

Electronic Supplementary Materials

Occurrence of *vanHAX* and related genes beyond the *Actinobacteria* phylum

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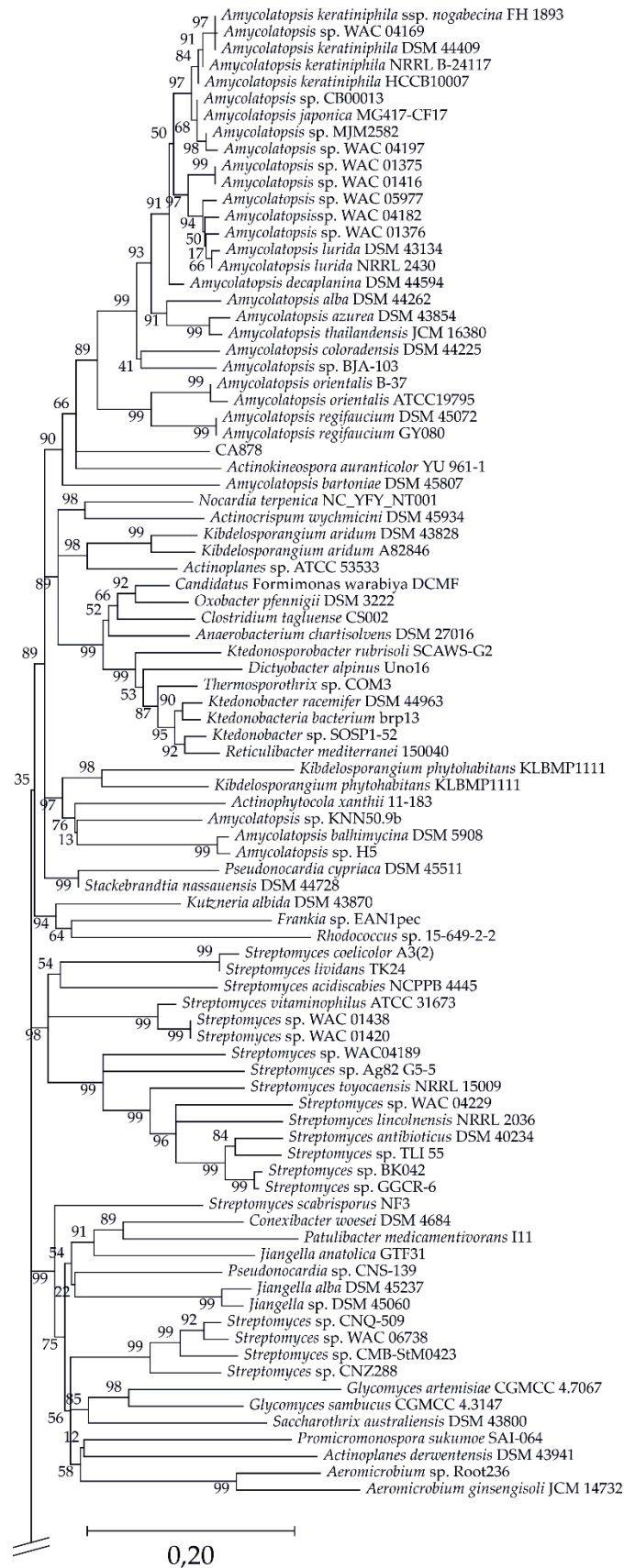
Inventory of Electronic supplementary materials

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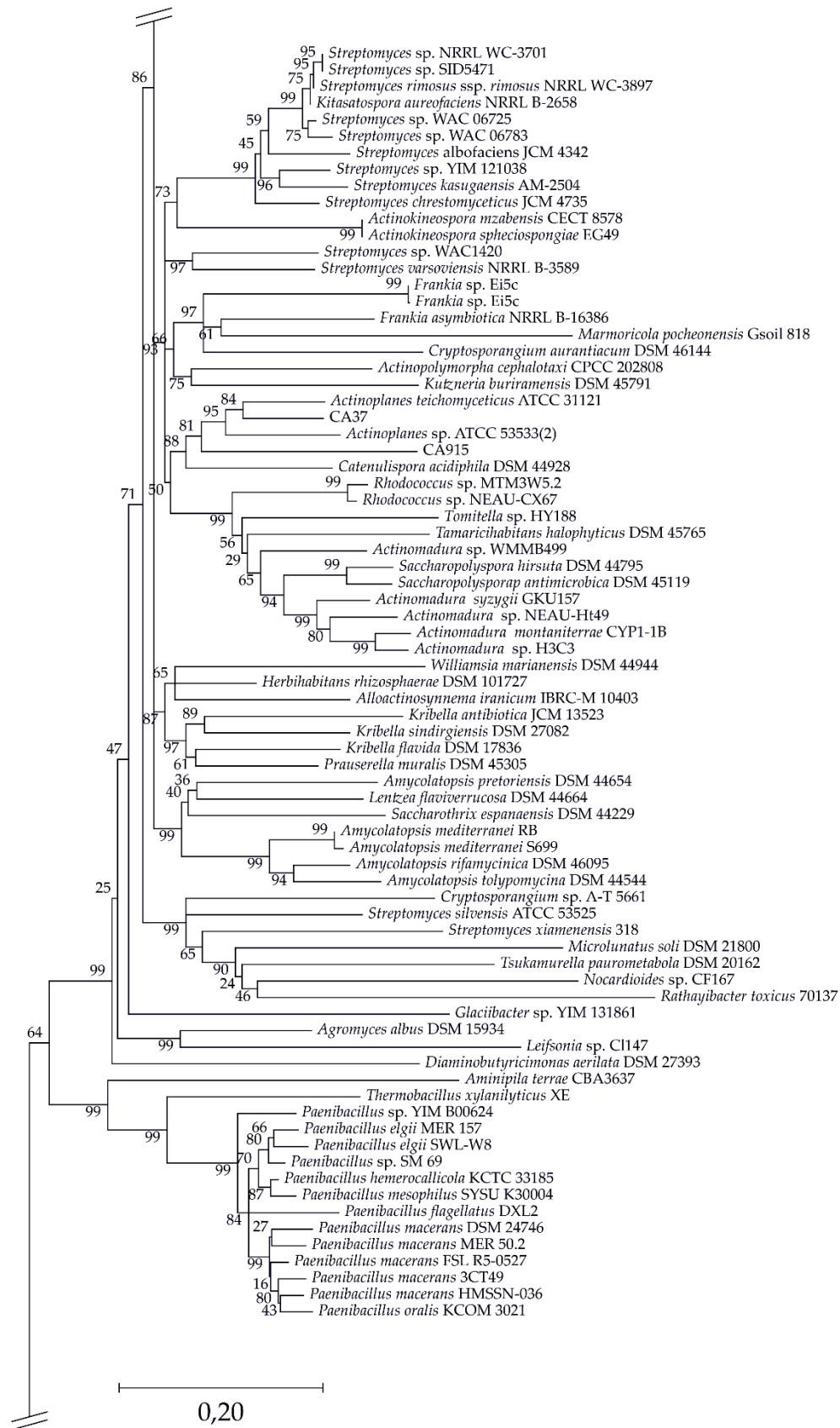
ESM Table S1. List of the bacterial species beyond *Actinobacteria* phylum described in the literature and from which sequencing data for *vanHAXRS* genes are available.

Taxonomic position	Accession number of corresponding nucleic acid sequence	Strain carrying <i>vanHAXRS</i> genes	Reference
Class <i>Bacilli</i>	CP022486	<i>Enterococcus (Ecc.) faecalis</i> ARO1/DG, plasmid pARO1.3	[1]
	PRJNA476469	Multiple <i>Ecc. faecalis</i> isolates, short reads covering parts of <i>van</i> genes available	[1]
	AB247327	<i>Ecc. faecalis</i> , plasmid pSL1	[2]
	GQ484956	<i>Ecc. faecalis</i> , plasmid pWZ1668	[3]
	GQ484955	<i>Ecc. faecalis</i> , plasmid pWZ7140	[3]
	GQ484954	<i>Ecc. faecalis</i> , plasmid pWZ909	[3]
	AF192329	<i>Ecc. faecalis</i> BM4382, transposon Tn1549	[4]
	NZ_AUWX01000187	<i>Ecc. faecium</i> E155	[5]
	NZ_AUWV01000199	<i>Ecc. faecium</i> E525	[5]
	FN424376	<i>Ecc. faecium</i> 399/F98/A4, partial sequence of plasmid pVEF4	[6]
	AM932524	<i>Ecc. faecium</i> BM4147, plasmid pIP816	[6]
	HQ115078	<i>Ecc. faecium</i> S177, plasmid pS177	[7]
	JQ663598	<i>Ecc. faecium</i> , plasmid pF856	[8]
	AM296544	<i>Ecc. faecium</i> 399/F99/H8, plasmid pVEF1	[9]
	AM410096	<i>Ecc. faecium</i> 399/F99/A9, plasmid pVEF2	[9]
	AM931300	<i>Ecc. faecium</i> 399/S99/A7, plasmid pVEF3	[10]
	NZ_RDPZ01000021, NZ_RDPZ01000080	<i>Ecc. faecium</i> HBSJRP	[11]
	AY655721	<i>Ecc. faecium</i> MLG229	[12]
	NZ_CP064436	<i>Ecc. faecium</i> PR00859-7, plasmid unnamed_4	[13]
	NZ_CP064428	<i>Ecc. faecium</i> PR01996-12 plasmid unnamed_4	[13]
	NZ_CP064448	<i>Ecc. faecium</i> PR02395-7 plasmid unnamed_5	[13]
	NZ_CP064419	<i>Ecc. faecium</i> PR02648-8 plasmid unnamed_2	[13]
	NZ_CP033206	<i>Ecc. faecium</i> RBWH1	[14]
	NZ_CP033209	<i>Ecc. faecium</i> RBWH1, plasmid pRBWH1.3	[14]
	NZ_JSET01000031	<i>Ecc. faecium</i> VRE3	[15]
	KY595962	<i>Ecc. faecium</i> VREfm1, plasmid pPEC286	[16]
	KY595966	<i>Ecc. faecium</i> VREfm81, plasmid pBUD102	[16]
	KY595968	<i>Ecc. faecium</i> VREfm87, plasmid pMIS10	[16]
	CM003134	<i>Ecc. gallinarum</i> A6981, plasmid pA6981	[17]
	PRJNA604849	<i>Enterococcus</i> sp. isolates, short reads covering parts of <i>van</i> genes	[18]
	AE017171	<i>Staphylococcus (Scc.) aureus</i> , plasmid pLW043	[19]
	NZ_LJOB01000059	<i>Scc. aureus</i> HOU1444-VR	[20]
	NZ_CP012594	<i>Scc. aureus</i> strain HOU1444-VR, plasmid pVR-MSSA_01	[20]
	NZ_AHBK01000092	<i>Scc. aureus</i> ssp. <i>aureus</i> VRS1	[21]
	NZ_AHBT01000037	<i>Scc. aureus</i> ssp. <i>aureus</i> VRS10	[21]

	NZ_AHBU01000043	<i>Sc. aureus ssp. aureus</i> VRS11a	[21]
	NZ_AHBV01000045	<i>Sc. aureus ssp. aureus</i> VRS11b	[21]
	NZ_AHBL01000065	<i>Sc. aureus ssp. aureus</i> VRS2	[21]
	NZ_AHBM01000055	<i>Sc. aureus ssp. aureus</i> VRS3a	[21]
	NZ_AHBN01000064	<i>Sc. aureus ssp. aureus</i> VRS4	[21]
	NZ_AHBO01000060	<i>Sc. aureus ssp. aureus</i> VRS5	[21]
	NZ_AHBP01000061	<i>Sc. aureus ssp. aureus</i> VRS6	[21]
	NZ_AHBQ01000050	<i>Sc. aureus ssp. aureus</i> VRS7	[21]
	NZ_AHBR01000049	<i>Sc. aureus ssp. aureus</i> VRS8	[21]
	NZ_AHBS01000053	<i>Sc. aureus ssp. aureus</i> VRS9	[21]
	DQ018711	<i>Paenibacillus (Pba.) apiarius</i> PA-B2B	[22]
	AF155139	<i>Pba. popilliae</i> ATCC 14706	[23]
	DQ018710	<i>Pba. thiaminolyticus</i> PT-2B1	[22]
	CP068595	<i>Pba. sonchi</i> LMG 24727	[24]
	CP019652	<i>Pba. larvae</i> subsp. <i>larvae</i> DSM 25430	[24]
	CP020557	<i>Pba. larvae</i> subsp. <i>pulvifaciens</i> strain SAG 10367	[24]
	CP019687	<i>Pba. larvae</i> subsp. <i>larvae</i> strain ATCC 9545	[24]
	CP020327	<i>Pba. larvae</i> subsp. <i>pulvifaciens</i> strain CCM 38	[24]
	CP019794	<i>Pba. larvae</i> subsp. <i>pulvifaciens</i> strain ATCC 13537	[24]
	CP003355	<i>Pba. larvae</i> subsp. <i>larvae</i> DSM 25430	[24]
	CP019717	<i>Pba. larvae</i> subsp. <i>larvae</i> strain Eric_V	[24]
	CP032410	<i>Brevibacillus (Bba.) laterosporus</i> E7593-50	[24]
	CP039710	<i>Thermoactinomyces (Tam.) vulgaris</i> 2H	[24]
	CP036487	<i>Tam. vulgaris</i> CDF	[24]
	Y15704-08, Y17303-05	<i>Bacillus (B.) circulans</i> VR0709	[25]
Class	KU558763	<i>Clostridioides (Cld.) difficile</i> AI0499, transposon Tn1549-like	[26]
<i>Clostridia</i>	AY655720	<i>Clostridium (Cli.)</i> sp. MLG245, transposon Tn1549-like	[12]
	AP008230	<i>Desulfitobacterium (Dsf.) hafniense</i> Y51	[27]
	JH414450	<i>Dsf. hafniense</i> DP7	
	CP001336	<i>Dsf. hafniense</i> DCB-2	



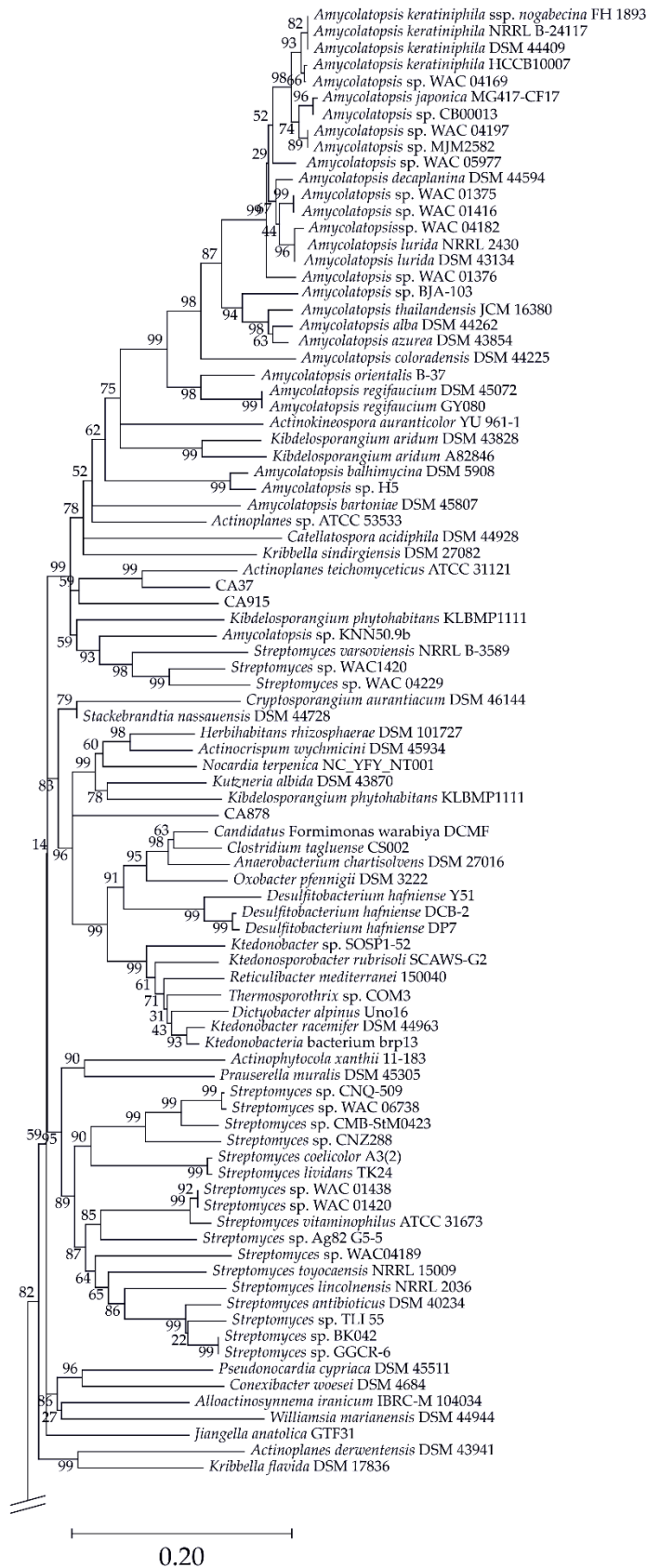
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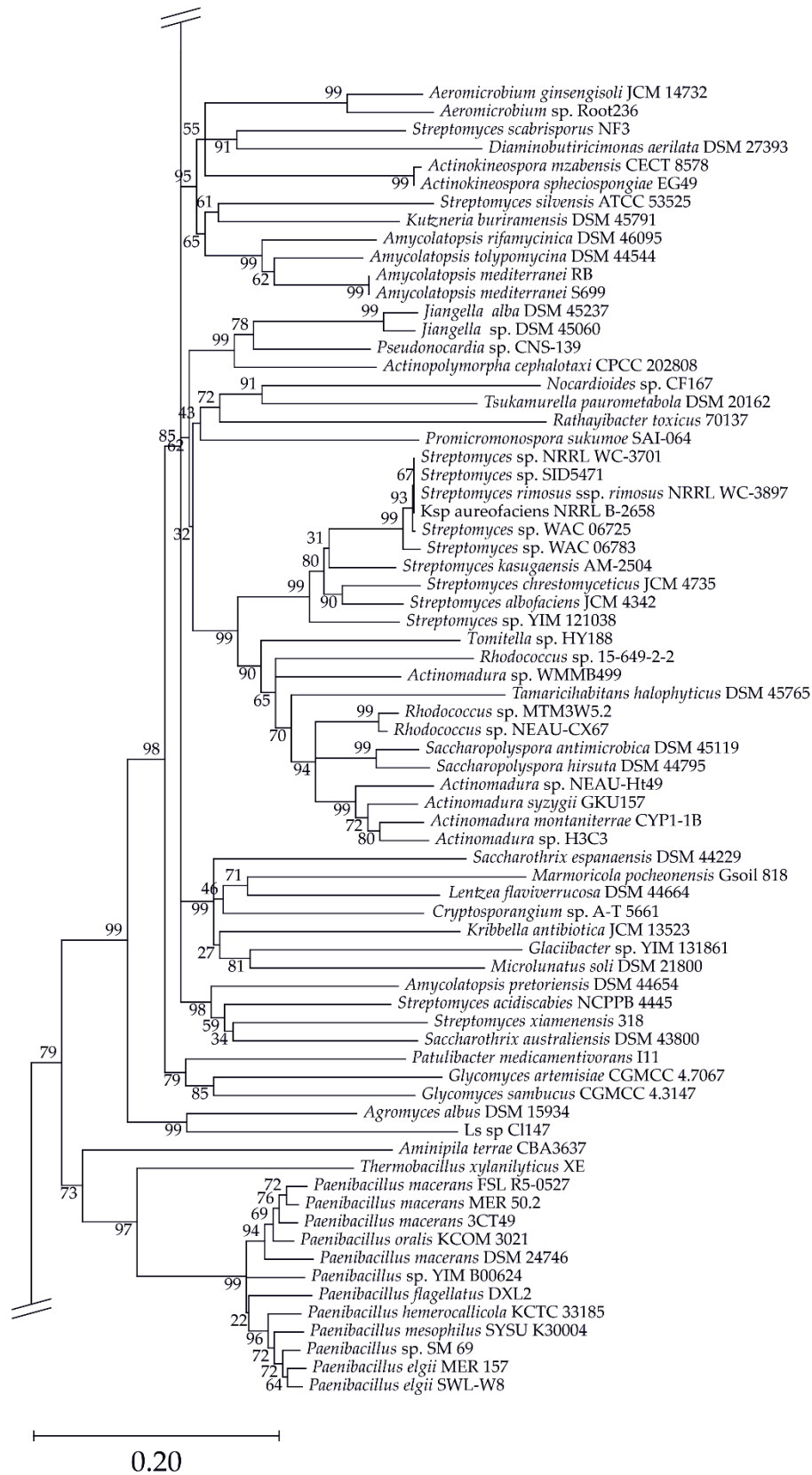
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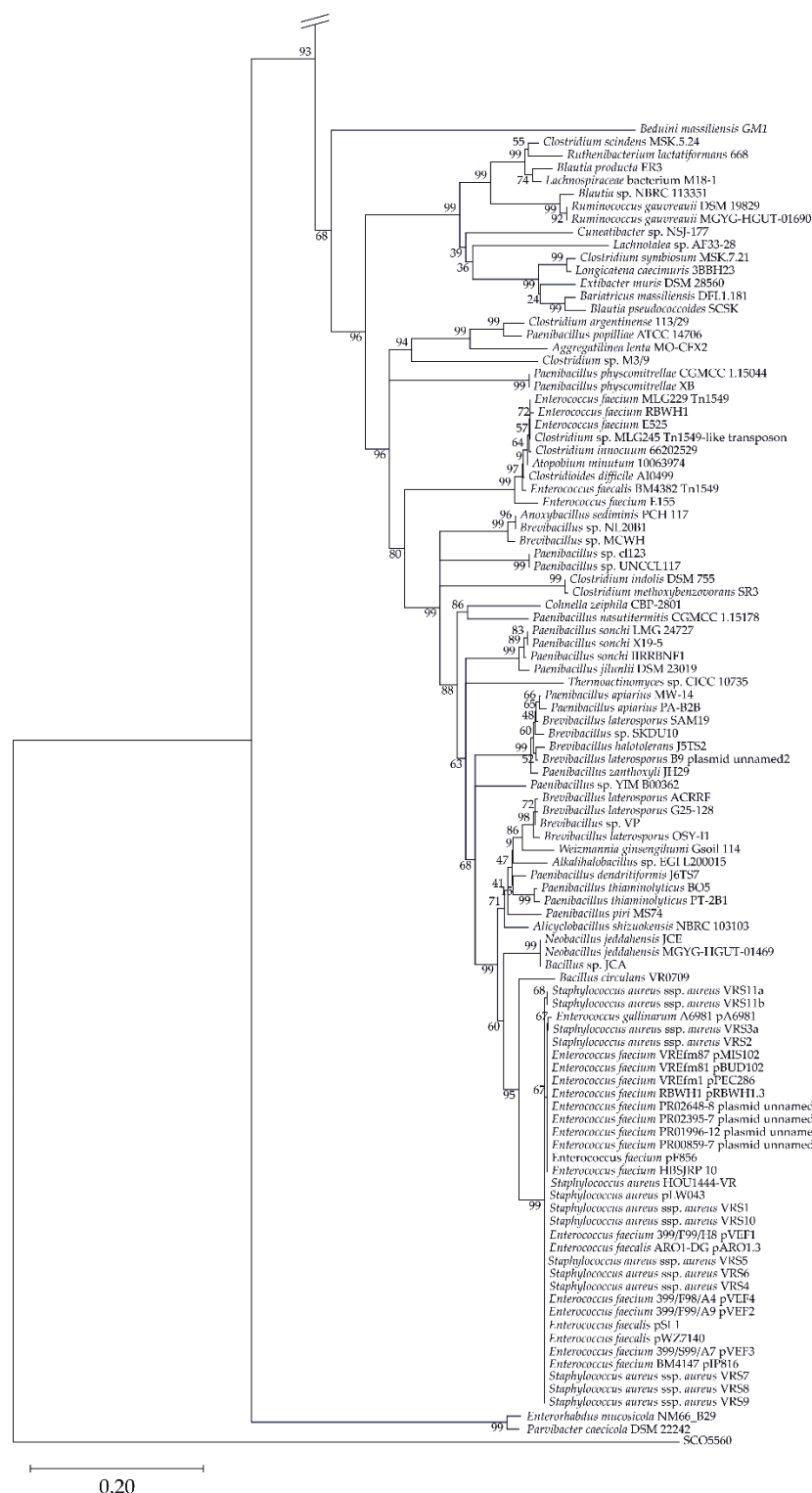
ESM Figure S1. Rooted neighbour-joining phylogenetic tree showing the overall phylogeny of 269 VanH-proteins coming from *Actinobacteria*, *Anaerolineae*, *Bacilli*, *Clostridia*, *Erysipelotrichia*, and *Ktedonobacteria*, including SCO2118 – a putative D-Lac dehydrogenase – from *Streptomyces coelicolor* A3(2) as an outgroup. Approach for the reconstruction of the tree is described in Methods section. Scale bar represents number of substitutions per site.



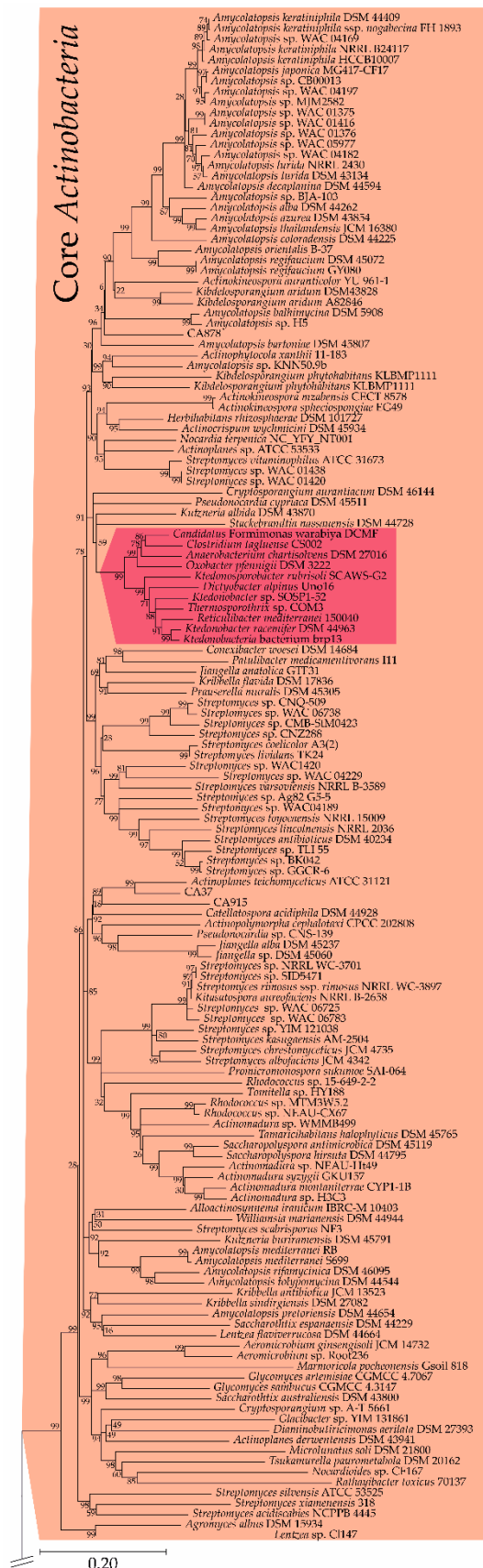
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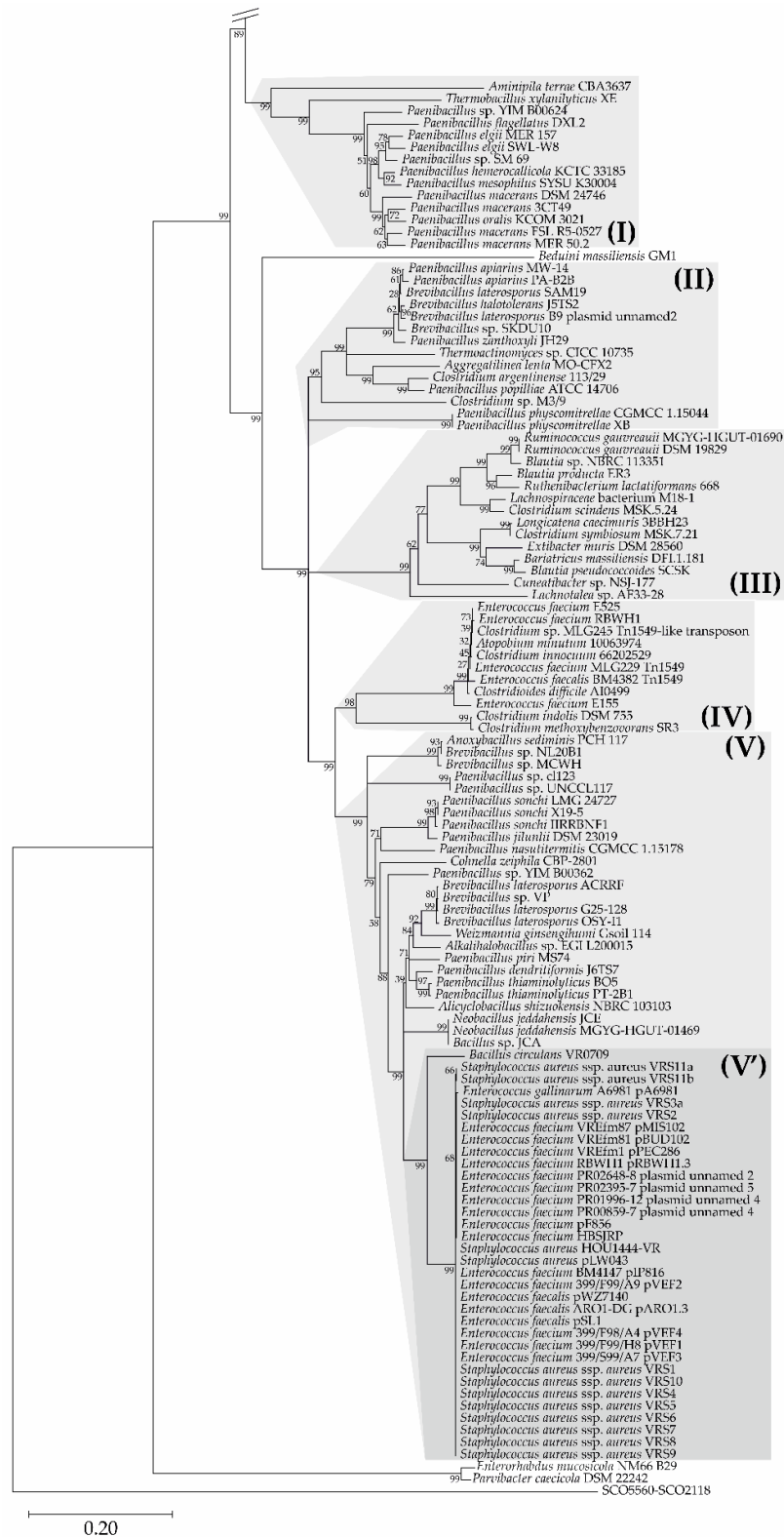
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ESM Figure S2. Rooted neighbour-joining phylogenetic tree showing the overall phylogeny of 265 VanA-proteins coming from *Actinobacteria*, *Anaerolineae*, *Bacilli*, *Clostridia*, *Erysipelotrichia*, and *Ktedonobacteria*, including SCO5560 – a putative D-Ala-D-Ala ligase – from *S. coelicolor* A3(2) as an outgroup. Approach for the reconstruction of the tree is described in Methods section. Scale bar represents number of substitutions per site.



ESM Figure S3. Continued on the next page.



ESM Figure S3. A version of a main text Figure 4 drawn to scale. Scale bar represents number of substitutions per site. “Core Actinobacteria” clade is highlighted in pink, while *Clostridia* and *Ktedonobacteria* subclade is in dark pink. Clades (I-V) discussed in main text are highlighted in grey.

Supplementary References

1. Rushton-Green, R.; Darnell, R.L.; Taiaroa, G.; Carter, G.P.; Cook, G.M.; Morgan, X.C. Agricultural origins of a highly persistent lineage of vancomycin-resistant *Enterococcus faecalis* in New Zealand. *Appl. Environ. Microbiol.* **2019**, *85*, e00137-19, 1-17, doi:10.1128/AEM.00137-19.
2. Lim, S.K.; Tanimoto, K.; Tomita, H.; Ike, Y. Pheromone-responsive conjugative vancomycin resistance plasmids in *Enterococcus faecalis* isolates from humans and chicken feces. *Appl. Environ. Microbiol.* **2006**, *72*, 6544–6553, doi:10.1128/AEM.00749-06.
3. Zhu, W.; Murray, P.R.; Huskins, W.C.; Jernigan, J.A.; McDonald, L.C.; Clark, N.C.; Anderson, K.F.; McDougal, L.K.; Hageman, J.C.; Olsen-Rasmussen, M.; et al. Dissemination of an *Enterococcus Inc18*-like *vanA* plasmid associated with vancomycin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **2010**, *54*, 4314–4320, doi:10.1128/AAC.00185-10.
4. Garnier, F.; Taourit, S.; Glaser, P.; Courvalin, P.; Galimand, M. Characterization of transposon Tn1549, conferring VanB-type resistance in *Enterococcus* spp. *Microbiology*. **2000**, *146*, 1481–1489, doi:10.1099/00221287-146-6-1481.
5. De Been, M.; Van Schaik, W.; Cheng, L.; Corander, J.; Willems, R.J. Recent recombination events in the core genome are associated with adaptive evolution in *Enterococcus faecium*. *Genome Biol. Evol.* **2013**, *5*, 1524–1535, doi:10.1093/gbe/evt111.
6. Sletvold, H.; Johnsen, P.J.; Wikmark, O.G.; Simonsen, G.S.; Sundsfjord, A.; Nielsen, K.M. Tn1546 is part of a larger plasmid-encoded genetic unit horizontally disseminated among clonal *Enterococcus faecium* lineages. *J. Antimicrob. Chemother.* **2010**, *65*, 1894–1906, doi:10.1093/jac/dkq219.
7. Halvorsen, E.M.; Williams, J.J.; Bhimani, A.J.; Billings, E.A.; Hergenrother, P.J. Txe, an endoribonuclease of the enterococcal Axe-Txe toxin-antitoxin system, cleaves mRNA and inhibits protein synthesis. *Microbiology* **2011**, *157*, 387–397, doi:10.1099/mic.0.045492-0.
8. Szakacs, T.A.; Kalan, L.; McConnell, M.J.; Eshaghi, A.; Shahinas, D.; McGeer, A.; Wright, G.D.; Low, D.E.; Patel, S.N. Outbreak of vancomycin-susceptible *Enterococcus faecium* containing the wild-type *vanA* gene. *J. Clin. Microbiol.* **2014**, *52*, 1682–1686, doi:10.1128/JCM.03563-13.
9. Sletvold, H.; Johnsen, P.J.; Simonsen, G.S.; Aasnæs, B.; Sundsfjord, A.; Nielsen, K.M. Comparative DNA analysis of two *vanA* plasmids from *Enterococcus faecium* strains isolated from poultry and a poultry farmer in Norway. *Antimicrob. Agents Chemother.* **2007**, *51*, 736–739, doi:10.1128/AAC.00557-06.

10. Sletvold, H.; Johnsen, P.J.; Hamre, I.; Simonsen, G.S.; Sundsfjord, A.; Nielsen, K.M. Complete sequence of *Enterococcus faecium* pVEF3 and the detection of an ω - ϵ - ζ toxin-antitoxin module and an ABC transporter. *Plasmid*. 2008, 60, 75–85, doi:10.1016/j.plasmid.2008.04.002.
11. Mello, S.S.; Van Tyne, D.; Lebreton, F.; Silva, S.Q.; Nogueira, M.C.L.; Gilmore, M.S.; Camargo, I.L.B.C. A mutation in the glycosyltransferase gene *lafB* causes daptomycin hypersusceptibility in *Enterococcus faecium*. *J. Antimicrob. Chemother.* **2020**, 75, 36–45, doi:10.1093/jac/dkz403.
12. Ballard, S.A.; Pertile, K.K.; Lim, M.; Johnson, P.D.R.; Grayson, M.L. Molecular characterization of *vanB* elements in naturally occurring gut anaerobes. *Antimicrob. Agents Chemother.* **2005**, 49, 1688–1694, doi:10.1128/AAC.49.5.1688-1694.2005.
13. Kinnear, C.L.; Hansen, E.; Morley, V.J.; Tracy, K.C.; Forstchen, M.; Read, A.F.; Woods, R.J. Daptomycin treatment impacts resistance in off-target populations of vancomycin-resistant *Enterococcus faecium*. *PLoS Biol.* **2020**, 18, 1–21, doi:10.1371/journal.pbio.3000987.
14. Bohlmann, L.; De Oliveira, D.M.P.; El-Deeb, I.M.; Brazel, E.B.; Harbison-Price, N.; Ong, C.L.Y.; Rivera-Hernandez, T.; Ferguson, S.A.; Cork, A.J.; Phan, M.D.; et al. Chemical synergy between ionophore PBT2 and zinc reverses antibiotic resistance. *MBio* **2018**, 9, e02391-18, 1-12, doi:10.1128/mBio.02391-18.
15. Khan, S.; Sung, K.; Marasa, B.; Min, S.; Kweon, O.; Nawaz, M.; Cerniglia, C. Draft genome sequence of multidrug-resistant *Enterococcus faecium* clinical isolate VRE3, with a sequence type 16 pattern and novel structural arrangement of Tn1546. *Genome Announc.* **2015**, 3, 1000000, doi:10.1128/genomeA.00871-15.
16. Meleghe, S.; Nyul, A.; Kovács, K.; Kovács, T.; Ghidán, Á.; Dombrádi, Z.; Szabó, J.; Berta, B.; Lesinszki, V.; Pászti, J.; et al. Dissemination of VanA-type *Enterococcus faecium* isolates in Hungary. *Microb. Drug Resist.* **2018**, 24, 1376–1390, doi:10.1089/mdr.2017.0296.
17. Eshaghi, A.; Shahinas, D.; Li, A.; Kariyawasam, R.; Banh, P.; Desjardins, M.; Melano, R.G.; Patel, S.N. Characterization of an *Enterococcus gallinarum* isolate carrying a dual *vanA* and *vanB* cassette. *J. Clin. Microbiol.* **2015**, 53, 2225–2229, doi:10.1128/JCM.03267-14.
18. Zaheer, R.; Cook, S.R.; Barbieri, R.; Goji, N.; Cameron, A.; Petkau, A.; Polo, R.O.; Tymensen, L.; Stamm, C.; Song, J.; et al. Surveillance of *Enterococcus* spp. reveals distinct species and antimicrobial resistance diversity across a One-Health continuum. *Sci. Rep.* **2020**, 10, 1–16, doi:10.1038/s41598-020-61002-5.
19. Weigel, L.M.; Clewell, D.B.; Gill, S.R.; Clark, N.C.; McDougal, L.K.; Flannagan, S.E.;

- Kolonay, J.F.; Shetty, J.; Killgore, G.E.; Tenover, F.C. Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. *Science*. **2003**, *302*, 1569–1571, doi:10.1126/science.1090956.
20. Panesso, D.; Planet, P.J.; Diaz, L.; Hugonnet, J.E.; Tran, T.T.; Narechania, A.; Munita, J.M.; Rincon, S.; Carvajal, L.P.; Reyes, J.; et al. Methicillin-susceptible, vancomycin-resistant *Staphylococcus aureus*, Brazil. *Emerg. Infect. Dis.* **2015**, *21*, 1844–1848, doi:10.3201/eid2110.141914.
 21. Kos, V.N.; Desjardins, C. a; Griggs, A.; Cerqueira, G.; Tonder, A. Van; Holden, M.T.G.; Godfrey, P.; Palmer, K.L.; Bodi, K.; Mongodin, E.F.; et al. Comparative genomics of vancomycin-resistant *Staphylococcus aureus* strains and their positions within the clade most commonly associated with methicillin-resistant *S. aureus* hospital-acquired infection in the United States. *MBio*. **2012**, *3*, e00112-12, 1–9, doi:10.1128/mBio.00112-12.Editor.
 22. Guardabassi, L.; Perichon, B.; Van Heijenoort, J.; Blanot, D.; Courvalin, P. Glycopeptide resistance *vanA* operons in *Paenibacillus* strains isolated from soil. *Antimicrob. Agents Chemother.* **2005**, *49*, 4227–4233, doi:10.1128/AAC.49.10.4227-4233.2005.
 23. Fraimow, H.; Knob, C.; Herrero, I.A.; Patel, R. Putative VanRS-like two-component regulatory system associated with the inducible glycopeptide resistance cluster of *Paenibacillus popilliae*. *Antimicrob. Agents Chemother.* **2005**, *49*, 2625–2633, doi:10.1128/AAC.49.7.2625-2633.2005.
 24. Andreo-Vidal, A.; Binda, E.; Fedorenko, V.; Marinelli, F.; Yushchuk, O. Genomic insights into the distribution and phylogeny of glycopeptide resistance determinants within the actinobacteria phylum. *Antibiotics* **2021**, *10*, 1533, 1-30, doi:10.3390/antibiotics10121533.
 25. Ligozzi, M.; Lo Cascio, G.; Fontana, R. *vanA* gene cluster in a vancomycin-resistant clinical isolate of *Bacillus circulans*. *Antimicrob. Agents Chemother.* **1998**, *42*, 2055–2059, doi:10.1128/aac.42.8.2055.
 26. Knight, D.R.; Androga, G.O.; Ballard, S.A.; Howden, B.P.; Riley, T. V. A phenotypically silent *vanB2* operon carried on a *Tn1549*-like element in *Clostridium difficile*. *mSphere*. **2016**, *1*, e00177-16, 1-4 doi:10.1128/msphere.00177-16.
 27. Kruse, T.; Levisson, M.; de Vos, W.M.; Smidt, H. *vanI*: a novel D-Ala-D-Lac vancomycin resistance gene cluster found in *Desulfitobacterium hafniense*. *Microb. Biotechnol.* **2014**, *7*, 456–466, doi:10.1111/1751-7915.12139.