Supporting information

Activities and Prevalence of Proteobacteria Members Colonizing *Echinacea purpurea* Fully Account for Macrophage Activation Exhibited by Extracts of This Botanical

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Materials and Methods

Cultures to determine isolate activity

Isolates were grown in 200 µL media in sterile 96-well microplates, incubated at 30°C and 200 rpm. Slow growing isolates were grown in a greater volume (400 µL) in sterile 2 mL microcentrifuge tubes under the same conditions. All bacteria were grown on tryptic soy broth (TSB), with the exception of isolates identified as *Pseudoxanthomonas*, *Rhizobium*, and *Dechloromonas hortensis*, which were grown on nutrient broth (NB), and *Stappia stellulata*, which was grown on Marine broth. Cultures were grown to an optical density of at least 0.5 at a wavelength of 600 nm. From each culture, a 60 µL aliquot was collected and stored at -20°C until analysis of macrophage activity. An additional 26 µL aliquot was collected and preserved for cell enumeration by mixing with 74 µL 95% ethanol to a final concentration of 70% ethanol and stored at 4°C until analysis. Aliquots were collected from uninoculated TSB, NB, Lactobacillus MRS, and Marine broth to use as blank controls in subsequent analyses.

Since both live and dead bacteria can elicit an immune response, a non-culture based method was used to determine the number of bacterial cells in each culture. Briefly, 16 µL were taken from each 70% ethanol aliquot and centrifuged at 10000 × *g* for 5 min. DNA was extracted from the resulting pellets and bacterial cells enumerated by PCR as described previously for bacteria in *Echinacea* tissue (Pugh et al., 2013).

Calculating total activity exhibited by the bacteria within the plant material

The amount of activity exhibited by plant extracts that was contributed by the bacteria within the plant material was estimated as follows: The prevalence of individual taxa in each plant sample was calculated by multiplying the estimated total bacterial load by the percent representation of

the taxa within the bacterial community. Activity contributed by each taxa was then calculated from its prevalence and EC_{25} value. The sum of the calculated activities contributed by the taxa comprising the community composition represents the total bacteria-dependent activity. A hypothetical example showing these calculations for a plant material containing only two bacterial endophytes is provided below.

• Plant extract EC_{25} = 100 μ g/mL. Based on the estimated total bacterial load and species structure, it is determined that this concentration of plant extract added to the culture well contains 500 bacteria from taxa A and 500 bacteria from taxa B.

• EC_{25} for taxa A = 5000 bacterial cells/well and EC_{25} for taxa B = 555 bacterial cells/well.

• Activity contributed by taxa A = [(500 bacteria/well ÷ 5000 (A EC_{25})] × 100 = 10% of the plant extract activity.

• Activity contributed by taxa B = [(500 bacteria/well ÷ 555 (B EC_{25})] × 100 = 90% of the plant extract activity.

• Sum of calculated activities contributed by both taxa equals 100%. This indicates that the identified bacteria account for all of the activity (EC_{25} value) exhibited by this plant extract.

Table 1S The amount of *in vitro* macrophage stimulatory activity exhibited by *E. purpurea* that is from different Proteobacteria subphyla. *E. purpurea* aerial plant material was obtained from fresh, wild, and cultivated plants and from dried material from six commercial growers. “Percent of activity due to Proteobacteria subphyla” represents the percent of macrophage stimulatory activity exhibited by an *E. purpurea* extract that can be accounted for by the sum of the activities contributed by the specified bacterial taxa contained in that plant material.

	Percent of activity due to Proteobacteria subphyla:				
Freshly harvested	**Gamma**	**Alpha**	**Beta**	**Delta**	**Unclassified**
Plant 1 (wild)	15	53	33	0	2
Plant 2 (wild)	35	43	11	0	1
Plant 3 (wild)	13	115	2	0	1
Plant 4 (wild)	46	46	2	0	1
Plant 1 (cultivated)	26	12	25	0	4
Plant 2 (cultivated)	7	15	6	0	0
Plant 3 (cultivated)	35	44	7	0	2
Plant 4 (cultivated)	38	31	12	0	0
Commercial material					
Company A	—	—	—	—	—
Company B	231	68	49	0	2
Company C	23	1	2	0	0
Company D	33	6	52	0	0
Company E	72	14	10	0	0
Company F	77	1	3	0	0
Mean (SE)	50 (16.1)	35 (9.0)	16 (4.9)	0 (0.0)	1 (0.3)

Table 2S Community composition (% bacteria identified) in commercial *E. purpurea* material at the genus level. Taxa listed make up > 2% of the total community in at least one sample. Taxa described as unclassified could only be identified down to the family, order, class, subphylum, phylum, or domain level. Gram-negative taxa are shaded in gray.

	Company				
	B	**C**	**D**	**E**	**F**
Pseudomonas	14.7	13.7	2.0	13.6	26.8
Pantoea	3.5	23.8	6.9	15.4	8.7
Bacillus	4.7	10.3	1.4	9.1	12.4
Flavobacterium	0.0	0.1	19.8	0.0	1.6
Pedobacter	3.1	2.7	3.0	5.4	6.6
unclassified Oxalobacteraceae	1.2	0.9	1.8	10.5	1.4
Hymenobacter	4.4	0.5	7.3	2.0	0.8
Diaphorobacter	2.0	1.7	8.5	0.4	0.4
Rathayibacter	5.4	0.8	4.9	1.4	0.2
Paenibacillus	0.2	5.0	2.0	2.7	2.7
Methylobacterium	5.6	0.7	1.2	3.2	0.5
unclassified Enteric bacteria cluster	0.8	1.7	0.8	1.9	5.1
Exiguobacterium	0.0	0.5	0.3	0.1	9.1
Erwinia	1.6	4.1	0.6	1.5	1.8
Massilia	0.5	0.6	0.6	5.4	1.0
Stenotrophomonas	3.2	0.6	2.3	0.1	1.0
Xanthomonas	0.9	4.5	0.4	0.4	0.4
Streptococcus	0.5	4.5	1.2	0.0	0.3
Stappia	2.0	0.1	0.9	2.8	0.2
Sphingomonas	4.1	0.1	0.4	1.2	0.1
unclassified Enterobacteriaceae	1.9	2.1	0.3	0.8	0.8
Citrobacter	0.0	0.2	0.0	3.1	2.4
Mucilaginibacter	1.2	0.3	2.9	0.2	0.8
Acidovorax	0.6	0.8	3.4	0.2	0.2
Curtobacterium	0.2	0.4	1.2	2.7	0.4
Carnobacterium	4.8	0.0	0.0	0.0	0.0
Clavibacter	0.5	0.5	2.6	0.6	0.5
Terribacillus	1.5	0.1	0.0	2.2	0.5
Total	69.0	81.2	76.5	86.7	86.7

Table 3S Community composition (% bacteria identified) in freshly harvested (wild and cultivated) *E. purpurea* material at the genus level. Taxa listed make up > 2% of the total community in at least one sample. Taxa described as unclassified could only be identified down to the family, order, class, subphylum, phylum, or domain level. Gram-negative taxa are shaded in gray.

	Wild plants				**Cultivated plants**			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Methylobacterium	18.7	19.1	15.5	26.3	1.4	2.4	35.9	15.4
Hymenobacter	6.0	9.2	12.7	9.7	30.4	16.1	13.4	15.4
unclassified Gammaproteobacteria	2.9	11.8	11.4	15.0	1.9	2.6	2.2	1.2
unclassified Comamonadaceae	6.7	4.6	2.9	0.6	5.7	8.5	0.9	1.2
unclassified Proteobacteria	2.4	2.7	3.9	1.6	4.7	1.9	2.3	0.9
unclassified Bacteria	3.0	3.1	1.6	0.7	3.4	3.7	2.9	1.0
Rhizobium	2.4	3.7	20.1	4.5	0.6	3.2	1.9	2.4
unclassified Xanthomonadaceae	2.4	0.0	0.9	1.4	0.0	0.7	0.0	0.1
unclassified Rhizobiales	3.0	1.1	3.4	3.3	0.2	3.3	2.2	2.6
Massilia	7.5	4.1	1.3	1.1	5.0	1.5	2.9	11.3
Variovorax	1.6	1.9	0.9	1.1	1.7	14.5	0.1	0.0
unclassified Oxalobacteraceae	10.9	2.7	0.5	0.4	7.1	0.6	1.3	2.2
unclassified Betaproteobacteria	0.9	0.9	0.2	0.2	2.9	0.3	2.3	0.7
unclassified Burkholderiales	2.7	1.8	0.7	0.6	2.5	1.8	0.3	1.2
unclassified Enteric bacteria cluster	0.7	6.7	1.8	2.6	0.2	0.3	3.1	3.6
unclassified Alphaproteobacteria	1.6	3.2	1.8	2.8	0.5	1.2	2.8	2.1
Pseudomonas	1.6	2.9	0.6	0.4	9.6	1.2	2.6	0.2
unclassified Sphingobacteriaceae	0.0	1.0	0.6	0.2	1.9	2.4	0.8	1.0
unclassified Enterobacteriaceae	0.0	2.9	0.4	1.3	1.2	4.3	2.2	4.0
Providencia	0.0	0.0	0.0	0.0	0.0	0.1	6.0	9.5
Erwinia	0.0	0.0	1.1	11.8	0.0	0.0	0.0	0.0
Stappia	0.4	0.4	4.3	3.0	0.0	0.2	0.0	0.3
Pedobacter	0.1	0.2	0.3	0.0	0.1	5.2	0.9	0.2
Sphingobacterium	0.0	0.2	0.3	0.0	0.1	4.4	0.2	2.1
Enterobacter	0.0	0.3	0.0	0.0	0.4	0.5	0.0	3.5
Deinococcus	0.1	0.0	0.0	0.0	2.9	1.0	0.0	0.1
Mitsuaria	2.6	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Total	78.3	85.0	87.4	88.9	84.4	81.7	87.1	82.2