2004

SAN LUIS VALLEY RESEARCH CENTER COMMITTEE (SLVRCC)

PROJECT OUTLINE

Project Title: Plant root exudates of potatoes: a new source of antibiotics

Principal Investigator: Dr. Jorge M. Vivanco (Department of Horticulture and Landscape Architecture)

Collaborators: Dr. David Holm (Colorado State University)
Dr. Herbert Schweizer (Colorado State University)
Dr. Fred Ausubel (Harvard Medical School)

Keywords: antimicrobial compounds, antibiotics, root exudates, human diseases.

I would like to respectfully request support from the San Luis Valley Research Center Committee (SLVRCC) to initiate a new project that will give new value-added properties to the potatoes grown in the San Luis Valley and provide a new way to market this crop. This proposed work is an outgrowth of the groundbreaking research currently underway in my laboratory, work which has identified several root-secreted compounds in various plants with exciting potential as antibiotics, herbicides, and antifungal agents (Walker et al. 2004).

INTRODUCTION

Plant roots, the very important "hidden half" of a plant system, are well-known for their primary functions in plants: they serve to anchor the plant to the ground, and they help the plant absorb water and nutrients from the soil. Recent studies conducted in my laboratory have explored various other functions of roots, including the production of secondary metabolites (such as rosmarinic acid) and roots' critical role in returning the sun's energy to the soil in the form of organic compounds. However, some processes involving roots, such as the secretion of various compounds into the soil surrounding the root (a process known as root exudation), are still largely unexplored (Walker et al. 2003a). For example, it is estimated that approximately 5-21% of all photosynthetically fixed carbon gets transferred to the rhizosphere through root exudation (Bais et al. 2004a), yet most of the work done to date has merely catalogued this process, without illuminating the mechanisms by which a plant controls this process, or why plants engage in such an apparently wasteful process. Until recently, root exudates had been simply categorized into two groups: low molecular weight compounds, such as amino acids, organic acids, sugars, phenolics and various other secondary metabolites, and high molecular weight compounds, mainly mucilage and proteins.

BACKGROUND AND PRELIMINARY RESULTS

Recent studies in our laboratory on root exudates have revealed their enormous potential as antibacterial and insecticidal agents (Walker et al. 2004, Bais et al. 2002). For instance, Walker et al. (2003b) have reported 289 secondary metabolites in the root exudates of *Arabidopsis*, several of which exhibit a wide range of anti-microbial activities against bacteria and fungi. In another report by Bais et al. (2002), fluorescent beta-carboline alkaloids from the root exudates of *Oxalis tuberosa* were shown to exhibit a strong insecticidal effect against the insect *Trichoplosia*. Within the past year, we have shown that root exudates of *Centaurea maculosa* (spotted knapweed) contains catechin, a powerful herbicide and fungicide. We have used this discovery to explore the ecological and evolutionary ramifications of root exudation (Bais et al 2003a), as well as to attain practical goals, such as the suppression of the highly invasive spotted knapweed. Our groundbreaking research has been highlighted in popular newspapers, magazines, and TV news shows, including *The New York Times*, *Scientific American Magazine*, *National Geographic*, *CNN News*, and the *Discovery Channel*.

In August 2002 we began work with the laboratory of Dr. Fred Ausubel (Harvard Medical School, Massachusetts General Hospital), to establish a new system for drug discovery that uses plant roots to develop new antibiotics and produce them in large quantities. This is one of our most exciting collaborative projects. Until 1995, it was believed that in general, plants were incapable of being infected by human pathogens. This belief changed when Dr. Ausubel reported that strain PA14 of the human pathogen *Pseudomonas aeruginosa* (the bacteria that affects Cystic Fibrosis patients) was an effective pathogen of a variety of plants, including *Arabidopsis thaliana* (Rahme et al. 1995). This discovery meant that it was possible to use plants to study the infection process of various human diseases, and to develop aggressive new treatments. One of the subjects our two laboratories have explored is why some (or most) plants are *not* infected by human pathogens; one of the important reasons for resistance appears to be the protective chemicals that plants exude into the soil. Different plant species produce differing arrays of compounds in their root exudates, and some of these compounds were found to be toxic to *P. aeruginosa*. This technology has recently been patented by Colorado State University and Massachusetts General Hospital.

The success of our project will increase the number of effective antibiotics available to medicine, but more immediately will allow the SLV Potato Growers Association to propose that eating potatoes may prevent and cure microbial diseases in humans because they've got naturally-occurring antibiotics. We are confident that the isolation of antibiotics in the potato root exudates predicts that these medicinal compounds will also be found in the edible part of the plant, the tubers. Furthermore, it's our hypothesis that SLV potatoes are particularly rich in these types of antibiotic compounds, as they tend to be produced in response to stressful growing conditions—such as those found at relatively high elevations, with increased exposure to UV light. We anticipate that this research will provide a new marketing and value-added tool for the potatoes.

We have reached the point where our lab's knowledge and expertise is sufficient to attempt research on the antibiotic potential of potato root exudates against various human microbial diseases (Walker et al 2004, Bais et al. 2003b). Various exudates' proven ability as antiviral and antimicrobial agents suggests that such research holds great promise.

RATIONALE

The main rationale behind this proposal is the proven potential of root exudates as anti bacterial, insecticidal and herbicidal agents, as well as the existence of certain qualities inherent to exudates, such as the potential to mass produce a particular compound in bioreactors. Furthermore, several plant secondary metabolites, such as taxol, vincristine and vinblastine, have already been shown to have anti-cancer properties. However, to date no one has tried using root exudates as antimicrobial agents. Thus it is proposed to explore the novel use of root exudates for their possible antibiotic potential.

We anticipate that potato varieties with reputed health benefits currently being developed by Dr. David Holm's program (CSU) will show antibiotic activity. The successful completion of this project will provide a unique marketing tool for Colorado potatoes. To the best of our knowledge, not a single potato program in the United States or in the world has ever proposed the screening of potatoes for unique antibiotic properties against human microbial diseases, even though the work funded by the SLVRCC in my laboratory has shown that potatoes produce several compounds known to have antifungal activity against *Phytophthora infestans*. The stature of Colorado State University as a national center of excellence for the studies of microbial diseases will further enhance the development of this project. Furthermore, the national and international recognition of our program and the media attention that surrounds our research will facilitate the rapid dissemination of our findings through scientific and popular publications. This will provide an additional marketing and advertisement tool for the potato growers in Colorado.

PAST EXPERIENCE WITH OTHER PLANTS AND METHODS

Traditionally, antimicrobial drug discovery has consisted of directly screening compounds from natural sources or those developed in the laboratory directly against target microorganisms. What our laboratory has done instead is to screen indirectly: we developed a system where we tested over fifty different plant species for their ability to survive *P. aeruginosa* infection. Those that survived we examined more closely, analyzing and characterizing the compounds they exuded from their roots; this technique allowed us to identify different potential compounds against this pathogen after just a few weeks. All of these potential antibiotics are currently undergoing further study.

We used classical plant culture techniques, growing plants in sterile nutritive liquid and adding *P. aeruginosa* (strain PA14) to the submerged roots; the liquid medium allows us to measure exactly what and how much is exuded by each root. We evaluated the different degrees of susceptibility, tolerance and resistance to *P. aeruginosa* (PA14) infection in all fifty plants. Susceptible plants died because of *P. aeruginosa* infection; tolerant plants survived but didn't substantially affect the growth of *P. aeruginosa*, and in the resistant plants both survived and actively killed the microbe (Walker et al., 2004; Bais et al., 2004).

We found that the fifty plants exhibited a wide range of responses to *P. aeruginosa*. In several cases we have been able to link the ability to tolerate or resist infection to the root exudates and in some cases to specific compounds found in the root exudates. The majority of the plants (~ 88%) were susceptible and succumbed to *P. aeruginosa* (PA14) infections within seven days. *Gaillardia aristata* (blanket flower) exhibited a potent resistance (~ 11.23 % mortality rate), and *Solanum melongena*, *Oxalis tuberosa*, *Ullucus tuberosum* and *Lithospermum esculentum* exhibited varying degrees of tolerance (~ 45.9% mortality rate).

These results confirmed the usefulness of root exudates as a continuous source for novel antimicrobials. For these particular experiments we used just the one human pathogen; our method, however, is endlessly flexible and could be adapted to any fungal, bacterial, or even viral pathogen, and any number of plants. We intend to use it to screen for potential antibiotics produced by the roots of potato plants.

MATERIALS AND OBJECTIVES

We will use the following varieties of potato that fall under three categories:

1) Health-related potatoes whose activity is related to color of the tubers and/or specialty potatoes (CO94165-3P/P, CO94183-1R/R, VC0967-2R/Y, Yukon Gold)
2) Advanced selections and varieties that are showing potential or are currently used in the San Luis Valley (Russet Norkotah, Russet Nugget, Russet Burbank, CO89097-2R, CO86218-2R, Sangre, AC89536-5RU, AC92009-4RU).

We aim to achieve the following objectives:

- Screen the above potato varieties for their ability to resist the attack of three important human pathogens: *Pseudomonas aeruginosa*, *Enterococus faecalis* and *Staphylococcus aureous*.
- Once appropriate potato varieties have been selected, we will test the root exudates of these plants for their ability to inhibit the growth of the human pathogens (or kill them outright).
- Next, we will isolate and characterize the active compounds from the root exudates by various chromatographic techniques, such as high liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance (NMR).

- Once active compounds have been isolated and characterized, we will test them on the microbes, and search for their presence in the potato tubers.

It is worth mentioning that all the procedures and methods are currently practiced at Dr. Vivanco's laboratory. Furthermore, Dr. Vivanco's laboratory is a Type II Containment facility. Thus, we are qualified and certified to work with Type II human microbes such as the ones described above.

Time Table

This project will take two years to be accomplished from start to finish.

Budget

Year 1 (2004): $30,000;Year 2 (2005): $30,000

Budget Justification

Research assistant/graduate student: To maintain a vigorous output of ideas, data and to coordinate the different projects proposed in this application, we request support for a research assistant/graduate student salary over the course of the project. We need a dedicated person with natural product/chemistry/physiology experience who will complement the biochemistry expertise at Dr. Vivanco's laboratory.

Supplies: Funds are requested for chemicals, supplies, limited small equipment and maintenance of equipment used as part of this project.

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