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**FINAL REPORT
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**Mode of Action of Yeast Biocontrol Agents of
Postharvest Diseases of Fruits**

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***Mode of Action of Yeast Biocontrol Agents of Postharvest Diseases
of Fruits***

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Abstract

Mode of Action of Yeast Biocontrol Agents of Postharvest Diseases of Fruits

In a previous BARD-supported study, three of the investigators of this research were involved in a study on biological control of postharvest diseases of citrus and deciduous fruits. Several naturally occurring, non-antibiotic producing yeast antagonists were identified. Application of some of these antagonists resulted in very high levels of biocontrol under laboratory conditions but lower efficacy in semi-commercial tests. It was felt that the lack of knowledge on the mode of action of the biocontrol agents was limiting their efficient use. The current study was aimed at narrowing this gap in our knowledge. Two specific objectives were outlined: to study the mechanism by which calcium salts enhance biocontrol activity and to determine the role, if any, of the yeast extracellular materials and/or enzymes which degrade fungal cell walls during the interaction between the antagonist, the pathogen and the host.

CaCl₂ but not MgCl₂ inhibited spore germination, and germ-tube elongation of *Botrytis cinerea*, *Penicillium expansum* and *P. digitatum* in culture. It also inhibited the pectinolytic activity of the pathogens. Biocontrol of apple decay by isolate 182 of *Candida oleophila*, an effective biocontrol agent, was enhanced by the addition of CaCl₂ whereas there was no effect on the biocontrol activity of isolate 247 of this yeast. Similarly, CaCl₂ enhanced efficacy of the US-7 isolate of *Pichia guilliermondii* in reducing infection of *P. digitatum* in citrus fruit. CaCl₂ by itself also reduced the infection of peel wounds and stimulated ethylene production by grapefruit peel. This antagonist exhibited a very high ability to maintain cytosolic Ca²⁺ homeostasis when exposed to high CaCl₂ concentrations. It is postulated, therefore, that enhanced biocontrol activity by calcium is the result of direct inhibition of the pathogen by calcium ions on spore germination and metabolism and indirectly due to the ability of the biocontrol agent to maintain normal metabolism in the presence of high levels of calcium.

The extracellular materials produced by *P. guilliermondii* in culture and on the fruit inhibited, at low concentrations, the pathogen in culture and reduced percent infection of the fruit. The direct inhibition of the pathogen by these materials may thus be involved in the mode of action of the antagonist.

This study contributed to our knowledge on the action of calcium salts and the yeast antagonist extracellular materials on biocontrol activity and will contribute to a more efficient use of this technology in the control of postharvest diseases of fruits.

Objectives of the Original Proposal

The general objective of the proposed study was to improve the efficacy of previously established yeast antagonists of postharvest diseases through the elucidation of their mode of action.

Two specific objectives were outlined:

- To study the mechanism by which calcium salts enhance biocontrol activity;
- to determine the role of the yeast extracellular material and/or enzymes which degrade fungal cell wall in the interaction between the antagonist, the pathogen and the host.

Relevant Data, Methodology, Results and Discussion

The data, methodologies, results and discussion are detailed in the following three manuscripts:

1. A published paper: Effect of Ca^{2+} and Mg^{2+} on *Botrytis cinerea* and *Penicillium expansum* *in vitro* and on the biocontrol activity of *Candida oleophila*. (9 p.).
2. A manuscript submitted for publication: Characterization of the effect of CaCl_2 on *Penicillium digitatum*, Grapefruit peel tissue and biocontrol activity of *Pichia guilliermondii*. (24 p.).
3. A manuscript to be submitted for publication: Antifungal activity of cell-wall surface materials of the yeast *Pichia guilliermondii* against postharvest diseases. (21 p.).

Description of Cooperation

Close cooperation between the investigators has existed throughout the project. It consisted of mutual visits, development of a joined strategy for work, joint planning of experiments, exchange of antagonists and joint publications and patents. Each of the cooperating scientists has contributed to the research by offering his specific experience and available methodologies.

Evaluation of the Research Achievement

An evaluation of the research achievements with respect to the original objectives of this study suggests that most of the research objectives were achieved: A better understanding of the processes involved in the enhancement of biocontrol activity in apple and citrus fruits by calcium ions enabled us to incorporate this enhancing agent in semi-commercial and commercial tests of the yeast antagonists. These tests can now be carried out following the registration by the US EPA last year of Aspire™ - the *C. oleophila* - based biofungicide product of Ecogen, Inc..

Of particular interest are the findings indicating that effective yeast antagonists may withstand high external calcium concentrations by their ability to maintain cytosolic Ca^{2+} homeostasis, compared with that of the pathogen. This finding may aid in the search for superior antagonists and in the development of methods to improve their efficacy.

As to the role of the yeast extracellular materials in the interactions between the yeast, the pathogen and the host, results of this study have clearly indicated at least one specific role for these materials that is not limited to one specific yeast-pathogen interaction. The activity of extracellular materials was considered new and warranted the application for a patent. Identity of the active component(s) of the extracellular materials are under investigation. Regardless of the results of this investigation it is felt that the use of these materials in the biocontrol procedure may have significant advantages.

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Conclusions

The objectives of this project were aimed at further studying some aspects revealed in a previous BARD project on biological control of postharvest diseases. It has produced meaningful results for the understanding of the complex interactions between the antagonist, the pathogen and the host. It has also helped in putting the information into practical use for more efficient control of postharvest diseases by biological means.

List of Publications

1. Wisniewski, M., Droby, S., Chalutz, E. and Eilam, Y. (1995).
Effect of Ca^{+2} and Mg^{+2} on *Botrytis cinerea* and *Penicillium expansum* in vitro and on the biocontrol activity of *Candida oleophila*.
Plant Pathol. 44: 1016-1024.
2. Droby, S., Wisniewski, M.E., Cohen, L., Weiss, B., Touitou, D., Eilam, Y. and Chalutz, E. (1996).
Characterization of the effect of CaCl_2 on *Penicillium digitatum*, Grapefruit peel tissue and biocontrol activity of *Pichia guilliermondii*.
Manuscript to be submitted for publication.
3. Droby, S., Chalutz, E., Wisniewski, M.E. and Wilson, C. (1996).
Antifungal activity of cell-wall surface materials of the yeast *Pichia guilliermondii* against postharvest diseases.
Manuscript to be submitted for publication.

Patents

The following patent applications and patents resulted from our research: The patent applications (# 1) relate to the use of the extracellular materials; The other patents (#2 and #3) are related to the use of the yeast antagonists.

1. Droby, S. and Chalutz, E. Wilson, C. and Wisniewski, M. (1994).
Fungicides and Method for using same.
a. Israel Patent Application No. 110, 441.
b. Australia Patent Application No. 74143 / 94.
c. U.S. Patent Application S.N. 08/309,959,.
d. European Patent Office No. 943 069 51.8.
2. McLaughlin, R., Wilson, C.L. and Chalutz, E. (1995).
Inhibiting plant pathogens with nonantibiotic antagonistic microorganism(s).
U.S. Patent No. 5,413,783,.
3. Wilson, C.L., Wisniewski, M.E. and Chalutz, E. (1995).
Biological control of diseases of harvested agricultural commodities using stains of the yeast *Candida oleophila*.
U.S. Patent No. 5,425,941,.