

23 **Table S1:** List of oligonucleotides used in this study.

| Primer | Sequence (5' → 3') | Purpose |
|-------------------------------------|---|--|
| pk19_fw | GTATGTTGTGTGGAATTGTGAG | Control primer flanking MCS pK19mobsacB |
| pk19_rev | CAGGGTTTTCCAGTCACGACG | |
| Do_crdA_fwd | CAAGCTTGCATGCCTGCAGGAAGAGGAACATTTGCCGC | Amplification of downstream region of <i>crdA</i> |
| Do_crdA_rev | GGTAGTCAGTTAGGTAATAACTAAAAATGATTGACG | |
| Up_crdA_fwd | TTAGTACCTAACTGACTACCTTCCATTCTTAGG | Amplification of upstream region of <i>crdA</i> |
| Up_crdA_rev | TTGTAAAACGACGGCCAGTGGAGGAAGAATGCCGGAGAAAAC | |
| CorePeptide- HTHHyd- LinL_fwd | AGCGAATTCGAGCTCGGTACCCTAGATTCTAAGATAAGGAGG TAAATAATGCTCGCTACTGCAGTTAATTC | Construction of pBAD33_crd |
| CorePeptide- HTHHyd-LinL_rev | TCAGTTCAGGCTCTTGCCGAGGAATGGG | Construction of pBAD33_crd |
| ABC-SDR_fwd | TCGGCAAGAGCCTGAACTGAGAAAATTGAC | Construction of pBAD33_crd |
| ABC-SDR_rev | CAAGCTTGCATGCCTGCAGGGGGAAATTACTCTGCTAAAAC | Construction of pBAD33_crd |
| pBAD33_fw | ATTATTTGCACGGCGTCA | sequencing of <i>crd</i> -locus |
| pBAD33_rev | GTTTTATCAGACCGCTTCT | sequencing of <i>crd</i> -locus |
| S1 | GAGCTCGGTACCCTAGATTC | sequencing of <i>crd</i> -locus |
| S2 | GTGGCTGTCTAGGTAATAAC | sequencing of <i>crd</i> -locus |
| S3 | TCCACCAATCGGCTTAAAGG | sequencing of <i>crd</i> -locus |
| S4 | GAGGTCGTGTTCCGGTCTTG | sequencing of <i>crd</i> -locus |
| S5 | CGCAATGACAGCCTTTAACCC | sequencing of <i>crd</i> -locus |
| S6 | GATTCAACCGAGAGCAGTTC | sequencing of <i>crd</i> -locus |
| S7 | GTATCGGTCCGGCAGTTGTG | sequencing of <i>crd</i> -locus |

24

25 **Table S2:** List of open reading frames contained in the *C. lactis* RW3-42 corynaridin gene cluster, the
 26 respective number of amino acids, putative TM-helices and homology to legonaridin biosynthesis
 27 proteins.

| orf ¹ | amino acids | TM-helices | legonaridin homolog | amino acid identity (%) to legonaridin homologs |
|------------------|-------------|------------|---------------------|--|
| <i>crdA</i> | 69 | - | <i>legA</i> | 25 |
| <i>crdG</i> | 275 | 1 | <i>legH</i> | 32 |
| <i>crdE</i> | 324 | - | <i>legE</i> | 21 |
| <i>crdL</i> | 182 | - | <i>legF</i> | 26 |
| <i>crdT</i> | 550 | 6 | <i>legB</i> | 31 |
| <i>crdC</i> | 243 | - | <i>legC</i> | 25 |

28 ¹orf = open reading frame

29

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LegA      1 MSVLAEFAN----TELV DV-EPGR LGSEATPTMI TPLATLAT-----PEATPVGFAATS 49
CrdA      1 --MLATAVNSAQDRGLIP ANYDGL SMSGAPMTCTPTVTVRITVRIMRATKRITVRGAEP-- 57

LegA     50 ATAAAVNMI THDVTRH                                     65
CrdA     58 AASDRVETLVA---V-                                       69

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30

31 **Figure S1:** CLUSTALW sequence alignment of LegA (Legonaridin) from *Streptomyces* sp. CT34 and
 32 CrdA (corynaridin) from *C. lactis* RW3-42.

33

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LegH      1 VASLGTLVSALEMLSQRKLDAGELLSAQLESTRPEFAKR---FPRLTRALSSKK---- 52
CrdG      1 -----MIDALKGL---QRVVQKP-LQA-----PLEFAERASAFSQLLATLEGISPKER 44

LegH     53 --AGV-----ALYGIQAGASAA--TMIW-----AHKRGVRAAGSA 83
CrdG     45 RFGGINDWDYTKYLFNSGGGISDAGVREIFVRCLATARIGASVVLLLP TGNNTRLVASS 103

LegH     84 VLA V TGAASRLRTPFGGDGADQLQQVINVVLAS-TGTFKDGDKGRDVAMRALALETTIS 141
CrdG    104 VSALS YLLGNRYT INGS DGAEQYSAI---ILASSALGRIDGGKNRDLAVDFIAAQTAFS 159

LegH    142 YVASGVVKLVSPVWLSGEAFSGVIRTHNYGDPNIYKLVHKYPM LGKLI TWTTVA AEVGF 200
CrdG    160 YFVAGAVKSLGREWRNGTAVERVV RTEVYGNRI FYRFLRRNPR LSESLTYS TVVVEMLF 218

LegH    201 PLV FVLPKPAAKAYLGSMTL FHLGIGQFMGLNRFVLAFAATHP ALLYVFDQSGRRP AVA 259
CrdG    219 PFLLLH-RGMRKVALASMF AFHAANVPLMGLGRFFIVFETS TYPAVMNSTNRLKGRFSD- 275

LegH    260 GNAVAALAPAAA                                         271
CrdG    -----

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34

35 **Figure S2:** CLUSTALW sequence alignment of LegH (HTTM-domain protein) from *Streptomyces* sp.
 36 CT34 and CrdG (HTTM-domain protein) from *C. lactis* RW3-42.

37

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LegE      1 -----MRF TGKRERARRYL THERPDGSLVEVDV- 28
CrdE      1 VTDFYRKTALITGGALGALKAGEMFGAFYADRF SRN-----WPRHMRKTCARNEVAVY 53

LegE     29 --NIPPNARRMVL LDNGLGT THEYWDWCEALP--ADMGYVRFNRPGYGLSTPSKR--Y 81
CrdE     54 KRAGVDDSQGCILLVHG MGNSSVSLVRLGEEISEL TGRTVIRYDRPGYGASRFCTDAPY 112

LegE     82 GLERHFAL LQE----LRETYVADLPLVLACHSLGGYFVAAYASLHPGAKEGVTGVVMID 136
CrdE    113 SVTQSVDELAEIIRWLHEQY---HWVTVVGH SFGLLAY--LALMGLEEPSWCNALLIE 166

LegE    137 ATDVAHLRSSRRAD I-----DRWSRQSM LME---Q-VFAVAGLSALRPALNQHKT 182
CrdE    167 PSH---LHEARRDPKRMLGVMLGVEELNRRHLLSPFGGELLDASIGPRALDGRRHPHVD 222

LegE    183 YRPEINRSYTAFLAQRRTWALAYRD-YRDAM-TYPELASVDSPLTVIT AENNKGDNAAH 239
CrdE    223 AVRRERRSSRCVRATRREFTTLAKLLFDGTVIRAPQ---PSAEVHVVASARSVGDVRQK 278

LegE    240 QKVQAKLATLSKRSRQRCIDGSDHESLLSIQPHAAQVAEII ADEPPTGRADAERRKSAG 298
CrdE    279 ELFAEYI---CSPADMTILGETSHDTIVLDKDAVNRISQIVNG----GSIHAA----- 324

LegE    299 ARP AVKSAADREKEL                                         313
CrdE    -----

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39 **Figure S3:** CLUSTALW sequence alignment of LegE (Alpha-beta hydrolase) from *Streptomyces* sp.
 40 CT34 and CrdE (Alpha-beta hydrolase) from *C. lactis* RW3-42.

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LegF      1 VTVRVNTERLKTAAVTTS LAAWLVATAVAQMPEQ--RFDNLLKRGKLRIPTPNWRFFGP 57
CrdL     1 -----MLRNVIEVVFSGWFTLTFLGHHPGGNHRSRGLLFRLSSTIFMPNWAFFAP 50

LegF     58 NPGVKDNHLLYRDVTDGKPGGEWQEIPIITRD--RAWYALAWNARNRSPKALFDAVQDVY 113
CrdL    51 NPGVYDDHLFYRVREGNEFSPWKEVVTSRNDDGPAWLSPFYSGASRRSKGIIDIFSTLE 109

LegF    114 VRSAAAYGTAMEPVVQSSGYQLLSGYIRHHLPHAE GASHSQFLVMYSYLAAPPEARQIEPI 172
CrdL   110 SLASPTLSRTDCNVIQAGRQAIANFMVNADEI-SPHTSEYEVMLVR-AAGYAQDEDPV 166

LegF    173 FVSREFPLEDEGVVQPEPYPVAA 195
CrdL   167 FYYR-FAVRNGIADAPH----- 182

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43 **Figure S4:** CLUSTALW sequence alignment of LegF (LinL protein) from *Streptomyces* sp. CT34 and
44 CrdL (LinL protein) from *C. lactis* RW3-42.

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LegF      1 -----MLLITGVLLGLLGVGASLTQPWAI GKLIEAAGKGESLAFFIVRLVGLF 48
CrdL     1 MTAFNLLRNNAGLITLGI LLSLLSTAVTLFQPALVGQLISGVSSGELQQ-PLMLLLLTV 58

LegF     49 CLGAVFSALQAYVIGRAGENIVYDVRQVLTGRLLRADLTFGKRPOQDILTRTVTDTSL 107
CrdL    59 LGTSVLTAAATMYVVSIAADRTVRDMRKKITNHLLYLVRVRELEKGGSGSFTRVTSDTSI 117

LegF    108 VKIALSQSLAQLIVSGATVIGGVLMFLIDVRLMLITMGCLGVASLLSLSIARKLRRVA 166
CrdL   118 VSTAFSSLTDFVGGFTVIGALIMAVVDWKL LLLVVI AVL LVALAIVSISSSLQNL 176

LegF    167 VQNRDDTGEFGTAVQRVLAALPTVKASRAETRETARIGRLAERARRSGIRVNAYNALFM 225
CrdL   177 SKVQDHLAALGEILQSALSAIRTIKAFRVE TKVIGNLSAEIDHAYRNRRRMSFVEAVLE 235

LegF    226 PSMNVGTQGALAVVVGAGMAWVARGEMNMADL TTFIMYLFQLVSP LVTF FMGVGQFIQG 284
CrdL   236 PLSTVASYMALLAVVLFSGIRLSNGDLSGEALTVFVTALFLMLAPIVQVSQSLGAFFEA 294

LegF    285 RAAIQRVDEL AQMPQENGGAQAARSSVPIGPLPQHLP AVEFHDVRFYRRGEEGETRTQV 343
CrdL   295 RGALDRINQLLRLEVEDSTE--SSSSLCTGPT--PLGTIEFDAYSVYR-----EKRTI 343

LegF    344 LHGISLTVPARGLTAVVGPSPGAGKTSMFQLIERFYALDGGVILLGGQNI GTLP LDTV RG 402
CrdL   344 LDNATVTVKSGEKVALTGASGSGKTSILSLL LKFYDVSAGHIRIGGKDLEDWNRRDLRA 402

LegF    403 LVGYVQQDSATMRGTVRENLT YAHPHASEDDIWEA-VEMAGLTSVVANLPKGLDTELGD 460
CrdL   403 MVTYVEQEPDLLSGTLRENLI LGTGESFDDETLIAMLGQFGL E--NFASVDGLSRSVGS 459

LegF    461 QGSGLSGGQRQRLCIARTLLQKPAVMLLDEATSNLSDSERDFRDV LRRVSQQCAVIAI 519
CrdL   460 GNSGLSGGERQRVAIIRAVLQNA PVILVDEPTSA LDSASAELSMSRL--LETDS TVIFT 516

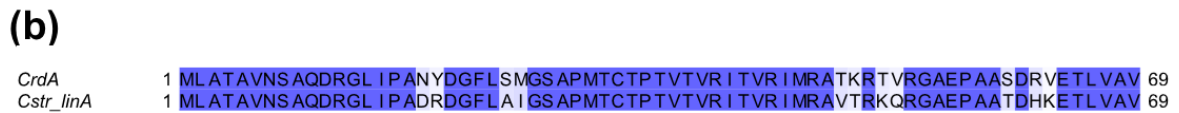
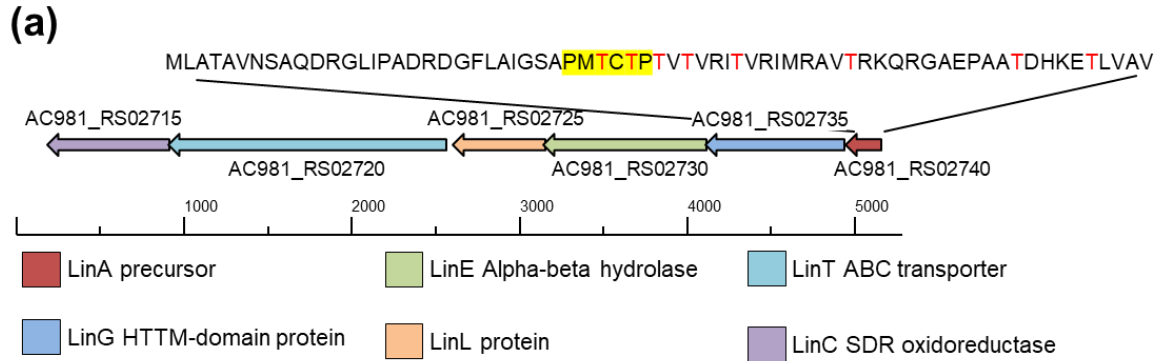
LegF    520 AHRISTVVD A EKIVVLEGGRRV RATGVHRELMEHDEL YRRLAGSQLHAGAAPVATATHW 578
CrdL   517 SHDANI VNL AERTLVVVDGRIVENSPNSRKSSNA----- 550

LegF    579 SGEPGAGPLASALGWHQWGSGLNEQTVRLRP IRIEEQW 616
CrdL   -----

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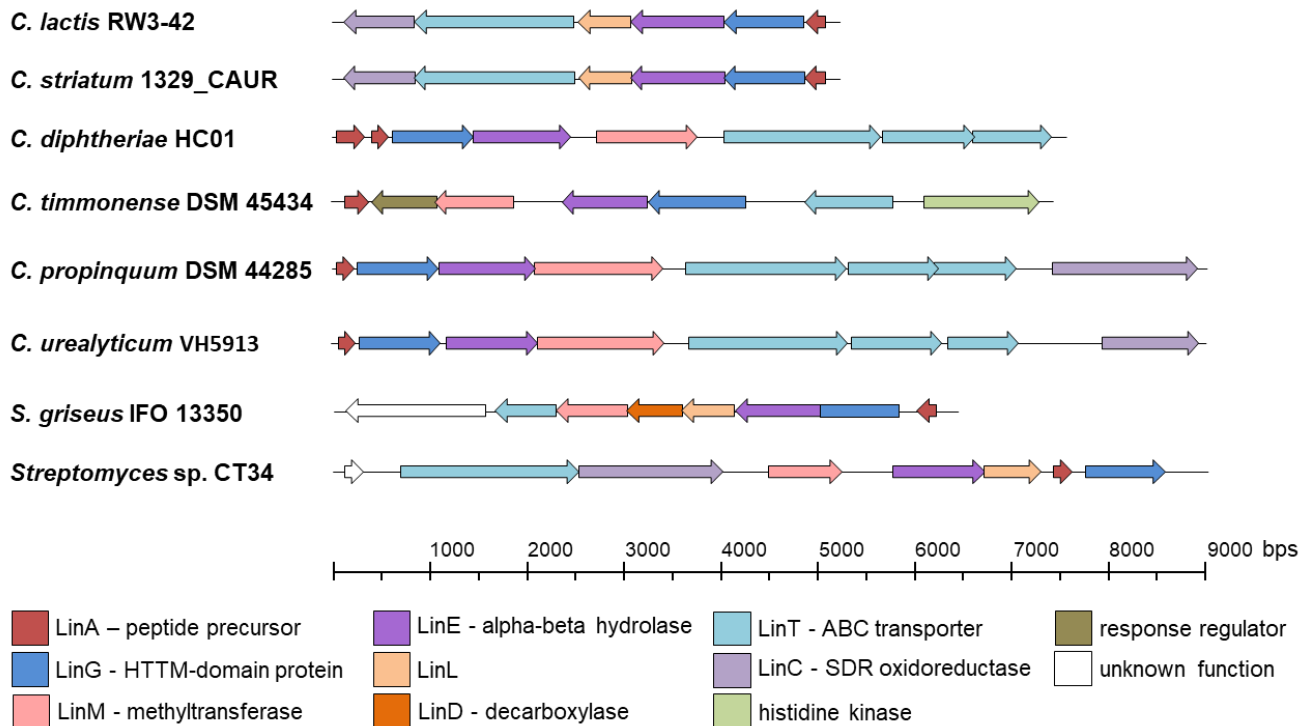
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47 **Figure S5:** CLUSTALW sequence alignment of LegB (ABC transporter) from *Streptomyces* sp. CT34
48 and CrdT (ABC transporter) from *C. lactis* RW3-42.



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Figure S7: Putative linaridin gene cluster in *C. striatum* 1329_CAUR. (a) Gene cluster and annotation according to BLASTP analyses. (b) Sequence alignment of the *C. striatum* precursor peptide AC981_RS02740 (Cstr_linA) and corynaridin (CrdA) of *C. lactis*.



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Figure S8: Putative linaridin gene clusters in *Corynebacterium* strains (1) compared with BGCs for other bacteriocins of the linaridin family. Gene cluster and annotation according to BLASTP analyses.

| | |
|---------------------------------------|--|
| <i>C. lactis</i> RW3-42 | MLATAVNSAQDRGLIPANYDGFLSMGSAPMTCPTVTVRITVRIMRA TKR TVRGAEPAA SDRVETLVAV |
| <i>C. striatum</i> 1329_CAUR | MLATAVNSAQDRGLIPADRDGFLAIGSAPMTCPTVTVRITVRIMRAVTRKQRGAEPAA TDHKE TLVAV |
| <i>C. diphtheriae</i> HC01 | MQPFSGFSLSSDERLAYFSIGHFILFQTTFGVDLSALSQLVNDNTAFRGRLDGMAETALP PVTATP AIAVTAAKAAGAVIGGGAVS GAAY AAYRAVIK MSRLSTLVNNDQITLEQVHSPVMA TPAAFGAGVVAGAKACGALVAAAGVGAAIASATKK |
| <i>C. timmonense</i> DSM 45434 | MRNNITKFADEADLQQVGSLYGDSEPVLATII TTTITATTA TTTAAGYHS SRPDEVMAGIDEDAPVSEMLAARKDAMLV |
| <i>C. propinquum</i> DSM 44285 | MSRLSTLVNNDQITLEQVHSPVMA TPAAFGAGVVAGAKACGALVAAAGVGAAIATATKE |
| <i>C. urealyticum</i> VH5913 | MSRLSTLVNNDQITLEQVHSPVMA TPAAFGAGVVAGAKACGALVAAAGVGAAIASATKK |
| <i>S. griseus</i> IFO 13350 | MRSEMTLTSTNSAEALAAQDFANTVLSAAAPGFHAD CET PAMA TPA TPTVAQFVIQGSTICLV C |
| <i>Streptomyces</i> sp. CT34 | MSVLAEFANTELVDVEPGR LGSEATPTMITPLATLATPEATPVGFAAT SATAAAVNMITHDVT RH |

62



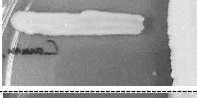


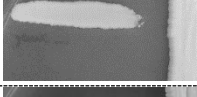


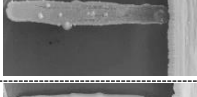
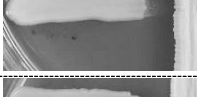


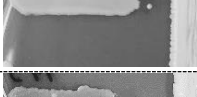


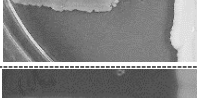


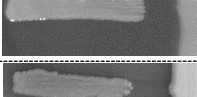


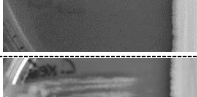


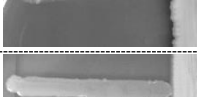


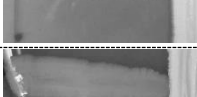



63 **Figure S9:** Predicted precursor peptide sequences in *Corynebacterium* strains (1) compared with BGCs

64 for other bacteriocins of the linaridin family. Threonine residues are displayed in red, serine residues in

65 blue letters. The predicted hexapeptide cleavage site PxxxTP is highlighted in yellow.

66

67 **Table S3:** Results of cross-streak and spot-on-lawn assays with different indicator bacteria. Cross
 68 streak assay with *C. lactis* RW3-42 streaked in the middle of a BHI agar plate and indicator bacteria
 69 lateral to it. Nisin (250 µg/mL) and corynaridin RPC-fraction (>300 µg/mL) were spotted in 10 µl drops
 70 onto the plates. Spots were documented using an iBright imaging device or a light-table and camera.
 71 n.d. = not detected.

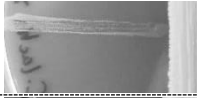

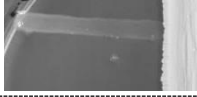


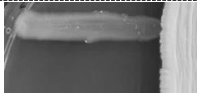


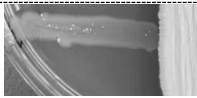

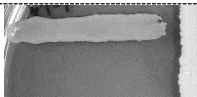




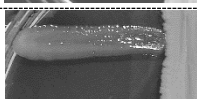
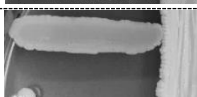

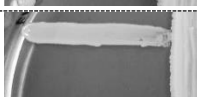

| Strain | Cross streak | Nisin | Corynaridin |
|--|---|---|---|
| <i>Bacillus subtilis</i> DSM 402 |  |  | n.d. |
| <i>Corynebacterium ammoniagenes</i> DSM 20306 |  |  |  |
| <i>Corynebacterium amycolatum</i> DSM 6922 |  |  |  |
| <i>Corynebacterium canis</i> DSM 45402 |  | n.d. | n.d. |
| <i>Corynebacterium casei</i> DSM 44701 |  |  |  |
| <i>Corynebacterium efficiens</i> DSM 44549 |  |  |  |
| <i>Corynebacterium glutamicum</i> ATCC 13032 |  |  |  |
| <i>Corynebacterium lipophiloflavum</i> DSM 44291 |  |  |  |
| <i>Corynebacterium striatum</i> DSM 20668 |  |  |  |
| <i>Corynebacterium xerosis</i> DSM 20743 |  |  |  |
| <i>Cutibacterium acnes</i> DSM 16379 |  |  |  |
| <i>Escherichia coli</i> K12 MG1655 |  | n.d. | n.d. |

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→ continued

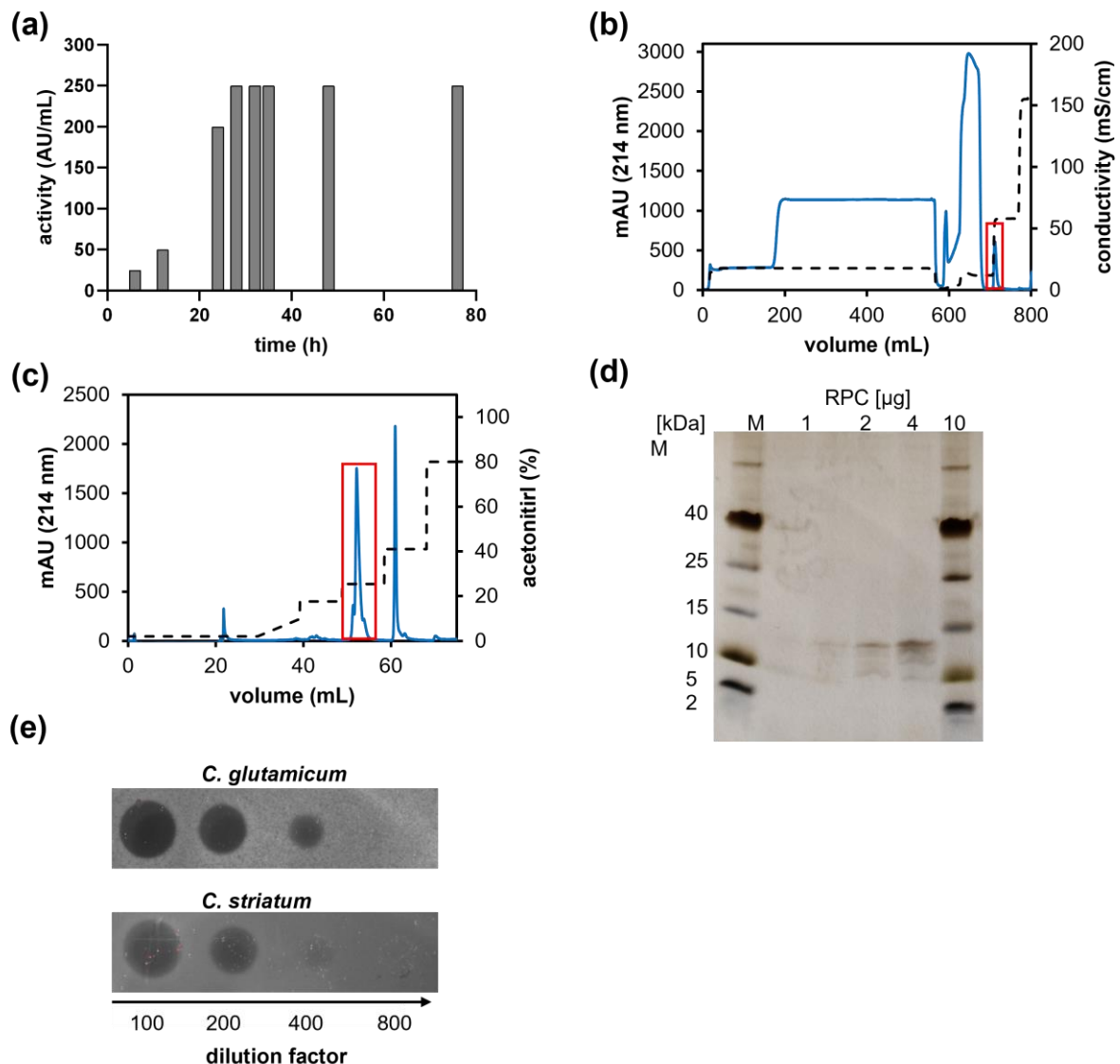
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77 **Table S3: continued.**

| strain | cross streak | nisin | corynaridin |
|--|---|---|---|
| <i>Lactobacillus plantarum</i> DSM 1055 |  |  | n.d. |
| <i>Lactococcus lactis</i> IL1403 |  |  |  |
| <i>Listeria innocua</i> LMG2785 |  |  |  |
| <i>Listeria monocytogenes</i> EGD-e |  |  | n.d. |
| <i>Micrococcus luteus</i> DSM 20030 |  |  |  |
| <i>Pediococcus acidilactici</i> 347 |  |  | n.d. |
| <i>Pseudomonas fluorescens</i> DSM 50090 |  | n.d. | n.d. |
| <i>Staphylococcus aureus</i> ATCC 29213 |  |  | n.d. |
| <i>Staphylococcus epidermidis</i> DSM 3269 |  |  | n.d. |

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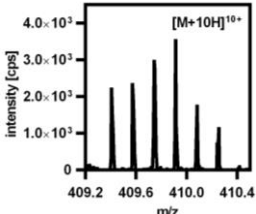
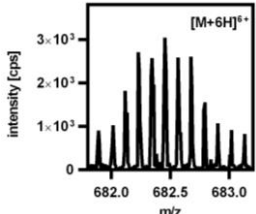
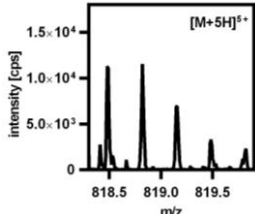
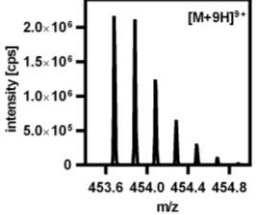
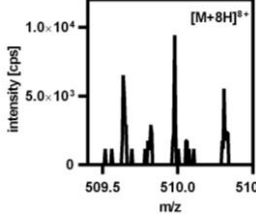
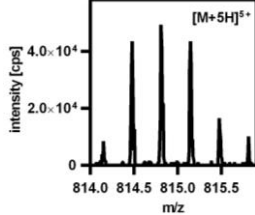
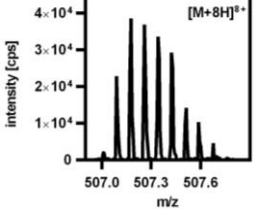
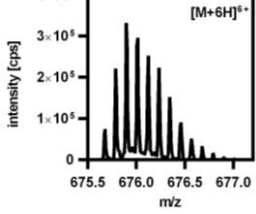
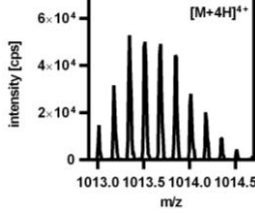
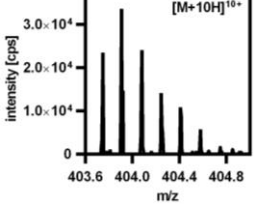
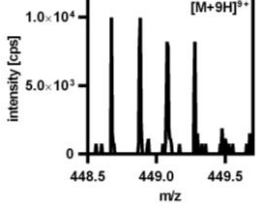
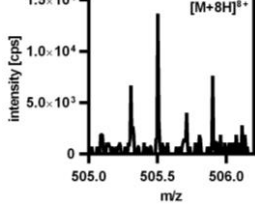
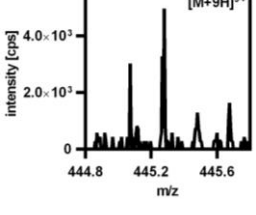
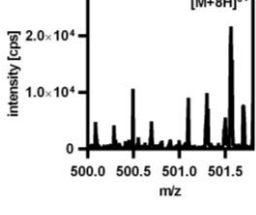
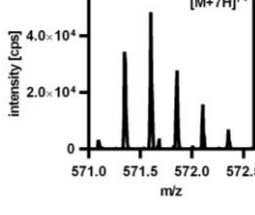
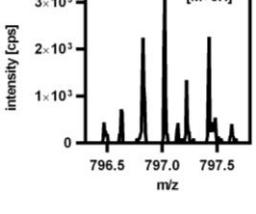
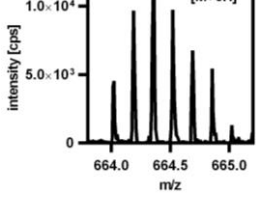
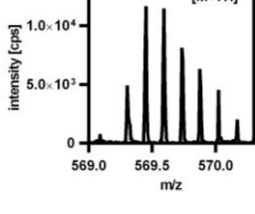
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82 **Figure S10: Improved procedure to obtain corynaridin in higher purity.** (a) Activity over time of *C.*
 83 *lactis* supernatants after growth in CLIV minimal medium with 1 % (w/v) glucose, tested via spot on lawn
 84 assay. Values represent mean and standard deviation of three biological replicates. (b and c)
 85 Purification of corynaridin from supernatants obtained after growth of *C. lactis* RW3-42 in CLIV minimal
 86 medium by ion exchange chromatography (b) and subsequent reversed phase chromatography (c). Red
 87 boxes indicate fractions that were used for further experiments. (d) Silver stained Novex™ 10-20% TRIS
 88 Tricine gel loaded with different amounts of RPC-purified corynaridin. (e) Activity of the RPC purified
 89 fraction against *C. glutamicum* and *C. striatum*. The pooled fractions were vacuum dried and
 90 resuspended in H₂O (HPLC grade) to a concentration of 200 μ g/mL.

91 **Table S4:** Complete list of all corynaridin variants with different number of dehydrated serine/threonine
 92 residues identified by LC-MS. Variants were considered identified if mass spectra for at least three
 93 charges with four isotopes had minimum signal intensities (counts per second; cps) of 10^3 .

| Peptide | | |
|---|----------------------|--|
| CT(Dhb)PT(Dhb)VT(Dhb)VRIT(Dhb)VRIMRAT(Dhb)KRT(Dhb)VRGAEPAAS(Dhas)DRVET(Dhb)LVAV | | |
| # dehydrated Ser/Thr | M _{mi} [Da] | Peaks |
| 1 | 4087.26 |    |
| 2 | 4069.25 |    |
| 3 | 4051.24 |    |
| 4 | 4033.23 |    |
| 6 | 3997.21 |    |
| 7 | 3979.20 |    |
| 8 | 3961.19 | See manuscript main text, Figure 5 |

95 **References**

- 96 1. Georgiou MA, Dommaraju SR, Guo X, Mast DH, Mitchell DA. 2020. Bioinformatic and reactivity-
97 based discovery of linaridins. *ACS Chem Biol* 15:2976–2985.

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