A Fluorescent Mutant of the Phosphorus-Solubilizing Fungus *Penicillium bilaiae* to Image Rhizosphere Growth

Shelagh Steckler, Caressa Caldwell, Kari Priest, and David Greenshields*

Novozymes BioAg Limited, 3935 Thatcher Avenue, Saskatoon, SK S7R 1A3
http://bioag.novozymes.com
*Corresponding author (dvgs@novozymes.com)

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Abstract

Novozymes manufactures and markets a phosphate inoculant, JumpStart®, based on the phosphorus-solubilizing fungus *Penicillium bilaiae*. We are developing a mutant of this fungus that expresses the DsRed-Express fluorescent protein, which will be used as a research tool to allow us to see where and how *P. bilaiae* interacts with plant roots.

Introduction

Novozymes manufactures and markets a phosphate solubilizing inoculant, JumpStart®, based on the phosphorus-solubilizing fungus *Penicillium bilaiae* (Kucey, 1983; Leggett et al., 2007). Strains of *P. bilaiae* can solubilize phosphate on agar plates and in liquid culture (Kucey, 1983; Wakelin et al., 2004), and can increase the growth and P uptake of various crops in both greenhouse and field trials (Kucey, 1987). To realize the full potential of phosphate-solubilizing fungi as inoculants, we need to better understand how *P. bilaiae* and similar organisms work to promote plant growth and increase yield.

The interaction between these types of beneficial organisms and plant roots is of particular interest. Colonization of roots by *P. bilaiae* can be observed microscopically, but this work is tedious and requires well-washed roots, which can be difficult to obtain from plants grown under field conditions. Root colonization by *P. bilaiae* has been confirmed using PCR (O'Gorman et al., 1998). While PCR is useful for determining presence or absence of an organism, it does not allow for visualization of the interaction between plant and fungus.

To better examine how *P. bilaiae* interacts with plant roots, we developed a mutant that expresses the DsRedExpress fluorescent protein. These fluorescent strains will be used as a research tool to allow us to study the *in* situ ecology of *P. bilaiae*. This project is part of Novozymes' commitment to on-going research using world-leading technology platforms.

Methods

1. Development of reliable protoplasting protocol

The protoplasting protocol was developed based on methods for *Aspergillus* spp. (Novozymes, unpublished) and other filamentous fungi (Turgeon et al., 2010). Young *P. bilaiae* was collected

by vacuum filtration, and digested with Glucanex® 200 G enzyme at 37 °C for 2 h. This solution was filtered through MiraCloth, overlaid with separation buffer, and protoplasts were isolated using differential centrifugation

2. Development of fluorescent/fungicide-resistant plasmid

The pBCphleo-DsRedExpress plasmid was made by cutting an EcoRV fragment containing pgpdA-DsRedExpress-TrpC from pPFJo181 (Novozymes, unpublished) and ligating it into the AleI site of pBCphleo (Silar, 1995).

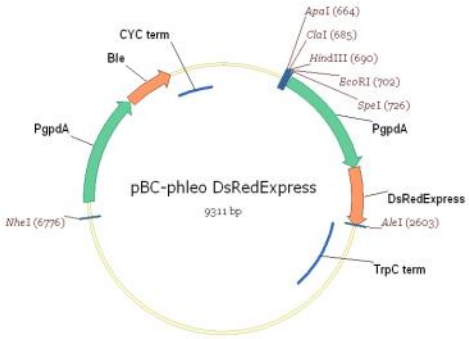

Figure 1. Plasmid construct containing red fluorescence and Bleocin resistance.

3. Transformation of *P. bilaiae* to express fluorescent proteins

The pBCphleop-DsRedExpress construct was randomly inserted into the genome of *P. bilaiae* strains according to El-Ganiny et al. (2010). Transformants were isolated from PDA + 100 μg mL^{-1} Bleocin and confirmed with a Nikon H600L epifluorescence microscope (Figure 3). Liquid spore stocks were produced from sporulating plates and stored in glycerol at -80 °C.

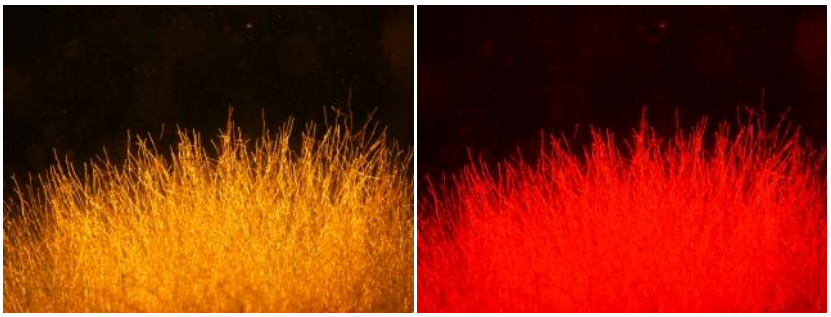

Figure 2. *Penicillium bilaiae* transformed with pBCphleo-DsRedExpress plasmid imaged with transmitted light (left) and epifluorescence (right).

4. Development of colonization assays to study root-fungi interactions

A variety of seeds (e.g., wheat, canola, corn) will be inoculated with wild-type *P. bilaiae* or pBCphleo-DsRedExpress transformants. Spore suspensions for on-seed inoculation are developed by growing strains on agar media, and collecting spores by washing plates with 0.1$ Tween80. The spore suspension is applied to pre-weighed seeds, which are planted into sterilized growth media (e.g., potting mix, turface, sand, etc.). After 1 to 2-weeks growth, plants are carefully removed from the growth media, and roots are washed in sterile water. Roots can then be mounted on slides and observed under red fluorescent light to improve visualization of colonization over standard staining methods.