The development of an expression system of endogenous serine proteases in basidiomycete fruiting body development, *Coprinus cinerea*.

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Summary

Mushrooms are of great importance to the economy and environment.

Agaricus bisporus is a button mushroom, the most grown and consumed; *Coprinus cinerea* is an ink cap mushroom.
Coprinus cinerea is the model organism for the study of basidiomycete fungi.

Cultivation of *Coprinus cinerea* FA2222, wild type and PG78, solid and liquid forms were performed, this is the strain of fungi that will uptake the vector, which is to be engineered with a new promoter.

Other task included designing primers for promoters , making competent cells to multiply up pGFPi004, DNA extraction to extract plasmid pGFPi004, restriction digests of the existing gpd promoter, electrophoresis to visual the results by sizing along size molecular weight ladder.

Introduction

The objective of the project was to monitor the expression of different promoters and investigating the role of endogenous serine proteases in basidiomycete fruiting body development *Coprinus cinerea*.

Studying SPR (serine proteases) is essential because it has a part in postharvest senescence (the growth phase in a plant or plant part from full maturity to death) of the mushroom.

The overall aim of this research is to lengthen the life of the mushroom by improving crop characteristics.

The value of the mushroom, to Ireland is considerable, valuing at €103 million in 2008 (Department of Agriculture, 2008). Increasing in to the value of mushrooms produced in the South to €112 million, and £31 million in the North of Ireland in 2013, (Teagasc, 2013)

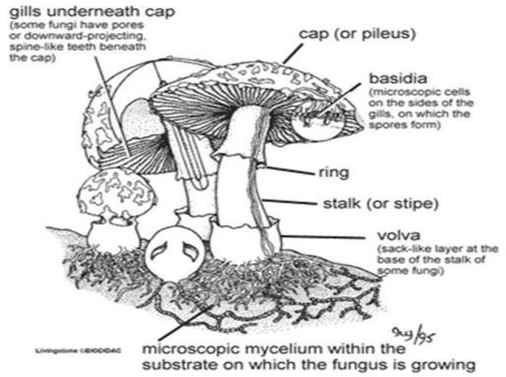

Figure 1: the parts of a mushroom.(Akers, 2010)

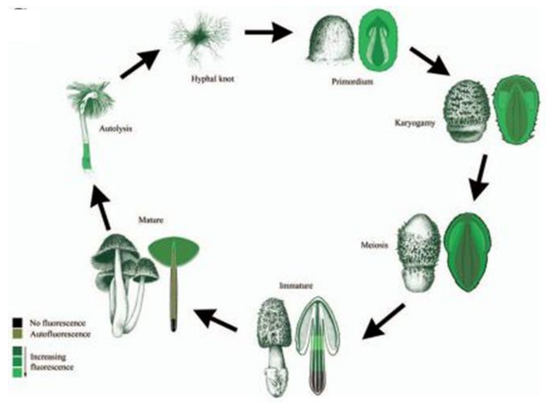

Figure 2: Stages of development of a mushroom; hyphal knot, primordium, karyogamy, meiosis, immature, mature, and autolysis. (Heneghan *et al* 2009).Serine proteases were studied by the strength of GFP fluorescence at different stages of fruiting body development.

Materials and Method

Cultivation of *Coprinus cinerea* FA2222, wild type and PG78, solid and liquid forms were performed, this is the strain of fungi that will uptake the vector, which is to be engineered with a new promoter.
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Figure 3: *Coprinus cinerea* mycelium, PG78 and FA2222 from Bristol,UK.

Other tasks included designing primers for promoters, making competent cells to multiply up pGFPi004,
DNA extraction to extract plasmid pGFPi004, restriction digests of the existing gpd promoter, electrophoresis to visual the results by sizing along size molecular weight ladder.

Results

Transformation

Competent cells TOP10 was used, cloning kit. Invitrogen's OneShot® TOP10 vials of chemically competent cells were successful and grew cells on both plates. More cells were counted on the GFPi004. More than 300 were counted on the GFPi004 plates and 21 colonies were on the pUC plate. DNA extraction….
Refer to figure 4

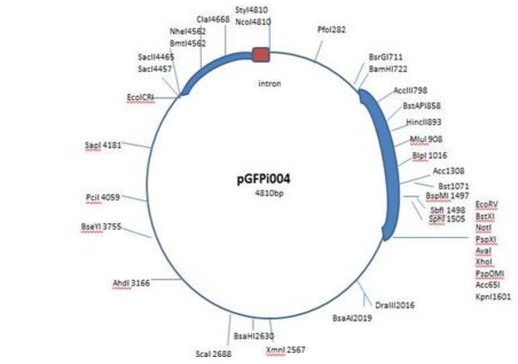

Figure 4: the backbone in this study is pGFPi004 which was based on the vector pMCSi004, which was constructed in Bristol UK.

Optimising gels

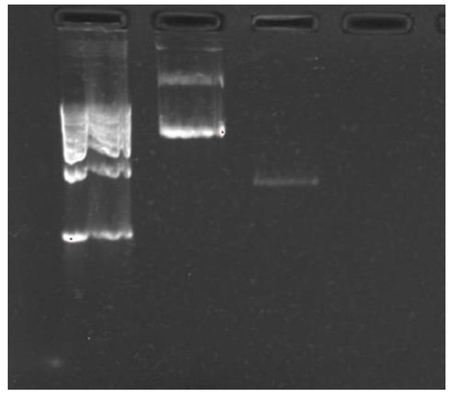

Figure 5: Running the plasmids on a gel and TBE buffer with lane 1: 1kb molecular weight ladder in, lane 2:pGFPi004 and lane 3: pUC as the control.

Restriction digest

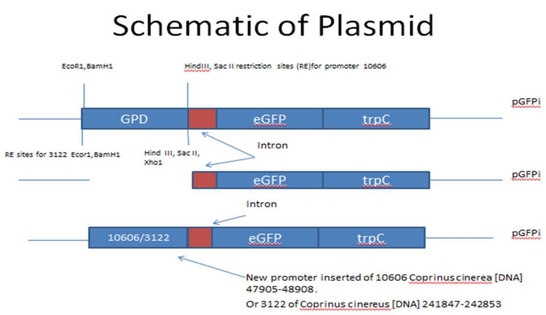

Figure 6: plasmid pGFPi004 based on pMCSi004, using restriction sites to cut out the promoter GPD, and inserting *Coprinus cinerea* promoters, 10606 between 47905-48908 or promoter 3122 between 241847-242853.

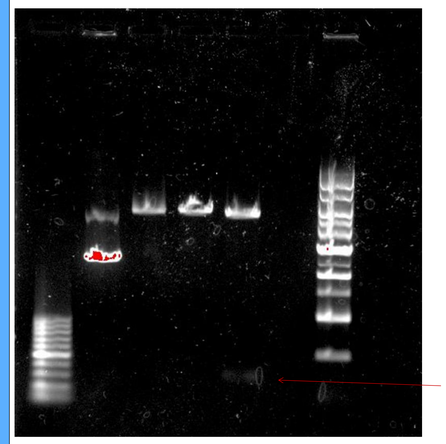

Figure 7: Restriction digests using EcoR1 and HindIII. The digests reagents were mixed together incubated at 37^{o}C for 3 hours, run on an agarose gel using TAE buffer. The expected band was to be ~300bp. Lane1:100bp, Lane 2: pGFPi004, Lane 3: Single digest HindIII, Lane 4: Single digest EcoR1, Lane 5: Double digest, Lane 6:1kb, Lane 7: Negative control.

Discussion

Optimising of agarose gel electrophoresis were the primary concern at the start of the project. The quality of the gel was grainy and looked like the gel wasn't dissolved in the buffer properly. The molecular weight ladders were smeary and not giving the result of crisp bands that was necessary (figure 5).
A lot of research was done to verify the reason for these undesirable traits of electrophoresis.

Buffers
Gel quality
Ladder quality
…
The digest was heated at 37^{o}C for 3 hours for a successful digest as seen in figure 5. Lane 5 shows a double digest successfully cut in figure 5, the picture was modified using the Bio-Rad to show up the cut plasmid.

Conclusion

It was concluded that changing the agarose and the procedure in dissolving it, using TAE buffer and not TBE and using one power pack per gel tank gave desirable results and molecular weight ladder and products were able to be sized correctly.

References

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Ireland, Department of Agriculture, Fisheries and Food (2009) Ireland's Horticulture Sector.