Abstract

Bacterial diseases of plants cause considerable losses in agriculture due to yield damages and deterioration of the quality of plant produce. Worldwide, agriculture relies primarily on chemical control methods (e.g. pesticides) and prevention to protect crops from pathogens. However, extensive usage of such substances in farming leads to severe ecological problems. Furthermore, it is postulated that the growing resistance of plant pathogens to chemicals used in agricultural applications will increase disease incidence in the near future. An excellent example of such a disease is potato soft rot caused by bacteria belonging to Soft Rot *Pectobacteriaceae* (SRP: *Pectobacterium* spp. and *Dickeya* spp.). Until now, control of potato soft rot relies mainly on prevention, good agricultural practices, and pathogen-free seed material usage.

Biological plant protection is an ecologically friendly alternative to chemical control methods to protect crops from diseases. This method utilizes the natural ability of microorganisms to limit the growth of each other. For example, beneficial bacteria, which produce a wide array of antimicrobial compounds, can be used to prevent the spread of pathogens. However, even though this method seems promising, its application encounters several technical difficulties.

Firstly, most of the research on using microorganisms for such purposes finishes before any bioactive product is developed and introduced to the market. Likewise, the selected beneficial strains are not usually broadly evaluated under natural conditions under which the crop is maintained. Therefore, even though microorganisms may have promising antimicrobial activity under laboratory conditions, the obtained results cannot be translated directly into an economic profit and marketed products. Additionally, most of the research presented so far employs only single strains of microorganisms, not their combinations or mixtures. The reason is that the products containing only one type of microorganism are easier to register

for marketing. However, they have a narrower activity range than products containing several beneficial strains combined in one product and supporting each other.

My doctoral dissertation aimed to develop an artificial (synthetic) consortium of bacterial strains effective against SRP bacteria. Furthermore, I developed a formulation to improve the shelflife of the consortium and subsequently tested the formulated mixture containing bacterial strains for the protective activity against SRP bacteria in potato tubers under real-life storage conditions.

During the experiments on potato tubers, the consortium of five strains of Gram-negative bacteria: *Serratia plymuthica* A294, *Lellitottia amnigena* A167, *Rahnella aquatilis* H145, *Serratia rubidaea* H440 and H469 was developed, based on the disease suppressing activity. Subsequently, I tested the preservation methods for increasing the survival of synthetic consortium during storage using wettable powder formulations. Afterwards, I evaluated the prepared formulations in 6-month experiments on potato tubers kept under storage conditions mimicking natural conditions used to store commercial potato tubers. Finally, I assessed the interactions of the selected bacterial strains and their antagonisms.

The obtained results led to the development of an artificial (synthetic) consortium with the proposed formulation, which could be applied on seed tubers under storage for protection against potato soft rot. The obtained results were published in three experimental research publications.

The first article describes the development and evaluation of the artificial (synthetic) consortium of microorganisms. The second publication deals with the design, development and evaluation of the formulation of the synthetic consortium. Finally, the last publication describes the genomes of the strains comprising artificial (synthetic) consortium and genome-based characteristics of their biological control activity.

The practical aspect of my PhD project has led to three patents (two Polish and one European patent) describing: (i) the usage of the designed synthetic consortium, (ii) the way to formulate the consortium to prolong its shelf life and (iii) a lyophilization reagent that was developed during my studies.

In summary, my research led to the development and evaluation of an innovative artificial (synthetic) consortium containing five bacterial strains that can be used to protect potato tubers against *Pectobacterium* spp. and *Dickeya* spp. under storage conditions. In a series of experiments, this consortium was evaluated for its activity and preparation stability to be introduced on the market. In addition, the members of the artificial (synthetic) consortium were tested to see whether they could negatively influence each other and whether the consortium's activity was based on the cooperative action of its members.

Such analysis of the microbial consortia can help us understand the complexity of interactions between antagonistic bacteria both in artificial and in natural settings.