

– Additional file 1 –

**Heterologous production and characterization of a pyomelanin of Antarctic *Pseudomonas* sp. ANT\_H4 – a metabolite protecting against UV and free radicals, interacting with iron from minerals and exhibiting priming properties toward plant hairy roots**

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**Table S1.** GC-MS data (retention times and characteristic ions of mass spectra) of identified steroids and triterpenoids

| Compound                    | Formula                                        | Molecular weight | Retention time [min] | Mass spectrum <i>m/z</i> (relative intensity)                                                              |
|-----------------------------|------------------------------------------------|------------------|----------------------|------------------------------------------------------------------------------------------------------------|
| cholesterol                 | C <sub>27</sub> H <sub>46</sub> O              | 386.6            | 31.06                | 386 (26), 107 (50), 105 (48), 91 (57), 81 (54), 79 (46), 69 (47), 57 (87), 55 (73), 43 (100), 41 (55)      |
| campesterol                 | C <sub>28</sub> H <sub>48</sub> O              | 400.6            | 33.59                | 400 (30), 107 (51), 105 (55), 95 (49), 83 (45), 81 (64), 71 (62), 57 (77), 55 (77), 43 (100), 41 (52)      |
| stigmasterol                | C <sub>29</sub> H <sub>48</sub> O              | 412.6            | 34.52                | 412 (36), 145 (64), 107 (52), 95 (100), 83 (66), 81 (90), 78 (60), 69 (67), 67 (85), 55 (69)               |
| sitosterol                  | C <sub>29</sub> H <sub>50</sub> O              | 414.7            | 36.15                | 414 (29), 145 (54), 107 (59), 105 (60), 95 (54), 91 (49), 81 (57), 57 (68), 55 (70), 43 (100)              |
| isofucosterol               | C <sub>29</sub> H <sub>48</sub> O              | 412.3            | 36.78                | 412 (5), 314 (100), 105 (47), 95 (50), 91 (42), 83 (40), 81 (51), 69 (61), 55 (96), 43 (49)                |
| β-amyrin                    | C <sub>30</sub> H <sub>50</sub> O              | 426.7            | 37.13                | 426 (27), 219 (18), 218 (100), 203 (49), 189 (17), 135 (11), 109 (13), 105 (12), 95 (15), 81 (18), 69 (14) |
| α-amyrin                    | C <sub>30</sub> H <sub>50</sub> O              | 426.7            | 38.62                | 426 (4), 219 (18), 218 (100), 203 (20), 189 (19), 135 (17), 133 (15), 122 (16), 119 (15), 95 (16)          |
| tremulone                   | C <sub>29</sub> H <sub>46</sub> O              | 410.7            | 39.20                | 410 (32), 187 (27), 174 (100), 161 (37), 159 (26), 91 (28), 57 (28), 55 (37), 43 (44), 41 (28)             |
| 24-methylenecycloartanol    | C <sub>30</sub> H <sub>50</sub> O              | 426.7            | 40.50                | 440 (5), 121 (60), 119 (55), 109 (62), 107 (76), 105 (57), 95 (98), 93 (64), 81 (72), 69 (99), 55 (100)    |
| oleanolic acid methyl ester | C <sub>31</sub> H <sub>50</sub> O <sub>3</sub> | 470.1            | 46.37                | 470 (1), 262 (48), 207 (13), 204 (16), 203 (100), 202 (21), 189 (22), 133 (17), 119 (13), 105 (14)         |

**Table S2.** The content of steroids and triterpenoid alcohols in hairy roots tissue

| Compound                  | Content [ $\mu\text{g/g DW} \pm \text{SD}$ ] |                     |                     |                     |
|---------------------------|----------------------------------------------|---------------------|---------------------|---------------------|
|                           | days                                         |                     |                     |                     |
|                           | 7                                            |                     | 14                  |                     |
|                           | C                                            | E                   | C                   | E                   |
| cholesterol               | 19.19 $\pm$ 3.64                             | 14.69 $\pm$ 1.16    | 30.31 $\pm$ 0.78    | 43.12 $\pm$ 1.87    |
| campesterol               | 126.80 $\pm$ 10.05                           | 231.32 $\pm$ 5.96   | 160.69 $\pm$ 6.98   | 190.97 $\pm$ 8.29   |
| stigmasterol              | 848.76 $\pm$ 67.30                           | 1284.40 $\pm$ 33.10 | 1342.80 $\pm$ 58.29 | 1609.26 $\pm$ 69.86 |
| sitosterol                | 492.72 $\pm$ 39.07                           | 799.88 $\pm$ 20.61  | 309.85 $\pm$ 13.45  | 461.86 $\pm$ 20.05  |
| isofucosterol             | 251.18 $\pm$ 19.92                           | 412.29 $\pm$ 10.63  | 299.95 $\pm$ 13.02  | 348.98 $\pm$ 15.15  |
| $\beta$ -amyrin           | 94.31 $\pm$ 7.48                             | 90.02 $\pm$ 2.32    | 25.15 $\pm$ 1.09    | 123.53 $\pm$ 5.36   |
| $\alpha$ -amyrin          | 85.21 $\pm$ 6.76                             | 135.32 $\pm$ 3.49   | 280.63 $\pm$ 12.18  | 361.45 $\pm$ 15.69  |
| tremulone                 | 163.86 $\pm$ 12.99                           | 106.80 $\pm$ 2.75   | 173.77 $\pm$ 7.54   | 380.70 $\pm$ 16.53  |
| 24-methylene-cycloartanol | 72.39 $\pm$ 5.74                             | 118.85 $\pm$ 3.06   | 104.84 $\pm$ 4.55   | 127.14 $\pm$ 5.52   |
| <b>Total</b>              | <b>2154.41</b>                               | <b>3193.56</b>      | <b>2727.99</b>      | <b>3647.00</b>      |

**Table S3.** The content of sterol glycosides in hairy roots tissue

| Compound     | Content [ $\mu\text{g/g DW} \pm \text{SD}$ ] |                   |                    |                    |
|--------------|----------------------------------------------|-------------------|--------------------|--------------------|
|              | days                                         |                   |                    |                    |
|              | 7                                            |                   | 14                 |                    |
|              | C                                            | E                 | C                  | E                  |
| cholesterol  | 66.86 $\pm$ 14.27                            | 50.61 $\pm$ 10.80 | 68.59 $\pm$ 14.64  | 62.99 $\pm$ 13.44  |
| campesterol  | 75.02 $\pm$ 16.01                            | 89.11 $\pm$ 19.01 | 128.20 $\pm$ 27.35 | 121.47 $\pm$ 25.92 |
| stigmasterol | 59.24 $\pm$ 12.64                            | 73.47 $\pm$ 15.68 | 130.15 $\pm$ 27.77 | 81.75 $\pm$ 17.44  |
| sitosterol   | 55.44 $\pm$ 11.83                            | 75.97 $\pm$ 16.21 | 67.37 $\pm$ 14.38  | 58.18 $\pm$ 12.41  |
| <b>Total</b> | <b>256.55</b>                                | <b>289.16</b>     | <b>394.31</b>      | <b>324.39</b>      |

**Table S4.** Free oleanolic acid (OA) content in hairy roots tissue

| Compound | Content [ $\mu\text{g/g DW} \pm \text{SD}$ ] |                      |                    |                        |
|----------|----------------------------------------------|----------------------|--------------------|------------------------|
|          | days                                         |                      |                    |                        |
|          | 7                                            |                      | 14                 |                        |
|          | C                                            | E                    | C                  | E                      |
| OA       | 87.03 $\pm$ 18.57                            | 1616.40 $\pm$ 307.08 | 176.25 $\pm$ 37.61 | 16037.34 $\pm$ 3046.72 |

**Table S5.** Oleanolic acid saponins (OA) released to the medium

| Compound | Content [ $\text{mg/L} \times \text{g DW} \pm \text{SD}$ ] |                 |                 |                 |
|----------|------------------------------------------------------------|-----------------|-----------------|-----------------|
|          | days                                                       |                 |                 |                 |
|          | 7                                                          |                 | 14              |                 |
|          | C                                                          | E               | C               | E               |
| OA       | 1.45 $\pm$ 0.27                                            | 1.98 $\pm$ 0.38 | 2.38 $\pm$ 0.45 | 2.91 $\pm$ 0.69 |

**Table S6.** Oleanolic acid saponins (OA) content in hairy roots tissue.

| Compound | Content [ $\mu\text{g/g DW} \pm \text{SD}$ ] |                    |                    |                    |
|----------|----------------------------------------------|--------------------|--------------------|--------------------|
|          | days                                         |                    |                    |                    |
|          | 7                                            |                    | 14                 |                    |
|          | C                                            | E                  | C                  | E                  |
| OA       | 313.30 $\pm$ 66.85                           | 116.27 $\pm$ 24.81 | 409.77 $\pm$ 87.44 | 139.82 $\pm$ 29.83 |