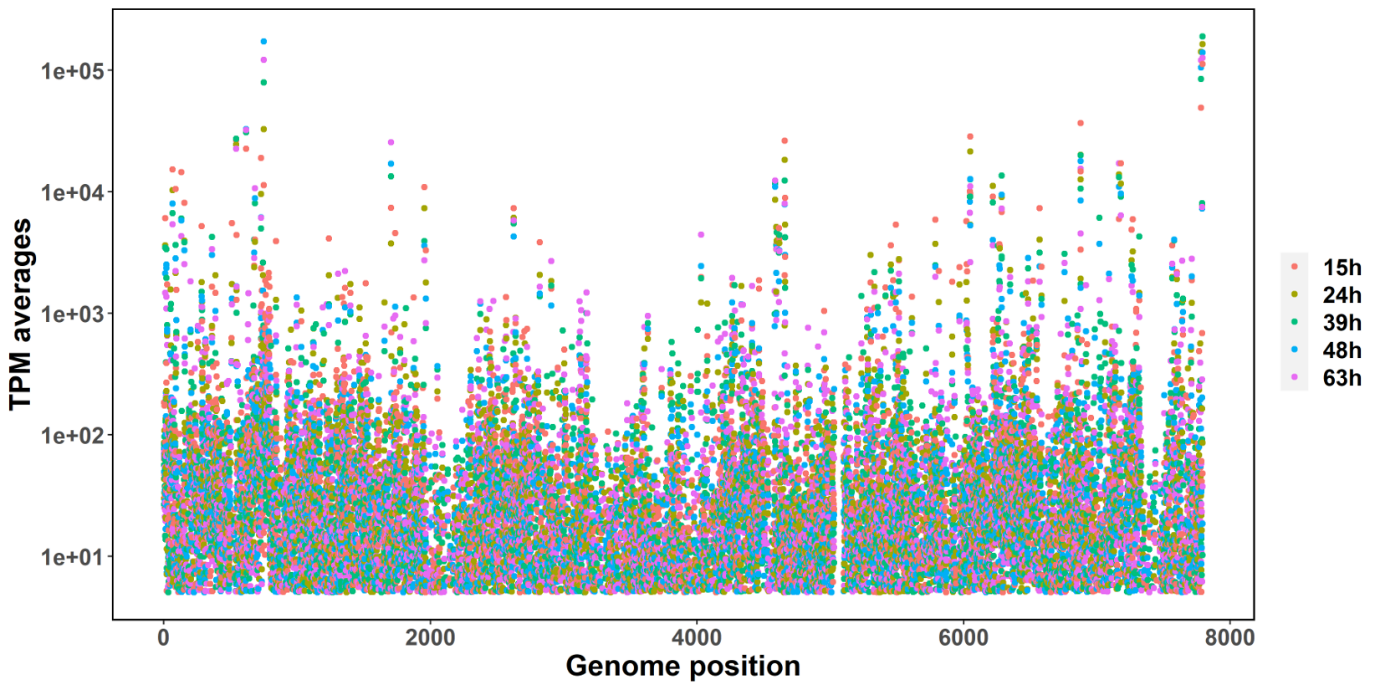
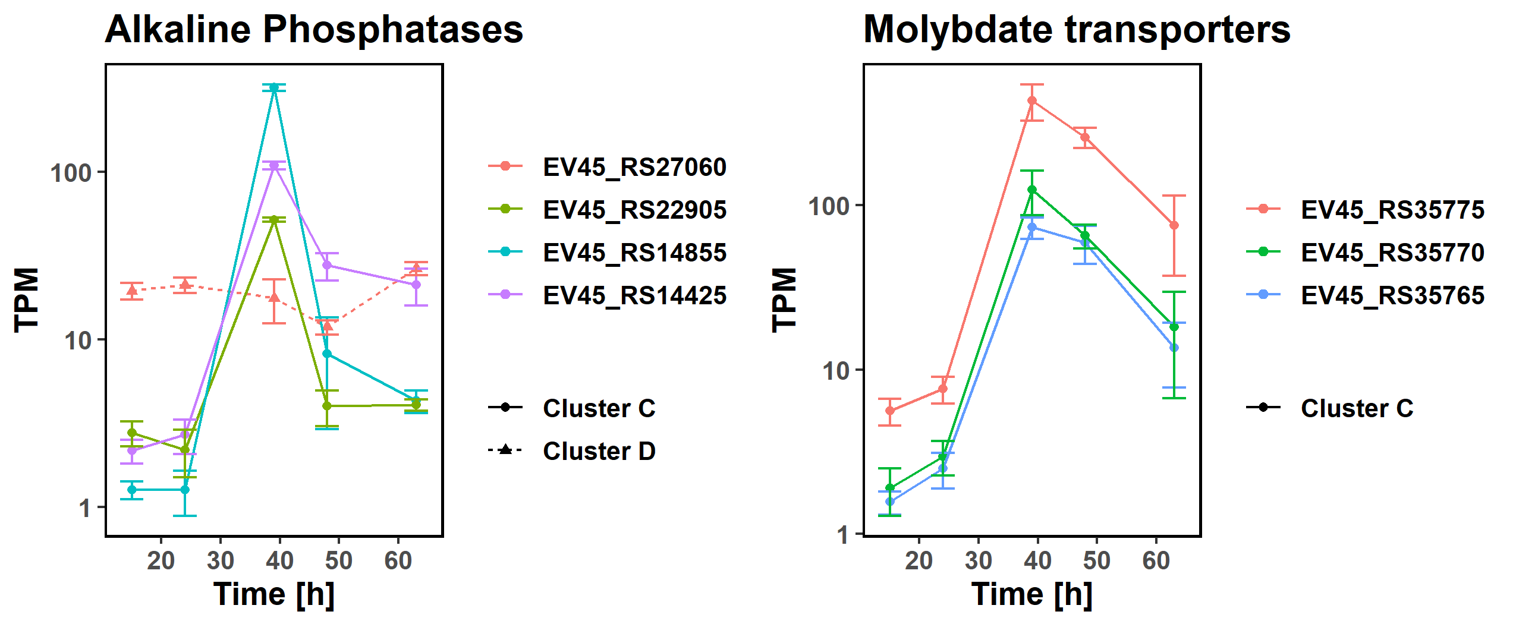
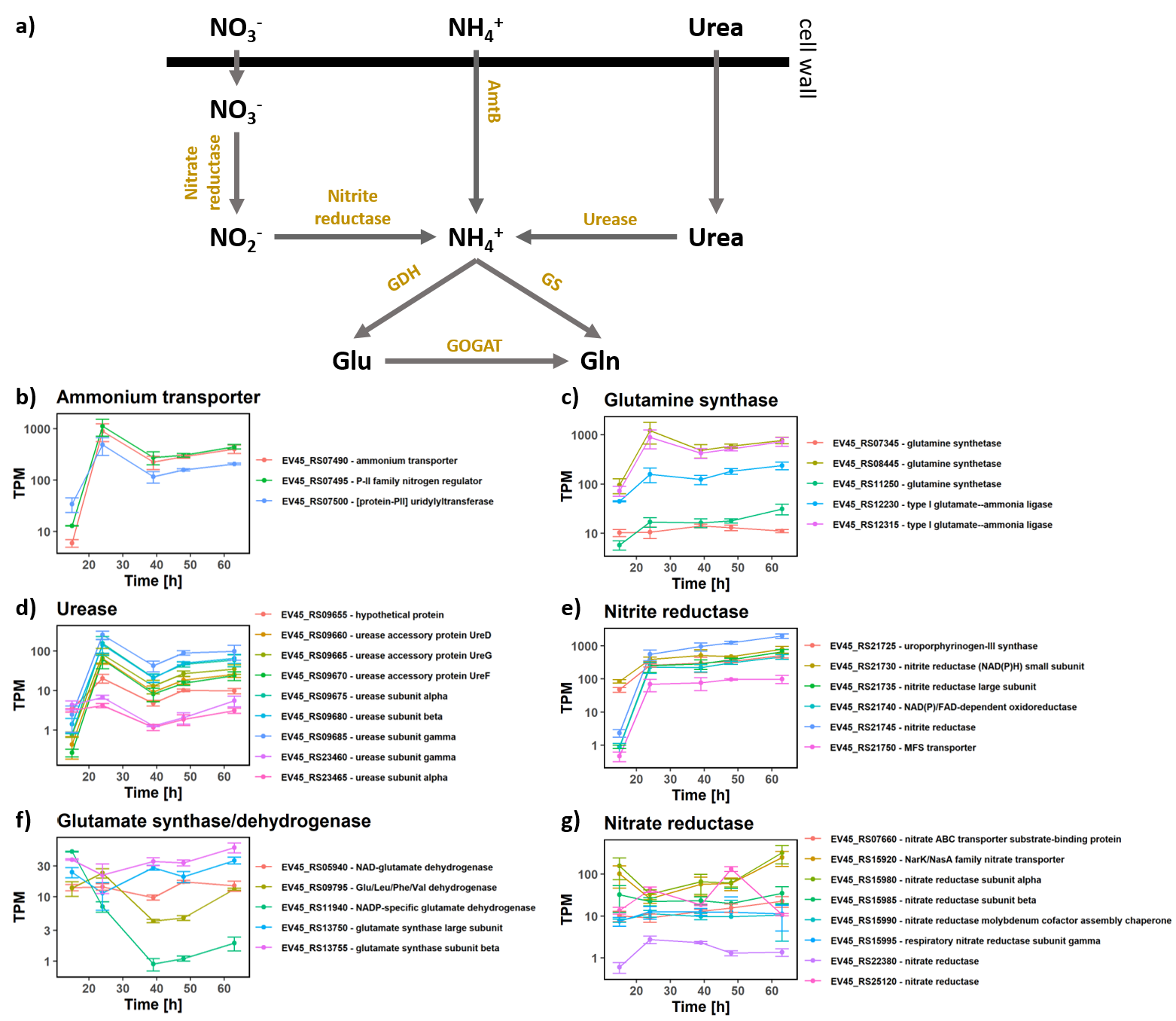
Supplementary material



**Supplementary Figure 1 –** Observed average TPMs at each time point as function of genes position.



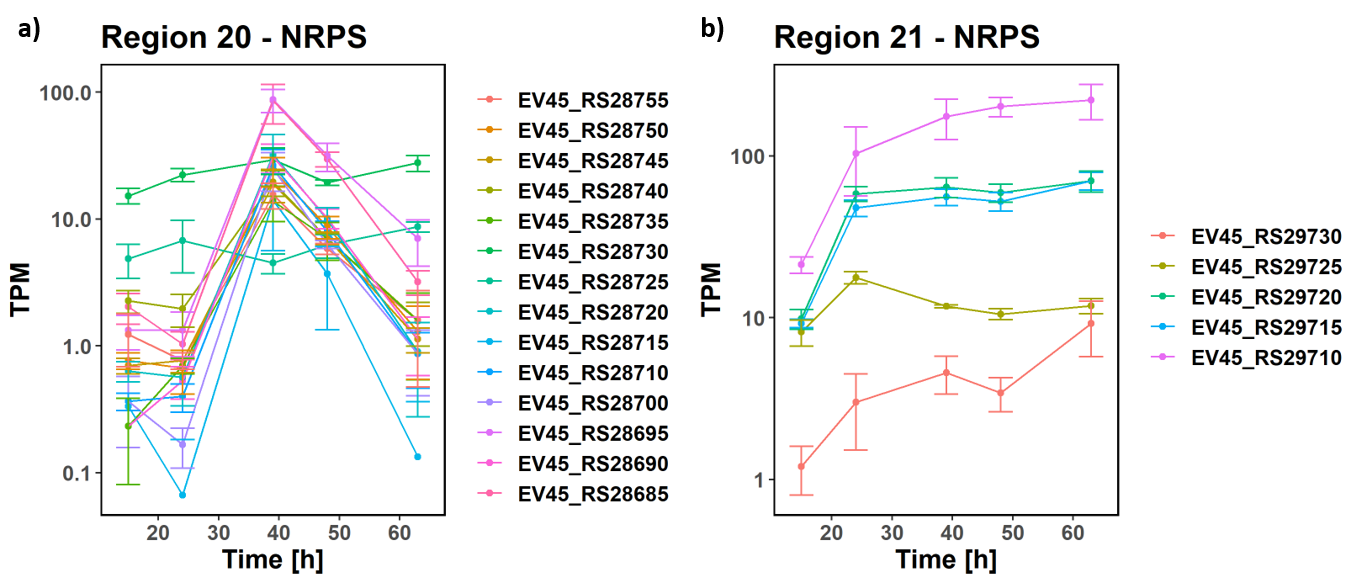
**Supplementary Figure 2 –** Gene expression of the alkaline phosphatases found in the *P. rosea* genome (left side) and the molybdate transport system following the same trend of phosphate and iron transporters. Data shown in logarithmic scale.



**Supplementary Figure 3 – a)** Genes involved in the nitrogen metabolism. GS stands for glutamine synthase, GDH stands for glutamate dehydrogenase and GOGAT stands for glutamate synthase. **b)** TPM values (in log scale) of the differentially expressed ammonium transporter and its two neighbouring genes. **c)** TPM values (in log scale) associated glutamine synthases. **d)** TPM values (in log scale) associated with the ureases genes. **e)** TPM values (in log scale) associated with the genes associated with nitrite uptake and reduction. **f)** TPM values (in log scale) associated glutamate synthases and dehydrogenases. **g)** TPM values (in log scale) associated with the genes associated with nitrate uptake and reduction. The error bars represent the standard deviation calculated from three replicates.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Region** | **Start** | **End** | **Compound class** | **Most similar known cluster** | **Similarity** |
| Region 1 | 102,380 | 111,920 | bacteriocin |  |  |
| Region 2 | 183,913 | 206,439 | lassopeptide | citrullassin D | 80% |
| Region 3 | 217,817 | 238,182 | LAP | murayaquinone | 3% |
| **Region 4** | **781,840** | **821,078** | **thiopeptide, LAP** | **GE2270A** | **100%** |
| Region 5 | 1,391,331 | 1,455,396 | NRPS | coelibactin | 36% |
| Region 6 | 1,857,003 | 1,907,498 | NRPS, T1PKS | crochelin A | 7% |
| Region 7 | 2,001,822 | 2,044,462 | NRPS | tetronasin | 5% |
| Region 8 | 3,219,426 | 3,239,019 | terpene | streptobactin | 11% |
| Region 9 | 3,432,943 | 3,476,008 | NRPS | aristeromycin | 9% |
| Region 10 | 3,634,741 | 3,657,603 | LAP, bacteriocin | A54145 | 5% |
| Region 11 | 3,691,229 | 3,756,669 | NRPS |  |  |
| Region 12 | 3,896,387 | 3,957,440 | NRPS, T1PKS | crochelin A | 11% |
| Region 13 | 4,312,406 | 4,332,834 | terpene | isorenieratene | 28% |
| Region 14 | 4,580,264 | 4,600,230 | terpene | geosmin | 100% |
| Region 15 | 4,861,587 | 4,902,660 | T3PKS | alkylresorcinol | 100% |
| Region 16 | 5,132,110 | 5,220,468 | NRPS, T1PKS | crochelin A | 7% |
| Region 17 | 5,414,011 | 5,436,635 | lanthipeptide | catenulipeptin | 60% |
| Region 18 | 5,525,854 | 5,594,004 | NRPS | glycinocin A | 9% |
| Region 19 | 5,912,385 | 5,933,383 | terpene |  |  |
| **Region 20** | **6,406,368** | **6,472,286** | **NRPS** | **streptobactin** | **58%** |
| Region 21 | 6,664,465 | 6,712,215 | NRPS | theonellamide | 13% |
| Region 22 | 6,742,733 | 6,770,809 | thiopeptide, LAP |  |  |
| Region 23 | 6,798,046 | 6,890,589 | T1PKS | sceliphrolactame | 56% |
| Region 24 | 7,266,706 | 7,280,076 | siderophore |  |  |
| Region 25 | 7,440,353 | 7,451,159 | bacteriocin |  |  |
| Region 26 | 7,716,564 | 7,763,022 | T1PKS | kistamicin A | 8% |
| Region 27 | 8,097,255 | 8,153,658 | NRPS | erythrochelin | 85% |

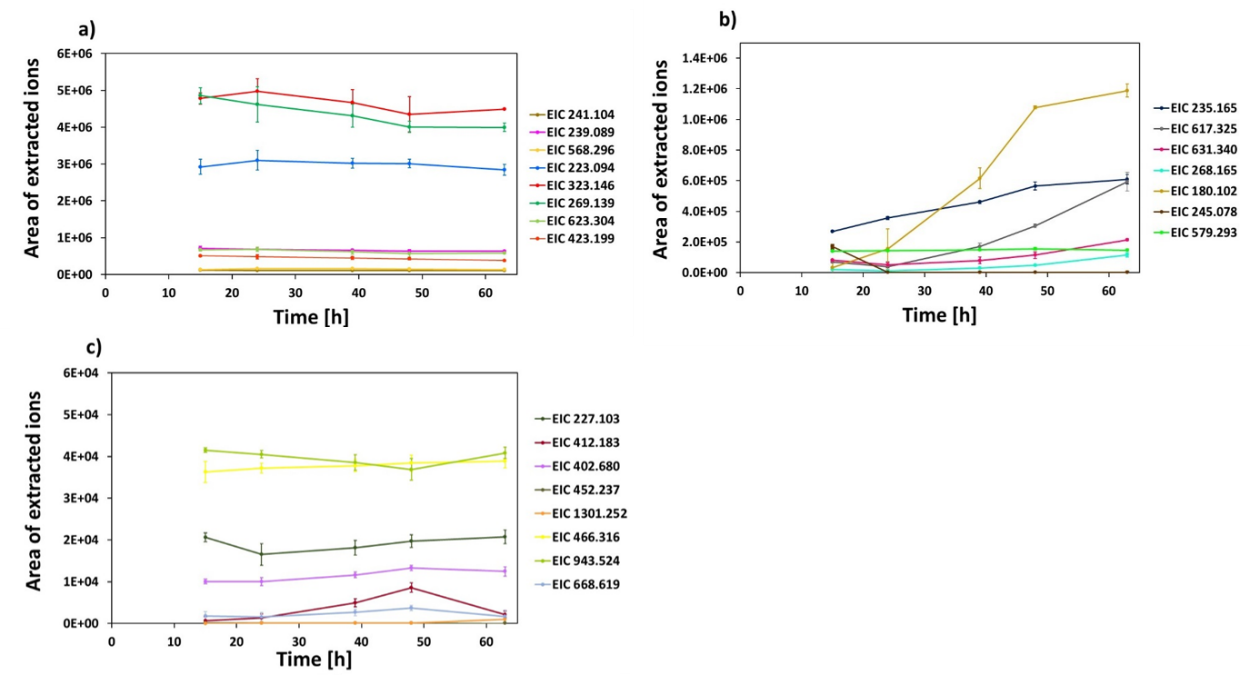
**Supplementary Table 1 –** Summary of the biosynthetic gene clusters predicted by antiSMASH v5.1.2 in *P. rosea*.



**Supplementary figure 4 – a)** Gene expression of a selection of genes found in the predicted BGC in region 20 (NRPS cluster, moderately similar to the streptobactin cluster). **b)** Gene expression of a selection of genes found in the predicted BGC in region 21 (NRPS cluster, very weakly similar the theonellamide cluster. The error bars represent the standard deviation calculated from three replicates. Data shown in logarithmic scale.

**Untargeted metabolomics analysis of whole-broth acetonitrile extracts**

The untargeted metabolomics experiment performed on the acetonitrile extracts detected several mass-to-charge ratios (*m/z*) associated with several metabolites present during fermentation. The metabolites detected were divided in three different groups. The first group contains metabolites that showed a roughly constant and high level over time. The three metabolites with the highest levels are associated with the following *m/z* values: 323.1462, 269.1386 and 223.0941 (**Supplementary figure 5a**).



**Supplementary figure 5 – a)** Areas of the extracted ions associated with the *m/z* values of metabolites which concentration is more or less constant over time and show high concentration. **b)** Areas of the extracted ions associated with the *m/z* values of metabolites that increased in the acetonitrile extract throughout fermentation. **c)** Areas of the extracted ions associated with the *m/z* values of metabolites which concentration is more or less constant over time with low concentration. The error bars represent the standard deviation calculated from three replicates.

None of the molecular formulae associated to [M+Na]+= 323.1462 (C15H24O6), and [M+H]+=269.1386 (C14H20O5 and C15H16N4O) could be identified as any known actinomycete metabolite in the Dictionary of Natural Products (DNP Version 29:1, June 2020 CRC Press). The molecular formula C10H16O4 was assigned to the adduct [M+Na]+= 223.0941 with 120 possible hits identified for this molecular formula (**Supplementary tables 2a-2d**).

The second group contains metabolites the level of which increases during fermentation (**Supplementary figure 5b**). In this group, the peak that reaches the highest level at of fermentation has a *m/z* value of 180.1019. The molecular formula C10H13NO2 associated to this peak matches several potential metabolites (**Supplementary tables 2a-2d**). The other two main peaks detected in this group have a *m/z* value of 235.1652 and 617.3253, respectively. The molecular formulae C10H22N2O4 and C24H48N9O14/C25H44N8O10 assigned to these *m/z* values could not be associated to any known metabolite suggesting potentially new metabolites produced by the strain. Finally, the third group contains metabolites showing low intensity and a roughly constant trend throughout fermentation (**Supplementary figure 5c**). One peak found in this group was annotated as benarthin (C17H25N5O7). This compound was detected as the ion [M+H]+ = 412.1830. This peak slightly increased from 15 to 48 hours, reaching the highest level at 48 hours.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| RT (min) | [M+H]+ | [M+2H]2+ | [M+H+NH4]2+ | [M+NH4]+ | [M+Na]+ | [M+3H]3+ | [M+2H+Na]3+ | MF | DPN | Identification by DNP |
| 1.18 | 235.1652 |  |  |  |  |  |  | C10H22N2O4 | No hits |  |
| 1.44 | 280.1388 |  |  |  |  |  |  | C11H21NO7 | \* | Fructose-valine |
| 1.56 | 235.1191 |  |  |  |  |  |  | C11H14N4O2 | \* | Cyclo(histidylproline) |
| 1.67 | 158.0924 |  |  |  |  |  |  | C6H11N3O2 | \* |  |
| 1.91 | 226.1549 |  |  |  |  |  |  | C11H19N3O2 | \* |  |
| 2.03 | 268.1038 |  |  |  |  |  |  | C10H13N5O4/ C9H17NO8 | \* | Adenosine/Fructose-serine |
| 2.38 | **617.3253** | 309.1661 |  |  |  |  |  | C24H48N9O14/ C25H44N8O10 | No hits |  |
| 2.52 | 254.161 |  |  |  |  |  |  | C11H19N5O2/ C10H23NO6 | \* | Cyclo(arginylpropyl) |
| 4.05 | 631.3401 |  |  |  |  |  |  | C25H50N4O14/ C23H38N18O4 | No hits |  |
| 4.29 | 169.0969 |  |  |  |  |  |  | C8H12N2O2 | \* |  |
| 4.68 | 227.1026 |  |  |  |  |  |  | C10H12N2O4 | 12 Hits | 2-Amino-4-hydroxy-4-(5-hydroxy-2-pyridinyl)-3-methylbutanoic acid (AA residue of nikkomycin Z) / Cyclo(glutamylprolyl) / Nikkomycin D |
| 5.05 | 412.183 |  |  |  |  |  |  | C17H25N5O7 | 1 Hit | Benarthin |
| 5.19 | 261.1315 |  |  |  |  |  |  | C8H16N6O4 | \* |  |
| 5.25 | 268.1654 |  |  |  |  |  |  | C13H21N3O3 | No hits |  |
| 5.51 | 283.175 |  |  |  |  |  |  | C12H26O7 | \* |  |
| 5.73 | 327.2011 |  |  |  |  |  |  | C14H30O8 | \* |  |
| 5.73 | 271.1233 |  |  |  |  |  |  | C14H16N2O3 | \* | Cyclo(2-hydroxyprolylphenylalanyl) / Cyclo(prolyltyrosyl) / N-Ac 2-methyltryptophan / Na-Ac methyl tryptophan |

**Supplementary Table 2a –** Summary of the untargeted metabolites detected in *P. rosea*.

\*Metabolites detected in the medium.

In those metabolites in which several adducts were detected, the most intense ion is indicated in bold.

Identification was done by searching the molecular formulae in the Dictionary of Natural Products (DPN).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| RT (min) | [M+H]+ | [M+2H]2+ | [M+H+NH4]2+ | [M+NH4]+ | [M+Na]+ | [M+3H]3+ | [M+2H+Na]3+ | MF | DPN | Identification by DNP |
| 5.95 | 371.2275 |  |  |  |  |  |  | C16H34O9 | \* |  |
| 6.03 | 180.1019 |  |  |  |  |  |  | C10H13NO2 | 92 Hits | Dihydro-N-hydroxyabikoviromycin / 2-Ethyl-6,7-dihydro-7-hydroxy-3(5H)-indolizinone / 3-Hydroxy-3-(2-methylphenyl) propano amide / alanine benzyl ester / alanine N-benzyl methyl ester / alanine N-benzyl |
| 6.15 | 415.2535 |  |  | **432.28** | 437.2354 |  |  | C18H38O10 | \* |  |
| 6.22 | 429.2322 |  |  | **446.2593** | 451.2146 |  |  | C18H36O11 | \* |  |
| 6.25 | 459.2797 |  |  | **476.3062** | 481.2613 |  |  | C20H42O11 | \* |  |
| 6.38 | 503.3057 |  |  | **520.3323** | 525.2874 |  |  | C22H46O12 | \* |  |
| 6.52 | 547.332 |  |  | **564.3586** | 569.3138 |  |  | C24H50O13 | \* |  |
| 6.62 | 591.3377 |  |  | **608.3845** | 613.3400 |  |  | C26H54O14 | \* |  |
| 6.72 | 635.384 |  |  | **652.4106** | 657.3655 |  |  | C28H58O15 | \* |  |
| 6.73 | 402.6802 |  |  |  |  |  |  | C34H38N10O13 | No hits |  |
| 6.81 | 679.4100 |  |  | **696.4369** | 701.3924 |  |  | C30H62O16 | \* |  |
| 6.89 | 723.4365 | **362.2219** | 370.7554 |  |  |  |  | C32H66O17 | \* |  |
| 6.96 | 767.4623 | **384.2349** | 392.7481 |  |  |  |  | C34H70O18 | \* |  |
| 7.04 | 811.4873 | **406.2479** | 414.7614 |  |  |  |  | C36H74O19 | \* |  |
| 7.09 | 855.5141 | 428.2612 | **436.7748** |  |  |  |  | C38H78O20 | \* |  |
| 7.16 | 899.5396 | 450.2743 | **458.7877** |  |  |  |  | C40H82O21 | \* |  |
| 7.23 | 943.5669 | 472.2876 | **480.8009** |  |  |  |  | C42H86O22 | \* |  |
| 7.30 | 987.5926 | 494.3006 | **502.8139** |  |  |  |  | C44H90O23 | \* |  |
| 7.35 | 1031.6182 | **516.3137** | 524.8271 |  |  |  |  | C46H94O24 | \* |  |
| 7.40 | 1075.6439 | **538.3267** | 546.8402 |  |  |  |  | C48H98O25 | \* |  |

**Supplementary Table 2b –** Summary of the untargeted metabolites detected in *P. rosea*.

\*Metabolites detected in the medium.

In those metabolites in which several adducts were detected, the most intense ion is indicated in bold.

Identification was done by searching the molecular formulae in the Dictionary of Natural Products (DPN).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| RT (min) | [M+H]+ | [M+2H]2+ | [M+H+NH4]2+ | [M+NH4]+ | [M+Na]+ | [M+3H]3+ | [M+2H+Na]3+ | MF | DPN | Identification by DNP |
| 7.43 | 1119.6802 | **560.3396** | 568.8534 |  |  |  |  | C50H102O26 | \* |  |
| 7.48 | 1163.697 | **582.3524** | 590.8665 |  |  |  |  | C52H106O27 | \* |  |
| 7.53 | 1207.7297 | **604.3653** | 612.8792 |  |  |  |  | C54H110O28 | \* |  |
| 7.57 | 1251.739 | 626.3777 | **634.8919** |  |  |  |  | C56H114O29 | \* |  |
| 7.62 | 1295.7725 |  |  |  |  | 432.5968 | **447.2721** | C58H118O30 | \* |  |
| 7.63 | 1339.7981 |  |  |  |  | **443.2723** |  | C60H122O31 | \* |  |
| 7.67 | 1383.8229 |  |  |  |  | **461.9473** |  | C62H126O32 | \* |  |
| 7.70 | 1427.8461 |  |  |  |  | **476.623** |  | C64H130O33 | \* |  |
| 7.74 | 1471.8663 |  |  |  |  | **496.9717** |  | C66H134O34 | \* |  |
| 7.77 | 1515.8921 |  |  |  |  | **505.9732** |  | C68H138O35 | \* |  |
| 7.82 | 1559.9285 |  |  |  |  | **520.6484** |  | C70H142O36 | \* |  |
| 7.84 | 1603.9453 |  |  |  |  | **535.3240** |  | C72H146O37 | \* |  |
| 8.09 | 255.0650 |  |  |  |  |  |  | C15H10O4 | 88 Hits | Daidzein |
| 8.16 | 241.1044 |  |  |  |  |  |  | C8H12N6O3/ C7H16N2O7 | No hits |  |
| 8.70 | 239.0888 |  |  |  |  |  |  | C8H11N6O3/ C7H14N2O7 | No hits |  |
| 8.92 | 452.2370 |  |  |  |  |  |  | C26H45NO5 | No hits |  |
| 8.92 | 271.0598 |  |  |  |  |  |  | C15H10O5 | 174 Hits | Genistein (biotransformation product from soy) |
| 9.10 | **1301.2517** | 651.1295 |  |  |  |  |  |  |  |  |
| 9.10 | 466.3158 |  |  |  |  |  |  | C26H43NO6/  C27H39N5O2 | 4 Hits/  No hits | No hits from actinomycetes |
| 9.41 | 551.2688 |  |  | **568.2958** | 573.2511 |  |  | C25H42O13 | 4 Hits | No hits from actinomycetes |

**Supplementary Table 2c –** Summary of the untargeted metabolites detected in *P. rosea*.

\*Metabolites detected in the medium.

In those metabolites in which several adducts were detected, the most intense ion is indicated in bold.

Identification was done by searching the molecular formulae in the Dictionary of Natural Products (DPN).

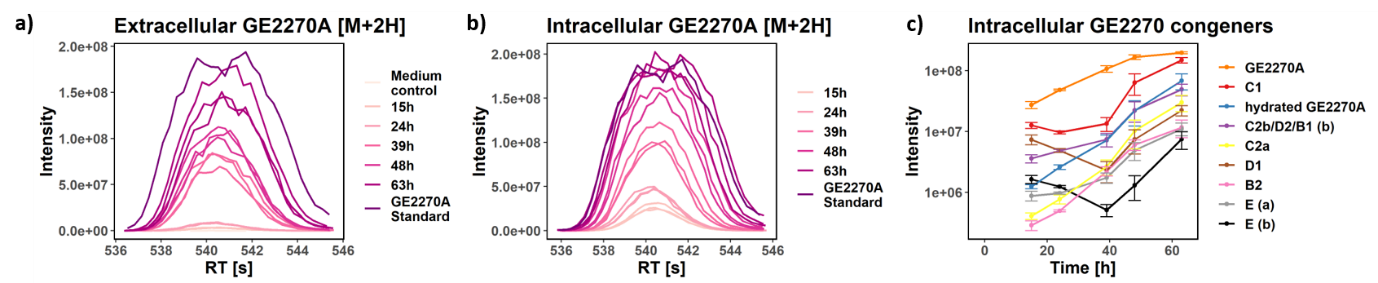
|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| RT (min) | [M+H]+ | [M+2H]2+ | [M+H+NH4]2+ | [M+NH4]+ | [M+Na]+ | [M+3H]3+ | [M+2H+Na]3+ | MF | DPN | Identification by DNP |
| 9.41 | **345.2745** |  |  |  | 367.2564 |  |  | C18H36N2O4 | No hits |  |
| 9.49 | 943.5243 |  |  |  |  |  |  | C44H74N6O16/ C45H70N10O12/ C48H78O18 | No hits |  |
| 10.27 | 201.1120 |  |  |  | **223.0941** |  |  | C10H16O4 | 120 hits | Attiamycin B/ 5-Hydroxy-3-(1-hydroxy-2-methylbutyl)-4-methyl-2(5H)-furanone |
| 10.71 | 245.0782 |  |  |  |  |  |  | C10H8N6O2/  C9H12N2O6 | No hits/ 12 Hits | Uridine/  Antibiotic F 2787 |
| 11.04 | 301.1639 |  |  |  | **323.1462** |  |  | C15H24O6 | 57 hits | No hits from actinomycetes |
| 11.20 | 623.3036 |  |  |  |  |  |  | C28H42N6O10/ C27H46N2O14 | No hits |  |
| 11.20 | 269.1386 |  |  |  |  |  |  | C14H20O5/  C15H16N4O | 54 Hits/  0 hits | No hits from actinomycetes |
| 11.82 | 401.2165 |  |  |  | **423.1988** |  |  | C20H32O8 | 109 hits | No hits from actinomycetes |
| 13.09 | 579.2928 |  |  |  |  |  |  | C30H38N6O6/ C29H42N2O10 | 0 Hits/  4 Hits | 4 (R),5-Dihydro-4-hydroxygeldanamycin/ 4 (S),5-Dihydro-4-hydroxygeldanamycin/ 4,5-Dihydro-15-hydroxygeldanamycin 15-h ydroxy-4,5-dihydrogeldanamycin/4,5-Dihydro-19-hydroxygeldanamycin |
| 13.68 | 668.6185 |  |  |  |  |  |  | C41H81NO5 | 4 Hits | No hits from actinomycetes |

**Supplementary Table 2d –** Summary of the untargeted metabolites detected in *P. rosea*.

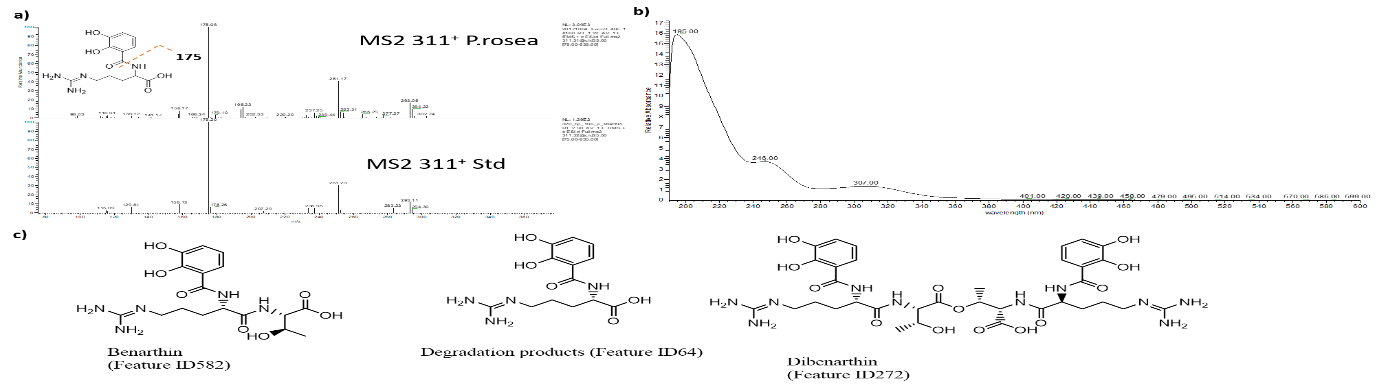
\*Metabolites detected in the medium.

In those metabolites in which several adducts were detected, the most intense ion is indicated in bold.

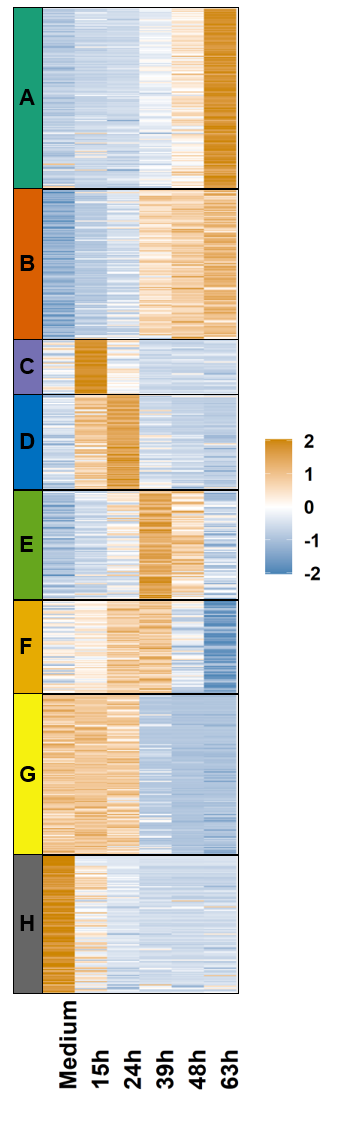
Identification was done by searching the molecular formulae in the Dictionary of Natural Products (DPN).



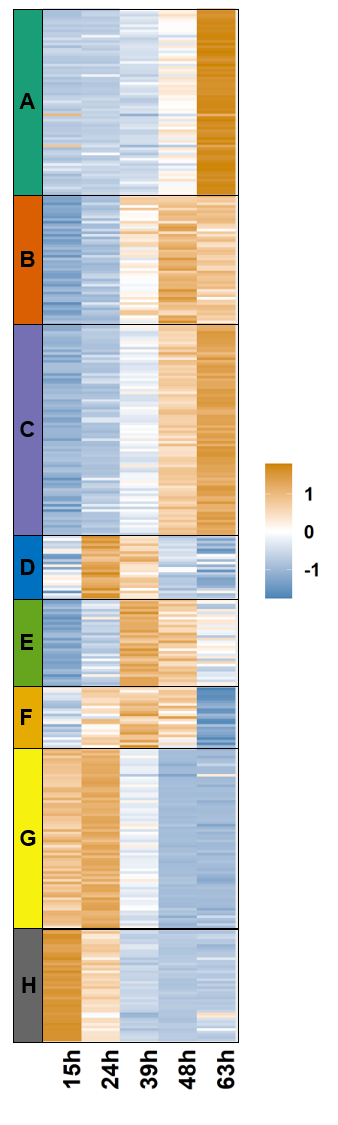
**Supplementary figure 6 – a)** Chromatogram of the main peak associated to GE2270A [M+2H]2+ in the extracellular environment. The comparison with the standard allows a certain identification. **b)** Chromatogram of the main peak associated to GE2270A [M+2H]2+ in the intracellular environment. The comparison with the standard allows a certain identification. **c)** Intensities over time associated with the peaks for which the most likely annotation is one of the GE2270A congeners in the intracellular environment. Data shown in logarithmic scale.



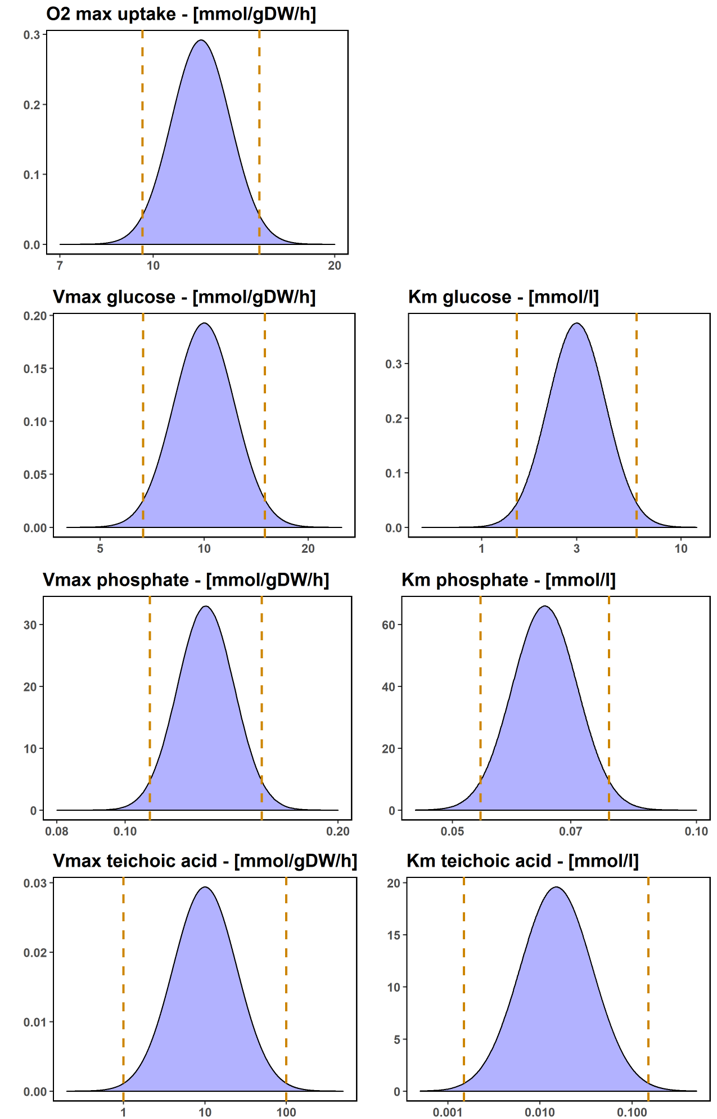
**Supplementary figure 7** – a) MS2 fragmentation spectrum of benarthin; b) UV spectrum benarthin. The UV absorbance is consistence with the one observed for benarthin in Hatsu et al. (1992) Journal of Antibiotics 45,7,1084-1087.



**Supplementary Figure 8 –** Heatmap showing normalized intensity values associated with the peaks, detected in negative mode in the extracellular environment, which levels show a statistically significant change during fermentation. The peaks are clustered into 8 groups with the k-means approach.



**Supplementary Figure 9 –** Heatmap showing normalized intensity values associated with the peaks, detected in negative mode in the intracellular environment, which levels show a statistically significant change during fermentation. The peaks are clustered into 8 groups with the k-means approach.

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**Supplementary figure 10** – probability distributions used to sample the parameters used for the ensemble modelling. Dashed lines represent 95% confidence intervals.