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Review

Proteomic studies of plant–bacterial interactions

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ABSTRACT

Plants and bacteria can interact with one another in a variety of different ways. The interaction may be beneficial, harmful or neutral for the plant, and sometimes the impact of a bacterium may vary as the soil conditions change. While a number of different soil bacteria are phytopathogenic, the majority of the more agronomically important plant disease-causing soil microorganisms are fungi. On the other hand, plant growth-promoting bacteria are typically of three general types: those that form a symbiotic relationship with the plant, those that are endophytic and colonize the inner tissues of the plant, and those of soil bacteria, which have competitive abilities to colonize efficiently the rhizosphere and the surface of plant roots.

While there have been significant advances in elucidating the mechanistic details of plant–bacterial interactions in recent years, many fundamental questions about these processes remain. Unfortunately, studies that focus on only a single biochemical pathway or mechanism often miss the multiplicity of effects that plants and bacteria have on one another, motivating the employment of broader proteome-wide approaches. On the other hand, using proteomics technology including high-resolution two-dimensional gel electrophoresis (2-DE) and high-sensitivity mass spectrometry (MS), it is possible to gain greater insight into the detailed impact that plants and soil bacteria have on one another.

In this regard, of all of the proteomic studies of plant–bacterial interactions, the symbiotic interaction between nitrogen-fixing bacteria and legumes has been studied in the greatest detail. Studies of the proteome of plant–pathogen interactions have also received considerable attention. However, there are currently very few proteomic studies of endophytic and rhizosphere associated plant growth promoting bacteria.

Here, some fundamental proteomic tools are introduced and the applications of one of these approaches (i.e., 2-DE coupled to MS) to the study of plant–bacterial interactions are discussed. This review specifically addresses the questions: what are the impacts of plants on the bacterial proteomes, and vice versa?

1. Introduction

Soil contains a large number of different types of microorganisms including bacteria, fungi, actinomycetes, protozoa and algae (Paul and Clark, 1989), with bacteria being by far the most common of these. Many soil bacteria interact with the roots of plants so that the concentration of bacteria that is found in the rhizosphere is generally much greater than the bacterial concentration in the rest of the soil (Dessaux et al., 2010; Lynch, 1990; Pinton et al., 2007), presumably reflecting the high levels of nutrients that are exuded from the roots of most plants and are then used to support bacterial growth and metabolism (Ahmad et al., 2008; Whipps, 1990).

The interaction between bacteria and plant roots may be beneficial, harmful or neutral for the plant, and sometimes the effect of a particular bacterium may vary as the soil and environmental conditions change (Lynch, 1990). For example, a bacterium that facilitates growth by providing plants with fixed nitrogen, which is often present in only limited amounts in the soil, is unlikely to provide any benefit to plants when large amounts of chemical nitrogen fertilizer is added to the soil.

While there are some soil bacteria that are pathogenic to plants, most of the more agronomically important plant disease-causing soil microorganisms are fungi, including the genera *Fusarium*, *Pythium* and *Rhizoctonia*.

The bacteria that provide some benefit to plants [i.e. plant growth-promoting bacteria (PGPB)] are of three general types: (i) those that form a symbiotic relationship, which involves formation of specialized structures or nodules on host plant roots, (ii) those

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that are endophytic and colonize the inner tissues of the plant without being pathogenic, and (iii) those that are able to competitively colonize the rhizosphere and plant root surface (Glick et al., 1999). While numerous soil bacteria are considered to be PGPB, not all bacterial strains of a particular species have identical metabolic capabilities. Thus, for example, some *Pseudomonas putida* strains actively promote plant growth while others have no measurable effect on plants.

While there have been significant advances in elucidating the details of plant–bacterial interactions in recent years, many fundamental questions about these processes remain to be resolved. However, approaches that examine only a single biochemical pathway often miss the plethora of effects that plants and bacteria have on one another, motivating the employment of broader proteome-wide approaches. Proteomic characterizations enable researchers to investigate the detailed response of plants and bacteria to various treatments and to one another. As a consequence of the development of various proteomics technologies, including high-resolution two-dimensional gel electrophoresis (2-DE) and high-sensitivity mass spectrometry (MS), there has been a significant increase in the amount of literature dealing with bacterial and plant proteomes (Jorrín-Novoa et al., 2009). To date, most of the reported studies have focused on the detection of protein expression changes in response to toxicity, nutrient changes, mutations, or over-expression of particular genes, with a more limited number of studies addressing plant–bacterial interactions. However, to establish a correlation between the functions of the proteins of interest and particular stimuli, it is necessary to use a variety of genetic and biochemical approaches in subsequent studies. Of the proteomic studies of plant–bacterial interactions, the symbiotic interaction between nitrogen fixing bacteria and legumes has been studied in the greatest detail (Jorrín-Novoa et al., 2009) with the proteome of plant–pathogen interactions also receiving considerable attention (Mehta et al., 2008; Quirino et al., 2010). In spite of their potential tremendous agricultural and environmental importance (Glick et al., 2007a,b; Lucy et al., 2004), there is a dearth of proteomic studies of endophytic and rhizosphere PGPB, likely in part because these organisms and their modes of action are less well characterized (Pühler et al., 2004).

Here, some fundamental technical aspects of certain proteomic tools are introduced and the applications of one of the key comparative proteomic profiling technologies (2-DE coupled to MS) in studies of plant–bacterial interactions are discussed. This review does not include details of the interactions between plants and other soil organisms (i.e., fungi, oomycetes, nematodes). Instead, the overall objectives of this review may be summarized as addressing the following questions: (i) what are the impacts of plants on the bacterial proteome?; (ii) what are the impacts of pathogenic bacteria on the plant's proteome?; and (iii) what are the impacts of beneficial bacteria on plants, both physiologically and proteomically?

2. Techniques in proteomics

There are currently a large number of proteomic techniques available for the analysis of various aspects of proteins, including their post-translational modification (PTM), expression profile, and interaction network (Pandey and Mann, 2000). The two most commonly utilized proteomic methods, two-dimensional gel electrophoresis (2-DE) and mass spectrometry (MS), are elaborated below. Then, the most broadly utilized differential display tool-difference gel electrophoresis (DIGE), which is an extension of the 2-DE technique to compare multiple samples simultaneously, is described. Other less common strategies that may be employed to elucidate plant–bacterial interactions are briefly discussed as well.

2.1. Protein separation using 2-DE

2-DE, a central tool in proteomic research, is a technique that was first developed in the 1970s for large-scale protein separation (Klose, 1975; O'Farrell, 1975). This technique begins with the separation of proteins based on their isoelectric points (in the first dimension) by isoelectric focusing (IEF), and then (in the second dimension) according to their subunit molecular masses by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The sequential combination of these methods in two perpendicular directions enables separation of thousands of proteins in a single gel. Separated protein spots may be subject to a variety of subsequent analyses, including western blotting; visualization by pre-electrophoresis fluorescence labeling; post-electrophoresis staining with coomassie blue, silver staining or SYPRO dyes; differential expression analysis; and identification by Edman degradation or mass spectrometry (MS). Protein spots of interest are excised from a gel and then digested with proteases (e.g. trypsin or GluC) before being analyzed by MS to determine their identities (Mann et al., 2001). High-resolution 2-DE remains the preferred method for protein separation because of its ability to simultaneously separate a large number of proteins and their isoforms, even though there are several technical problems inherent with this approach including inadequate consistency of protein separation and poor resolution of proteins that are not highly abundant, basic (e.g. ribosomal and nuclear proteins) or hydrophobic (e.g. membrane proteins). The reproducibility of 2-D gels is no longer a problem since the equipment and reagents that are commercially available include the use of immobilized pH gradients (IPG) (Görg et al., 1988, 1995, 2000, 2009). In addition, the establishment of more or less standardized proteomic methodology has decreased the variability of protein separations and increased the reliability of this technique (Görg et al., 1988, 1995, 2000, 2009).

The above mentioned improvements notwithstanding, the quality of 2-D gels is still heavily dependent on the expertise of the individual experimenter. Another technical problem with 2-D gels is the difficulty in detecting low abundance proteins, including regulatory proteins, signal transduction proteins and receptor proteins. For example, the predicted dynamic range of protein concentrations in plasma is ~12 orders of magnitude (Corthals et al., 2000), making it extremely difficult to analyze relatively low abundance proteins. However, many more proteins can be displayed and analyzed if samples are pre-fractionated or enriched (Corthals et al., 2000; Görg et al., 2009; Stasyk and Huber, 2004), or separated on narrow-range or ultra-narrow-range immobilized pH gradient strips (Corthals et al., 2000; Görg et al., 2009). Membrane and alkaline proteins, both of which are particularly difficult to resolve, have also been sucessfully analyzed by 2-D gel studies. Even though the hydrophobicity of the membrane proteins is problematic for every step in 2-DE, from protein sample extraction to entering the second dimension polyacrylamide gel, they have been successfully analyzed by 2-DE by incorporating thiourea, acetonitrile or detergents such as tetradecanoylamide-propyl-dimethyl ammonio-propane-sulforate in the 2-D sample buffer (Görg et al., 2009; Nouwens et al., 2000). Effective 2-D separation of alkaline proteins has also been made possible by the combination of various strategies such as the addition of isopropanol to the 2-D rehydration buffer and the utilization of pHs gradients up to pH 12 (Görg et al., 1997, 1998, 1999, 2009; Hoving et al., 2002). Low abundance and basic proteins may be well resolved in 2-D gels (e.g. Klose, 1975; Klose and Kobalz, 1995; O'Farrell et al., 1977) by the application of an alternative IEF method, nonequilibrium pH