**SUPPLEMENTARY MATERIAL – TEXT, TABLES, FIGURES TO MS Peer\_J\_24535 RESUBMISSION**

**Supplementary Material, S\_Text\_1** to line 191 of the resubmitted MS:

**Some Introductory Remarks on the *Agrobacterium* System**

We originally started to work on *Agrobacterium* as a plant pathogenic target (**Fodor et al., 2012**) of EMA PF, and found *Agrobacterium* strains of different S/R phenotypes to cell-free culture media of EMA and EMC in both overlay and agar-diffusion tests.

This discovery made it necessary to get an insight into *Agrobacterium* as a genetic system. The *Agrobacterium* cell contain a circular and a linear bacterial chromosome **([S\_Ref\_1]** **Van Larebeke et al., 1974)**; [**S\_Ref\_2] (Allardet-Servent, et al., 1993)** and the dispensable accessory genome comprising several compatible plasmids.

(The classic name of this collection of DNA is “plasmone”). The dispensable non-chromosomal (“plasmone”) DNA of the different wild type (natural) isolates of *Agrobacterium* species are very different. The virulent *Agrobacterium* carries large intact tumor-inducing (Ti) plasmids **[S\_Ref\_1]** (Van Larebeke et al., 1974). When a virulent *Agrobacterium* infects a sensitive plant through wounded tissue, a neoplasmic disease develops. The molecular basis of the tumori - genesis is stable incorporation of T-DNA of the Ti plasmid into the genome of plant cells **[S\_Ref\_3] (Chilton et al, 1977).** The numerical taxonomic analysis [**S\_Ref\_4] [ (Kerstens et al., 1973)** defines *Agrobacterium* species, (such as *A. tumefaciens*, *A. rhizogenes,* *A. radiobacter*, which also be delineated by genome (chromosome) sequences. **[S\_Ref\_5] (Wood et al., 2001),** **[S\_Ref\_6] (Slater et al, 2009)**.

Two *A. tumefaciens*, chromosomes (C58 and Ach5) have been sequenced and annotated **[S\_Ref\_5** (Wood et al., 2001) **[S\_Ref\_7]** Henkel et al., 2014).

The respective large circular plasmid in *A. tumefaciens* is called a tumor-inducing or Ti plasmid, and in *A. rhizogenes* a (“hairy”) root inducing Ri plasmid Ri is responsible for both virulence and tumor induction [**S\_Ref\_8]** (Currier & Nester, 1976). The essential *vir* genes are located in the *vir* region of the Ti plasmid, activated in the infecting bacterium; organized and regulated in a “prokaryotic” manner and never inserted into the chromosome of the infected plant. Contrarily*,* the tumor- promoting genes are inserted into the plant chromosome, and expressed and regulated in a eukaryotic manner **[S\_Ref\_9]** (Koncz and Shell (1986). No but one report was found in the literature if any gene located within the T region were expressed in the vegetative *Agrobacterium* cell **[S\_Ref\_10]** (Schröder et al., (1983) existing and propagating in the rhizosphere outside the infected plants.

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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 Biotechnology  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 Sciences, 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.

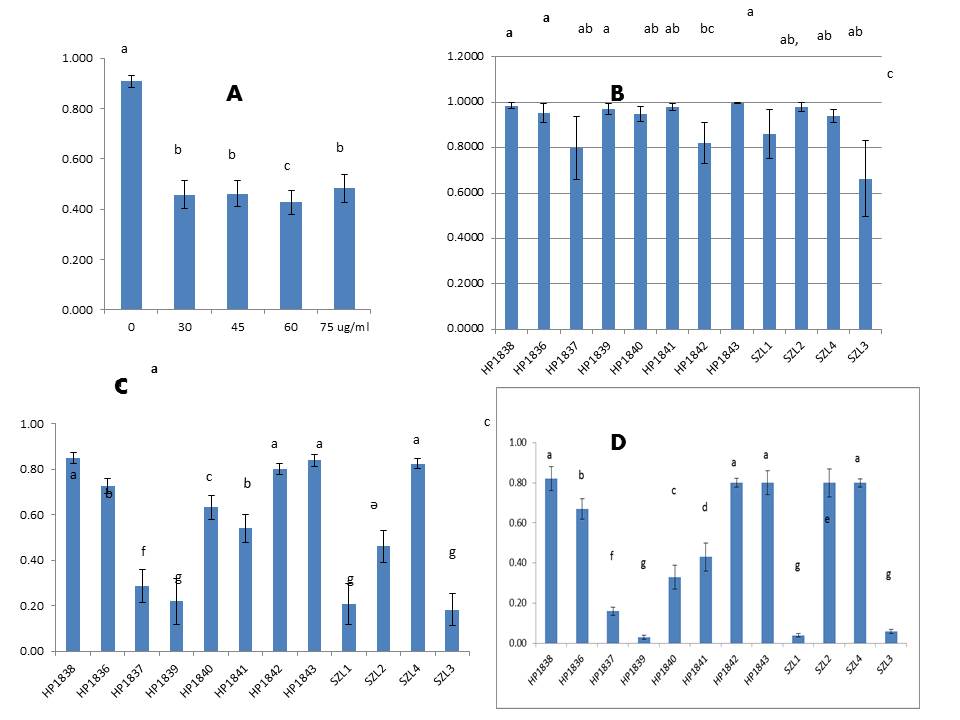
**Table S2** *Xenorhabdus* Antimicrobial Peptide-Rich Fractions Separated from EMA CFCM. Two of which (EMA\_PF2 and EMA30 were selected for Liquid Bioassays in *Agrobacterium* Bioassays

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Name Of Preparation | | Origin | WAY OF PURIFICATION | Agar Diffusion Bioassay on | | | |
| SAR  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.

**Fig S1**

OD values obtained in liquid bioassays of EMA\_PF2 in *Agrobacterium* strains: grouping according to different aspects.

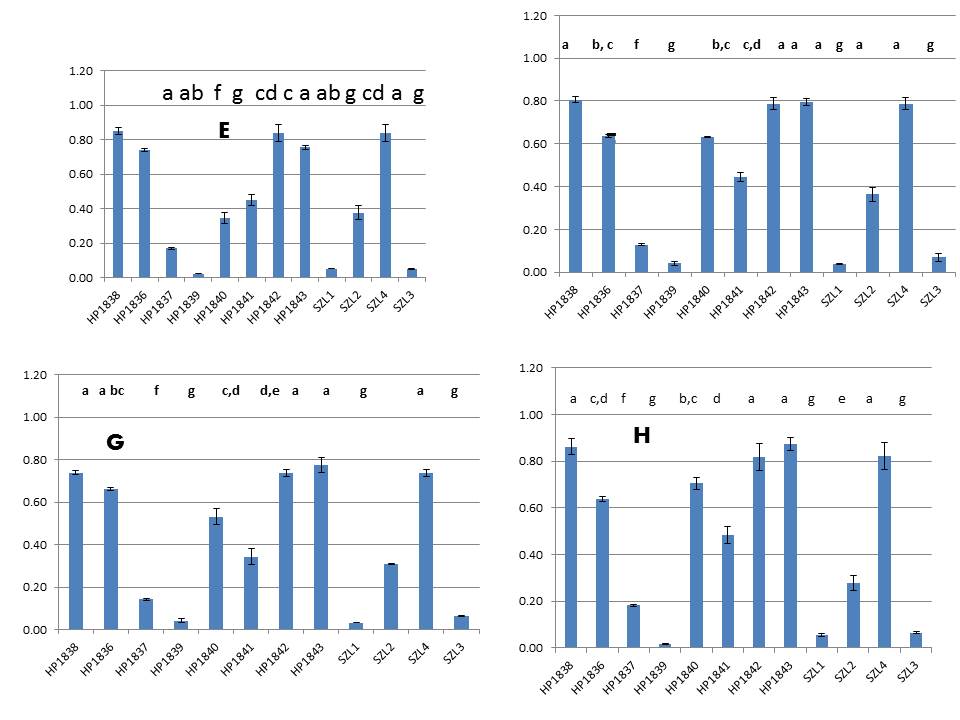


Grouping of OD values (Means +/- Standard Errors of the Means (S.E.)Raw Data are available)

* according to EMA\_PF2 concentrations (A); control values, (determined at 0 µg /ml doses: **A**;
* according to as EMA\_PF2 control values, (determined at 0 µg /ml doses): **B**; “
* according to average OD values of each strain, measured at each (0, 30, 45, 60 and 75 µg /ml) doses: **C**;
* According to average OD values of each strain, when treated (at 30, 45, 60 and 75 µg /ml doses) with EMA\_PF2: **D**.

**Fig S2**

OD values of Agrobacterium strains at different EMA\_PF2 doses (Raw Data are available)



**Fig S2E**  Means +/- Standard Errors of the Means (S.E,) of the OD values of the 12 *Agrobacterium* strains measured at 30 ug /ml EMA dose.

**Fig S2 F:** Means +/- Standard Errors of the Means (S.E,) of the OD values of the 12 *Agrobacterium* strains measured at 45 ug /ml EMA dose.

**Fig S2 G:** Means +/- Standard Errors of the Means (S.E,) of the OD values of the 12 *Agrobacterium* strains measured at 60 ug /ml EMA dose.

**Fig S2 H:** Means +/- Standard Errors of the Means (S.E,) of the OD values of the 12 *Agrobacterium* strains measured at 75 ug /ml EMA dose

Compare graphs on FigS1 and FigS2 and note that C – H (and especially the D –H) graphs look almost the same. This indicates that the between 30 – 75 ug ml dose range the effect of EMA PF was not dose-dependent. The antimicrobial activity was at the top at 30 ug ml, but it was toxic only for HP1839, SZL1, and SZL3 and almost toxic for HP1837. The rest of the strains were not uniformly but resistant to EMA PF. In these strains only cytostatic but not cytotoxic effects were detectable.

Legends to **Fig S1 and Fig S2**: Note that the first column (of OD values determined in untreated control cultures) is unambiguously higher than dose determined in the treated cultures. The latter four do not seem to be different. Statistical analysis confirms this observation. **Fig S1 B**: Means +/- Standard Errors of the Means (S.E,) of the OD values of the 12 *Agrobacterium* strains determined in the untreated control cultures (at 0 µg /ml EMA PF dose). Note that despite of detectable variability the pattern of the OD values is rather uniform, the OD values are all above 0.75, with the only exception of SZL3 (of 0.66) forming practically a single (2 overlapping) Duncan’s Group. **Fig S1 C**: Means +/- Standard Errors of the Means (S.E,) of the OD values of the 12 Agrobacterium strains measured at 0, 30, 45, 60 and 75 µg /ml EMA PF doses; **Fig S1 D**: Means +/- Standard Errors of the Means (S.E,) of the OD values of the 12 *Agrobacterium* strains measured in the treated cultures, that is, only at 30, 45, 60 and 75 µg /ml EMA PF doses; **Fig S2 E:** Means +/- Standard Errors of the Means (S.E,) of the OD values of the 12 *Agrobacterium* strains measured at 30 µg /ml EMA PF dose**; Fig S2 F**: Means +/- Standard Errors of the Means (S.E,) of the OD values of the 12 *Agrobacterium* strains measured at 45 µg /ml EMA PF dose. **Fig S2 G**: Means +/- Standard Errors of the Means (S.E,) of the OD values of the 12 Agrobacterium strains measured at 60 µg /ml EMA PF dose; **Fig S2 H**: Means +/- Standard Errors of the Means (S.E,) of the OD values of the 12 *Agrobacterium* strains measured at 75 µg /ml EMA PF dose.

Note that the graphs C – H (and especially the D –H) look are almost the same.

This indicates that the between 30 – 75 µg ml dose range the effect of EMA PF was not dose-dependent. The antimicrobial activity was highest at the top at 30 µg ml, but it was toxic only for HP1839, SZL1, and SZL3 and almost toxic for HP1837.

The rest of the strains were not uniformly but resistant, but not uniformly, to EMA PF. In these strains, only cytostatic, but not cytotoxic, effects were detectable.

**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

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Table S3 A** The ANOVA Table: Analysis of Variances of All OD Values in 0 -75 µg/ml dose range | | | | | | | |
| **Source** | | **DF** | | **Sum of Squares** | **Mean Square** | **F Value** | **Pr > F** |
| **Model** | | 60 | | 20.43434197 | 0.34057237 | 83.43 | <.0001 |
| **Error** | | 119 | | 0.48574969 | 0.00408193 |  |  |
| **Corrected Total** | | 179 | | 20.92009166 |  |  |  |
|  | | **R-Square** | | **Coeff Var** | **Root MSE** | **odv Mean** |  |
| 0.976781 | | 11.60944 | 0.063890 | 0.550328 |
| **Source** | | **DF** | | **Anova SS** | **Mean Square** | **F Value** | **Pr > F** |
| **trtmt** | | 11 | | 11.87884732 | 1.07989521 | 264.56 | <.0001 |
| **conct** | | 4 | | 5.81619747 | 1.45404937 | 356.22 | <.0001 |
| **trtmt\*conct** | | 43 | | 2.61170554 | 0.06073734 | 14.88 | <.0001 |
|  | | | | | | | |
| **Table S3B**The ANOVA Table – Analysis of Variances of – OD values at 0 and 30 µg/ml doses | | | | | | | |
| **Source** | | | **DF** | **Sum of Squares** | **Mean Square** | **F Value** | **Pr > F** |
| **Model** | | | 25 | 7.81897965 | 0.31275919 | 40.79 | <.0001 |
| **Error** | | | 45 | 0.34502095 | 0.00766713 |  |  |
| **Corrected Total** | | | 70 | 8.16400059 |  |  |  |
|  | **R-Square** | | | **Coeff Var** | **Root MSE** | **odv Mean** |  |
| 0.957739 | | | 12.87412 | 0.087562 | 0.680141 |
| **Source** | | **DF** | | **Anova SS** | **Mean Square** | **F Value** | **Pr > F** |
| **trtmt** | | 11 | | 2.48568542 | 0.22597140 | 29.47 | <.0001 |
| **conct** | | 1 | | 3.60911799 | 3.60911799 | 470.73 | <.0001 |
| **rep** | | 2 | | 0.13535422 | 0.06767711 | 8.83 | 0.0006 |
| **trtmt\*conct** | | 11 | | 1.58882201 | 0.14443836 | 18.84 | <.0001 |
| (Dependent Variable: odv) | | | | | | | |
| Class Levels Values: **trtmt** =12: HP1836; HP1837; HP1838; HP1839; HP1840; HP1841; HP1842; HP1843; SZ001; SZL002; SZL003; SZL004. Conct = 5: [0, 30, 45, 60, 75 µg/ml]; **rep** = 3:1, 2, 3; Number of Observations Read: 180; Number of Observations Used 180. | | | | | | | |

**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… 2

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | | | |
| **Table S3C** EMA PF Liquid Bioassay on *Agrobacterium* strains: Analysis of Dose / Effect relations by Duncan's Multiple Range Test for All ODV0-75 Values | | | | |
|  | Alpha | | 0.05 |  |
|  | Error Degrees of Freedom | | 118 |  |
|  | Error Mean Square | | 0.004094 |  |
| Number of Means | 2 | 3 | 4 | 5 |
| Critical Range | .02987 | .03143 | .03247 | .03324 |
| Conct | Mean | N |  | Duncan Grouping |
| 0 | 0.90767 | 36 |  | A |
| 75 | 0.48333 | 36 |  | B |
| 45 | 0.46094 | 36 |  | B |
| 30 | 0.45783 | 36 |  | B |
| 60 | 0.42636 | 36 |  | C |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| .**Table S3D** EMA PF Liquid Bioassay on *Agrobacterium* strains: Analysis of Dose / Effect relations by  T Tests (LSD) for All ODV0-75 Values | | | | | | | | |
|  | | 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 t | | 1.98010 | | |
|  | | Least Significant Difference | | 0.0298 | | |
|  | | | | | | | | |
| Concts / Comparison | | | Difference Between Means | | 95% Confidence Limits | | |
|  | 0 - 30 | | 0.44983 |  | 0.42001 | 0.47965 | |
|  | 0 - 45 | | 0.43122 |  | 0.40140 | 0.46104 | |
|  | 0 - 60 | | 0.48131 |  | 0.45149 | 0.51112 | |
|  | 0 - 75 | | 0.42433 |  | 0.39451 | 0.45415 | |
|  | 30 - 45 | | -0.01861 |  | -0.04843 | 0.01121 | |
|  | 45 - 60 | | 0.05008 |  | 0.02026 | 0.07990 | |
|  | 60 - 75 | | -0.05697 |  | -0.08679 | -0.02715 | |
|  | BUT: 45 - 75 | | -0.00689 |  | -0.03671 | 0.02293 | |
|  | And: 30 - 75 | | -0.02550 |  | -0.05532 | 0.00432 | |

**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 ODV0-75 | | | | | |
| 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 | |

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| --- | --- | --- | --- | --- | --- | --- |
| **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 instructionspropositions of the SAS 9.4 Software. The design of the experiment was a randomized complete block, design with the 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) waswere 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) *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”, trtm); at 5 different (0, 30, 45, 60 and 75 ug / ml) concentrations; in 3 replicates. It shows that the (at least in treated – untreated relastions) 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 test (Table S3C) scored the controls to Group A but the grouping of thhe 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 measured in cultures of untreated (at 0) and treated differently treatedtreated (with (30, 45, 60 and - 75 µg / ml doses) Agrobacterium cultures handled as independent, separate data pools, and compared. We didaccomplished 4 different ANOVA procedures restricted only 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 doses 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 experimental-wise error, which could not influence the conclusions, that within the range of of 30-75 µg/ml EMA PF doses, no significant dose-effect relations should be considered, and we are pooled the OD values measured in this range ofof each strains forand comparisonng. We have been considering the Duncan’s Multiple Range test as the most accurate to distingish between experimental groups reacting differently to the same treatmemnts. The means wihithin a given Duncan’s Group labelled with a 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, labelled, say, letter B, are significvantly different at the P=0.05 level. We overcheacked each case with the t(LSD) test as well (data are not given), and found that the Duncan’s Multiple Range Tests were completely fair.

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**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 |  |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **TABLE S4 B**: Duncan’s Multiple Range test of the OD 30-75 VALUES  Alpha = 0.05; Error Degrees of Freedom: 22; Error Mean Square = 40.23677; | | | | | | | | | | | | | | | | | |
| No of Means | | | | 2 | | | | | 3 | | | | 4 | | | | |
| Critical Range | | | | 6.201 | | | | | 6.512 | | | | 6.710 | | | | |
| No of Means | 2 | 3 | 4 | | 5 | | 6 | 7 | | | 8 | 9 | | 10 | | 11 | 12 |
| Critical Range | 10.74 | 11.28 | 11.62 | | 11.86 | | 12.04 | 12.18 | | | 12.29 | 12.38 | | 12.45 | | 12.50 | 12.55 |
|  | | DC | | | |  | | | |  | | | | |  | | |
| Duncan’s Groups | | | | | |  | | | |  | | | | |  | | |
| Super group | | Duncan’s Groups | | | | STRAINS | | | | N | | | | | MEAN | | |
| EMA RESISTANT | | DC | | | | STRAINS | | | | N | | | | | MEAN | | |
| A | | | | HP1838 | | | | 12 | | | | | 0.81525 | | |
| A | | | | HP1843 | | | | 12 | | | | | 0.79933 | | |
| A | | | | SZL004 | | | | 12 | | | | | 0.79658 | | |
| B | | | | HP1836 | | | | 12 | | | | | 0.66883 | | |
| C | | | | HP1840 | | | | 12 | | | | | 0.55350 | | |
| D | | | | HP1841 | | | | 15 | | | | | 0.43513 | | |
| E | | | | SZL002 | | | | 12 | | | | | 0.33242 | | |
| EMA SENSITIVE | | F | | | | HP1837 | | | | 12 | | | | | 0.15592 | | |
| G | | | | SZL003 | | | | 12 | | | | | 0.06183 | | |
| G | | | | SZ001 | | | | 12 | | | | | 0.04375 | | |
| G | | | | HP1839 | | | | 12 | | | | | 0.03092 | | |

**Footnotes to 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. **Abreviation**s: EMA PF: Antimicrobially Peptid Rich Fraction from the cell-free culture media (CFCM) of *Xenorhabdus budapstensis* (EMA) (AF13), see Vozik et al., 2015. MIC: minimal inactivation concentration. For HP1837: MIC75; for HP1839, SZL1 & SZL3: MIC90. We have been considering the Duncan’s Multiple Range test as the most accurate to distingish between experimentl groups reacting differently to the same treatemts. The means wihitin a give Duncan’s Group labelled with a a letter, say, with letter A, may differ from each other, but the SD values overlap; but iffer significantly from those belonging to another Duncan’s Group, labelled, say,letter B, are significantly different at P=0.05 level. We overchecked each case with t(LSD) tests as well (data are not given), and found that the Duncan’s Multiple Range Tests were completely fair.

The ANOVA-based Duncan’s Multiple Range test of the OD (30-75) of the EMA\_PF treated *Agrobacterium* strains scored them to **7 Duncan’s Groups (DG);** and we scored the 7 Duncan’s Groups to 2 clearlyunambiguously separateble “Super-Groups”, (**Table S3B**).

Four *Agrobacterium* strains of S phenotype, including HP1837 of Duncan’s Group F); HP1839, SZL1, and SZL3 of Duncan’s Group G) were scored to **Duncan’s Super-Group I**.

The rest of the *Agrobacterium* strains are of the R phenotype, including the wild-type (TDNA) (+) AGR strain HP1838 (of Duncan’s Group A); the pMP90 helper-plasmid harboring SZL4 and plasmid-cured (HP1836, HP1840; HP1841; HP1842; HP 1843)), all nopaline catabolizing strains belonging to Duncan’s Groups A-D); as well as one (SZL2) of the two octopine catabolizing strains examined, were scored to **Duncan’s Super-Group II**.

**Table S5 EMA PF Liquid Bioassay of EMA PF on L, L, - Succinamopine (AGR) *Agrobacterium* strains**: ANOVA Procedure for OD(30-75) Values (in cultures of 30, 45, 60 & 75 µg /ml Doses (A). Duncan’s Multiple Range Test and Grouping of AGR strains at each EMA\_PF Dose (B)

. **Table S5B**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Class Levels Values: trtmt = 4: HP1838; HP1839; SZ001; SZL003. conct = 1 [30-75]; rep 12 1 2 3 4 5 6 7 8 9 10 11 12; Number of Observations Read 48; Number of Observations Used 48 | | | | | | | | |
| **Strain** | **Dose (**µg/ml)  **of EMA-PF** | **N** | **OD Values** | | **Duncan Grouping** | | | |
| **Mean** | **Std Dev** | Super-groups | Duncan Groups | | |
| HP1838 | 0 | 3 | 0.98533333 | 0.02454248 | I. |  | A | |
|  |  |  |  |  |  | A | |
| HP1839 | 0 | 3 | 0.97033333 | 0.04142865 |  | A | |
|  |  |  |  |  |  | A | |
| SZL1 | 0 | 3 | 0.85933333 | 0.18558107 | B | A | |
|  |  |  |  |  | B |  | |
| SZL3 | 0 | 3 | 0.66333333 | 0.29173333 | B |  | |
| HP1838 | 30 | 3 | 0.98533333 | 0.02454248 | I |  | | A |
|  |  |  |  |  |  | |  |
| SZL1 | 30 | 3 | 0.05100000 | 0.00264575 | II | B | |  |
| SZL3 | 30 | 3 | 0.04766667 | 0.00568624 | B | |  |
| HP1839 | 30 | 3 | 0.02166667 | 0.00152753 | B | |  |
| HP1838 | 45 | 3 | 0.80666667 | 0.02173323 | I |  | | A |
|  |  |  |  |  |  | |  |
| SZL3 | 45 | 3 | 0.07033333 | 0.03300505 | II |  | | B |
| HP1839 | 45 | 3 | 0.04200000 | 0.01800000 | C | | B |
| SZL1 | 45 | 3 | 0.05100000 | 0.00264575 | C | |  |
| HP1838 | 60 | 3 | 0.73933333 | 0.01665333 | I |  | | A |
|  |  |  |  |  |  | |  |
| SZL3 | 60 | 3 | 0.06400000 | 0.00458258 | II |  | | B |
| HP1839 | 60 | 3 | 0.04200000 | 0.01907878 | C | | B |
| SZL1 | 60 | 3 | 0.03233333 | 0.00057735 | C | |  |
| HP1838 | 75 | 3 | 0.86266667 | 0.06110101 | I |  | | A |
|  |  |  |  |  |  | |  |
| SZL3 | 75 | 3 | 0.06533333 | 0.00763763 | II | B | |  |
| SZL1 | 75 | 3 | 0.05533333 | 0.01379613 | B | |  |
| HP1839 | 75 | 3 | 0.01800000 | 0.00400000 | B | |  |

**Footnotes to Table S5**. EMA PF Liquid Bioassay of EMA PF on L, L, - Succinamopine (AGR) *Agrobacterium* strains: ANOVA Procedure for OD 30-75) Values (in cultures of 30, 45, 60 & 75 µg /ml Doses) (A). Duncan’s Multiple Range Test and Grouping of AGR strains at each EMA\_PF Dose (B). Note that at each dose but 0, the OD values of the wild type (TDNA) (+) plasmid-harboring HP1838, and those of the helper ((TDNA) (-) strains (HP1839; SZL1; SZL3), were significantly different.

Note that the OD values measured in the controls (at 0 µg/ml MA PF) form practically one data-pool (of two overlapping Duncan’s Group) while those measured at each of the treated cultures sharply separated into two super-groups.

The statistical evaluation of the results on AGR strains: Within the frame of the Anova Procedure for the OD 30-75 values (Anova Table: **Table S5A; Duncan’s Multiple Range Test: Table S5B**) of the four AGR strains the Duncan’s Multiple Range Test scored them to 2 large non-overlapping Super-Groups: R (Duncan’s Group A and HP1838 whereas scored to it) and S (including two overlapping Duncan’s Group, A (SZL1, SZL3) and B (SZL3, HP1839).

In order to learn whether the spectacular differences between the OD values of the wild type (TDNA)(+) AGR (HP1838) strain and those of the helper (TDNA)(-) plasmid harboring derivatives (HP1839, SZL1) , and other (A4TC) helper- (TDNA)(-) plasmid harboring (SZL3) strains were significant, we compared the distribution of the OD values of the untreated and those which were treated with 30, 45, 60 and 75 µg/ml doses separately.

For comparison we used ANOVA-based Duncan’s Multiple Range tests. Results are presented in Table S5B). We found that no super-grouping in the untreated controls could be made; while at each of the EMA\_PF2 concentrations the (TDNA)(+) AGR (HP1838) strain separated to one, while all the helper-plasmid harboring (TDNA)(-) strains were into another Duncan’s Group.

The effect of the EMA\_PF on the helper-plasmid harboring (TDNA) (-) AGR strains is unambiguously cytotoxic at any concentration, but hardly act in the (TDNA) (+) AGR (HP1838) strain.

This analysis also confirmed that the small differences between the helper-plasmid- carrying strains at each of the 4 (30, 45, 60 and 75 µg/ml) treated groups do not have any biological significance. (We got exactly the same results when the control (N=3) and the treated (N=12) data were subjected to GLM analysis followed by the Duncan’s Multiple Range Test. Data are not given here).

**Table S6**

**Table S6A** GLM Procedure for OD values of Liquid Bioassay of EMA PF on *Agrobacterium tumefaciens* Octopine Metabolizing (OCT) strains HP1837 and SZL2: comparison of the data with untreated cultures

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| The GLM Procedure for OD values of Control vs. Treated cultures of *A. tumefaciens* OCT strains | | | | | |
| Dependent variable: OD Values | | | | | |
| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
| Model | 14 | 2.30285300 | 0.16448950 | 26.72 | <.0001 |
| Error | 15 | 0.09235300 | 0.00615687 |  |  |
| Corrected Total | 29 | 2.39520600 |  |  |  |
|  | R-Square | Coeff Var | Root MSE | odv Mean |  |
|  | 0.961443 | 21.03638 | 0.078466 | 0.373000 |  |
|  | R-Square | Coeff Var | Root MSE | odv Mean |  |
|  |  |  |  |  |  |
| Source | DF | Type I SS | Mean Square | F Value | Pr > F |
| Trtmt (Strains) | 1 | 0.23585333 | 0.23585333 | 38.31 | <.0001 |
| Conct (Doses) | 1 | 1.99176333 | 1.99176333 | 323.50 | <.0001 |
| Rep | 11 | 0.07521550 | 0.00683777 | 1.11 | 0.4157 |
| trtmt\*conct | 1 | 0.00002083 | 0.00002083 | 0.00 | 0.9544 |
|  |  |  |  |  |  |
| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
| Trtmt (Strains) | 1 | 0.15308163 | 0.15308163 | 24.86 | 0.0002 |
| Conct (Doses) | 1 | 1.13406008 | 1.13406008 | 184.19 | <.0001 |
| Rep | 11 | 0.07521550 | 0.00683777 | 1.11 | 0.4157 |
| trtmt\*conct | 1 | 0.00002083 | 0.00002083 | 0.00 | 0.9544 |

**Table 6S B Comparison of the OCT strains HP 1837 and SZL2**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| t Tests (LSD) for OD Values | | | | | | | | |
| t-(LSD) test | | |  | Tukay’s (HSD) test | | | |  |
| Alpha | | | 0.05 | Alpha | | | | 0.05 |
| Error Degrees of Freedom | | | 15 | Error Degrees of Freedom | | | | 15 |
| Error Mean Square | | | 0.006157 | Error Mean Square | | | | 0.006157 |
| Critical Value of t | | | 2.13145 | Critical Value of Studentized Range | | | | 3.01432 |
|  | | | | | | | | |
| conct Comparison | Comparison | LSD | | | | | Comparisons significant at the 0.05 level are indicated by \*\*\*. | |
| Difference of Means | | | 95% Confidence Limits | |
| Untreated –Treated | 0 -3075 | 0.56783 | | | 0.64417 | 0.72050 | NS | |
| Treated -Untreated | 3075 - 0 | -0.56783 | | | -0.64417 | -0.72050 | NS | |
|  | HSD | | | | | | | |
| Untreated –Treated | 0 - 3075 | 0.64417 | | 0.72050 | | 0.56783 | \*\*\* | |
| Treated -Untreated | 3075 - 0 | -0.64417 | | -0.56783 | | -0.72050 | \*\*\* | |

**Footnote to Table S6:** Statistical evaluation of the results on OCT strains.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 GLM Procedures following the directions of the SAS 9.4 Software**.** First, we measured the OD values in the control and treated cultures of SZL2 and HP1837, and compared them by the t (Least Significant Difference, LSD) test (for comparison-wise errors); and by the Tukay’s Studentized Range (HSD) Test (for experimental errors). Second, we compared the OD30-75 values of the two strains by ANOVA Procedure, followed by LSD and HSD tests. The OD values of the untreated and treated cultures were significantly different. Furthermore, the OD30-75 values of the two strains were also different.

Fig S3 OD values of EMA\_PF2 in vitro liquid bioassays on NOP strains

1. HP1843 C58C1\* Nal (R) (Duncan’s Group A)

B. SZL4 GV3101::pMP90RK C58 C1\*Rif **(R)** (pTiC58DT-DNA) (Duncan’s Group A)

C. HP1842 C58C1\* Nal (R) (Duncan’s Group A)

D. HP1836 C58C1\* Nal**(R)** (Duncan’s Group B)

.

E. HP 1840 C58 C1\*Rif (R) (Duncan’s Group C )

**Fig S3 OD Values of EMA PF Resistant Nopaline – Catabolizing (NOP) *Agrobacterium* strain**

**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 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 o C 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. On the basis of their significantly different OD values, these strains could be scored to different Duncan’s Groups (Duncan’s Group A, B, C, D, respectively), which reflects differences in the cytostatic effect of EMA PF on them. The Duncan Multiple Range test was carried out as a part of the ANOVA Procedure conductedarried out withby SAS 9.4 software.

A Comment on Results of NOP strains:

The OD patterns of the two *Agrobacterium* nopaline strains (HP1843, SZL4, HP1842, HP1836, HP1840 and HP1841 treated with EMA PF are shown in **FigS3 A, B, C, D, E, F**, respectively.

When comparing the six NOP strains, it could be seen that the OD values both in the control, (measured at 0 µg/ml dose), and in those measured in the treated (with 30, 45, 60 and 75 ug/ml doses) cultures are high and variable. But the distribution patterns of the control and in the treated cultures are not the same. Furthermore, the distribution of the OD values determined the within the 30 -75 µg/ml dose range are very similar to each other. (Compare (Fig 6007 A, B and C, respectively).

This indicates that EMA PF was unambiguously were active in the NOP strains too, but definitely were less active than in the OCT or ARG strains examined.

The OD values of the same strain measured at 30, 45, 60 and 75 ug/ml doses practically did not differ from each other significantly (**FigS3A**) providing a single pool of data (OD30-75) to compare them to those of the untreated controls.

The statistical comparison (GLM procedure) of the OD values of control and treated A. tumefaciens nopaline (NOP) strains are given in Table **FigS3**B.

It unambiguously proves that EMA PF exerted significant but quantitatively different cytostatic, but no detectable cytotoxic effect on each of the NOP strains examined.

The comparison of the results obtained in liquid cultures of the Ti-free (plasmid-cured) HP1843, HP1842, HP1836, HP1840 and HP1841) and the helper plasmid. (pMP90) harboring (SZL4) strains, no significant difference could be demonstrated in their sensitivities to EMA PF. But in the agar diffusion assay both SZL4 and SZL5 was sensitive to the CFCM of EMA and EMC (**FigS3**)

The missing information is the S/RF phenotype of a wild NOL strain to EMA PF.

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