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Effect of Tannic Acid on the Transcriptome of the Soil Bacterium *Pseudomonas protegens* Pf-5

Chee Kent Lim,[a]* Anahit Penesyan,[a] Karl A. Hassan,[a] Joyce E. Loper,[b] Ian T. Paulsen[a]

Department of Chemistry and Biomolecular Sciences, Macquarie University, North Ryde, NSW, Australia[a]; USDA-ARS Horticultural Crops Research Laboratory and Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, USA[b]

Tannins are a diverse group of plant-produced, polyphenolic compounds with metal-chelating and antimicrobial properties that are prevalent in many soils. Using transcriptomics, we determined that tannic acid, a form of hydrolysable tannin, broadly affects the expression of genes involved in iron and zinc homeostases, sulfur metabolism, biofilm formation, motility, and secondary metabolite biosynthesis in the soil- and rhizosphere-inhabiting bacterium *Pseudomonas protegens* Pf-5.

Tannins are polyphenolic compounds produced in the leaves, roots, bark, galls, fruits, and buds of many plants (1). Tannins can be divided into two types: condensed and hydrolysable. Condensed tannins are made up of flavonol polymers (2), whereas hydrolysable tannins are composed of a central polyol, esterified to gallic acids to form gallotannins (3). From these basic structures, plants synthesize many derivatives with diverse functions, including defense against herbivores and pathogens (4). An important property of tannins is their ability to form complexes with metals such as iron (5, 6), copper (7), and zinc (8, 9). Tannins can also bind proteins (10), scavenge free radicals (1), and inhibit microbial growth (4, 11, 12), possibly through tannin-polymer complexation, membrane disruption, and/or chelation of metal ions (13).

Tannins are among the most abundant organic compounds in plants, but knowledge of their concentrations in soil is vague due to differences in physical, chemical, and biotic properties of the soils and in methods for tannin quantification (14–16). Estimated concentrations of phenolic compounds in soil and humus vary between 0.18 and 37.6 mg/g dry weight (17–20). At these concentrations, tannins are likely to affect the physiology of microorganisms inhabiting the soil or rhizosphere.

Here we describe the effect of tannic acid (TA), a form of hydrolysable tannin, on the transcriptome of the model biocontrol bacterium *Pseudomonas protegens* Pf-5 (previously called *Pseudomonas fluorescens* Pf-5) (21, 22). *P. protegens* Pf-5 was isolated from soil and can colonize root and seed surfaces (23), protecting them from fungal, oomycete, and bacterial pathogens, primarily through the secretion of a range of bioactive secondary metabolites and exoenzymes (24, 25). Given the wide distribution of tannins in soil and their high abundance in some roots and seeds (26, 27), these compounds could have an important influence on the gene expression and secondary metabolism of soil and rhizosphere bacteria such as Pf-5. To date, few studies have investigated the role of plant-derived phenolic compounds in gene expression by biocontrol bacteria (28, 29).

In Mueller-Hinton (MH) broth (Oxoid, Thermo Fisher Scientific, Adelaide, SA, Australia), TA (Sigma-Aldrich, St. Louis, MO) inhibited the growth of *P. protegens* Pf-5 at concentrations exceeding 20 μg/ml (Fig. 1). Concentrations of TA above 300 μg/ml also influenced cell morphology, inducing filament formation (see Fig. S1 in the supplemental material), as observed previously in *P. fluorescens* (30). Experiments evaluating the Pf-5 transcriptome were done in MH amended with two TA concentrations: 20 μg/ml (low TA), which did not significantly affect growth, and 160 μg/ml (high TA), which resulted in significantly lower cell density (Fig. 1).

The transcriptomic effects of TA were determined using whole-genome microarrays as described previously (31, 32). Amendment of MH medium with TA had a broad influence on the Pf-5 transcriptome, with the transcript abundance of 64 genes altered significantly, by at least 2-fold, at the low TA concentration and 575 genes at the high TA concentration (Fig. 2; see also Table S1 in the supplemental material). The low TA concentration induced expression of many genes in two functional-role categories, transcription and central intermediary metabolism, whereas the high TA concentration significantly affected genes in 19 of the 24 role categories (see Fig. S2 in the supplemental material). Quantitative reverse transcriptase PCR (qRT-PCR) validation was performed with a set of gene-specific primers as described previously (31) (see Table S2 in the supplemental material), and the results correlated highly with the microarray data (see Fig. S3 in the supplemental material).

TA had a strong effect on the transcription of genes involved in iron homeostasis of Pf-5, enhancing the expression of genes encoding heme uptake and biosynthesis and transport of the siderophores pyoverdine and enantio-pyochelin (33, 34). Pyoverdine production by Pf-5 also increased in a dose-dependent manner in response to TA (see Fig. S4 in the supplemental material). Genes having putative roles in iron storage (such as PFL_4769, PFL_4859, and PFL_5555) were downregulated, whereas PFL_4858, which encodes a bacterioferritin-associated ferredoxin that mobilizes iron stored in bacterioferritin B (35), was upregu-

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Address correspondence to Ian T. Paulsen, ian.paulsen@mq.edu.au, or Joyce E. Loper, Joyce.Loper@ars.usda.gov.
*Present address: Chee Kent Lim, Department of Biology, University of North Carolina, Charlotte, North Carolina, USA.
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