ISOLATION OF SOME ENDOPHYTIC BACTERIAL STRAINS FROM POTATO TUBERS

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Abstract. Endophytic bacteria and fungi occur in different wild and cultivated plants, being a wide distributed phenomenon. The potato tubers contain in their vascular and pulp areas bacteria that can be used for beneficial purposes including antagonists of pathogenic bacterial and fungi strains. The work presents some preliminary results of this isolation, with the purpose to identify strains of interest for agriculture.

Key words: *potato tubers, endophyte bacteria*

INTRODUCTION

The endophytic fungi and bacteria are microorganisms associated with the plant tissues without causing an apparent disease. Endophytic bacterial strains were isolated from potato varieties Zagadka and Nigru, the strains showed to have a beneficial action on plants. The introduction of *Pseudomonas fluorescens* inside the plants tissues looks like to enhance the activity of bacteria from tissues (Podolich et al. 2007).

Many wild and cultivated plants were investigated for endophytes, finding there a lot of bacteria and fungi strains by cultivation and culture independent methods. Many authors showed the presence of such bacteria and fungi in different species of plants. For example, the herbaceous plants used in traditional Indonesian medicine, contains about 18 strains of bacteria endophytes and about 32 strains from phyllosphere, identified by molecular methods, PCR and ARDRA (Yogiara et al., 2012). Some bacteria have proteolythic and lipolythic properties, but the strains were not characterized. Isolation of about 214 bacterial endophytic strains was reported (Ngama et al. 2012) and characterized by molecular and physical methods (MALDI-TOF), clustered in 53 genotypes and tested for enzymatic activity. The strains were identified as belonging to the *Pseudomonas, Yersinia, Serratia, Klebsiella, Rahnella, Enterobacter, Bacillus, Ewingella* and *Raoultella* genera. The beneficial effect of some endophytic bacteria was demonstrated. The bacteria release cytokines-like substances (Bhore & Satisha, 2010) with the role of plant growth regulator.

The screening of such isolates with beneficial characteristics resulted in huge biological material (bacterial strains) some of them having bio-control properties against different plant pathogens like *Dickeya* sp. (identified in stem rot of potato) demonstrating antibiosis (Chzaykowski al., 2009). Important agricultural plant species like *Glycine* sp. harbors about 65 bacterial strains some of them demonstrating cellullase and pectinase activity (Figueredo et al., 2004). The synthesis of useful metabolites by endophytic microorganism was presented and demonstrated by Strobel and Daisy (2003).

The endophytic strains looks like to be able to increase the resistance of the potatoes to different diseases and to enhance growth of the plants (Sturz et al., 1999; Ardanov et al., 2011).

The purpose of this study is to isolate bacteria strains with potential as antagonists of pathogenic fungi of cultivated plants.

MATERIALS AND METHODS

The potato tubers were provided by the National Institute of Research and Development for Potato and Suger Beet from Brasov. The variety *Santé* from this institute and the variety *Picasso* originated from Portugal were investigated. The tubers were disinfected and cut in two parts. From the tuber the samples from vascular and pulp areas were divided and grinded with a sterile metal crusher in sterile 1.5 ml Eppendorf tubes and suspended in sterile water. The mixture water- tuber pulp, was diluted with PBS and aliquots of 100µl, plated on LB agar media in Petri dishes, and incubated overnight at 26°C (figure 1). After about 30h of incubation, the colonies were picked up and each of them re-insemination on separate Petri dishes in order to obtain pure cultures.

The bacterial strains isolated from tubers were randomly selected and identified by biochemical characteristics pattern with BIOLOG system (according to the producer) using protocol A, after that the micro plates were red with the Biolog system. The strains were then inserted in cryotubes with 20% glycerol solution and stored in -80^{0}C freezer in RDIPP culture collection for further experiments in order to identify the possible antagonists against plant pathogens.

RESULTS AND DISCUTIONS

The screening of potato tuber resulted in about 37 new isolated strains, out of which only 8 were practically identified. The isolated bacterial strains were not exclusively endophytes, most of them being isolated from different other environments. Most of the tubers were practically sane, being not affected by rot or other diseases. The strains showing to be endophytic bacteria but not only most of them being isolated from different other environment. The tuber was practically sane, not affected by rot or other disease, as shown in photo. Only one of the tubers was affected by a ring rot-like area, but the presence of *Clavibacter michiganense* fv *sepedonicus* could not be demonstrated. In the same time a yeast strain of a dark mauve color was isolated but was not identified. Most of the strains were prepared according to cryopreservation protocol using as cryoprotectant the glycerol 20% and then stored at -80°C.

The main identified bacterial strains in Santé variety were: *Gordonia terrae, Pseudomonas corrugata, Rhodococcus erithropolis, Paenibacillus soli, Rhizobium radiobacter*, *Mycobacter phlei*, and *Bacillus subtillis ssp. subtillis* and in Picasso variety the bacteria *Micrococcus luteus* according to database match of Biolog software identification system (table 1).

CONCLUSIONS

The isolation of non-pathogenic and non-symbiotic bacteria confirmed their presence in association with plant tissues, but their role is still uncertain. The presence of

such bacteria which can live outside of the tubers may be a positive factor for plants. Also, the endophytic bacteria seem to have special correlation with plants and with rhizosphere bacteria. This kind of bacterial strains can be a source of several enzymes and precious metabolites for different human activities.

In conclusion, it is necessary to continuing the researches and the screening of the endophytic bacterial strains for useful products. A problem is that we do not know if the microbial endophytic community is dependent on the type of plants or is dependent of climate or some biogeographic considerations including the area of cultivated plants.

Performing researches with some intensive metagenomic analysis on each principal cultivated plant will help the understanding the role played by the endophytic bacterial strains. These strains can be considered a solution for farmers, especially for organic farm systems.

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Table 1. Identified strains isolated from potato tubers

Nr.crt	Strain name	Origin	Short description (colonies on plates)	Identification with BIOLOG System
1	CBR	Santé variety	Red colonies medium size	*Gordonia terrae*
2	PII2	Santé variety	White creamy and medium size colonies	*Pseudomonas corrugata*
3	SV1	Santé variety	Medium size cream colonies	*Rhodococcus erithropolis*
4	Pc.51	Picasso variety	Big irregular cream colonies	*Micrococcus luteus*
5	SE2	Santé variety	Little whitish colonies	*Paenibacillus soli*
6	SE 1	Santé variety	White-yellowich medium size colonies	*Rhizobium radiobacter*
7	P10.1.	Santé variety	Medium size red colonies convexes with smooth aspect	*Mycobater phlei*
8.	P44.2	Santé variety	Whitish colonies	*Bacillus subtilis ss subtilis*

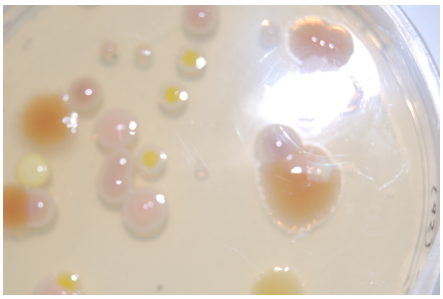

Figure 1. Bacterial strains isolated from *Santé* variety potato tubers.