SUPPLEMENTARY MATERIAL

Volatile profile of Echinacea purpurea plants after in vitro endophyte infection
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The differences in volatile profile of Echinacea purpurea plants not-inoculated (EpC) and inoculated with their endophytes from roots (EpR) and stem/leaves (EpS/L) were analysed and compared by GC-FID/GC–MS in an in vitro model. Non-terpenes and sesquiterpene hydrocarbons were the most abundant classes with an opposite behaviour of EpS/L showing a decreased emission of sesquiterpenes and an increase of non-terpene derivatives. The main compounds obtained from EpS/L were (Z)-8-dodecen-1-ol and 1-pentadecene, while germacrene D and β-caryophyllene were the key compounds in EpC and EpR. For the first time, this work indicates that bacterial endophytes modify the aroma profiles of infected and non-infected in vitro plants of the important medicinal plant E. purpurea. Therefore, our model of infection could permit to select endophytic strains to use as biotechnological tool in the production of medicinal plants enriched in volatile bioactive compounds.

Keywords: volatile organic compounds; Echinacea purpurea; endophytes; plant-bacteria interaction
Experimental

Bacterial cultures and plant material

Bacterial endophytes were separately isolated from the roots (R) and the aerial compartment (stem and leaves, S/L) of *E. purpurea* plants grown at the “Il Giardino delle Erbe”, Casola Valsenio, Italy, as previously reported (Chiellini et al. 2014). Stock cultures were grown at 30°C on tryptone soy agar (TSA; Bio-Rad, USA) solid medium or tryptone soy broth (TSB, Bio-Rad, USA) liquid medium. *E. purpurea* seeds were provided by the “Il Giardino delle Erbe”, Casola Valsenio, Italy. Seeds were surface sterilized in order to prevent any microorganism growth and then dark germinated at 24±1°C in De Wit Culture tubes (LAB Associates BV, The Netherlands) containing 5 ml of Linsmaier & Skoog Medium (LS) including vitamins (Duchefa Biochemie, The Netherlands) (Maggini et al. 2017). After root formation, the seedlings were transferred in sterile capped glass flasks, containing 30 ml of LS solid medium, supplemented with 3% sucrose, and placed in a growth chamber at 24±1°C with a photoperiod of 16 h light a day.

Plant-bacteria interaction model

In this work, we used a modified version of the *in vitro* model developed in Maggini et al. (2017). *Inocula* of bacterial endophytes, isolated from R and S/L compartment of *E. purpurea* plants, were respectively incubated for one and three days at 30 °C. The bacterial suspensions were then adjusted to 8x10⁸ CFU/ml (OD₆₀₀=1). The R and S/L pools generated from 100 μl of each diluted 1:10 OD₆₀₀ suspension cultures were then centrifuged at 4000 rpm for 20 minutes and the pellet suspended in a correspondent volume of 0.9% saline solution. Three 2-months old *E. purpurea* plants were infected with 100 μl of each bacterial pool. Three plants (control) were infected with 100 μl of sterilized saline solution. Plants were then incubated in the growth chamber at 24±1 °C for 30 days. The experiment was performed in triplicate.
**Sample preparation for VOC analysis**

The phytochemical analysis of E. purpurea volatiles was focused only on echinacea leaves (from control plants, plants infected with root endophytes and plants infected with stem/leaf endophytes) since it is known that E. purpurea leaves contain higher amount of terpenoids in comparison with the roots. The analyses of the volatile organic compounds (VOCs) were performed using Supelco SPME device coated with polydimethylsiloxane (PDMS, 100 μm). All samples of *E. purpurea in vitro* plants were analysed immediately after harvesting. The fresh plant material was introduced separately into a glass vial (5 ml, filled up with 1.5 ml of sample) covered with aluminium foil and left to equilibrate for 30 min at room temperature. Multiple experiments were done to find the best condition of SPME analysis and then a double measurement was performed. Fibers were conditioned before analysis, following the manufacturer indications. After the equilibration time, the fibre was inserted into the vial with sample and exposed to the headspace for 20 min at room temperature. Then, the fibre was withdrawn into the needle and transferred to the injector of the GC–MS instrument where the fibre was desorbed for 30 min. All the plant samples were analyzed without culture medium, using leaves of the same size and with similar weight. The time of contact between SPME fiber (PDMS) and each sample was always the same.

**Volatile analysis (GC-FID/GC–MS) and compound identification**

All the analyses were performed following the method previously described (Bandeira Reidel et al. 2016; Giovanelli et al. 2017).

**In planta bacterial growth analysis**

In order to evaluate endophytes multiplication into host tissues, 1.0 g of each sample was homogenized and serially diluted up to $10^{-7}$/ml cells as previously described (Maggini et al.
Five replications of each dilution were plated on TSA medium. Bacterial growth was scored after two, three and four days of incubation of the plates at 30 °C.

**Statistical data analysis**

To evaluate whether the VOCs identified were useful in reflecting the chemical relationships between samples, a principal component analysis (PCA) was conducted (Cserhati 2010). Differences between control and infected plants were compared by Kruskall-Wallis test, followed by individual Mann-Whitney U Tests (MWU). Bonferroni corrected P values were reported: \( P < 0.05 \) was considered significant. The analyses were performed by using the modules present in the PAST program, version 3.15 (Hammer et al. 2001).
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**Table S1.** Bacterial strains from *E. purpurea* stem/leaf (S/L; Maggini et al., 2017) and roots (R) used in this work.

| Strain code | Genus               | GenBank accession of partial 16S rRNA gene sequence |
|-------------|---------------------|-----------------------------------------------------|
| EpS/L1      | Curtobacterium sp.  | KJ642423                                            |
| EpS/L2      | Curtobacterium sp.  | KJ642424                                            |
| EpS/L4      | Microbacterium sp.  | KJ642438                                            |
| EpS/L5      | Bacillus sp.        | KJ642422                                            |
| EpS/L16     | Arthrobacter sp.    | KJ642432                                            |
| EpS/L17     | Staphylococcus sp.  | KJ642469                                            |
| EpS/L18     | Arthrobacter sp.    | KJ642419                                            |
| EpS/L20     | Pseudomonas sp.     | KJ642444                                            |
| EpS/L22     | Staphylococcus sp.  | KJ642476                                            |
| EpS/L25     | Pseudomonas sp.     | KJ642442                                            |
| EpS/L27     | Arthrobacter sp.    | KJ642420                                            |
| EpS/L31     | Rhodobacter sp.     | KJ642453                                            |
| EpS/L32     | Sphingomonas sp.    | KJ642455                                            |
| EpS/L34     | Staphylococcus sp.  | KJ642465                                            |
| EpS/L35     | Arthrobacter sp.    | KJ642421                                            |
| EpS/L37     | Pseudomonas sp.     | KJ642443                                            |
| EpS/L39     | Pseudomonas sp.     | KJ642336                                            |
| EpS/L40     | Staphylococcus sp.  | KJ642466                                            |
| EpS/L43     | Pseudomonas sp.     | KJ642443                                            |
| EpS/L50     | Sphingomonas sp.    | KJ642457                                            |
| EpS/L54     | Frigoribacterium sp.| KJ642425                                            |
| EpS/L59     | Frigoribacterium sp.| KJ642426                                            |
| EpS/L62     | Staphylococcus sp.  | KJ642472                                            |
| EpS/L64     | Frigoribacterium sp.| KJ642427                                            |
| EpS/L65     | Microbacterium sp.  | KJ642440                                            |
| EpS/L70     | Sphingomonas sp.    | KJ642460                                            |
| EpS/L80     | Frigoribacterium sp.| KJ642428                                            |
| EpS/L81     | Agrococcus sp.      | KJ642418                                            |
| EpS/L82     | Methylobacterium sp.| KJ642437                                            |
| EpS/L83     | Sphingomonas sp.    | KJ642462                                            |
| EpS/L84     | Frigoribacterium sp.| KJ642430                                            |
| EpS/L87     | Kineococcus sp.     | KJ778698                                            |
| EpS/L89     | Staphylococcus sp.  | KJ642473                                            |
| EpS/L91     | Frigoribacterium sp.| KJ642429                                            |
| EpS/L95     | Staphylococcus sp.  | KJ642470                                            |
| EpS/L96     | Staphylococcus sp.  | KJ642474                                            |
| Sample Code | Species         | Accession Number |
|-------------|----------------|------------------|
| EpS/L102    | *Staphylococcus* sp. | KJ642464         |
| EpR1        | *Pseudomonas* sp.   | KJ642508         |
| EpR2        | *Pseudomonas* sp.   | KJ642504         |
| EpR5        | *Pseudomonas* sp.   | KJ642499         |
| EpR9        | *Agrobacterium* sp  | KJ642533         |
| EpR10       | *Agrobacterium* sp  | KJ642528         |
| EpR11       | *Agrobacterium* sp  | KJ642484         |
| EpR12       | *Agrobacterium* sp  | KJ642529         |
| EpR14       | *Agrobacterium* sp  | KJ642531         |
| EpR15       | *Agrobacterium* sp  | KJ642530         |
| EpR16       | *Agrobacterium* sp  | KJ642532         |
| EpR17       | *Pseudomonas* sp.   | KJ642498         |
| EpR18       | *Agrobacterium*     | KJ642487         |
| EpR19       | *Pseudomonas* sp.   | KJ642512         |
| EpR21       | *Pseudomonas* sp.   | KJ642524         |
| EpR23       | *Rhizobium* sp.     | KJ642527         |
| EpR24       | *Pseudomonas* sp.   | KJ642503         |
| EpR25       | *Pseudomonas* sp.   | KJ642505         |
| EpR28       | *Pseudomonas* sp.   | KJ642513         |
| EpR29       | *Pseudomonas* sp.   | KJ642511         |
| EpR32       | *Pseudomonas* sp.   | KJ642502         |
| EpR34       | *Pseudomonas* sp.   | KJ642497         |
| EpR36       | *Pseudomonas* sp.   | KJ642510         |
| EpR37       | *Pseudomonas* sp.   | KJ642522         |
| EpR39       | *Pseudomonas* sp.   | KJ642497         |
| EpR41       | *Pseudomonas* sp.   | KJ642519         |
| EpR45       | *Rhizobium* sp.     | KJ778700         |
| EpR58       | *Pseudomonas* sp.   | KJ642491         |
| EpR61       | *Pseudomonas* sp.   | KJ642509         |
| EpR67       | *Achromobacter* sp. | KJ642479         |
| EpR68       | *Achromobacter* sp. | KJ642477         |
| EpR69       | *Achromobacter* sp. | KJ642482         |
| EpR70       | *Achromobacter* sp. | KJ642478         |
| EpR73       | *Pseudomonas* sp.   | KJ642494         |
| EpR74       | *Pseudomonas* sp.   | KJ642495         |
| EpR76       | *Pseudomonas* sp.   | KJ642525         |
| EpR77       | *Pseudomonas* sp.   | KJ778699         |
| EpR81       | *Pseudomonas* sp.   | KJ642492         |
| EpR84       | *Pseudomonas* sp.   | KJ642509         |
|     | Microbacterium sp. | Pseudomonas sp. | Achromobacter sp. | Pseudomonas sp. | Pseudomonas sp. | Microbacterium sp. |
|-----|-------------------|-----------------|------------------|-----------------|-----------------|-------------------|
| EpR85 | KJ642489          | KJ642516        | KJ778696         | KJ642518        | KJ642517        | KJ642490          |

Table S2. Total viable count (TVC) as Colony Forming Units (CFU)/g into the host root (R) and stem/leaf (S/L) plant tissues.

| Host | TVC (log CFU/g) |
|------|----------------|
|      | R              | S/L            |
| EpR  | 6.40 ± 5.27    | 5.60 ± 4.35    |
| EpS/L| 6.01 ± 5.64    | 6.97 ± 5.05    |

Table S3. Chemical classes of aroma profile from *E. purpurea* control and infected plants.

| Class of Components | Class° | Samples (%) |
|--------------------|--------|-------------|
|                    | EpC    | EpR         | EpS/L        |
| Non terpenes       | NT     | 29.25       | 21.80        | 44.20          |
| Monoterpene hydrocarbons | MH | -- | 1.9 | -- |
| Sesquiterpene hydrocarbons | SH | 66.00 | 73.70 | 53.15 |
| Oxygenated sesquiterpenes | OS | 0.35 | -- | -- |
| Apocarotenoids | AC     | 0.30       | --            | --             |
| Total              | 95.90  | 97.40       | 97.35         |

EpC = control plants; EpR = plants infected with endophytes from roots; EpS/L = plants infected with endophytes from stem/leaves