A rapid microscale assay for determining growth of wood-inhabiting fungi against *Lactobacillus* metabolites

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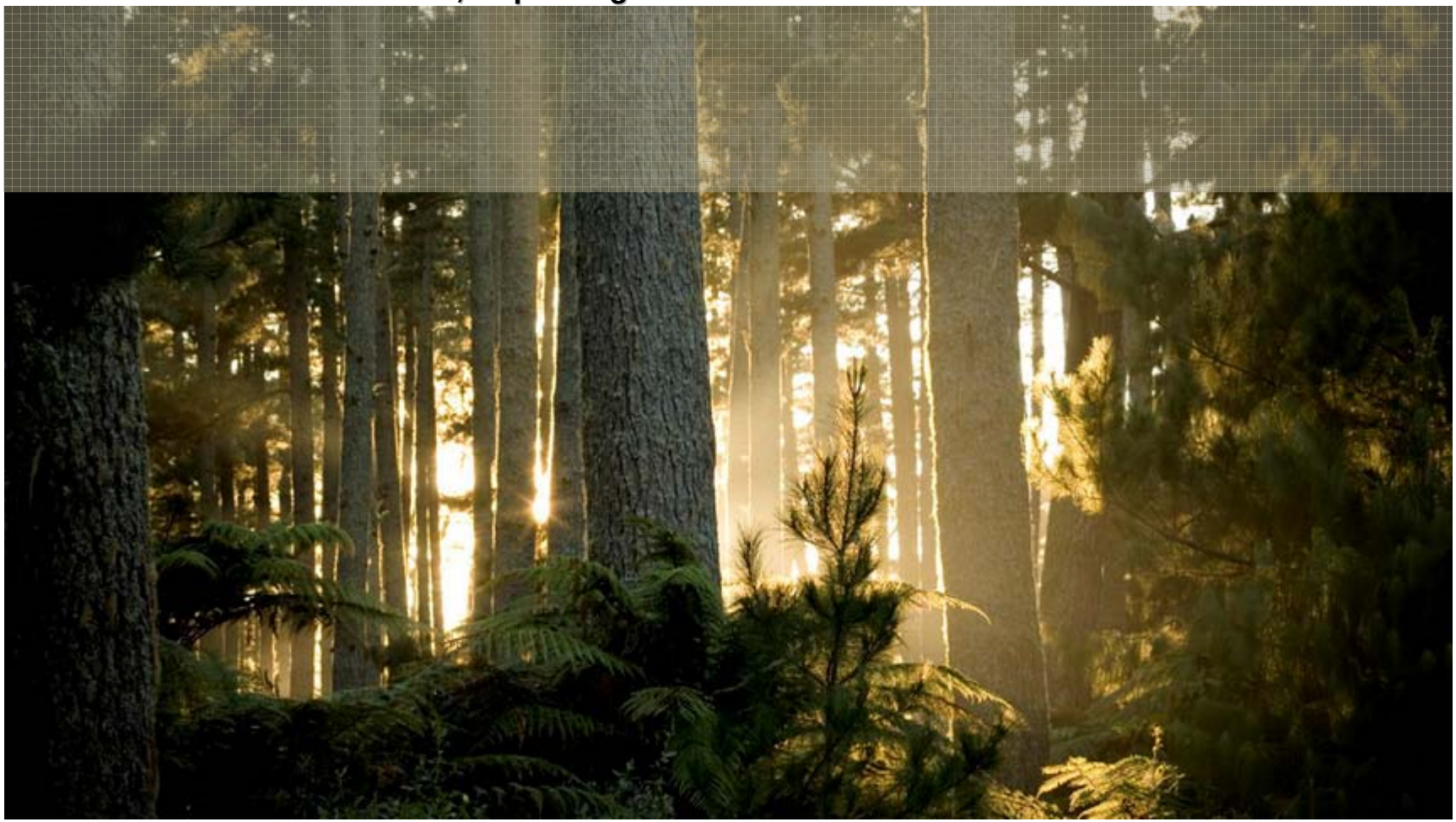

Overview

- Aim
- Background
- Methods
- Comparison of methods
- Conclusions

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Aim

To develop a rapid optical density assay using 96-well microplates to assess the activity of *Lactobacillus* metabolites against wood decay fungi.

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Background

- Why?
  - Want to look at bacterial secondary metabolites for antifungal activity
  - Previously recognised that *Lactobacillus* isolates may have activity against sapstain fungi
  - Needed quick method for screening lots of variables at one time

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Methods

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Cultures

- Fungi
  - *Oligoporus placenta*
  - *Antrodia xantha*
  - *Coniophora puteana*

- Bacteria
  - *Lactobacillus rhamnosus*

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Media

- MRS (de Man, Rogosa, Sharp)
- Sabouraud Dextrose
- Yeast Malt
- MRS minus salts

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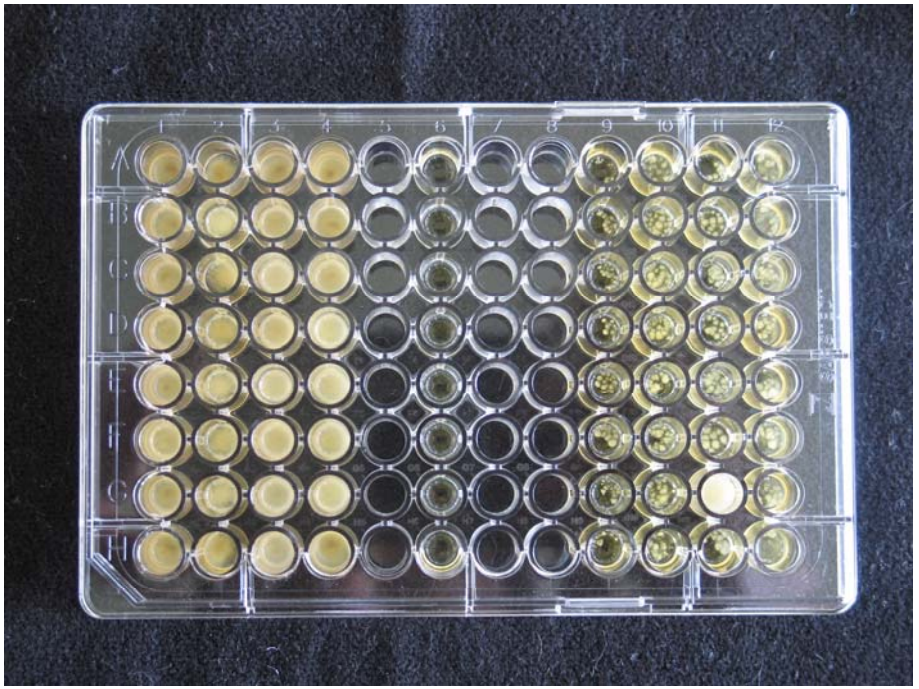
Microscale absorbance assay (MAA)

Fungal inoculum

- Grow fungi in liquid malt media (~ 2weeks)
- Homogenise in Waring blender
- Pipette 50µL into well of 96-well plate (covered)



Microscale absorbance assay (MAA)

Bacterial supernatant

- Grow *Lactobacillus* in MRS broth for required time and temperature
- Centrifuge at 5600 rpm for 10 mins (room temp)
- Filter through 0.22µm filter (Millipore Steritop)
- Dilute with MRS $\Rightarrow$ 4:1, 2:1, 1:1, 1:2, 1:4, 0:1 (MRS:supernatant)
- Add 100µL to 96-well plate

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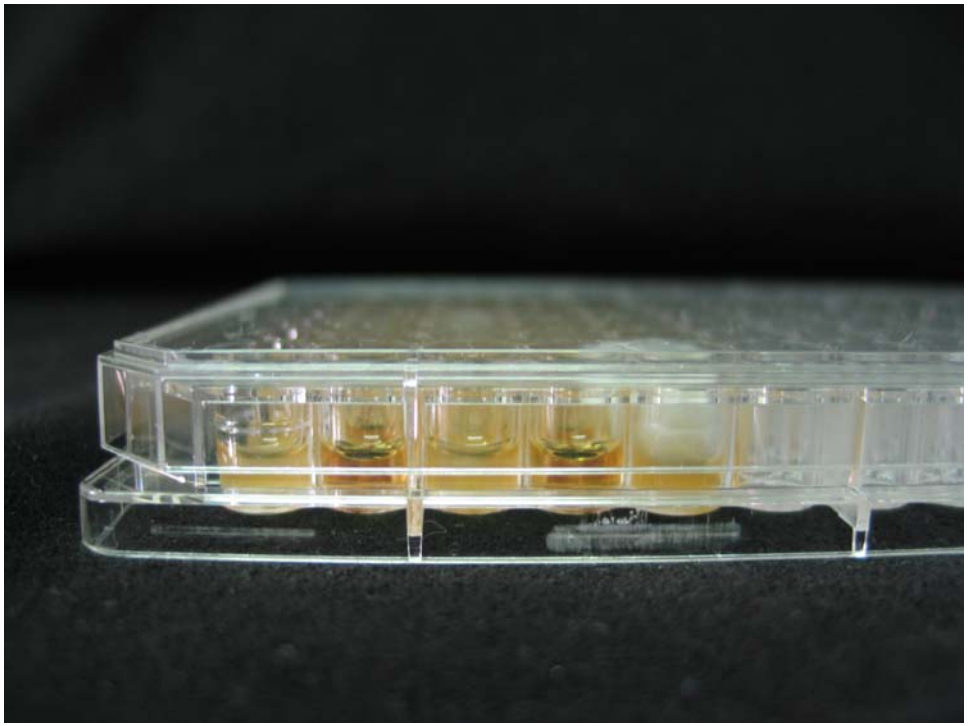
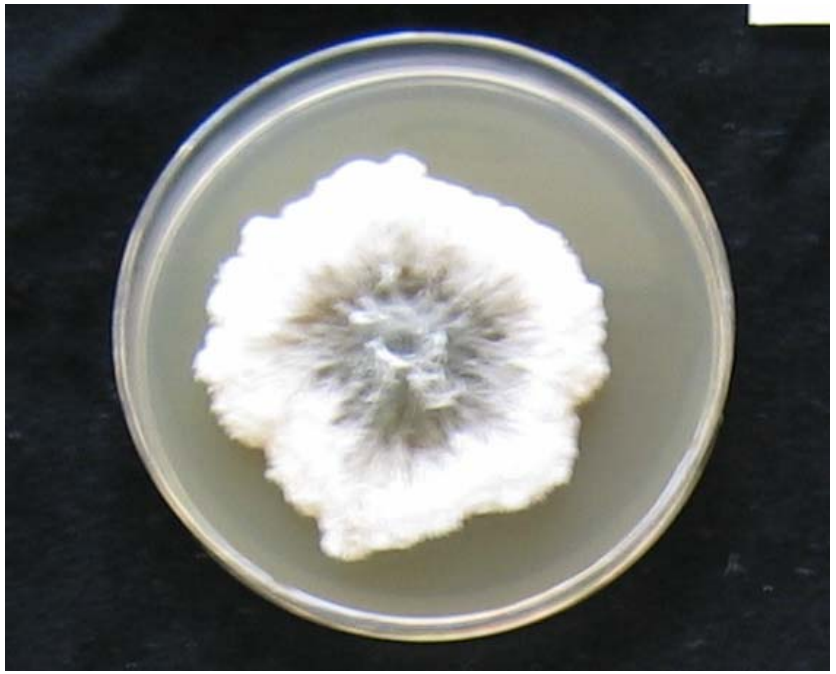
Microscale absorbance assay (MAA)

- Measure absorbance on plate reader at 550nm
- Incubate 4 – 7 days at 25^{o}C / 75% RH
- Re-measure absorbance
- Calculate change in absorbance



Growth Rate Assay (GRA)

- Amend solid media with supernatant
- Inoculate with 5mm fungal plug
- Incubate 25^{o}C / 75%RH
- Measure colony diameter after 1 week



Comparison of methods

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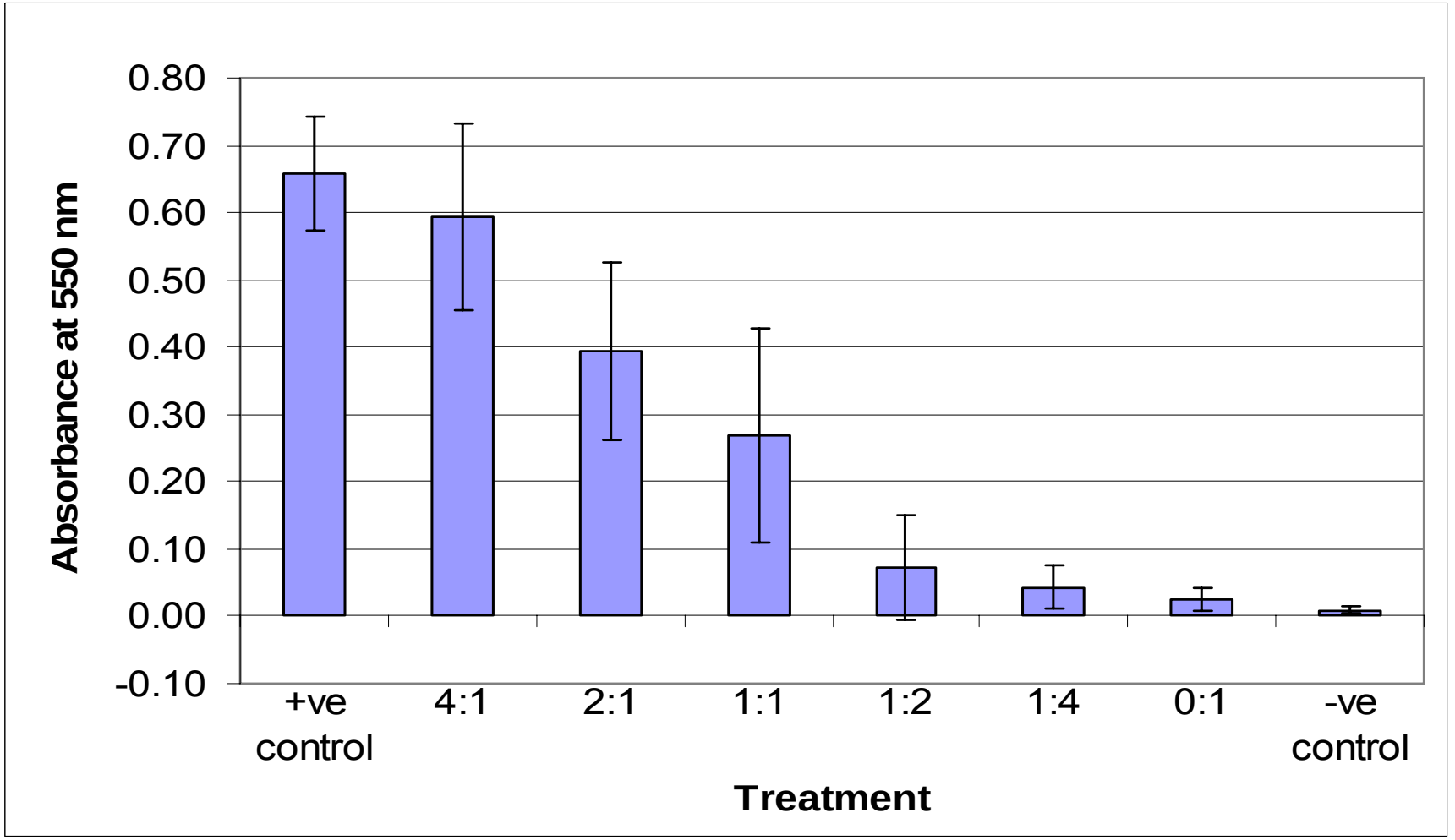

Effect of *Lactobacillus rhamnosus* supernatant on growth of *Oligoporus placenta* in MRS media (MAA)

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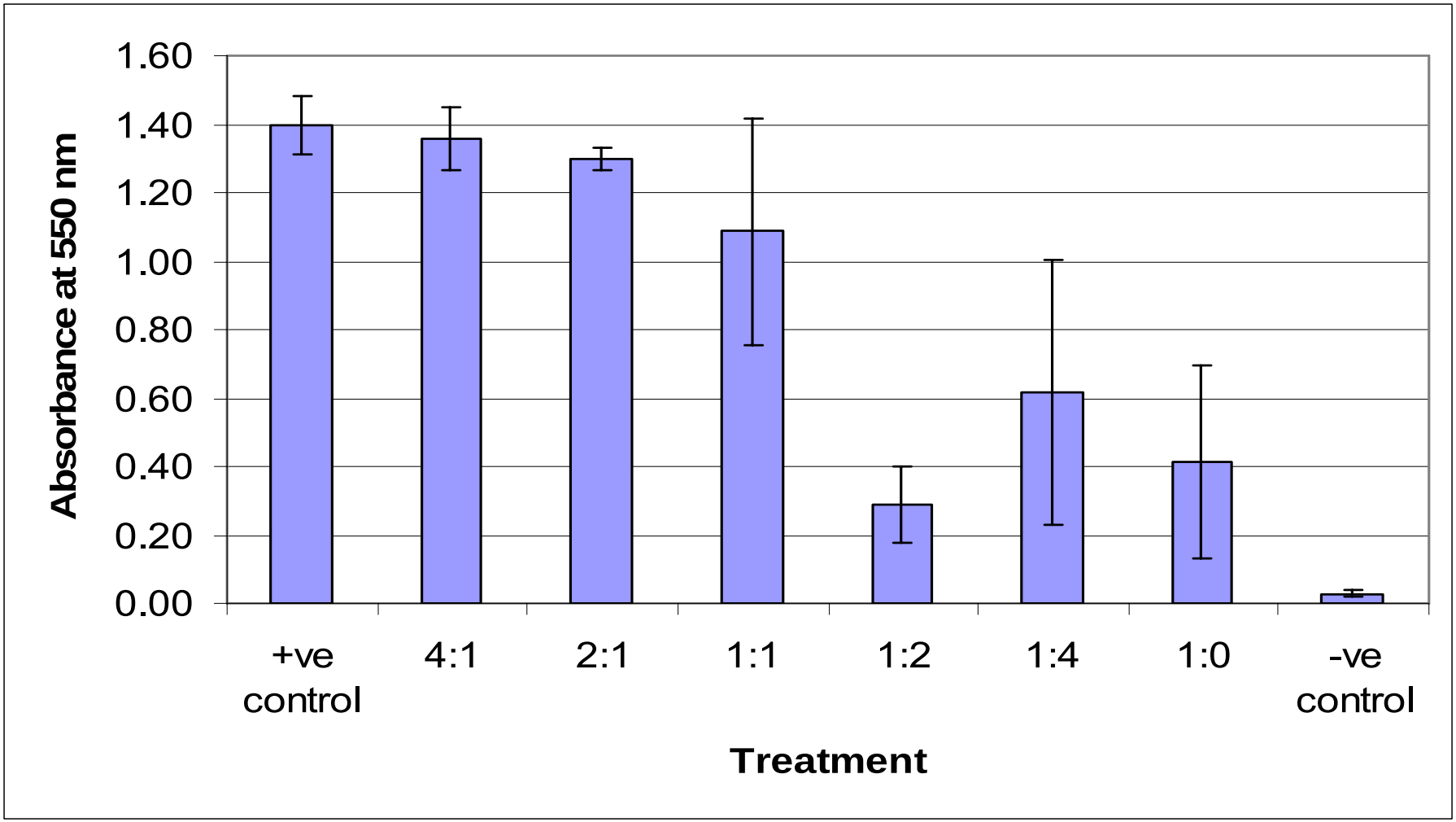

Effect of *Lactobacillus rhamnosus* supernatant on growth of *Antrodia xantha* in MRS media (MAA)

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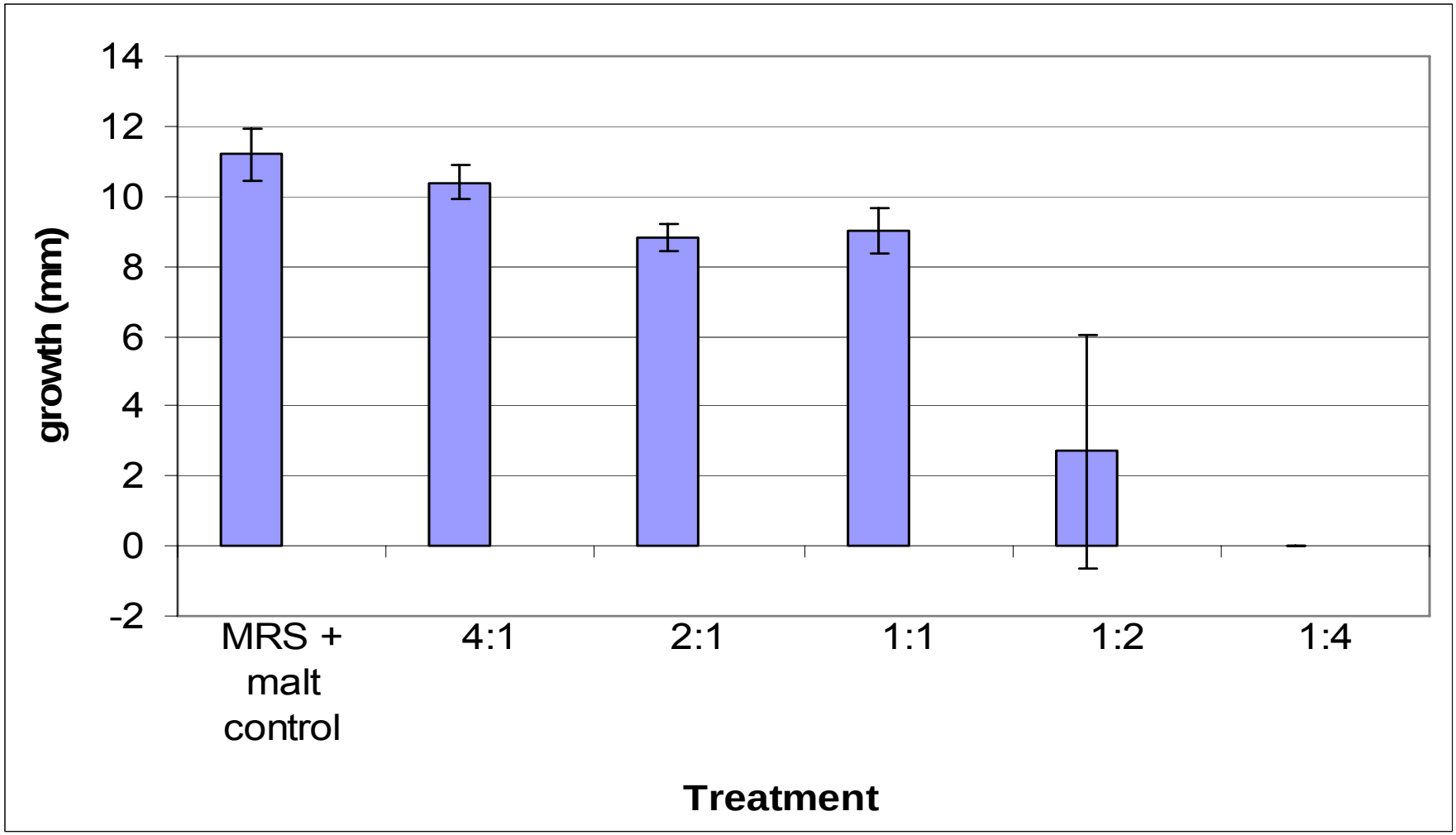

Growth of *Oligoporus placenta* after 6 days on MRS agar amended with *Lactobacillus rhamnosus* supernatant dilutions (GRA)

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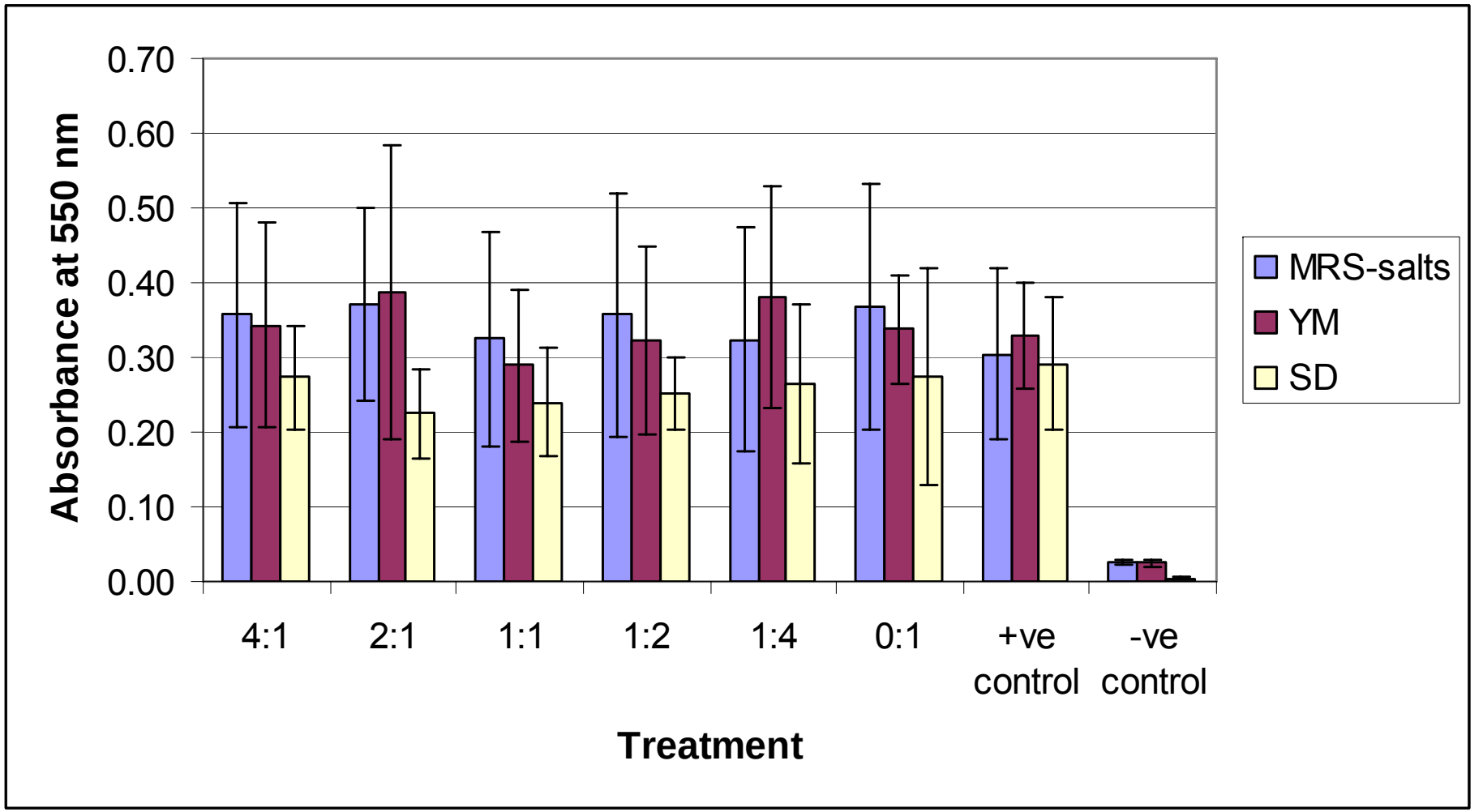

Effect of *Lactobacillus rhamnosus* supernatant on growth of *Oligoporus placenta* in MRS-salts, YM, and SD media.

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Conclusions

- Correlation between growth rate assay and microscale assay
- MRS broth supports growth of both *Lactobacillus* and some decay fungi and supports production of metabolites
- *Lactobacillus rhamnosus* produces antifungal metabolites effective against decay fungi
- Method is easy to set up and provides relatively fast results

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