REVIEW ARTICLE

Distribution of PASTA domains in penicillin-binding proteins and serine/threonine kinases of *Actinobacteria*

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PASTA domains (penicillin-binding protein and serine/threonine kinase-associated domains) have been identified in penicillinbinding proteins and serine/threonine kinases of Gram-positive Firmicutes and *Actinobacteria*. They are believed to bind β -lactam antibiotics, and be involved in peptidoglycan metabolism, although their biological function is not definitively clarified. *Actinobacteria*, especially *Streptomyces* species, are distinct in that they undergo complex cellular differentiation and produce various antibiotics including β -lactams. This review focuses on the distribution of PASTA domains in penicillin-binding proteins and serine/threonine kinases in *Actinobacteria*. In *Actinobacteria*, PASTA domains are detectable exclusively in class A but not in class B penicillin-binding proteins, in sharp contrast to the cases in other bacteria. In penicillin-binding proteins, PASTA domains distribute independently from taxonomy with some distribution bias. Particularly interesting thing is that no *Streptomyces* species have penicillin-binding protein with PASTA domains. Protein kinases in *Actinobacteria* possess 0 to 5 PASTA domain and 4 PASTA domain-containing groups. The 4 PASTA domain-containing groups can be further divided into two subgroups. The serine/threonine kinases in different groups may perform different functions. The pocket region in one of these subgroup is more dense and extended, thus it may be involved in binding of ligands like β -lactams more efficiently. *The Journal of Antibiotics* (2016) **69**, 660–685; doi:10.1038/ja.2015.138; published online 13 January 2016

INTRODUCTION

PASTA domains (penicillin-binding protein and serine/threonine kinase-associated domains) were first identified in the C terminus of Streptococcus pneumoniae penicillin-binding protein PBP2x.¹ In the crystal structure, van der Waal's interactions between the ß-lactam ring of cefuroxime, a second-generation cephalosporin, and one of two PASTA domains in PBP2x were observed.² In view of the structural similarity of the β-lactam ring of cefuroxime to the D-alanyl-D-alanine residues of the stem pentapeptide of peptidoglycan precursors, Yeats et al.3 proposed that PASTA domains bind uncrosslinked peptidoglycan. Subsequently, these domains were found in a variety of high MW penicillin-binding proteins (PBPs) as well as in serine/threonine kinases (STPKs) mainly from Gram-positive Firmicutes and Actinobacteria. The domains consist of 60-70 aminoacid residues and occur singly or as a few successive copies. Although PASTA domains show low amino-acid sequence similarity, they share strong structural conservation. Each domain has a globular fold consisting of three β -strands and one α -helix.

As for the interaction between PASTA domains in PBPs and β -lactam compounds, several studies have been published. The PASTA domains in PBP2x of *S. pneumoniae* bind the β -lactam antibiotic cefuroxime and a fluorescent penicillin Bocillin FL^{2,4} Furthermore, localization of PBP2x together with FtsZ and FtsW to cell division sites depends on its PASTA domains, but not on its transpeptidase activity.^{5,6} As PASTA domains bind uncross-linked peptidoglycan,⁷

it is suggested that localization of PBP2x is dependent on the localization of its substrate. In addition, alanine707 within the PASTA domain is important for stabilization.⁸ On the other hand, the PASTA domain of *Mycobacterium tuberculosis* PBP PonA2 does not bind muropeptide nor does it bind the two β -lactam antibiotics cefuroxime and cefotaxime, or polymeric peptidoglycan.⁹ In *Bacillus subtilis*, among 16 different PBPs only 2 of them, PBP2b and SpoVD, contain PASTA domain in SpoVD. However, the PASTA domain in SpoVD is not essential for cortex biosynthesis and not important for targeting SpoVD to the forespore outer membrane during sporulation.¹⁰ Therefore, functionality of PASTA domains in PBPs remains controversial.

The functions of the PASTA domains in STPKs are more clear.^{11,12} S. pneumoniae possesses only a single STPK StkP with four PASTA domains in its C-terminal region, and its corresponding phosphatase PhpP forms a functional pair with StkP.^{13,14} The PASTA domains of StkP in S. pneumoniae were shown to bind synthetic and native peptidoglycan and β -lactam antibiotics.⁷ In response to the binding, the PASTA domains are involved in the activation of StkP and substrate recognition.¹⁵ Activated StkP phosphorylates cell division proteins DivIVA and FtsZ. FtsZ is a prokaryotic tubulin homolog.^{15–18} Depending on the extracellular PASTA domains, StkP is recruited to cell division and bacterial growth.^{18–20} Therefore, cell wall

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biosynthesis and uncross-linked peptidoglycan are the signals for StkP to localize to cell division sites and stimulate its autophosphorylation activity.¹⁶

PknB (Rv0014c) is one of 11 STPKs but the only PASTA-containing STPK in M. tuberculosis.²¹⁻²³ The PknB of M. tuberculosis has four PASTA domains.²⁴ The extracellular PASTA domains bind muropeptides depending on the presence of specific amino acids at the second and third positions of the stem peptide.²⁵ In addition, the extracellular PASTA domain is required for proper localization of PknB to the mid-cell. The PASTA domains also have a role in stimulating growth by binding exogenous peptidoglycan fragments and are suggested to be a sensor for a signaling molecule that promotes initial growth.²⁴ PknB phosphorylates the DivIVA ortholog Wag31 in M. tuberculosis and Corynebacterium glutamicum,^{26,27} and this phosphorylation may trigger remodeling of bacterial morphology.²⁸ Peptidoglycan fragments can induce germination of dormant B. subtilis spores through interactions with the PASTA domain-containing STPK, PkrC in B. subtilis and the serine/threonine kinase PrkC from Staphylococcus aureus can induce germination of dormant spores in B. subtilis through interaction with peptidoglycan fragments.^{11,29}

Actinobacteria, especially Streptomyces species, are unique in that they are filamentous, soil-dwelling, Gram-positive bacteria and are characterized by their ability to undergo complex cellular differentiation similar to filamentous fungi.³⁰ Furthermore, Streptomyces species produce a wide variety of secondary metabolites including β -lactam antibiotics and enzymes.³¹ Previously, roles of PBPs in Actinobacteria were reviewed³² and STPKs in Streptomyces coelicolor A3(2) were discussed from an evolutionary point of view.³³ PASTA domains, as the name implies, are domains that suggest some type of relationship between PBPs and STPKs in Actinobacteria is of interest from the point of views of their functional role in peptidoglycan metabolism and their evolutionary origins. This review paper deals with this topic.

DISTRIBUTION OF PASTA DOMAIN IN PBPS

Table 1 summarizes the genome size (Mb), G+C%, the number of proteins, the number of putative class A and class B PBPs, PBPs with PASTA domain, the number of putative STPKs and STPKs with PASTA domain(s) in 95 species of *Actinobacteria*. PBP and STPK genes were selected at two steps. From the database (Microbial Genomes in NCBI, http://www.ncbi.nlm.nih.gov/genomes/MICROBES), putative PBP and STPK genes were screened with keywords 'PBP', 'penicillin-binding protein', 'STPK' and 'protein kinase' at the first step. For each species, the screened sequences were analyzed at the second step by aligning and constructing phylogenetic trees by using ClustalW as implemented by MEGA together with the representative sequences of PBPs and STPKs.

It is intriguing that PBPs with PASTA domains are detected only in class A PBPs in *Actinobacteria*. No class B PBPs in *Actinobacteria* contain PASTA domains. This is in sharp contrast to the cases in *B. subtilis, Clostridium perfringens* and *S. pneumonia*. All of the PBPs with PASTA domains in these bacteria, that is, PBP2b (GenBank accession number: BSU15160, the same thereafter) and SpoVD (BSU15170) in *B. subtilis,* SpoVD (BAB80270), SpoVD (BAB81569) and SpoVD (BAB81586) in *C. perfringens* and PBP2x in *S. pneumoniae* belong to the class B PBPs. Class A PBPs have both transplycosylase and transpeptidase domains, whereas class B PBPs have only transpeptidase domain. Therefore, it is interesting to know the interaction between transglycosylase and PASTA domains in *Actinobacteria*.

In Actinobacteria, most PBPs with PASTA domains possess only one PASTA domain, with the exceptions of Beutenbergia cavernae DSM 12333 BCAV 4182, Clavibacter michiganensis subsp. michiganensis NCPPB 382 CMM_0919, Isoptericola variabilis 225 ISOVA_3000, Leifsonia xyli subsp. xyli str. CTCB07 LXX_03600, Tropheryma whipplei str. Twist TWT_0705 and T. whipplei TW08/27 TW_0722, where each has two PASTA domains. In contrast, BSU15160 in B. subtilis subsp. subtilis str. 168, BAB80270, BAB81569 and BAB81586 in C. perfringens str. 13 and PBP2x in S. pneumoniae R6 each possess two PASTA domains, whereas only BSU15170 in B. subtilis subsp. subtilis str. 168 has one PASTA domain. These two features, that is, that PBPs with a PASTA domain belong absolutely to class A PBPs, and that there is only one PASTA domain in PBPs, are quite characteristic properties in PBPs in Actinobacteria. In addition, none of the Streptomyces species analyzed here has PBPs with PASTA domains (Figure 1 and Table 1) as discussed later.

Genome sizes and G+C contents are not apparently related to the presence of PBPs with PASTA domain(s). For example, some bacterial species with small genome sizes such as Bifidobacterium adolescentis (2.09 Mb), Mobiluncus curtisii (2.15 Mb) and Olsenella uli (2.05 Mb) have PBPs without PASTA domains, but Propionibacterium acnes (2.49 Mb) and T. whipplei (0.93 Mb) each have a PBP with a PASTA domain. On the other hand, bacterial species with large genome sizes such as Amycolatopsis mediterranei (10.24 Mb), Catenulispora acidiphila (10.47 Mb) and Streptosporangium roseum (10.37 Mb) have no PBP with PASTA domains, whereas Actinosynnema mirum (8.25 Mb) and Actinoplanes missouriensis (8.77 Mb) each have a PBP with a PASTA domain. Similarly, some bacterial species with high G+C contents such as Clavibacter michiganensis (72.5%), I. variabilis (73.9%) and Nocardiopsis dassonvillei (72.7%), and some with low G+C contents such as T. whipplei (46.3%) have a PBP with PASTA domains, whereas others with high G+C contents such as Geodermatophilus obscurus (74.0%), Micrococcus luteus (73.0%) and Streptomyces griseus (72.2%) or with low G+C contents such as Atopobium parvulum (45.7%) and Gardnerella vaginalis (41.2%) have PBPs without PASTA domains. Moreover, the presence of PBPs with PASTA domains seems to be distributed independently from taxonomic classification with some distribution bias (Figure 1). All species belonging to Propionibacteriales, Corynebacteriales and Micromonosporales possess PBPs with a PASTA domain. However, PBPs in Micrococcales species are divided into two groups: PBPs with a PASTA domain and those without a PASTA domain. Similarly, in Pseudonocardiales (Saccharopolyspora erythraea NRRL 2338 and A. mirum DSM 43827) and Coriobacteriales (Eggerthella lenta DSM 2243), some PBPs have PASTA domains but others do not. Of particular interest is that none of the Streptomyces species analyzed here has PBPs with PASTA domains (Figure 1 and Table 1). However, antibiotic production, which is a notable feature of many Streptomyces spp., is presumably not related to this phenomenon, because Saccharopolyspora erythraea, which produces erythromycin, possesses a PBP with a PASTA domain. The reason for the lack of PASTA domains in the PBPs of Streptomyces spp. remains to be clarified. Furthermore, no PBP with PASTA domain was detectable in orders Bifidobacteriales and Pseudonocardiales.

The PASTA domains were searched for in the PBPs of all of the *Actinobacteria* analyzed in this paper by Blast analysis of NCBI. The secondary structures of these PASTA domains were analyzed by using PSIPRED software (http://bioinf.cs.ucl.ac.uk/psipred/). The 51 PASTA domains detected by these analyses each comprise a small globular fold consisting of three β -sheets and one α -helix (Figure 2). The lengths of amino-acid residues in the PASTA domains are similar to

							PBP with	No. of	
		Genome	G+C	No. of			PASTA	protein	
Bacteria	Prefix	size (Mb)	%	protein	Class A PBP	Class B PBP	<i>domain</i> ^a	kinase	Protein kinase with PASTA domain ^a
Acidimicrobium ferrooxidans DSM 10331	AFER	2.16	68.3	1964		0089, 0769, 1250	None	4	0087(4), 0088(2), 1553(4)
Acidothermus cellulolyticus 11B	ACEL	2.44	66.9	2157	2004, 2135	0020, 0751, 1004	2004(1)	4	0019(3), 0986(4)
Actinomyces urogenitalis DORA_12	Q605	2.6	68.4	3034	AUC00015G0001, AUC00927G0001, AUC01040G0002	AUC00266G0006 AUC00451G0003	None	Q	Q605_AUC00266G0004(4), Q605_AUC00956G0003(4),
Actinoplanes missouriensis 431	AMIS	8.77	70.8	8124	10850, 39800, 60090, 78300, 80960	00450, 15180, 71610	60090(1), 78300(1)	26	470(3), 14740(4)
Actinosynnema mirum DSM 43827	AMIR	8.25	73.7	6916	0235, 5121, 7034	0023, 5772, 5886	0235(1)	29	0021(4), 1396(4)
Amycolatopsis mediterranei U32	AMED	10.24	71.3	9228	3027, 8853, 9287, 9288	2045, 2965	None	25	0051(4), 2318(4)
Amycolicicoccus subflavus DQS3-9A1	AS9A	4.86	62.2	4705	0253, 2577	0033, 4146, 4510	0253(1)	13	0031(4), 1301(4)
Arcanobacterium haemolyticum DSM20595	ARCH_RS	1.99	53.1	1732	08675	00530, 03395	None	б	00525(3), 03830(4)
Arthrobacter aurescens TC1	AAUR	5.23	62.4	4588	3369, 3416	0030, 1704, 3184, 4181	None	D	0028(3), 1694(4)
Atopobium parvulum DSM 20469	Apar	1.54	45.7	1353	1010	0480.0673.1344	None	m	1345(4)
Beutenbergia cavernae DSM 12333	BCAV	4.67	73.1	4197	0604, 4182	0028, 0389, 2416	0604(1), 4182 (2)	9 6	0026(3), 1908(4)
Bifidobacterium adolescentis ATCC 15703	BAD	2.09	59.2	1632	0157, 1336	0040, 1107	None	٢	0038(4), 0143(1), 1014(3)
Brachybacterium faecium DSM 4810	Bfae	3.61	72.0	3068	26280, 31830	06090, 10750, 26970	26280(1)	6	14290(4), 26990(4)
Brevibacterium casei S18	C272	3.66	68.1	3216	05329, 06024, 15065	05334	None	7	12727(1), 12732(3), 13024(3)
<i>Catenulispora acidiphila</i> DSM 44928	CACI	10.47	69.8	8913	1390	0037, 1307, 1448, 1656, 5826, 6659, 7282	None	52	6001(3)
Cellulomonas flavigena DSM 20109	Cfla	4.12	74.3	3678	3099, 3701	0027, 1590, 3460	3099(1), 3701 (1)	6	0025(3), 2064(4)
Clavibacter michiganensis subsp. michiganensis NCPPB 382	CMM	3.4	72.5	3078	0915, 0919	0017, 1865	0919(2)	00	1873(4)
Conexibacter woesei DSM 14684	Cwoe	6.36	72.7	5914	3542	0016, 1104, 1801, 2661, 3775, 5329	None	9	0017(4)
Corynebacterium diphtheriae NCTC 13129	DIP	2.49	53.5	2272	0298, 2294	0055, 1497, 1604,	0298(1)	4	0053(4), 1615(5)
Corynebacterium glutamicum ATCC 13032	NCGL	3.31	53.8	2959	0274, 2884	0042, 1933, 2084	0274(1)	വ	0040(4), 2095(5)
<i>Cryptobacterium curtum</i> DSM 15641	Ccur	1.62	50.9	1357	02290	06100, 09550	None	ς	03300(4)
Eggerthella lenta DSM 2243	ELEN_RS	3.63	64.2	3049	02953, 11730	06285, 10550, 12605, 15235	02953(1)	7	15240(3)
<i>Frankia alni</i> ACN14a	FRAAL	7.50	72.8	6700	1281, 6546, 6857	1919, 2190, 5852, 6753	6546(1)	50	3851(3), 4020(3), 5081(4), 6755(3)
<i>Frankia</i> sp. CcI3 Gardnerella vaoinalis HMP9231	FRANCCI3 HMPRFF9231	5.43 1 73	70.1 41.2	4499 1299	0754, 4277, 4526 1162-1288	1214, 1409, 3641, 4434 0015_1089	4277(1) None	29	3077(4), 3623(2), 4436(3) 0017(4)_0948(4)
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Table 1 The numbers and types of putative PBP and protein kinase genes

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							PBP with	No. of	
		Genome	C+C	No. of			PASTA	protein	
Bacteria	Prefix	size (Mb)	%	protein	Class A PBP	Class B PBP	domain ^a	kinase	Protein kinase with PASTA domain ^a
Gardnerella vaginalis ATCC 14019	HMPREF0421	1.67	41.4	1365	20263, 20394	20139, 20438	None	Ð	20142(4), 20603(4)
Geodermatophilus obscurus DSM 43160	GOBS_RS	5.32	74.0	4811	24855	16015, 19365	None	9	00320(1)
Gordonia polyisoprenivorans VH2	GPOL	5.67	67.0	5110	07230, 49200	00240, 20210, 27800	07230(1)	23	00220(4)
Isoptericola variabilis 225	ISOVA	3.31	73.9	2881	2701, 3000	0021, 1284, 2892	3000(2)	9	0019(4), 1812(4)
Janibacter sp. HTCC2649	JNB	4.23	68.4	4100	13593	00615, 05649, 12079	None	6	13208(3)
Jonesia denitrifican DSM 20603	JDEN_RS	2.75	58.4	2481	02160, 12675	05355	None	D	00860(4), 07220(4)
Kineococcus radiotolerans	KRAD	4.96	74.2	4681	0429, 4341	0073, 0475, 3205	0429(1)	9	0072(3), 3232(4)
SKSJUZIO	LON	0 70		7500	01110 26120 20060 E0010			C L	
Nitasatuspura setae NM-0034		0.10	14.1	1 200	2113U, 3043U, 3030U, 3304U	20130, 33410, 40100, 40130, 11000, 00050	N	7 r C	ZIDZU(3), 39400(3)
Kocuria rhizophila UCZZUI	KKH 	7.7	/1.2	/952	04490	1488U, ZU65U	None	4 0	149/U(3), 2U66U(1), 2U6/U(3)
Kribbella flavida DSM 17836	Kfla	7.58	70.6	6943	0444, 2401, 7017	0061, 2302, 2880, 6892	0444(1)	20	0060(3), 2845(4)
Kytococcus sedentarius DSM 20547	KSED	2.79	71.6	2554		00200, 09230, 16620	None	m	00180(1)
Leifsonia xyli subsp. xyli str. CTCB07	LXX	2.58	67.7	2028	02090, 03600, 05450, 23190	00230, 15320	03600 (2),05450(1)	9	00210(3), 00220(1), 15400(4)
Micrococcus luteus NCTC 2665	Mlut	2.5	73.0	2236	18460	00770, 13660	None	9	00750(3), 00760(2), 13750(3)
Micromonospora aurantiaca ATCC	MICAU	7.03	72.8	6222	3350, 4230, 4961, 5144, 5927,	0098, 4478, 5070	5927(1)	17	0100(3), 4509(4)
27029					6271				
Mobiluncus curtisii ATCC 43063	HMPREF0573	2.15	55.4	1909	10708, 10751	10644, 11576	None	с	10646(2), 11797(4)
Mycobacterium avium 104	MAV	5.48	69.0	5120	0071, 0446	0020, 2330, 3723	0446(1)	11	0017(4)
Mycobacterium bovis BCG str. Tokyo	ЛТΥ	4.37	65.6	3944	0051, 3742	0016, 2174, 2881	3742(1)	11	0014(4)
172									
Mycobacterium leprae TN	ML	3.27	57.8	1605	2308, 2688	0018, 0908, 1577	2308(1)	4	0016(4)
Mycobacterium tuberculosis H37Rv	RV	4.41	65.6	3906	0050, 3682	0016c, 2163c, 2864c	3682(1)	11	0014c(4)
Nakamurella multipartita DSM	NAMU	6.06	70.9	5240	0707	0079, 2190, 3930	0707(1)	17	0077(4), 2392(5), 3222(5),
44233									4030(1)
Nocardia farcinica IFM 10152	NFA	6.29	70.7	5934	03390, 55490, 55570	820, 17600, 18430, 41160	03390(1)	21	800(4), 17330(4)
Nocardioides sp. JS614	NOCA	5.29	71.5	4909	0326, 4676	0024, 3069, 3462, 4600	0326(1)	12	0022(3), 3086(4), 3705(1)
Nocardiopsis dassonvillei subsp.	Ndas	6.54	72.7	5497	0250, 0388, 4845, 5307	0890, 2552, 3385, 3720, 5248	5307(1)	35	0036(3), 31139(4), 5246(3)
dassonvillei DSM 43111									
Olsenella uli DSM 7084	OLSU_RS	2.05	64.7	1727	02875	04295, 05540, 08820	None	4	08825(4)
Propionibacterium acnes 266	PAZ	2.49	60.0	2345	01380, 22310	01980, 08010	22310(1)	4	c01970(3)
Propionibacterium acnes KPA171202	PPA	2.56	60.0	2297	0126, 2149	0185, 0752	2149(1)	4	0184(3)
Renibacterium salmoninarum ATCC	RSAL33209	3.16	56.3	3507	2795	2241, 2500, 2891	None	Ð	1800(2), 2512(4), 2892(2),
33209									2893(3)
Rhodococcus erythropolis PR4	RER	6.90	62.3	6437	04630, 58380, 58990	00300, 10660, 25560, 35580	04630(1)	∞	00280(4), 35760(4)
Rothia dentocariosa ATCC 17931	HMREF0733	2.51	53.7	2217	10478	10948, 11665	None	4	10946(3), 10947(2)
Rubrobacter xylanophilus DSM 9941	Rxyl	3.23	70.5	3140	1310, 2308	0022, 1138, 1498	None	00	0021(4)
Saccharomonospora azurea NA-128	SACAZ	4.76	70.0	4376	02388, 02721	01210, 02813	None	21	01512(4), 02811(4)
Saccharomonospora viridis DSM 43017	SVIR	4.31	67.3	3828	36250, 39340	24950, 33330	None	16	00360(4), 27990(4)

Table 1 (Continued)

PASTA domains in Actinobacteria H Ogawara

Table 1 (Continued)									
							PBP with	No. of	
		Genome	C^{+C}	No. of			PASTA	protein	
Bacteria	Prefix	size (Mb)	%	protein	Class A PBP	Class B PBP	domain ^a	kinase	Protein kinase with PASTA domain ^a
Saccharopolyspora erythraea NRRL 2338	SACE	8.21	71.1	7197	0314, 0385, 6352, 7356	0046, 5864, 5990	0314(1)	28	0044(4), 1710(4)
Salinispora arenicola CNS-205	SARE	5.79	69.5	4917	3240. 3923. 4021. 4796. 5078	0051. 3444	4796(1)	10	0053(3). 3480(4)
Salinispora tropica CNB-440	STROP	5.18	69.5	4536	3015.3548.3639.4354.4560	0046. 3218	4354(1)	0	0048(3), 3253(4)
Sanguibacter keddieii DSM 10542	Sked	4.25	71.9	3710	04860.37820	00200. 22860. 36830	37820(1)	9	00180(4), 15820(4)
Segniliparus rotundus DSM 44985	SROT RS	3.16	66.8	2999	05555	01875, 11430	05555(1)	4	01870(4)
Slackia heliotrinireducens DSM 20476	SHEL	3.17	60.2	2766	08350	08800, 14210	None	13	27070(4)
Stackebrandtia nassauensis DSM 44728	Snas	6.84	68.1	6379	1056, 5713	2665, 6473	None	43	1251(1), 1807(1), 2077(1), 2079(1),
									2132(1), 2149(1), 2236(1), 3561(1),
									5130(1), 5305(1), 6471(3)
Streptomyces albulus NK660	DC74	9.37	72.3	8086	3326, 4231, 4477, 5204, 7647	3128, 3135, 3598, 4185, 5459	None	40	2595(4), 4186(4)
Streptomyces albus J1074	XNR	6.84	73.3	5832	1770, 2736, 2983, 4127	1496, 2096, 2097, 3038, 4337, 4789	None	25	3037(4), 3064(1), 4768(4)
Streptomyces avermitilis MA-4680	SAV	9.12	70.7	7676	3225, 4294, 4423, 4583, 5179, 7219	2952, 3603, 3604, 4339, 5458, 6116, 6387	None	33	4338(4), 4371(1), 6092(4)
Streptomyces bingchenggensis	SBI	11.94	70.8	10 022	03076, 04174, 05361, 05810,	02283, 04376, 05407, 06233,	None	67	05406(4), 07851(4)
BCW-1					06697, 09068	07119, 07873			
Streptomyces cattleya NRRL8057	SCAT	8.09	73.0	7470	1929, 2889, 3140, 3906	0768, 1207, 1730, 1901, 3088, 4153,5676	None	18	1232(4), 3053(1), 3089(4)
Streptomyces clavuligerus	SCLAV	8.56	72.4	7287	2006, 2887, 3942	1087, 1301, 1774, 2276, 2947,	None	33	1326(4), 2946(4), 2991(1)
ATCC27064						4154, 4179, 4180, 4198			
Streptomyces coelicolor A3(2)	SCO	9.05	72.0	8152	2897, 3580, 3901, 5039	1875, 2090, 2608, 3156, 3157, 3771, 3847, 4013, 5301	None	37	2110(4), 3821(1), 3848(4)
Streptomyces collinus Tu365	B446	8.38	72.6	7113	15140, 19060, 23580	09755, 10960, 13820, 16355, 19320, 24955	None	39	11080(4), 19315(4), 19450(1)
Streptomyces davawensis JCM4913	BN159	9.56	70.6	8616	3357, 4150, 4546, 5391	3075, 4478, 5121, 5122, 5684, 6352, 6632	None	35	4396(1), 4479(4), 6328(4)
Streptomyces ghanaensis ATCC 14672	SSFG	8.51	72.2	7891	02387, 02608, 03635, 04479	02394, 03587, 04216, 04217, 04765, 05266	None	35	03552(1), 03588(4)
Streptomyces griseoflavus Tu4000	SSRG	8.05	71.7	6337	02182, 02879, 03203, 03961	01957, 03076, 03158, 03705, 03706 04177 04634 04850	None	35	03115(1), 03159(4), 04610(4)
Streptomyces griseus subsp. griseus NBRC 13350	SGR	8.55	72.2	7136	2494, 3341, 3679, 4647	2203, 3726, 4232, 4340, 4934, 5621	None	30	3725(4), 5391(4)
Streptomyces hygroscopicus subsp.	SHJG	10.38	71.9	9107	3853, 4373, 5171, 5432, 6136	3336, 4100, 4627, 4628, 5219, 6411	None	43	3594(4), 5218(4), 5252(1)
Jiriggangerisis 3000 Stroctomicos linidons TK2A	21 IV	0 25	002	2067	12100 10760 20300 22205	11966 19346 10036 10460	Nono	20	18030(4) 18175(1) 27165(4)
ouchiminares invitation invest		000	1 1 1		10170, 10100, 20200, 20200	21910, 21915, 24635, 28340		0	10000(4), 10110(1), 5/100(4)
Streptomyces rimosus subsp. rimo- sus ATCC10970	SRIM	9.5	71.9	8411	00295, 08328, 13873, 22689	00065, 04191, 06646, 15770, 26297, 31850, 31885	None	40	00070(4), 07563(1), 24996(4)

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							PBP with	No. of	
		Genome	G+C	No. of			PASTA	protein	
Bacteria	Prefix	size (Mb)	%	protein	Class A PBP	Class B PBP	domain ^a	kinase	Protein kinase with PASTA domain ^a
Streptomyces scabiei 87.22	SCAB	10.15	71.5	8746	33601, 41401, 56801, 64431	10101, 29591, 45551, 53611, 53621, 60051, 70631	None	43	0931(1), 45201(1), 67711(4)
Streptomyces sp. PAMC26508	F750	7.63	71.1	7073	2719, 3337, 3596, 4580	1743, 2434, 2998, 3546, 4834, 6320	None	27	1975(4), 3512(1), 3547(4)
Streptomyces sp. SirexAA-E	SACTE	7.41	71.7	6357	2371, 3027, 3329, 4291	1307, 1519, 2029, 2618, 2701, 3283, 4532	None	32	1542(4), 3284(3)
Streptomyces sviceus ATCC 29083	SSEG	9.31	69.8	8205	01073, 07525, 03439, 04164	00010, 00011, 00733, 01896, 09019, 09517,	None	40	02705(4), 06024(4)
Streptomyces venezuelae ATCC 10712	SVEN	8.23	72.4	7448	2646, 3350, 3677, 4705	1522, 1745, 2386, 2985, 3631, 4995	None	37	1769(4), 3592(1), 3632(4)
Streptomyces violaceusniger Tu4115	STRVI	11.14	71.0	8985	1350, 2314, 3845, 8252, 9005	0275, 1135, 3190, 7171, 7897, 7904	None	38	0274(4), 7194(4)
Streptomyces viridochromogenes DSM 40736	SSQG	8.65	71.1	7714	02328, 02941, 03901, 04279, 05113	01781, 02628, 03242, 03243, 03958, 05348	None	37	02054(4), 03956(4), 03996(1)
Streptomyces zinciresistens K42	SZN	8.22	72.5	7579	06389, 16730, 18682, 28493	02952, 10458, 13352, 17932, 18819, 22026	None	39	08009(4), 17937(4), 21616(1)
Streptosporangium roseum DSM 43021	SROS	10.37	70.9	8975	2902, 3010, 8177, 9363	0113, 1441, 1456, 2864, 3583, 4062, 7279, 7683,	None	71	0111(3), 1975(3), 2780(4), 8314(1)
Thermobifida fusca YX	TFU	3.64	67.5	3087	0570, 3097	1416, 2475	None	23	1041(4), 3066(3)
Thermobispora bispora DSM 43833	TBIS	4.19	72.4	3546	0195, 1426, 3106, 3566	0053, 0796, 1401, 1685, 1727, 2465	None	29	0055(3), 1337(4), 2915(1)
Thermomonospora curvata DSM 43183	TCUR	5.64	71.6	4890	1026, 1268, 4921, 4955	0065, 1542, 2932, 4002	1026(1), 4921(1)	49	0063(3), 3036(4)
Tropheryma whipplei str. Twist	TWT	0.93	46.3	808	0705	0222, 0776	0705(2)	4	216(3), 778(2)
Tropheryma whipplei TW08/27	ΤW	0.93	46.3	783	0722	0548, 0787	0722(2)	4	555(4), 789(2)
Tsukamurella paurometabola DSM 20162	TPAU	4.48	68.4	4242	3939, 4192	0029, 0349, 1690, 2652, 3973	3939(1)	12	0027(4), 2669(5)
Xylanimonas cellulosilytica DSM 15894	XCEL_RS	3.83	72.5	3413	07030, 15260, 16845	06420, 16455	16845(1)	9	00100(3), 10040(4)
Abbreviation: PASTA domain, penicillin-bi- ^a The number within parenthesis is the nur	nding protein and s nber of PASTA dom	erine/threonine 1ains.	kinase-a	ssociated do	omains.				



Figure 1 Phylogenetic tree of 95 species of *Actinobacteria* on the basis of nucleotide sequences of their 16S ribosomal RNA. Bacterial species having penicillin-binding proteins (PBPs) with PASTA domain (penicillin-binding protein and serine/threonine kinase-associated domains) are marked with red. The tree was constructed by using ClustalX 2,⁵⁴ and *B. subtilis* ribosomal RNA as the outgroup. Bootstrap probabilities are indicated at the nodes. The names of actinobacterial species, strain numbers, accession numbers and number of base pairs of 16S ribosomal RNAs are listed in Supplementary Table S2. A full color version of this figure is available at the *Journal of Antibiotics* journal online.

Leifsonia_xyli_03600_717_776_6	– IPD–LAGKTPAEAKSILT––GLGLDSA–DGGPQDSAQPAGT VSATDP–ATGTPVSKGTQVT VYTS–	
Nocardia_farcinica_03390_697_7	Q V P – D V V G M S Q S E A T T A L V – – S A G F Q V S A V – – N G S G P P P K G S V M S M A P – – N G S A I P – G S V I T I Y I S –	
Kribbella_flavida_0444_696_756	– L P F – V N G M N V Q D A T N R L Q – – Q A G F Q V T V S P E Q V R S T E A A G T V A Y T S P – R Q R D G A P E G S M V T L H I S –	
Eggerthella_lenta_02953_668_71	PS-AVGKTFEEAIQILNGYKAGYIEEYSDTVPEGTVISQSVQEGKVVIVVS	
Corynebacterium_diphtheriae_02	SYVGREGESVRKELSAKGYKVTMTS-APGDGIPKGSVVNVIAP-VPLKPGGTIELFISD	
Nakamurella_multipartita_0707_	Q V P – N V V G K N V D D A K T L L T – A A G F T V A V A P – K Q D T G A S V N I V V D Q N P – K T T A L P – G G Q I T L G V S –	
Ylanimonas_cellulosilytica_16	E V P R N L T G R D I A K V T Q T L E – – K L G L K V – – R P S P V D S D Q R K G T V L A V S G – – E G S T V P P G S T I T V D V S –	
Kineococcus_radiotolerans_0429	- V P S - V V G R S V A S A T S E L Q A A G F K V R V A G G T V S S Q S V P K G R V	
Actinoplanes_missouriensis_783	– – – – D V T C D S L G S A R D R L E – – G Q G F S V S V G S – T V D S K C P A G T V A S T N P – – S G R T I K – G G V V V L E I S –	
Sanguibacter_keddieii_37820_77	– VPDGLVGSSRDAAANAIA – AAGLTPAVT – EQFDVNPAGTVIGVNP-GSGAEVEPGSTVTIIVS –	
fropheryma_whipplei_0722_690_7	– V P N – V I G M T K E E A K L A L E – – Q I G L V Y E E K – – P G E E V T N D Q T V T N T D P – S P G S S V P Q G T S V T V – – –	
Actinoplanes_missouriensis_600	– I P – D V E C D P L G R A S N R L E – – R A G F T V D I G R – E V D S N C P A G T A A G T D P – S G R T V K – N G V V T I E V S –	
Salinispora_arenicola_4796_725	– I P – N V K C I S V E K A K S R I S – – G A G F V A V V G R N Q V E S N C P A G T A A G T S P – – D G R T A K – G S V V T I K V S –	
Acidothermus_cellulolyticus_20	T V P D – V A G Q D P Q T A V T T L Q – – Q L G L V V T V D P T P V Y S D Q P A N T V A G T D P – P A G S T V Y V G N T I T V H L S –	
Propionibacterium_acnes_2149_7	K I P - S T D G M T Y P Q A K A A I L K A G F R T K V W H V W D D S E P G T Y L G T D P E G S A H A - G A A V T L K I S -	
Leifsonia_xyli_05450_735_795_6	– V P D – V S G K T F A E A R D L L Q – – G L G L N V A P D P I P T M S Y Q P A G M V A G T N P – A A G T L V D G G A S V T I Q V S –	
Propionibacterium_acnes_22310_	KIP-STDGMTYPQAKAAILKAGFRTKVWHVWDDSEPGTYLGTDPEGSAHA-GAAVTLKIS-	
Frankia_alni_6546_792_851_60aa	VLPN-VVGQPQQVAEQILVQLGFHVTSQPVPAPVAPGIVVYMSP-APGGPVSINTEIRLSVS-	
Frankia_sp_CcI3_4277_753_811_5	– L P D – V V G Q P Q Q V A E Q T L V – – Q L G F H V R – – S Q P V P H P A P P G T V V A M S P – A P G S E V S L N T E V T L Q I S –	
[ropheryma_whipplei_0705_750_8	– V P N – V I G M T K E E A K L A L E – – Q I G L V Y E E K – – P G E E V T N D Q T V T N T D P – S P G S S V P Q G T S V T V – – –	
Beutenbergia_cavernae_4182_684	- V P D - V V G L D E A T A T A Q I G E V G L V A S V S Q E P S T Q P A G T V V H T D P - G A G T E V V V G S T V T L V V S -	
Thermomonospora_curvata_4921_6	E V P D – V R G R R V R E A I A I L R – – R A G L T W E I V P R R V P S D R P R G T V A T T S P – G P G D E I E P G G K V M L Y I S –	
Beutenbergia_cavernae_4182_770	P V P – D T T G M E A T A A Q V Q L S – – L A G F T V V R A D – E A S D Q Q P A G F V V R Q D P – – A G G T A P R G S T I T I V V S –	
[soptericola_variabilis_3000_6	E V P – N L V G M D I A R A T R E L E – – R L G L V L G – A P V A Q D S D Q P R G T V L A Q S S – – T G – Q V P V G S T I T L T V S –	
[soptericola_variabilis_3000_7	– V V P E V S G M T R T A A E Q Q L R – – N A G L R S S V R E – E E N A D V A P G T V I S T D P – G G G A T V E P G S T V T L I V A –	
fropheryma_whipplei_0705_814_8	TIPN-VIGMTKEEAVRLLSGQKLNISEIKVSSPTLAGKVISVLP-GVGTSISSNSVITITV	
Amycolicicoccus_subflavus_0253	Q V P – N V A G L T Q S T A I R R L E – – S S G Y S Y N T V – – W T S S A E S R G V V T S Y S P – – N G F A A P – G A T I T L Y V S –	
Rhodococcus_erythropolis_04630	Q V P – E V V G L S Q S A A T S R L Q – – A S G F T V N S V – – T T A S G A R A G T V T G V S P – – S G S A I P – G S T V T I Y I S –	
Salinispora_tropica_4354_728_7	SVEKAKSRITEAGFAVVVSPNRVESNCPAGTAAGTTPGGRTAK-GSVVTIKVS-	
Mycobacterium_bovis_3742_703_7	- V P - S V A G L D V D A A R Q R L K D A G F Q V A D Q T N S V N S S A K Y G E V V G T S P S G Q T I P - G S I V T I Q I S -	
Mycobacterium_avium_0446_680_7	- V P - S V A G L D I D A A R Q R I K E A G F Q V A D Q N N F V N S S A K Q G E V V G T T P S G A T I P - G S I I T I Q V S -	
Aicromomonospora_aurantiaca_59	– IP – G V K C E S I E T A K S R L K – – G A G F E P V V S S N K V P S E C P A G T A A G T S P – – D G R T I K – G G V V M I E V S –	
Mycobacterium_tuberculosis_368	– VP – SVAGLDVDAARQRLK – – DAGFQVADQTNSVNSSAKYGEVVGTSP – SGQTIP – GSIVTIQIS –	
Corynebacterium_glutamicum_027	– – – – – V V G Q S E A S A R R T L E – – A K G Y K V T T R S – V S G A G S A R G T V V S A T P – – Q G A V L I D G G T V I L D I S –	
Clavibacter_michiganensis_0919	T V P – N V Q G Q D M T G A R Q T L R – – S A G F D V T M A Q E Q V Q D N S Q I G K A T R T D P – – A A G Q Q S – S G P V T L Y I – –	
Actinosynnema_mirum_0235_716_7	- V P - D V V G K S V N D A T R V L Q E A G Y K V A Q Q S R N S A Q A K G T V V S Q T P R G S A L Q - G T L I T L L V S -	
Brachybacterium_faecium_26280_	V V P D – V S G M V L S E A E A T I E – – A A G Y K H E V K R E K N D D V G – Q W Y V I G T D P – T P G T D L P E G E T V V I R Q S –	
[ropheryma_whipplei_0722_754_8	TIPN-VIGMTKEEAVRLLSGQKLNISEIKVSSPTLAGKVISVLP-GVGTSISSNSVITITV	
Gordonia_polyisoprenivorans_07	– V P – D V T G L T A S D A T A R L Q – – A A G F K V T Q L – – E I D S P R P Q G T V V F T A P – – S D S A L P – G S T I T I Y V S –	
Beutenbergia_cavernae_0604_732	Q V P G – V I G Q D L A A A Q Q T L T – – A A G F Q V Q V – A E P V T S D Q A A G T V A E M D P – A E G S R V R P G T T I T L – – –	
Segniliparus_rotundus_05555_70	– I P N – V I G Q L K D D A V R Q L K – – D L D F P V T V V – – T D Y R WE P K D T V I S Q T P P G G G N S V V P G V A V T I H V S –	
Saccharopolyspora_erythraea_03	Q V P – N V V G H T A Q R A T E I L Q – – K A G Y R V Q E K – – S V N S Q R R Q G T V T S Q T P – – R G F A L P – G E L V T I A I S –	
[sukamurella_paurometabola_393	KIP-NIIGLNVNAAKARLESAGFKTTVSYVNNTAPKDTVVSTSPSGFTQP-GSTITLNLS-	
Mycobacterium_leprae_2308_702_	Q V P – S V T G L D L D A A R Q R L K – – G A G F Q V A D Q P T L I N S T A K L G A V V G T T P – – S G – – – – – – – – – – – – – – – –	
Leifsonia_xyli_03600_782_837_5	T I P N – V V G M K V S D A Q K T M A – – D A G F V I K L T – – G E S – – S P D A I V Q A E E P – G A G S A A R P G T <mark>Q V T L</mark> T – – –	
Cellulomonas_flavigena_3099_74	– V P S – V V G Q N E Q R A R E I L E – – G A G F R V S V S G E Q V P S S Y P A G T V A E Q S A – T L – – – A P R G S S I S L K I S –	
Streptococcus_pneumoniae_R6_63	PMPS-VKDISPGDLAEELRRNLVQPIVVGTGTKIKNSSAEEGKNLAPNQQVLILS	
Nocardioides_0326_704_764_61aa	T V P T – T S G M S V D Q A I S T L K – – A A G F N A I E G G T – A A S G N P A G T V A Y T A P – A E G S S A P E G S V T V Y E S –	
Thermomonospora_curvata_1026_6	E L P D – V R G L K L P D A M E R L R – – R A G F <mark>R – V V V G A A V D S E Y P A G T V A E T</mark> S P – G P G S R V E P G I <mark>T V T</mark> L – – –	
Nocardiopsis_dassonvillei_5307	– V P D – V I G R S E Q E A V S V L E – – A A G Y T A N V S A T R V R S P E P E G T V A A V N P – D P G T R L P E G A T V N V F L S –	
Clavibacter_michiganensis_0919	Q V P D – V S G M S P A D A Q S A I E – – G A G L A F A Q G G A R A S S S V P A G Q V A G S D P – G A G A N A A R G S T V T V F T S –	
Cellulomonas_flavigena_3701_70	Q V P S G L A G R T E A D A S G A L Q -	

Figure 2 Amino-acid alignment of 51 PASTA domains (penicillin-binding protein and serine/threonine kinase-associated domains) in penicillin-binding proteins (PBPs) of Actinobacteria. The amino-acid sequences are aligned by using ClustalX 2.54 The conserved amino acids are marked with red and colon. α-Helix are marked with yellow, and three β-sheets are with cyan, green and magenta, respectively. A full color version of this figure is available at the Journal of Antibiotics journal online.

other PASTA domains, that is, from 40 (Kineococcus radiotolerans KRAD_0429 719-758, the numbers at the end indicate the number of amino-acid sequences of the PASTA domain, the same hereafter) to 62 (Acidothermus cellulolyticus ACEL_2004 703-764; Cellulomonas flavigena Cfla_3701 701-762; C. michiganensis CMM_0919 710-771 and Thermomonospora curvata TCUR_4921 666-727) (Supplementary Table S1). In addition, conserved amino-acid residues, shown in red and marked with colons in Figure 2, are detectable in most PASTA domains. Moreover, helical domains are also preserved (Figure 2). A

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> phylogenetic tree was constructed on the basis of their amino-acid sequences (Figure 3). Many PASTA domains from the same order form clusters in the tree: PASTA domains within the Micrococcales group into four clusters with T. whipplei (four domains) forming one cluster, C. michiganensis and C. flavigena in a second cluster, Xylanimonas cellulosilytica, I. variabilis and B. cavernae (one domain each) in a third cluster and B. cavernae (two domains) and C. michiganensis (one domain) in a fourth cluster; Micromonosporales are represented in a single cluster comprising Salinispora tropica,

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PASTA domains in Actinobacteria

H Ogawara





Figure 4 Phylogenetic tree of 677 PASTA domains (penicillin-binding protein and serine/threonine kinase-associated domains) in serine/threonine kinases (STPKs) on their amino-acid sequences. These sequences are tentatively classified into 21 clusters. Position 1 PASTA domain (PASTA 1) were marked with yellow, position 2 PASTA (PASTA 2) with blue, position 3 PASTA domains (PASTA 3) with green, position 4 PASTA domains (PASTA 4) with red and PASTA 5 with white, respectively. The numbering of the PASTA domains are from N- to C-terminal regions in STPKs. The tree was constructed by using ClustalX 2.⁵⁴ A full color version of this figure is available at the *Journal of Antibiotics* journal online.



Figure 4 Continued



















Cluster 15

Figure 4 Continued

oacenaroporyspora_crynnaca_oace_0044_0_007_002_00aa 0.1 Corynebacterium glutamicum NCgl0040 3 507 564 58aa Corynebacterium diphtheriae DIP 0053 3 530 587 58aa Acidothermus_cellulolyticus_Acel_0019 1 355 415 61aa Stackebrandtia nassauensis Snas 6471 3 486 548 63aa Nocardioides JS614 Noca 0022 3 488 548 61aa Kitasatospora setae KSE 21520 3 523 578 56aa Streptosporangium roseum Sros 0111 2 448 506 60aa Leifsonia xyli Lxx15400 4 577 628 52aa Clavibacter michiganensis CMM 1873 4 586 643 58aa Acidimicrobium ferrooxidans Afer 0087 4 570 626 57aa Streptomyces viridochromogenes SSQG 03956 2 448 508 62aa Streptomyces ghanaensis SSFG 03588 2 447 507 61aa Streptomyces sviceus SSEG 02705 2 445 505 61aa Streptomyces davawensis BN159 4479 2 445 505 61aa Streptomyces lividans SLIV 19030 2 452 510 59aa Streptomyces coelicolor Sco3848 2 452 510 59aa Streptomyces_hygroscopicus_SHJG_5218_2_446_505_60aa Streptomyces_collinus_B446_19315_2_447_505_59aa Streptomyces zinciresistens SZN 17937 2 425 483 Streptomyces griseoflavus SSRG 03159 2 448 507 60aa Streptomyces_avernitilis_Sav_4338_2_447_505_59aa Streptomyces albus Xnr 3037 2 452 512 61aa Streptomyces rimosus SRIM 00070 2 446 506 61aa Cluster 17 Streptomyces albulus 4186 2 447 507 61aa Streptomyces venezuelae SVEN 3632 2 460 519 60aa Streptomyces SirexAA E SACTE 3284 2 448 507 60aa Streptomyces sp PAMC26508 F750 3547 2 450 509 60aa Streptomyces griseus SGR 3725 2 450 509 60aa Streptomyces clavuligerus Sclav 2946 2 447 507 61aa Rubrobacter xylanophilus Rxyl 0021 2 411 468 58aa Tsukamurella paurometabola Tpau 0027 3 534 592 59aa Streptomyces venezuelae SVEN 3632 3 525 585 61aa Streptomyces SirexAA E SACTE 3284 3 516 572 57aa Streptomyces sp PAMC26508 F750 3547 3 515 575 61aa Streptomyces griseus SGR 3725 3 515 575 61aa Brachybacterium faecium Bfae 26990 3 542 602 61aa Streptomyces rimosus SRIM 00070 3 513 573 61aa Streptomyces albulus 4186 3 514 574 61aa Streptomyces clavuligerus Sclav 2946 3 513 572 61aa Streptomyces cattleva Scat 3089 3 515 575 61aa Actinoplanes missouriensis AMIS 14740 4 606 658 53aa Rhodococcus erythropolis Rer 00280 3 504 562 59aa Nocardia farcinica Nfa 800 3 499 558 60aa Amycolicicoccus subflavus AS9A 0031 3 492 550 59aa Frankia alni FRAAL 3851 1 580 640 61aa Acidimicrobium ferrooxidans Afer 0087 2 437 496 60aa Stackebrandtia nassauensis Snas 2236 1 463 522 60aa Stackebrandtia nassauensis Snas 2077 1 426 490 65aa Stackebrandtia nassauensis Snas 5305 1 496 555 61aa Stackebrandtia nassauensis Snas 1807 1 505 561 57aa Cluster 18 Stackebrandtia_nassauensis_Snas_2079_1_427_487_61aa Stackebrandtia nassauensis Snas 3561 1 414 475 62aa Stackebrandtia nassauensis Snas 2132 1 420 488 69aa Stackebrandtia nassauensis Snas 1251 1 497 555 59aa Saccharomonospora viridis Svir 27990 4 600 656 57aa Saccharomonospora azurea Sacaz 01512 4 601 657 56aa Nocardioides JS614 Noca 3705 1 330 386 57aa Corynebacterium diphtheriae DIP 1615 5 672 725 54aa Tsukamurella_paurometabola_Tpau_2669_4_607_665_59aa Corynebacterium glutamicum NCgl2095 4 619 671 53aa Corvnebacterium diphtheriae DIP 1615 4 605 668 64aa Frankia sp Francci3 3623 1 565 625 61aa Cluster 19 Catenulispora_acidiphila_Caci_6001_3_547_601_55aa Tropheryma whipplei TW 555 4 542 598 57aa Stackebrandtia_nassauensis_Snas_5130_1_449_508_60aa Stackebrandtia nassauensis Snas 2149 1 408 468 61aa Tsukamurella paurometabola Tpau 2669 5 669 725 57aa Mohiluncus curtisii HMPREE0573 11797 4 607 662 56aa





Figure 4 Continued

Salinispora arenicola, Micromonospora aurantiaca and A. missouriensis (two domains). Corynebacteriales group into three clusters with C. glutamicum and Corynebacterium diphtheria domains in one subgroup, Nocardia farcinica, Rhodococcus erythropolis and Amycolicicoccus subflavus domains in a second subgroup, and Mycobacterium leprae, Mycobacterium avium, Mycobacterium tuberculosis and Mycobacterium bovis domains as the third subgroup. Finally, P. acnes (two domains) forms another subgroup (the order Propionibacteriales). Interestingly, there are numerous examples: two domains each in C. flavigena, C. michiganensis, I. variabilis and T. curvata, and three domains in L. xyli of domains that are found in a single species, but are located within different clusters. In addition, PBPs in Micrococcales and Corynebacteriales form several branches in the tree, respectively. Taken together with the fact that conserved amino-acid residues appear in most PASTA domains (Figure 2), these results suggest that some domains evolve independently from taxonomic classification, whereas others evolve vertically along with the species. Similar topological trees were obtained by using maximum parsimony and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) methods.

On the other hand, a phylogenetic tree constructed by using the amino-acid sequences of PBPs excluding PASTA domain shows clusters following taxonomic classification (Supplementary Figure S1). This is also the cases with trees constructed using transglycosylase (Supplementary Figure S2) as well as transpeptidase (Supplementary Figure S3) domains, confirming that at least some PASTA domains evolve independently of taxonomy and the amino-acid residues in both transglycosylase and transpeptidase domains are more conserved than those in PASTA domains.

DISTRIBUTION OF PASTA DOMAIN IN STPKS

In comparison with the PBPs, all bacterial species analyzed in this paper contain 1 to 11 protein kinases,³⁴ and each of these can have 0 to 5 PASTA domains (Table 1). That is, STPKs can be divided into two groups: PASTA-containing STPKs and those without PASTA domains. In general, however, most STPKs do not have PASTA domains. The STPKs in *S. coelicolor* SCO4423 (AfsK), SCO6681 (RamC), SCO3821 (PksC), SCO2110 (PkaF) and SCO3848 (PknB) have 0, 0, 1, 4 and 4 PASTA domains, respectively. Figure 4 shows a phylogenetic tree constructed by using amino-acid sequences of the 677 PASTA domains of STPKs in *Actinobacteria*. These PASTA domains were searched for in the STPKs of all of the *Actinobacteria* analyzed in this paper by Blast analysis. The secondary structures of

these PASTA domains were analyzed by using PSIPRED software (http://bioinf.cs.ucl.ac.uk/psipred/). Similar to the STPKs with PASTA domains of other bacteria, all of the STPKs with PASTA domains in Actinobacteria detected in this work comprise the serine/threonine protein kinase domain in the N-terminal region, and the PASTA domains locate in the far most C-terminal region. The number of amino-acid residues in PASTA domains and the composition of the secondary structures in PASTA domains are also similar (e.g. Figure 5). When these 677 sequences are tentatively categorized into 21 clusters, PASTA domains from the same relative positions within the STPKs in Actinobacteria have a tendency to be located within the same clusters. For example, many PASTA domains from position 1 (PASTA 1) fall into clusters 1, 6, 9, 10 and 13, with clusters 1 and 13 being especially prominent; those from position 2 (PASTA 2) fall into clusters 3, 4, 8, 9, 12 and 17, with 4, 9, 12 and 13 being especially prominent; those from position 3 (PASTA 3) fall into clusters 2, 3, 5, 7, 10, 11, 14, 16 and 17, with 5, 11, 14 and 16 especially prominent; and those from position 4 (PASTA 4) fall into clusters 8, 15, 19, 20 and 21, with 19, 20 and 21 being prominent. This is in accordance with the suggestion of Jones and Dyson⁵ that individual PASTA domains evolve position-dependently. Cluster 18 consists of only PASTA domains from Stackebrandtia, but these STPKs contain only one PASTA domain. STPKs, P. acnes Ppa_0181, P. acnes_Paz c01970 missouriensis AMIS_470 from cluster and Α. 20 and M. luteus Mlut_13750 in cluster 21 have only three PASTA domains. Therefore, their amino-acid sequences are more similar to those of position 4 (PASTA 4) instead of position 1 (PASTA 1) or 3 (PASTA 3), although they group into cluster 20 or 21, which consists of many position 4 (PASTA 4) sequences. Similarly, position 2 PASTA (PASTA 2) and position 1 PASTA (PASTA 1) domains of M. luteus Mlut_13750 belong to clusters 14 and 4, respectively. Similarly, STPKs, Stackebrandtia nassauensis Snas_5130, S. nassauensis Snas_2149 and Nocardioides JS614 Noca 3705 group with cluster 19, although they have position 1-type PASTA (PASTA 1) domains. These STPKs possess only one PASTA domain. Although some position 2 and position 3 PASTA domains of Streptomyces violaceusniger (Strvi_7194 and Strvi_7194, respectively) and of Streptomyces bingchenggensis (Sbi_07851 and Sbi_07851, respectively) cluster together with other position 2 or position 3 PASTA domains of Streptomyces species (cluster 12 and cluster 16, respectively), other position 2 and position 3 PASTA domains of S. violaceusniger (Strvi_0274 and Strvi_0274, respectively) and of S. bingchenggensis (Sbi_05406 and Sbi_05406,

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			PASTA 1		PASTA 2
S_cattleya_1232_361_636aa S_bingchenggensis_07851_361_649a S_violaceusniger_7194_361_646aa	VCGGAVVWYVGS IVVGLVLALGLGVGIWYINS VLATALAAGVWYISA	GQFTSVPPVLALTQDEAGKRLRÄAGLGV- GQFTKMPYLLGKSEADARKKLKDAGLEV- GQFTEVPPVLRKTQAEAAKKLHGAGLDV-	SFGEA-WSETVAKGRVVSTDPGPGAR KITRE-FSDVVDKGKVIKTDPGGGKQ KVVNE-FSDVIPKGKVIGSKPGPGER	IRGN-GTVRVVVSRGPHRVAVPDVTGQPLDAARDR IRNT-GTVTIHISDGPPRTQVPNVVGMPLKQARRQ IVRDT-GTVTIRVSQGPPRAEVPNVVGMPLAEARRA	SGAGLT-PGTVAKAFSDSVPAGSVISTDPA SDANLS-VGEVTKEFRDETPQGSVISTDPR ADQGLT-IGKVIRRFSSETAQDSILSTSPV
S_albulus_2995_01_2078a S_rimosus_24996_361_668aa S_venezuelae_1769_361_648aa S_albuluizgous_1226_261_644aa	ATGRSGAVAGLLRARRKAVSIVAAVLLVLGVGAGVWYINS 		SVKHA-FSPNVERGHVMATTPENGKR STKRA-FSDVYERGTVMAVDPAPGER	AV PDLAGT PLADARKAL IRGT-GTVVITLSKGPEIVQVPELAGTPVADAKKII IRGN-GTVTLTLSRGPEIVKVPNLKNKPLAEAKRII IPCN-CSVTIVISPCEUVDVDVGDVGPTIADARDKI	RDLGLV=POTEIRIPSDEVARGSVIRTDPA RDLGLT=VGTVRKEPSDEVARGSVISTDPA EREGLA=TGVVTTAFSEGVPQGAVISSDPE
S_c1avd11gerus_1320_361_644aa S_griseus_5391_361_644aa S_PAMC26508_1975_361_640aa S_SiroxA4_1542_361_639aa			SVRNG F SE I DIGA V SI DFG IGER SVERV-FSDTVERGSVVSSDPASGDR SVERA-YSDTVERGEV I SSDPASGER	IRGN-GSVILVVSRGFEVVVVFPVRGRILAAAEREL IRGN-GSVKLVVSRGPEVVVPVAGRSLADARREL IRGN-DAVKLVISRGPEVVKVPDVEGLALADARREL	ERVOLATION I REPNALDINGS VIRTERE KKVGLV-PGMATREFSEE I ARGEVIRTDPR KKAGEE-OCMVTRESSEE I ARGEVIRTDPA
S_3110AA_1042_301_035aa S_albus_4768_361_624aa S_davawensis_6328_361_646aa	VAVLLVLGVGAGVWYINS	GQFTRVPPLLAKTEDEARQRLADAGLEVG GQFTRVPPLLAKTEDEARQRLADAGLEVG COPTRVPALLSQPEAKAADRLEDAGLEVG	SVDKA-YSDTADRGTVTKTDPGTGER KVDRA-FSDTVKRGQVISTDPAVGER	VRGN-GSVNLTVSRGP ETVNTPVEGLASDARNEL VRGN-GSVNLTVSLGPRVVTVPRVEGMALDKAKARI IRQN-DSVNLVVSKGPEVVKVPDLGCVLDKARELI IRQN-DSVNLVSSCP	RDAGLE-PGMVTREFSDSVAKGEVVKTDFA RDAGLE-PGMVTRAFSDSVAKGEVVSTDPS AGAGLE-PGMVTREFSDSVARGSVISTDPT
S_zinciresistens_06009_661_0554a S_viridochromogenes_02054_361_65 S_griseoflavus_04610_61_346aa		GQFTEVPPLLAKTQAQAEDRLEDAGLDIG GQFTEVPPLLAKTQAQAEDRLEDAGLDLG GQFTKVPPLLAKTEKEARDRLATAGLDVG	VVGRA-TSDTVRRGTVISSDPGTGAR VVEHA-YSDTVDRGTVISSDPKTGAR ZVNEA-FSDTVERGTVISTDPRA	IRGN-DSVRLTISSOFRIVALFALGOFLAAAR BL IRGN-DSVSLTISDGPRIVRVPALAGQKLDEARSLI IRSH-DPVALTLSKGPETVRLPDLDGYPLDKARDVI IRSH-DPVALTLSKGP	KDDGLE-PGMVTREFSDD1TRGSVVSSDFG KDDGLE-PGMVTREFSEDVPKGSVISARPA KDEGLE-PGMVTREFSDGVPRGSVISTKPG
S_coeffcolor_2110_361_667aa S_lividans_27155_361_667aa S_avermitilis_6092_361_648aa		GQFTKVPPLLSKTEAQARDRLDDAGLDVG GQFTKVPPLLSKTEAQARDRLDDAGLDVG GQFTKVPPVLAKTQVEATAQLEKAGLDVK	VVRHA-1SD1VERGKV1STDPGVGDR VVRHA-YSDTVERGKV1STDPGVGDR QVRHD-YSDTVKRGTV1RSDPKPGAR	IRKN-DSVSLIVSDOFDIVKLPDVIGTKLDKARILL IRKN-DSVSLTVSDGPDTVKLPDVTGYKLDKARTLI IRDN-DSVTLTISDGPRTVKVPDLAGLPLSKARAEL	EDEGLE-PGMVTRAPSDEVARGPVISTRPG EDEGLE-PGMVTRAPSDEVARGPVISTRPG KADGLA-PGMVSRAVSDDVPKGSVISTDPA
S_collinus_11080_301_629_aa S_hygroscopicus_3594_361_655aa S_cattleya_3089_361_673aa			QVERE-ISDIVKSGIVIKIDPNPGAR QVKRA-YSDIVERGIVIGSDPAPGAR (GGDD-YCD-QDKGKICSQDPQSGR	IRSN-DSVILVISROPEVVKVPDLROVPLAKARARI IRDH-DSVTLTVSLGPQTVDVPDVAGQSLAKARARI 4DKG-QTITVVISKGAKPVEIPDESGKSFSDAQQDI	KAAGEE-PGLVNRAFSDSVARGSVVGTDPG KADDLE-PGMVTRAFSEDVPRGSVIGTTPE QSKGFTNIARKDDSGNSGKSPGTVLSQDPS
S_01ngcnenggens1s_05406_051_076a S_violaceusniger_0274_361_670aa S_albulus_4186_361_666aa	LVGAIFIGKSLFGG		VIGSPIPCDJARKIVIVCRQSAVGENA VVGSK-FCENADKDTVCEQNPESGGE KQGSK-QCDNAKKGQITEQDPAADEK	LERD TO I VELT INSKOP REPARTVE FED V LIVI QUE SAKDAL IKRY-GVVELTMSKGPKPARTVEVPDVLIVVQ ASAKDQL VDKG-TTVKVTMCSGA	EAKGFR-VIQKIEE-SDQIFGKVIDQDFK EAKGFK-VEQKTQQ-SDQTAGKVIDQNPK KSKGFQ-NITKTEEVSDRTFGVVTKQDPK
S_r1iiosus_00010_361_013aa S_albus_3037_361_662aa S_coelicolor_3848_361_673aa	GAIFIGKSLF6G		VSATE-ECEDQPAKHICSQDPEPGGGGAK IFKQQ-ECEDQPKGNICAQDPKQGTD	VERG-TFVRVINCIGFEKVIIPDVIGLIFDRAKED VERG-STVSVVVSTGAPKITVPDVQGLSFEKAEEQU VDKE-STVNLVVSTGAPKVAVPNVLDKNIDEAKKQL	KAKGFE-DVGATERVSDAFEGTVVDGDFA KAKGFTDIEQKAEE-SDQTPGTVIGQDPD EDKGFE-VETKQTE-SSQDEGTILSQNPD
S_11V10ans_15060_301_010aa S_collinus_19315_361_664aa S_hygroscopicus_5218_361_664aa			PPTRK-PCANQPKGNVCSQDPVAGSD AVTKK-PCEDQATGNVCSQDPQPDAK	VKKN-DTINIVVSTGA PKIDVPDVRGIQFDKAKSRI VKKN-STVNLVISTGA-PKIDVPDVRGIQFDKAKSRI VEKN-STVNLVISTGA PKIQVPDVRGIQFDQAKSQI	EAKGFT-VDKKTEV-SAQTPGVVIAQDPA KEKGFE-VEKKTQV-STQTPDVVISQDPE DVCEB-VEKKTQV-STQTPDVVISQDPE
S_avermittillS_4338_301_007aa S_zinciresistens17937_301_652aa S_davawensis_4479_361_666aa	DDGGYGYDDRVDRRRQKKSNTSTILLAVAGVLVLVGAILIGKWVFAG	D-RAANDI VPAPAR VGLI LADAKQQA VNGDVKL- E-SASNNSVAAPSLVGET QDSAKELLANVDLKLG V-GVGNTSTKAPALIGLT LPEAEKLANVAEVKL-	STIKK-PCEDQAKGNVCSQSPDAKIK 2VTIK-ECDN0PKNRICSQNPEAKIK 2STEK-PCEDQIKGKICSQIPTQGTV	VKKN-SLVNLVLSIGAPKIAVPDVIGLIPUKAKSQL IDKG-STIDVVVSTGAPKVSVPDVTGLKFNKAKDEL VDKN-STIEIVVSTGAPKVAVPSVLGDDFEDAKATI	ED&GFR-VERKTEV-SDQTPGVVTAQDPS ER&GFV-VEQKTKE-SDRTPGVVTAQDPE EGD&YA-FVVKKKEEVSSEPEGTVTAQDPE
5_gr1seoi1avus_03159_361_067aa S_ghanaensis_03588_361_667aa S_viridochromogenes_03956_361_67		DGVDNORVAAF NY VGE I KADAERLAV NGDLKL - GVSNDGVTVPNFVGQTKADAQERADNVELKL- - GVGNDTVPAPNLVGETVATARQLAKNSDLKL-	SFVER-PCEDQSKGA1CDQDFEAGTA SFEEK-PCENQSKGR1CEQDLKPETP STTNK-PCENQSKGK1CDQDPNAGVK	AEKG-DTVNLIVSTGAPKVVPPDVGGLTERAKSUL AEKG-DTVNLVVSTGAPKVAVPSVIGLSLEDAKAKL VKKG-DTINLVVSTGAPKVAVPSVVGDSLQDAKATL	EDRGF1-VEGANEV-SD4TFGVVIGQDFG EGDDYQ-LKIETQARESSETPNSVLEQNPP TGDRYQ-FEVKTESKESAEDPGTVLEQNPT
S_c1avu11gerus_2946_361082_aa S_venezue1ae_3632_361_673aa S_SirexAA_3284_361_655aa			QSGAE=RCD=QFKGR1CSQ1PADG1 EAGTE=RCD=QFKNS1CRQSPAADGTSK /GAKE=ECENQAEGK1CSQDPAADA1	VSKG-DIVITYLSEGAPKVEYPDVIEKSLUSARDAL METG-GTIQVYVSEGAPLIEVPDVIEQSRERAEESL MKG-DTVTVVVSTGAPKVEVPDVLEKSQESARDAL	TRRGFK-VEVDEVE-SISEDPGIVIEQRPR KGKGFK-VKVEQEE-SDEDPGTVLRQNPQ LEDKGFT-VKVESVE-SDSTPDTVTKQSPE
5_gr1seus_3725_361_964aa S_PAMC26508_3547_361_661aa	GAILIGNTLFSS	ADDDGQVNAPALVGSI VGQABALAARIDVIVK VDDGADGKVDVPNNVGST <u>VEQAQRLAEN</u> SEIVLK	VGAEE-PCEEQEKGEICSQDPAADAL VGSKE-PCEAQEKGKICSQSPGSQEQ	UUKN-GIVIVVSAGAPKIEIPNVLEKSEUGAREEL MATG-ETVTVVVSTGAPKVEVPDVLEKS <u>EESARKAI</u> :* * *	EKKOFT-VNVTTEE-SEKTEGTVIQQDPK EDKGFT-VNVKTAE-SEKTPDSVISQDPE . : *
S_cattleya_1232_361_636aa	KGTARKSGDAVAFTVSK-GRPVPVPDVTGKTVSDAEQALTGAG-LK-	PASIA 3 -VEVSGTPVYSDAVPKDSVAQQSP-GHG-TQAAA	GDTVTLTVSKGQQLFKVP-DVTGKDVD	PASTA 4 EATRILQQAGF-KVSVIQVFFTGKVFNQSPGGGSTG	PKNTTITLWAR
S_bingcnenggensis_0/851_361_646aa S_violaceusniger_7194_361_646aa S_albulus_2595_61_257aa	AGT TRRPAT I VGTVV SK-GCE DJ PDVVGESEDDARSDLEDAG-FK- SGSVRRPETPVS I VVSK-GCPVG I PDVVGDSVDEARSKLEDEG-FK- AGSTRRPDT AVAL TVSR-GAPVDVPNVVGSAREDAASL RQAG-FR- LOCKDRPATAL TVSR-GAPVDVPNVVGSAREDAASL RQAG-FR-	-VKIAQERVFSDE-DAGSVAEQSS-SDGDIRGAN -VKIADRQVFSEE-DKGSVAEQSPAGGGNGEGAK -VQFADQPVFSPQ-DKDAVARQSP-GAG-TTLGK	JULITERS CONTRACTOR CONTRACT	DAKQILEEAGF-EVDVDKPLL-FPGDIVIDQSVDGGDEA DAKAQLEEAGF-KVNVEKALL-FPGDKVQDQSVHGGDEA DATKALTALGF-KVEVDKPFL-FPQDSVASQSVAAGQA	PKGSSIIIKFK-GVF PKGSTITIKIKGGVF PKGSTITIRLK-GAL
5_r1mosus_24999_361_008aa S_venezuelae_1769_361_648aa S_clavuligerus_1326_361_644aa	AGSKREPDTAVALIVSK-GAVDPDVTGRDRADAEATLRGAG-FD- PGTGRAPDSAIALVVSK-GAPIDVPDVTGETVDAATATLQDAG-LK- AGVRRGPESAVALVVSK-GAPVEVPGVTGSALQEARDELEGAG-FT-	-VKLADGIVHSPH-EAGKVARQSP-GQG-SRQGK -VVLAPERINSSE-AAGSVAAQSL-AEG-SRAAK -VEVASGRVHSAQ-EPGTVARQSA-PAG-TELAT	JDTVTLTLSSGPEMVAVP-DVTGKSTD GDTVTLTVSKGPKLVEVP-DVTGEKLD GDTVTLTVSKGPRTATVP-DVTGQGAG	DARRAL IGAGF-EVEVKARFEL-FFRDAVESQSVAGGDA DARTALEDEGF-EVEVKKSFP-YLGDTVASQSVEGGSTA AARDTLESAGF-RVKTEKNFP-YLGDTVESQSPGGDRA	PEGSTVTITIK-GL PEGSTVTIKIK-GL
S_gr1Seds_3591_301_041aa S_PAMC26508_1975_361_640aa S_SirexAA_1542_361_649aa	AGTERNITOSAVAF VSK-GAPTOT POVIGESREEATAELEARG-EK- AGTERNITOSAVALVVSK-GSPVDVPDVTGESVDDATAALDEAG-EK- IGTERNAGSAVALVVSK-GSPVDVPDVTGEDVEDATAALEEKEAG-EK-	-AEVLFORVINSAE-AEGUVAEQSF-GGG-AEAAE -AEVLPERVNSAE-AQGEIARQTP-GSG-AEAAE -AEVLPDRVESAQ-AAGDIARQAP-GGG-AEAAE	3DTIELTSKGPRALDVF-DVTGKDVD 3DTVELTVSKGPRMLQVP-DVTGKDVD 3DTVELTVSKGPRMVAVP-DVTGKDVD	EARSTLEDEAGF-DVRVDRFL-SFSDTTASQSVDGGEA EARSTLEDAGF-EVKVDRPFL-SFSDTVGSQSVEGGEA EARETLEEAGF-EVKVDRPFL-SFSDTVASQSVKGGDA	AEGSTITIRTK-AL AEGSRITVKTK-GF
S_alous_4108_501_62484 S_davawensis_6328_361_646aa S_zinciresistens_08009_361_638aa	AGTERRSGSAVALVVSK-GRPVDVPDVTGESLDDARAELDEVG-LK- GGTERRSGSAVALVVSK-GRPVDVPDVTGESLDDARAELDEVG-LK- DGTKVRSGSAVALVVSK-GSPVDVPDVSGESPADARAALEESG-LK- DGTKVRSGSAVALVVSK-GSPUDDTGSAV-UNDEVEGAL	-VKVAAEQVTSPDFEKGEVAAQSP-KPG-TELAK -VKVAAEQVTSPDFEKGEVAAQSP-AEG-GQAAE -VRIDSARVNSEF-DAGQAARQSP-AEG-GQAAE	GEVELTLSKG PDITEVF-DVVGGNVD GDTVTLTLSKG PEMVEVP-DVVGGNVD GDTVTLTLSKG PEMIEVP-DVVGAGAS	DAKAELEAAGF-EVEEDRGLIGLFGDTVEGQSVEGGDTA DAKAELEAAGF-EVEEDRGLIGLFGDTVEGQSVEGGDTA DATARLEAAGF-SVEEDRGLIGLFGDTVKKQSVKGGKTA	PKGSTITIEIR PKGSTITIEIR PKGSTITITIR
S_viiiidone_27155_261_657aa	GTRYRAGAAALVYSK GSFIDIT DYTGDDLADAGGLEDAG LK KGTKVRAGSAVALTVSK-GSPVDVPDVTGDDLADARAGLEGAG-LK- SGTTVRAGSAVALVVSK-GSPVDVPDVTGDDLGARAGLEGAG-LK- SGTTVRAGSAVALVVSK-GSPVDVPDVTGDDLDALGALEGAG-LK-	-VKTADERVNSEH-DAGQVARQNP-GEG-RQVAE -VKTADERVNSEH-DAGQVARQNP-GEG-RQVAE -VKTADERVNSEV-DSGRVARQTP-EPG-GRAE	DTVTLTVSKG PEMVEVP-DVVGDSVD DTVTLTVSKG-PEMVEVP-DVVGDSVD DTVTLTVSKG-PRMLEVP-DVVGDSVD	DARSRLEGAGF-RVKEDRGLLGLFGDTVRSQSVEGGDTA DAKQKLEDAGF-EVDEDRGLLGLFGDTVRKQSVDGGDTA DAKQKLEDAGF-EVDEDRGLLGLFGDTVKKQSVDGGDTA	PRGSTITIEIR PEGSTVTITIR
S_11V10ants_27105_301_0574a S_avermitilis_6092_361_648aa S_collinus_11080_361_629_aa S_bwwwwwwwwwwwwwwwwwwwwwwwwwww	AGTERRGGSATALLVSK-GSPVDFDVTDDDDDDDAAAALL20AG-LK- AGTERRGGSATALLVSK-GSPVDVTGEDLDSARQDLEGAG-LK- TGTERHAGSATALLVSK-GSPUDVPDVTGEDLDSARQDLEGAG-LK- ACTEPUACSATALTVSK-GSPUDVPDVTGEDLDSARQDLEGAG-LK-	-VRIADERINSEL-DSONVARGIF-EFG-ORAAE -VRISSQRVTSAEFEKGQVAQQSP-GADDDKVAE -VSVSARQVHSDF-DKGQVAQQSP-ANG-RAAE -VRVACQNUSEV_DECEVAAQSP-CDC-DDAAE	JDTVTLTVSRG—PEMVEVP-DVVGDSVD JDTVTLTISKG—PEMVEVP-DVVGDSVA JDTVTLTLSLG—PEMVEVP-DVVGSSVD	DAQQTLEDAGF-EVDEDROLLGEFGDEVKDQSVEGGEKA DAQQTLEDAGF-EVDKDRGLLGFFGDEVKDQSVEGGEKA DATKRLEDAGF-KVEKDRGLLGLFGDTVSKQSVDAGQKA	PKGSTITITIR PKGSTITITIR
S_cattleya_3089_361_673aa S_bingchenggensis_05406_361_676a	AGSSVAKDTKITLTVAAAPQQLTVPDVTNKPYSDAQAQLQQMG-FQVG GGEKVAPCTFITLTVAAAQSVTVPDVTTKKIADATSTIFDIG-IK-	TKTQESTTVAPDTVISQSP-GPN-NQAPVI -VOTTEVENAD-V-FAGTVISQNP-OPN-FOVIP	NSTITLTVAKAPSGGGTVPGDILGHKLSI ATTVTLTVAKAPAQQQNVAVP-QLRGQKLK	VQSELSSLSG-VTVNVQGPQ-DENAVVVGSDPKPGSQV DAKKALFAVGI-TVGTVSGSQ-NENSTVLFSDPTFGSQV	QPGQTITLQTLDNPMGGGGDQNNNGGK
S_violaceusniger_0274_361_670aa S_albulus_4186_361_666aa S_rimosus_00070_361_673aa	GGKKVAKGATITLTVAEAQQKVTVPDVTTQKVADATAALQGKG-LK GQAETTKDTQITLTVAKAAPKSTVPDVTGQDFNAAQKQLQDLG-FT GQSECTKKSKITLVVAKKSPTTTVPDVTGQDFNAAQKQLTDIG-FQ	VQTQEVDNGDVEAGTVTDQTPKGG-QEVLP -VNRTDQDVSDPG-QNGKVISQSP-TGNQQAAQ -VTKNEQETSDPNQVGKVISQSPNGNTDAPP	SSTVTLTVAKAAAENVPVP-NLGGQKLK SSTVTLTVGKAAAQAQVP-GVTGKKVK SSTITLTVGKAAEQKTVP-GVVGOTVK	VAKKALQDLGL-NVGNITGPQ-DDEATVVASQPGAGTQV VAKQMLQQAGFNNTQFAGGSSQDDNAMVISQDPQPGTGG VAKKALQSAGFTNTQFANGSSQDDXARVJTQDPQPNTPS	PKGQSVNLITVAGQGNGGNGN DP-ASTTVTLTTMGGNNDGNNDG DPGSTTVTLTTVGSSPSGDDGGDDG
S_albus_3037_361_662aa S_coelicolor_3848_361_673aa S_lividans_19030_361_673aa	RDSEVEKGTTITLTVAKEKAMTAVPDVSGKSCEEATSQLEGSD-LK PGKELEKGSTVTLEVAKAEEKATVPDVVGRTCDEAKAQVESGGDLT PGKELEKGSTVTLEVAKAEEKATVPDVVGRTCDEAKAQVESGGDLT	-ANCVEEENEAEAGKVFSQTP-AAQ-AQVQP AVCTDQPTNDPN-QVGKVISTTP-QSS-TQVDP AVCTDQPTNDPN-QVGKVISTTP-QSG-TQVDP	STVTLKIAKAAEKVKVPGGLRGQKLG SKVTIVVGKAVEKTKVP-EVRGKTLA SKVTIVVGKAVEKTKVP-EVRGKTLA	WKKALTDAGL-TVGNVSGSP-DDNALVAALDPGEDAEV SARQTLQQSGFTNVQVAQGSPGDDNAKVFASNPQPGSEV SARQTLQQSGFTNVQVAQGSPGDDNAKVFASNPQPGSEV	PKGTAVNLTTV-GPPGEDGG DDPAATPITLMTV-PGDGGNGNGGN DDPAATPITLMTV-PGDGGNGNGGN
S_collinus_19315_361_664aa S_hygroscopicus_5218_361_664aa S_avermitilis_4338_361_667aa	GGTSKEKGTTITLTVAKAEEKVSVPDVTGKSCDEAKSILQSKG-LAP GGSSKEKGTTITLTVAKPEEKVTVPDVTGKSCDEAKAELQAKG-LAP GDSERQKGTTITLTVAEAQKVTVPDVTGKSCDEAKQQMQANN-LT	VANCNDVAVTDPG-QDGKVQNTTP-AAG-QQVSK VSSCTETPVTDPA-QDGKVQNTNP-SAG-QQVSK VGNCTEVDTSDAS-QAGKV1QTSP-TAG-TEVKK	NTPVAINVGKAQG-QQQVQVP-QVQNRSLK NTPIAINVGKAQQ-PQQKPVP-NVAGQPLK NSAVDIQVGKAPQQVAVP-NLGGQSLK	XARKMLEQLGL-QVNV-QGAQ-DDNAVVLTSNPAPGSNV XAQQ1LQQQGFTQIQ-VNGPN-DDKARVIQQTPPQGTQA XAKKALQDAGL-NVGNITGSQ-DDNAIVIASDPQAGTQV	NQGDTVTLFTVDQGGGNNGNND DP-AGTQIVLTTADFGGGNNGGGD AQGTAVNLTAAGGNNNNGGNNN
S_zinciresistens_17937_301_652aa S_davawensis_4479_361_666aa S_griseoflavus_03159_361_667aa	GSTEQEKGSTITLTVAKEEAKSTVPDVAGRTCDEAKAQMQANN-LT LGEEVEKGSTITLTVAKAEEKATVPDVIGQTCDAAKTQMTASD-LV GDTEQEKGSTITLTVAKEADKATVPDVTNRTCDEAKAQMTGNE-LV	-GTCTEVETQDPN-QVGKVISTSP-EAG-SEADR -GECTEVDTQDPN-QVGKVIATTP-GAG-QTADP -GNCVDVETDNPA-LVGKVIGTDP-QQG-TQLAK	<pre>wSTVTIQVGKQAQQKTQVP-NVVGQTVG WSTVNIQIGKQAV-NEQVQVP-QIVGQKLK WSPVTIQIGKAKE-EQKFAMP-KVTGMTVAN</pre>	QAKQILQGGGFTNIQFENGSDQGDNALVTKQDPGAGQQV XAKKMLEQAGL-APGNIQGSQ-NDDATVFSSNPTPGTTV XAKQVLAGAGL-QLDKVQGGG-DDNAVVLQSNPQEGQEV	DNPGGTQIKLTTV-CFGGNNGGNNG NKGQTVDIIAV-EDGGGNGGND KKGDKVTLVALGGQQGGNGG
S_ghanaensis_03588_361_667aa S_viridochromogenes_03956_361_67 S_clavuligerus_2946_361682_aa	SGKEVEKGSTITLVIAKEEARSTVPDVIGKSCDEAKAQUSAND-LV LGEEVEKGSTITLTIAKAEAKSTVPDVVGQSCDQAKAQUTAND-LV GASMAEKNTTVTLTVAK-QALKTVPFVEGNPYPSAQQQLQVNG-PQ	-GNCTEQETDDPN-QVGKVISTTP-SAN-TPIGK GNCTEVDTQDDN-QVGKVIATTP-QAG-SSVDK -VNVEYVDSDKPRDQVLTQKP-AGN-SQAPE	SQVQIVVGKAKQ-EEKFGMP-KVTQMTVG SSVNIQIGKKQKQKTRVP-QVVGQTVG STVTLTVSKGPEQVITVP-PLTGKTLG	NAKQALAQAGL-QLERVEGVD-NDNAVVIQSVPAEGQEV NAKQTLAQAGFTNIQFANGSDQSDNALVAGQDPQPNQEV NAKLLISQAGL-ELGDVTGPD-DDNARVIDQNPKGGGQP	KKGDKVTLIAIGQQGGNGGNNG DDPAGTTVTLQTIGAGGNNNGGNG GEEKKVDVTTLDLGGGDNNGNNSD
S_venezuelae_3632_361_673aa S_SirexAA_3284_361_655aa S_griseus_3725_361_664aa	EGTKAEQNSEVTITVAK-QKLSQLPDVKNRTYDQAVLQLNGVG-FT PGDKAERESEVTITVAK-EKILDLP-VLNRTYDEAVSQLNGIG-FS GGEKAEDGSEITITVAV-QATDPMPDVRTRQYDAAVAQLNQLG-FT	KITREDVDSDQ-PAGTVIDQTP-QGP-SDQPK NISKQEVDADQ-PAGTVVGQTP-EGP-SKQAK NVSRADVDSDK-PAGEVIEQTP-AGP-SKQAK	DVQITLKVSKGPQ-KTQVQIPLDIGGKKFK DVQIVLKVSKGPPQPEQVAVP-DLSNKTLA DAQIVLKVSKGPAQPEQVQIPGDIGNRPYQ	DVKQQLEALGLVVVQQGSD-KEDATVFGSTPPAGSMV AKAALAQAGL-QISV-QGVD-EDNARVLGFQPQAGQMV DVKGQLEGLGF-VVALAPNSVDKPNARVITSTPAPTTQA	NTGDTVNVMTV-GGGGGDGRNG- DKNSTVNVQTMPGG-NG- PKGSTVTLVTI-EGGGNGG-NG-
S_PAMC26508_3547_361_661aa	GGSKVEKDTEVTITVAK-KTTEPMPDVRTRPYDAAVQQLNGLG-FT :::::** * ::	NVSRQDVDSEE-AAGTVIDQSP-AGP-SKQSKI ;	DVQIVLKVSKGPAQPEQVTVPGDIVGKRFQ : : :, :* :	DAKGALEGLGL-VVALAPNSSDKPNALVMQADPGANSQV *	DKGSTVTLTTI-GGGGGDGG : :
S_cattleya_1232_361_636aa S_bingchenggensis_07851_361_649a S_violaceusniger_7194_361_646aa					
S_albulus_2595_61_257aa S_rimosus_24996_361_668aa S_venezuelae_1769_361_648aa					
S_clavuligerus_1326_361_644aa S_griseus_5391_361_641aa S_PAMC26508_1975_361_640aa					
S_SirexAA_1542_361_639aa S_albus_4768_361_624aa S_davawensis_6328_361_646aa					
S_zinciresistens_08009_361_638aa S_viridochromogenes_02054_361_65 S_griseoflavus_04610_61_346aa					
S_coelicolor_2110_361_667aa S_lividans_27155_361_657aa S_avermitilis_6092_361_648aa					
S_collinus_11080_361_629_aa S_hygroscopicus_3594_361_655aa S_cattleya_3089_361_673aa	CKGHGGGG-WGGIFGG				
S_briggenenggens1s_05406_361_676a S_violaceusniger_0274_361_670aa S_albulus_4186_361_666aa S_signer_00070_261_676a	GGGDGGGI-FGGQNGF				
S_rimosus_00070_301_673aa S_albus_3037_361_662aa S_coelicolor_3848_361_673aa S_liidaaa 10020_361_673aa	GAL SU				
S_rtvtdans_19030_301_073aa S_collinus_19315_361_664aa S_hygroscopicus_5218_361_664aa S_nucrmitil/	VI 0 707-1004707				
S_avermitilis_4538_361_667aa S_zinciresistens_17937_301_652aa S_davawensis_4479_361_666aa S_gmigosflor02160	GNNGG-GF-FGGUTGTSTRTEH G-GI-FGGVNGVTFRTED				
S_griseofiavus_03159_361_667aa S_ghanaensis_03588_361_667aa S_viridochromogenes_03956_361_67	SP "0"01 F00 F300"-K0DD C-GF-F6GLT6GR0DD GNG-G1-F6GLT-GRNED				
S_craverigerus_2940_301062_aa S_venezuelae_3632_361_673aa S_SirexAA_3284_361_655aa S_griseus_3725_361_654aa	NF-FGGGGGFFD				
S_griseus_3725_361_664aa	CEGP-1GGLGGDD				

Figure 5 Amino-acid alignment of 41 PASTA domains (penicillin-binding protein and serine/threonine kinase-associated domains) in serine/threonine kinases (STPKs) of *Streptomyces*. The amino acid are aligned by using MUSCLE.⁵⁶ Approximately four PASTA regions are indicated. Four α -helix are marked with yellow, and the two classes in Table 2 are shown in green and blue, respectively. The conserved amino-acid residues are indicated with dot, colon or star. A full color version of this figure is available at the *Journal of Antibiotics* journal online.

PASTA	domains	in	Actinobacteria
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Table 2 Distribution of PASTA domains of STPKs of Streptomyces species in the phylogenetic tree

STPK	Position 1	Position 2	Position 3	Position 4
Class 1				
Streptomyces albulus 4186	6 ^a	17	17	19
Streptomyces albus 3037	6	17	7	8
Streptomyces avermitilis 4338	6	17	14	8
Streptomyces bingchenggensis 05406	6	20	20	8
Streptomyces cattleya 3089	6	15	17	8
Streptomyces clavuligerus 2946	6	17	17	19
Streptomyces coelicolor 3848	6	17	14	19
Streptomyces collinus 19315	6	17	14	8
Streptomyces davawensis 4479	6	17	14	8
Streptomyces ghanaensis 03588	6	17	14	19
Streptomyces griseoflavus 03159	6	17	14	19
Streptomyces griseus 3725	6	17	17	8
Streptomyces hygroscopicus 5218	6	17	14	19
Streptomyces lividans 19030	6	17	14	19
Streptomyces rimosus 00070	6	17	17	19
Streptomyces PAMC26508 3547	6	17	17	8
Streptomyces SirexAA 3284	6	17	17	None ^b
Streptomyces venezuelae 3632	6	17	17	8
Streptomyces violaceusniger 0274	6	20	20	8
Streptomyces viridochromogenes 03956	6	17	14	19
Streptomyces zinciresistens 17937	6	17	14	19
Class 2				
Streptomyces albulus 2595	13	12	16	20
Streptomyces albus 4768	13	12	16	20
Streptomyces avermitilis 6092	13	12	16	20
Streptomyces bingchenggensis 07851	13	12	16	20
Streptomyces cattleya 1232	13	12	16	21
Streptomyces clavuligerus 1326	13	12	16	20
Streptomyces coelicolor 2110	13	12	16	20
Streptomyces collinus 11080	13	12	16	20
Streptomyces davawensis 6328	13	12	16	20
Streptomyces griseoflavus 04610	13	12	16	20
Streptomyces griseus 5391	13	12	16	20
Streptomyces hygroscopicus 3594	13	12	16	20
Streptomyces lividans 27155	13	12	16	20
Streptomyces rimosus 24996	13	12	16	20
Streptomyces PAMC26508 1975	13	12	16	20
Streptomyces SirexAA 1542	13	12	16	20
Streptomyces venezuelae 1769	13	12	16	20
Streptomyces violaceusniger 7194	13	12	16	20
Streptomyces viridochromogenes 02054	13	12	16	20
Streptomyces zinciresistens 08009	13	12	16	20

Abbreviations: PASTA domains, penicillin-binding protein and serine/threonine kinase-associated domains; STPK, serine/threonine kinases.

^aNumber of cluster in Figure 4. ^bPosition 4 is missing.

respectively) behave independently from other position 2 or position 3 PASTA domains of *Streptomyces* species and belong to cluster 20.

C. diphtheria DIP_1615, *C. glutamicum* NCgl_2095, *Nakamurella multipartite* Namu_2392, *N. multipartite* Namu_3222 and *Tsukamurella paurometabola* Tpau_2669 each have five PASTA domains. The position 5 PASTA domains of these species typically fall into cluster 19 or cluster 20, which are otherwise dominated by position 4 PASTA domains, indicating that most position 5 PASTA domains are similar to those of position 4. Only *N. multipartite* Namu_2392 forms an exception by falling into cluster 7.

The position 1 PASTA domains (PASTA 1) of *Streptomyces* cluster into clusters 1, 6 and 13 in Figure 4; position 2 domains fall into

clusters 12 and 17; position 3 domains into clusters 14, 16 and 17; and position 4 domains into clusters 8, 19 and 20. Interestingly, all STPKs having position 1-type PASTA domains that cluster into cluster 1, in Figure 4, retain only one PASTA domain. The other STPKs of *Streptomyces* can be divided into two classes (Table 2): PASTA 1 of one class group with cluster 6 (see Figure 4), while those of the second class cluster into cluster 13 in Figure 4. For those STPKs in which PASTA 1 cluster to cluster 6, their PASTA 2 clusters to cluster 17 or 20, their PASTA 3 cluster to clusters 7, 14, 17 or 20 and their PASTA 4 cluster to cluster 13, the PASTA 2 clusters exclusively to cluster 12, PASTA 3 absolutely to cluster 16 and PASTA 4 to cluster 20 or 21 (see

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	778 580. 570. 410. 410. 420. 420.
Predicted Secondary structure	
Template Sequence	G T R D V Q V P D V R G Q S S A DAI A T L Q N R G F K I R T L Q K P D S T I P P D H V I G T D P A A N T S V S A G D
Template Predicted Secondary structure	· · · · · · · · · · · · · · · · · · ·
	355 360
Predicted Secondary structure	438 . 440
Query Sequence Template Sequence	TVNLVVSTGAPKVAVPNVIDKNIDEAKKQLEDKGFEV. ETKQTESSQDEGTILSQNPDP
Template Known Secondary structure	S
Template Predicted Secondary silucture	415 420
Desite a la constante de la constante	496 500
Query Sequence	GKELEK GST VTLEVAKAEEKAT VPD VVG RT CDEAKA QVESGGDLTAVCT DQPT NDPNQVG
Template Known Secondary structure	SS TT SSB
Template Predicted Secondary structure	475 480
	556 560
Predicted Secondary structure Query Sequence	K V I S T T P Q S S T Q V D P G S K V T I V V G K A V E K T K V P E V R G K T L A E A R Q I L Q Q S G F T N V Q V
Template Sequence Template Known Secondary structure	E E V T G T N P P A G T T V P V D S V I E L Q V S K G N Q F V M P D L S G M F W V D A E P R L R A L G W T G M L D K G A B S S - T T
Template Predicted Secondary structure	533
	613 620
Predicted Secondary structure	
Template Known Secondary structure	
Template Predicted Secondary structure	
	222
b	
Des dista di Casa a dans atas atas	339400

Predicted Secondary structure		
Ouerv Sequence NSGOFTKVPPLL	L S K T E A O A R D R L D D A G L D V G K V R H A Y S D T V E R G K V I S T D P G V G D R I R K	N
Template Sequence GITRDVOVPDVR	R G O S S A Ď A I A T L O N R G F K I R T L O K 🗬 P D S T I P P D H V I G T D P A A N T S V S A	G
Template Known Secondary structureS ST	TTS	S
Template Predicted Secondary structure		
355 360		
459460	470	
Predicted Secondary structure		
Query Sequence D S V S L T V S D G P D	D T V K L P D V T G Y K L D K A R T L L E D E G L E P G M V T R A F S D E V A R G F V I S T K P	G
Template Sequence DEITVNVSTGPE	E Q R E I P D V S T L T Y A E A V K K L T A A G F G R F K Q A N S P S T P E L V G K V I G T N P	Р
Template Known Secondary structure	——————————————————————————————————————	-
Template Predicted Secondary structure		
414 420	430	•
519520 · · · · · · · · · · · · · · · · · · ·	530	
Predicted Secondary structure		0
Templete Sequence S G T T V K A G S A V A		G
Template Known Secondary structure S.S.S. T.T.	IIIVGS GPATKDIPDVA QLVDVA QKNENVTGFTKPS QASVDSPKA	
Template Predicted Secondary structure		_
474 490		
578 . 580	. 590	
Predicted Secondary structure		
Query Sequence RVARQTPEPGGR	R A A E G D T V T L T V S K G P R M I E V P D V V G D S V D D A K O K L E D A G F E V D E D R G	6 - C
Template Sequence, E,V,T,G,T,N,P,P,A,G,T,T		16
	T V P V D S V I E L Q V S K G . N Q F V M P D L S G M F WV D A E P R L R A L G WT G M L D K G	A
Template Known Secondary structure B S S - T T -	T V P V D S V I E L Q V S K G N Q F V M P D L S G M F W V D A E P R L R A L G W T G M L D K G T T T S	A
Template Known Secondary structure B S S – T T – Template Predicted Secondary structure	T V P V D S V I E L Q V S K G IN Q F V MP DL S G M F WV D A E P R L R A L G WT G M L D K G T T S . T T B . T T	A
Template Known Secondary structure Template Predicted Secondary structure	T V P V D S V I E L Q V S K G N Q F V M P D L S G M F WV D A E P R L R A L G WT G M L D K G T T S	A
Template Known Secondary structure B S S T T Template Predicted Secondary structure 533	T V P V D S V I E L Q V S K G N Q F V M P D L S G M F WV D A E P R L R A L G WT G M L D K G T T T S T T B	A
Template Known Secondary structure B S S T T Template Predicted Secondary structure 533 540 533 540	T V P V D S V I E L Q V S K G N Q F V M P D L S G M F WV D A E P R L R A L G WT G M L D K G T T S T T B	A .
Template Known Secondary structure B S S — T T Template Predicted Secondary structure 533 540 Predicted Secondary structure 633 640 Outro Functionary structure 633 640	T V P V D S V I E L Q V S K G N Q F V M P D L S M F WV D A E P R L R A L G WT G M L D K G T T S T T B	A • • •
Template Known Secondary structure B S S T T Template Predicted Secondary structure 533 540 540 Predicted Secondary structure 638 640 540 Query Sequence L L F G T	T V P V D S V I E L Q V S K G N Q F V M P D L S G M F WV D A E P R L R A L G WT G M L D K G T T S T	ю.
Template Known Secondary structure B S S T Template Predicted Secondary structure 533 540	TVP V DS VI ELQVSKG.NQF V MP DLSG MF WV DA EPRLRALGWTGMLDKG TTS TTB	λο .
Template Known Secondary structure B S S — T T — Template Predicted Secondary structure 533	T V P V D S V I E L Q V S K G N Q F V M P D L S G M F WV D A E P R L RAL G WT G M L D K G T T S T T B 550 550	λο .

Figure 6 Comparison of the secondary and quaternary structures of serine/threonine kinases (STPKs) of *S. coelicolor* SC03848 and *S. coelicolor* SC02110 by using Phyre2,³⁷ and *M. tuberculosis* of PknB (PDB code number, 2KUI) as a template. (a) Comparison of the secondary structure of *S. coelicolor* SC03848 and the template. Blue arrows indicate β -sheets, and green coils show α -helix. Light brown and brown indicate inserted and deleted residues. (b) Comparison of the secondary structure of *S. coelicolor* SC02110 and the template. Symbols are the same as in **a.** (c) ProQ2 quality assessment of *S. coelicolor* SC03848. ProQ2 is a model quality assessment algorithm that uses support vector machines to predict local as well as global protein models.⁵⁷ N and C terminals are indicated. (d) ProQ2 quality assessment of *S. coelicolor* SC02110. (e) Pocket detection of *S. coelicolor* SC03848 by using fpocket2 program.³⁹ A full color version of this figure is available at the *Journal of Antibiotics* journal online.



Figure 6 Continued

Figure 4). A *S. coelicolor* STPK (SCO3848, PknB) in class 1 and that (SCO2110, PkaF) in class 2 (Table 2) are reported to be involved in the regulation of central carbon metabolism, carbon flux and of biosynthesis of the antibiotic actinorhodin and in the regulation of morphogenesis and actinorhodin production, respectively,^{35,36} indicating that STPKs in class 1 and class 2 in Table 2 show different functions and those in the same group may carry out similar biological functions. These STPKs have four PASTA domains. No report has been published on the biological function of another STPK (SCO3821, PksC) in *S. coelicolor* carrying one PASTA domain.

A phylogenetic tree constructed by using the amino-acid sequences of these PASTA domains in Streptomyces shows that these sequences are clearly divided into two classes (Supplementary Figure S4), which correspond to the classes in Table 2. Furthermore, the amino-acid sequence alignment of the PASTA domains of these STPKs also shows two classes (Figure 5), indicating that some parts of the amino-acid sequences evolved from at least two origins. However considering that certain amino-acid residues are conserved throughout the sequences (Figure 5), one original PASTA domain is presumed to have been quadruplicated to form the four prototype PASTA domains, and then some parts were further modified in two independent directions, indicating that the PASTA domains in STPKs of Streptomyces species evolved position-dependently. However, when a phylogenetic tree was constructed with PASTA domains in STPKs of five Streptomyces species, some close interrelationships were observed between different positions (Supplementary Figure S5), that is, PASTA domains in different positions were located close together in the phylogenetic tree. For example, PASTA domains in position 1 (cluster 6 in Figure 4) and position 3 (cluster 14) of class 1 and, those in position 3 (cluster 16 in Figure 2) and position 4 (cluster 20 in Figure 4) and those in position 2 (cluster 17 in Figure 4) and position 3 (cluster 17 in Figure 4) of class 2 (Table 2). Similar phenomena are also observed in Figure 4. These results indicate that the PASTA domains in STPKs of *Streptomyces* species evolved position-independently as well and support the idea that one original PASTA domain is presumed to have been quadruplicated to form the four prototype PASTA domains.

Another interesting observation is that almost the entire amino-acid sequences of two STPKs, Streptomyces albulus DC74_2595 and Streptomyces griseoflavus SSRG_04610, are occupied by four PASTA domains. The secondary structures of these PASTA domains are similar to other PASTA domains, that is, that they are composed of one α -helix and three β -sheets. However, in the case of S. albulus DC74_2595 (Supplementary Figure S6A), the gene sequence for this kinase is preceded on the chromosome by another protein kinase encoding gene and the genes overlap by 3 bp. (Supplementary Figures S6A–C), suggesting that these protein kinases interact with each other. On the other hand, in the case of S. griseoflavus SSRG_04610 (Supplementary Figure S7A), the gene encoding this protein kinase has an atypical start codon (GAG) (Supplementary Figures S7B and C) and the preceding putative open reading frame ends (TAG) just before the putative start codon (GAG) of SSRG_04610 (Supplementary Figure S7C). This preceding putative open reading frame (Supplementary Figure S7D) apparently also encodes a protein kinase from its Blast analysis (Supplementary Figure S7E). These results suggest that S. albulus DC74_2595 and S. griseoflavus SSRG_04610 are not protein kinases but complex PASTA domains, and have their roles by acting in concert with the preceding protein kinases, respectively.

SECONDARY AND QUARTERNARY STRUCTURE PREDICTION

When the secondary and quarternary structures of *S. coelicolor* STPKs SCO3848 and SCO2110 were analyzed using Phyre2,³⁷ as

representatives of these two classes, the NMR structure of the PASTA domain of *M. tuberculosis* of PknB (PDB code number, 2KUI) was selected as a best fit template in both cases. In addition, the secondary structures of both sequences fit very closely to that of template in both cases (Figures 6a and b), and the ProQ2 quality assessments³⁸ were also very similar (Figures 6c and d). Significant difference were only observed in Pocket detection,³⁹ where the pocket region in SCO2110 was more compact and extended than that in SCO3848 (Figure 6f), suggesting that the SCO2110 pocket may bind β -lactam antibiotics more efficiently. These pocket regions are within the PASTA 4 domains.

CONCLUSION

PASTA domains are detected in PBPs as well as STPKs. They are proposed to bind β-lactam antibiotics and peptidoglycan fragments, and also to be involved in cell wall metabolism. However, the functions of PASTA domains in PBPs and those in STPKs are thought to be different. For example, the PASTA domain of S. pneumoniae PBP2x (2 PASTA domains) binds to β-lactam antibiotics as well as peptidoglycan fragments,² whereas that of *M. tuberculosis* PBP PonA2 (1 PASTA domain) does not bind β-lactam antibiotics, muropeptides, nor polymeric peptidoglycan. No report has been published on the binding property of other PBPs. However, it is interesting to know whether the PASTA domains in PBPs with two PASTA domains such as B. cavernae DSM 12333 BCAV_4182, I. variabilis 225 ISOVA_3000 bind β-lactam antibiotics and muropeptides. On the other hand, PASTA domains of STPKs such as M. tuberculosis PknB with four PASTA domains are proposed to bind β-lactams and peptidoglycan fragments.9,40 While the PASTA domains of S. pneumoniae PBP2x form a compact conformation, those of STPKs display an extended conformation.²⁴ The conformational differences between PASTA domains in PBPs and those in STPKs and the number of PASTA domains may reflect their functional differences. Considering that Actinobacteria, especially Streptomyces species, show complex morphogenesis and produce various antibiotics including β-lactam antibiotics, and that STPKs with PASTA domains are involved in the signal transduction leading to morphogenesis, cell wall metabolism^{17,40-44} and microbial resistance,^{45–50} it is of interest to know the distribution of PASTA domains in PBPs and STPKs in Actinobacteria. The results showed that PASTA domains in PBPs distribute independently of taxonomy with some distribution bias. Intriguingly though, no Streptomyces species possess PBPs with PASTA domains. In contrast, STPKs in Streptomyces do contain PASTA domains, and on this basis, can be divided into three groups: one PASTA-containing STPKs, four PASTA-containing STPKs and those without PASTA domain. Four PASTA-containing STPKs can be further resolved into two classes.

Only a few reports have been published on their biological function. SCO4423 (AfsK) without PASTA domain is a global regulator of secondary metabolism in *S. coelicolor*.⁵¹ SCO6681 (RamC), another STPK without PASTA, is reported to be involved in aerial mycelium formation and sporulation but not in secondary metabolism.^{52,53} SCO2110 (PkaF), a 4 PASTA-containing STPK, is related to morphogenesis and actinorhodin biosynthesis but not to undecylprodigiosin production,³⁵ and SCO3848 (PknB), another 4 PASTA-containing STPK, is reported to deregulate central carbon metabolism, with carbon flux diverted to biosynthesis of actinorhodin.³⁶ PknB (Rv0014c) of *M. tuberculosis*, a 4 PASTA domain-containing STPK, phosphorylates Wag31 and triggers remodeling of bacterial morphology.²⁸ No report has been published on the function of SCO3821 (PksC), a 1 PASTA domain-containing STPK. Therefore, available data are too limited to conclude definitely the functional roles

of PASTA domains in PBPs as well as in STPKs at the present time. The pocket region in SCO2110 (PkaF) was more compact and extended than that in SCO3848 (PknB) and might bind β -lactam antibiotics more efficiently. Further research is needed on PASTA domain to clarify the biological functions of PBPs and STPKs in *Actinobacteria*, especially in the field of cell wall metabolism and antibiotic resistance.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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