

Biosynthesis of a broad-spectrum nicotianamine-like metallophore in Staphylococcus aureus

Ghassan Ghssein, Catherine Brutesco, Laurent Ouerdane, Clémentine Fojcik, Amélie Izaute, Shuanglong Wang, Christine Hajjar, Ryszard Lobinski, David Lemaire, Pierre Richaud, et al.

▶ To cite this version:

Ghassan Ghssein, Catherine Brutesco, Laurent Ouerdane, Clémentine Fojcik, Amélie Izaute, et al.. Biosynthesis of a broad-spectrum nicotianamine-like metallophore in Staphylococcus aureus. Science, 2016, 352 (6289), pp.1105-1109. 10.1126/science.aaf1018 . hal-03133612

HAL Id: hal-03133612 https://hal.science/hal-03133612v1

Submitted on 13 Feb 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Title: Biosynthesis of a broad-spectrum nicotianamine-like metallophore in Staphylococcus aureus

3

4	Authors: Ghassan Ghssein ^{1,2,3,*} , Catherine Brutesco ^{1,2,3,*} , Laurent Ouerdane ^{4,*} , Clémentine
5	Fojcik ⁵ , Amélie Izaute ^{1,2,3} , Shuanglong Wang ⁴ , Christine Hajjar ^{1,2,3} , Ryszard Lobinski ⁴ , David
6	Lemaire ^{2,3,6} , Pierre Richaud ^{2,3,7} , Romé Voulhoux ⁸ , Akbar Espaillat ⁹ , Felipe Cava ⁹ , David
7	Pignol ^{1,2,3} , Elise Borezée-Durant ⁵ , Pascal Arnoux ^{1,2,3} ,†

8 Affiliations:

- 9 ¹CEA, DSV, IBEB, Laboratoire de Bioénergétique Cellulaire, Saint-Paul-lez-Durance, France
- ¹⁰ ²CNRS, UMR 7265 Biologie Végétale et Microbiologie Environnementales, Saint-Paul-lez-
- 11 Durance, France-
- ¹² ³Aix Marseille Université, CEA, CNRS, BVME UMR 7265, 13108, Saint Paul-Lez-Durance,
- 13 France
- ⁴CNRS-UPPA, Laboratoire de Chimie Analytique Bio-inorganique et Environnement,
- 15 UMR5254, Hélioparc, 2, Av. Angot, 64053 Pau, France
- ⁵Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France
- 17 ⁶CEA, DSV, IBEB, Lab Interact Protein Metal, Saint-Paul-lez-Durance, France
- 18 ⁷CEA, DSV, IBEB, Lab Bioenerget Biotechnol Bacteries & Microalgues, Saint-Paul-lez-
- 19 Durance, France
- 20 ⁸CNRS & Aix-Marseille Université, Laboratoire d'Ingénierie des Systèmes Macromoléculaires
- 21 (UMR7255), Institut de Microbiologie de la Méditerranée (IMM), Marseille, France
- 22 ⁹Laboratory for Molecular Infection Medicine Sweden, Department of Molecular Biology, Umeå
- 23 Centre for Microbial Research, Umeå University, Umeå, Sweden
- 24
- 25 *These authors contributed equally to this work.
- 26 [†]Correspondence to: pascal.arnoux@cea.fr.
- 27

1 Abstract: Metal acquisition is a vital microbial process in metal-scarce environments such as 2 inside a host. Using metabolomic exploration, targeted mutagenesis, and biochemical analysis, 3 we discovered an operon in Staphylococcus aureus that encodes the different functions required for the biosynthesis and trafficking of a broad-spectrum metallophore related to plant 4 5 nicotianamine (here called staphylopine). The biosynthesis of staphylopine reveals the 6 association of three enzyme activities: a histidine racemase, an enzyme distantly related to 7 nicotianamine synthase, and a staphylopine dehydrogenase belonging to the DUF2338 family. 8 Staphylopine is involved in nickel, cobalt, zinc, copper and iron acquisition, depending on the growth conditions. This biosynthetic pathway is conserved across other pathogens, thus 9 10 underscoring the importance of this metal acquisition strategy in infection.

11

1 Main Text:

2 Metals are required in many life processes and, consequently, all organisms have 3 developed mechanisms for their uptake and homeostasis. Pathogenic bacteria must face an 4 additional barrier of metal limitation that is usually set by the host, precisely in order to prevent 5 bacterial growth (1). This strategy of "nutritional immunity" by the hosts also extends to many 6 micronutrients such as iron, manganese or zinc with dedicated mechanisms of sequestration (2, 7 3). The paradigm of metal acquisition in metal-deprived environments is associated with iron: 8 whereas one strategy consists in transporting the reduced form, the most common scheme 9 involves the synthesis of high affinity ferric siderophores, their extracellular release prior to capture and import of the siderophore- Fe^{3+} complex (4). These siderophores are frequently 10 11 associated with virulence and may also be used for the transport of metals other than iron (5, 6), 12 with only a few examples of other types of microbial metallophores. Methanobactin, for 13 example, is a peptide-based chalkophore used for copper scavenging by methane oxidizing 14 bacteria (7). Free L-Histidine is used as a nickelophore by some bacteria, and more complex 15 molecules are possibly used by S. aureus or H. pylori, although their identity or functional relevance are unknown (8, 9). Recently, a nickel/cobalt uptake system (called CntA-F) was 16 17 uncovered in *S. aureus* and was found to play a role in the virulence of this strain (10).

18 Plants have also evolved their own metal chelators and among them, nicotianamine is an 19 important metabolite that is essential in the homeostasis of iron, copper, nickel and zinc (11, 12). 20 Nicotianamine is also the first precursor of phytosiderophores, a family of molecules chemically 21 different but functionally equivalent to siderophores (13). Nicotianamine is enzymatically 22 synthesized by nicotianamine synthase (NAS), which catalyzes the condensation of three α -23 aminobutyric acid moieties from S-adenosylmethionine (SAM) with the cyclization of one of 24 them to form an azetidine ring (14-16). Nicotianamine and its phytosiderophore derivatives seem 25 to be restricted to plants and some fungi, although some archaea apparently produce a 26 nicotianamine-related molecule called thermonicotianamine, of which the functional role 27 remains unknown (17).

28 Trying to better understand this family of plant NAS and archaeal NAS-like enzymes, we 29 found a gene putatively coding for a NAS-like enzyme in bacterial genome, including *S. aureus*. 30 The NAS-Like gene from S. aureus (sav2469), here called cntL, shares 12% sequence identity 31 with one of the four NAS from Arabidopsis thaliana, which corresponds to a very distant 32 homology. However, threading-based structure prediction programs detected a structural 33 homology of CntL with eukaryotic NAS and archaeal NAS-like enzymes (Fig. S1). The gene 34 coding for CntL is systematically located upstream of a gene whose product belongs to the 35 DUF2338 family (cntM in S. aureus, sav2468; Figs. 1, S2). Threading-based prediction indicates 36 with high confidence that this family of protein is related to Octopine-dehydrogenase, a 37 NAD(P)H dependent enzyme which reductively condensates a free amino acid, such as arginine, 38 with an α -ketoacid, such as pyruvate (18). This family of enzymes is known to be involved in the 39 escape of scallops from their starfish predators (19), or in the growth of parasitic bacteria, such 40 as Agrobacterium tumefaciens at the expense of plants in which they induce opine-producing 41 tumors, offering bacteria a selective growth advantage (20). In S. aureus, the gene upstream of 42 cntL (cntK, sav2470) appears restricted to the phylum of Firmicutes and encodes a protein that is 43 distantly related to a diaminopymelate epimerase (DapF).

1 On the basis of these predictions, the putative activity of each of these three enzymes 2 could be joined together to form a biosynthetic machinery: the CntK epimerase would produce 3 an amine substrate of proper stereochemistry that would be modified by the CntL NAS-like 4 enzyme through addition of one or two α -aminobutyric acid moiety(ies), and CntM would 5 reductively condensate an α -ketoacid to the previously formed product, leading to the formation of a candidate molecule. Although their putative activity was not recognized at the time, the 6 7 three corresponding genes (*cntKLM*) were shown to belong to an operonic structure involved in 8 the import of nickel and cobalt ions through an ABC transporter named *cntA-F* (8, Fig. 1A). Two 9 independent genetic studies targeting either the solute binding protein (SBP; cntA) or the MFS 10 transporter (Major Facilitator Superfamily, here called *cntE*) indicated that this operon is important for the virulence and fitness of S. aureus (10, 21). The phenotype of these mutations 11 could be reconciled by the hypothesis of a siderophore-like system with a biosynthetic 12 13 machinery (CntKLM), a putative exporter (CntE) and a putative importer of a metal-14 metallophore complex (CntABCDF).

15 We attempted to identify the candidate metallophore through an *ab-initio* approach using E. coli or S. aureus strains, and through a knowledge-based approach using purified proteins 16 17 (22). For the first approach we cloned the entire 10 kb cnt operon (cntKLMABCDFE) in E. coli 18 and induced gene expression and protein production. After adding nickel we tracked small 19 molecules co-eluting with nickel by metabolomic studies using hydrophilic interaction liquid 20 chromatography (HILIC; Fig. S3). We also constructed a mutant strain of S. aureus devoid of the cntL gene and cultivated both the WT and this mutant strains in chemically defined media 21 22 (CDM), a media with low metal concentration in which expression of the operon is known (10). 23 We then detected metal complexes in the extracellular fractions by coupling HILIC separation 24 with inductively coupled plasma-mass spectrometry (ICP-MS) and electrospray ionization-mass 25 spectrometry (ESI-MS) (Fig. 1). A main peak was found to elute between 30 to 33 min in the 26 HILIC/ICP-MS experiment, with the retention time depending on the detected metal (Fig. 1B). 27 In all the cases these peaks were absent in the *cntL* mutant strain, therefore corroborating our central hypothesis of the existence of a direct link between these metal complexes and the central 28 29 NAS-like enzyme. Investigation of these metal complexes by coupling HILIC and ESI-MS led 30 us to find a candidate molecule showing the characteristic metal isotopic patterns (Fig. 1C, Fig. 31 S3). The retention times of these compounds in HILIC/ESI-MS match those of the metals elution 32 found by HILIC/ICP-MS, therefore indicating that the same metal complexes were observed by 33 the two techniques. The knowledge-based approach consisted in the "fishing" of the nickel-34 metallophore complex: we expressed and purified the SBP (CntA) from E. coli and incubated it 35 in a supernatant of a *cntA* mutant of *S*. *aureus* grown in CDM media. We then supplemented this 36 mix with nickel to force nickel-metallophore complex formation and binding to CntA. After re-37 purification of the SBP and further analysis by MS we looked for the characteristic nickel 38 isotopic pattern and found a candidate molecule with the same mass as found by the 39 metabolomic approaches (Fig. S4). Using our set of accurate masses with molecular formula finder softwares we proposed an empiric formula of $C_{13}H_{19}N_4O_6$ -Metal. 40

41 We attempted to fit this formula by using simple rules derived from our proposed 42 enzymes activities and putative substrates (22), and we finally propose the structure of a 43 candidate molecule that would be the result of three biosynthetic enzyme activities: 1) 44 racemization of L-histidine in D-histidine by CntK, 2) nucleophylic attack of one α -45 aminobutyric acid moiety from SAM onto D-histidine to produce an intermediate (noted xNA) by CntL and, 3) reductive condensation of the xNA intermediate with a molecule of pyruvate by
CntM (Fig. 2A). Gas-phase fragmentation of zinc and nickel metal complexes confirmed the
proposed structure of the discovered molecule (Fig. S5), which we name staphylopine to recall
its origin in *S. aureus* and its inclusion in the opine family (23).

5 We then tested and refined this hypothetical biosynthetic pathway by cloning, expressing 6 and purifying the three enzymes and by testing their respective activities (Figs. 2B-D, S6). First, 7 we incubated CntK with L- or D-histidine and found that it indeed produces D-histidine from the L- stereoisomer and vice versa, therefore establishing CntK as a histidine racemase (Fig. 2B). 8 9 Because co-incubation with L-histidine and competing concentration of either L-alanine or L-10 methionine did not inhibit the histidine racemase activity, CntK appears specific for histidine (Fig. S7). CntK is therefore not a diaminopimelate epimerase as its sequence similarity could 11 12 suggest, but rather represents a novel enzyme family of histidine racemase. With regard to CntL 13 we determined a dissociation constant of 68 µM for SAM, which corroborates our central 14 hypothesis that CntL binds to SAM (Fig. S8). We then tested our prediction suggesting the 15 binding of NAD(P)H to CntM by incubating either NADH or NADPH with the purified enzyme. 16 This indicated a preference of the enzyme for NADPH, and fluorescence Resonance Energy Transfer (FRET) between the tryptophan residue of the protein (excitation 280 nm, emission 340 17 nm) and NAD(P)H (340nm and 460 nm, respectively) further revealed that CntM is specific for 18 19 NADPH with a dissociation constant of about 50 µM (Fig. 2C), a value that was confirmed using microcalorimetric titrations (Fig. S8). Therefore, CntM has the biochemical characteristics of 20 21 members of the opine synthase enzyme family, as suggested by the results of threading 22 programs.

23 We then investigated the combined enzymatic activities of CntK, CntL and CntM 24 through the use of a carboxyl-[¹⁴C] labeled SAM substrate. The use of this radiolabelled 25 substrate allowed us to follow the α -aminobutvric acid moiety of SAM after product separation by thin layer chromatography (TLC) and autoradiography. We found that [¹⁴C]-labeled SAM 26 27 alone gave one strong band with three bands of lower intensity, likely corresponding to SAM and some degradation products, such as, e.g. S-adenosyl-L-homocysteine (Fig. 2D). Interestingly, 28 29 although the incubation of [¹⁴C]-SAM with CntL and L-His did not change this SAM pattern, the 30 incubation with D-His led to a novel prominent band which migrated below the lowest SAM 31 band (Fig. 2D). According to our model, this band corresponds to the intermediate called xNA. 32 We then tested the effect of co-incubating both CntL and CntM enzymes together with their 33 putative substrates: L- and D-His, pyruvate and NADPH (Fig. 2D, lanes 4 and 5). Here again the 34 SAM pattern stayed unchanged using L-His. However, using D-His we could detect two novel 35 bands: one corresponding to the previous intermediate xNA, and one that migrated between the 36 two upper bands of SAM and would correspond to staphylopine. This identification is further 37 supported by the fact that the staphylopine band appears at the expense of the xNA intermediate, 38 as expected in such a case. We also confirmed the histidine racemase activity of CntK by co-39 incubating all three enzymes together with their substrates. In this case, the incubation with L- or 40 D-His both led to the appearance of the xNA and staphylopine bands, in accordance with the 41 CntK activity, which produces both L- and D-His when supplied with either stereoisomer (Fig. 42 2D, lanes 6 and 7). Incubation of the three enzymes with different aminoacids, α -keto acids and 43 reducing agents confirms that the histidine racemase CntK is indeed specific for histidine and 44 that CntM could use neither NADH nor α -keto glutarate in place of NADPH and pyruvate, respectively (Fig. S9). Overall, these in vitro studies of the three biosynthetic enzymes show that 45

they sequentially synthesize staphylopine according to our proposed scheme using common and
 defined substrates.

3 Finally, we confirmed that staphylopine biosynthesis is impaired in the *cntL* mutant of *S*. 4 aureus and explored the effect of mutations of several genes in the cnt operon on metal 5 homeostasis and staphylopine production and trafficking (Fig. 3). When compared to WT strain 6 of S. aureus, both the xNA and the staphylopine bands disappeared in the cntL mutant, 7 confirming that both bands are dependent on CntL activity (Fig. 3A). Furthermore the bands 8 corresponding to xNA and staphylopine migrated at the same distance in E. coli or S. aureus cell 9 extracts, therefore confirming that both molecules are produced heterologously in E. coli. We 10 next sought to determine the effect of the *cntL* mutation with regard to intracellular metal accumulation in various culture conditions: CDM, where metal concentration is rather low, 11 12 Chelex-demetalated CDM (dCDM) where metal level is even lower, NTA-demetalated CDM 13 supplemented with metalated NTA resin to supposedly mimic a chelating environment (rCDM), 14 and LB where metal concentration is high. We note that the growth of the *cntL* mutant is nearly identical to that of the WT strain in these media (Fig. S10), therefore indicating that metal 15 16 content would be attributable to the absence of staphylopine and not to a growth defect. In all 17 these culture conditions we established that the *cnt* genes, transcribed from two promoters 18 upstream *cntKLM* and *cntA-E*, are highly expressed in metal scarce media, whereas their 19 activities is lower but still significant in LB (Fig. S11). In CDM and dCDM, the cntL mutation 20 caused a significant decrease in intracellular accumulation of nickel, copper and cobalt as determined by ICP-MS (Figs. 3B, S12). This metal accumulation defect is comparable to the one 21 22 observed in the *cntA-F* mutant, indicating that metal or metal-staphylopine complexes are 23 transported via CntA (Fig. 3B). This defect in metal accumulation is complemented when 24 reintroducing the *cntL* gene in the mutant, although using slightly different conditions adapted 25 for ICP-AES metal content determination (Fig. S13). In this case there is no significant decrease in intracellular copper, but a significant decrease in intracellular zinc, nickel and cobalt. In 26 27 rCDM, the tendency observed in CDM is confirmed, although in this condition iron, zinc, 28 copper, nickel and cobalt content are all affected in the mutant strain as compared to the WT. 29 Finally, in a metal rich medium such as LB, the *cntL* deletion mutant accumulated less iron, copper and cobalt. Taken together, these sets of experiments suggest that, depending on the 30 31 nature of the metal, its concentration, and the bacterial growth status, staphylopine is involved in 32 the import of a large array of divalent metals in S. aureus. Testing metal transport over 10 33 minutes supports the involvement of both staphylopine and CntA-F in the import of all these 34 metals. Indeed, import of iron, zinc, nickel and cobalt are all significantly decreased in both *cntL* and *cntA-F* mutants (Fig. 3C). The significant short-term transport of iron and zinc is consistent 35 36 with their known regulatory effect exerted on the cnt operon (10, 21). The transport of copper is 37 somehow different as WT cells efficiently export this metal during this short time, probably in 38 response to its toxicity. In this case the absence of CntL or CntA-F appears to decrease this 39 toxicity and its consecutive efflux. In order to investigate the transport of metal-staphylopine 40 complexes through CntA, we exploited the sensitivity of S. aureus to high cobalt concentration. 41 We found that *cntL* and *cntA-F* mutant strains are resistant to an otherwise toxic concentration of 42 cobalt to WT, whereas supplementation of synthetic staphylopine in the media restores its toxicity in cntL but not in cntA-F strain (Fig. S14). This suggests a co-transport of cobalt-43 44 staphylopine complexes via CntA-F, as the ABC transporter is still present in the cntL (hence the 45 cobalt toxicity) whereas it is absent in the *cntA-F* mutant (where cobalt-staphylopine cannot enter the cell). Finally, we measured the staphylopine level in the intracellular and supernatant 46

1 fractions of the WT, *cntABCDF*, *cntL* and *cntE* mutants (Fig. 3D). This experiment confirmed 2 the absolute need of CntL in the biosynthesis of staphylopine, further supported the function of 3 CntA-F in the import of staphylopine-metal complexes, and clearly indicated that CntE is 4 involved in the export of staphylopine. Overall, despite being distantly related, staphylopine 5 apparently mirrors the effect that nicotianamine has on the same range of metal but in the context 6 of metal homeostasis and transport in plants (17). Chemically synthesized staphylopine has the 7 same range of metal affinity than nicotianamine (similar K_d and affinity order: $Cu^{2+} > Ni^{2+} >$ 8 $Co^{2+} > Zn^{2+} > Fe^{2+}$; Fig. S15), further supporting their functional comparison as broad-spectrum 9 metallophores.

10 The mechanisms of staphylopine biosynthesis export and recovery of the metal complexes are summarized in Fig. 4. The biosynthesis of staphylopine aggregates three 11 unprecedented enzyme activities with the use of D-histidine, the fusion of a single α -12 13 aminobutyric acid moiety from SAM, and the fabrication of an opine derivative. This latter 14 activity was so far restricted to plant's bacterial transfection or anaerobic glycolysis in marine 15 invertebrates (19, 23). The use of histidine as a building block is interesting as the free amino 16 acid has a relatively high affinity for nickel on its own and is directly used for nickel uptake by bacteria (8, 24-26). However, the use of the D- stereoisomer of histidine is surprising because D-17 amino acids are mainly used in cell wall biosynthesis and have been mostly restricted to alanine 18 19 and glutamate (27), although other "non-canonical" D-amino acids are reported to be involved in 20 the composition of the cell wall (28, 29), in biofilm stability (30), virulence (31) or sporulation 21 (32). Another peculiarity of the staphylopine biosynthetic machinery resides in the NAS-like 22 CntL enzyme that only uses one α -aminobutyric acid moiety from SAM, whereas the NAS-like from *M. thermotautrophicum* uses two, and plant enzymes use three such moieties. Interestingly, 23 24 the evolution from bacterial staphylopine to plant's nicotianamine preserves a large part of the 25 molecule's backbone, therefore suggesting a conservative chemical evolution going together 26 with enzymatic simplifications wherein plant nicotianamine is synthesized by a single enzyme. 27 Finally, homologous genes involved in staphylopine biosynthesis are conserved in other 28 pathogenic bacteria such as Yersinia pestis or Pseudomonas aeruginosa underscoring the likely 29 influence of this metallophore in bacterial pathogenicity. Indeed, the export pathway of this 30 metallophore was recently identified as a critical function for the growth of *P. aeruginosa* in 31 airway mucus secretions where metal access is severely limited (33).

32

1 **References and Notes:**

- M. L. Reniere, G. Pishchany, E. P. Skaar, in *Iron uptake and homeostasis in microorganisms*. (2010).
- 4 2. B. D. Corbin *et al.*, *Science* **319**, 962 (2008).
- 5 3. M. I. Hood, E. P. Skaar, *Nat Rev Microbiol* **10**, 525 (2012).
- 6 4. Z. Ma, F. E. Jacobsen, D. P. Giedroc, *Chem Rev* **109**, 4644 (2009).
- 7 5. A. G. Bobrov *et al.*, *Mol Microbiol* **93**, 759 (2014).
- 8 6. I. J. Schalk, M. Hannauer, A. Braud, *Environ Microbiol* **13**, 2844 (2011).
- 9 7. H. J. Kim *et al.*, *Science* **305**, 1612 (2004).
- 10 8. H. Lebrette *et al.*, *Metallomics* 7, 613 (2015).
- K. Schauer, B. Gouget, M. Carriere, A. Labigne, H. de Reuse, *Mol Microbiol* 63, 1054 (2007).
- 13 10. L. Remy *et al.*, *Mol Microbiol* **87**, 730 (2013).
- 14 11. C. Curie et al., Ann Bot 103, 1 (2009).
- 15 12. E. L. Walker, B. M. Waters, *Curr Opin Plant Biol* 14, 318 (2011).
- 16 13. R. Hell, U. W. Stephan, *Planta* **216**, 541 (2003).
- 17 14. A. Herbik *et al.*, *Eur J Biochem* **265**, 231 (1999).
- 18 15. K. Higuchi et al., Plant Physiol 119, 471 (1999).
- 19 16. H. Q. Ling, G. Koch, H. Baumlein, M. W. Ganal, *Proc Natl Acad Sci U S A* 96, 7098
 (1999).
- 21 17. C. Dreyfus, D. Lemaire, S. Mari, D. Pignol, P. Arnoux, *Proc Natl Acad Sci U S A* 106, 16180 (2009).
- 23 18. S. H. Smits, A. Mueller, L. Schmitt, M. K. Grieshaber, J Mol Biol 381, 200 (2008).
- 24 19. M. Harcet, D. Perina, B. Plese, *Biochem Genet* **51**, 666 (2013).
- 25 20. M. W. Bevan, M. D. Chilton, Annu Rev Genet 16, 357 (1982).
- 26 21. Y. Ding, Y. Fu, J. C. Lee, D. C. Hooper, *J Bacteriol* **194**, 6586 (2012).
- 27 22. Materials and methods available on Science online.
- 28 23. J. Thompson, J. A. Donkersloot, Annu Rev Biochem 61, 517 (1992).
- 29 24. P. T. Chivers, E. L. Benanti, V. Heil-Chapdelaine, J. S. Iwig, J. L. Rowe, *Metallomics* 4, 1043 (2012).
- 31 25. H. Lebrette, M. Iannello, J. C. Fontecilla-Camps, C. Cavazza, *J Inorg Biochem* 121, 16
 (2013).
- 33 26. M. M. Shaik, L. Cendron, M. Salamina, M. Ruzzene, G. Zanotti, *Mol Microbiol* 91, 724 (2014).
- 35 27. W. Vollmer, D. Blanot, M. A. de Pedro, *FEMS Microbiol Rev* **32**, 149 (2008).
- 36 28. F. Cava, M. A. de Pedro, H. Lam, B. M. Davis, M. K. Waldor, *Embo J* 30, 3442 (2011).
- 37 29. H. Lam et al., Science **325**, 1552 (2009).
- 38 30. I. Kolodkin-Gal *et al.*, *Science* **328**, 627 (2010).
- 39 31. A. T. Anfora, B. J. Haugen, P. Roesch, P. Redford, R. A. Welch, *Infect Immun* 75, 5298
 40 (2007).
- 41 32. K. A. O'Connor, D. R. Zusman, *Mol Microbiol* **24**, 839 (1997).
- 42 33. M. Gi et al., Sci Rep 5, 14644 (2015).
- 43 44
- 44
- 45
- 46

1 Acknowledgments: We thank the Commissariat à l'Energie Atomique et aux Energies 2 Alternatives (CEA), the Agence Nationale de la Recherche (ANR) and the CEA program 3 ToxNuc-E for financial support. Support was also provided by the HélioBiotec platform (funded 4 by the European Regional Development Fund, the Région Provence Alpes Côte d'Azur, the 5 French Ministry of Research, and the "Commissariat à l'Energie Atomique et aux Energies Alternatives"). G. G. was supported by the Région PACA. Work at the F. C. lab was supported 6 7 by MIMS, KAW, the Swedish Research Council and a Kempe foundation scholarship to A. E. 8 We wish to thank J. Lavergne for careful reading of the manuscript and we also thank A.

- 9 Verméglio, C. Cavazza and H. Lebrette for fruitful discussions.
- 10



3 Fig. 1. The cnt operon and detection of a broad-spectrum metallophore. (A) The *cnt* operon 4 encodes three putative biosynthetic genes together with the genes coding for a full ABC 5 transporter and a transporter belonging to the MFS family. (B) HILIC/ICP-MS chromatograms 6 of extracellular fractions of S. aureus and comparison of the WT strain (plain line) with the cntL 7 mutant (dotted lines). (C) Mass spectra of a chelating compound present in its free and 8 complexed forms (here shown in complex with Zn) in the extracellular fraction of the WT strain 9 but absent in the *cntL* mutant as observed in chromatogram around 31 min during HILIC/ESI-10 MS analyses. The empirical molecular formula was deduced from exact masses. (D) HILIC/ESI-11 MS extracted ion chromatograms (XIC) of some metal-ions observed to form a complex with the 12 above-mentioned chelator. Chromatograms were traced using the exact masses (\pm 3 ppm) of the 13 expected complex and these masses were absent in the extracellular fraction of the *cntL* mutant. 14 (E) Proposed chemical structure of staphylopine (top) and comparison with nicotianamine (bottom).

15

2



2 Fig. 2. Biosynthesis of staphylopine and biochemical characterization of the biosynthetic 3 enzymes. (A) Biosynthesis pathway for the assembly of staphylopine from L-histidine, SAM and pyruvate. (B) Epimerase assay of CntK and comparison with L- and D-histidine standards. (C) 4 5 Titration of NADH (red) and NADPH (blue) binding to CntM (5 µM) followed by fluorescence 6 energy transfer between tryptophan excitation (280 nm) and NAD(P)H emission (460 nm). 7 Fitting of the data obtained for NADPH binding lead to a K_d of 50 μ M. (**D**) TLC separation of reaction products incubating [¹⁴C]-SAM (labeled on its α-aminobutyric acid moiety; marked 8 9 with a * in panel A) with various purified enzymes, pyruvate, NADPH and either L- or D-10 Histidine.



2 Fig. 3. Effect of *cnt* mutations in metal content and staphylopine localization. (A) TLC 3 separation of co-incubation of E. coli cell extracts separately expressing CntK, CntL and CntM 4 with $[^{14}C]$ -SAM and comparison with the cell extracts from WT S. aureus and cntL mutant 5 strains. (B) Intracellular metal content of the S. aureus WT (left), cntL (middle) and cntA-F 6 (right) strains grown in CDM, and determined by ICP-MS. Error bars, mean \pm s.d. ***P*<0.01 and 7 ***P<0.001. (C) Intracellular metal content change of the S. aureus WT (left), cntL (middle) and 8 cntA-F (right) strains grown in dCDM until late exponential phase (T0) and then incubated 10 9 minutes with 0.3µM Fe(II), Zn, and 0.1µM Mn, Ni, Co, Cu. Metal content variation was 10 determined by ICP-MS and expressed as the differential of atoms after 10 min. as compared to T0. Error bars, mean \pm s.d. *P<0.05, **P<0.01 and ***P<0.001. (**D**) Staphylopine level in the 11 intracellular (left; note the logarithm scale) and supernatant (right) fractions of the WT, 12 13 *cntABCDF*, *cntL* and *cntE* mutant strains. Error bars, mean \pm s.d. **P*<0.05 and ***P*<0.01, ND: 14 Not detected.



Figure 4: Model of staphylopine biosynthesis, export and recovery of staphylopine-metal complexes through the ABC transporter. IM: Inner Membrane, CW: Cell Wall.

1 Supplementary Materials

- Materials and Methods 2
- Figs. S1-S16 3
- 4 Tables S1-S3
- 5 6 Refs (34-49)

1 Supplementary Materials:

2 Materials and Methods

3 Bioinformatic analysis

Sequences from CntK (encoded by *sav2470*), CntL (*sav2469*) and CntM (*sav2468*) were
analyzed by searching for homologues using PsiBLAST (*34*) and the Pfam database (*35*).
Sequence alignment was done using the Muscle program (*36*) and visualized in the Jalview suite
(*37*). The threading program Phyre2 (*38*) was used to detect distant homologues. Gene syntheny

- 8 were inspected using the MaGe MicroScope web interface (39).
- 9

10 Gene cloning, protein expression and purification

11 The entire operon from S. aureus (9,3 kb; from cntK to cntE; sav2470 to sav2462) was amplified by PCR (See Table S1 for oligonucleotides used in this study). The resulting fragment was 12 13 digested by NdeI and XhoI and ligated in plasmid pET22b+. The three biosynthetic genes (*cntK*, 14 cntL and cntM) and the cntA gene were separately cloned in pET100, pET-SUMO, pET101 and 15 pET100, respectively, according to standard protocols. After co-transformation with plasmid 16 pRARE (encoding for rare codon in E. coli BL21) strains were cultivated in LB media 17 supplemented with appropriate antibiotics. When the OD of the culture was about 0.6 they were 18 transferred at 16 °C and protein expression was induced by adding 0.1 mM IPTG. Bacteria were 19 recovered after overnight growth by centrifugation at 7,000 g for 15 min. Cells were resuspended 20 in buffer A (20 mM Na,K PO₄ (pH 7.5), 500 mM NaCl, 20 mM imidazole) and disrupted using a 21 French press operating at 1000 psi. Cell debris were removed by centrifugation at 8,000 g for 20 22 min. The supernatant was centrifuged at 100,000 g for 1 h at 4°C to remove cell wall debris and 23 membrane proteins. The resulting soluble fraction was loaded on a nickel affinity column 24 (HisTrap 1 ml column, G.E. Healthcare), and the protein was eluted stepwise with imidazole (75 25 mM wash and 250 mM elution). Collected fractions were loaded onto a gel filtration column 26 (Hiload 26/60 Superdex 200, G.E. Healthcare), equilibrated with buffer B (20 mM HEPES, 50 27 mM NaCl pH 8.0) for CntA, CntK and CntL, or buffer C (buffer B + 500 mM NaCl) for CntM. Before gel filtration, the tag of CntL was removed by incubating the protein (1 mg/mL) with the 28 29 SUMO (Ulp-1) protease using a protein / protease ratio of 1/100 (w/w) and an overnight 30 incubation at room temperature. Protease, free tag and uncleaved protein were then removed by a 31 second passage through a Ni-NTA column equilibrated with buffer A.

32

33 Construction of cntL and cntE deletion mutants of S. aureus and complementation

34 A deletion mutant of S. aureus RN6390 was obtained as described (40). Briefly, two DNA 35 fragments corresponding to upstream and downstream regions of *cntL* or *cntE* gene were amplified from RN6390 chromosomal DNA using primers pairs listed in Table S1. Deletion 36 37 fragments were generated using splicing by overlap extension PCR and cloned into BamHI (for 38 cntL mutant) or BamHI and XmaI (for cntE mutant) sites of shuttle vector pMAD (41). The 39 resulting plasmid was introduced into S. aureus RN4220 by electroporation, extracted and 40 subsequently introduced into S. aureus RN6390. Deletion of the chromosomal region of interest 41 was subsequently obtained by double-crossing over events as previously described (42). To 42 complement the cntL deletion strain, the cntL gene was amplified by PCR (Table S1) and

1 inserted into BamHI site of shuttle vector pOS1-Plgt to generate pCntL in which *cntL* is under 2 the control of the *lgt* promoter (43). Recombinant vectors (pOS1-Plgt and pCntL) were 3 introduced into *S. aureus* strains as described above.

4

5 *Cell culture conditions*WT and mutant strains of *S. aureus* were grown at 37°C with aeration in 6 either CDM (40, 44) deprived of added metals except magnesium (1 mM; Table S2), 7 demetalated CDM (CDM treated with chelex; dCDM) or LB. For cultures in CDM with metal-8 NTA resin (rCDM), NTA-treated CDM and metalated resin were prepared as follows: Ni-NTA 9 His-bind resin (EMD Millipore, Billerica) was extensively washed with 100mM EDTA (~15 10 time the resin volume) to eliminate nickel linked to NTA, and then with double distilled water to remove EDTA. CDM was then incubated overnight with the demetalated NTA resin and further 11 12 filtrated to obtain NTA-treated CDM. The resin (now metalated by the traces of metals present in 13 CDM) was then placed into Amicon centrifugal filter units (Amicon Ultra-0.5ml 3KDa; Merck 14 Millipore, Tullagreen) inserted into eppendorf tubes. After having cut the bottom of these tubes, they were introduced into 50 ml Falcon tubes containing the NTA-treated CDM. Despite 15 16 demetalation by EDTA, the total amounts of metal in rCDM treated cells seems to indicate that 17 residual amounts of Mn, Cu and Ni remained trapped by the resin as the uptake of these metals 18 was found higher in rMCD cells than CDM cells. For short time uptake experiments, strains 19 were grown in demetalated CDM until late exponential phase, then supplemented with a metal cocktail (0.3µM Fe(II) and Zn; 0.1µM Ni, Co, Cu, Mn) and incubated for 10 minutes prior to 20 21 metal level determination by ICP-MS.

22

23 Analysis of metal concentrations by ICP-MS

24 Metal content of the media used in this study are reported in Table S3. After growing until late 25 exponential phase or overnight, the cultures (15 mL) were centrifuged and pellets were washed three times with ice cold 1mM EDTA and then once with double distilled water. Cells were then 26 27 dried overnight at 100 °C. Metal contents were determined in cell fraction by resuspending the 28 dried pellet with a 200 µL mixture of 70% HNO₃ (100 µL) and 30% H₂O₂ (100 µL). The mixture was heated at 80°C for 3 h and then diluted with water to reach a final volume of 2 mL. 29 30 Acidified sample solutions were then analyzed by ICP-MS (Agilent 7500cs) operating in 31 hydrogen collision gas mode (H₂) to remove possible interferences.

32

33 *Cell fractionation for staphylopine quantification and TLC assay*

34 WT and mutant strains of S. aureus were grown in CDM at 37°C with aeration, centrifuged, and 35 the supernatants were collected, filtrated and stored at -20°C. The pellets were resuspended in 2 36 ml lysis buffer (0.1 mg /ml lysostaphin in Hepes 20 mM) and incubated at 37°C for 1 h by 37 shaking (150 rpm). Lysates were then transferred into tubes containing 0.1 mm glass beads (MP 38 Biomedicals, Solon, OH) and subjected to mechanical disruption by homogenization using a 39 FastPrep FP120 instrument (Thermo Scientific, Waltham) in three 45 s intervals at a speed of 40 6.0, with cooling on ice between intervals. The lysates were centrifuged at 14,000 g for 20 min at 4°C and supernatants were collected. Staphylopine level determination is described further 41 below. 42

1 Analysis of metal complexes using HILIC/ICP-MS

2 This separation method preserves metal-ligand complexes and facilitates their downstream 3 identification (45, 46). Analytical reagent grade chemicals such as acetonitrile, acetic acid, 4 formic acid, nitric acid and ammonia were purchased from Sigma-Aldrich (Saint-Quentin-5 Fallavier, France). Ultrapure water (18 MQ.cm) was obtained from a Milli-Q system (Millipore, 6 Bedford, MA). Microbore HILIC separations were performed using an Agilent 1100 capillary HPLC system (Agilent, Tokyo, Japan) equipped with a 100 µl min⁻¹ splitter module. ICP-MS 7 8 detection was achieved using a model 7500cs instrument (Agilent) fitted with platinum cones, 1 9 mm i.d. injector torch and a T-connector allowing the introduction of 5% O₂. The HILIC/ICP-10 MS coupling was done via an Isomist interface (Glass Expansion, Melbourne, Vic, Australia) consisting of a 20-ml Cinnabar cyclonic spray chamber cooled to 2 °C and fitted with a 50 µl 11 12 min⁻¹ Micromist U-series nebulizer.

13 The column used for HILIC separation was a TSK gel amide 80 (250 mm \times 1 mm i.d., 5 μ m) 14 from Tosoh Biosciences (Stuttgart, Germany). Gradient elution, at a flow rate of 50 μ l min⁻¹, was

15 carried out using eluent A, acetonitrile, and eluent B, 5 mM ammonium formate (pH 5.5). The

16 gradient program was: 0–5 min 10% B, 5–45 min up to 50% B, 45–50 min 50% B, 50–52 min up

to 65% B, 52–55 min 65% B, 55–60 min down to 10% B, 60–65 min 10% B. Samples were diluted with acetonitrile and water to obtain a 1 : 2, sample to acetonitrile ratio, and centrifuged.

19 A 7- μ l aliquot of the supernatant was injected into the HILIC column each time.

20

21 Analysis for metal complexes using HILIC/ESI-MS

22 For HILIC/ESI-MS, the HPLC systems were connected to an LTQ Orbitrap Velos mass 23 spectrometer (Thermo Fisher Scientific, Bremen, Germany). The coupling was achieved via a 24 heated electrospray ionization source (H-ESI II) (Thermo Fisher Scientific. The ion source was 25 operated either in the positive ion mode at 3.0 kV. The vaporizer temperature of the source was 26 set to 120 °C and the capillary temperature to 280 °C. In full MS mode, the resolution was set at 27 100 000 (FWHM at m/z 400) whereas in MS/MS mode it was set at 30 000. MS data were 28 processed using Xcalibur 2.1 software and MetWorks 1.2.1 software (Thermo Fischer Scientific) 29 to screen spectra for metal containing molecules. To get accurate masses, MS and MS/MS spectra were recalibrated offline using precursor/fragment ions with known formula. Putative 30 metal species were fragmented during a subsequent chromatographic run with collision induced 31 32 dissociation (CID) mode at 35% energy. In order to fit the molecular formula that we obtained 33 from accurate masses, we used simple chemical rules as follow: 1/ each time there is a reductive 34 condensation there is a mass loss of 16 Da between the two reactants. 2/ each time there is an α -35 aminobutyric acid moiety transfer from SAM to an amino acid there is a mass loss of 2 Da 36 between the two reactants. This way, the "simplest" molecule would consist of pyruvate (88Da) 37 reductively condensated with glycine (75 Da) that would be modified by two α -aminobutyric 38 acid moieties (103 Da), which would give a molecular weight of 349 Da, above the 328 Da 39 measured. The only possibility is therefore to use only one α -aminobutyric acid moiety from 40 SAM; and only using histidine (155 Da) and pyruvate we can obtain the expected 328 Da.

41

42 Determination of metal binding affinity and quantification of synthetic staphylopine

1 Synthetic staphylopine (N-[3-(N-L-Alanyl)-amino-3-carboxypropyl]-D-histidine) was ordered to 2 Toronto Research Chemical Inc (Toronto, Canada) and its NMR spectrum is provided in Fig. 3 S16. Metal content of the studied elements in staphylopine synthetic standard was measured by 4 ICP MS and was found to be lower than 0.1% of staphylopine mass. Analysis of this synthetic 5 standard by HILIC/ESI-MS showed that it has the same retention time, chromatographic peak 6 shapes, fragmentation behavior in MS instrument and metal affinity than the natural molecule 7 found in culture supernatants. It was then used also to quantify staphylopine in spent culture 8 media through standard addition technique, which resulted in staphylopine peak increases during 9 HILIC/ESI-MS analysis and which allow to determine staphylopine concentration and confirmed 10 results obtained by HILIC/ICP-MS analysis. We tested metal affinity of staphylopine standard by mixing different metals (Fe(II), Fe(III), Cu, Zn, Co, Ni) with this molecule prior to direct 11 analysis by ESI-MS. It was found that metal affinity for staphylopine was in the following order: 12 13 Cu(II) > Ni > Co > Zn > Fe(II) > Mn(II), which is identical to what was observed for 14 nicotianamine. Fe(III) affinity for staphylopine relative to other metals is pH dependent and, whereas it has a higher pKd value than Cu(II), Fe(III) has a poor affinity for staphylopine at 15 16 neutral pH, a phenomenon already described for nicotianamine (47). Stability constants for 17 staphylopine:metal complexes were estimated by metal competition experiments with 18 nicotianamine. Mixtures of nicotianamine:staphylopine:metal were analyzed in physiological 19 conditions (pH = 7) by infusion in ESI MS. Were analyzed: 1) a mixture of only nicotianamine 20 and staphylopine at 100µM each to establish maximal signal intensity for these free ligands, 2) a mixture of nicotianamine (100 µM), staphylopine (100µM) and an excess of metal (1mM, each 21 22 metal was tested in a different experience) to establish maximal signal intensity for the 23 complexed ligands and 3) a mixture of nicotianamine (100 µM), staphylopine (100µM) and 24 limited level of metal (80µM, each metal was tested in a different experience) to created 25 competition and observed the proportion between metal complexes formed. The estimated pKd 26 values are given in Fig. S15C. Like nicotianamine also, the complex of staphylopine with metal 27 is only observed as a 1:1 complex during ESI MS analysis. All these data lead to consider that 28 staphylopine is very similar to nicotianamine in terms of metal affinity and structure. A putative 29 model for metal:staphylopine complex can then be proposed (hexadentate structure with 3 amino 30 groups (the ones spaced by 3 carbons) and the 3 carboxylic groups, Fig. S15A).

31

32 Fishing and Metabolomic studies

33 The CntA protein, predicted to bind the metallophore-nickel complex, was purified by 34 conventional HisTrap and incubated in 50 mL of a culture supernatant from a *cntA* mutant of S. 35 aureus cultivated in CDM. Nickel was then added to a final concentration of 50 µM to allow the nickel-metallophore complex to form and to bind to CntA. After 2 h of incubation the CntA 36 protein was purified again, exchanged against a buffer containing 10 mM ammonium acetate, 37 38 and finally analyzed by MS. Cell fraction of an E. coli strain expressing the entire cnt operon 39 was also analyzed by HILIC/ICP-MS for metal detection and HILIC/ESI-MS for metabolite and 40 metal complex identification.

41

42 *Metal content using ICP-AES (atomic emission spectroscopy)*

43 *S. aureus* strains were grown at 37 °C with aeration in LB medium or in CDM until late 44 exponential phase. In CDM condition, nickel, copper, zinc or cobalt chloride was then added (1

µM final). Cultures were then grown overnight and harvested by centrifugation at 6,000 g at 4°C 1 2 for 15 min. Pellets were washed three times with ice-cold 1 mM EDTA and once in double 3 distilled water. Cells were dried overnight at 100°C and then digested with 500 µl of nitric acid 4 (70 % HNO₃ ICP Grade, JT Baker) overnight at 80°C. Samples were further diluted by addition 5 of distilled water (final volume 6 mL). The element measurements were performed with 6 Inductively Coupled Plasma - Absorption Emission Spectrometer (ICP-AES Vista MPX, 7 Varian). The metal concentrations were determined using standard curves obtained by analyzing 8 ICP grade element standard solutions. The metal contents were calculated assuming 4 X 10^8 9 CFU (Colony Forming Unit) for an OD600 of 1 unit, as determined by plating diluted cultures 10 grown in CD medium.

11

12 Fluorescence studies and microcalorimetry

13 Fluorescence studies were performed using a Cary Eclipse spectrophotometer (Agilent). The

14 FRET experiment was done using a protein concentration of 5 μ M an excitation wavelength at 15 280 nm. The signal was recorded between 400 and 500 nm. Five spectra were averaged in order

to increase signal to noise ratio. The dissociation constants were obtained by directly fitting the

10 to increase signal to noise ratio. The dissociation constants were obtained by directly fitting in 17 experimental data to a binding equation in SigmaDlat

17 experimental data to a binding equation in SigmaPlot.

18 Microcalorimetry was used to determine the dissociation constants for the binding of NADPH to

19 CntM using a MicroCal iTC200 system (MicroCal). The experiments were performed at 25 °C in

20 buffer C. Titration experiments consisted of 13 injections (3 μ L) of NADPH (1 mM in buffer C)

21 to 200 μ L of protein (97 μ M). Dissociation constant was obtained using one set of sites fitting in

22 Origin (MicroCal).

23

24 Epimerase/Racemase assay

25 The product from a racemization reaction (30 µg of purified CntK incubated during 30 min at 26 room temperature in 100 mL of buffer (Na,K phosphate 50 mM pH 8.0, DTT 1 mM) and 27 supplemented with 20 mM L- or D-amino acids) was derivatized with L-FDAA (1-fluoro-2-4-28 dinitrophenyl-5-L-alanine amide, Marfey's reagent, Thermo Scientific). First, an equal volume 29 of NaHCO₃ 0.5 M was added to the racemization reaction; then, 6 µl of this reaction were 30 reacted with L-FDAA (10 mg/mL in acetone) at 80°C for 3 min. The reaction was stopped with 2 31 N HCl and the samples were filtered. The products were separated at 2 mL/min with a linear 32 gradient of triethylamine phosphate/acetonitrile in HPLC for 45 min with an Aeris peptide 33 column (250 x 4.6 mm; 3.6 µm particle size) (Phenomenex, USA) and detected at 340 nm. For the co-incubation with alanine or methionine, the flow was 1 mL/min and the gradient length 34 35 was set to 105 min. Background noise was removed from the L,D-His racemization reactions 36 graphics.

37

38 Activity assay followed by TLC

39 Cellular fractions of *S. aureus* WT and *cntL* mutant strains were supplemented with EDTA and

40 DTT (1 mM final) prior to the TLC assay. [¹⁴C]-SAM (BioTrend, Köln, Germany) was added to

41 the cell fraction(s) at a final concentration of 20 μ M, together with 1mM pyruvate and 100 μ M

42 NADPH in order to ensure that there was enough substrate during the assay. The assay using

purified proteins consisted in incubating the enzymes, either separately or by co-incubation, at a 1 final concentration of 3 µM and using [¹⁴C]-SAM (20 µM), NAD(P)H (30 µM), L- or D-histidine 2 3 (10 μ M) and α -keto acid (pyruvate or α -keto glutarate; 1mM). The total volume was 100 μ L in 4 buffer D (HEPES 50 mM, DTT 1 mM, EDTA 1 mM, pH 9.0). The mixture was incubated for 30 5 min at 28 °C. The reactions were stopped by adding ethanol to a final concentration of 50 %(v/v)6 and the products were then separated by TLC. An aliquot of 10 µL of the reaction mixtures were 7 spotted on HPTLC (High Performance TLC) Silica Gel 60 Glass Plates (Merck KGaA), and the 8 plates were developed with a phenol:n-butanol:formate:water (12:3:2:3 vol/vol) solvent system. 9 These separation parameters were used in the initial biochemical characterization of plant 10 nicotianamine synthase (48). HPTLC plates were dried and exposed to a $[^{14}C]$ -sensitive imaging plate for 2-3 days. Imaging plates were then scanned on a Typhoon FLA 7000 phosphorimager 11 12 (GE Healthcare).

13

14 Construction of promoter-reporter gene fusion and luciferase assays.

15 The reporter gene *luxAB* from *Vibrio ficheri* cloned into shuttle vector pCN58 was used for gene 16 expression analysis. The cntK upstream region (569bp) was cloned using oligonucleotides indicated in Table S1, as previously described for the cntA promoter (10). To follow the cnt 17 18 promoter-luciferase reporter activity, CDM and LB media were inoculated with an overnight 19 culture to obtain an optical density (OD_{600}) of 0.1 or 0.05, respectively. Culture aliquots were 20 removed, monitored for growth (OD₆₀₀), and submitted to luminescence assays. Measurements 21 of luminescence were performed immediately after mixing 450 µl of cell suspension with 50 µl 22 of 0.1 % (vol/vol in 40 % ethanol) nonanal (Sigma, St Louis, MO). Luciferase activity, expressed 23 in relative luminescence units (RLUs), was measured using a Biolumat LB9500T Luminometer (Berthold, Bad Wildbad, Germany), based on 10 s measurements in the integrate data mode. 24 25 Specific luciferase activities were calculated by dividing the RLUs by absorbance (RLU/OD₆₀₀). The promoterless pCN58 plasmid was used as control in reporter gene assays. 26

Supplementary Figures and Table 1

Α

Cntt DapF DapF (100%) CntL Alcohol dehydrogenases of the MDR Family (Ribosomal Methydransferase) NAS/NAS-like (100%) CntM DUF2338 Octopine dehydrogenase (100%) Vashdopsir, thaliana/1-320 1 MAC ONNLVVKO IDLYOQIS-KLKSLKPSKNVDT LFCQLVSTCLPTDTN IDVTNMCE Worosporg, casaa/1-302 Vashdopsir, thaliana/1-320 1 MAC ONNLVVKO IDLYOQIS-KLKSLKPSKNVDT LFCQLVSTCLPTDTN IDVTNMCE Worosporg, casaa/1-302 Vashdopsir, thaliana/1-320 1 MAC ONNLVVKO IDLYOQIS-KLKSLKPSKNVDT LFCQLVSTCLPTDTN IDVTNMCE Worosporg, casaa/1-302 Vashdopsir, thaliana/1-327 1 MAC ONNLVVKO IDLYOQIS-KLKSLKPSKNVDT LFCQLVSTCLPTDTN IDVTNMCE Worosporg, casaa/1-302 Vashanabacteming, estin/1-328 1 MAC + MARINE KL LOQYLKE EQUAL & LEVAPE KS LUBA EXEMPT Worosporg, casaa/1-302 Vashanabacteming, estin/1-273 1 MAC + MS CKKYEWC ELLAN & LEVAPE KS LUBA EXEMPT Worosporg, casaa/1-302 Vashanabacteming, estin/1-265 1 MMN FN KL KL COC EXEC + ILK KL LUBA LEA WARB VE LEA	Protein			Threading	
Criti Determinants Find MDPT family (Ribosomal Methyltransferase) NAS/NAS-like (100%) Criti DUF2338 Octopine dehydrogenase (100%) Validopsis, falana/-320 1 MAC Q NN LVVKQI DLVDQI S - KLKSLKP SK NVDT LFGQLVSTCLPTDT N I DVTNMCE Weurospord, cassa/1-320 1 MSC (XWL RALL AN UPP CE EI NR LLGRLVHTCV IIP FS - V IQQI LNFP Weurospord, cassa/1-320 1 MSC (XWL RALL AN UPP CE EI NR LLGRLVHTCV IIP FS - V IQQI LNFP Weurospord, cassa/1-327 1 MSC (YWL RALL RALL AN UPP CE EI NR LLGRLVHTCV IIP FS - V IQQI LNFP Weurospord, cassa/1-327 1 MSC (YWL RALL RALL SSYCDL DN LSSK SKI HALFKR LDL LAA - LEVDEKS IDA ILKST Weurospord, cassa/1-273 1 MSC (YWL RK RALL SSYCDL DN LSSK SKI HALFKR LDL LAA - LEVDEKS IDA ILKST Weurospord, cassa/1-273 1 MSC (YWL RK RALL SSYCDL DN LSSK SKI HALFKR LDL AA - LEVDEKS IDA ILKST Weurospord, cassa/1-202 5 % KDM RANI ILK CC EAECYL QWFST LLGS (DDD N LDOHLIN PF YS NNL YNK ILGK LLGA - LEVDEKS IDA ILKST Vabidopsit, Jubian/1-228 5 % KDM RANI ILK CC EAECYL QWFST LLGS (DD DN LDOHLIN PF YS NNL KLCK LL FOULQUENDA Vabidopsit, Jubian/1-227 6 % NM RANI ILK CC EAECYL QWFST LLGA FW RD - COCYL LLKR KLD AA - LEVPEKS NULL LLAA - LEVPEKS NULL L	CntK	DapF (Diaminonimelate enimerase)	-	DapF (100%)	
Introduction (Cutodsoftime intering) (Cutodsoftime intering) rabidopsis_thalians/1-320 1 MAC QNNLVVKQI DLY DQI 5 - KLKS LKP 5 KNVDT LFCQL VST CLP TDTN IDVTNMCE supports_crasss/1-302 1 MAC QNNLVVKQI DLY DQI 5 - KLKS LKP 5 KNVDT LFCQL VST CLP TDTN IDVTNMCE supports_crasss/1-302 1 MAC QNNLVVKQI DLY DQI 5 - KLKS LKP 5 KNVDT LFCQL VST CLP TDTN IDVTNMCE supports_crasss/1-302 1 MSC YI WOKK IK LIS AR LEG (SAVYKGP CEE EL LOSS SCK. HALFKK DE LAA - LEVDEK SI DAI LKS MSC YI WOKK IK RI AS RLEGNYKFD - EMDTS SCW P LLDE IE I LAND ST DF ESAKI ILDDA LLEGT	CntL	Alcohol dehydrogenases of the	FmrO (Pibosomal Motbultransforaso)	NAS/NAS-like (100%)	
Validapit, Hallany 1-320 1 MAC QNLLYKOI IDIYDQ IS KLKSLKPSKNDT LFGUYSTCLPTDTN IDVTNMCE Validapit, Hallany 1-320 1 MGSCANTAGMIASI IDTKGLK-KSKNDT LFGUYSTCLPTDTN IDVTNMCE Validapit, Hallany 1-320 1 MGSCANTAGMIASI ISTAGMIASI ISTAGMI	CntM	DUE2338	DUF2338	Octopine dehydrogenase (100%)	
trabidoptis_thilana/1-320 1 MAC QNNL VYKQI DLYDQIS - KLKSLKPSKNVDT LFQQLVSTCLPTDTN IDVTNMCE teurospor_crassa/1-328 1 MAG C QNNL VYKQI DLYDQIS - KLKSLKPSKNVDT LFQQLVSTCLPTDTN IDVTNMCE teirobacterium, termosubtorphicm/1-261 1 MAG C + LPSLSPSPEVDCE E INR LLCNLVHTCVQI PP S - VIQQILNFF teirbanobacterium, termosubtorphicm/1-263 1 MAG C + LPSLSPSPEVDCE E INR LLCNLVHTCVQI PP S - VIQQILNFF teirbanobacterium, termosubtorphicm/1-263 1 MSC Y LYWOK IK RIASE LGMNY HFO - LDDS S SCK LHALFKRLDE LAA. LEVDEKSIDA ILKSP teirbanobacterium, termosubtorphicm/1-263 1 MMC RTPLLETIR LEE E I ANKS LS SV COLDN - ECISALCO TO ID EITK ILDE IE VIANDE ID FD SAKHILDDD termina_pests/1-263 1 MMC RTPLLETIR LEE E I ANKS LS SV COLDN - ECISALCO TO ID EITK ILDE IE VIANDE ID FD SAKHILDDD trabindoccosca_averol/1-273 1 MMC RTPLLETIR LEE E IAKK LS LAVCO QA QA G SHFUL E R R SR LQ SFNOE POLLOPYK LD E R - COCY E ILRRKLDPN - SC LG GEOS SRQ WAG SD DD VIANDE IN LARGE LS LAVCO QA QA G SHFUL E R R SR LQ SFNOE POLLOPYK LD E R - COCY E ILRRKLDPN - LC LS LA SECK LE POLLOPYK LD E R - COCY E ILRRKLDPN - COCY E ILRRKLDPN - LC LA AND QA LAVCO DE SKQ WAG SD DD VIANDE IN LICS SA SC LAVCO QA QA SI SKY VIANDE SC LG GEOS SRQ	L.	2000	2000	Colopine denyarogenade (100%)	
<pre>rabidopsig_thaliana/1-320 1MCSCANTKAQMIALSILDTIKQLK.KLPAYQPCGEINSKNDTECQLVSTCLPTDTN IDVTMCEC termsopar_assal-1-302 1MCSCANTKAQMIALSILDTIKQLK.KLPAYQPCGEINSLLGALVTTCQLIPPSPVDVTKLSP terhanostaria_activatoriphicum/1-26 1MSCCANTKAQMIALSILDTIKQLK.KLPAYQPCGEINLQLAALLEVALLEVSLPSPVDR</pre>					
 Heurosporg, crassal /-302 I MS CG XIN TK AQMI AL SI LDTHKQLK - KLP AY OP CE EINR LLCNL VHTCVQ IHP P S - V 10Q ILNF P fethanostarcim, acetivoran/1-273 I MS CG YINOK EV AAL IE KLA GLQAA IA - ELP SLS PS P EV DR LLCD LE IE IANDS TI DFS SK HIA LD KR LD S SK GLHAD. FK R, DE LAAD. LEV DE KS IDA ILK SP fethanostarcim, acetivoran/1-273 I MS CG YINOK IK RI AS REL COMY HED - LDD S SK GLHAD. FK R, DE LAAD. LEV DE FS SK HIA LD KR LD S SK GLDDN - ECLS ALCOTO ID CI TK ILDE IE LIANDS TI DFS SK HIA LD KR LD SY COLDN - ECLS ALCOTO ID CI TK ILDE IE LIANDS TI DFS SK HIA LD KR LD SY COLDN - ECLS ALCOTO ID CI TK ILDE IE LIANDS TI DFS SK HIA LD KR LD SY COLDN - ECLS ALCOTO ID CI TK ILDE IE LIANDS TI DFS SK HIA LD KR LD SY COLDN - ECLS ALCOTO ID CI TK ILDE IE LIANDS TI DFS SK HIA LD KR LD SY COLDN - ECLS ALCOTO ID CI TK ILDE IE LIANDS TI DFS SK HIA LD KR LD SY COLDN - ECLS ALCOTO ID CI TK ILDE IE LIANDS TI DFS SK HIA LD KR LD SY KIANDS ALS SY COLDN - ECLS ALCOTO ID CI TK ILDE IE LANDS TI DFS SK HIA LD KR LD SY KIANDS ALS SY COLDN - ECLS ALCOTO ID CI TK ILDE IE LANDS TI DFS SK HIA LD KR LD SY KIANDS ALS SY COLDN - ECLS ALCOTO ID CI TK ILDE IE LANDS TI DFS SK HIA LD KR LD SY KIANDS ALS SY COLDN - ECLS ALCOTO ID CI TK ILDE IE LANDS TI DFS SK HIA LD KR LD SY KIANDS ALS SY COLDN - ECLS ALCOTO ID CI TK ILDE IE COLD SK AL WQADS DP KIANDS ALS SY KIANDS ALS SY COLDN - LS KI KI LD KANT ALS SY KIANDS ALS ALS SY KIANDS ALS	krabidopsis_thaliana/1-320	1 MAC QN	NLVVKQIIDLYDQIS-KLKSLKPSI	KNVDT L F GQ L V S T C L P T D T N I	DVTNMCEEV
 Increase Nylane (1-228 Increase Nylane (leurospora_crassa/1-302	1 MG S C A N I T K A	QMIALSILDTHKQLK - KLPAYQPGI	EEINR LLGNLVHTCVQIHPPS-VI	QQILNFPGL
tethanabacterium, thermoutorophicum/1-263 1 MNN IQ K IE DAF LVAE LRQ IS AVV KCP G EEE LLQS S S CK LHAL FKR LDE LAA - LEVDEKS IDA ILKS Y tethanabacterium, thermoutorophicum/1-273 1 MSC Y LYWD KI KR IA S R LE GMNY HPD - EMDT S CVMP L DE E I AND S T ID F IS AKH IL DD A ILKS Y tethanabacterium, thermoutorophicum/1-263 1 MSC Y LYWD KI KR IA S KI LC GMNY HPD - EMDT S CVMP L DE I AND S T ID F IS AKH IL DD F S AKH IL D	lordeum_vulgare/1-328	1 MDAQNKEV	AALIEKIAGIQAAIA-ELPSLSPS	P E V D R L F T D L V T A C V P P S P V	DVTKLSPEH
<pre>tethanobecketerium; thermountorophicum/1-266 1 MSCV 1YWDR iK RI AS R LEGMNY HF D-EMITS CVMP LDE LE LA HDS TI DF ES AKH LL DD F tethanobeckuter; ruminanitum/1-273 1 MSCV YWGK IE EI AWK LS SY GL DDE TK I, LDE TK I, LDE TV ANDE ALS ST ALD DE TK I, LDE JF SA KH LL DD F seudomona_serginoss/1-263 1 WT LMK ADV LAQ LA CLOQ LE SQ L HDAANQ - QNO SH FV LLER</pre>	lethanosarcina_acetivorans/1-273	1 MNNIQKIED	DAFLVAELRQ I SAVVKGPGEEEILQ	S S S G K L H A L F K R L D E L A A - L E V D E K S I	DAILKSPTF
<pre>tethanobrevbacter_unimantum/1-273 1 MSCYKYWGKIEEIANKLSSYCDLDN - EGLSALDGTDIDEITK ILDE JEVIAHDKEID FDSAKHILDD FFILAPWGCXX, AUREVJ-265 1 MSCYKYWGKIEEIANKLSSYCDLDN - EGLSALDGTDIDEITK ILDE JEVIAHDKEID FDSAKHILDD FFILAPWGCXX, AURADOV SAFILLER RSRLQSINDEPQLQDYRLQLEHDAWARDOV FFILAPGGCX, AURADOV SAFILLER RSRLQSINDEPQLQDYRLQLEHDAWARDOV FFILAPGGCX, AURADOV SAFILLER RSRLQSINDEPQLQDYRLQLEHDAWARDOV FFILAPGGCX, AURADOV SAFILLER LETTV + KARLDR LSCLG GEDCSALA PKISSYLLKLGKLE FDLLSALAALPP NL GQ GULPS LR TICSEAESCLETIWAEHTLALAANGGKETVLKAVQTD FFY FQNY IDLARL LSA IRAALPP NL GQ GULPS LR TICSEAESCLETIWAEHTLALAANGGKETVLKAVQTD FFY FQNY IDLARL LSA IRAALPP NL GQ GULPS LR TICSEAESCLETIWAEHTLALAANGGKETVLKAVQTD FFY FQNY IDLARL LSA IRAALPP NL GQ GULPS LR TICSEAESCLETIWAEHTLALAANGGKETVLKAVQTD FFY FQNY IDLARL LSA IRAALPP NL GQ GULPS LR TICSEAESCLETIWAEHTLALAANGGKETVLKAVQTD FFY FQNY IDLARL LSA IRAALPP NL GQ GULPS LR TICSEAESCLETIWAEHTLALAANGGKETVLKAVQTD FFY FQNY IDLARL LSA IRAALPP NL GQ GWARLASL IK R FK FK</pre>	lethanobacterium_thermoautotrophic	<i>cum/1-266</i> 1 M S C Y I Y W D K I	KRIASRLEGMNYHFD-EMDTSGVM	P LLDEIEEIAHDSTIDFESA	KHILDDAEN
taphylococcus, aureus/1-272 1 - MNN FNN EI K LI LQQY LEK F EAHY ERV L - QDQY I EA LET FN MODY SEFILNP I Y EQ QF NAWR DV V Serving a serving noss/1-263 rabidopsis, thalian/1-263 1 MY T LMK ADU LAQLAC LQQ EI SQL HDNAQ QNQ SHF V LLET FN Y SNY LK LGK LE FD LQDY FD LQDY RLOL HDDA NG QC PQ SHF V LK RX LDP	fethanobrevibacter_ruminantium/1-2	273 1 M S C Y K Y W G K I	EEIANKLSSYGDLDN-EGLSALDG	TDIDEITKILDE EVIAHDKEIDFDSA	KHILDDEKN
ersinia. petis /1-265 IM CONTROL AND CARLENDER OF A CONTROL OF A CONTR	taphylococcus_aureus/1-272	1 - MNNFNNEIKLIL	.QQYLEKFEAHYERVL-QDDQYIEAI	L ET LMDDY S E F I L NP I Y EQ - Q F	NAWRDVEEK
<pre>seudomonas_aeruginosa/1-263</pre> 1 MG CRTP LLETLR LLE CE FIR LLTYYAR EC - CGCY EILR RLLD LS CLIGE DCSRAQWQ ADS DDP xtabidopsis_thalana/1-320 59 KDMRANLIK LCE AE GY LE GY LE GY LE GU PF STIL LGS LO CCY EILR RLLD LS CLIGE DCSRAQWQ ADS DDP trabidopsis_thalana/1-320 59 KDMRANLIK LCE AE GY LE GY LE GY LE GU PF STIL LGS LO CCY EILR RLLD LS CLIGE DCSRAQWQ ADS DDP trabidopsis_thalana/1-328 59 KDMRANLIK LCSAA EGX LE AHYADLLA AAHQG HETV LKAYOT DF WF GAN'I DLAR LE LSA RAALPP NH- tethanosactorophicum/1-273 66 AQLIPS LIFK FY LYNLKLENEK KAKN LLE SSD PWETLR SFY KP WY NL SRLEY ELLAR HVPC tethanosactor activorans/1-273 66 AQLIPS LIFK FY WINLG KLE RE KAKN LLE SSD PWETLR SFY KP WP RX LELKK NEAALGR tethanosevacacter ruminantium/1-273 70 NKALGLIRK FY YNL CAR LE RE NAL COY EK HQV VL LOGA STTGFY KP WP RX LELLK NEAALGR tethanosevacactivorans/1-263 66 AQLIPS LQY LTAQCYKQYEV IR ARR LLG CGASTTGFY KP WP RX LELKK NEAALGR tervina_pestif/-263 66 AQLIPS LQY LTAQCYKQYEV IR AR	ersinia_pestis/1-265	1 MYTLMKADVLAQL	AGLQQEISQLHDNAQ-QNQSHFVLI	LER R F S R LQ S F N D E P Q L Q D Y R L	QLEHDDAVV
rabidopsis_thalana/1-320 59 K DWR AN LIKL CG EA EGY LEQHEST LLGS DEQ NP LDHLHI F PW GNY KL CK LEFDLLSDH S skurospor_crass/1-302 66 QQ LP S LRT LC SE AE SC LEQHEST HWA EHT LA LAAHQCH ET VLKA VQT DF PW GNY NL LAR LE LSA FAA AL PP NN ordemu, vidjang, crass/1-328 66 QQ LP S LRT LC SE AE SC LEQHEND LLAT FDNP LDHLCL F PW GNY NL LSK LE FE LLAAH PP NN fethanosactim_intermoautotophicum/1-266 69 NAL IDS ISR FR LYN LKLEN KKAK NL ES SDPWET LR N FT VP NN LQ LART YT GS fethanosketim_intermoautotophicum/1-267 69 NAL IDS ISR FR LYN LKLEN KKAK NL ES SDPWET LR N FT VP NN LQ LART YT GS seudomosa_serus/1-273 70 NKA LQ LIR KF YVN LGAK LE RAK KAN NL ES SDPWET LR N FT VP NN LQ LART YT GS seudomosa_serus/1-263 65 QA LG LR KD YT YA CVO VE VIR AR RL LDGODA STG FD NN SLOQU IL LART YN SLOQU IL LAN KAN NL ES SDPWET LR N FT VP NN LQ LART YT GS seudomosa_serus/1-263 65 QA LG LR ND AVOAL CCI LEKHLGOU GV HHP GERGR LL ST NN SLOQU IK LA CO rabidopsis_thaliana/1-320 124 VPT KI A TV G SOPM LT S VL AK FHL PNT TH KN B ID SHANT LA SN LV SQ D DP - LSK NI FHT TD seudomosa_serus/1-328 128 CCENAV IC GCP LL T SWC LI E IK KL GO DNI PT I CN D MS PT AI DV SS QL NG ALGP VE CON VIC CE CO CON VIC LE KHL LCOU SU HHP GE GUE AN RANK NV LA PNE DT TD OX SQ LNG ALGP VE CON VIC CE CO CON VIC LI EN KT LG QUE AN ALLOY IC CON VIC LE CON VIC VIC LE DO LA LSK NV LA NE DE CON VIC LG CAN NE RANK NV LA PNE DE TD TO XQ LO CON VIC LA LY CON VIC LE CON VIC LI LA VC CON VIC LE CON VIC LE CON VIC	seudomonas_aeruginosa/1-263	1 MQGRTPLLETLRE	LECEIRLLTVYAREC-CGCYEILR	K L D R L S G L I G E D C S R A Q W	QADSDDPAL
leurosporcrassa/1-30266 QQ L L P S R T I C S E A E S C L E T HWA EHT LA AHQ CH E T V LKAV QT D F P Y FQ NY ID LAR L E L S A I RAA L P P NOrotedum, vujaret 1-32860 Q N L P S R T I C S E A E S C L E T HWA EHT LA AHQ CH E T V LKAV QT D F P Y FQ NY IN LS R L E S A I RAA L P P NOrotedum, vujaret 1-32860 Q N L P S R T I C S E A E S C L E T HWA EHT LA AHQ CH E T V LKAV QT D F P Y FQ NY IN LS R L E S A I RAA L P P NOrotedum, vujaret 1-32769 NA L I D S I S R F L Y N L K L E N E KAK L L F S D P W ET I L R F Y F P NY I L L L NA E AA L C P K E HANDAN L C N L E K K V Y I L C K L M E K AA L C Y L E L K N E AA L C Y F N N L L L N E R C AA L I R K Y NY I C A R L R E R NA L T L L B D D P K V L E L L N E R AA L C Y F N Y N L E L L N E R C AA L I R K Y N Y I C A R L R E R NA L Y L E L X N E AA L C D F K K I I C V I C A C L R K Y V Y I C A R L R E R NA L Y L I L X N D S D Q E I K L A A L C O Y K V C V Q V V I N A R R R L D G C A S T C Y L S F N D L C E I K K I A O Q L Q L R D A V Q A L C E L E K H L C Q	rabidopsis_thaliana/1-320	59 K DMR ANLIKLCGE	AEGYLEQHFSTILGSLQEDQNPLD	H L H I F P Y Y S NY L K L G K L E F D L L S	QHSSH
iordeum, vulgare/1-328 61 Q RMR EA LIRLCSAAEGKLEAHYADLLATFDNPLDHLGLPFYYFNYVLGLARE YELLARHVP iordeum, vulgare/1-328 61 Q RMR EA LIRLCSAAEGKLEAHYADLLATFDNPLDHLGLPFYYFNYULGLARE HE KLARV iordeum, thermoutotrophicum/1-266 69 NALIDS ISRFEFLYNLKLENEKKAKNLLESSDPWETLRSFFYPRYULGLARE HE REAL iordeum, thermoutotrophicum/1-273 69 NALIDS IRKFEFLYNLKLENEKKAKNLLESSDPWETLRSFFYPRYULGLANE RALA iordeum, vulgare/1-263 68 KQIA-QLRQVANAALCDYEKHQVCALCSPSKTDDYLTSFNQSLQQEIKLAG iordeum, vulgare/1-328 69 QALCRR PAAVQALCELEKHLQQCVLHPFGEMGRYLTSFNQSLQQEIKLAG iordeum, vulgare/1-328 124 V PTKTAFVCSGPMFLTSTVLAKFHLPNTTFHNFDIDSHANTLASNLVSRDPD-LSKRMIFHTTD ieurospora_crass/1-302 124 V PTKTAFVCSGPMFLTSTVLAKFHLPNTTFHNFDIDSHANTLASNLVSRDPD-LSKRMIFHTTD ieurospora_crass/1-328 125 P - RKVFVCGGPLFLSLVLAKFHLPNTTFHNFDIDSHANTLASNLVSRDPD-LSKRMIFHTTD ieurospora_crass/1-328 125 P - RKVFVCGGPLFLSLVLAKFHLPNTTFHNFDIDSHANTLASNLVSRDCGARMSFHTAD ieurospora_crass/1-328 125 P - RKVFVFCGGPLFLSLVLAKFHLPNTTFHNFDIDSHANTLASNLVSRDCGARMSFHTAD ieurospora_crass/1-328 128 CERKAVF IGGCPLFLSLVL	leurospora_crassa/1-302	66 QQILPSLRTICSE	AESCLETHWAEHTLALAAHQGHET	V L K A V Q T D F P Y F Q N Y I D L A R L E L S A I R	AALPPNNTA
tethanaarcima_acetivorany/1-273 69 NA LIDS ISERFLVNLKLENEKAK NLESSDPWETLENFTYYPRYLGLAKTEYTGS C tethanabrevibacer_ruminantium/1-273 69 NA LIDS ISERFLVNLKLENEKAK EVIESDSPWETLESFYYPRYLGLAKTEYTGS R tethanabrevibacer_ruminantium/1-273 70 NK ALQL RKFYVYIGAELEREKALYIESDSPWETLESFYYPRYLGLLKNEAALGYFORDEKALGSYFORDEKALGS	lordeum_vulgare/1-328	61 Q R M R E A L I R L C S A	AEGKLEAHYADLLATFDNPLD	HLGLFPYY SNY VNLSRLEYELLA	RHVPGIA
tethanobacterium, thermosutorophicum/1-263 63 NHALSL RKFYVNLCMKLEMEKAQ EVISDSPWETLRSFYFYPRYLELNKEAALGR tethanobreviacer, ruminantium/1-273 70 NKALQL RKFYVYLGARLERKAQ EVISDSPWETLRS	lethanosarcina acetivorans/1-273	69 NALIDSISRFRFL	YNLKLENEKAK NLLESSDPWET	FLRNFTYYPNYLQLARTEYTGS-	GLK
tethanobrevibacter_ruminantum/1-273 70	lethanobacterium thermoautotrophic	um/1-266 63 NHALSLIRKFYVN	LGMKLEMEKAQ EVIESDSPWET	TLRSFYFYPRYLELLKNEAALG-	R F R
taphylococcut_aureux/1-272 66 A Q L IK S Q Y IT AQ C V K Q Y V I A A R R L D G Q A STTG Y F D N I E H C I D E E F Q C S seudomonas_aeruginosa/1-263 65 K Q I A - Q R Q V A NA AL C O Y E K M Q V C A L C S P S S X T D Y L T S F N S L Q G I K I A S rabidopsis_thaliana/1-320 124 V P T K I A F V C S G P M L I S I V L A K F H L P N T F H N F D I D S H A N T L A S N L Y S R D P D - L S K R M I F H T D eurospor_crassi/1-302 139 P L K I T F I G S Q L C L R V A A AL C O Y E K M Q C M D I P T (C N I D M S P T A I D Y S Q I N G AL C G A R K R A K L F G A T AD C Y G K M V Y K G G C P L X I V V C A A H H L P F T D I D S H A N L A S N L Y S R D P D - L S K R M I F H T D fethanosacterium, thermosutorophicum/1-263 128 P C R V Y K G G Q F L I L I S W C L D E I R T A G Q N D I P T (C N I D M S P T A I D Y S Q I N G A L G L S F N X V V G G Q F L X I V L A H H H P T D I A E L S K N K I L G C V C V N V I T C D tethanosacterium, thermosutorophicum/1-273 129 F O R V Y I G G C P L I L I I M F N N V T G C K C I I E Q D P Q A V D L S K V K K I L C P K E V T I T Q C X N R K K K V I E C C V E V V V I T C Q S N D K L L V G S Q L T T T A V U A K E T G C A N R A R K I K I E C O C V V V V I T C Q S N D K L L V G S Q L T T T A V U A K E T G C Y I C Q I D P Q A V D L S K K V K K I L D D E C I E I V T G N A L V S R V F I C Q C P L V X I T C Q S N D K L L V G S Q L T T T A V U A K E V G A U L X G X V K I D P A Q U L S K V K K I L D D E C I V Y V X V X V Y Y Y Y Y Y Y Y Y Y Y Y Y	lethanobrevibacter ruminantium/1-2	73 70 NKALQLIRKFYVY	IGARLERENAL TILEADDPKVA	ALDSFHFYDRYIGLIDNERQLA-	NFM
ersina pestis/1-265 68 KQ I A - Q R QVANA ALCOY EKHQVC ALCS P S KTDD V LT F NOS LQQ E IKIA C seudomonas_aeruginosa/1-263 65 Q A LG LR R DA AVQALCE LE KHL CQ C V LH EP G EM GR V LG S LE S IR GE LD SA C S Q A LG LR R DA AVQALCE LE KHL CQ C V LH EP G EM GR V LG S LE S IR GE LD SA C seudomonas_aeruginosa/1-263 124 V P T KIAF V G S C P LF LT S WC LL DE IR KT AG QND MI P T I CN ID MS P TA I DV S S Q LM CA L C V G C ME F LC G Drodeum, vulgare/1-328 125 P - A RV A F V G S C P LF S S V L A A HH LP KT T F HN F B I D S HANT LA S N L V S R D P - L S KR M I F HT T D Henrospora_crass/1-302 139 P LK KI T F I G S C P L F S S V L A A HH LP KT Q F D N U LC GA AN ER AR KL F GAT AD GV GA EM S F H AD 125 P - A RV A F V G S C P L F S S V L A A HH LP KT Q F D N U LC GA AN ER AR KL F GAT AD GV GA EM S F H AD 126 P - A RV A F V G S C P L T I S L V L A K H LP KT Q F D N U LC GA AN ER AR KL F GAT AD GV GA EM S F H AD 127 P - A RV A F V G S G C P L T I G L L S N V T G M N V V E I EP D I A LS R KV I KE LG V D C V N V I T C 128 P G C V L F L G S G P L L T I M F N N T G C K C I G I D P Q AV D LG R K V K N L A D LE G I E I V T C Herbanobreater ruminatimu	taphylococcus aureus/1-272	66 AOLIKSLOVITAO	CVKOVEVIRAR RLLDGOASTT	GYEDNIEHCIDEEFGOC-	SIT
seudomona_aeruginosa/1-263 65 QALGLRURDAAVQALCELEKHLCQ CYLHEPGEMGR YLGSLLESIRGELDSA C rabidopsis_thaliana/1-320 124 VPTKIAFVGSQPMLLTSIVL AKFHLPNTTFINFFIDSHANTLASNLYSRDPD -LSKRMIFHTTD eurospora_crassa/1-302 139 PLKKITFIGSQPLPLTSWCLLDEIRKTAGQNDMIPTICNIDSHANTLASNLYSRDPD -LSKRMIFHTTD fethanosarcina_acetivorans/1-273 125 P - ANVAFV GSQPLPLTSWCLLDEIRKTAGQNDMIPTICNIDAGAANERAKLFGATADCVGARMSFHTAD fethanosarcina_acetivorans/1-273 128 FGCVLFLGSQPLPLTSILVL AAHHLPFTIGDVDLGGAANERAKLFGATADCVGARMSFHTAD fethanosarcina_acetivorans/1-273 129 ENKTFVFLGSQPLPLTINFF 120 SNDKLLVSGALPTTGLUV SGPLAULTIGLL	ersinia pestis/1-265	68 KOLA-OLROVANA	ALCOYEKHOVC ALCSPSSKTDI	DYLTSENOSLOOEIKLA-	GMO
rabidopsis_thaliana/1-320 124 V P T K I A F V G S G P M L T S I V L A K F H L P NT T F H N F D I D S H AN T L A S N L V S R D P D - L S K R M I F H T T D leurospac_crassa/1-302 139 P L K X I T F I G S Q P L F S S L V L	seudomonas_aeruginosa/1~263	65 QALGLRLRDAAVQ	ALCELEKHLCQ GVLHEPGEMGE	R	GID
Labudapa_Unital/_320 Labudapa_Unital/_320 Labudapa_Unital/_320 Labudapa_Unital/_320 Usinspar_carsal/-302 Lab P LK X T F LG G C P L L SWC L LD E LK X T A GONDMI PT L ON D LG AN LEX A K L F GAT A DC V G A K M S F T A DC V C K C I G F Q D D V A DL S K K V L K L D C S C V V V I C C G C L L L I L M F A C C C C L C L C C C C C C C C C C C C	rabidoncie thaliana (1. 220	124 VETKIAEVESER		HNEDIDSHANTIASNEVSPORD-ISKR	MICHTTOVI
Hurosput, Jussa (1-326) 139 FLKK N HV G G QFL F S SLVL AAHHL F TIG DNY D LG GAANEGAR KL F GAT ADCV G GRM F HT AD Herhanobacterium, thermoutorophicum/1-263 128 F - AKVA FV G G G QFL T S LV L AAHHL F TIG DNY D LG GAANEGAR KL F GAT ADCV G GRM F HT AD Herhanobacterium, thermoutorophicum/1-263 128 F - G KV AFV G G G QFL T L S LV L AAHHL F FT Q D NY D LG GAANEGAR KL F GAT ADCV G GRM F HT AD Herhanobacterium, thermoutorophicum/1-263 128 F - G KV AFV G G G QFL T L S LV L S HVY C MW NVY E FD I A ELSK KV I E GL C V D C VNV I T CD Herhanobacterium, thermoutorophicum/1-263 129 S NDK T FV F LG S QFL L T T I M F S HVY C MW NVY C E G L Q D P QAV D L S R KV I KK I L D - L D E G L E I V T KO Heurosport, crassa (1-302) 139 NA R V L FV G S G A L P T S L V L X K L G A T FC Y D HP AAQ Q L AR Q L VQ S L C L E R X VQ Y I D - N KE T G C A N C A KE T G A T AD V G S Q K V Y L V K S G A L P T AL V L V G S G K L T T AL V L X K L C A T V F C Y D HP AAQ Q L AR Q L VQ S L C L E R X VQ Y I T D A C A ME Y A KL G A T F G A L V Y R S A R P - N KE I V Y V V L V Y G S Q K L Y K S A L P - A E V G X KU K Y K S A L P - N KE Y K Y K L V G S Q K D E E I F K V Y K T M K P G A L V Y R S A R P - R G L - Y P I V D Y E D I R R Q - L Y N Y Y K Y Y Y Y Y Y Y Y Y Y Y Y Y Y Y	laurospora_srassa (1_202	120 0 1 4 4 1 7 5 1 6 5 6 0 1	PLT SWCI LDE LPKTACONDMIDTIC	CNIDMSDIALDVSSOLNCALCD-WCEC	MEELCCEAC
Distribution 128 PGCVVFICSCPLLSLVL	lordaum vulgare /1-328	125 P - APVAEVCSCPI	PESSIVI AAHHIPETOEL	ONY DI CCAANERARKI ECATADOVCAR	MSEHTADVA
<pre>Humbandarding_lectronals/1-2/3 is the form of the second sec</pre>	athanararcina acativorane (1-272	128 PCDCVIELCSCPI	PISITVI		IN TIECNHE
Hernandszeiterum 128 ENKT FV FLÖSGLUL I LIVIGS LLUK FLÖSGLUL I LIVIGS Hernandszeiterum 129 ENKT FV FLÖSGLUL I LIVIGS FLÖSGLUL I LIVIGS Handbreider 129 ENKT FV FLÖSGLUL I LIVIGS FLÖSGLUL I LIVIGS Handbreider 129 ENKT FV FLÖSGLUL I LIVIGS FLÖSGLUL I LIVIGS Handbreider 129 ENKT FV FLÖSGLUL I LIVIGS FLÖSGLUL I LIVIGS Standbreider 120 SNDKL LIVIGSGLUT TALVI	Aethanobacterium thermoautotrophic	120 PODCVEPLOSOFL	PLTCILL	NVVELEPDIAELSPKVIECICVDC	VNVITCDE
Heinandorwakter_inimitation/1223 125 KNK KULLUKGSGALP MILLQV Heinandorwakter_inimitation/1223 125 KNK KULVGSGALP MILLQV Heinandorwakter_inimitation/1223 125 KNK KULVGSGALP MILLQV Heinandorwakter_inimitation/1223 128 NATEGLQQVDVFLAALVGMDKESKVKATEHLEKHMAPGALLVIRSA-HALRAFL-YPIVDSDLKG-F Heinandoreinimitation/1223 129 NATEGLQQVDVVFLAALVGMDKESKVKATEHLEKHMAPGALLVIRSA-HALRAFL-YPIVDSDLKG-F Heinandoreinimitation/1223 190 DLTGLGAVDVYFLAALVGMDKESKVKATEHLEKHMAPGALLVIRSA-HALRAFL-YPIVDSDLKG-F Heinandoreinimitation/1223 190 DLTGLGAVDVYFLAALVGMDKESKVKATEHLEKHMAPGALLVIRSA-HALRAFL-YPIVDSDLKG-F Heinandoreinimitation/1223 190 DLTGLGAVDVYFLAALVGMDKESKVKATEHLKKVNTTERKUVRSA-RF-CFLPVNVTEKLGUNRSA-WGLRTERLEVPVDFDIRTERLGC-F Heinandoreinimitation/1223 190 DLTGLGAVDVYFLAALVGMDAACFRKEVFRLAKILPVOTETRITITTERLGUNRSA-WGLRTERLEVPVDFDVSVDIDT-G-F Heinandoreinimitation/12263 182 TUTOGLE-FOULWWAAQAFRKEVFRLAKILPVOTETRITITTERLGUNRSALVVSVDIDTFVVYRSVSVDIDTERLEVVSDIDTIT-G-F Heinandoreinister/12263 182 ELTORP-VDHILVASLVDICGLVVFRAVMOIVODTVVVKRGLUVVSTRALLSVFKDESLVKRYG-DGLAEKSELTAKELAF Heinandoreinister/12263 182 ELTORP-VDHILVASLVQOLSA	athanobacterium_chermoautocrophic				LEIVTONEN
approposition 211 M GEV LIV GG GALL TIALV	ternanobrevibacter_ruminantium/1-2		PATILOV AVETCASV	ICIDI DROAVDI CRRIVNVIA RNED	TITONER
estimal pesity/1-263 111 MOLEN LEV GGALLETSALVI AR LUA FUGLAND FANUGLAND TANUNGLAND TANUNGLAN	laphylococcus_aureus/1-272				VOELD NIK
rabidopsis_thaliana/1-320 189 NATEGLDQYDVYELAALVGMDKESSVKATEHLEKHMAPGAVLMIRSA-HALRAFL-YPIVDSDLKG-F eurospor_crassi,11-302 211 SPS IS LEDSDVVYALVGMSQKDREEIFLKVVRTMRPGALLVVRSA-WGLRTCL-YPEVNVTTERLLGU ordeum_vujare/1-328 190 DLTGELGAYDVYELAALVGMSQKDREEIFLKVVRTMRPGALLVVRSA-HALRAFL-YPEVNVTTERLLGU fethanosiarcina_acetivorans/1-273 190 NLPLGTR-COLYMVAAQA EPKKVFRLAKLUVRSA-RP-REGL-YPUVDFDIRRGC-F fethanosiarcina_acetivorans/1-273 190 TLTGELGAYDVYENALA EPKKVFRLAKLUPGGTKVSVRUKEKGLRRL-OGNSIFELPSG-F rethanoosacterium_thermoautotrophicum/1-260 182 TV IDGLE - FDVLMVAAQA EPKKVFRLHRKVDTETRITVTY-TGMRAIL-YAPVSDDDITG-F rethanoosacterium_thermoautotrophicum/1-263 182 TV IDGLE - FDVLMVAAQA EPKKVFRLHRKVDVVDETTRITVTY-TGMRAIL-YAPVSDDDITG-F rethanoosacterium_thermoautotrophicum/1-263 182 TV IDGLE - FDVLMVAAQA EPKKVFRLHRKVVDTETRITVTYTTY-TGMRAIL-YAPVSDDDITG-F rethanoosevacureus/1-272 182 ELKDIKOVTHITFSTT PLKYSIEELVDITNENVVVANKGG-GCIXAIESYPVEKDTETRGK seudomonas_eruginosa/1-263 182 ELTDRPVDHITVSVV-AVAVGLV DCGAVLAQIRQVMRADAKVLLHG-SGIKGLFNYP-VEPAELEG-W rabidopsis_thaliana/1-320 257 LTIYHPTDDVVNSVVIAKKAVEQGLSGCPQN-GDAHARCAVPLVSPCNFSTKMEASALEKSEELTAKELAF rethanosacterium_thermoautotrophicum/1-263 254 VKNVYSVVIAKVGVARVGC 254 VLXVPCPPPINTDVVVNYVVKVAKVGCG 254 VLXVPCVFFLVVCVXKVXVAKVGCG rabidopsis_thaliana/1-320 252 LXVHPVVVXVXVXVAKVG	seudomonas_aeruginosa/1-263	119 ADARVLFVGSGAL	PTSALVLAREVGAHL	CCLDIDEEALGYAREIARCQGLEAR	MQFSSLPPA
trainingst; thaitan/1-320 189 NATECLOUTOVE CAALVGMUKES VAALELEKENMAAP GAVLMUKSA-HALKAAL-FTFTOUSSDUKC-FTEUROSPACEASAI-1302 teurospor_carsain_1-320 115 PS 15 LED SDVVYVAALVGMUKES VAALGKER FLEKENMAAP GAVLMUKSA-HALKAAL-FTFTOUSSDUKC-FTEUROSPACEASAI-1302 teithanosarcial accetivorans/1-273 190 NLP LCT R- CDLYWVAALVGMUKES VAALKER KVT KAREGALVUKSA-HALKAAL FTC L-YPEVDYT ETR LLGVL teithanosarcial accetivorans/1-273 190 NLP LCT R- CDLYWVAALVGMUKES VAALKER KVT KAREGALVUKSA-RP-R CFL -YPIVDP EDIRGC-FTE teithanostacterial accetivorans/1-273 191 TI AE - LO -YDI LM VAAQAFPIK EVFEHLAKI LP CGT KVSK IV EKG KE RL L-OKSI FELP S-R VFEKDR-R-GFL teithanosterial accetivorans/1-272 191 TI AE - LO -YDI LM AA FAAFPIK EVFEHLAKI LP CGT KVSK IV EKG KE RL L-V YPI VDY DDIDTGFL teithanosterial accetivorans/1-265 182 ELKDI KOVT HI FSSTIPIK EVFEHLAKI LP CGT KVW KG KI SI FKLP YFEKDR-R-GF teithanosterial accetivorans/1-263 181 ELAF SR D-ATHFLIAS LV ADG QA LLAQL VPVTR SS KLWR YG - NGCI KAI FNY PS QE TA EDK HV seudomonas_aeruginosa/1-263 252 CAVVHP TDVV NS VI AR K LG CPT TF GV NGTR GCM FMPCN-CSK I HA I INNNR GK KMNI EEFS A varospace/1-328 252 CAVVHP TDVV NS VV AR KG C- 254 VLR VP F PINVY NS VV VAR KAY CC- 254 VLR VP F PINVP FV KS KAY VAQU SC CO ordeam vulgare/1-328 254 LAVHHP EG EVIN NS VV AR KAY EQ LSC PQ N- GDAHARGAY PLVS PC NF ST KM EASALEKS EELT AK ELAF tethanosacteriam, themosutorophicum/1-273 <t< td=""><td></td><td></td><td></td><td></td><td></td></t<>					
eurosporz, crassa/1-302 211 SPS IS LEDS DVV VAAL VGM SQL DE ET FLAV VKI MAR GALL LVVK SA. WULK LL-PEVNV TI EKLLGU eurosporz, crassa/1-302 211 SPS IS LEDS DVV VAAL VGM SQL DE ET FLAV VKI MAR GALL LVVK SA. WULK LL-PEVNV TI EKLLGU ethanobacterium, thermoautorophicum/1-263 190 DLT GE LCA YD VV FLA AL VGM SQL ALE KAK VI AAL GALK WV SAA. SV KI KL CL PE VNV TI EKLLGU ethanobacterium, thermoautorophicum/1-263 190 DLT GE L- FOU LWN AALA EP RK VF RNI HAR VD ET ET RIVY TT KM KAL L-V SP VS DD DI TG-F ethanobacterium, thermoautorophicum/1-263 191 TI AE - LO-YD I LMI AA FA EP RK VF RNI HAR VD TE TRI IV TT TG KA AL L - V SP VT EK DT R ersinia_pestis/1-263 191 TI AE - LO-YD I LMI AA FA EP RK VF RNI HAR VD TE TRI IV KA AL FN YF NS DD DI TG-F esting pestis/1-263 181 ELK DV TH IF SS TI PL RY SI LEELYD TT FOLV NA FG - GO LK SI FN CP YC HE VK S W seudomonas_aeruginosa/1-263 181 ELAF SR D- ATHFLI AS LV ADV GALL AQ LV PV YT RS SK LVM RY G- NGL KSI FN CP YC HE VK S W seudomonas_aeruginosa/1-263 257 LT I YH P T DDV VS SV LAR K LGG PT TP GV NG TR GCM FM P CN - C SK I HAI MNNR GK KNM I EE FS A eurosporz_erossa/1-302 257 LT I YH P T DDV VS SV VAR VK G LGG PT TP GV NG TR GCM FM P CN - C SK I HAI MNNR GK KNM I EE FS A ethanobacterium, thermoautorophicum/1-263 254 L XV NY F KK R LGG PT TP GV NG TR GCM FM P CN - C SK I HAI MNNR GK KNM I EE FS A ethanobacterica, cruninatinum/1-273 258 VK VM Y	rabidopsis_thaliana/1-320	189 NATEGLDQYDVVF	LAALVGMDKESKVKATEHLEKHMAN	GAVLMLKSA-HALKAFL-YPIVDSSD	LKG-FQI
Ordeum, yugare1-328 190 DL I (JE LGA YOVY I GA LV GMA A E LGA KV MAL GA MWY EGA SL VVK SA -K H - KG FL - TP I OVP ED I KK G - Fethanostacting, activorans/1-273 190 NL P (GT - CD LYM VA QA F FK KV FE HLAK I LP GGT KVYK SA -K H - KG FL - TP I OVP ED TP VVK SY LY EK GL RR I L - OGN SL FE LP S - G - F Fethanostacterium, thermoautotrophicum/1-276 187 TV I D G LE - F D V LIV A QA F FK KV FE HLAK I LP GGT KVYK SY LY EK GL RR I L - OGN SL FE LP S - G - F FK KV FE HLAK I LP GGT KVYK SY LY EK GL RR I L - OGN SL FE LP S - G - F rethanostrotophicum/1-273 191 T I A E - D - Y D LIM I A FA F P GR VF FH I HR Y VD T ET R I I Y GT Y - TGM RA I L - Y AP Y S DDD I T - G - F raphylococcus, aureus/1-272 182 EL KD I KOVT HI I F S T I P L I Y S I LE L Y D L T N F N W Y AM R G - G G I KA I F NY P S Q E T A ED K N D KQ A LLAQ L VP Y T X S S K LWA Y G - N G K S S I KO S I F KL Y F H L XK S I F KL Y F H L XK S I F KL Y F H E V X S I Y K A D KQ A LLAQ L VP Y T X S S K LWAY G - G G I K G L F NY P - V E P A E L E G W seudomonas_aeruginosa/1-263 181 E LA F S R O - A T H F LI A S LV Q G S AV LAQ I R Q VM R A D A K V L R H G - S G I K G L F NY P - V E P A E L E G W validopisis, thaliana/1-320 257 L T I Y H P T D VV N S V V A R K A C C L G G T T F G V M K A D A K V L R H G - S G I K G L F NY P - V E P A E L E G W validopisis, thaliana/1-320 258 L A V H H P E O V I N S V V A R K A C C L G G C T T G C M G A A R G A V P L Y P C N S T K M E A S A L E K S E E L T A K E L A F tethanostroid a c c t i N S V I V R K K A Y E A V A Q L S C Q V H O A R A V E Q L S C Q V H	eurospora_crassa/1-302	211 SP ST SLEDSDVVY	VAALVGMSQKDKEETFLKVVKIMKI	GALLVVKSA-WGLKICL-YPEVNVII	ERLLGVLEG
tethanabaterium_thermoautorophicum/1-263 190 NLP LCI R - CDLYMV AALA EP KR RV FEN LAK I LP COL KK NL - VG NS LP ELP S C + Ethanabaterium_thermoautorophicum/1-263 tethanabaterium_thermoautorophicum/1-273 191 TI A E - LD - YD I LM I AA FA EP KR RV FEN LAK I LP VD ET RT I I YT Y - TGM RA I L - Y SP VT EK DT R C + EP KR RV FEN LAK I V VD ET RT I YT Y - TGM RA I L - Y SP VT EK DT R C + EP KR RV FEN LAK I V VD ET RT I YT Y - TGM RA I L - Y SP VT EK DT R C + EP KR RV FEN LAK I V VD ET RT I YT Y - TGM RA I L - Y SP VT EK DT R C + EP KR RV FEN LAK I V VD ET R I Y VD AK I KA I FN Y P SO DD I T G - F tethanabaterium_thermoautorophicum/1-263 192 TL KD VT HI I FS ST I P LC Y SI LE EL YD I TK N Y VVAR K FG - DC LK SI FN CP YC HE VNCS + U seudomonas_aeruginosa/1-263 181 E LA FS R D - AT HF LI A S LV A DK QA LL Q LP P VY TR SS K LVM RYG - SG LK GL FN Y P V E P A EL E - G - G seurospora_crassi/1-302 257 LT I Y HP TD DV V S VV I AR KA L GG P TT P G VNG TR GCM FM P CN - C S K I HA I MNNR GK KNM I E EF SA seurospora_crassi/1-302 252 LA VH P F D DV V S S V V I AR KA L GG P TT P G VNG TR GCM FM P CN - C S K I HA I MNNR GK KNM I E EF SA seurospora_crassi/1-302 253 LA VH HP F D DV V S S V V I AR KA L GG P TT P G VNG TR GCM FM P CN - C S K I HA I MNNR GK KNM I E EF SA seurospora_crassi/1-302 254 LR VQ P EP I I N TV V F LK R L GG P TT P G VNG TR GCM FM P CN - C S K I HA I MNNR GK KNM I E EF SA seurospora_crassi/1-302 254 L X VH P F D EV V S V V AR KA E A LA G L S C P Q - G DA HA R GA V P L S P C N F S T KM E A SA LE K S E	ordeum_vulgare/1-328	190 DLIQELGAYDVVF	LAALVGMAAEEKAKVIAHLGAHMVI	EGASLVVKSA-KP-KGFL-YPIVDPED	TRRGG-FET
ethanobacterium_thermosulotrophicum/1–268 182 TV IDCLE – FDV LMV AALA EP KRV FNN HKY VDI EI KI IYK IY - TGMKAL L-YAPYS DDDI C + ethanobrevatorer_ruminatium/1–273 191 TI AE - ID - YD I LMI AE A EP KRV FAN VMN IVOEDTP V/ YT Y - TGMKAL L-YAPYS DDDI C + paphylococcus_aureus/1–272 182 ELKDI KDV THI IF SS TI P LI YS ILEE LYDLTME NV VAME FG - DCI KALF NY PSQETA EDK NE ELKDI KDV THI IF SS TI P LI YS ILEE LYDLTME NV VAME FG - DCI KALF NY PSQETA EDK NE ELKDI KDV THI IF SS TI P DCI ALLAQ LYV YT KS SK LVKNY G - NGL KS I FNC PY CHEVNCS - H- W teudomonas_aeruginosa/1–263 181 ELAF SR D - ATHFLI AS LV LG GY TIP GV NGTR GCM FM P CN - C SK I HALI NNNR GK KNM I EEF SA eurospora_crassa(1-302 257 LT I YH PT DDV VN SV VI AR K LG GY TIP GV NGTR GCM FM P CN - C SK I HALI NNNR GK KNM I EEF SA eurospora_crassa(1-302 258 LX VH PT DDV VN SV VI AR K LG GY TIP GV NGTR GCM FM P CN - C SK I HALI NNNR GK KNM I EEF SA eurospora_crassa ectivoran(1/-273 258 259 LX VH MY VG KV R K LG GY TIP GV NGTR GCM FM P CN - C SK I HALI NNNR GK KNM I EEF SA ethanobacterium_thermosutotrophicum/1-273 253 EVML INP CEV IN SV VAR VR C LG GY TIP GV NGTR GCM FM P CN - C SK I HALI NNNR GK KNM I EEF SA ethanobacterium_thermosutotrophicum/1-273 253 EVML INP CEV IN SV VAR VR C LG GY TIP GV NGTR GCM FM P CN - C SK I HALI NNNR GK KNM I EEF SA ethanobacterium_thermosutotrophicum/1-273 253 EVML INP CEV IN SV V K K CP D	ethanosarcina_acetivorans/1-273	190 NLPLGIR-CDLYN	VAAQAEPKKEVFEHLAKILP	JGIKVSYKLYEKGLKKIL-DGNSLFEL	PSG-FEI
teltanobrevbacter_ruminantum/1-273 191 TIAE - LD - YD ILMIAAFA EPKDRV YEANVWDIVDEDTPVLYBLY - TGMRAIL - YS PYTEKDTR G teltanobrevbacter_ruminantum/1-273 191 TIAE - LD - YD ILMIAAFA EPKDRV YEANVWDIVDEDTPVLYBLY - TGMRAIL - YS PYTEKDTR F terstina_pestis/1-265 182 ELKDIKDVTHIIFS STI PLRYSILEELYDTKENVVVAMRFG - DGLKSIFNCPYCHEVNCS H- w seudomonas_aeruginosa/1-263 181 ELAFS R D - ATHFLIAS LV ADVGALLAQ LV PYVTRSSK LVMRYG - SGLKSIFNCPYCHEVNCS H- w seudomonas_aeruginosa/1-260 257 LTIYHPTDDVVSSVVARVRC LGGPTTPGVNGTRCGMFM PCN - CSKIHAIMNNRGKKNMIEEFSA seurospora_crass/1-302 257 LTIYHPTDDVVSSVVARVRC LGGPTTPGVNGTRCGMFM PCN - CSKIHAIMNNRGKKNMIEEFSA seurospora_crass/1-302 259 LAVHPFCEVINSVVARVRC LGGPTTPGVNGTRCGMFM PCN - CSKIHAIMNNRGKKNMIEEFSA seurospora_crass/1-302 259 LAVHPFCEVINSVVARVRC LGGPTTPGVNGTRCGMFM PCN - CSKIHAIMNNRGKKNMIEEFSA seurospora_crass/1-302 259 LAVHPFCEVINSVVARVRC LGGPTTPGVNGTRCGMFM PCN - CSKIHAIMNNRGKKNMIEEFSA setrospora/1-232 259 LAVHPFCEVINSVVVARVRC LGGPTTPGVNGTRCGMFM PCN - CSKIHAIMNNRGKKNMIEEFSA setrospora/1-233 259 LAVHPFCEVINSVVVARVRC LGGPTTPGVNGTRCGMFM PCN - CSKIHAIMNNRGKKNMIEEFSA setrospora/1-233 259 LAVHPFCEVINSVVVARVRC LGGPTTPGVNGTRCGMFM PCN - CSKIHAIMNNRGKKNMIEEFSA setrospora/1-233 254 LKVVPVVVVVVVVVVVVVVVVVVRVC - LGGPTTPGVNGTRCGMFM PCN - CSKIHAIMNNRGKKNMIEEFSA <td>lethanobacterium_thermoautotrophic</td> <td>um/1-266 182 TVIDGLE-FDVLN</td> <td>IVAALAEPKRRVFRNIHRYVD</td> <td>FETRIIYRTY - TGMRAIL - YAPVSDDD</td> <td>ITG - FRF</td>	lethanobacterium_thermoautotrophic	um/1-266 182 TVIDGLE-FDVLN	IVAALAEPKRRVFRNIHRYVD	FETRIIYRTY - TGMRAIL - YAPVSDDD	ITG - FRF
aphylococcus_aureus/1-262 182 ELKDIKDVTHIFSSTIPLWSSLEELYDLTNENVVVANKEG-DGIKALENVPSQETAEDKW servina_pestis/1-265 182 ELKDP-VDHIVSVVVAVKEVDOYQALLAQLVPVTRSSKUKNYG-GOGIKALENVPSQETAEDK seudomonas_aeruginosa/1-263 181 ELAFSRD-ATHFLIASUVQQISAVLAQIRQVMRADAKVLLEHG-SGIKGLENVP-VEPAELEG-W rabidopsis_thaliana/1-320 257 LTIYHPTDDVVNSVVIARKGLGGPTTPGVNGTRGCMFMPCN-CSKIHAINNNRGKKNNIEEFSA eurospora_crassi-1-302 282 CAVVHPTDVVNSVVIARKG- 282 CAVVHPTDVVNSVVVARVRG- cordeum_yulgare/1-328 253 LAVHHPEGEVINSVVARVRG- 254 LAVHPECVINSVVARVRG- tethanosizeria_activorant/1-273 254 VLNQPEPPINTVFKKR- 294 LAVUPEPPINTVFKKR- LGGPTTPGVNGTRGCMFMPCN-CSKIHAINNNRGKKNNIEEFSA tethanosizeria_activorant/1-273 255 VNNTSVVARVRG- 295 LVNNTSVVFKCPD- 245 AGVVPFPINTVFKKR- tethanosizeria_activorant/1-273 251 VNNTSVVFKCPD- 295 VNNTSVVFKCPD- 245 AGVVPSVTFKCPD- tethanosizeria_activorant/1-272 255 VNNTSVVFKCPD- 295 VNKHMRPQQIFDIALYKKAAIKVGITDV- 245 ASKPVTTG-LYDLIILEKAGR- teudomonas_aeruginosa/1-263 245 ASKPVTG-LYDLIILEKAGR-	lethanobrevibacter_ruminantium/1-2	191 T I A E - L D - Y D I L N	IIAAFAEPKDRVFANVWDIVDI	EDTPVLYRTY - TGMRAIL - Y SPVTEKD	TRG - FHI
restinia_pestis/1-265 182 ELTDRP VDHTIVASLV ADVQALLAQLVPYVTRSSKLVMRYG-NGLKSJFFNCPYCHEVNCSH- residomonas_aeruginosa/1-263 181 ELAFSRD-ATHFLIASLV ADVQALLAQLVPYVTRSSKLVMRYG-NGLKSJFFNCPYCHEVNCSH- rabidopsis, fhalana/1-320 257 LTIYHPTDDVVNSVVIARKLGCPTTPGVNGTRGCMFMPCN-CSKIHAIMNNRGKKMMIEEFSA eurospora_crassa/1-302 282 CAVVHPTDDVVNSVVIARKLGCPTTPGVNGTRGCMFMPCN-CSKIHAIMNNRGKKMMIEEFSA ordeum vulgare/1-328 259 LAVHPPEGEVINSVVVARKAVEAQLSGPQN-GDAHARGAVPLVSPPCNFSTKMEASALEKSEELTAKELAF ethanostacterium_thermoautotrophicum/1-263 245 GAVVNTSVVIRKAIKAVEKCP ethanobacterium_thermoautotrophicum/1-273 255 EVMLPYCKKPN 255 EVMLPPCKVNNTSVVIRKAAIKVCID 245 AGVVLPSGKVNNTSVVIRKAAIKVCIDV- etsinia_pestis/1-265 245 SKPVTG-LYDLILLENAAIKVCITDV- etsinia_pestis/1-263 244 CAERVSQP-LYDTLILEKAGR-	aphylococcus_aureus/1-272	182 ELKDIKDVTHIIF	SSTIPLKYSILEELYDLTNI	ENVVVAMRFG-DGIKATENYP SQETAE	DK WQ (
seudomonas_aeruginosa/1-263 181 E LAF S R D - AT HF LI AS LV Q Q S AV LAQ IR Q VMR AD AK V LL HG - S G I K G L EN Y P - V E P A E LE G - W rabidopsis_thaliana/1-320 257 LT I Y HP T D D V V N S V V I A R K L G G P T T P G V NG T R G C M FM P C N - C S K I HA I MN NR G K K NM I E E F S A eurospora_crassia/1-302 282 C AV V HP T D D V V N S V V I A R K L G G P T T P G V NG T R G C M FM P C N - C S K I HA I MN NR G K K NM I E E F S A eurospora_crassia/1-302 282 C AV V HP T D D V V N S V V I A R K L G G P T T P G V NG T R G C M FM P C N - C S K I HA I MN NR G K K NM I E E F S A eurospora_crassia/1-302 253 L AV HHP E G E V I N S V I V A R K AV E AQ L S C P Q N - G D AH AR G AV P L V S P P C N F S T KM E A S A L E K S E E L T A K E L A F tethanostacterium_thermosutotrophicum/1-263 245 A G V V P S G K V N NT S V L V K C P D - tethanostacteric ruminatium/1-273 253 E V N L I P R G K T M T S V L V K C P D - tethanostacteric ruminatium/1-273 253 E V N L I P R G K T M T S V L V K K A A I K V G I T D V - 245 G V N K HMR P Q Q I F D I A L Y K K A A I K V G I T D V - ersinia_pestis/1-263 244 C A E R V S Q P - L Y D T I L E K A G R -	ersinia_pestis/1-265	182 ELTDRP VDHII	VASLVADKQALLAQLVPYVT	R S S K L V M R Y G - NG L K S I F N C P Y C H E V N	C S H - WR 1
abidopsis_thaliana/1-220 257 LT Y HP T D DVV \$ SVV I A R K L G G PT F G V NG T R C C M F M P C N - C S L I H AI IMNN R G K K M I E E F S A urospora_crassal - 302 abidopsis_thaliana/1-302 258 L A V HP F D DV V S V V A R K L G G PT F G V NG T R C C M F M P C N - C S L I H AI IMNN R G K K M I E E F S A volume, volume, volume - 1-268 abidopsis_thaliana/1-273 259 L A V HP P C D V I N S V V A R K A V E AQ L S G PQ N - G D A H AR G A V P L V S P P C N F S T K M E A S A L E K S E E L T A K E L A F ethanobactelium_thermoautotrophicum/1-266 abidopsis_thaliana/1-273 253 E V M L L P R G K T NN T S V L V F K C P ethanobactelium_thermoautotrophicum/1-273 253 E V M L L P R G K T NN T S V L V F K C P abidopsis_thaliana/1-273 254 S G V V L P S C K N N T S V L V F K C F abidopsis_thaliana/1-273 254 S K V V T G L V L Y R G K T NN T S V L V F K C F abidopsis_thaliana/1-265 245 S K V V T G L V L Y L K K A L K V C I T D V abidopsis_thaliana/1-263 244 C A E R V V T G L V D L I L E K A G R	eudomonas_aeruginosa/1-263	181 ELAFSRD-ATHFL	IASLVQQKSAVLAQIRQVMR	ADAKVLLEHG-SGIKGLENYP-VEPAE	LEG-WQ1
eurospora_crass/1-302 282 CAVVH PFTDVV N SV VVAR VRG- ordeum_volgare/1-328 259 LAVHHP FTDVV N SV VVAR VRG- ethanosacteria_acetivorans/1-273 254 YLR VVP ECPV I N SV I VARKAV EAQLSGPQN-GDAHARGAVPLVSPPCNFSTKMEASALEKSEELTAKELAF ethanosacteria_acetivorans/1-273 254 YLR VVP EVRKPD- ethanobacteria_cruinantium/1-273 251 EVMLLPR CKTINTSV EVKCPD- ethanobacteria_cruinantium/1-273 245 VNKHMRPQQ I FDIALYKKAAI KVCI TDV- eudomonas_aeruginosa/1-263 244 CAERVSQP - LYDTLILEKAGR-	rabidopsis_thaliana/1-320	257 LTIYHPTDDVVNS	VVIARKLGGPTTPGVNGTR	GCMFMPCN-CSKIHAIMNNRGKKN	MIEEFSAIE
ordeum, yulgare/1-328 259 L A V H HP E C E V I N S V I VAR KA V E AQ L S C PQ N - C D AH AR C A V P L V S P P C N F S T K M E A S A L E K S E E L T A K E L A F ethanostacterium, thermoautotrophicum/1-268 254 Y L R V Q P E P I I N T V V F K K R ethanostacterium, thermoautotrophicum/1-268 245 A G V V L P S G K V N T S V L V K C P D - ethanobacterium, thermoautotrophicum/1-267 253 E V M L L P R G C T N T V L V K C P D - ethanobacterium, thermoautotrophicum/1-273 251 E V M L L P R G C T N T V L V K K A A I K V G I T D V - aphylococcus, aureus/1-272 245 V N K HM R PQQ I F D I A L Y K K A A I K V G I T D V - estimane 245 A S K P V T T G L Y D L I L L E N G R - eudomona, acreuginosa/1-263 244 C A E R V S Q P - L Y D L I L E K A G R -	eurospora crassa/1-302	282 CAVVHP FTDVVNS	V V V A R V R G		
ethanosarcina_acetivorans/1-273 254 Y L R VQ P E P P I NN T V Y F L K R R - ethanobacterium_thermoautotrophicum/1-266 245 A G V V L P S G K V N N T S V L Y F K C P D - ethanobreviatorer ruminantium/1-273 253 E V M L L P R G K T N N T S V L Y F K C P D - phylococcus, aureus/1-272 245 V N K H M R P QQ I F D I A L Y K K A I K V G I T D V - risnia_pstis/1-265 245 A S K P V T G - L Y D L I L L E N A H A - ieudomonas_aeruginosa/1-263 244 C A E R V S Q P - L Y D T L I L E K A G R -	ordeum vulgare/1-328	259 LAVHHPEGEVINS	VIVARKAVEAQLSGPQN-GDAHAR	GAVPLVSPPCNFSTKMEASALEKSEEL	TAKELAF-
ethanobacterium, thermosutotrophicum/1-266 245 ACVV LP SCK VN NT SV LV K CP D- ethanobrewibacter, ruminantum/1-273 25 EVMLLP R GKTN NT SV LV K CP D- ethanobrewibacter, ruminantum/1-273 245 VN KHM R PQQ I FD I A LY K K A I K V G I TD V- rstmia_pestis/1-265 245 AS KP V TT G - LY D LI I LE PN HHA - eudomona_serupinssi/1-263 244 CA E R V SQP - LY D LI I LE R AGR -	ethanosarcina acetivorans/1-273	254 Y LR VQP EPPINNT	V V F L K R R		
ethanobrevibacter_ruminantium/1-273 253 EVMLLPRGKTNNTSVLIRKIS- aphylococcus, aureus/1-272 245 VNKHMRPQQIFDIALYKKAAIKVGITDV- sevidomonas_aeruginosa/1-263 245 CKRVSQP-LYDTLILEPNHAA- LEVDOMONAS_aeruginosa/1-263	ethanobacterium thermoautotrophic	um/1-266 245 AGVVLPSGKVNNT	SVLVFKCPD		
aphylococcus, aureus/1-272 245 VNK HMR PQQ. I FD I A L Y K KA A I K V G I T D V- rstnia_pestis/1-265 245 A S K P V TT G - L Y D L I L E P N HHA - eudomonas_aeruginosa/1-263 244 C A E R V SQP - L Y D T L I L E K A G R -	ethanobrevibacter ruminantium/1-2	253 EVMLLPRGKTNNT	SVLIRKIS		
rsinia_pestis/1-265 245 A S K P V T T G - LY D L I T L E P N H H A	aphylococcus aureus/1-272	245 VNKHMRPOOLEDI	ALYKKAAIKVGITDV		
eudomonas_aeruginosa/1-263 244 CAERVSQP - LYDTLILEKAGR-	ersinia pestis/1-265	245 ASKPVTTG-LYDI	IILEPNHHA		
	eudomonas_aeruginosa/1-263	244 CAERVSQP - LYDT	L I L E K A G R		



2

3 Figure S1: Sequence analysis of the three putative enzymes located in the *cnt* operon. (A)

4 Summary of sequence analysis of CntK, CntL and CntM protein sequences through PSI-Blast,

Pfam and Phyre2. (B) Sequence alignment of three eukaryotic NAS, three archaeal NAS-Like 5

1 and three bacterial NAS-Like enzymes, including CntL from S. aureus. Threshold for the 2 ClustalX coloring scheme correspond to 30 % sequence conservation as defined in Jalview. 3 Residues marked by a red dot are conserved residues that are located near the 4 thermonicotianamine (tNA) product in the structure of an archaeal NAS-Like enzyme shown in 5 C. (C) Structure of an archaeal NAS-Like enzyme (pdb code 3FPE) in complex with its product 6 tNA (represented in green stick). The N-Terminal domain is colored in blue (helices) and salmon 7 (loops), and the C-Terminal domain in light gray. Side chains of conserved residues in the 8 sequence alignment (labeled with a red dot in B) are represented in stick. Most of the conserved residues are located closed to the tNA product, therefore clearly indicating that bacterial NAS-9 10 like enzymes are related to the NAS family.



1

3 Figure S2: Synteny of the NAS-like encoding gene in selected bacteria. Genes are color-

4 coded as a function of the Pfam family to which the product belongs, as indicated in the grey

5 inset. Y. pestis, P. aeruginosa and F. varium does not possess a close CntK homologue but a

6 distantly related protein that is annotated as diaminopymelate epimerase (DapF).





Figure S3: Metabolomic study of *E. coli* strains expressing the *cnt* operon or the empty vector. (A) HILIC/ICP-MS chromatograms of intracellular fractions of *E. coli* strain (supplement with 0.1 μ M nickel to ensure a nickel complexation of the metallophore) and expressing the *cnt* operon from *S. aureus* (blue line) and comparison with the strain expressing the empty vector (red line). (B) Mass spectra of a chelating compound present in its complexed forms with nickel. Molecular formula was deduced from the exact mass of the ⁵⁸Ni complex. (C) HILIC/ESI-MS extracted ion chromatograms of *m*/*z* 385.06.



Figure S4: Fishing of the metallophore through a knowledge based approach. (A) Scheme of the "knowledge based approach". (B) Final mass spectra of CntA after incubation and repurification.



Figure S5: MS/MS fragmentation of Staphylopine-Metal complexes. MS/MS fragmentation of Staphylopine-Zn (A) and Staphylopine-Ni complexes (B). The mass of the ions are indicated as well as their interpretation with the fragmentation scheme shown in (C). (C) Proposed

- 1 fragmentation scheme of Staphylopine-Zn and Staphylopine-Ni complexes. The theoretical masses of the ions are indicated on the right of the proposed ion. The underlined masses were detected in the observed fragmentation pattern shown in (A) and (B).
- 2 3 4



Figure S6: SDS-PAGE gel of purified CntK, CntL and CntM. The expected molecular weights are 35 kDa, 31 kDa and 51kDa for CntK, CntL and CntM respectively. The final purification yields are 2, 5 and 20 mg of pure protein per liter of culture.



1 2 3 Figure S7: Activity of CntK in the presence of competing amino acids. (A) CntK was incubated with 20 mM of L-histidine and 40 mM of L-alanine. (B) CntK was incubated with 20 mM of L-histidine and 40 mM of L-methionine.

4





2 Figure S8: Microcalorimetric titration of CntL with SAM and CntM with NADPH. (A) 3 Representative data (raw data on top and curve fit below) from an ITC experiment where SAM 4 was titrated into the reaction cell containing CntL. The first addition (not used in the fit) was half 5 the volume of the other additions. Thermodynamic values obtained from the curve fit are: $\Delta S =$ 6 1.29 cal/mol/K, $\Delta H = -5212 \pm 211.5$ cal/mol, $K_d = 68 \pm 5 \mu M$, $n = 1.07 \pm 0.03$, where n is the 7 stoichiometry of the binding. (B) Representative data (raw data on top and curve fit below) from 8 an ITC experiment where NADPH was titrated into the reaction cell containing CntM. The first 9 addition (not used in the fit) was half the volume of the other additions. Thermodynamic values 10 obtained from the curve fit are: $\Delta S = 6.91$ cal/mol/K, $\Delta H = -3831 \pm 530.1$ cal/mol, $K_d = 48 \pm 4$ μ M, $n = 0.929 \pm 0.09$, where *n* is the stoichiometry of the binding. 11 12



- 1
- 2 **Figure S9: Substrate specificity of CntK, CntL and CntM.** (A) Test of seven different L-3 aminoacids with divers properties on the activity of CntKLM using NADPH and pyruvate as

4 substrates of CntM. (**B**) Test of substrate specificity of CntM using all three biosynthetic 5 enzymes and different CntM substrates (NADPH *vs* NADH and pyruvate *vs* α -keto glutarate; α -6 KG).



metalated NTA resin (rCDM) in 50 ml tubes at 37°C with aeration.

demetalated CDM (dCDM) in microplates, or (B) in CDM and demetalated-NTA CDM with





Figure S11: Luciferase activities of WT strain harbouring fusion of *PcntA–luxAB* (PcntA)

and PcntK-luxAB (PcntK) in various media. (A) LB medium. (B) CDM. (C) dCDM. (D)
 rCDM.



¹

2 Figure S12: Import of divalent metals in various culture conditions. (A) Intracellular metal 3 content determined by ICP-MS of S. aureus WT (left bars) and cntL (right bars) mutant strains 4 grown in dCDM. Error bars, mean \pm s.d. **P*<0.05 and ***P*<0.01. (**B**) Intracellular metal content 5 determined by ICP-MS of S. aureus WT (left bars) and cntL (right bars) mutant strains grown in 6 rCDM. Error bars, mean \pm s.d. *P<0.05, **P<0.01 and ***P<0.001. (C) Intracellular metal 7 content determined by ICP-AES of S. aureus WT (left bars) and cntL (right bars) mutant strains 8 grown in LB medium without further metal supplementation. Error bars, mean \pm s.d. *P<0.05 9 and **P<0.01. (D) HILIC/ESI-MS extracted ion chromatograms (XIC) of the Staphylopine-Fe 10 complex detected in the extracellular fraction of S. aureus grown in CDM. Chromatogram was 11 traced using the exact mass of the complex (\pm 3 ppm). This mass was absent in the extracellular 12 fraction of the *cntL* mutant.





Figure S13: Metal content of WT S. aureus, cntL mutant and complemented strains. Cells were grown in CDM supplemented with the indicated metal (1µM), and subsequently grown

4 5 overnight, prior to determination of their intracellular metal concentration by ICP-AES. Error 6 bars, mean \pm s.d. ***P*<0.01 and ****P*<0.001.



Figure S14: Toxicity of cobalt and CntA-F / staphylopine-dependent transport of cobalt.
(A) WT, (B) *cntL* and (C) *cntA-F* strains were grown at 37°C in microplates in CDM
supplemented or not with 2mM cobalt (Co), 10μM of staphylopine (Sp), or both (Co+Sp).
Growth in CDM +/- Sp alone was similar for all strains, indicating that staphylopine alone is not toxic or beneficial in these conditions.



6

Figure S15: Putative structure and estimated pKd for staphylopine by comparison with nicotianamine. Putative chemical structure of staphylopine-metal complex (A) and nicotianamine-metal complex (B). (C) pKd values for staphylopine complexes were estimated by metal competition experiments with nicotianamine by ESI MS.





2 Figure S16: NMR spectrum of synthetic staphylopine.

Name	Sequence (5'-3')	Targeted
		plasmid
CntA-Fw	CACCATGAGAAAACTAACTAAAATG	pET100
CntA-Rv	TTATTTATACTGCATTTCATTGAA	
CntK-Fw	CACCATGAATCGGCAGGTTATAGA	pET100
CntK-Rv	TTATTCTATATATGCTTTTCCTGT	
CntL-Fw	AATAACTTTAATAATGAAATCAAA	pET
CntL-Rv	TTAGACATCCGTAATACCTACCTTTATA	SUMO
CntM-Fw	CACCATGTCTAAATTATTAATGATAGGCAC	pET101
CntM-Rv	TGAAAGCGTTCTATTGATTTCCA	
Cntop-Fw	AGAAGGAGATATACATATGAATCGGCAGGTTATAG	pET22b+
	AATTTTCT	
Cntop-Rv	GGTGGTGGTGCTCGAGAAGACTACTCGCTGGACGT	
	GGTGTT	
	CGGGATCCTGGCCGCAACACATGTATGCTGTG	pMAD
Mut-cntL5'A		
Mut-cntL3'A	CATCCGTAATACCTACAAAGTTATTCATTGTAGGCT	pMAD
	CCTTATTC	
Mut-cntL5'B	TACAATGAATAACTTTGTAGGTATTACGGATGTCTA	pMAD
	AAT	
Mut-cntL3'B	CGGGATCCGCTAAAGCAGAGATTCGTTGACAC	pMAD
Mut- <i>cntE</i> -5'A	CGCGGATCCTGTTGGAAGAAGTCGGTCTATC	pMAD
Mut- <i>cntE</i> -3'A	ACTACTCGCTCAAGCCATTGCACCTTTCAT	pMAD
Mut- <i>cntE</i> -5'B	GCAATGGCTTCAGCGAGTAGTCTTTAATGAAGT	pMAD
Mut- <i>cntE</i> -3'B	TCCCCCGGGTAATACATGGTGGGAATTCATTCC	pMAD
pOS-cntL5	CGCGGATCCGCCTACAATGAATAACTTTAATAATG	pOS1-Plgt
pOS-cntL3	CGCGGATCCGGATTGCGACCGGACCAGTGC	pOS1-Plgt
pLUX-cntK5	CGCGGATCCGAAGTTTATGGAAGGATTATC	pCN58
pLUX-cntK3	CCGGAATTCCGACACTCCTTTAGATGTA	pCN58

- Table S1: Oligonucleotides used in this study

Ingredient	Final medium concentration (g/l)
D-glucose	4
Amino acids	
L-cysteine	0,48
L-aspartic acid	2,4
L-glutamic acid	2,4
L-proline	2,4
L-arginine	0,36
L-glycine	2,4
L-histidine	0,48
L-lysine HCL	0,6
L-serine	2,4
L-valine	0,48
L-threonine	2,4
L-alanine	2,4
L-isoleucine	0,6
L-leucine	0,6
L-tryptophan	0,06
L-methionine	0,18
L-tyrosine	0,18
L-phenylalanine	0,2
Salts	
Na ₂ HPO ₄	5,7
(NH4) ₂ SO ₄	6,84
KH ₂ PO ₄	1,34
MgSO ₄ 7H ₂ O	0,247
Vitamins	
Nicotinic acid	0,01194
Thiamine HCL	0,0006
Ca panthothenate	0,002
Biotine	0,0001

Table S2: Composition of chemically defined medium. The pH is adjusted to 7 with KOH and the volume to 1L with milliQ water. The medium is then sterilized by filtration through a

0,22µm filter.

Culture media concentration, nM						
	Mn	Fe	Zn	Cu	Ni	Co
LB	116 ± 9	4450 ± 356	5320 ± 426	206 ± 16	25 ± 2	134 ± 11
CDM	17 ± 3	171 ± 14	105 ± 8	7 ± 2	12 ± 2	< 5
dCDM	< 5	20 ± 3	22 ± 3	< 5	< 5	< 5

Table S3: Metal concentrations in culture media and in the intracellular fraction of WT

2 3 cells in different culture conditions.

1 Supplementary references

- 2 34. S. F. Altschul *et al.*, *Nucleic Acids Res* 25, 3389 (1997).
- 3 35. R. D. Finn *et al.*, *Nucleic Acids Res* **42**, D222 (2014).
- 4 36. R. C. Edgar, *Nucleic Acids Res* **32**, 1792 (2004).
- 5 37. M. Clamp, J. Cuff, S. M. Searle, G. J. Barton, *Bioinformatics* 20, 426 (2004).
- 6 38. L. A. Kelley, S. Mezulis, C. M. Yates, M. N. Wass, M. J. Sternberg, *Nat Protoc* 10, 845
 7 (2009).
- 8 39. D. Vallenet *et al.*, *Nucleic Acids Res* **41**, D636 (2013).
- 9 40. A. Hiron, E. Borezee-Durant, J. C. Piard, V. Juillard, J Bacteriol 189, 5119 (2007).
- 10 41. M. Arnaud, A. Chastanet, M. Debarbouille, *Appl Environ Microbiol* **70**, 6887 (2004).
- 11 42. C. Rigoulay et al., Infect Immun 73, 563 (2005).
- 12 43. J. Bubeck Wardenburg, W. A. Williams, D. Missiakas, *Proc Natl Acad Sci U S A* 103, 13831 (2006).
- 14 44. D. Taylor, K. T. Holland, J Appl Bacteriol 66, 319 (1989).
- 15 45. L. Grillet *et al.*, *J Biol Chem* **289**, 2515 (2014).
- 46. L. Ouerdane, S. Mari, P. Czernic, M. Lebrun, R. Lobinski, *Journal of Analytical Atomic Spectrometry* 21, 676 (2006).
- 18 47. N. von Wiren et al., Plant Physiol 119, 1107 (1999).
- 19 48. K. Higuchi, K. Kanazawa, N. K. Nishizawa, S. Mori, *Plant and Soil* 178, 171 (1996).
- 20 49. I. Benes, S. K., H. Ripperger, A. Kircheiss, *Experientia* **39**, 261 (1983).