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Proteome studies in Legume (*Phaseolus vulgaris L.*)

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Abstract

Proteomics techniques were used to identify the underlying mechanism of the early stage of symbiosis between the common bean (*Phaseolus vulgaris L.*) and bacteria. Proteins from roots of common beans inoculated with bacteria were separated using two-dimensional polyacrylamide gel electrophoresis and identified using mass spectrometry. From 483 protein spots, 29 plant and 3 bacterial proteins involved in the early stage of symbiosis were identified. Of the 29 plant proteins, the expression of 19 was up regulated and the expression of 10 was down regulated. Up regulated proteins included those involved in protein destination/storage, energy production, and protein synthesis; whereas the down regulated proteins included those involved in metabolism. Many up regulated proteins involved in protein destination/storage were chaperonins and proteasome subunits. These results suggest that defense mechanisms associated with induction of chaperonins and protein degradation regulated by proteasomes occur during the early stage of symbiosis between the common bean and bacteria.

Keywords: *Phaseolus vulgaris*, Symbiosis, Proteomics, *Rhizobium etli*, Root

Introduction

Legumes can establish intracellular interactions with symbiotic as well as pathogenic microbes. Such intracellular accommodation of bacteria always leads to the formation of a membrane compartment, the interface between the cytoplasm of the host and the bacterial cell [1-12]. In exchange for plant photosynthates, the endosymbiotic rhizobia convert nitrogen to ammonia which is supplied to the plant for incorporation into amino acids and ultimately proteins. In root endosymbiotic, bacteria-derived lipochito oligosaccharides with an N-acetyl glucosamine backbone, the Nod factors, play a key role in mediating recognition [4]. The perception of Nod factors through LysM domain receptor kinases [13-16] induces a chain response in host roots that includes ion flux changes [17], membrane depolarization [16], calcium spiking [11], cytoskeleton modifications [18], activation of cortical cell division [14], and root hair curling. During the early stage of Nod factor recognition, swelling of the root hair tip occurs, and is followed by new tip outgrowth, root hair branching [2], nodule primordium initiation, and formation of curled root hairs [9]. The bump-shaped nodule primordia become visible on the root surface 3 days after inoculation [15]. Nodulins, plant proteins expressed during infection and nodule maturation, play important roles in cell signaling, differentiation, and cycle reactivation [13]. Global analytical procedures applied to the study of symbiosis in leguminous plants have elucidated the roles of some symbiosis related proteins, including amylases [16], membrane trans-porters [10], glycolytic enzymes [19], malate dehydrogenase, phosphoenol pyruvate carboxylase [3], and chaperonins [12]. Although many studies of symbiosis have been carried out to date, most of these have focused on model legumes. The common bean is one of the most ancient of food crops [6]; but, no genomic sequence data is yet available for this important plant. As with other legumes, beans can enter a nitrogen-fixing endosymbiotic with prokaryotes of the genus *Rhizobium*, allowing them to grow and produce protein-rich seeds even on nitrogen poor soil. Common beans thus play a crucial role in the introduction of fixed nitrogen into both agricultural and natural systems. During the early stage of symbiotic interaction between bean plants and bacteria, root hair deformation occurs [1], and this is the first visual evidence of symbiosis [7], but the molecular mechanisms underlying the symbiotic response in legumes, such as the perception of Nod factors and next signal transduction and nodule organogenesis, stay unknown, especially in the common bean.

In this study proteins from roots of common beans inoculated with bacteria were separated using two-dimensional polyacrylamide gel electrophoresis and identified using mass spectrometry. From 483 protein spots, 29 plant and 3 bacterial proteins involved in the early stage of symbiosis were identified. Out of the 29 plant proteins, the expression of 19 was up regulated and the expression of 10 was down regulated. Up regulated proteins included those involved in protein destination/storage, energy production, and protein synthesis; whereas the down regulated proteins included those involved in metabolism. Many up regulated proteins involved in protein destination/storage were chaperonins and proteasome subunits. These results suggest that defense mechanisms associated with induction of chaperonins and protein degradation regulated by proteasomes occur during the early stage of symbiosis between the common bean and bacteria.

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