

istecs – europe & PPI Prancis

Proceeding

ISSN 0855-8692



The 5th Indonesian Student's Scientific Meeting
October 6th – 7th, 2000
Paris, France

Isolation and Determination of Indigenous Endophytic Bacteria from Greenhouse Tomato Plants

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Abstract

Rhizosphere bacteria represent the largest part of the rhizosphere microbial community. Basic knowledge including isolation, identification and characterization of the bacteria is very important in order to utilize the beneficial of rhizobacteria as plant growth and health promoting. Endophytic bacteria, bacteria that reside within living internal plant tissue, may be considered as a plant-growth and plant-health promoting rhizobacteria. In this experiment, indigenous endophytic bacteria from tomato plants in the greenhouse were isolated and identified. The isolation of bacteria was based on the trituration and surface sterilization method. The Bacteria were identified using Fatty Acid Analysis (FAME) and Gas Chromatography (GC).

Key words : indigenous endophytic bacteria, tomato plants, isolation

Introduction

Studies on the beneficial of plant-associate microorganisms have been conducted for many years and some of them are well known to increase nutrient availability, produce growth hormones, or deter plant pathogens [1]. Endophytic bacteria are plant-associate bacteria that are able to colonize and persist in various healthy plant tissues, including fruits, vegetables, stem and roots [2]. Indigenous endophytic bacteria isolated from the internal plant tissue of healthy plants comprise over 129 species of 54 genera and the most commonly isolated bacterial genera are *Pseudomonas*, *Bacillus*, *Enterobacter* and *Agrobacterium* [3].

Endophytic bacteria have been defined by Kado [4] as " bacteria that reside within living plant tissue without doing substantive harm or gaining benefit other than securing residency". Hallmann *et al.* [3] have defined that any bacterium as an endophytes if it can be isolated from surface disinfected plant tissue or extracted from inside the plant. Since bacterial endophytes have a natural association with plant and can colonize plant tissues without inciting disease, they are potential candidates for use as agricultural inoculants with provide plant growth-promoting or biological control of plant diseases [5; 3].

It is very important to know a general overview of root colonization by endophytic bacteria and to have a quantitative understanding of the indigenous endophytic bacterial community to help assess endophytes as potential sources of effective strains for plant-growth and plant-health promoting. Objectives of this experiment were to determinate and characterize physiologically of indigenous endophytic bacteria from greenhouse tomato plants

Materials and Methods

Isolation of endophytic bacteria

Bacteria were isolated from the root and stem of tomato plants. Two weeks of tomato

CV. "Hellfruch Fruchstamm" were planted singly on pot consists of soil, sand and compost (2:2:1). After 2 months the plants were harvested. Roots were washed and then sterilized in 3% NaOCl and 0.01% Tween 20 for 3 minutes [3]. Then the roots were washed three times in 0.1 M Potassium phosphate buffer and were homogenized with mortar and pestle in buffer solutions under aseptic condition. An appropriate dilution of bacterial cells was plated on 10% tryptic soy agar (TSA). Strains of bacteria were randomly selected and purified on 100% TSA. The bacteria were stored in tryptic soy broth (TSB) plus 20% glycerol at -80°C . The bacteria were identified using fatty acid analyses (FAME) and MIDI system.

Identification of endophytic bacteria

Harvesting. The bacteria from -80°C were growth on special tryptic soy broth agar (TSBA) and incubated at 28°C for 24 hours. A large loopful of bacteria (about 0.05g) was placed into a 13 x 100 ml culture tube.

Saponification. One ml of 15% NaOH in 50% methanol (Reagent 1) was filled into the tube and the tube was sealed with a teflon-lined cap. Fatty acids are saponified by heating the tube for 30 minutes in a 100°C waterbath, with two brief periods of mixing during the heating and the tube was cooled.

Methylation. Following cooling, 2 ml of 6 N HCL in 50% methanol (Reagent 2) was added, the tube recapped and heated at 80°C for 10 minutes to methyl esters of the free Fatty acids and cooled rapidly.

Extraction and wash. Extraction into 1 ml of hexane/MTBE (1:1) (Reagent 3) was followed by discarding of the aqueous phase and washing of the organic extract with 3 ml of 1.2 % aqueous NaOH (Reagent 4). The Fatty acids in the hexane/ether extract were then assayed with a gas chromatograph.

Results and Discussion

Bacteria were recovered from surface-disinfected root and stem of tomato plants on media Tryptic soy broth agar (TSA). The plants were growth in different growth media; soil & sand, soil and composts and field soil only. Total endophytic bacteria populations from tomato roots were higher than from tomato stems. The populations of bacteria from tomato roots or stems with different growth media did not vary. Total populations of bacteria in roots were between 10^4 - 10^5 cfu/g fresh weight, and populations in stems were between 10^2 - 10^4 cfu/g fresh weight.

A total 102 bacteria, 62 bacterial species were isolated from tomato roots and 40 species were isolated from stem. The endophytic bacteria isolated comprised 15 species of 8 genera; 14 species were found in root and 10 species were found in stem.

The most frequently isolated groups were *Pseudomonas putida* from tomato roots and *Bacillus megaterium* from tomato stems. Some endophytic bacteria were found to be associated either in the root or in the stem. Because some endophytic bacteria are able to colonize in the root and stem of the plant systematically.

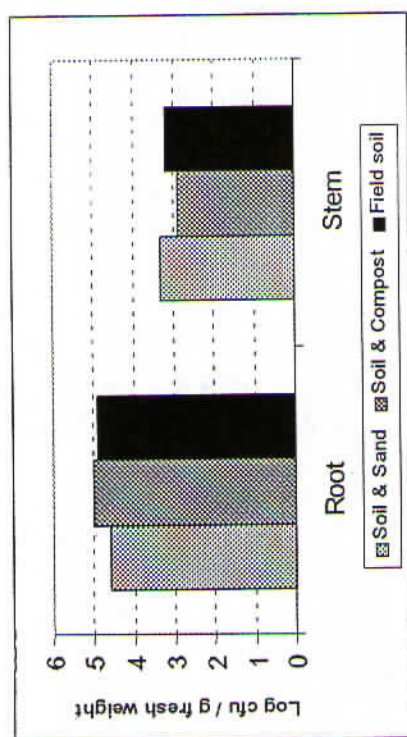


Figure 1. Population of endophytic bacteria isolated from tomato roots and stems after surface-disinfected sterilization with NaOCl 1.5%.

Table 1. Identification and isolation frequency of endophytic bacteria from tomato root and stems

Bacterial species	Root	Stem
<i>Bacillus megaterium</i>	6.45 %	30.00 %
<i>Bacillus pumilus</i>	-	10.00 %
<i>Chryseobacterium balustinum</i>	6.45 %	-
<i>Chryseobacterium indologens</i>	3.23 %	-
<i>Curtobacterium flaccumifaciens</i>	3.23 %	-
<i>Erwinia amylovora</i>	6.45 %	-
<i>Kluyvera cryocrescens</i>	8.06 %	7.50 %
<i>Micrococcus varians</i>	3.23 %	2.50 %
<i>Neisseria flavescens</i>	8.06 %	2.50 %
<i>Pseudomonas fluorescens</i>	6.45 %	2.50 %
<i>Pseudomonas chlororaphis</i>	6.45 %	5.00 %
<i>Pseudomonas putida</i>	29.03 %	7.50 %
<i>Pseudomonas savastanoi pv. nerium</i>	1.60 %	-
<i>Pseudomonas syringae</i>	1.60 %	-
<i>Stenotrophomonas maltophilia</i>	3.23 %	7.50 %
Unknown	6.45 %	7.50 %

Chryseobacterium balustinum, *Chryseobacterium indologens*, *Curtobacterium flaccumfaciens*, *Erwinia amylovora*, *Pseudomonas savastanoi* pv. *nerium* and *Stenotrophomonas maltophilia* were isolated only from tomato roots; *Bacillus pumilus* was only from tomato stems. These data indicate that internal tomato root support a more diverse microbial flora than in the tomato stem.

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