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**Isolation and assessment of attachment
bacteria and yeasts for the biological control
of *Botrytis cinerea*.**

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of the requirements for the degree of
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Abstract

The biological control of *Botrytis cinerea* Pers. infection by microbial agents applied to the host surface has been based on a wide range of mechanisms of which resource competition, antibiosis and induced host resistance have been considered the most important. A 1995 review of antagonistic mechanisms concluded that biocontrol agent (BCA) colonisation of the plant host was critical for successful biocontrol but that few isolates appear to achieve this. Recent research has shown a reduced epiphytic growth prior to penetration of *B. cinerea* when conidia are applied as dry spores. Such pre-penetration infection morphology would provide little opportunity for antibiosis, resource competition or induced host resistance. Contemporary *in vivo* plant tissue assays and *in vitro* agar plate-based-assays have perpetuated the traditional biocontrol model based on such mechanisms hence an alternative approach was required. BCA selection based on microbial adhesion to the pathogen itself appeared to offer such an approach.

An investigation of methods of *B. cinerea* conidial application showed that disease incidence was increased and development advanced from aerosol application of spores. Aerosol application was used as the standard technique for biocontrol experiments in the remainder of this study.

A total of 12 bacterial and eight yeast candidates were obtained from the attachment assay. *In vivo*, 15 reduced disease by more than 90% in at least one combination of incubation temperature (1°C, 7°C or 15°C) and BCA concentration (three-times to 60-times the *B. cinerea* population applied). When BCA application followed *B. cinerea* inoculation by up to 48 h, high biocontrol activity was observed. The five yeasts tested postharvest on kiwifruit conferred high biocontrol (>90%) when applied simultaneously or up to 48 h after *B. cinerea* inoculation. All eight bacterial and seven yeast BCA candidates also reduced disease incidence in stem wounds by more than 80% in glasshouse tomato plants.

In vitro investigations into antagonistic mechanisms suggested that antibiosis was unlikely to be important in all but two of these bacterial BCAs. Production of

endochitinase was common among the yeasts but there was no single presumptive mechanism for bacterial biocontrol. Variable levels of adhesion by BCA isolates were detected by light and electron microscopy and indicate that biocontrol may not be correlated quantitatively to the number of adhesion events. Adhesion of yeast and biocontrol activity were not affected by a monoclonal antibody to *B. cinerea*. However, bacterial adhesion and biocontrol activity were dramatically reduced indicating that the antibody blocked bacterial adhesion sites and that bacteria and yeast adhere to different sites on the pathogen.

A monoclonal antibody-based ELISA immunoassay was developed to measure vegetative biomass of *B. cinerea* in infected tomato stem tissue with or without BCAs. The key to the successful application of this ELISA assay was the extraction of the pathogen antigen from the plant tissue using 0.1M copper sulphate and salts solution. Significant reductions in pathogen growth were detected in host tissue co-inoculated with *B. cinerea* and BCA.

The attachment assay was an efficient isolation method that optimised use of laboratory resources and could be employed in future programmes as a presumptive test for biocontrol. With this determinative selection criterion, BCAs with desirable characteristics such as reduced importance of BCA application dose and timing were obtained. A comparison of these results with those in the literature led to the proposal for an alternative biocontrol model for *B. cinerea* that could supplement existing technologies.

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To Him who is the creator of all things that we seek to understand with our tools we call science and philosophy.

"He makes the grass grow for the cattle,
and plants for men to cultivate-
bringing forth food from the earth:
wine that gladdens the heart of man,
oil to make his face shine,
and bread that sustains his heart".

(Psalm 104: 14-15)

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