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2 **SUPPLEMENTARY MATERIAL**

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4 **Article Title**

5 **Mutations improving production and secretion of extracellular lipase by**
6 ***Burkholderia glumae* PG1**

7 **Journal Name**: *Applied Microbiology and Biotechnology*

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30 **SUPPLEMENTARY MATERIALS AND METHODS**

31 **β-Lactamase activity assay.** The β-lactamase activity was measured with the
32 chromogenic substrate CENTA (Bebrone et al. 2001) dissolved in 100 mM potassium
33 phosphate buffer (pH = 7.2) to a concentration of 150 µM. 10 µl of the substrate was
34 combined with 150 µl cell-free supernatant or sonicated cell extract. Enzymatically
35 degraded CENTA was continuously recorded by the absorbance at 405 nm and 30 °C.

36 **Construction of signal peptide fusions with PhoA.** *PhoA* genes fused to *B.*
37 *glumae* PG1 or LU8093 signal peptide sequence, respectively, was synthesized by Life
38 technologies (Darmstadt, Gemany) with terminal restriction sides and *Nde*I-*Hind*III
39 hydrolyzed. Resulting fragments were used to replace *lipAB* operon in *Nde*I-*Hind*III
40 hydrolyzed plasmid pBBR-*lipAB*. The resulting plasmids pBBR-*wtSP-phoA* and pBBR-
41 *LUSP-phoA* encode the fusions under the control of wild-type *lipAB* promoter and
42 include the sequence of one of the LipA signal peptides (32 amino acid residues)
43 followed by the PhoA sequence lacking its own signal peptide (21 residues).

44 **Alkaline phosphatase activity assay.** The alkaline phosphatase activity from
45 PhoA in cells grown under standard conditions was measured with 4-
46 methylumbelliferylphosphate (MUP, Sigma-Aldrich, Deisenhofen, Germany) as
47 described in (Monds et al. 2006). Cells (0.4 ml) were pelleted and resolved in 0.4 ml
48 TSP salts (2 g/liter $(NH_2)SO_4$, 2 g/liter NaCl, and 1.65 g/liter KCl). Cells were lysed by
49 adding 10 µl 0.1% (w/v) sodium dodecyl sulfate, 20 µl chloroform and incubation at 30°C
50 for 10 min. Cell debris was pelleted (10,000 *x* g, 10 min) and the supernatant extracted.
51 Subsequently, 100 µl 1 M Tris-HCl (pH 8.0) and 10 µl of 10 mM MUP (in Tris-HCl) was

52 added to the supernatant and incubated at 30°C for 30 min. Reaction was terminated by
53 adding 20 µl 3 M NaOH. The cleavage of MUP to fluorescent 4-methylumbelliferone by
54 alkaline phosphatase was detected in an *Infinite M1000 Pro* fluorescence photometer
55 (Tecan, Männedorf, Germany) set at 360 nm excitation and 450 nm emission. Obtained
56 relative fluorescent units (RFU) was correlated to cell density at $O.D._{580nm}$.

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58 **SUPPLEMENTARY FIGURES**

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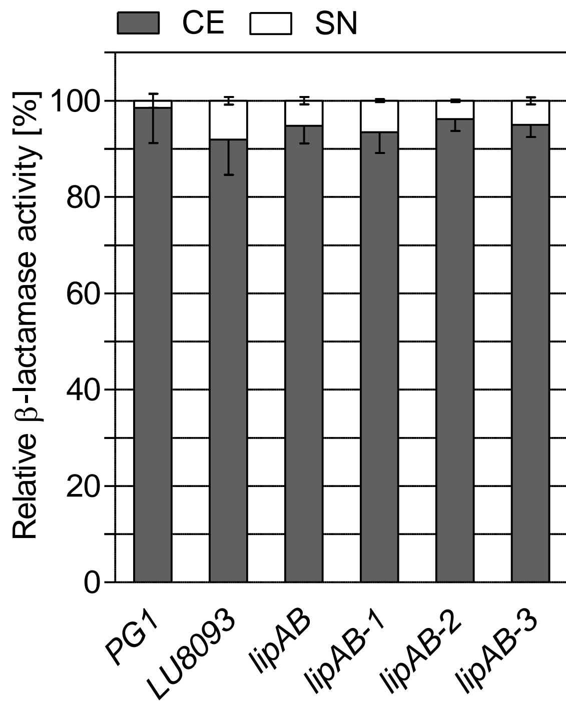

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61 **Fig. S1** Determination of relative β-lactamase activities in cell extracts (CE) and
62 supernatants (SN) of different *B. glumae* strains as indicated at the X-axis. Lactamase
63 activities were determined separately in cell-free supernatants and cell extracts of each
64 culture with the chromogenic substrate CENTA. The activities in cell extract and
65 supernatant of each strain were set as 100 % activity and each column shows the
66 proportionate activities in the cell extract and supernatant.

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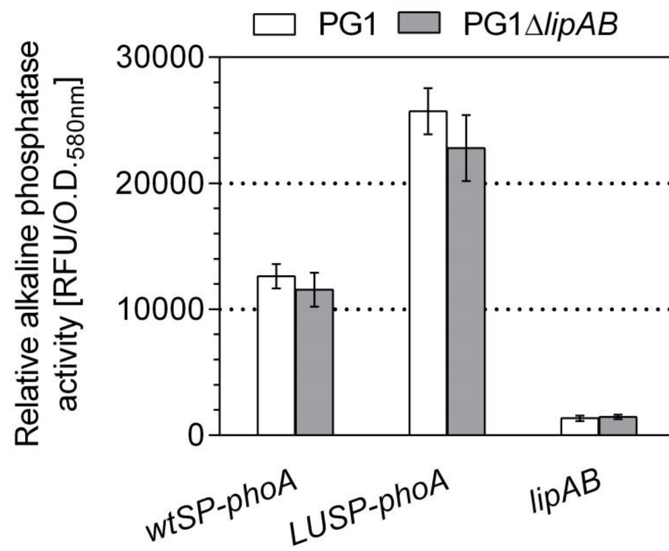

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69 **Fig. S2** Relative PhoA activity in *B. glumae* PG1 (PG1) and PG1Δ*lipAB* (PG1Δ*lipAB*)
70 harboring different *phoA* fusion plasmids. PhoA exhibits phosphatase activity only in the
71 periplasm and is therefore a reporter enzyme for transport across the inner membrane.
72 The *phoA* gene is fused to the wild-type signal peptide sequence of *lipA* (*wtSP-phoA*) or
73 the signal peptide derived from *B. glumae* LU8093 (*LUSP-phoA*), respectively. As a
74 control, basal alkaline phosphatase activity was detected in cultures expressing plasmid-
75 based *lipAB* (*lipAB*).

76 **TABLE S1** Identified secondary metabolite gene clusters in *B. glumae* PG1. Cluster distribution code:

77 B = *Burkholderia spp.*;[P] = [Polyangium]; A = *Acinetobacter spp.*; R = *Ralstonia spp.*; M = *Myxococcus spp.*;

78 V = *Verrucosispora spp.*; P = *Pseudomonas spp.*; H = *Halothiobacillus spp.*; Mt = *Methylobacter spp.*

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Cluster #	Type	Location		Key genes	Known distribution in other species
		From, bp	To, bp		
The following clusters are from Chromosome 1:					
Cluster 1	Bacteriocin	455,203	466,027	BGL_1c04090 - BGL_1c04100	
Cluster 2	T1pks	717,666	765,267	BGL_1c06330 - BGL_1c06490	R,B
Cluster 3	Hserlactone (*bgal3*)	889,059	909,637	BGL_1c07910	
Cluster 4	Nrps	1,344,640	1,366,545	BGL_1c11770 - BGL_1c11810	
Cluster 5	Bacteriocin	1,665,493	1,676,437	BGL_1c14410	B
Cluster 6	Nrps-t1pks	2,163,831	2,183,517	BGL_1c18980 - BGL_1c19040	B,[P]
Cluster 7	T1pks	2,222,887	2,270,579	BGL_1c19550 - BGL_1c19660	B
Cluster 8	Nrps (Pyochelin)	2,234,577	2,369,342	BGL_1c20500 - BGL_1c20630	B
Cluster 9	Terpene	2,749,579	2,760,412	BGL_1c23780 - BGL_1c23860	B
Cluster 10	Other	3,043,761	3,055,615	BGL_1c26420 - BGL_1c26510	V
The following clusters are from Chromosome 2:					
Cluster 11	Nrps-t1pks	88,975	102,059	BGL_2c00870 - BGL_2c00970	P
Cluster 12	Nrps-pks	103,295	133,241	BGL_2c00980 - BGL_2c01100	B
Cluster 13	Terpene	287,742	305,843	BGL_2c02440 - BGL_2c02630	B
Cluster 14	Nrps-hserlactone (*bgal2*)	457,994	507,439	BGL_2c03860 - BGL_2c03970	M
Cluster 15	Hserlactone (*bgal1*)	1,227,844	1,248,455	BGL_2c09850 - BGL_2c09870	A,Mt,H
Cluster 16	Phosphonate	1,443,527	1,479,157	BGL_2c11750 - BGL_2c11890	B
Cluster 17	Bacteriocin	1,852,110	1,858,332	BGL_2c14830 - BGL_2c14870	B
Cluster 18	Nrps-t1pks	1,974,105	2,031,300	BGL_2c15950 - BGL_2c16080	R,B,P
Cluster 19	Other	2,040,810	2,084,400	BGL_2c16410 - BGL_2c16520	B,P
Cluster 20	Nrps	2,104,675	2,187,993	BGL_2c16900 - BGL_2c17050	B
Cluster 21	Nrps	2,198,446	2,262,022	BGL_2c17350 - BGL_2c17480	B
Cluster 22	Nrps	2,256,808	2,301,211	BGL_2c17690 - BGL_2c17700	B
Cluster 23	Blactam	3,465,755	3,487,245	BGL_2c27580 - BGL_2c27720	
Cluster 24	Terpene	3,567,421	3,588,491	BGL_2c28290 - BGL_2c28460	B
Cluster 25	Terpene	3,712,608	3,732,771	BGL_2c29420 - BGL_2c29550	B

Comparison of certain secondary metabolite clusters (for Table S1)

We analyzed the genome for the presence of biosynthetic gene loci putatively involved in secondary metabolism and identified 25 putative clusters with antiSMASH (Blin et al. 2013). Secondary metabolites represent a versatile source of new drugs, for example antibiotics (Clardy et al. 2006). Cluster 2 shows homologies to polysaccharide gene clusters of several *Burkholderia ssp.* and especially to an ancient capsule biogenesis cluster described for the avirulent *B. thailandensis* (Yu et al. 2006). Cluster 5 shows homology to bacteriocin gene clusters from other *Burkholderia* species and cluster 6 represents a NRPS/PKS hybrid showing significant homology to those involved in the formation of natural products of the glidobactin and syringolin proteasome inhibitor families (Ramel et al. 2009; Schellenberg et al. 2007) leading to the speculation that *B. glumae* PG1 can produce such compounds as well. Cluster 8 should be regarded as NRPS cluster showing high similarity in gene structure and arrangement to many species and can be expected to be involved in the formation of an iron chelating compound with similarity to pyochelin produced by *Pseudomonas* sp. (Reimmann et al. 2001). Analysis of cluster 9 reveals a terpene biosynthetic gene locus with close homology to many other *Burkholderia* species. Cluster 18 represents a mixed NRPS/PKS gene cluster which is highly conserved among the genera *Ralstonia* and *Pseudomonas*. Cluster 19 is an atypical NRPS cluster not containing any condensation domain. It is conserved among several *Burkholderia cenocepacia* strains and can also be found in pseudomonads, although with slightly different organization of genes. Clusters 20 and 21 are other NRPS type gene clusters, both highly conserved in *B. glumae* BGR1 and cluster 21 also in *B. gladioli* BSR3. Cluster 23 represents β-lactam type gene loci without any homologs in the database. Clusters 24 and 25 represent

104 additional terpene synthase containing gene loci and are again highly conserved
105 between the Bptm group and phytopathogenic *Burkholderia* species.
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107 ## SUPPLEMENTARY REFERENCES

108 Bebrone C, Moali C, Mahy F, Rival S, Docquier JD, Rossolini GM, Fastrez J, Pratt RF, Frere JM, Galleni M
109 (2001) CENTA as a chromogenic substrate for studying beta-lactamases. Antimicrob Agents Ch
110 45:1868-1871 doi:10.1128/AAC.45.6.1868-1871.2001
111 Blin K, Medema MH, Kazempour D, Fischbach MA, Breitling R, Takano E, Weber T (2013) antiSMASH 2.0 -
112 a versatile platform for genome mining of secondary metabolite producers. Nucleic Acids Res
113 41:W204-212 doi:10.1093/nar/gkt449
114 Clardy J, Fischbach MA, Walsh CT (2006) New antibiotics from bacterial natural products. Nat Biotechnol
115 24:1541-1550 doi:10.1038/nbt1266
116 Monds RD, Newell PD, Schwartzman JA, O'Toole GA (2006) Conservation of the Pho regulon in
117 *Pseudomonas fluorescens* Pf0-1. Appl Environ Microbiol 72:1910-1924
118 doi:10.1128/AEM.72.3.1910-1924.2006
119 Ramel C, Tobler M, Meyer M, Bigler L, Ebert MO, Schellenberg B, Dudler R (2009) Biosynthesis of the
120 proteasome inhibitor syringolin A: the ureido group joining two amino acids originates from
121 bicarbonate. BMC Biochem 10:26 doi:10.1186/1471-2091-10-26
122 Reimmann C, Patel HM, Serino L, Barone M, Walsh CT, Haas D (2001) Essential PchG-dependent
123 reduction in pyochelin biosynthesis of *Pseudomonas aeruginosa*. J Bacteriol 183:813-820
124 doi:10.1128/JB.183.3.813-820.2001
125 Schellenberg B, Bigler L, Dudler R (2007) Identification of genes involved in the biosynthesis of the
126 cytotoxic compound glidobactin from a soil bacterium. Environmental microbiology 9:1640-1650
127 doi:10.1111/j.1462-2920.2007.01278.x
128 Yu Y, Kim HS, Chua HH, Lin CH, Sim SH, Lin D, Derr A, Engels R, DeShazer D, Birren B, Nierman WC, Tan P
129 (2006) Genomic patterns of pathogen evolution revealed by comparison of *Burkholderia*
130 *pseudomallei*, the causative agent of melioidosis, to avirulent *Burkholderia thailandensis*. BMC
131 Microbiol 6:46 doi:10.1186/1471-2180-6-46

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