Effects of Co-cultured Bacteria and Fungi on *Pseudomonas protegens* or *Pseudomonas chlororaphis* and *Saccharomyces cerevisiae*

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Abstract

Pseudomonas chlororaphis and *Pseudomonas protegens* are two bacteria commonly found in the environment. They are known to produce antifungals and secondary metabolites that can affect fungi and other organisms to protect the roots of plants. *Saccharomyces cerevisiae* is a fungus found on the surface of fruits and used in baking. These bacteria and fungi exist in the same environment but the interactions between the two have not been studied. In this study, *P. chlororaphis* and *P. protegens* were cocultured with *S. cerevisiae* to figure out how the bacteria affects yeast cell growth and physical morphology. Based on the cocultures, *P. chlororaphis* and *P. protegens* were able to coexist equivalently with *S. cerevisiae* with no changes to the morphology of the yeast cells. However, when the bacteria and yeast cells were streaked overlapping each other, the bacteria colonies appeared mucoid. *P. protegens* began to connect via cell-to-cell junctions at each end of the filament giving the cells an elongated appearance and allowing them to share nutrients thus increasing survival. These results support the two organisms coexisting in an environment potentially helping plant growth.

Background and Significance

- Bacterial-fungal interactions could be the reason why some ecosystems thrive.
- The interactions between plant-specific *Pseudomonas* and *Saccharomyces cerevisiae* is not well documented.
- Characterizing the interactions between various *Pseudomonas* strains and *S. cerevisiae* could help us understand why bacteria strains can antagonize or promote fungal growth
- Bacterial-fungal interactions are cooperative or competitive and can vary based on species, strains, the ratio of bacteria to fungi, and the environment they are found in.
- *P. protegens* and *P. chlororaphis* are plant specific strains found near the roots of plants that have positive effects on plants
- *Saccharomyces cerevisiae* is a highly adaptive, dominant fungi found on the surface of fruits
- *Pseudomonas* strains primarily use secondary metabolites and secretion systems to produce antifungal which negatively impact fungal growth
- Past studies have shown mucoid bacteria growth due to *S. cerevisiae* and increased viability in the stationary growth phase
- Lactic acid bacteria has increased fungal growth while other bacteria introduces toxins and signaling molecules that antagonize fungal growth

Coexistance of viable *Pseudomonas* and *Saccharomyces* when cocultured

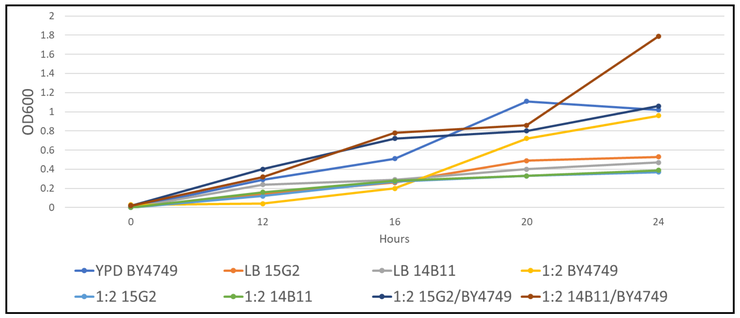

Figure 1: Growth curve for coculture *Pseudomonas* strains (*P. protegens* 15G2 and *P. chlororaphis* 14B11) and *S. cerevisiae* (BY4749) colonies in mixed media (BY4749 and either 14B11 or 15G2 in a 1:2 ratio of LB to YPD media) and controls (15G2 and 14B11 in LB media and BY4749 in YPD) using OD600 readings.

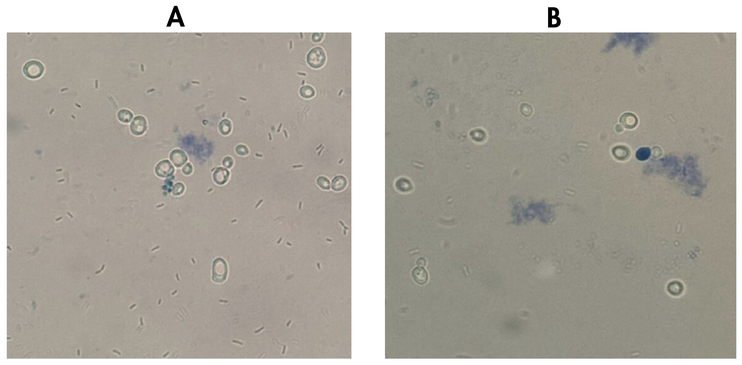

Figure 2: Fluorescent microscopy of methylene blue stained cocultures confirming any dead yeast cells. Image A) S. cerevisiae and *P. protegens* B) *S. cerevisiae* and *P. chlororaphis*. All fluorescent microscopy was taken at 40x.

Morphological Differences in Cocultures

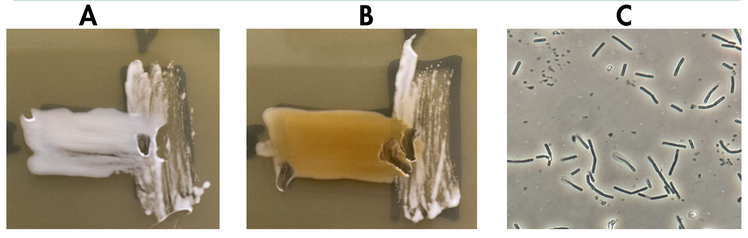

Figure 3: Cross-streak assay showed mucoid growth on the bacteria sections and no yeast cell growth in the cross sections. Image A) Cross-streak of *P. protegens* and *S. cerevisiae* B) Cross streak of *P. chlororaphis* and *S. cerevisiae B) P. protegens cell-to-cell fusion taken from the cross-streak in image A.*

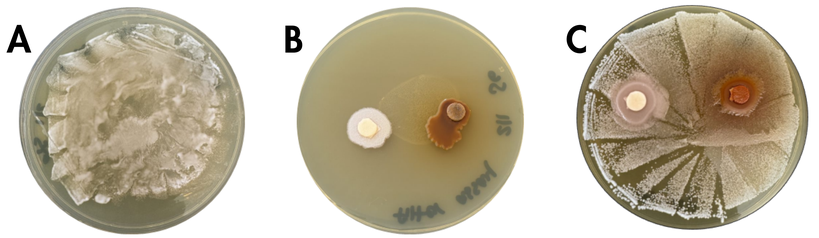

Figure 4: Filter paper assay A) Control yeast cell growth pattern B) Control *Pseudomonas* strains growth pattern atop filter paper, white is *P. protegens* and orange is *P. chlororaphis*. C) Filter paper assay with *Pseudomonas* strains growing on *S. cerevisiae*.

Analysis of Results

- Both cocultures had both organisms that grew together (Fig 1) The two curves on the graph for the cocultures are greater than and follow a different pattern of growth than the ones with just fungi or bacteria suggesting both organisms are in the cultures.
- Viability of *S. cerevisiae* in the cocultures was confirmed through methylene blue staining (Fig 2)
- Mixed media had no influence on growth rate (Fig 1) since all the cultures grew in mixed media equivalently to the cultures with just YPD or LB media
- Cross-streaking and filter paper assay had mucoid, puffy bacterial growth (Fig 3 & 4)
- *P. protegens* from the cross-streaking appeared to have direct cell-to-cell fusion when cross-streaked, potentially to increase the bacteria's odds of survival (Fig 3C)

Conclusions

- The two strains of *Pseudomonas* are capable of coexisting with *S. cerevisiae*
- The antifungals usually produced by *Pseudomonas chlororaphis* and *Pseudomonas protegens* had no effect on *Saccharomyces cerevisiae*
- The coexistence of *Pseudomonas* and *Saccharomyces* could promote plant growth and survival

Future Works

Continue coculturing the two organisms at varying ratios, temperatures, and strains to see if the patterns hold for plant-specific strains of *Pseudomonas*