonomic position. PBPs of genus *Frankia* form distinct subclusters VII-1 and VII-2, and those of genus *Propionibacterium* construct other discrete subclusters IX-1 and IX-2.

Like the class B PBPs, class A PBPs of *Streptomyces* species form large five subclusters, VIII-1, VIII-2, VIII-3, VIII-4 and VIII-5. Similarity analyses of class A PBPs from *Streptomyces* indicate that those inherent in the same subcluster have very low E-values irrespective of different species, especially PBPs in subcluster VIII-1 (Table 3). In addition, E-values between PBPs belonging to different subclusters but from the same species are not so different from those from different species. The amino-acid sequence similarity among class A PBPs are generally higher than those among class B PBPs. Another interesting fact clarified by amino-acid alignment analysis is that among 13 PBPs in subcluster VIII-3, 4 PBPs (SGM_0550, SHJG_3853, STRVI_2314 and SBI_03076) do not have essential serine residues in the motif SXXK. In addition, except two PBPs (SRIM_22689 and KSE_38960), they do not possess essential lysine residues, although other features[18,26] requisite for PBPs are conserved (Figure 3), suggesting that it is doubtful whether these PBPs function as transpeptidases or the transpeptidase activity is very low even though they retain penicillin-binding properties.

Class A PBPs from Gram-positive bacteria are classified into five subclasses,[26] A1 (whose prototype is *Escherichia coli* PBP1A), A2 (whose prototype is *E. coli* PBP1B), A3 (whose prototype is *Streptococcus pneumoniae* 1A), A4 (whose prototype is *S. pneumoniae* 2A) and A5 (whose prototype is *S. pneumoniae* 1B). Phylogenetic and similarity analyses indicate that all the class A PBPs from *Streptomyces* analyzed in this paper form a completely different cluster in a phylogenetic tree from these five clusters, where E-values are in the range of 3.4e−16 to 4.9e−31, indicating very low similarities (see Supplementary Figure 2). These results, together with the results in class B PBPs where E-values range from 9.3 to 7.7e−39, suggests strongly that the gene transfer and/or gene conversion occurred very rarely between PBPs in *Streptomyces* and those in Gram-positive and Gram-negative bacteria.

PBPS WITH LOW AFFINITY TO PENICILLINS

Ogawara and Horikawa[37] reported over 30 years ago that β-lactam-producing *Streptomyces* species possessed PBPs of very low affinity to benzylpenicillin. Later, two PBPs, that is, SCLAV_4179 and SCLAV_4198 were reported to have low affinity to penicillins.[23,27] A mutant disrupted in SCLAV_4198 gene exhibited a significant decrease in its resistance to benzylpenicillin and cephalosporins.[27] Moreover, a probe containing SCLAV_4198 hybridized to genomic DNAs from β-lactam producers, *S. jumonjinensis* NRRL 5741, *S. griseus* NRRL 3851 and *S. lipmanii* NRRL 3584, suggesting that SCLAV_4198-like sequences and SCLAV_4198-mediated resistance mechanisms are likely to be present in these β-lactam-producing species. Table 4 lists low-affinity PBPs and some of the closely related PBPs in *Streptomyces*. The PBPs belonging to subclusters VIII-6 and VIII-7 in Figure 1 are assumed to have low affinity to penicillins.

CONCLUSION

The work on self-resistance to β-lactam antibiotics in *Actinobacteria* in my research career started by the findings that most of the *Streptomyces* species constitutively produced β-lactamase independent of their resistance to β-lactam antibiotics and β-lactam production,[25,38] and the detection of PBPs in *Streptomyces* species by autoradiography took over 6 months instead of a few days in *E. coli*[16] and *B. subtilis*.[37] When I visited Dr Hamao Umezawa, my boss at that time, for the proofreading of the paper, he immediately said that 'The avoidance of the contamination of *Streptomyces* species was the most important and absolute necessity in the fermentation of *Penicillium* for the production of benzylpenicillin. It caused the complete destruction of benzylpenicillin because of their production of β-lactamases.' He knew by experience that most *Streptomyces* species produced β-lactamases. On the basis of these two findings, I proposed about 35 years ago in Antimicrobial Agents and Chemotherapy[37] and Microbiological Reviews[39] that low-affinity PBPs were the main cause of self-resistance to β-lactam antibiotics in *Streptomyces*. Since then, supporting evidence is gradually accumulating. This review offers some substantiating evidence from the points of PBPs for self-resistance and resistance in general to β-lactam antibiotics in *Streptomyces* even though they are Gram-positive bacteria.

1 Fleming, A. On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of *B. influenza*. Br. *J. Exp. Pathol.* **10**, 226–236 (1929).
2 Bush, K. & Jacoby, G. A. Updated functional classification of β-lactamases. *Antimicrob. Agents Chemother.* **54**, 969–976 (2010).
3 Spratt, B. G. The 2011 Garrod Lecture: from penicillin-binding proteins to molecular epidemiology. *J. Antimicrob. Chemother.* **67**, 1578–1588 (2012).
4 Gao, B. & Gupta, R. S. Phylogenetic framework and molecular signatures for the main clades of the phylum *Actinobacteria*. *Microbiol. Mol. Biol. Rev.* **76**, 66–112 (2012).
5 Ventura, M. *et al.* Genomics of *Actinobacteria*: tracing the evolutionary history of an ancient phylum. *Microbiol. Mol. Biol. Rev.* **71**, 495–548 (2007).
6 Chater, K. F., Biró, S., Lee, K. J., Palmer, T. & Schrempf, H. The complex extracellular biology of *Streptomyces*. *FEMS Microbiol. Rev.* **34**, 171–198 (2010).
7 Liras P., Martin J. F. Gene clusters for β-lactam antibiotics and control of their expression: why have clusters evolved, and from where did they originate?. *Int. Microbiol.* 2006; **9**: 9–19.
8 Ochi, K., Hosaka, T. New strategies for drug discovery: activation of silent or weakly expressed microbial gene clusters. *Appl. Microbiol. Biotechnol.* **97**, 87–98 (2013).
9 Núñez, L. E., Méndez, C., Braña, A. F., Blanco, G. & Salas, J. A. The biosynthetic gene cluster for the β-lactam carbapenem thienamycin in *Streptomyces cattleya*. *Chem. Biol.* **10**, 301–311 (2003).
10 Cundliffe, E. How antibiotic-producing organisms avoid suicide. *Annu. Rev. Microbiol.* **43**, 207–233 (1989).
11 Cundliffe E. & Demain A. L. Avoidance of suicide in antibiotic-producing microbes. *J. Ind. Microbiol. Biotechnol.* **37**: 643–672 (2010).
12 Davies, J. & Davies, D. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* **74**, 417–433 (2010).
13 Thaker, M. N. *et al.* Identifying producers of antibacterial compounds by screening for antibiotic resistance. *Nat. Biotechnol.* **31**, 922–927 (2013).
14 Macheboeuf, P., Contreras-Martel, C., Job, V., Dideberg, O. & Dessen, A. Penicillin binding proteins: key players in bacterial cell cycle and drug resistance processes. *FEMS Microbiol. Rev.* **30**, 673–691 (2006).
15 Typas, A., Banzhaf, M., Gross, C. A. & Vollmer, W. From the regulation of peptidoglycan synthesis to bacterial growth and morphology. *Nat. Rev. Microbiol.* **10**, 123–136 (2012).
16 Spratt, B. G. Distinct penicillin binding proteins involved in the division, elongation, and shape of *Escherichia coli* K12. *Proc. Natl Acad. Sci. USA* **72**, 2999–3003 (1975).
17 Suginaka, H., Blumberg, P. M. & Strominger, J. L. Multiple penicillin-binding components in *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli*. *J. Biol. Chem.* **247**, 5279–5288 (1972).
18 Goffin, C. & Ghuysen, J.-M. Biochemistry and comparative genomics of SxxK superfamily acyltransferases offer a clue to the mycobacterial paradox: presence of penicillin-susceptible target proteins versus lack of efficiency of penicillin as therapeutic agent. *Microb. Mol. Biol. Rev* **66**, 702–738 (2002).
19 Pratt, R. F. Substrate specificity of bacterial DD-peptidases (penicillin-binding proteins). *Cell Mol. Life Sci.* **65**, 2138–2155 (2008).
20 Sauvage, E., Kerff, F., Terrak, M., Ayala, J. A. & Charlier, P. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiol. Rev.* **32**, 234–258 (2008).
21 Ogawara, H. Self-resistance to β-lactam antibiotics in *Streptomyces* (in Japanese). *Bull. Meiji Pharmaceut. Univ* **43**, 1–20 (2014).
22 Zhi, X. Y, Li, W. J. & Stackebrandt, E. An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class *Actinobacteria*, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. *Int. J. Syst. Evol. Microbiol.* **59**, 589–608 (2009).
23 Ishida, K. *et al.* Characterization of *pbpA* and *pbp2* encoding penicillin-binding proteins located on the downstream of clavulanic acid gene cluster in *Streptomyces clavuligerus*. *Biotechnol. Lett.* **28**, 409–417 (2006).
24 National Institutes of Health Protein clusters: PCLA_902386. Available at http://www.ncbi.nlm.nih.gov/proteinclusters/?term=PCLA_902386. Accessed on 24 September 2014.
25 Ogawara, H. Production and properties of beta-lactamase in *Streptomyces*. *Antimicrob. Agents Chemother.* **8**, 402–408 (1975).
26 Goffin, C. & Ghuysen, J.-M. Multimodular penicillin-binding proteins: an enigmatic family of orthologs and paralogs. *Microb. Mol. Biol. Rev.* **62**, 1079–1093 (1998).
27 Paradkar, A. S, Aidoo, K. A, Wong, A. & Jensen, S. E. Molecular analysis of a β-lactam resistance gene encoded within the cephamycin gene cluster of *Streptomyces clavuligerus*. *J. Bacteriol.* **178**, 6266–6274 (1996).
28 Hoch, J. A. & Varughese, K. I. Keeping signals straight in phosphorelay signal transduction. *J. Bacteriol.* **183**, 4941–4949 (2001).
29 Molle, V. & Kremer, L. Division and cell envelope regulation by Ser/Thr phosphorylation: *Mycobacterium* shows the way. *Mol. Microbiol.* **75**, 1064–1077 (2010).
30 Urabe, H. & Ogawara, H. Cloning, sequencing and expression of serine/threonine kinase-encoding genes from *Streptomyces coelicolor* A3(2). *Gene* **153**, 99–104 (1995).
31 Morlot, C. *et al.* Interaction of penicillin-binding protein 2x and Ser/Thr protein kinase StkP, two key players in *Streptococcus pneumoniae* R6 morphogenesis. *Mol. Microbiol.* **90**, 88–102 (2013).
32 Fiuza, M. *et al.* Phosphorylation of a novel cytoskeletal protein (RsmP) regulates rod-shaped morphology in *Corynebacterium glutamicum*. *J. Biol. Chem.* **285**, 29387–29397 (2010).
33 Munshi, T. *et al.* Characterisation of ATP-dependent Mur ligases involved in the biogenesis of cell wall peptidoglycan in *Mycobacterium tuberculosis*. *PLoS ONE* **8**, e60143 (2013).
34 Shah, I. M., Laaberki, M. H., Popham, D. L. & Dworkin, J. A eukaryotic-like Ser/Thr kinase signals bacteria to exit dormancy in response to peptidoglycan fragments. *Cell* **135**, 486–496 (2008).
35 Hempel, A. M. *et al.* The Ser/Thr protein kinase AfsK regulates polar growth and hyphal branching in the filamentous bacteria *Streptomyces*. *Proc. Natl Acad. Sci. USA* **109**, E2371–E2379 (2012).
36 Yeats, C., Finn, R. D. & Bateman, A. The PASTA domain: a β-lactam-binding domain. *Trends Biochem. Sci.* **27**, 438–440 (2002).
37 Ogawara, H. & Horikawa, S. Penicillin-binding proteins of *Streptomyces cacaoi, Streptomyces olivaceus, and Streptomyces clavuligerus*. *Antimicrob. Agents Chemother.* **17**, 1–7 (1980).
38 Ogawara H., Horikawa S., Shimada-Miyoshi S. & Yasuzawa K. Production and properties of β-1actamases in *Streptomyces*.Comparison of the strain isolated newly and thirty years ago. *Antimicrob. Agents Chemother.* 1978; **13**: 865–870.
39 Ogawara H. Antibiotic resistance in the pathogenic and the producing bacteria, with special reference to β-lactam antibiotics. *Microbiol. Rev.* 1981; **45**: 591–619.
40 Larkin, M. A. *et al.* Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947–2948 (2007).
41 Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797 (2004).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)