Supplementary:

Supplementary information:

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Supplementary Material, Text, Tables, and Figures

Supplementary material includes supplementary information on Agrobacterium strains; details of preparation and purification of antimicrobial peptides from EMA_CFCM; data and statistic analysis of the data obtained in *Agrobacterium* strains other than those belonging to opine groups other than the AGR. Tables and Figures from the Supplementary material (see also in separate supplementary files).

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Additional information about the *Agrobacterium* strains used in this study

Origin and the most important practical information on them.

DOI: 10.7287/peerj.preprints.26900v1/supp-2

PURIFICATION OF ANTIMICROBIAL PEPTIDE FRACTIONS FROM EMA_CFCM

Table S2: +++ = very strong antimicrobial activity; Abbreviations: EMA= *X, budapestensis* HGB033; CFCM = Cell-Free Culture Medium; PF = Peptide Rich Fraction; * = Name of HPLC Sample; RPCC = Reverse Phase Column Chromatography; Test organisms; CA =*Candida albicans*; SA = *.S, aureus*; EC = *E. coli* HGB2226; XN = a *Xenorhabdus nematophila* lab isolate which is extremely sensitive to *Xenorhabdus* antibiotics. **HGB1795** is a transposon-induced insertion mutant of the XNC1_2022 gene (Gene ID: 9430524; Gene Page Link: NCBI UniProtKB; Locus Tag: XNC1_2022 see gene page for GenePage for the XNC1_2022 gene EcoGene-RefSeq) from *X. nematophila* (strain ATCC 19061 / provided by Prof. Helge Bode via Prof. Heidi Goodrich-Blair. responsible for the biosynthesis of Bicornutin A (Fuchs et al., 2012).

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ANOVA PROCEDURE OF OD VALUES DETERMINED IN *IN VITRO* BIOASSAYS OF EMA_PF2 IN *AGROBACTERIUM* STRAIN I

Table S3: The data analysis was performed using [SAS/STAT] software, Version [9.4] of the SAS System for [Windows X 64 Based Systems]; (Copyright © [2013 of copyright]; SAS Institute Inc. SAS, Cary, NC, USA. We used ANOVA and GLM Procedures alternatively following the propositions of the SAS 9.4 Software. The design of the experiment was a randomized complete block, design with a number of the respective treatments, concentrations, and replicates. Data have been averaged as to allow the analysis of variance (ANOVA). The significance of differences of the means (α = 0.05) was determined by using t (LSD) tests or Duncan's Multiple Range Tests, depending upon the experiment Anova Table S3A summarizes the results of Anova Procedure for all the 180 OD values of (36 untreated control and 144 treated).

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ANOVA ANALYSIS: COMPARISON OF OD VALUES OF THE EMA-PF2 TREATED *AGROBACTERIUM* CULTURES

Table S4: The data analysis was performed using [SAS/STAT] software, Version [9.4] of the SAS System for [Windows X 64 Based Systems]; (Copyright © [2013 of copyright]; SAS Institute Inc. SAS, Cary, NC, USA, see Footnotes to Table S3. The significance of differences of the means (α=0.05) were determined here by using Duncan's Multiple Range Tests, depending upon the experiment as a part of the Anova Procedure. Duncan's Multiple Range Test of OD30-75 values measured in Liquid Culture Bioassay of EMA PF on *Agrobacterium* strains.

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RAW DATA PRESENTED IN FIG 1

Raw data of Fig 1 A and B.

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RAW DATA: OD VALUES OF EMA_PF2 *IN VITRO* LIQUID BIOASSAYS (ALL) FOR FIG 5, ALSO FOR Fig 7 AND Fig S3. (FOR BOTH REVIEW AND PUBLIC INFORMATION)

This Excel file contains the most important data for this Publication. For both review and public information. Data are presented in Fig 5 and are grouped, from different aspects in Fig7 and FigS3. The detailed statistical analyses are given in the Supplementary material, Tables S3, and S4.

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RAW DATA PRESENTED IN TABLE 2

Raw data on agar-diffusion bioassays (on four sensitive targets) of antimicrobial active peptide fractions isolated from EMA_CFCM by amberlite absorption, (followed by methanol purification and elution, ultrafiltration (EMA_PF1, EMA_PF2), then HPLC fractionation or RPLC purification; and RPLC) EMA30, also followed by HPLC purification (AF103-40,- 43 and 44 fractions).

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THE OD VALUES OF THE OBTAINED *IN VITRO* LIQUID BIOASSAY OF EMA_PF2 IN NOP STRAINS.

Legends /Footnotes to Fig S3: Comparison of OD values obtained in liquid bioassays of EMA PF (antimicrobial active peptide fraction from cell-free media of early stationary phase cultures of *Xenorhabdus. budapestensis*, EMA) on nopaline–catabolizing (NOP) Agrobacterium strains HP1843, HP1842, HP1836, HP1840, HP1841, and SZL4. The tests were carried out in LB liquid cultures of 200 µl final volumes, inoculated with 5 µl O/N culture of the respective test bacterium, and incubated at 30 oC for 24h. Note that although the OD values of the PF-treated cultures were significantly lower than those in the respective untreated (control) ones, there was no detectable dose dependence within the range of 30 -75 µg/ml. None of the doses 30, 45, 60, and 75 µg/m exerted a cytotoxic but cytotoxic effect on them. Based on their significantly different OD values, these strains could be scored in different Duncan's Groups (Duncan's Group A, B, C, and D, respectively), which reflects differences in the cytostatic effect of EMA PF on them.

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ROW DATA (IN EXCEL) PRESENTED IN TABLE 3

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Table S1 Origin of the *Agrobacterium* Strains (To Line 345 of the Resubmitted MS Peer_J_24535

Name	From	Deposited by	Opine	Plasmid	S /R Phenotype To EMA_CFCM in Agar Diffusion Test
HP1836	NAIK Biotech-nology Institute Gödöllő Hungary Dr. Olasz	B. Dudás	Nopaline	Cured	R
HP1837		B. Dudás	Octopine	Helper	S
HP1839		B. Dudás	Nopaline	Helper	S
HP1840		B. Dudás	Nopaline	Cured	R
HP1841		D. Silhavy	Nopaline	Cured	R
HP1842		V. Tisza	Nopaline	Cured	R
HP1843		G.B. Kiss	Nopaline	Cured	R
HP1838		B. Dudás	Agropine	Intact	R
SZL1	BRC Hungarian Academy of Sci-ences, Szeged, Hungary, Dr. Szabados	Szabados	Agropine	Helper + Bin	S
SZL2		Szabados	Octopine	Helper + Bin	R
SZL3		Szabados	Agropine	Helper + Bin	S
SZL4		Szabados	Nopaline	Helper + Bin	S

Footnotes to Table S1: For all other information, see MS_Peer_J_34535 Table 1.

Table S2 *Xenorhabdus* Antimicrobial Peptide-Rich Fractions Separated from EMA CFCM. Two of which (EMA_PF2 and EMA_{30} were selected for Liquid Bioassays in *Agrobacterium* Bioassays

Name Of Preparation	Origin	WAY OF PURIFICATION	Agar Diffusion Bioassay on			
			SA^R JE	EC HGB2226	XN HGB 1975	CA JE
EMAPF		AmberlitR XAD1180; Methanol elution	+++	+++	+++	+++
EMAPF1	EMAPF	Ultrafiltration; MW > 10,000 D fraction	+++	+++	+++	+++
EMAPF2		Ultraliltration; MW < 10,000 D fraction;	+++	+++	+++	+++
$EMA_{(30)}$ AF103*	CFCM	RPCC; Eluted with 30 % AN / 0.1% TFA	+++	+++	+++	+++
HPLC Fraction 40	AF103*	HPLC	+++	+++	+++	+++
HPLC Fraction 43		HPLC	+++	+++	+++	+++
HPLC Fraction 44		HPLC	+++	+++	+++	+++

Footnotes to Table S2: +++ = very strong antimicrobial activity; Abbreviations: EMA= *Xenorhabdus budapestensis* HGB033; CFCM = Cell-Free Culture Medium; PF = Peptide Rich Fraction; * = Name of HPLC Sample; RPCC = Reverse Phase Column Chromatography; Test organisms; CA =*Candida albicans*; SA = .*Staphylococcus aureus*; EC = *Escherichia coli* HGB2226; XN = a *Xenorhabdus nematophila* lab isolate which is extreme sensitive to *Xenorhabdus* antibiotics. **HGB1795** is a transposon-induced insertion mutant of the XNC1_2022 gene (Gene ID: 9430524; Gene Page Link: NCBI UniProtKB; Locus Tag: XNC1_2022 see gene page for GenePage for the XNC1_2022 gene EcoGene-RefSeq) from *X. nematophila* (strain ATCC 19061 / DSM 3370 / LMG 1036 / NCIB 9965 / AN6), provided by Prof. Helge Bode via Prof. Heidi Goodrich-Blair. We used this mutant since previously Bicornutin A was believed as the active EMA antibiotic molecule (Böszörményi et al., 2009) and the XNC1_2022 gene of *X. nematophila* was believed to be a homologue of *X. budapestensis Nrp*S (nrpS) gene, (GenBank: Accession Number is JX424818.1; gene synonym="bicA) which is responsible for the biosynthesis of Bicornutin A (Fuchs et al., 2012). It turned out that it is not the case. However, some role in the scenario related to antibiotics activity and self-resistance cannot be ruled out, since Bicornutin A and fabclavine coexist in our peptide-preparations.

Table S3 EMA_PF2 Liquid Culture Bioassay on *Agrobacterium* strains ANOVA Procedure for OD Values Including Those Determined in Untreated Control and Each Treated Cultures --- continued… 3

...**Table S3 E**: EMA PF Liquid Bioassay on *Agrobacterium* strains Analysis of Dose / Effect relations by Tukay's Studentized Range (HSD) Test for All $ODV_{0\text{-}75}$			
Alpha		0.05	Comparisons significant at the 0.05 level are indicated by ***.
Error Degrees of Freedom		119	
Error Mean Square		0.004082	
Critical Value of Studentized Range		3.91744	
Minimum Significant Difference		0.0417	

	Trtmt Comparison	Difference Between Means		95% Confidence Limits
	0 – 30	0.44983	0.40812	0.49155
	30 – 45	-0.01861	-0.06033	0.02310
	45 – 75	-0.00689	-0.04860	0.03483
	30 – 60	0.03147	-0.01024	0.07319
	30 – 75	-0.02550	-0.06721	0.01621

Table S3 F EMA PF Liquid Bioassay on Agrobacterium strains: Duncan's Multiple Range Test for ODV Values measured at 0 and 30 at 0 and 30 µg/ml Doses and Grouped by Doses (Respective ANOVA Table: Table 28B)			
	Alpha	0.05	
	Error Degrees of Freedom	45	
	Error Mean Square	0.007667	
	Harmonic Mean of Cell Sizes	35.49296	
Note:	Cell sizes are not equal.		
Number of Means	2		
Critical Range	.04186		

Duncan Grouping	**Mean**	**N**	**conct**
A	0.90880	36	0
B	0.45783	36	30

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Footnotes to / Captive to Table S3: The data analysis was performed using [SAS/STAT] software, Version [9.4] of the SAS System for [Windows X 64 Based Systems]; (Copyright © [2013 of copyright]; SAS Institute Inc. SAS, Cary, NC, USA. We used ANOVA and GLM Procedures alternatively following the instructions of the SAS 9.4 Software. The design of the experiment was a randomized complete block, design with several respective treatments, concentrations, and replicates. Data have been averaged to allow the analysis of variance (ANOVA). The significance of differences of the means (α = 0.05) was determined by using t (LSD) tests or Duncan's Multiple Range Tests, depending upon the experiment Anova Table S3A summarizes the results of the ANOVA Procedure for all the 180 OD values of (36 untreated control and 144 treated) *Agrobacterium* cultures, (as dependent variable), measured in Liquid Culture Bioassay of EMA PF on 12 *Agrobacterium* strains (HP1836 HP1837 HP1838 HP1839 HP1840 HP1841 HP1842 HP1843 SZL1 SZL2 SZL3 SZL4, as "treatment", true); at 5 different (0, 30, 45, 60 and 75 ug/ml) concentrations; in 3 replicates. It shows that

(at least in treated – untreated relations) the PF acted in a dose-dependent manner (F= 360.59; Pr>F; <.0001) and the strains responded differently (F= 263.25; Pr>F; <.0001). The Duncan Multiple Range tests (Table S3C) scored the controls to Group A but the grouping of the treated cultures did not seem to prove dose–effect relations within the range of 30-75 μg/ml EMA PF doses. To learn more about the dose–effect relations, OD values were measured in cultures of untreated (at 0) and treated differently treated (with (30, 45, 60, and - 75 μg / ml doses) Agrobacterium cultures handled as independent, separate data pools, and compared. We accomplished 4 different ANOVA procedures restricted only to 0 & 30; 0 & 45; 0 & 60 and 0 & 75 μg/ml EMA PF doses. Since the results were very similar, we present here the results of only one of them. Anova Table S3B restricted to OD values determined at 0 and 30 μg/ml EMA PF dose concentrations confirm that the OD values measured at untreated (at 0) and treated with 30 μug / ml concentrations comprise different data pools. This was confirmed by Duncan's Multiple Range Test (Table S3F). The Duncan's Multiple Range test for all OD values (OD 0-75) measured in 0, 30, 45, 69, and 75 μg / ml doses in in the Liquid Bioassay of EMA PF on Agrobacterium by Duncan Multiple Range Test (Table S3C), showed that the OD values of the controls (Mean: 0.90767) sharply separated (Duncan Group A) from those of the rest: Means = 0.48333 (for 75); 0.46094 (for 45); 0.45783 (for 30) (scored Duncan's Group B) to and from 0.42636 (for 60 μg ml), scored to Duncan's Group C. Despite the minor differences between the means of the OD values of the 4 treated groups, the lowest value (0.42636 (in 60 μg ml) was statistically lower than those of the other 3 treated groups, and this was confirmed by t (LSD) tests as well (Table S3D). The HSD test did not show significant differences between the (30, 45, 60, and 75 μg and ml) treated Agrobacterium cultures. Tukay's (HSD) test ((Table S3E). We considered as an experimental-wise error, which could not influence the conclusions, that within the range of 30-75 μg/ml EMA PF doses, no significant dose-effect relations should be considered, and we have pooled the OD values measured in this range of each strain forand comparison. We have been considering Duncan's Multiple Range test as the most accurate to distinguish between experimental groups reacting differently to the same treatments. The means within a given Duncan's Group labelled with a letter, say, with letter A, may differ from each other, but the SD values overlap; but differ significantly from those belonging to another Duncan's Group, labeled, say, letter B, are significantly different at the P=0.05 level. We overheated each case with the t(LSD) test as well (data are not given), and found that Duncan's Multiple Range Tests were completely fair.

. **TABLE S4 A**: OD 30-75 Values: Means ± Standard Deviations

Agrobacterium strains	N	OD_{30-75} Mean +/-SE		
HP1836	12	0.66883333	± 0.04599769	
HP1837	12	0.15591667	± 0.02306693	
HP1838	12	0.81525000	± 0.06015000	
HP1839	12	0.03091667	± 0.01625623	
HP1840	12	0.55350000	± 0.14573232	
HP1841	12	0.43513333	± 0.06826824	
HP1842	12	0.79608333	± 0.07247502	
HP1843	12	0.79933333	± 0.05940054	
SZ1	12	0.04375000	± 0.01183312	
SZL2	12	0.33241667	± 0.04599769	
SZL3	12	0.66883333	± 0.06230199	
SZL4	12	0.06183333±	0.0172459	