Genome sequence of the soil bacterium *Saccharomonospora azurea* type strain (NA-128^T)

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Abstract
Saccharomonospora azurea Runmao *et al*. 1987 is a member to the genomically so far poorly characterized genus *Saccharomonospora* in the family *Pseudonocardiaceae.* Members of the genus *Sacharomonosoras* are of interest because they originate from diverse habitats, such as leaf litter, manure, compost, surface of peat, moist and over-heated grain, where they might play a role in the primary degradation of plant material by attacking hemicellulose. They are Gram-negative staining organisms classified among the usually Gram-positive actinomycetes. Next to *S. viridis, S. azurea* is only the second member in the genus *Saccharomonospora* for with a completely sequenced type strain genome will be published. Here we describe the features of this organism, together with the complete genome sequence with project status 'permanent draft', and annotation. The 4,763,832 bp long chromosome with its 4,472 protein-coding and 58 RNA genes was sequenced as part of the DOE funded Community Sequencing Program (CSP) 2010 at the Joint Genome Institute (JGI).

Introduction
Strain NA-128^T (= DSM 44631 = ATCC 43670 = NBRC 14651) is the type strain of the species *Saccharomonospora azurea* [1], one out of currently nine members in the genus *Saccharomonospora* [51]. The strain was originally isolated in the course of screening for new antibiotics from a soil sample collected near Guangyun City, Sichuan (China) [1]. The genus name *Saccharomonospora* was derived from the Greek words for *sakchâr*, sugar, *monos*, single or solitary, and *spora* a seed or spore, meaning the sugar (-containing) single-spored (organism) [5]; the species epithet was derived from the Latin adjectiv *azurea*, azure, referring to the color of the areal mycelium [1]. Yoon *et al.* [2] showed in 1999 *via* DNA-DNA hybridization that '*S. caesia*' [3] (formerly known as '*Micropolyspora caesia*' [4]), which was neglected from the approved list [20], was indeed a synonym of *S. azurea*. *S. azurea* and the other type strains of the genus *Saccharomonospora* were selected for genome sequencing in a DOE Community Sequencing Project (CSP 312) at Joint Genome Institute

(JGI), because members of the genus (which originate from diverse habitats, such as leaf litter, manure, compost, surface of peat, moist and over-heated grain) might play a role in the primary degradation of plant material by attacking hemicellulose. This expectation was underpinned by the results of the analysis of the genome of *S. viridis* [16], one of the recently sequenced GEBA genomes [46]. The *S. viridis* genome, so far the sole sequenced genome from the genus *Saccharomonospora*, contained an unusually large number (24) of genes for glycosyl hydrolases (GH) belonging to 14 GH families, which were identified in the Carbon Active Enzyme Database [47]. Hydrolysis of cellulose and starch were also reported for other members of the genus (that are included in CSP 312), such as *S. marina, S. halophila*, *S. saliphila*, *S. paurometabolica*, and *S. xinjiangensis* (see original descriptions of the type strains in *IJSEM*). Here we present a summary classification and a set of features for *S. azurea* AN-128^T, together with the description of the genomic sequencing and annotation.

Classification and features

A representative genomic 16S rRNA sequence of *S. azurea* NA-128^T was compared using NCBI BLAST [9,10] under default settings (e.g., considering only the high-scoring segment pairs (HSPs) from the best 250 hits) with the most recent release of the Greengenes database [11] and the relative frequencies of taxa and keywords (reduced to their stem [12]) were determined, weighted by BLAST scores. The most frequently occurring genera were *Saccharomonospora* (47.9%), *Kocuria* (17.7%), *Corynebacterium* (9.4%), *Kibdelosporangium* (6.0%) and *Prauserella* (5.5%) (176 hits in total). Regarding the eight hits to sequences from members of the species, the average identity within HSPs was 99.5%, whereas the average coverage by HSPs was 99.8%. Regarding the 42 hits to sequences from other members of the genus, the average identity within HSPs was 97.0%, whereas the average coverage by HSPs was 98.3%. Among all other species, the one yielding the highest score was *Saccharomonospora xinjiangensis* (AJ306300), which corresponded to an identity of 98.9% and an HSP coverage of 100.1%. (Note that the Greengenes database uses the INSDC (= EMBL/NCBI/DDBJ) annotation, which is not an authoritative source for nomenclature or classification.) The highest-scoring environmental sequence was FN667533 ('stages composting process pilot scale municipal drum compost clone PS3734'), which showed an identity of 100.0% and an HSP coverage of 97.9%. The most frequently occurring keywords within the labels of all environmental samples which yielded hits were 'feedlot' (7.9%), 'top' (4.1%), 'beef, cattl, coli, escherichia, habitat, marc, neg, pen, primari, secondari, stec, surfac, synecolog' (3.9%), 'feedbunk' (2.3%) and 'compost' (1.7%) (74 hits in total). Environmental samples which yielded hits of a higher score than the highest scoring species were not found.

Figure 1 shows the phylogenetic neighborhood of *S. azurea* in a 16S rRNA based tree. The sequences of the three identical 16S rRNA gene copies in the genome do not differ from the previously published 16S rRNA sequence (Z38017).

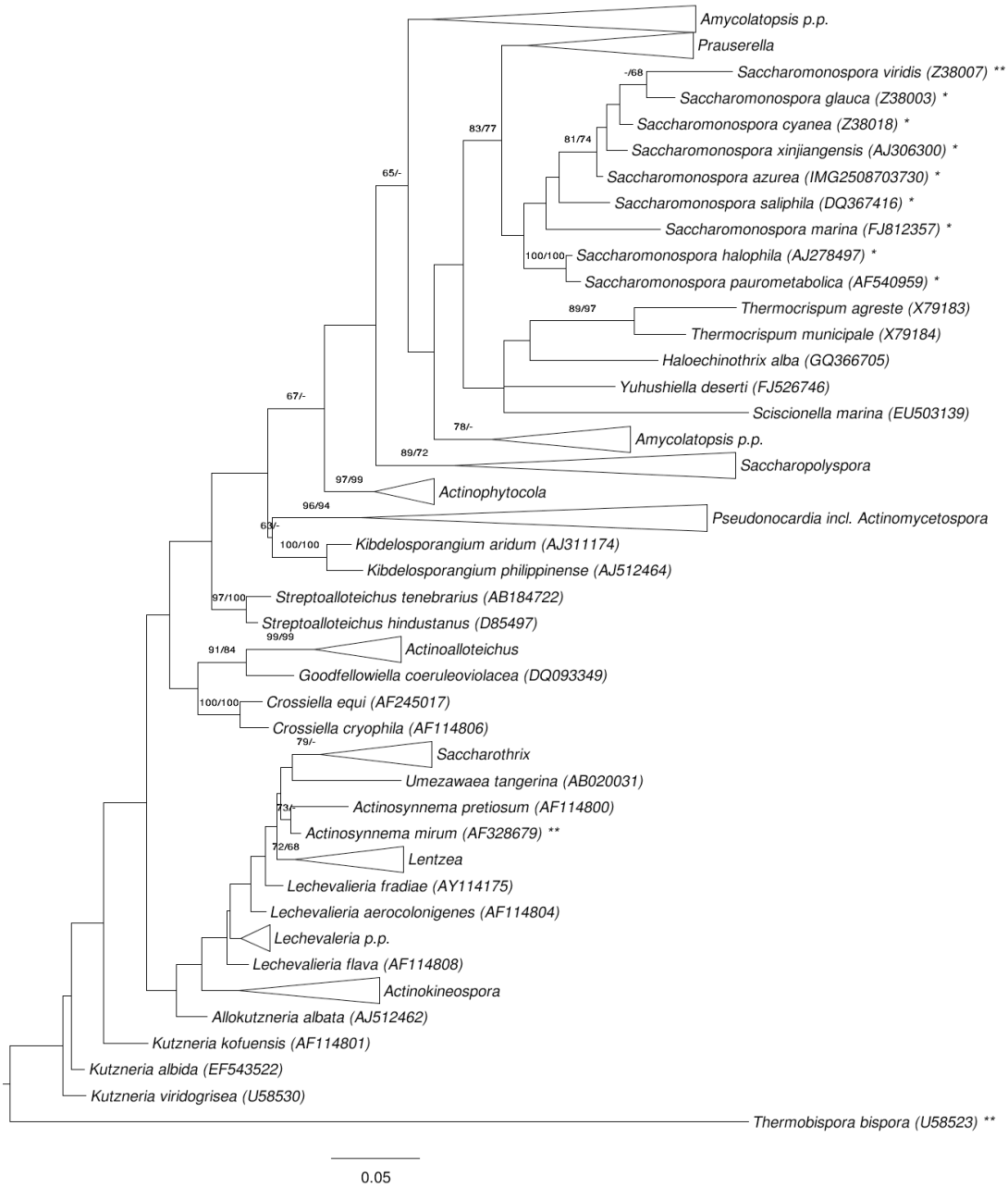

Figure 1. Phylogenetic tree highlighting the position of *S. azurea* relative to the type strains of the other species within the family *Pseudonocardiaceae*. The tree was inferred from 1,386 aligned characters [13,14] of the 16S rRNA gene sequence under the maximum likelihood (ML) criterion [15]. Rooting was done initially using the midpoint method [8] and then checked for its agreement with the current classification (Table 1). The branches are scaled in terms of the expected number of substitutions per site. Numbers adjacent to the branches are support values from 550 ML bootstrap replicates [17] (left) and from 1,000 maximum-parsimony bootstrap replicates [18] (right) if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [19] are labeled with one asterisk, those also listed as 'Complete and Published' with two asterisks [16,48,49]. *Actinopolyspora iraqiensis* Ruan *et al*. 1994 was ignored in the tree, because a proposal for the transfer of this species to the genus *Saccharomonospora* [6] was recently rejected on formal criteria [5].

Cells of *S. azurea* NA-128^T stain Gram-negative and form an irregularly branched vegetative mycelium of 0.3 to 0.4 μm diameter (Figure 2) [1]. The monopodally branching aerial

mycelium has a diameter of 0.3 to 0.6 μm [1]; the mature mycelium and the spores are azure to cyaneus when grown on Oatmeal agar (ISP3) or on Czapek sucrose agar [1]. Smooth, round spores are 0.8 to 1.0 mM long, mostly found on the aerial mycelium, but rarely on the substrate mycelium [1]. No distinct soluble pigment was detectable [1]. The growth range of strain NA-128^T spans from 24-40°C, with an optimum at 28-37°C [1]. Strain NA-128^T grows well in up to 7% NaCl containing medium, but is inhibited at 10% NaCl [1]. Physiological characteristics such as growth substrates, gelatin formation and peptonization of milk are described in detail by Runmao (1987) [1].

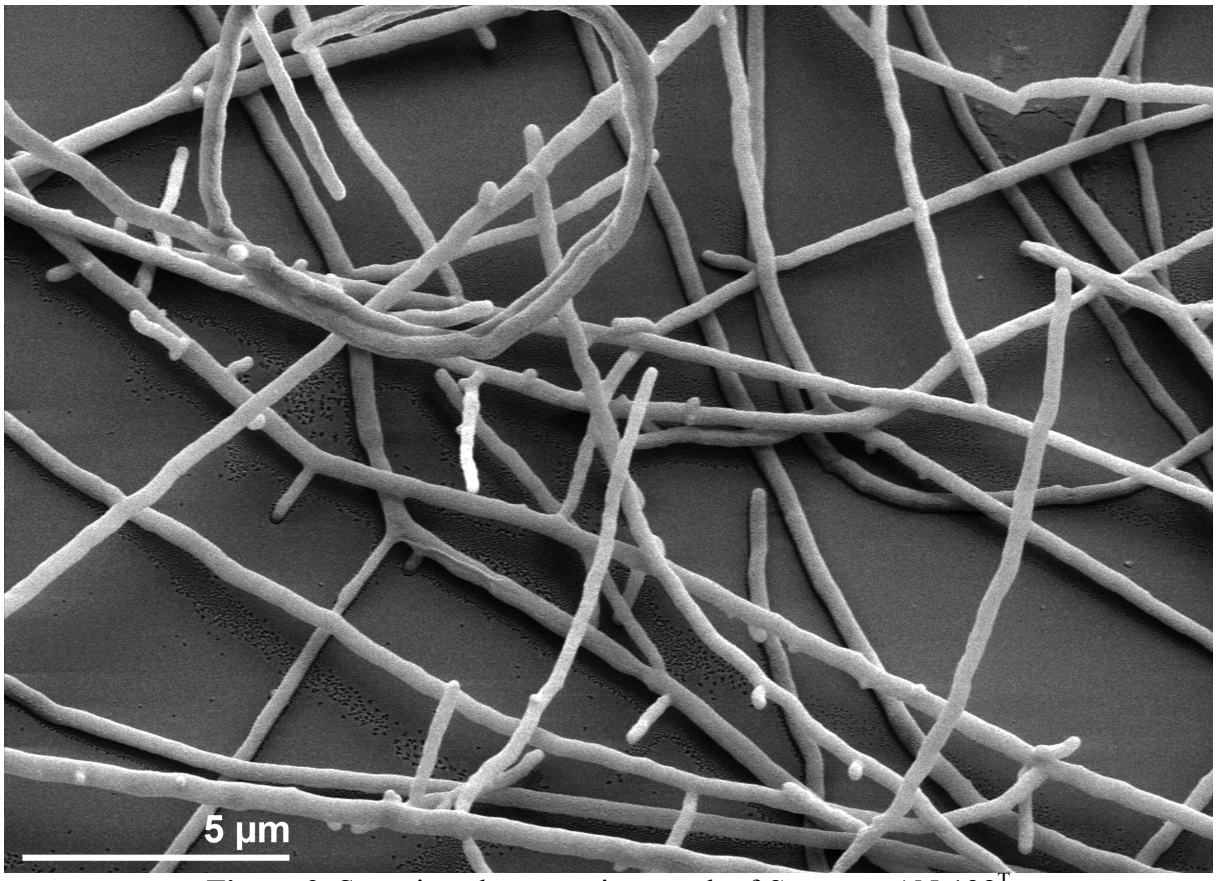

Figure 2. Scanning electron micrograph of *S. azurea* AN-128^T

Chemotaxonomy

The cell wall of strain AN-128^T contains *meso*-diaminopimelic acid; Galactose and arabinose are present, indicating a type IV cell wall / type A whole cell sugar pattern [1]. The fatty acids spectrum is dominated by almost 80% hexadecanoic acids: *iso*-$C_{16:0}$ (27.0%), $C_{16:1\ cis\text{-}9}$ (17.0%), *iso*-$C_{16:0\ 2\text{-}OH}$ (14.0%), $C_{16:0}$ (palmitic acid, 13.0%), *iso*-$C_{16:1\ H}$ (7.0%), *anteiso*-$C_{16:0}$ (1.0%) [50]. There are no data available for polar lipids and quinines of this strain.

Table 1. Classification and general features of *S. azurea* AN-128 ^{T} in accordance with the MIGS recommendations [7].

MIGS ID	Property	Term	Evidence code
	Current classification	Domain *Bacteria*	TAS [21]
		Phylum *Actinobacteria*	TAS [45]
		Class *Actinobacteria*	TAS [24]
		Subclass *Actinobacteridae*	TAS [24,44]
		Order *Actinomycetales*	TAS [20,24,43,44]
		Suborder *Pseudonocardinae*	TAS [25,26,44]

		Family *Pseudonocardiaceae*	TAS [22-25,44]
		Genus *Saccharomonospora*	TAS [20,26]
		Species *Saccharomonospora azurea*	TAS [1]
		Type-strain AN-128	TAS [1]
	Gram stain	negative	NAS
	Cell shape	variable	NAS
	Motility	non-motile	NAS
	Sporulation	single spores with smooth surface, mainly on aerial mycelium	TAS [1]
	Temperature range	mesophile, 24–40°C	TAS [1]
	Optimum temperature	28–37°C	TAS [1]
	Salinity	grows in up to 7% NaCl; 10% is inhibitory	TAS [1]
MIGS-22	Oxygen requirement	aerobic	TAS [1]
	Carbon source	mono, di- and trisaccharides	TAS [1]
	Energy metabolism	chemoheterotrophic	NAS
MIGS-6	Habitat	soil	TAS [1]
MIGS-15	Biotic relationship	free living	NAS
MIGS-14	Pathogenicity	none	NAS
	Biosafety level	1	TAS [27]
MIGS-23.1	Isolation	soil	TAS [1]
MIGS-4	Geographic location	Guangyuan City, Sichuan (China)	TAS [1]
MIGS-5	Sample collection time	1986 or before	NAS
MIGS-4.1 MIGS-4.2	Latitude – Longitude	32.45 – 105.84	NAS
MIGS-4.3	Depth	not reported	
MIGS-4.4	Altitude	not reported	

Evidence codes - TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from of the Gene Ontology project [28].

Genome sequencing and annotation

Genome project history

This organism was selected for sequencing as part of the DOE Joint Genome Institute Community Sequencing Program (CSP) 2011, CSP 312, "Whole genome type strain sequences of the genus *Saccharomonospora* – a taxonomically troubled genus with bioenergetic potential". The genome project is deposited in the Genomes On Line Database [19] and the complete genome sequence is deposited in GenBank. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI). A summary of the project information is shown in Table 2.

Table 2. Genome sequencing project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	Improved high quality draft
MIGS-28	Libraries used	Three genomic libraries: one 454 pyrosequence standard library, one 454 PE library (12 kb insert size), one Illumina library
MIGS-29	Sequencing platforms	Illumina GAii, 454 GS FLX Titanium

MIGS-31.2	Sequencing coverage	1,025.0 × Illumina; 8.6 × pyrosequence
MIGS-30	Assemblers	Newbler version 2.3, Velvet version 1.0.13, phrap version SPS - 4.24
MIGS-32	Gene calling method	Prodigal
	INSDC ID	*not yet available*
	GenBank Date of Release	*not yet*
	GOLD ID	Gi07579
	NCBI project ID	62037
	Database: IMG	2508501044
MIGS-13	Source material identifier	DSM 44631
	Project relevance	Bioenergy and phylogenetic diversity

Strain history

The history of strain NA-128^T starts in 1985 with Hu Runmao (Sichuan Industrial Institute of Antibiotics, People's Republic of China) who deposited the strain in the American Type Culture Collection (accession ATCC 43670), from where it was later deposited in the German Collection of Microorganisms and Cell Cultures (accession DSM 44631), where cultures of the strain are maintained freeze dried as well as in liquid nitrogen.

Growth conditions and DNA isolation

Strain NA-128^T, DSM 44631, was grown in DSMZ medium 83 (Czapek Peptone Medium) [41] at 28°C. DNA was isolated from 0.5-1 g of cell paste using Jetflex Genomic DNA Purification Kit (GENOMED 600100) following the standard protocol as recommended by the manufacturer with the following modifications: extended cell lysis time (60 min.) with additional 30µl Achromopeptidase, Lysostaphin, Mutanolysin; proteinase K was applied in 6-fold the supplier recommended amount for 60 min. at 58°C. The purity, quality and size of the bulk gDNA preparation were assessed by JGI according to DOE-JGI guidelines. DNA is available through the DNA Bank Network [42].

Genome sequencing and assembly

The genome was sequenced using a combination of Illumina and 454 sequencing platforms. All general aspects of library construction and sequencing can be found at the JGI website [29]. Pyrosequencing reads were assembled using the Newbler assembler (Roche). The initial Newbler assembly consisting of 215 contigs in one scaffold was converted into a phrap [30] assembly by making fake reads from the consensus, to collect the read pairs in the 454 paired end library. Illumina GAii sequencing data (5,162.6 Mb) was assembled with Velvet [31] and the consensus sequences were shredded into 1.5 kb overlapped fake reads and assembled together with the 454 data. The 454 draft assembly was based on 80.3 Mb 454 draft data and all of the 454 paired end data. Newbler parameters are -consed -a 50 -l 350 -g -m -ml 20. The Phred/Phrap/Consed software package [30] was used for sequence assembly and quality assessment in the subsequent finishing process. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible mis-assemblies were corrected with gapResolution [29], Dupfinisher [39], or sequencing cloned bridging PCR fragments with subcloning. Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR primer walks (J.-F. Chang, unpublished). A total of 158 additional reactions were necessary to close gaps and to raise the quality of the finished sequence. Illumina reads were also used to correct potential base errors and increase consensus quality using a software Polisher developed at JGI [40]. The error rate of the completed genome sequence is less than 1 in 100,000. Together, the combination of the Illumina and 454 sequencing platforms provided 1,033.6 x coverage of the genome. The final assembly contained 345,324 pyrosequence and 64,928,268 Illumina reads.

Genome annotation

Genes were identified using Prodigal [32] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI GenePRIMP pipeline [33]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) non-redundant database, UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. These data sources were combined to assert a product description for each predicted protein. Non-coding genes and miscellaneous features were predicted using tRNAscan-SE [34], RNAMMer [35], Rfam [36], TMHMM [37], and signalP [38].

Genome properties

The genome consists of a 4,763,852 bp long chromosome a 70.1% G+C content (Table 3 and Figure 3). Of the 4,530 genes predicted, 4,472 were protein-coding genes, and 58 RNAs; 96 pseudogenes were also identified. The majority of the protein-coding genes (73.8%) were assigned a putative function while the remaining ones were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 4.

Table 3. Genome Statistics

Attribute	Value	% of Total
Genome size (bp)	4,763,852	100.00%
DNA coding region (bp)	4,287,642	90.00%
DNA G+C content (bp)	3,331,901	70.08%
Number of replicons	1	
Extrachromosomal elements	0	
Total genes	4,530	100.00%
RNA genes	58	1.28%
rRNA operons	3	
tRNA genes	47	1.04%
Protein-coding genes	4,472	98.72%
Pseudo genes	96	2.12%
Genes with function prediction (proteins)	3,342	73.77%
Genes in paralog clusters	2,354	51.96%
Genes assigned to COGs	3,312	73.11%
Genes assigned Pfam domains	3,450	76.16%
Genes with signal peptides	1,332	29.40%
Genes with transmembrane helices	1,070	23.62%
CRISPR repeats	0	

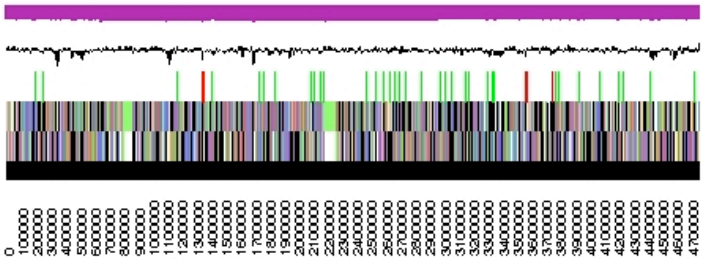

Figure 3. Graphical map of the chromosome. From bottom to the top: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.

Table 4. Number of genes associated with the general COG functional categories

Code	value	%age	Description
J	171	4.6	Translation, ribosomal structure and biogenesis
A	1	0.0	RNA processing and modification
K	394	10.6	Transcription
L	175	4.7	Replication, recombination and repair
B	2	0.1	Chromatin structure and dynamics
D	35	0.9	Cell cycle control, cell division, chromosome partitioning
Y	0	0.0	Nuclear structure
V	58	1.6	Defense mechanisms
T	190	5.1	Signal transduction mechanisms
M	156	4.2	Cell wall/membrane biogenesis
N	6	0.2	Cell motility
Z	0	0.0	Cytoskeleton
W	0	0.0	Extracellular structures
U	36	1.0	Intracellular trafficking and secretion, and vesicular transport
O	134	3.6	Posttranslational modification, protein turnover, chaperones
C	245	6.6	Energy production and conversion
G	259	7.0	Carbohydrate transport and metabolism
E	313	8.4	Amino acid transport and metabolism
F	91	2.4	Nucleotide transport and metabolism
H	194	5.2	Coenzyme transport and metabolism
I	179	4.8	Lipid transport and metabolism
P	176	4.7	Inorganic ion transport and metabolism

Q	152	4.1	Secondary metabolites biosynthesis, transport and catabolism
R	478	12.8	General function prediction only
S	282	7.6	Function unknown
-	1,218	26.9	Not in COGs

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References

1. Runmao H. *Saccharomonospora azurea* sp. nov., a new species from soil. *Int J Syst Bacteriol* 1987; **37**:60-61.
2. Yoon JH, Kim SB, Lee ST, Park YH. DNA-DNA relateness between Saccharomonospora species: '*Saccharomonospora caesia*' as a synonym of *Saccharomonospora azurea*. *Int J Syst Bacteriol* 1999; **49**:671-673.
3. Kurup VP. Taxonomic study of some members of *Micropolyspora* and *Saccharomonospora*. *Microbiologica* 1981; **4**:249-259.
4. Kalakoutskii LV. A new species of the genus *Micropolyspora – Micropolyspora caesia* n. sp. *Microbiology* (English translation of *Microbiologiya*) 1964; **33**:765-768.
5. Euzéby JP, List of Bacterial Names with Standing in Nomenclature: a folder available on the internet. *Int J Syst Bacteriol* 1997; **47**:590-590.
6. Tang SK, Wang Y, Klenk HP, Shi R, Lou K, Zhang YJ, Chen C, Ruan JS, Li WJ. *Actinopolyspora alba* sp. nov. and *Actinopolyspora erythraea* sp. nov., isolated from a salt field, and reclassification of *Actinopolyspora iraqiensis* Ruan *et al*. 1994 as a heterotypic synonym of *Saccharomonospora halophila*. *Int J Syst Evol Microbiol* 2011; **61**, 1693-1698.
7. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Thomson N, Allen MJ, Angiuoli SV, *et al.* The minimum information about a genome sequence (MIGS) specification. *Nat Biotechnol* 2008; **26**:541-547.
8. Hess PN, De Moraes Russo CA. An empirical test of the midpoint rooting method. *Biol J Linn Soc* 2007; **92**:669-674.
9. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Bascic local alignment search tool. *J Mol Biol* 1990; **215**:403-410.
10. Korf I, Yandell M, Bedell J. BLAST, O'Reilly, Sebastopol, 2003.
11. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, *et al*. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Env Microbiol* 2006; **72**:5069-5072.
12. Porter MF. An algorithm for suffix stripping. Program: *electronic library and information systems* 1980; **14**:130-137.

13. Lee C, Grasso C, Sharlow MF. Multiple sequence alignment using partial order graphs. *Bioinformatics* 2002; **18**:452-464.

14. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 2000; **17**:540-552.

15. Stamatakis A, Hoover P, Rougemont J. A rapid bootstrap algorithm for the RAxML web servers. *Syst Biol* 2008; **57**:758-771.

16. Pati A, Sikorski J, Nolan M, Lapidus A, Copeland A, Glavina Del Rio T, Lucas S, Chen F, Tice H, Pitluck S, *et al*. Complete genome sequence of *Saccharomonospora viridis* type strain (P101^T). *Stand Genomic Sci* 2009; **1**:141-149.

17. Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A. How many bootstrap replicates are necessary? *Lect Notes Comput Sci* 2009; **5541**:184-200.

18. Swofford DL. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0 b10. Sinauer Associates, Sunderland, 2002.

19. Liolios K, Chen IM, Mavromatis K, Tavernarakis N, Kyrpides NC. The genomes on line database (GOLD) in 2009: Status of genomic and metagenomic projects and their associated metadata. *Nucleic Acids Res* 2010; **38**:D346-D354.

20. Skerman VBD, McGowan V, Sneath PHA. Approved Lists of Bacterial Names. *Int J Syst Bacteriol*1980; **30**:225-420.

21. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms. Proposal for the domains *Archaea* and *Bacteria*. *Proc Nat Acad Sci USA* 1990; **87**:4576-4579.

22. List Editor. Validation List no. 29. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. *Int J Syst Bacteriol* 1989; **39**:205-206.

23. Embley MT, Smida J, Stackebrandt E. The phylogeny of mycolate-less wall chemotype IV Actinomycetes and description of *Pseudonocardiaceae* fam. nov. *Syst Appl Microbiol* 1988; **11**:44-52.

24. Stackebrandt E, Rainey FA, Ward-Rainey NL. Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* 1997; **47**:479-491. doi:10.1099/00207713-47-2-479.

25. Labeda DP, Goodfellow M, Chun J, Zhi X-Y, Li W-J. Reassessment of the systematics of the suborder *Pseudonocardineae*: transfer of the genera within the family *Actinosynnemataceae* Labeda and Kroppenstedt 2000 emend. Zhi *et al*. 2009 into an emended family *Pseudonocardiaceae* Embley *et al*. 1989 emend. Zhi *et al*. 2009. *Int J Syst Evol Microbiol* 2011; **61**:1259-1264. doi:10.1099/ijs.0.024984-0 [PubMed]..

26. Nonomura H, Ohara Y. Distribution of actinomycetes in soil. X. New genus and species of monosporic actinomycetes in soil. *J Ferment Technol* 1971; **49**:895-903.

27. BAuA 2010, Classification of bacteria and archaea in risk groups. http://www.baua.de TRBA 466, p. 194.

28. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, *et al*. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000; **25**:25-29.

29. The DOE Joint Genome Institute. www.jgi.doe.gov

30. Phrap and Phred for Windows. MacOS, Linux, and Unix. www.phrap.com

31. Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 2008; **18**:821-829.

32. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal Prokaryotic Dynamic Programming Genefinding Algorithm. *BMC Bioinformatics* 2010; **11**:119.

33. Pati A, Ivanova N, Mikhailova N, Ovchinikova G, Hooper SD, Lykidis A, Kyrpides NC. GenePRIMP: A Gene Prediction Improvement Pipeline for microbial genomes. *Nat Methods* 2010; **7**:455-457.

34. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 1997; **25**:955-964.

35. Lagesen K, Hallin PF, Rødland E, Stærfeldt HH, Rognes T, Ussery DW. RNammer: consistent annotation of rRNA genes in genomic sequences. *Nucleic Acids Res* 2007; **35**:3100-3108.

36. Griffiths-Jones S, Bateman A, Marshall M, Khanna A, Eddy SR. Rfam: an RNA family database. *Nucleic Acids Res* 2003; **31**:439-441.

37. Krogh A, Larsson B, von Heijne G, Sonnhammer ELL. Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. *J Mol Biol* 2001; **305**:567-580.

38. Bendtsen JD, Nielsen H, von Heijne G, Brunak S. Improved prediction of signal peptides: SignalP 3.0. *J Mol Biol* 2004; **340**:783-795.

39. Han C, Chain P. Finishing repeat regions automatically with Dupfinisher. *In:* Proceeding of the 2006 international conference on bioinformatics & computational biology. Arabnia HR, Valafar H (*eds*), CSREA Press. June 26-29, 2006: 141-146.

40. Lapidus A, LaButti K, Foster B, Lowry S, Trong S, Goltsman E. POLISHER: An effective tool for using ultra short reads in microbial genome assembly and finishing. AGBT, Marco Island, FL, 2008.

41. List of growth media used at DSMZ: http://www.dsmz.de/catalogues/catalogue-microorganisms/culture-technology/list-of-media-for-microorganisms.html.

42. Gemeinholzer B, Dröge G, Zetzsche H, Haszprunar G, Klenk HP, Güntsch A, Berendsohn WG, Wägele JW. The DNA Bank Network: the start from a German initiative. *Biopreserv Biobank* 2011; **9**:51-55. [doi:10.1089/bio.2010.0029]

43. Buchanan RE. Studies in the nomenclature and classification of bacteria. II. The primary subdivisions of the *Schizomycetes*. *J Bacteriol* 1917; **2**:155-164. [PubMed].

44. Zhi XY, Li WJ, 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* 2009; **59**:589-608. doi:10.1099/ijs.0.65780-0 [PubMed].

45. Garrity GM, Holt JG. The Road Map to the Manual. *In:* Garrity GM, Boone DR, Castenholz RW (*eds*), Bergey's Manual of Systematic Bacteriology, Second Edition, Volume 1, Springer, New York, 2001, p. 119-169.

46. Wu D, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, Kunin V, Goodwin L, Wu M, Tindall BJ, *et al*. A phylogeny-driven Genomic Encyclopaedia of *Bacteria* and *Archaea*. *Nature* 2009; **462**:1056-1060.

47. Carbon Active Enzyme Database. www.cazy.org.

48. Land M, Lapidus A, Mayilraj S, Chen R, Copeland A, Glavina Del Rio T, Nolan M, Lucas S, Tice H, Cheng JF, *et al.* Complete genome sequence of *Actinosynnema mirum* type strain (101^T). *Stand Genomic Sci* 2009; **1**:46-53.

49. Liolios K, Sikorski J, Jando M, Lapidus A, Copeland A, Glavina Del Rio T, Nolan M, Lucas S, Tice H, Cheng JF, *et al.* Complete genome sequence of *Thermobispora bispora* type strain ($R51^T$). *Stand Genomic Sci* 2010: **2**:318-326.

50. Wink JM. Compendium of Actinobacteria. http://www.dsmz.de/microorganisms/wink_pdf/DSM44631.pdf.

51. Garrity G. NamesforLife. BrowserTool takes expertise out of the database and puts it right in the browser. *Microbiol Today* 2010; 37:9.

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