

## **Additional Material**

### **AMB Express**

#### **Characterization of two *Pantoea* strains isolated from extra-virgin olive oil**

Graziano Pizzolante<sup>1</sup>, Miriana Durante<sup>2</sup>, Daniela Rizzo<sup>3</sup>, Marco Di Salvo<sup>1</sup>, Salvatore Maurizio Tredici<sup>1</sup>, Maria Tufariello<sup>2</sup>, Angelo De Paolis<sup>2</sup>, Adelfia Talà<sup>1</sup>, Giovanni Mita<sup>2</sup>, Pietro Alifano<sup>1#</sup>, Giuseppe Egidio De Benedetto<sup>3#</sup>

<sup>1</sup>Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Via Provinciale Monteroni 165, 73100 - Lecce, Italy; <sup>2</sup>Istituto di Scienze delle Produzioni Alimentari - CNR, Via Provinciale Monteroni 165, 73100 - Lecce, Italy; <sup>3</sup>Laboratory of Analytical and Isotopic Mass Spectrometry, Department of Cultural Heritage, University of Salento, Lecce, Italy

#To whom correspondence should be addressed:

Pietro Alifano, Tel: 0039 0832 298856; e-mail: [pietro.alifano@unisalento.it](mailto:pietro.alifano@unisalento.it)

Giuseppe E. De Benedetto, Tel: 0039 0832 299067; e-mail: [giuseppe.debenedetto@unisalento.it](mailto:giuseppe.debenedetto@unisalento.it)

**Table S1.** Phenotypic traits of *Pantoea septica* OOWS-10, OOYS-10 and related type strain.

Strains: 1, *Pantoea septica* OOWS-10 ; 2, *Pantoea septica* OOYS-10 ; 3, *Pantoea septica* LMG 5345<sup>T</sup> (Brady et al. 2010). +, positive reaction; -, negative reaction, (+); weakly positive reaction; ND, not determined. W, white; Y, yellow; B, beige.<sup>a</sup>

| Tests                        | 1   | 2   | 3   |
|------------------------------|-----|-----|-----|
| Colony colour                | W   | Y   | B   |
| Motility                     | +   | +   | +   |
| <b>Enzymatic activities:</b> |     |     |     |
| Oxidase                      | -   | -   | -   |
| Catalase                     | -   | -   | -   |
| Arginine dehydrolase         | -   | -   | -   |
| Lysine decarboxylase         | -   | -   | -   |
| Ornithine decarboxylase      | -   | -   | -   |
| Phenylalanine deaminase      | (+) | (+) | (+) |
| <b>Production of:</b>        |     |     |     |
| Indole                       | -   | -   | -   |
| H <sub>2</sub> S             | -   | -   | -   |
| Acetoin                      | +   | +   | +   |
| Nitrite                      | +   | +   | ND  |
| <b>Hydrolysis of:</b>        |     |     |     |
| Gelatin                      | -   | -   | ND  |
| Urea                         | -   | -   | -   |
| <b>Acid from:</b>            |     |     |     |
| Amygdalin                    | +   | +   | ND  |
| L-Arabinose                  | +   | +   | +   |
| Dulcitol                     | -   | -   | -   |
| D-Glucose                    | +   | +   | ND  |
| Inositol                     | +   | +   | ND  |
| Lactose                      | +   | +   | +   |
| D-Mannitol                   | +   | +   | +   |
| Melibiose                    | +   | +   | ND  |
| L-Rhamnose                   | +   | +   | +   |
| Sorbitol                     | +   | +   | ND  |
| Saccharose                   | +   | +   | ND  |
| <b>Assimilation of:</b>      |     |     |     |
| L-Arabinose                  | +   | +   | +   |
| Capric acid                  | -   | -   | -   |
| Citric acid                  | -   | -   | +   |
| Gluconate                    | +   | +   | +   |
| D-Glucose                    | +   | +   | +   |
| D-Malate                     | +   | +   | +   |
| D-Maltose                    | +   | +   | +   |
| D-Mannitol                   | +   | +   | +   |
| D-Mannose                    | +   | +   | +   |
| N-acetyl-Glucosamine         | +   | +   | +   |

<sup>a</sup>Data source:

Brady CL, Cleenwerck I, Venter SN, Engelbeen K, De Vos P & Coutinho TA (2010) Int J Syst Evol Microbiol 60:2430-2440.

**Table S2** Phenotypic traits of *Stenotrophomonas rhizophila* OOOWS-2 and OOOWS-9 and related reference strain. The two isolated strains were not able to utilize (as carbon source): adipic acid, D- and L-arabinose, capric acid, dulcitol, erythritol, D-and L-fucose, glycerol, inulin, D-melezitose, methyl- $\alpha$ D-glucopyranoside, methyl- $\alpha$ D-mannopyranoside, methyl- $\beta$ D-xylopyranoside, phenylacetic acid, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, L-sorbose, starch, D-tagatose. Strains: 1, *Stenotrophomonas rhizophila* OOOWS-2; 2, *Stenotrophomonas rhizophila* OOOWS-9; 3, *Stenotrophomonas rhizophila* DSM 14405<sup>T</sup> (Wolf et al. 2002). +, positive reaction; -, negative reaction, (+); weakly positive reaction; ND, not determined. OW, opaque white; Y, yellow.<sup>a</sup>

| Tests                        | 1   | 2   | 3  |
|------------------------------|-----|-----|----|
| Colony colour                | OW  | OW  | Y  |
| Motility                     | +   | +   | +  |
| <b>Enzymatic activities:</b> |     |     |    |
| Oxidase                      | -   | -   | -  |
| $\beta$ -Galactosidase       | +   | +   | ND |
| Arginine dehydrolase         | -   | -   | -  |
| <b>Production of:</b>        |     |     |    |
| Nitrite                      | (+) | (+) | +  |
| <b>Hydrolysis of:</b>        |     |     |    |
| Aesculin                     | +   | +   | +  |
| Gelatin                      | +   | +   | +  |
| Urea                         | -   | -   | -  |
| <b>Acid from:</b>            |     |     |    |
| D-Glucose                    | -   | -   | -  |
| <b>Assimilation of:</b>      |     |     |    |
| N-acetyl-Glucosamine         | +   | +   | +  |
| D-Adonitol                   | +   | +   | ND |
| Amygdalin                    | +   | +   | ND |
| D-Arabitol                   | +   | +   | ND |
| L-Arabitol                   | +   | +   | ND |
| Arbutin                      | +   | +   | ND |
| D-Cellobiose                 | +   | +   | +  |
| Citric acid                  | +   | +   | +  |
| D-Fructose                   | +   | +   | +  |
| D-Galactose                  | +   | +   | +  |
| Gentioniose                  | +   | +   | +  |
| D-Glucose                    | +   | +   | +  |
| Glycogen                     | +   | +   | +  |
| Inositol                     | +   | +   | ND |
| D-Lactose                    | +   | +   | +  |
| D-Lyxose                     | +   | +   | ND |
| D-Malate                     | +   | +   | +  |
| D-Maltose                    | +   | +   | +  |
| D-Mannitol                   | +   | +   | ND |
| D-Mannose                    | +   | +   | +  |
| D-Melibiose                  | +   | +   | ND |
| D-Saccharose                 | +   | +   | +  |
| Salicin                      | +   | +   | +  |
| D-Trehalose                  | +   | +   | +  |
| D-Turanose                   | +   | +   | +  |
| Xylitol                      | +   | +   | ND |
| D-Xylose                     | +   | +   | +  |
| L-Xylose                     | +   | +   | +  |

<sup>a</sup>Data source:

Wolf A, Fritze A, Hagemann M, Berg G (2002) Int J Syst Evol Microbiol 52:1937-1944.

**Table S3** Phenotypic traits of *Pseudomonas* sp. OOBS-2 and related reference strains. The isolated strain was not able to utilize (as carbon source): adipic acid, amygdalin, D-arabinose, arbutin, D-cellobiose, dulcitol, D- and L-fucose, gentiobiose, glycogen, inulin, D-lactose, D-maltose, D-melezitose, methyl- $\alpha$ D-glucopyranoside, methyl- $\alpha$ D-mannopyranoside, methyl- $\beta$ -D-xylopyranoside, phenylacetic acid, D-raffinose, L-rhamnose, salicin, D-sorbitol, L-sorbose, starch, D-tagatose, L-xylose.

Strains: 1, *Pseudomonas* sp. OOBS-2; 2, *Pseudomonas gessardii* CIP 105469<sup>T</sup> (Verhille et al. 1999); 3, *Pseudomonas cedrina* subsp. *cedrina* CIP 105541<sup>T</sup> (Dabboussi et al. 1999; Behrendt et al. 2009). +, positive reaction; -, negative reaction, (+); weakly positive reaction; ND, not determined. B, brown; NP, non pigmented.<sup>a</sup>

| Tests                        | 1   | 2  | 3  |
|------------------------------|-----|----|----|
| Colony colour                | B   | ND | NP |
| Motility                     | +   | +  | +  |
| <b>Enzymatic activities:</b> |     |    |    |
| Oxidase                      | +   | +  | +  |
| $\beta$ -Galactosidase       | -   | -  | -  |
| Arginine dehydrolase         | +   | -  | +  |
| <b>Production of:</b>        |     |    |    |
| Nitrite                      | (+) | +  | +  |
| <b>Hydrolysis of:</b>        |     |    |    |
| Aesculin                     | -   | -  | -  |
| Gelatin                      | +   | -  | +  |
| Urea                         | (+) | -  | -  |
| <b>Assimilation of:</b>      |     |    |    |
| D-Adonitol                   | +   | +  | +  |
| N-acetyl-Glucosamine         | +   | +  | +  |
| D-Arabitol                   | +   | +  | +  |
| L-Arabitol                   | +   | +  | ND |
| Capric acid                  | +   | +  | +  |
| Citric acid                  | +   | +  | +  |
| D-Fructose                   | +   | +  | +  |
| D-Galactose                  | +   | +  | +  |
| D-Glucose                    | +   | +  | +  |
| Inositol                     | +   | +  | +  |
| D-Lyxose                     | +   | -  | +  |
| D-Malate                     | +   | +  | +  |
| D-Mannose                    | +   | +  | +  |
| D-Mannitol                   | +   | +  | +  |
| D-Melibiose                  | +   | -  | -  |
| Phenylacetic acid            | +   | -  | -  |
| Potassium gluconate          | +   | +  | +  |
| Potassium 2-ketogluconate    | +   | ND | +  |
| Potassium 5-ketogluconate    | +   | ND | -  |
| D-Saccharose                 | +   | -  | -  |
| D-Trehalose                  | +   | +  | +  |
| D-Turanose                   | +   | -  | -  |
| Xylitol                      | +   | +  | ND |

<sup>a</sup>Data source:

Behrendt U, Schumann P, Meyer JM, Ulrich A (2009) *Int J Syst Evol Microbiol* 59:1331-1335.

Dabboussi F, Hamze M, Elomari M, Verhille S, Baida N, Izard D, Leclerc H (1999) *Res Microbiol* 150:303-316.

Verhille S, Batda N, Dabboussi F, Hamze M, Izard D, Leclerc H (1999) *Int J Syst Bacteriol* 49:1559-1572.

**Table S4** Phenotypic traits of *Pseudomonas stutzeri* OOOYW-9 and related type strain. The isolated strain was not able to utilize (as carbon source): N-acetyl-glucosamine, D-adonitol, amygdalin, D- and L-arabinose, L-arabitol, arbutin, D-cellobiose, dulcitol, erythritol, D- and L-fucose, D-galactose, gentiobiose, inositol, inulin, D-lactose, D-lyxose, D-mannose, D-melezitose, D-melibiose, methyl- $\alpha$ D-glucopyranoside, methyl- $\alpha$ D-mannopyranoside, methyl- $\beta$ D-xylopyranoside, phenylacetic acid, potassium 2-ketogluconate, D-raffinose, L-rhamnose, D-ribose, D-saccharose, salicin, D-sorbitol, L-sorbose, D-tagatose, D-trehalose, D-turanose, xylitol.

Strains: 1, *Pseudomonas stutzeri* OOOYW-9; 2, *Pseudomonas stutzeri* ATCC 17588<sup>T</sup> (Lehmann and Neumann 1896; Sijderius 1946); 3, *Pseudomonas balearica* DSM 6083<sup>T</sup> (Bennasar et al. 1996). +, positive reaction; -, negative reaction; (+), weakly positive reaction, d, diverse reaction; ND, not determined. Y, yellow; P, pale; NP, non pigmented.<sup>a</sup>

| Tests                        | 1 | 2  | 3  |
|------------------------------|---|----|----|
| Colony colour                | Y | P  | NP |
| Motility                     | + | +  | +  |
| <b>Enzymatic activities:</b> |   |    |    |
| Oxidase                      | + | +  | +  |
| $\beta$ -Galactosidase       | - | -  | -  |
| Arginine dehydrolase         | - | -  | -  |
| <b>Production of:</b>        |   |    |    |
| Nitrite                      | + | +  | +  |
| <b>Hydrolysis of:</b>        |   |    |    |
| Aesculin                     | - | -  | -  |
| Gelatin                      | - | -  | -  |
| Urea                         | - | -  | -  |
| <b>Acid from:</b>            |   |    |    |
| D-Glucose                    | - | -  | -  |
| <b>Assimilation of:</b>      |   |    |    |
| D-Arabitol                   | + | ND | ND |
| Capric acid                  | + | d  | ND |
| Citric acid                  | + | +  | ND |
| D-Fructose                   | + | d  | ND |
| D-Glucose                    | + | +  | ND |
| Glycerol                     | + | +  | ND |
| Glycogen                     | + | -  | ND |
| D-Malate                     | + | +  | +  |
| D-Maltose                    | + | +  | +  |
| D-Mannitol                   | + | d  | -  |
| Potassium gluconate          | + | d  | ND |
| Starch                       | + | +  | +  |
| D-Xylose                     | - | -  | +  |
| L-Xylose                     | - | -  | +  |

<sup>a</sup>Data source:

Bennasar A, Rossellò-Mora R, Lalucat J, Moore ERB (1996) Int J Syst Evol Microbiol 46:200-205.

Lehmann KB, Neumann RO (1896) Atlas und Grundriss der Bakteriologie und Lehrbuch der speciellen bakteriologischen Diagnostik. München.

Sijderius R (1946) Heterotrophe bacterien, die thiosulfaat oxydeeren. Thesis. Amsterdam: University Amsterdam.

**Table S5** Phenotypic traits of *Sporobolomyces roseus* OOPS-1, OOPS-10 and related reference strain. Strains: 1, *Sporobolomyces roseus* OOPS-1; 2, *Sporobolomyces roseus* OOPS-10; 3, *Sporobolomyces roseus* MUCL 30251<sup>T</sup> (Kluyver 1925). +, positive reaction; -, negative reaction, (+), weakly positive reaction; ND, not determined. P, pink.<sup>a</sup>

| Tests                              | 1   | 2   | 3  |
|------------------------------------|-----|-----|----|
| Colony colour                      | P   | P   | P  |
| <b>Assimilation of:</b>            |     |     |    |
| D-Adonitol                         | -   | -   | -  |
| L-Arabinose                        | -   | -   | -  |
| Calcium 2-keto gluconate           | -   | -   | -  |
| D-Cellobiose                       | +   | +   | +  |
| D-Galactose                        | +   | +   | +  |
| D-Glucose                          | +   | +   | +  |
| Glycerol                           | +   | +   | +  |
| Inositol                           | -   | -   | -  |
| D-Lactose                          | -   | -   | -  |
| D-Maltose                          | +   | +   | +  |
| D-Melezitose                       | +   | +   | +  |
| Methyl- $\alpha$ D-glucopyranoside | (+) | (+) | d  |
| N-acetyl-glucosamine               | -   | -   | -  |
| D-Raffinose                        | +   | +   | +  |
| D-Saccharose                       | +   | +   | +  |
| D-Sorbitol                         | (+) | (+) | ND |
| D-Trehalose                        | +   | +   | +  |
| D-Xylitol                          | -   | -   | -  |
| D-Xylose                           | (+) | (+) | d  |

<sup>a</sup>Data source:

Kluyver AJ (1925) Über Spiegelbilder erzeugende Hefenarten und die Hefengattung *Sporobolomyces*. Zentralbl Bakteriol, II Abt 63:1-20.

**Table S6.** Growth of microbial isolates with Alàì<sup>®</sup> extra-virgin olive oil as sole carbon and energy source.

| Microbial isolate                          | Growth on solid M9-OO | Growth on solid M9-glucose | $\mu$ (h <sup>-1</sup> ) in M9-liquid OO <sup>a</sup> | $\mu$ (h <sup>-1</sup> ) in M9-liquid glucose <sup>a</sup> |
|--|-----------------------|----------------------------|---|--|
| <i>Pantoea septica</i> OOWS-10             | +++                   | ++++                       | 0.25  | 0.48   |
| <i>Pantoea septica</i> OOYS-10             | +++                   | ++++                       | 0.30  | 0.50   |
| <i>Stenotrophomonas rhizophila</i> OOOWS-2 | ++                    | ++++                       | 0.15  | 0.53   |
| <i>Stenotrophomonas rhizophila</i> OOOWS-9 | ++                    | ++++                       | 0.18  | 0.56   |
| <i>Pseudomonas stutzeri</i> OOYW-9         | +++                   | ++++                       | 0.20  | 0.45   |
| <i>Pseudomonas cedrina</i> OOBS-2          | +                     | ++++                       | 0.10  | 0.47   |
| <i>Sporobolomyces roseus</i> OOPS-1        | +++                   | ++++                       | 0.40  | 0.51   |
| <i>Sporobolomyces roseus</i> OOPS-10       | +++                   | ++++                       | 0.37  | 0.49   |

<sup>a</sup>  $\mu$ , specific growth rate during the logarithmic phase. Values are means of five independent experiments. +, very poor growth; ++, poor growth; +++, good growth; +++++, very good growth.

**Table S7.** Fatty acid profiles from *Pantoea septica* isolates OOYS-10 and OOWS-10<sup>a</sup>.

| <b>Fatty acid</b>                      | <b><i>Pantoea septica</i> OOWS-10</b> | <b><i>Pantoea septica</i> OOYS-10</b> |
|--|---------------------------------------|---------------------------------------|
|  | % of total fatty acids                |                                       |
| C <sub>14:0</sub>                      | 3.8                                   | 10.1                                  |
| C <sub>15:0</sub>                      | 1.3                                   | 6.7                                   |
| C <sub>16:0</sub>                      | 34.7                                  | 32.9                                  |
| C <sub>18:0</sub>                      | 2.3                                   | 3.3                                   |
| C <sub>17:0</sub> cyclo                | 8.8                                   | 4.3                                   |
| C <sub>16:1<math>\omega</math>7c</sub> | 26.5                                  | 22.3                                  |
| C <sub>18:1<math>\omega</math>7c</sub> | 22.6                                  | 20.4                                  |

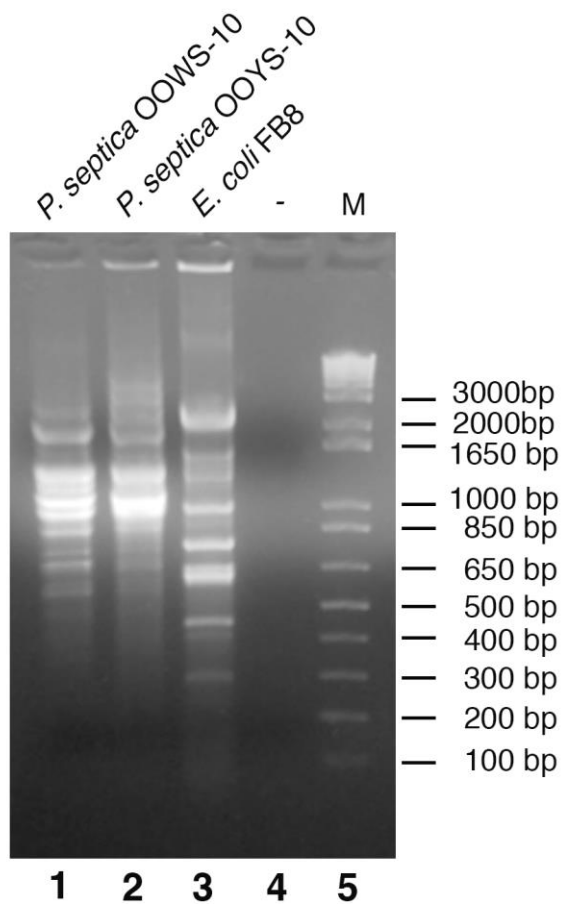
<sup>a</sup>Values are percentage of total fatty acids. Components representing less than 0.5% in all strains were omitted.



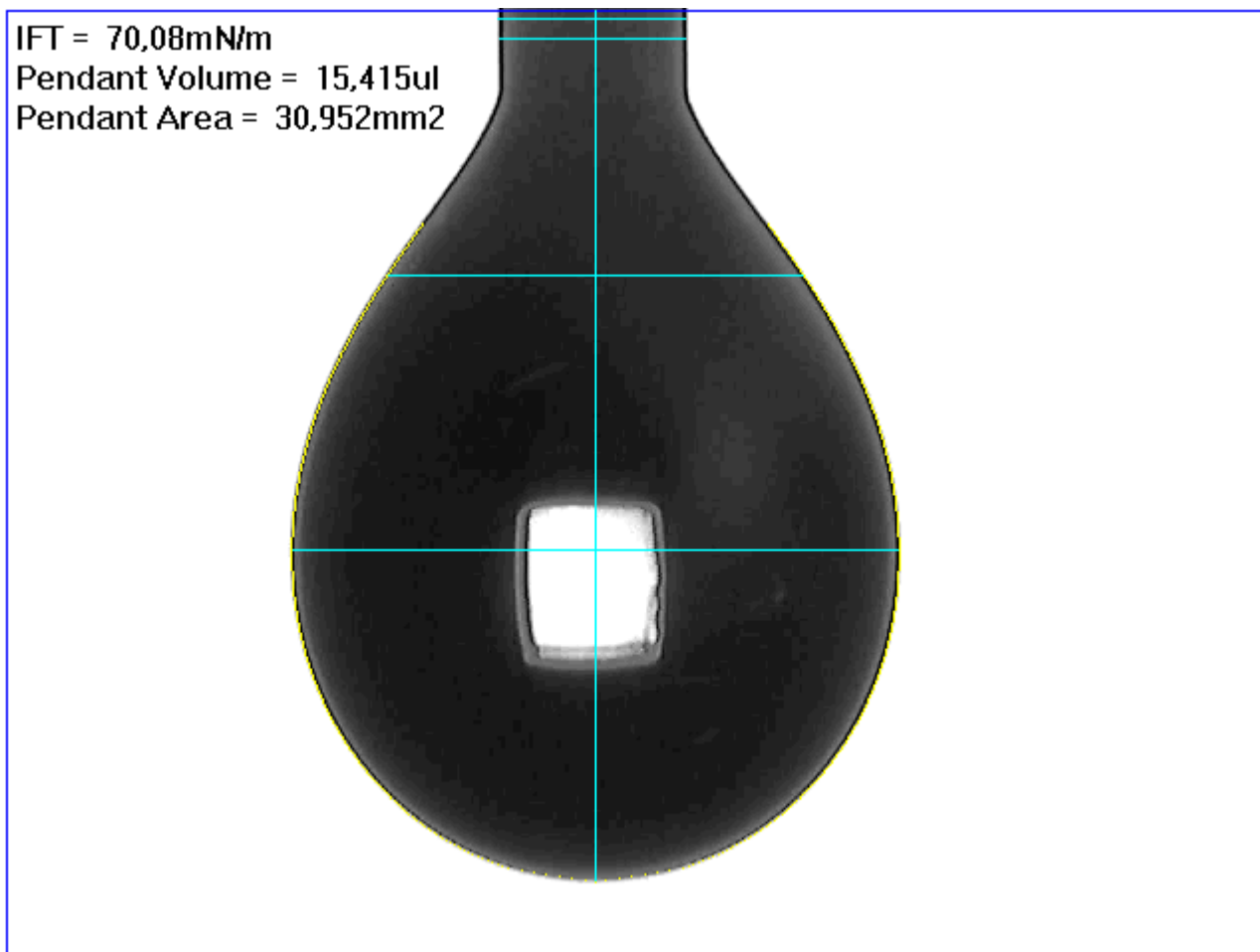
**Table S8.** Carotenoids and isoprenoid quinones from *Pantoea septica* isolates OOYS-10 and OOWS-10<sup>a</sup>

|                            | <i>Pantoea</i>          | <i>septica</i> | <i>Pantoea septica</i> |
|----------------------------|-------------------------|----------------|------------------------|
|                            | OOWS-10                 |                | OOYS-10                |
|                            | $\mu\text{g g}^{-1}$ dw |                |                        |
| <b>Carotenoids</b>         |                         |                |                        |
| Lutein                     | 0.8±0.02                |                | 1.6±0.2                |
| $\beta$ carotene           | 0.7±0.01                |                | 1.2±0.1                |
| <b>Isoprenoid quinones</b> |                         |                |                        |
| Quinone 8 (Q-8)            | 109.9±1.1               |                | 199.9±1.8              |
| Quinone 10 (Q-10)          | 2.3±0.1                 |                | 6.3±0.7                |

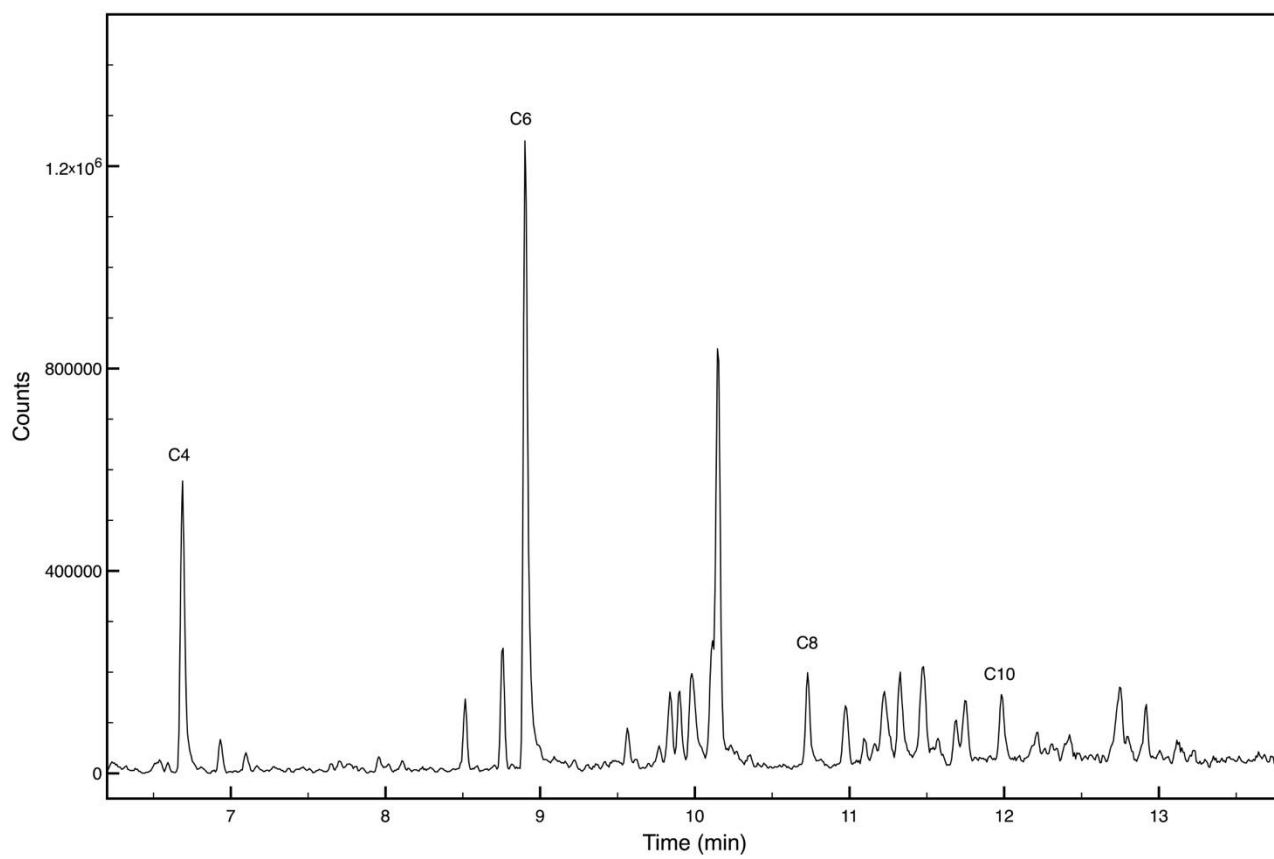
<sup>a</sup>Values are expressed as  $\mu\text{g g}^{-1}$  dw.



**Fig. S1.** BOX-PCR genomic fingerprinting of *P. septica* isolates OOWS-10 and OOYS-10. Bacterial DNA was extracted from *P. septica* isolates OOWS-10 (lane 1) and OOYS-10 (lane 2) and reference *E. coli* K12 strain FB8 (lane 3), and BOX-PCR genomic fingerprinting was carried out as described in the Material and Methods section. Negative control (-, no DNA) and molecular weight ladders (M) were loaded, respectively, in lanes 4 and 5.



**Fig. S2.** Surface tension measurements with pendant drop tensiometry: grayscale image, drop data and resulting surface tension for the PBS control.



**Fig. S3.** Extracted ion chromatogram in the 250-350 mass range where the identified  $\beta$ -hydroxy fatty acids have been labelled.