BIOREMEDIATION OF STICKY COTTON

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Abstract

Sticky cotton occurs when late season insect infestations cause honeydew to deposit on the lint. Bioremediation offers a possible method of removing this contamination. The purpose of this research was to examine the ability of plant associated yeast to digest the sugars found in aphid and whitefly honeydew and to evaluate CO_2 flux as a means of monitoring microbial activity on cotton lint. When assessed across all 250 strains with *in vitro* testing, sucrose supported the highest average rate of growth followed closely by glucose and fructose, then melezitose and trehalose. When ranked by the maximum observed growth rate, fructose and glucose supported the highest rate followed closely by melezitose and sucrose with the maximum rate observed on trehalose being considerably lower. These results indicate that suitable strains of yeast could readily be selected from local phyllosphere yeast populations. A CO_2 flux measuring system consisting of a sample chamber, infrared analyzer, and gas handling circuits was evaluated as a technique to measure microbial activity on cotton lint. The system could readily detect the release of CO_2 through respiration of *S. cerevisiae* or a wild phyllosphere yeast growing on sucrose treated lint. Tests with seed cotton responded similar to tests with ginned cotton, indicating that seed respiration would not interfere with measurements of microbial respiration.

Introduction

Sticky cotton occurs when aphids and whiteflies excrete honeydew onto the lint while feeding on the foliage. Honeydew is a sugar rich mixture that is somewhat variable in composition depending on which insect is present (Hendrix et al., 1992). The predominant sugars found on sticky cotton are melezitose, trehalulose, sucrose, fructose, glucose, and trehalose (Brushwood and Perkins, 1995; Hendrix, Wei et al., 1992; Tarczynski et al., 1992). Other unique sugars such as bemisiose can also occur (Hendrix and Wei, 1994). Proper late season insect management is the best way of avoiding sticky cotton but when insect control measures fail, lint becomes contaminated with honeydew creating the need for remedial measures. These remedial measures vary from processing aids sprayed on the lint (Perkins, 1986) to enzymes applied to break down the sugars (Hendrix et al., 1993; Henneberry et al., 1997). However, none of these approaches has been entirely successful. Bioremediation offers a possibility of eliminating stickiness by using microorganisms to consume the sugars. Several attempts to use various bacteria for bioremediation have been evaluated with limited success (Balasubramanya et al., 1985; Heur and Plant, 1985). Yeasts have several characteristics that would be useful in a bioremediation agent for sticky cotton. Yeasts readily consume sugars, grow over a range of temperature and moisture, and withstand desiccation. Yeasts have been successfully used to reduce honeydew contamination on other crops (Dik et al., 1991). The present research was initiated to survey the ability of various plant associated yeasts to utilize the sugars found in honeydew. A second purpose of this research was to develop and test a monitoring method for microbial activity on cotton lint. Monitoring the microbial activity during bioremediation presents problems because cotton lint is a solid substrate and not amenable to the usual optical methods of determining microbial development and activity. To overcome this problem a system to measure carbon dioxide release by microbial respiration was developed and tested.

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Materials and Methods

Carbohydrate Utilization

Naturally occurring yeasts were collected from cotton leaves, lint, and from other plants growing in the San Joaquin Valley of California. A sub-sample of 250 strains was selected to evaluate the ability of these yeast to metabolize some of the sugars found in insect honeydew. The yeast were grown on Bacto YM agar (Difco Laboratories, Detroit MI) for 48 hr then suspended in sterile water and adjusted to a standard optical density. Solutions of sugars (5.0 g/l) (either sucrose, glucose, fructose, melezitose, or trehalose) were prepared in a solution of Bacto yeast nitrogen base (6.7 g/l) (Difco Laboratories, Detroit MI), filter sterilized, then dispensed into 96 well microplates. Two controls of either water or the nutrient base without added sugar were also tested to check for growth on endogenous stored nutrients. (Trehalulose was not available at this time and therefore will be evaluated in later tests). Each strain by sugar combination was tested in two replicates. Plates were incubated at 28 C and growth was determined by periodically measuring optical density over a 48 hour period. Growth rate was measured as the change in optical density over time.

CO_2 Flux Measurements

A CO_2 measuring system consisting of a sample chamber, an infrared gas analyzer, and a gas handling circuit was setup by modifying a Li-cor 6200 portable photosynthesis system (Li-cor Inc., Lincoln, NE). The control unit was programmed to measure the CO_2 concentration over time and calculate a rate of CO_2 flux from the sample into the chamber. To test the sensitivity of the system, yeast suspensions and a sucrose solution in Bacto yeast nitrogen base were applied to cotton lint and placed in the sample chamber. A commercial strain of *Saccharomyces cerevisiae* was used as a standard in some

of these trials. Tests were then set up to determine if the evolution of CO_2 from microbial respiration could be measured in a reliable and timely manner. Comparisons were also made using filter paper as the matrix since further screening could be simplified by using filter paper in place of lint. Seed cotton was also compared to ginned lint to determine if seed respiration would interfere with measurements of microbial respiration since advanced evaluations of bioremediation will be conducted with seed cotton.

Results and Discussion

Carbohydrate Utilization *in Vitro*

All the sugars evaluated could be degraded to some degree by many of the strains tested. When assessed across all 250 strains, sucrose supported the highest average rate of growth followed closely by glucose and fructose, then melezitose and trehalose. Essentially no growth occurred in water or the nutrient base controls. The distribution of growth rates on any given sugar was positively skewed with some strains showing much higher rates than the average. When ranked by the maximum rate, fructose and glucose supported the highest rate followed closely by melezitose and sucrose. The maximum rate on trehalose was considerably lower. Many strains could utilize more than one sugar and within the same strain, growth rates on the different sugars tended to be correlated. These results indicate that the naturally occurring yeast population in the San Joaquin Valley will be a suitable source for selecting bioremediation agents for whitefly and aphid honeydew. The positively skewed distribution indicates that selecting strains for rapid rates of utilization will be successful. Trehalulose has yet to be tested but given its prevalence in honeydew, it is likely that strains will be found that can degrade this sugar. Ultimately, selected strains will have to be tested for the ability to reduce or eliminate stickiness as measured by the thermal detector test and lint processing trials.

CO_2 Flux Measurements

The carbon dioxide measuring system was sensitive enough to detect the microbial respiration. A sample with *S. cerevisiae* growing on sucrose had a CO_2 flux of 0.05 ppm/s while a control of lint and sucrose media without S. *cerevisiae* showed a CO_2 flux of less than 0.01 ppm/s. When filter paper was substituted for lint, no differences in CO_2 flux were observed. Tests comparing ginned lint with seed cotton showed no differences in CO_2 flux, indicating that seed respiration would not be a confounding factor. In further evaluations, a wild strain of yeast growing on sucrose had a flux of 0.012 ppm/s compared to *S. cerevisiae* with a flux of 0.017 ppm/s under the same conditions. The same wild strain growing on crude whitefly honeydew in the nutrient base had a rate of 0.013 ppm/s which was comparable to growth on sucrose. These results indicate that CO_2 flux will be a good method of monitoring microbial respiration during the screening and development of bioremediation agents. A reliable reading could be obtained within one to three minutes after placing the sample in the test chamber. The comparable result with filter paper or lint indicates that filter paper can be substituted as needed in later tests which will simplify screening of various strains and growth conditions. Seed respiration did not influence the results in these test which indicated that this technique will be applicable to later bioremediation trials. Several different chamber sizes are adaptable to this system and a probe configuration for monitoring modules in the field is possible.

Conclusions

Based on a sample of 250 yeast strains collected from the phyllosphere of cotton and other plants in the San Joaquin Valley, the ability to utilize the sugars in honeydew seems to be a rather common and variable trait in this yeast population. These results indicate that further selection might yield good bioremediation strains. The ability of the CO_2 measurement system to rapidly detect yeast metabolism on the lint substrate means that this system will be useful in monitoring microbial activity during bioremediation.

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