Bacteria and bacteriophages based biocontrol product against Soft Rot *Enterobacteriaceae* in potato tubers

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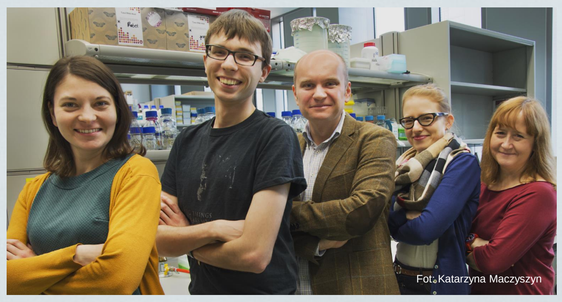
Working group



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Introduction

Soft rot *Enterobacteriaceae* (SRE) (pectinolytic *Pectobacterium* and *Dickeya* spp. (formerly known as pectinolytic *Erwinia* spp.)) are economically important plant pathogenic bacteria of various agricultural crops including potato (*Solanum tubersoum* L.). SRE are responsible for soft rot and blackleg diseases and cause increasing damage in potato production worldwide. Effective management to control blackleg and soft rot disease has not been developed and validated detection methods for SRE do not exist.

We attempt to develop a biological control product containing wild type strains of antagonistic bacteria and lytic bacteriophages to be used against SRE in potato tubers during storage and transit.

In the presented part of the project, we aimed to obtain an appropriate method for measuring development of soft rot in potato tubers, as well as on potato slices. Further we examined the antagonistic interactions of the biocontrol agents to be able to effectively design protecting combinations of antagonists.

Materials & Methods

In whole tuber experiments, potato tubers were vacuum infiltrated with 10^6 cfu of SRE bacteria (mixture of 6 strains) and 10^8 cfu of antagonistic bacteria (mixture of up to 5 strains). Each tuber was cut and assigned 0-5 mark (Fig.1). The whole set was washed and weighed to assess macerated tissue mass.

Figure 1 shows representative tubers for soft rot symptom index (0 – 5 scale)

In the potato slice experiment, the same bacteria concentrations were used. Bacteria were put in 2 repeats into 3 slices, 3 wells each. Development of soft rot was measured by radius of macerated tissue around well (Fig. 2), and by weighing of macerated tissue of the set.

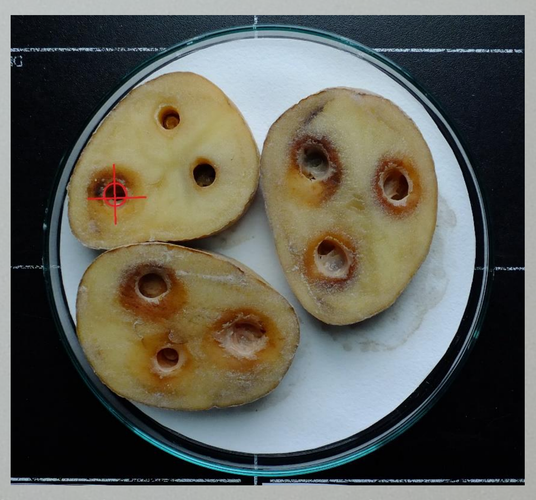

Figure 2 shows a set of potato slice experiment. Mean of two soft rot diameters were measured, well diameter was subtracted, and then measurement was divided by 2. Leading to obtaining soft rot radius (red line outside the red circle).

Interactions between antagonistic bacteria were assessed using dual culture agar assay with individual antagonistic strain. Inhibition zones were measured and mean value is presented.

Results & Discussion

In order to statically analyze results of soft rot suppression, we developed 6 stage soft rot symptom index in which we assess tuber maceration. Mean S = 183.38, p-value = 1.026e-06 rho = 0.8621184 and median mark S = 299.32, p-value = 6.007e-05 rho = 0.774951 in our scale strongly correlate with soft rot mass of a setup (Fig. 3).

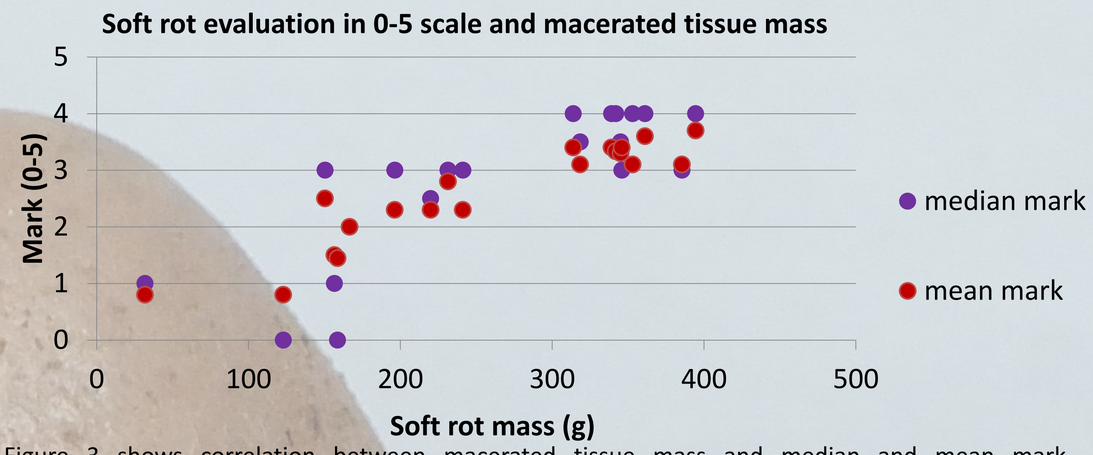

Figure 3 shows correlation between macerated tissue mass and median and mean mark of 10 potato tubers kept in one box under disease promoting conditions 28°C 96h of high humidity.

For high throughput analyzes, we have used a potato slice assay. Disease symptoms are assessed based on soft rot radius and mass, values that strongly correlate R_s = 0.951715 t = 15.501 df = 25 p = 2.487e-14 (Fig. 4).

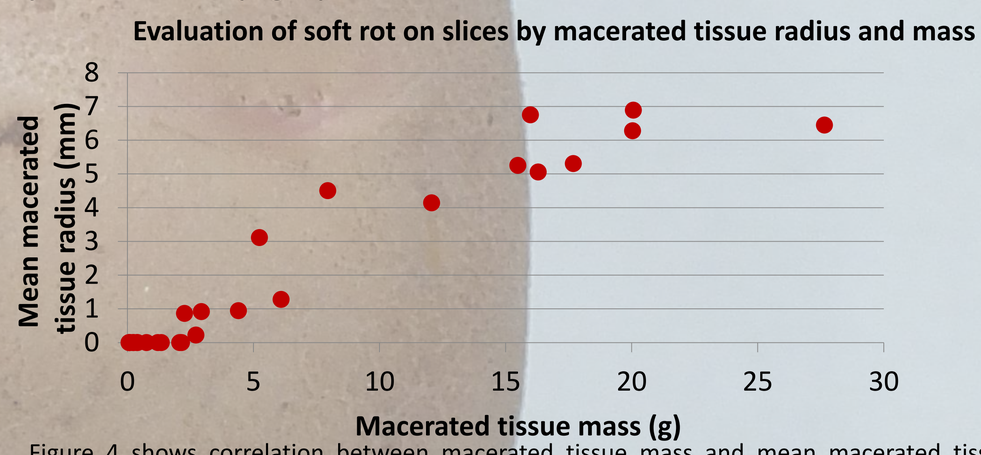

Figure 4 shows correlation between macerated tissue mass and mean macerated tissue radius of 3 potato slices with 3 wells each, kept under disease promoting conditions, 28°C 48h of high humidity.

Antagonistic interactions between chosen biocontrol agents may occure. Here, we present a network of such interactions between five selected strains (fig. 5).

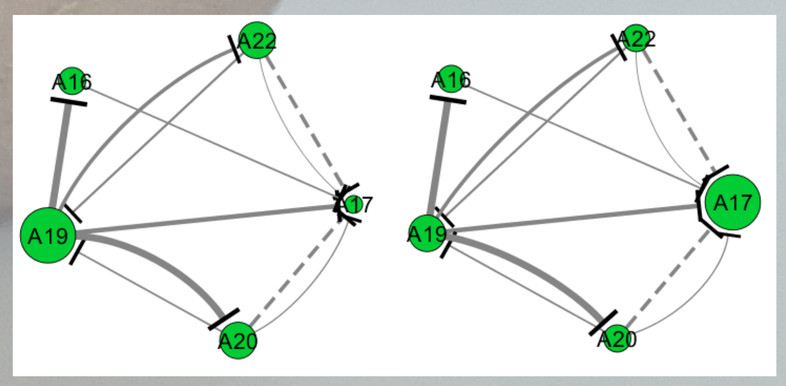

Figure 5 shows antagonistic interactions between chosen biocontrol agents. The knot size of a given strain indicates how many strains it inhibits (on the left) or is inhibited by (on the right). The thickness of connecting lines is proportional to the radius of inhibition. A dashed line (not in scale) refers to a bigger zone of suppressed growth. Strains identification is not to be reviled before patent application is accepted.

Conclusion

1. A soft rot symptom index is an easy and an efficient system for the evaluation of soft rot symptoms.
2. In a potato slice assay, macerated tissue mass and radius strongly correlate, first being more accurate, but the second an easier method.
3. Several strategies of antagonistic bacteria interaction are possible, whereas being a strong inhibitor does not always protect from being inhibited.

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