Cellulolytic soil Chytrid:

A fungus and its bacterial interaction

Charlotte Joynson (C.joynson@ncl.ac.uk)

British Mycological Society promoting fungal science

Newcastle University

Aims

To locate and culture *Rhizophlyctis rosea*, in different soil types

To extract, multiply and sequence *R.rosea* DNA

To find a symbiotic bacterial partner associated with *Rhizophlyctis rosea*

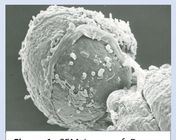

Figure 1: SEM Image of *R.rosea* covered by its symbiotic bacterial partner

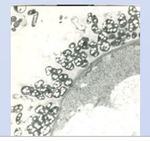

Figure 2: Light microscope Image of *R.rosea* covered by its symbiotic bacterial partner

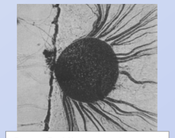

Figure 3: Microscope image of R.rosea encysting cellophane

Introduction

Chytrids are zoospore producing fungi that have little or no mycelium, and have a cell wall composed of chitin. *Rhizophlyctis* (Karlingia) is a Chytrid genus in the order Spizellomycetales, an order that is well adapted to the soil environment. *R.rosea* is an orange pigmented cellulolytic chytrid found in soil, which preferentially hydrolyse cellulose. They are monocentric and have branched tapering tubular rhizoids on their sporangium. An experiment was carried out in order to observe soils from different geographical regions, to see which types inhabited *R.rosea*, and to then culture them, via different methods. *R.rosea* has also been thought to have a symbiotic partnership with bacteria, and so the experiment was also carried out in attempt to find this symbiotic relationship and potentially identify the bacterial partner.

Materials and methods

The various soil samples (Quenca, Cyprus, Mallorca, Turkey, Qaroun lake, The Red sea (x5), Borg El A (x2), W Ryan (x4) , WS (x2), and Minrea) were sieved, weighed, and poured out (approx. 6g) into labelled petri dishes, and moistened with sterile distilled water before having 5 autoclaved cellophane square (10mm) placed on each plate. Two repeats were carried out for each soil type, and they were incubated at 28°C. Cellophane was used to selectively bait the cellulolytic *R.rosea* acting as a nutrient source.

After 3 days the cellophane squares were placed in water and observed under an inverted microscope. Squares with what appeared to be *R.rosea* growth were cultured on different media including; Liquid XY and DT Salt media and XY agar and DT Salt agar. **DNA extraction** was carried out using liquid from a successful DT media plate, containing free floating *R.rosea*, and also using edges cut straight from cellophane squares, containing *R.rosea* and finally using liquid containing spores.

These extractions involved a lot of centrifugation and vortexing and the addition of the following; glass beads (0.2g), 0.5ml CTAB extraction buffer, (0.4ml) chloroform/isoamyalcohol, the bottom layer of phenol/chloroform/isoamyalcohol 30% PEG 600% 6M Nacl, 0.2ml 70% ice cold Et OH. This resulted in a pellet that was then resuspended in 10µl sterile distilled water..

A master mix was made up and DNA in the pellet from the DNA extraction was amplified by PCR for 90 minutes at specified settings. The PCR products were then separated against a DNA ladder by gel electrophoresis on a gel containing 2.5 µl Ethidium Bromide. Once complete the gels were placed in a UV gel imager to take a photo of the results (figure 7) and the DNA was sent off for sequencing to check the identity.

Successful squares were also prepared for observation under the scanning electron microscope (figures 4-6).

Results

R.rosea was only found in Quenca soil.

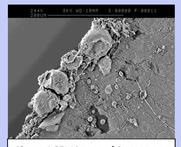

Figure 4 SEM image of *R.rosea* on the cellophane edge

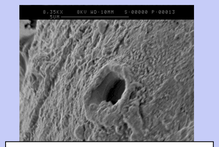

Figure 5 SEM image of *R.rosea* exit papillae (unplugged) after releasing its zoospores

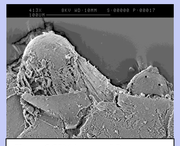

Figure 6 SEM image of *R.rosea* on the cellophane edge

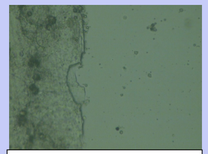

Figure 7 Inverted microscope image of the effect of *R.rosea* on the edge of the cellophane

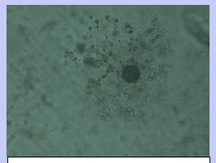

Figure 8 Inverted microscope image of floating *R.rosea*

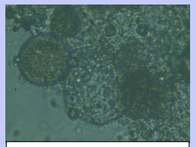

Figure 9 Inverted microscope image of *R.rosea* full of spores (left) and after spore release (right)

Table 1 Results of cellophane observation, from different cultures, taken from Quenca soil.

Plate	Observation
DT L1/2/4/5	Spores inoculated the new cellophane squares well.
DT L3	Transferring squares to liquid agar appeared to activate release of zoospores. *Rhizophlyctis* on original square released spores that infected the new cellophane squares well.
DT A3/4/5	Spores inoculated the new cellophane squares well Only a small amount of protists found and no other fungi or bacteria.
DT A1	Spread of *Rhizophlyctis* but also other microorganisms
DT A2	Spread of *Rhizophlyctis* only
XY L 1/2/3, XY A1/2/3	Small amount of *Rhizophlyctis* spread, but a large amount of growth of other fungal microorganisms, with mass amounts of mycelium.
W1	Transferring squares to water appeared to activate release of zoospores. No spread of *Rhizophlyctis* to cellophane squares through water.

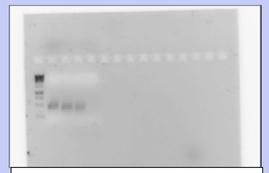

Figure 10 Gel electrophoresis image from isolate 3.

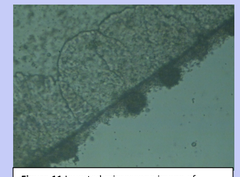

Figure 11 Inverted microscope image of R.rosea on the cellophane edge.

The sequencing results recorded *Rhizophlyctis rosea* as 98% similar to the PCR products sequenced.

Discussion

Growth of *R.rosea* occurred mainly on the edge of the cellophane, with the mycelium growing into the cellophane (Figure 11), potentially due to the edges of the cellophane providing easier access to cellulose.

Growth of many other fungi and bacteria occurred on the XY plates whereas minimal contamination occurred on DT Salt plates. This could be due to the specific salts in the XY Media being favoured by other fungi, which then out-competed *R.rosea.*

Transfer into water/liquid media appeared to initiate zoospores release. These zoospores were shown to be good at infecting other cellophane squares, excepted when in water. This could be due to water lacking nutrients required for survival away from the cellophane.

DT Salt media such as plate DT L2 was shown to host a large amount of free floating *R.rosea* (Figure 8), as it contains nutrients to allow growth away from cellophane and its liquid nature allows free floating.

R.rosea has a large effect on the cellophane (Figure 7), resulting in the edges looking disintegrated. Figure 9 shows many *R.rosea* growing together, showing that it is self parasitizing.

The presence of bands at 700bp (except for the negative control) in Gel electrophoresis (Figure 10) confirm the presence of fungal DNA, as fungal SSU primers of 700bp were used when extracting the DNA. The species identification was also confirmed as sequencing results were returned at 98%.

Overall the experiment was relatively successful, as *R.rosea* was found and cultured, many good photos were taken, zoospore release was seen and DNA was extracted and amplified. However the bacterial twin was not identified, this could be due to the fact that it does not grow in the soils tested, or had died out, or many other possible reasons.