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Uptake of non-pathogenic *E. coli* by Arabidopsis induces downregulation of heat shock proteins

Chanyarat Paungfoo-Lonhienne,* Susanne Schmidt and Thierry G.A. Lonhienne
School of Biological Sciences; The University of Queensland; Queensland, Australia

We recently demonstrated that non-pathogenic and non-symbiotic microbes *E. coli* and yeast are taken up by roots and used as a source of nutrients by the plant. Although this process appears to be beneficial for the plant, the nutritional gain of microbe incorporation has to exceed the energy expense of microbe uptake and digestion, and the question remains whether the presence of microbes triggers pathogen- and other stress-induced responses. Here, we present evidence that digesting microbes is accompanied by strong downregulation of genes linked to stress response in Arabidopsis. Genome-wide transcription analysis shows that uptake of *E. coli* by Arabidopsis roots is accompanied by a pronounced downregulation of heat shock proteins. Plants upregulate heat shock proteins in response to environmental stresses including temperature, salt, light and disease agents including microbial pathogens. The pronounced downregulation of heat shock proteins in the presence of *E. coli* indicates that uptake and subsequent digestion of microbes does not induce stress. Additionally it suggests that resources devoted to stress resistance in control plants may be re-allocated to the process of microbe uptake and digestion. This observation adds evidences to the notion that uptake of microbes is an active, purposeful and intentional behavior of the plant.

Key words: heat shock proteins, Arabidopsis, plant-microbe interactions, stress response

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*Correspondence to: Chanyarat Paungfoo-Lonhienne;
Email: Chanyarat@uq.edu.au

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Interactions between microbes and plants include beneficial and detrimental relationships for the plant. Beneficial relationships include symbioses[1] such as diazotrophic endophytes that supply nitrogen[2,3] and other endophytic associations that promote plant growth.[4] Detrimental relationships involve fungal, bacterial and virus pathogens.[1] Beside these well established interactions, there is evidence for a new type of interaction that is beneficial for the plant only. Plants take up and digest non-pathogenic and non-symbiotic microbes such as *E. coli* and yeast and use them as a nutrient source.[5] The incorporation of microbes into roots occurs by mechanisms that appear to be controlled by the plant and include the generation of an extracellular cell wall-like structure for enclosing microbes at the root surface and induction of genes encoding cell wall synthesizing, loosening and degrading enzymes, to facilitate incorporation of microbes into root cells.[5]

We therefore concluded that in the absence of pathogenic or symbiotic relationships, plants coordinate the entry of *E. coli* and yeast into root cells with an apparent expenditure of energy.[5] However, for this process to be of evolutionary significance, benefits of accessing microbes as a nutrient source have to outweigh the energy expense. Such notion is in agreement with plant behavior being active and purposeful and relying on cost-benefit assessment.[6] Therefore, to fully demonstrate the benefits of this process for the plant, an analysis of nutritional gain versus energy expenditure is required. We commenced this analysis by scrutinizing the processes involved in the incorporation and digestion of microbes via the plant's metabolic changes.

Arabidopsis plants cultivated in axenic hydroponic culture for three weeks with

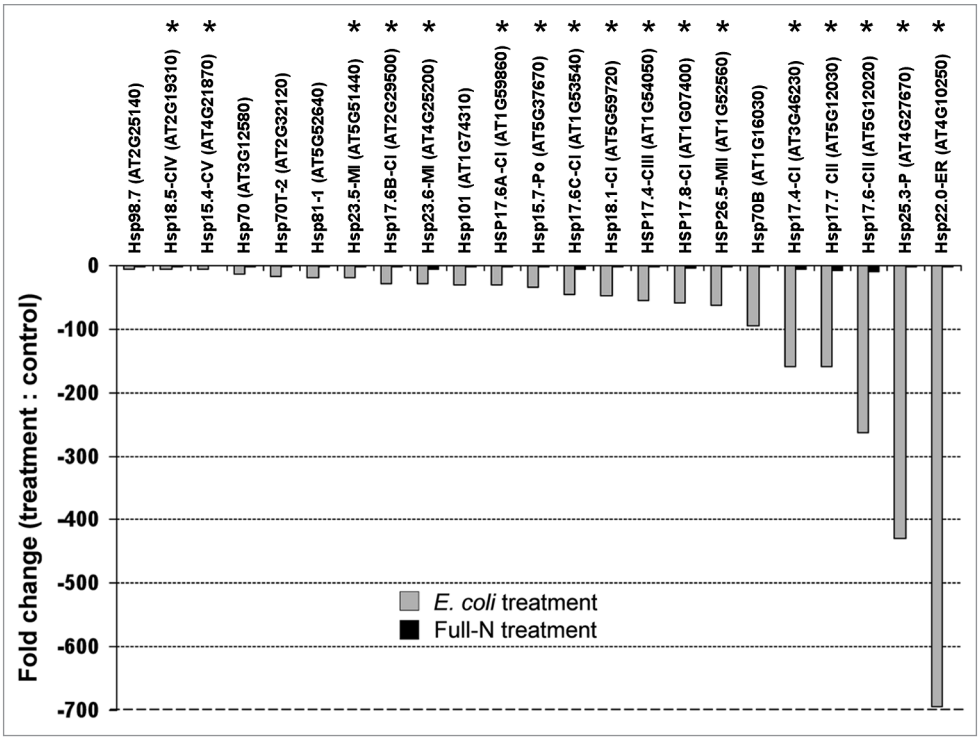

Figure 1. Differential expression of heat shock protein genes in Arabidopsis roots incubated with *E. coli* Bl21 (gray bar) or ammonium nitrate (black bar). Expression values correspond to treatments versus control (plants grown without N). Small heat shock proteins (sHSPs) are indicated by asterisk. Gene expressions greater than 2-fold change compared to control are shown for *E. coli* treatment. For microarray experiments and analysis, refer to reference 5.

full-N supplied (10 mM NH_4NO_3) were grown without N for 3 days and incubated for a further 24 h in the presence of *E. coli* Bl21 (final OD_{600} = 2, *E. coli* treatment) or 10 mM NH_4NO_3 (full-N treatment), or without any addition of nutrients (control). RNA was extracted from roots to probe an Agilent microarray (Agilent Technologies, USA). Expression values corresponded to treatments versus control. Comparative analysis of gene expression between treatments revealed that the expression of heat shock proteins (HSPs) was dramatically downregulated in plants treated with *E. coli* Bl21, compared to full-N treatment (**Fig. 1**). Heat shock proteins contribute to stress tolerance and their expression is induced in response to heat stress and multiple environmental stresses arising from biotic and abiotic stimuli.[7-9] HSPs act as molecular chaperones that regulate the folding, localization, accumulation and degradation of proteins.[10] HSPs are classified into a number of families based on their molecular mass (HSP100, HSP90, HSP70, HSP60 and small HSPs (sHSPs)).[10] Our results show that 17 sHSPs out of the 19 members of the sHSP superfamily that constitute the majority of HSPs, are downregulated in *E. coli* treatment (**Fig. 1**). All sHSP genes in plants are induced in response to environmental stresses,[7] with the exception of two genes (Hsp21.7-CVI and Hsp14.7-CVII) that are involved in specific housekeeping functions of plant cells and are constitutively expressed.[9] These two sHSPs were not downregulated in *E. coli*-treated plants confirming that the downregulation of HSPs observed in our experiments is caused by a suppressed stress response.

Thus, we argue that the absence of an induction of HSPs upon *E. coli* uptake is an indication that *E. coli* incorporation into roots does not cause stress, corroborating the notion that this process is directed by the plant and not microbes. The downregulation of numerous HSPs may be an indication that the plant undergoes resources re-allocation. Plants trade-off resources as required to remain competitive.[6,11] Resistance response to abiotic and biotic stress requires substantial resources,[12,13] providing potential for resource re-allocation. It is worth mentioning that full-nitrogen supplied plants did not significantly downregulate HSPs compared to *E. coli* treated plants (**Fig. 1**), which excludes the possibility that a replete N status is the cause of HSPs downregulation. That resources generally devoted to stress resistance may be used for the metabolic systems involved in uptake and digestion of microbes and could be crucial for optimizing the cost/benefit balance of microbial digestion.

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