Received date: 15-Nov-2013

Accepted date: 12-Dec-2013

Transcriptional profiling of Gram-positive *Arthrobacter* in the phyllosphere: induction of pollutant degradation genes by natural plant phenolic compounds

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Running title: *Arthrobacter* gene expression on leaf surfaces

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1462-2920.12375

SUMMARY

Arthrobacter chlorophenolicus A6 is a Gram-positive, 4-chlorophenol degrading soil bacterium that was recently shown to be an effective colonizer of plant leaf surfaces. The genetic basis for this phyllosphere competency is unknown. In this paper, we describe the genome-wide expression profile of *A. chlorophenolicus* on leaves of common bean (*Phaseolus vulgaris*) compared to growth on agar surfaces. In phyllosphere-grown cells, we found elevated expression of several genes known to contribute to epiphytic fitness, for example those involved in nutrient acquisition, attachment, stress response and horizontal gene transfer. A surprising result was the leaf-induced expression of a subset of the so-called *cph* genes for the degradation of 4-chlorophenol. This subset encodes the conversion of the phenolic compound hydroquinone to 3-oxoadipate, and was shown to be induced not only by 4-chlorophenol but also hydroquinone, its glycosylated derivative arbutin, and phenol. Small amounts of hydroquinone, but not arbutin or phenol, were detected in leaf surface washes of *P. vulgaris* by gas chromatography–mass spectrometry. Our findings illustrate the utility of genomics approaches for exploration and improved understanding of a microbial habitat. Also, they highlight the potential for phyllosphere-based priming of bacteria to stimulate pollutant degradation, which holds promise for the application of phylloremediation.

38

39 INTRODUCTION

40 Plant leaf surfaces (collectively referred to as the phyllosphere) provide a large and unique habitat
41 for microbial life. Even though the phyllosphere can be a harsh and stressful environment with rapid
42 changes in temperature, relative humidity and harmful ultraviolet radiation, it is typically colonized
43 by large populations and diverse communities of bacteria, fungi, and other microorganisms (Leveau,
44 2006; Meyer and Leveau, 2012; Vorholt, 2012; Rastogi *et al.*, 2013). A relatively understudied aspect
45 of phyllosphere microbiology is the ability of several phyllosphere bacteria to degrade aromatic
46 pollutants, such as toluene, phenol and phenanthrene (De Kempeneer *et al.*, 2004; Sandhu *et al.*,
47 2007; Waight *et al.*, 2007; Sandhu *et al.*, 2009; Yutthammo *et al.*, 2010), as well as various foliar
48 pesticides (Ning *et al.*, 2010; Zhou *et al.*, 2011). Such bacteria have potential towards
49 phylloremediation (Sandhu *et al.*, 2007), i.e. the removal of foliage-associated organic pollutants by
50 members of the phyllosphere community.

51

52 Representatives of the genus *Arthrobacter* (high GC Gram-positive, family Micrococcaceae, order
53 Actinomycetales, class Actinobacteria, phylum Actinobacteria) are well known for their exceptional
54 resistance to various stresses and their ability to degrade a wide variety of organic pollutants
55 (Mongodin *et al.*, 2006). *Arthrobacter* species are common members of phyllosphere communities
56 (Rastogi *et al.*, 2012) and they were recently shown to exhibit a high level of epiphytic fitness
57 (Scheublin and Leveau, 2013). This combination of properties make *Arthrobacter* a target genus for
58 studies on phylloremediation.

59

60 Little is known about the genes underlying phyllosphere competency in *Arthrobacter*. From the few
61 studies that are available for other bacterial genera (Marco *et al.*, 2005; Gourion *et al.*, 2006; Fink *et*
62 *al.*, 2012; Yu *et al.*, 2013), it has become clear that phyllosphere exposure affects the expression of
63 genes involved in motility, chemotaxis, biofilm formation and attachment, as well as genes related to

nutrient starvation, and osmotic, oxidative and desiccation stresses. For the plant pathogen *Pseudomonas syringae*, genes involved in virulence, such as toxin production genes also showed different transcript levels (Yu *et al.*, 2013). The proteome of *Methylobacterium extorquens* featured several induced proteins during epiphytic growth, including enzymes involved in methanol utilization, stress proteins, and regulatory proteins (Gourion *et al.*, 2006). In a metaproteomic study of the total phyllosphere community, proteins related to carbohydrate transport, carbon and nitrogen metabolism, motility and stress were among the most abundantly expressed (Delmotte *et al.*, 2009).

In the study we describe here, we employed whole-genome transcriptome arrays of *Arthrobacter chlorophenolicus* A6 to gain a better understanding of its phyllosphere competency. Strain A6 is a 4-chlorophenol-degrading isolate from soil (Westerberg *et al.*, 2000). Recently, strain A6 was demonstrated to be an excellent phyllosphere colonizer (Scheublin and Leveau, 2013). It has been studied extensively with regard to the genes that contribute to 4-chlorophenol degradation and its complete genome sequence is available (Nordin *et al.*, 2005; Unell *et al.*, 2009). We designed transcriptome arrays to investigate which genes were induced in the phyllosphere of common bean (*Phaseolus vulgaris*) as compared to growth on agar surfaces. Since water availability is an important factor in phyllosphere survival and activity (Beattie, 2011), we included both high and low relative humidity treatments for the phyllosphere-grown cells. In addition, we compared the expression profiles of strain A6 on agar surfaces with or without 4-chlorophenol. These analyses revealed an unexpected connection between epiphytic growth and 4-chlorophenol exposure, which we followed up on in more detail by quantifying the expression of 4-chlorophenol degradative genes in response to plant phenolic compounds using reverse-transcriptase real-time PCR and identification of naturally occurring phenolic compounds on bean leaf surfaces by gas chromatography–mass spectrometry (GC-MS).

88

89 RESULTS

90 Using custom-made microarrays, we determined and compared the transcriptional profiles of *A.*
91 *chlorophenolicus* A6 cells that were recovered in quadruplicate from 1) bean leaf surfaces after
92 incubation for 48 h at 97% relative humidity (PhylH, for Phyllosphere High humidity), 2) bean leaf
93 surfaces after incubation for 48 h, of which the first 24 hours were at 97% relative humidity and the
94 second 24 hours at 50% relative humidity (PhylL, for Phyllosphere Low humidity), 3) the surface of a
95 1/10 strength tryptic soy agar plate supplemented with 1 mM 4-chlorophenol after incubation for 48
96 h at 97% relative humidity (A+CP, for Agar plus 4-ChloroPhenol), and 4) the surface of a 1/10 strength
97 tryptic soy agar plate after incubation for 48 h at 97% relative humidity (A-CP, for Agar without 4-
98 ChloroPhenol).

99

100 Clustering of the transcriptome microarray data showed a clear separation between phyllosphere
101 samples (PhylH and PhylL) on the one hand and agar samples (A+CP and A-CP) on the other (Figure
102 1). Among the agar samples, A+CP replicates also clearly separated from A-CP replicates. Such a
103 separation was less obvious for the PhylH and PhylL samples (Figure 1). We observed a strongly
104 positive correlation between the expression of individual genes at high and low relative humidity in
105 the phyllosphere (Figure 2a), which suggests a similar bacterial experience under these two
106 conditions. In fact, for only three genes that were differentially expressed in the phyllosphere
107 compared to growth on agar, the expression was significantly different ($p < 0.05$) between the PhylH
108 and PhylL samples. The first, AchI_4566, is part of the so-called *cph* gene cluster for 4-chlorophenol
109 degradation and will be discussed in more detail below. Like AchI_4566, AchI_0518 was expressed
110 more highly under conditions of low humidity. It encodes a putative substrate transporter belonging
111 to the major facilitator superfamily MFS_1, with high sequence similarity to proline/betaine
112 transporters of other *Arthrobacter* species. The expression of the third gene, AchI_2563, was lower
113 under conditions of low humidity compared to high humidity. Its predicted product is also annotated

as a MFS_1 protein. With 41% sequence similarity to a valanimycin resistance gene of *Streptomyces viridifaciens* (accession number AAN10244), AchI_2563 might be involved in antibiotic efflux, but why its expression is suppressed at low relative humidity is not evident.

A weak but significant positive correlation was observed between the PhylH and A+CP treatment (Figure 2b) and between the PhylL and A+CP treatment (not shown), suggesting that there were more genes that responded in the same way to these two conditions than there were genes that responded in opposite directions. Included in this list of genes are several that contribute to the degradation of 4-chlorophenol, as will be detailed below. A complete list of differential gene expression is given in Table S1. A number of specific differences (Table 1) will be highlighted further below, organized primarily by gene function. Unless otherwise noted, any reference to up- or down-regulation of genes is relative to gene expression on agar surfaces in the absence of 4-chlorophenol (i.e. A-CP).

Chlorophenol degradation genes

All but one of the 11 genes in the *cph* gene cluster (AchI_4564-4574) (Nordin *et al.*, 2005) were induced in response to 4-chlorophenol (Figure 3, black bars). The exception was AchI_4571 (*cphR*), which is annotated as a transcriptional activator protein. We found that three genes in the cluster, AchI_4564, AchI_4565 and AchI_4566, were also induced during growth on bean leaf surfaces (Figure 2; Figure 3, white and grey bars). These genes form a putative operon coding for the three-step conversion of hydroquinone to 3-oxoadipate as follows: AchI_4564 codes for CphC-II converting hydroquinone to hydroxyquinol (Nordin *et al.*, 2005), AchI_4566 codes for CphA-II which is a predicted hydroxyquinol 1,2-dioxygenase, and the product of AchI_4565 is CphF-II which is predicted to catalyze the conversion of maleylacetate to 3-oxoadipate. Not induced in the phyllosphere were AchI_4570 (*cphB*) and AchI_4573 (*cphC-I*), both of which are predicted to code for the production of

hydroquinone from 4-chlorophenol, or AchI_4569 and AchI_4574, whose gene products are CphA-I and CphF-I, presumed paralogs of CphA-II and CphF-II, respectively.

We confirmed by RT-qPCR that AchI_4564 and AchI_4566, but not AchI_4569, were induced during epiphytic growth (Figure 4a). In liquid culture, the expression of AchI_4569 was stimulated only by 4-chlorophenol (1 mM), while AchI_4564 and AchI_4566 were induced by 4-chlorophenol (1 mM), phenol (1 mM), hydroquinone (1 mM, 10 μ M, and 100 nM, but not 10 nM) and arbutin (1 mM and 10 μ M, but not 100 nM). No induction was observed upon exposure to 1 mM concentrations of the following (plant) phenolic compounds: 4-hydroxybenzoic acid, protocatechuic acid, coumaric acid, caffeic acid, ferulic acid, quercetin, catechol, or resorcinol (Figure 4b). Arbutin is a glycosylated form of hydroquinone and has been identified in leaf extracts of several plant species (see DISCUSSION). Using GC-MS, we were unable to detect arbutin in leaf washes from bean plants that were used in our experiments. However, we consistently found hydroquinone in these leaf washes in an amount of 1.5 ng per leaf averaged (Figure S1). Other phenolic compounds that we identified in at least one of three replicate samples included caffeic acid, ferulic acid, 4-hydroxybenzoic acid, and protocatechuic acid; we did not detect 4-CP in the leaf washes.

Nutrient acquisition

Besides various phenolics, several other compounds were detected by GC-MS analysis of the bean leaf surface washes. These included compounds that one expects to find in the phyllosphere environment, such as intermediates of the citric acid cycle, tartrate, glycol, pentoses, hexoses, disaccharides, polar amino acids, long chain alcohols, and fatty acids (Table S2). Glucose, along with fructose and sucrose, is known to be the most abundant carbon source on bean leaf surfaces (Leveau and Lindow, 2001), but the microarray data did not support the notion that *A. chlorophenolicus* A6 utilizes these sugars during epiphytic growth. Perhaps the expression of genes for catabolism of glucose went undetected due to the fact that growth on leaf surfaces was compared to growth on

165 tryptic soy agar, which features glucose as the main carbon source. However, the microarray data did
166 show phyllosphere-induced expression of genes involved in the acquisition of other nutrients,
167 specifically phosphate, nitrogen, iron, and sulphur, as explained below.

168
169 Cluster AchI_0362-0365 codes for subunits of a phosphate ABC transporter system and was highly
170 expressed in the phyllosphere. The fold-change in transcript level for AchI_0362, which encodes a
171 periplasmic phosphate-binding protein, was highest of all differentially expressed phyllosphere genes
172 (Table S1). AchI_2231 and AchI_2232, which encode a nitrogen regulatory protein P-II and an
173 ammonium uptake transporter, respectively, were also induced in the phyllosphere. Under nitrogen
174 limiting conditions, the P-II protein is involved in deadenylation of glutamine synthetase type I, which
175 activates the enzyme. Glutamine synthetase type III, encoded by AchI_3525, also had an increased
176 expression in the phyllosphere, as did the gene for a second ammonium transporter (AchI_3518),
177 located several genes upstream. Ammonium transporters, the P-II protein and glutamine synthetases
178 are all key enzymes in the acquisition of nitrogen at low ammonium concentrations (Javelle *et al.*,
179 2004).

180
181 Cluster AchI_3731-3735 includes genes involved in iron uptake and showed increased expression in
182 the phyllosphere, as well as in response to 4-chlorophenol. The cluster features a periplasmic binding
183 protein, two transport system permease proteins and an ABC transporter which are all part of an iron
184 complex transport system. In addition, the cluster codes for a siderophore-interacting protein. In
185 close proximity, three genes predicted to encode for a membrane-associated ferric iron reductase
186 (AchI_3724-3726) also showed increased levels of expression in the phyllosphere.

187
188 Several sulfur assimilation genes were induced in the phyllosphere, as well as on agar with 4-
189 chlorophenol. These genes included AchI_1844-1849 which code for components of the sulfur
190 assimilation (SUF) system iron-sulfur (FeS) cluster. The SUF system operates under iron starvation

191 and oxidative stress (Outten *et al.*, 2004). Genes AchI_2817-2820 code for assimilatory sulfate
192 reduction via 3'-phosphoadenylylsulfate (PAPS) and were expressed more highly in the phyllosphere,
193 as were genes coding for incorporation of sulfide, the product of the PAPS assimilation pathway, i.e.
194 5-methyltetrahydropteroyltriglutamate/homocysteine S-methyltransferase (AchI_1726) and cysteine
195 desulfurase (AchI_2389).

196 197 Attachment and motility

198 The AchI_2643-2650 gene cluster is predicted to be involved in surface attachment. The genes
199 encode Flp pilus assembly proteins. Flp pili belong to a subfamily of the type IV pilin family which
200 mediates unspecific attachment to surfaces and the formation of micro-colonies (Kachlany *et al.*,
201 2001; Pelicic, 2008). Most genes of this cluster were highly expressed in the phyllosphere.

202 Interestingly, this gene cluster is flanked upstream by another Flp pili cluster with a paralogous set of
203 genes, but those genes were not induced in the phyllosphere. AchI_4158, coding for a prepilin
204 peptidase, was expressed 4-fold higher in the phyllosphere than on agar surfaces, while other pilin
205 associated genes were not differentially expressed. There is evidence that the presence of type IV pili
206 increases the phyllosphere fitness of bacteria (Suoniemi *et al.*, 1995; Roine *et al.*, 1998).

207
208 AchI_0159 and AchI_2284 are two other genes with elevated expression in the phyllosphere and with
209 involvement in attachment. Their predicted gene products contain a fasciclin-like (FAS1) domain,
210 which is found in proteins from bacteria to mammals and considered an ancient cell adhesion
211 domain (Ulstrup *et al.*, 1995). This finding is in agreement with a metaproteomic study where
212 bacterial proteins with a fasciclin domain were consistently recovered from the phyllosphere of
213 soybean, clover and *Arabidopsis* (Delmotte *et al.*, 2009).

214
215 The *A. chlorophenolicus* genome contains a cluster of 31 genes involved in flagellar synthesis
216 (AchI_2971-3001). These genes were collectively induced in response to 4-chlorophenol, while only

217 two of them were significantly higher expressed in the phyllosphere, namely Achl_2990 and
218 Achl_2991, encoding a flagellar motor switch protein and a flagellar M-ring protein, respectively.

219

220 Stress

221 Gene cluster Achl_3258-3266 was one of the most highly expressed in the phyllosphere. This cluster
222 consists mainly of genes coding for hypothetical proteins, two of which (Achl_3258 and Achl_3265)
223 contain an Asp23 domain, which is an alkaline shock protein family (Kuroda *et al.*, 1995). The same
224 cluster also codes for a CsbD family protein (Achl_3263) and a sigma-24 factor (Achl_3264). CsbD is a
225 bacterial general stress response protein, but its role in stress response is unclear (Pragai and
226 Harwood, 2002). The A6 genome contains three *csbD* homologs, all of which showed increased
227 expression in the phyllosphere. The RNA polymerase sigma-24 subunit belongs to the
228 extracytoplasmic function (ECF) subfamily of sigma factors. These sigma factors are involved in
229 responses to extracytoplasmic stresses, such as oxidative stress and desiccation (Testerman *et al.*,
230 2002; Cytryn *et al.*, 2007). Nine such sigma-24 genes are present in the *A. chlorophenolicus* genome
231 and four of them were expressed significantly higher in the phyllosphere than on agar.

232

233 Another stress related gene is *phoU* (Achl_0848) which showed elevated expression in the
234 phyllosphere. PhoU acts as a global negative regulator that increases resistance against multiple
235 antibiotics and stresses by a decrease in cellular metabolism (Li and Zhang, 2007). PhoU, as well as
236 CsbD and Asp23 are expressed in a sigma-B dependent manner. The alternative sigma factor SigB is a
237 master regulator in general stress response in *Bacillus subtilis* and related gram-positive bacteria
238 (Hecker *et al.*, 2007). However, the *A. chlorophenolicus* A6 genome does not appear to contain genes
239 annotated as sigma-B factors.

240

241 Also induced under stress is the phage shock protein C (Darwin, 2005). Cluster Achl_2712-2714
242 encodes two such phage shock C proteins and one hypothetical protein, all three of which were

243 higher expressed on leaf surfaces than on agar surfaces. Like the EFC sigma factors, the phage-shock-
244 protein system reacts to extracytoplasmic stress (Darwin, 2005).

245
246 The *A. chlorophenolicus* genome contains six drug resistance transporters of the EmrB/QacA
247 subfamily. Four of these were significantly higher expressed in the phyllosphere (14-, 7- and two
248 times 3- fold). In addition, AchI_1744, which is annotated to encode for a daunorubicin resistance
249 ABC transporter, showed 6-fold higher expression in the phyllosphere. These data suggest that *A.*
250 *chlorophenolicus* A6 cells encountered adverse compounds in the phyllosphere that needed to be
251 transported out of the cell.

252 253 Horizontal gene transfer

254 It has been shown previously for *Pseudomonas syringae* and for *Pseudomonas putida* that the
255 phyllosphere stimulates horizontal gene transfer (Normander *et al.*, 1998; Bjorklof *et al.*, 2000). We
256 observed phyllosphere-induced expression of genes encoding relaxase and mobilization proteins
257 (AchI_4629 and AchI_4630). Both genes were also expressed to a higher level in the presence of 4-
258 chlorophenol. They are located on plasmid pACHL02, which is the same plasmid that harbors the *cph*
259 gene cluster for 4-chlorophenol degradation.

260 261 Glyoxylate bypass

262 The AchI_0710 and AchI_0711 genes coding for key enzymes (malate synthase and isocitrate lyase,
263 respectively) in the glyoxylate bypass were induced in the phyllosphere. Elevated levels of the same
264 two enzymes were found during growth of *A. chlorophenolicus* A6 on phenol (Unell *et al.*, 2009),
265 which the authors took as an indication of 'insufficient energy'. Activation of the glyoxylate bypass on
266 plant leaf surfaces suggests that strain A6 is assimilating carbon from C2 compounds, possibly acetyl-
267 CoA acquired through catabolism of plant derived compounds.

268

Hypothetical proteins

The genes with the second and third most highly increased expression levels in the phyllosphere, Achl_3864 (95-fold) and Achl_1321 (82-fold), are annotated to encode for hypothetical proteins conserved in *Arthrobacter* species but with unknown function. They are not part of an operon with known genes. Achl_1321 has a transmembrane helix and a signal peptide cleavage site outside the cell, suggesting that this protein is secreted by the cell. Similarly, the gene with the highest degree of repression in the phyllosphere, Achl_4451, is a hypothetical protein. Investigation of the function of these three genes would be particularly interesting in the context of phyllosphere colonization.

Another gene cluster of interest is Achl_0049 to Achl_0052. These genes were induced in the phyllosphere compared to agar and expressed higher at low than high relative humidity. Again, these genes are all hypothetical proteins with unknown functions. Upstream of this cluster is a MarR regulatory gene which was also higher expressed in the phyllosphere. MarR regulators can control a variety of functions, such as resistance to antibiotics, organic solvents and oxidative stress (Aleksun and Levy, 1999).

DISCUSSION

Where previous studies of the phyllosphere transcriptome have focused on Gram-negative plant and human pathogens (Fink *et al.*, 2012; Yu *et al.*, 2013), we here present to the best of our knowledge the first transcriptional profile of a Gram-positive phyllosphere-competent strain. Our data set highlights many similarities and differences to the transcriptional profiles of leaf-associated bacteria published to date. We found an increased expression of genes related to nutrient acquisition, attachment, stress response and horizontal gene transfer in the phyllosphere, which is to a large extent in accordance with previous studies that investigated gene and protein expression in the phyllosphere (Marco *et al.*, 2005; Delmotte *et al.*, 2009; Fink *et al.*, 2012; Yu *et al.*, 2013). A surprising finding of the present study was the leaf-induced expression of part of the chlorophenol degradation

295 pathway. This will be discussed in greater detail below. Another unique finding was the induction of
296 Flp pili genes. Although attachment is considered to be an important factor in phyllosphere
297 colonization, and a role of pili has been suggested (Leveau, 2006), this is the first time that Flp pili
298 genes have been identified as phyllosphere-inducible. In contrast, leaf exposure of *P. syringae*
299 increased the expression of a large number of genes involved in flagellar synthesis and chemotaxis
300 (Yu *et al.*, 2013). Therefore, attachment could be an important phyllosphere survival strategy for
301 *Arthrobacter*, as opposed to motility for *P. syringae*. The expression profile of *A. chlorophenolicus* A6
302 in the phyllosphere was minimally affected by humidity levels. Moreover, we did not find evidence
303 for (increased) production of osmoprotectants such as trehalose in the phyllosphere, which is in
304 contrast with findings for *P. syringae* (Yu *et al.*, 2013). However, one of the two significantly higher
305 expressed genes under low versus high relative humidity is annotated to code for a transporter that
306 may aid in osmoadaptation by allowing uptake of compatible solutes including betaine and proline
307 (Axtell and Beattie, 2002). Many epiphytes can produce these types of compounds, which would
308 suggest that survival of A6 on leaf surfaces may be hardwired to depend in part on the presence and
309 activity of other microbes on the leaf surfaces.

310

311 We observed substantial similarities between the transcriptional response of *A. chlorophenolicus* to
312 the phyllosphere and upon exposure to 4-chlorophenol. Genes involved in phosphate and iron
313 uptake, sulfur assimilation, plasmid mobilization genes and several 4-chlorophenol degradation
314 genes were significantly higher expressed under both conditions. Out of 337 genes with a
315 significantly altered expression under both conditions, 91% changed in the same direction, i.e. either
316 up or down, compared to growth on agar in the absence of chlorophenol. Those similarities suggest a
317 priming effect, where exposure to the phyllosphere could lead to a pre-adaptation of bacteria for
318 growth on organic pollutants such as 4-chlorophenol. Interestingly, a higher expression of genes
319 involved in xenobiotic degradation in the phyllosphere compared to liquid growth medium was also
320 found for the *P. syringae* transcriptome, although this species is not known as a pollutant degrader

321 (Yu *et al.*, 2013). In *A. chlorophenolicus* A6, stress-related genes that were induced in the
322 phyllosphere were not responsive to the presence of 4-chlorophenol. This indicates that the stress
323 response to 4-chlorophenol was different from phyllosphere-induced stresses. Under water stress
324 induced by sodium chloride or polyethylene glycol, none of the genes for 4-chlorophenol degradation
325 were induced (Moreno & van der Meer, unpublished data), suggesting that osmotic stress is not a
326 trigger for their expression. A more likely explanation is that these genes react to phenolic
327 compounds that are naturally present in the phyllosphere.

328
329 Our data demonstrated the phyllosphere-induced expression of three genes within the *cph* gene
330 cluster for 4-chlorophenol degradation. These three genes are thought to constitute a subcluster
331 (cluster II) which evolved independently from the rest of the *cph* gene cluster and were combined by
332 a more recent horizontal gene transfer event (Nordin *et al.*, 2005). Based on our qPCR results (Figure
333 4), we hypothesize that leaf surface-induced expression of the cluster II genes was triggered by the
334 presence of hydroquinone or derivatives thereof on leaf surfaces. Indeed, we could detect
335 hydroquinone in bean leaf washes and our data suggest that hydroquinone is available to bacteria
336 such as *A. chlorophenolicus* A6 on the leaf surface in concentrations sufficiently high to stimulate the
337 expression of genes that code for the degradation of hydroquinone. Hydroquinone and its
338 glycosylated form arbutin have been identified in the leaves of a broad range of plant species, such
339 as pear, bearberry, *Polygonella myriophylla* and several species in the family Lamiaceae (Pedersen,
340 2000; Parejo *et al.*, 2001; Jin and Sato, 2003; Weidenhamer and Romeo, 2004). In the resurrection
341 fern, hydroquinone and arbutin are important for the plant to deal with desiccation stress (Suau *et*
342 *al.*, 1991). Hydroquinone has also been listed as a compound with antimicrobial (Jin and Sato, 2003)
343 and surface-wetting (Wieckowska *et al.*, 2007) properties.

344
345 Our findings are of potential interest for phyllosphere-based bioremediation studies. The ability of
346 phyllosphere bacteria to degrade airborne aromatic pollutants such as phenol has been previously

established (Sandhu *et al.*, 2007, 2009). Although the exact relationship between genes involved in (or induced by) hydroquinone and phenol remains to be investigated, our observation that exposure to the phyllosphere induces many of the same degradation genes as exposure to phenol indicates that the phyllosphere might prime bacteria for pollutant degradation. Such priming, for example through a process of 'phyllo-augmentation', could potentially result in increased degradation of and/or a faster response to aromatic pollutants in bio-based environmental cleanup operations.

In summary, we demonstrated that the phyllosphere competency of *A. chlorophenolicus* A6 is linked to the expression of a number of specific gene functions that support epiphytic survival. Most unforeseen and exciting was the discovery that the best studied genes of this bacterium so far, namely the *cph* genes for the degradation of the pollutant 4-chlorophenol, were induced during leaf colonization and that their elevated expression *in planta* concurs with our demonstration that hydroquinone, an inducer of these genes, is present in the leaf environment.

EXPERIMENTAL PROCEDURES

Experimental setup and sample preparation for transcriptome microarrays

Arthrobacter chlorophenolicus strain A6 was grown at 28 °C and 250 rpm in Lysogeny Broth (LB). At mid-exponential phase, the bacterial culture was centrifuged for 10 min at 3,838 ×g. The pellet was resuspended in sterile demineralized water to obtain a bacterial suspension with an optical density at 600 nm (OD₆₀₀) of 0.15, which corresponded to approximately 8x10⁷ colony forming units (CFU) per ml. The above-ground portion of two-week old bean plants (*Phaseolus vulgaris*, green snap bean, variety Blue Lake Bush 274) with the first two leaves fully expanded were dipped into this bacterial suspension. Five plants were incubated at 97% air humidity for two days in a growth chamber; we refer to this as the high humidity phyllosphere or PhylH treatment. Five other plants were incubated for 1 day at 97% air humidity, followed by a second day at 50% air humidity; we refer to this as the reduced or low humidity phyllosphere or PhylL treatment. In addition, 500 µl of bacterial suspension

373 was spread on 1/10 strength Tryptone Soy Agar (TSA; Oxoid, Cambridge, UK) with 15 g agar per liter
374 and supplemented with 1 mM 4-chlorophenol (4-CP); we refer to this as the agar plus 4-CP (or A+CP)
375 treatment. Another 500 µl was spread on 1/10 strength TSA without the 4-chlorophenol; we refer to
376 this as the A-CP treatment. Both agar treatments were incubated at 97% humidity for two days. We
377 chose agar surfaces as a control (rather than liquid cultures) so as to avoid picking up genes that are
378 surface-induced but not phyllosphere-specific. All plants and plates were incubated in a growth
379 chamber that was maintained a day-night cycle of 16 h and 8 h at 21 °C and 16 °C, respectively.
380 Bacterial population sizes on leaves were estimated at 0, 24, and 48 hours post-inoculation to show
381 that they increased during the first 24 hours but stabilized during the second 24 hours following
382 inoculation (Figure S2). Similarly, the bacteria on plate established a lawn during the first 24 hours,
383 without apparent growth after that and until time of harvest. For each treatment, we prepared four
384 independent replicate experiments. For each replicated experiment, bacteria were recovered from
385 leaves by sequentially putting five leaves from five different plants in 20 ml RNA protection solution
386 [two parts RNeasy Protect Bacteria reagent (Qiagen; Venlo, The Netherlands) and one part phosphate-
387 buffered saline (PBS)] with 5 sec vortexing, 7 min sonication and 5 sec vortexing for each leaf. The
388 solution was centrifuged for 20 min at 3,838 × *g* and bacterial pellets were frozen at -80 °C until RNA
389 extraction. Bacteria from agar plates were washed from the surface with 1 ml RNeasy Protect solution
390 according to the manufacturer's instructions (Qiagen) and frozen at -80 °C until RNA extraction.

391 392 Microarray design

393 YODA software (Nordberg, 2005) was used to design 50-mer probes that target genes from the
394 chromosome and both plasmids of *A. chlorophenolicus* A6. The microarray design has been
395 deposited in the NCBI Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo>) under accession
396 number GSE48198 (platform GPL17332). The majority of probes (99.5%) were designed with the
397 following parameters: 1 to 3 non-overlapping probes per gene, a maximum of 70% identity to non-
398 target sequences, a maximum of 15 consecutive matches to non-target sequences, a melting

399 temperature range of 8° C, and a GC content range of 12%. The remaining 0.5% of probes were
400 designed with the following less stringent parameters: a maximum of 80% identity to non-target
401 sequences, a melting temperature range of 15°C, and a GC content range of 30%. In total, 13589
402 probes were designed that target 99.8% of the predicted protein-coding A6 genes (4581 out of
403 4590). An additional 7 positive control probes were included in the design. Probes were synthesized
404 on microarrays by Agilent Technologies (Santa Clara, CA) using the 8 × 15,000 format.

405

406 RNA extraction and microarray procedures

407 Bacterial pellets were thawed at room temperature and resuspended in 200 µl TE buffer (30 mM
408 Tris-Cl, 1 mM EDTA, pH 8.0) containing 15 mg of lysozyme and 2 mg of proteinase K per ml. The
409 suspension was incubated at room temperature for 30 min with regular vortexing. Then 700 µl lysis
410 solution of the Aurum Total RNA Mini Kit (Bio-Rad Laboratories; Veenendaal, The Netherlands) was
411 added and the total volume was transferred to a 2 ml screw cap tube containing 100 mg of 0.1:0.5
412 mm beads (1:1) (Merlin Bioproducts; Breda, The Netherlands). Tubes were shaken in a Mini-
413 Beadbeater (Biospec Products, Breukelen, The Netherlands) twice for 1 min at 5,000 rpm with a 1
414 min interval on ice. Tubes were centrifuged for 10 sec and the solution without beads was
415 transferred to a fresh tube. After the addition of 500 µl 70% isopropanol, samples were further
416 treated according to the Aurum Total RNA Mini Kit protocol. Columns were eluted with 80 µl elution
417 buffer. An additional 30 min DNase treatment was performed with Ambion TURBO DNA-free (Applied
418 Biosystems; Nieuwerkerk a/d ijssel, The Netherlands). After the DNase treatment, the RNA was
419 precipitated with 1/10 volume of 7.5 M ammonium acetate (Sigma-Aldrich; Zwijndrecht, The
420 Netherlands), 1/50 volume glycogen (5 mg/ml) (Fermentas; St. Leon-Rot, Germany) and 2.5 volumes
421 of ethanol. RNA pellets were washed with 80% ethanol and resuspended in 12 µl nuclease-free
422 water. RNA quality was verified with Experion RNA StdSens (Bio-Rad Laboratories).

423

424 The procedures for cDNA synthesis and labeling and for array hybridization were based on a protocol
425 described elsewhere with slight modifications (Johnson *et al.*, 2011). An amount of 2-5 µg of RNA was
426 mixed with 1.25 µl random primers (500 µg/ml; Promega) in a total volume of 12 µl and incubated at
427 70 °C for 10 min followed by 4 °C for 5 min. Each tube received 13 µl of mastermix containing 0.6 µl
428 Cyanine 3-dCTP (1 mM; Perkin-Elmer), 0.6 µl Superase-In (20 U/µl; Ambion), 1 µl Superscript II (200
429 U/µl; Invitrogen), 5 µl 5x 1st strand buffer, 2.5 µl DTT (100 mM), 0.25 µl dATP-dGTP-dTTP mixture (10
430 mM each), 0.1 µl dCTP (5 mM) and 2.9 ml nuclease-free water. Labeled cDNA was produced by
431 incubation at 42 °C for 120 min, followed by 70 °C for 10 min and 4 °C for 5 min. RNA was hydrolyzed
432 under alkaline conditions as follows. After the addition of 2.5 µl 1 M NaOH, samples were heated at
433 65 °C for 20 min, allowed to cool to room temperature for 10 min and neutralized by the addition of
434 2.5 µl 3 M Na-acetate (pH 5.2) and 2.5 µl 1 M HCl. The labeled cDNA was then purified using the
435 MinElute PCR Purification Kit (Qiagen) according to the manufacturer's instructions. The product was
436 eluted in 20 µl EB buffer and quantity and incorporation efficiency were determined using the
437 MICROARRAY function on a NanoDrop spectrophotometer (ThermoFisher Scientific, Waltham, MA).
438 Sixty ng of labeled cDNA with incorporation efficiencies between 2 and 3% was loaded onto each
439 microarray, hybridized for 17 hours at 65°C, and washed and scanned as described for labeled cRNA
440 in the One-Color Microarray-Based Gene Expression Analysis Manual (Agilent Technologies, Santa
441 Clara, CA). The fragmentation step (heating to 60°C for 30 minutes) was omitted. Hybridization signal
442 intensities were extracted from scanned images using the AGILENT FEATURE EXTRACTION software
443 package (version 10.7.1.1; Agilent Technologies, Santa Clara, CA).

444

445 To corroborate the array results, three replicate RNA samples each were obtained following the
446 procedures as described above for the agar surface treatments, the high humidity phyllosphere
447 treatment and the low humidity phyllosphere treatment. These RNA samples were subjected to
448 reverse transcription quantitative PCR (RT-qPCR) as described below.

449

Microarray data analyses

The expression data were analyzed using the Genespring GX software version 11 (Agilent technologies, Santa Clara, CA) as described elsewhere (Johnson *et al.*, 2011). In short, data were log₂-transformed, normalized by quantile and scaled with the baseline to the median of all samples. All genes were filtered by expression level and were retained when the signal intensity was above the 20th percentile in at least one of the samples. Samples were clustered by hierarchical clustering using Euclidian distances and the average linkage rule; they were clustered on both entities and conditions. All treatments were compared pairwise to the agar surface control treatment using a Welch's t-test with asymptotic p-value computation and Benjamini Hochberg False Discovery Rate (FDR) for multiple testing correction. In addition, the high and low humidity phyllosphere treatments were compared with each other in a Welch's t-test. Genes that were at least 2-fold differentially expressed between treatments with a corrected p-value lower than 0.05 were considered statistically different. Gene annotations were retrieved from the IMG database (<http://img.jgi.doe.gov/>).

Array information is available in the NCBI Gene Expression Omnibus database under accession number GSE48198.

Gene expression in the presence of plant phenolic compounds

A. chlorophenicus A6 cells were harvested from an LB overnight culture by centrifugation for 10 min at 3,838 ×g. Cells were resuspended in Brunner mineral medium (MM; DSMZ medium no. 457, Braunschweig, Germany) with 5 mM fructose, adjusted to an OD₆₀₀ of 0.15 and incubated at 28 °C and 150 rpm. When the OD₆₀₀ reached 0.35, 1 mM of phenolic compound (see below) was added and the flasks were incubated for another 2 hours at 28 °C and 150 rpm. There were triplicates for each of the 18 treatments. We tested 12 phenolic compounds: phenol, 4-chlorophenol, 4-hydroxybenzoic acid, protocatechuic acid, coumaric acid, caffeic acid, ferulic acid, quercetin, arbutin, hydroquinone, catechol, and resorcinol. In the controls, no phenolic compound was added. Arbutin was also tested

476 at concentrations of 10^{-2} and 10^{-4} mM and hydroquinone at 10^{-2} , 10^{-4} and 10^{-5} mM. After 2 hours, 3
477 ml culture was treated with RNAprotect (Qiagen) according to the manufacturer's instructions and
478 cells were frozen at -80°C until RNA extraction. In addition, the OD_{600} and number of colony forming
479 units per ml (CFU ml^{-1}) of the cultures were determined. RNA was extracted according to the protocol
480 described above and resuspended in 35 μl nuclease-free water.

481 482 RT-qPCR

483 Table 2 summarizes the qPCR primers that were designed for the *A. chlorophenolicus* A6 genes
484 AchI_4564, AchI_4566, AchI_4569 and AchI_1611, using the primer3 software
485 (<http://primer3.sourceforge.net/>) or SciTools of Integrated DNA Technologies
486 (<http://eu.idtdna.com/scitools/Applications/RealTimePCR/>). Plasmid-encoded genes AchI_4564,
487 AchI_4566, and AchI_4569 are part of the *A. chlorophenolicus* gene cluster for 4-chlorophenol
488 degradation, while AchI_1611 serves as a reference gene; it is chromosomally located and encodes
489 the RNA polymerase sigma factor RpoD. Appropriate annealing temperatures (Table 2) were
490 optimized by testing a range of temperatures.

491
492 Two-step RT-qPCR was performed on RNA samples from phyllosphere bacteria (see 'RNA extraction
493 and array hybridization') and RNA samples from bacteria that were exposed to different phenolic
494 compounds (see 'Gene expression in the presence of plant phenolic compounds'). An amount of 420
495 ng RNA was converted to cDNA with random hexamer primers in a reaction volume of 20 μl
496 (RevertAid First Strand cDNA Synthesis Kit, Fermentas). The cDNA product was diluted 50 times.
497 qPCR mixtures contained 12.5 μl ABsolute™ QPCR SYBR® green mix (ABgene, Fisher Scientific,
498 Landsmeer, The Netherlands), 10 μg BSA, 6.25 pmol of each primer and 5 μl of diluted cDNA
499 template in a total volume of 25 μl . The qPCRs were performed on a Corbett Research Rotor-Gene
500 3000 thermal cycler (Westburg, Leusden, The Netherlands) with a regime of one step of 15 min at 95
501 $^{\circ}\text{C}$, and 40 cycles of 60 sec at 95 $^{\circ}\text{C}$, 40 sec at the respective annealing temperature (Table 2), and 60

502 sec at 72 °C. Gene expression was calculated relative to the Achl_1611 *rpoD* gene using the $2^{-\Delta\Delta Ct}$
503 method (Livak and Schmittgen, 2001).
504
505 Analysis of leaf surface washes by gas chromatography – mass spectrometry (GC-MS)
506 For each of three independent samples, 32 primary leaves of two-week old *P. vulgaris* plants were
507 sequentially dipped into 100% methanol for 30 sec. The methanol extract was then divided in two
508 parts, both parts received 50 ng of 3,5-dihydroxybenzoic acid as an internal standard and one part
509 was spiked with 50 ng of hydroquinone. The methanol extract was filtered over Whatman filter
510 paper and the solvent evaporated under a flow of nitrogen. Dried samples were dissolved in 50 µl
511 acetonitrile and subsequently derivatized with 100 µl BSTFA [N,O-bis(trimethylsilyl)
512 trifluoroacetamide] and 10 µl TMCS (trimethylchlorosilane) overnight at room temperature. Samples
513 were diluted with 500 µl acetonitrile and 1-10 µl were injected in an Agilent GC 7890 (Agilent Inc.,
514 Santa Clara, CA, USA) with BPX-5 column (30 m × 0.32 mm × 0.25 µm, SGE, Darmstadt, Germany) in
515 either split or split-less mode. The temperature program from 50°C to 300°C was as follows: 50 °C (5
516 min) to 100 °C (30 min) at 30 °C/min, to 175 °C (5 min) at 10 °C/min, to 250 °C (5 min) at 10 °C/min,
517 and to 300 °C (15 min) at 30 °C/min. The injector was set at 280 °C and the He flow was 1.7 ml/min.
518 Mass spectra were recorded using an Agilent MS 5975C inert XL MSD and analysed with an MSD
519 ChemStation G1701 EA.E.02.00.493. External standards were run for arbutin, caffeic acid, ferulic acid,
520 4-hydroxybenzoic acid, protocatechuic acid, hydroquinone, catechol and resorcinol.

522 ACKNOWLEDGMENTS

523 We thank David R. Johnson for his help with the microarray design. This work was supported by the
524 BACSIN project within the 7th framework program of the European Union (KBBE-211684). This is
525 NIOO-KNAW publication xxxx.

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657

660 **Table 1:** Differential expression of selected genes in *A. chlorophenolicus* A6

Locus Tag ¹	PhyIH ²	PhyIL ²	A+4CP ²	Predicted function
Achl_0049	7.6	21.6	1.6	hypothetical protein
Achl_0050	16.8	25.7	-1.0	hypothetical protein
Achl_0051	26.2	81.1	1.5	protein of unknown function DUF1469
Achl_0052	18.6	84.5	1.9	hypothetical protein
Achl_0159	4.1	2.4	-2.1	beta-Ig-H3/fasciclin
Achl_0362	111.8	101.3	4.4	phosphate ABC transporter, periplasmic phosphate-binding protein
Achl_0363	21.1	18.7	1.8	phosphate ABC transporter, inner membrane subunit PstC
Achl_0364	14.6	14.9	1.8	phosphate ABC transporter, inner membrane subunit PstA
Achl_0365	2.4	2.9	-2.5	phosphate ABC transporter, ATPase subunit(EC:3.6.3.27)
Achl_0518	1.4	11.3	-1.5	general substrate transporter
Achl_0710	10.2	5.9	-96	malate synthase A (EC:2.3.3.9)
Achl_0711	4.5	4.3	-211	Isocitrate lyase (EC4.1.3.1)
Achl_0848	7.4	8.8	1.5	phosphate uptake regulator, PhoU
Achl_1321	81.6	81.9	-2.0	hypothetical protein
Achl_1726	3.6	11.0	1.6	2-hydroxypropyl-CoM lyase(EC:2.1.1.14)
Achl_1744	6.2	6.7	1.8	daunorubicin resistance ABC transporter ATPase subunit
Achl_1844	3.2	3.1	3.0	putative transcriptional regulator
Achl_1845	2.6	2.2	2.3	FeS assembly protein SufB
Achl_1846	2.7	2.2	2.7	FeS assembly protein SufD
Achl_1847	2.1	1.9	3.6	Rieske (2Fe-2S) domain protein
Achl_1848	1.8	1.6	2.3	FeS assembly ATPase SufC
Achl_1849	2.1	2.1	2.5	protein of unknown function DUF59
Achl_2231	22.3	6.2	-1.1	nitrogen regulatory protein P-II
Achl_2232	70.3	13.5	-1.4	ammonium transporter
Achl_2284	2.5	2.3	-1.7	beta-Ig-H3/fasciclin
Achl_2389	2.0	7.3	1.6	cysteine desulfurase
Achl_2563	-4.1	-17.0	2.1	major facilitator superfamily MFS_1
Achl_2643	20.6	225.1	1.6	Flp/Fap pilin component
Achl_2644	9.1	53.9	-1.6	TadE family protein
Achl_2645	5.5	35.2	-1.8	hypothetical protein
Achl_2646	4.5	22.9	-1.3	SAF domain protein
Achl_2647	2.7	5.7	-1.1	Flp pilus assembly protein ATPase CpaE-like protein
Achl_2648	2.2	12.6	1.2	type II secretion system protein E
Achl_2649	1.0	3.5	-1.6	type II secretion system protein
Achl_2650	1.7	33.3	1.2	type II secretion system protein
Achl_2712	13.0	3.4	-2.0	phage shock protein C, PspC
Achl_2713	6.8	1.9	-2.4	hypothetical protein
Achl_2714	3.2	1.6	-1.7	phage shock protein C, PspC
Achl_2817	4.3	8.0	6.7	sulfate adenylyltransferase, large subunit(EC:2.7.7.4)
Achl_2818	5.4	9.5	8.0	sulfate adenylyltransferase, small subunit(EC:2.7.7.4)
Achl_2819	3.3	5.1	2.8	phosphoadenosine phosphosulfate reductase(EC:1.8.4.8)
Achl_2820	8.6	12.9	12.2	Sulfite reductase (ferredoxin)(EC:1.7.7.1)
Achl_2971	1.8	1.7	2.6	hypothetical protein
Achl_2972	1.1	-1.1	7.2	flagellar biosynthesis protein FlhA
Achl_2973	-2.3	-2.3	-1.2	type III secretion exporter
Achl_2974	1.8	1.8	3.4	flagellar biosynthetic protein FliR
Achl_2975	1.1	-1.2	4.4	flagellar biosynthetic protein FliQ
Achl_2976	1.5	-1.0	1.9	flagellar biosynthetic protein FliP
Achl_2977	1.3	1.1	2.8	flagellar biosynthesis protein FliO
Achl_2978	1.7	2.0	2.8	flagellar motor switch protein FliN
Achl_2979	1.1	-1.1	7.2	surface presentation of antigens (SPOA) protein
Achl_2980	2.4	1.9	2.9	OmpA/MotB domain protein
Achl_2981	1.4	-1.1	4.5	MotA/TolQ/ExbB proton channel
Achl_2982	1.4	1.3	3.2	flagellar FlbD family protein
Achl_2983	1.2	1.2	5.4	protein of unknown function DUF1078 domain protein
Achl_2984	-1.1	-1.2	5.3	flagellar hook capping protein
Achl_2985	2.3	2.5	2.2	hypothetical protein
Achl_2986	1.3	-1.1	4.3	NLP/P60 protein
Achl_2987	2.2	1.8	1.8	flagellar export protein FliJ
Achl_2988	2.7	1.9	6.8	ATPase, FliI/YscN family(EC:3.6.3.14)

Locus Tag ¹	PhyIH ²	PhyIL ²	A+4CP ²	Predicted function gene product
Achl_2989	1.2	1.3	3.2	hypothetical protein
Achl_2990	2.2	1.9	2.7	flagellar motor switch protein FltG
Achl_2991	4.4	4.0	6.9	flagellar M-ring protein FltF
Achl_2992	2.4	2.1	2.3	flagellar hook-basal body complex subunit FltE
Achl_2993	1.8	1.3	3.6	flagellar basal-body rod protein FlgC
Achl_2994	1.8	1.3	5.3	flagellar basal-body rod protein FlgB
Achl_2995	1.4	-1.1	4.6	hypothetical protein
Achl_2996	-1.0	-1.2	3.8	flagellar protein FltS
Achl_2997	-1.6	-2.0	3.6	flagellar hook-associated 2 domain protein
Achl_2998	-1.1	-1.6	7.6	flagellin domain protein
Achl_2999	1.2	-1.1	5.7	FlgN family protein
Achl_3000	2.0	1.3	6.3	flagellar hook-associated protein FlgK
Achl_3001	1.3	1.3	5.6	flagellar hook-associated protein 3
Achl_3258	28.2	3.0	1.2	protein of unknown function DUF322
Achl_3259	10.0	1.9	-1.6	hypothetical protein
Achl_3260	64.7	5.7	2.6	hypothetical protein
Achl_3261	26.6	3.3	1.1	hypothetical protein
Achl_3262	60.3	2.8	2.4	hypothetical protein
Achl_3263	14.1	2.7	-1.2	CsbD family protein
Achl_3264	9.4	1.9	-1.3	RNA polymerase, sigma-24 subunit, ECF subfamily
Achl_3265	6.7	1.3	-1.6	hypothetical protein
Achl_3266	4.4	1.5	-1.4	hypothetical protein
Achl_3518	17.4	7.0	1.7	ammonium transporter
Achl_3525	7.6	5.4	1.5	glutamine synthetase, type III(EC:6.3.1.2)
Achl_3724	31.2	16.7	-1.3	Ferric reductase domain protein transmembrane component domain protein
Achl_3725	25.7	10.0	-1.1	FMN-binding domain protein
Achl_3726	17.0	7.7	1.3	ApbE family lipoprotein
Achl_3731	3.8	4.3	2.6	Siderophore-interacting protein
Achl_3732	3.1	3.0	2.4	ABC transporter related(EC:3.6.3.34)
Achl_3733	4.0	4.5	3.1	transport system permease protein
Achl_3734	5.9	6.7	5.3	transport system permease protein
Achl_3735	6.9	7.0	7.3	periplasmic binding protein
Achl_3864	94.6	18.4	1.1	hypothetical protein
Achl_4158	3.7	2.8	-1.1	peptidase A24A prepilin type IV(EC:2.1.1.-,EC:3.4.23.43)
Achl_4451	-30.3	-36.1	-2.0	hypothetical protein
Achl_4564	5.1	46.0	27.7	monooxygenase FAD-binding(EC:1.14.13.20)
Achl_4565	3.3	27.5	21.0	iron-containing alcohol dehydrogenase(EC:1.3.1.32)
Achl_4566	4.1	51.9	35.5	intradiol ring-cleavage dioxygenase(EC:1.13.11.1)
Achl_4567	1.3	5.2	3.1	hypothetical protein
Achl_4568	-1.3	-1.3	79.0	hypothetical protein
Achl_4569	-1.5	-2.0	77.1	intradiol ring-cleavage dioxygenase(EC:1.13.11.1)
Achl_4570	-2.5	-2.7	79.5	flavin reductase domain protein FMN-binding
Achl_4571	-1.9	-1.9	-1.8	transcriptional activator domain protein
Achl_4572	-1.7	-1.1	237.6	protein of unknown function DUF1486
Achl_4573	-2.0	-1.7	137.0	4-hydroxyphenylacetate 3-hydroxylase(EC:1.14.13.3)
Achl_4574	1.6	1.9	20.6	iron-containing alcohol dehydrogenase
Achl_4629	3.7	2.7	2.1	Relaxase/mobilization nuclease family protein
Achl_4630	2.1	1.8	3.7	mobilisation protein

¹Only those genes referred to explicitly in the text are listed here. For a complete list, see Table S1. ²The values shown are fold-changes in expression of *A. chlorophenolicus* A6 genes on leaves at high relative humidity (PhyIH), on leaves at low relative humidity (PhyIL) or on agar plates supplemented with 4-chlorophenol (A+4CP), compared to agar plates without 4-CP. Genes with a more than two-fold change in gene expression and a corrected p-value <0.05 are indicated in bold.

Table 2: Primer specifications

Primer name	Target gene	Sequence	Annealing temperature
1611-f	Achl_1611	GTGGAAGTCATCAACAAG	54
1611-r	Achl_1611	GTCATGTCCAGTTCTAGTG	54
4564-f	Achl_4564	ATATCCCTCAGACGTACC	59
4564-r	Achl_4564	AGACATACTCAGTGGAGAAC	59
4566-f	Achl_4566	GCTTCTATGACGTCCAAT	59
4566-r	Achl_4566	AGTCCCCAGAAGGAGTAT	59
4569-f	Achl_4569	CCAGCACCTACACAACTTCG	59
4569-r	Achl_4569	AAGGCCTAGAACGTCAGAAAG	59

Table S1: Fold-change in gene expression and corrected p-values of *A. chlorophenolicus* genes on transcriptome arrays. Genes with a more than two-fold change in gene expression and a corrected p-value lower than 0.05 are indicated in bold. Comparisons were made between 1) high humidity phyllosphere and agar surface treatment, 2) low humidity phyllosphere and agar surface treatment, and 3) chlorophenol and agar surface treatment. Genes with a significantly different expression between high and low phyllosphere humidity treatments are indicated with asterisks (** = $p < 0.05$, * = $p < 0.1$).

Table S2: Compounds identified in three independent methanol extracts from the surface of bean leaves.

FIGURE LEGENDS

Figure 1: Cluster diagram of *A. chlorophenolicus* A6 transcriptome array samples. Samples were organized by hierarchical clustering using Euclidian distances and the average linkage rule. PhylH, high humidity phyllosphere treatment; PhylL, low humidity phyllosphere treatment; A-CP, agar surface treatment; A+CP, agar plus 4-chlorophenol treatment.

Figure 2: Correlations between the fold-change in gene expression under high and low humidity conditions in the phyllosphere (a), and between high humidity in the phyllosphere and 4-chlorophenol exposure (b). All changes in gene expression are relative to agar surface without chlorophenol. Black symbols represent genes that were significantly ($P < 0.05$) more than 2-fold differentially expressed in both treatments. The other genes are indicated in grey symbols. Indicated by a circle and arrow are the *cph-II* genes, i.e. *cphC-II*, *cphA-II*, and *cphF-II*, which form a putative operon and have been implicated in 4-CP degradation, coding for the conversion of hydroquinone to oxoadipate.

Figure 3: Fold-change in expression of *A. chlorophenolicus* 4-chlorophenol degradation genes on the transcriptome arrays. White bars represent the comparison between the high humidity phyllosphere and agar surface treatment, grey bars between the low humidity phyllosphere and agar surface treatment, and black bars between the 4-CP and no 4-CP agar surface treatment.

Figure 4: Fold-change in expression of *cph* genes in *A. chlorophenolicus* A6 as determined by RT-qPCR. Gene expression in the phyllosphere at high and low air humidity as compared to an agar surface (a), and gene expression in mineral medium in presence versus absence of several phenolic

705 compounds (b). White bars, AchI_4564 (*cphC-II*); grey bars, AchI_4566 (*cphA-II*); black bars,
706 AchI_4569 (*cphA-I*).

707
708 **Figure S1:** GC-MS analysis of *Phaseolus vulgaris* leaf surface wash extracts. A peak with the retention
709 time (19.6 min) of silylated hydroquinone is indicated in a chromatogram section by an arrow (a). The
710 corresponding mass spectrum (b) matched that of silylated hydroquinone from the NIST Mass
711 Spectrometry Data Center (c) and of an authentic standard (not shown).

712
713 **Figure S2:** Population sizes of *A. chlorophenolicus* A6 on bean leaves that were sampled for RNA
714 extraction and subsequent microarray analysis. 'Wet' refers to the PhylH treatment, while 'dry' refers
715 to the PhylL treatment.

Figure 1

Figure 2

a)

b)

Figure 3

Figure 4