An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

14S865 - A genomic approach to understanding and improving mushroom compost utilisation

Final Report

This project was funded under the Department of Agriculture, Food and the Marine Competitive Funding Programme.

SUMMARY

The aim of this project was to use genomic and molecular tools to investigate how the commercial mushroom, Agaricus bisporus, obtains its nutrition from the substrate it grows on. The main ingredient in the substrate is wheat straw, which is broken down by enzymes to provide nutrition to the growing mushrooms. The enzymes are coded for by genes in the mushroom mycelium and they are switched on or off as they are needed. Identifying the key enzymes, and how they work together, will help us to understand how we may improve the utilisation of the substrate and increase crop yields in the future. The key objectives were: (1) to characterise what A. bisporus genes are active during the production of mushrooms, signifying their importance in terms of crop yield and (2) to understand more about the 'promotor' regions that control gene expression.

Results from the project showed that forty clusters of A. bisporus genes were identified during the crop production phase, each with a different pattern of gene expression. The genes within each cluster all had a similar pattern of expression, suggesting that they were possibly working together. The pattern of expression in some clusters of genes rose and fell, similar to the pattern of mushroom production in flushes, while other clusters of genes were switched on or off during some stages of the crop but not others. The study on the promoter regions generated interesting data regarding what substrates induced them, and at what time points. This is the largest gene expression data set ever generated for Agaricus bisporus. All mushroom breeders will have access to the dataset, which will facilitate strain-breeding to develop more productive strains. Data for the forty clusters of genes will be available through various online databases and referenced in peer reviewed publications.

KEYWORDS

genomics, sustainability

ACRONYM	**AgGenes**
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Section 1

Start Date

01 June 2015

End Date

31 August 2019

Select Research Programme

Research Stimulus Fund

Please select the appropriate TRL Scale

TRL 4: Technology validated in lab

NRPE Priority area

Sustainable Food Production and Processing

Total DAFM Award

€395,200.80

Total Project Expenditure

€394,101.73

Rationale for undertaking the Research

The mushroom industry in Ireland is acknowledged to be one of the best in the world, growing high quality Agaricus bisporus mushrooms for export. It is the largest horticultural sector in Ireland with a farm gate value of €119 million in 2019, and with over 80% exported to the UK. As profit margins are very tight, there is a need to increase efficiency wherever possible. The industry has improved productivity through harvester training, efficiencies in crop management and increases in compost and casing quality to a point where average yields are in the region of 30 kg of top class fresh mushroom product/m2 - some of the best yields in the world. To continue to retain its position as a major player in the European mushroom world, the industry must continue to push the boundaries of production efficiency.

Mushroom Stakeholders believe that Irish growers can increase productivity to 35 kg/m2 and more, through improvements to current systems of production but also through increasing compost utilisation by existing commercial strains and/or through the development of new more efficient strains by strain breeders. Since the publication of the Agaricus bisporus genome sequence (Morin et al. 2012), Teagasc has embraced the enormous potential that this offers and has been conducting genomic research to advance Ireland’s role in the global development of the next generation of mushroom strains. This project has advanced Irelands expertise and standing in mushroom science and Agaricus genomics research.

Methodology

Microarray analysis of gene expression in the compost over the course of a crop was done using custom designed Agilent microarrays for A. bisporus, which cover the whole genome. Compost samples were obtained every 48 hours from Day 11 of the crop cycle until the end of the third flush (Day 37) – fourteen time points in total. Labelled cRNA was extracted from the compost samples, hybridized to the microarrays, and gene transcript levels calculated using the appropriate software. Putative temporal gene expression profiles were then identified, by comparing transcript levels at each time point with the level detected at Day 11 before the production of mushrooms had started. Using statistical and bioinformatics analysis, the profiles were grouped into clusters of genes with similar expression profiles.

Gene expression profiles of three genes of interest were compared for two contrasting commercial strains, white strain 'A15' (from Sylvan spawn company) - and brown strain 'Heirloom' (from Amycel spawn company). Brown strains are usually lower-yielding compared to white strains and it would be interesting to see if this was reflected in the gene expression data.

Biostatistical and bioinformatics analysis was done on the A. bisporus genome to identify Transcription Factor Binding Sites (TFBS) associated with the promotor regions of genes, which have a key role in gene expression.

Compost samples for protein extraction were obtained for seven time points from the microarray study, taken before, during and after the 1st flush (Day 13-25). Proteins were extracted, and purified for Liquid Chromatography/Mass Spectrometry analysis to identify all of the A. bisporus proteins present. Using bioinformatic analysis these proteins were functionally annotated. Fold-change calculations were used to identify significantly up- and down-regulated proteins in each sample when compared to the earliest sample (D13) to examine differential protein abundances before, during and after the 1st flush.

Project Results

The gene expression profiles identified by microarray analysis were confirmed and verified. Around 2,000 A. bisporus genes (representing almost 20% of the 10,000+ genes in A. bisporus) were differentially expressed during the period when mushrooms were being actively produced and these could be aggregated into forty clusters, each with a distinct expression pattern (see figure in Grogan et al. TResearch Spring 2021, attached). Some clusters correlated with the rise and fall of mushroom yield associated with the 1st, 2nd and 3rd flushes of mushrooms (Cluster 26) while some gene clusters were switched on (Cluster 20) or off (Cluster 19) during the third flush, when mushroom yield was rapidly decreasing. This provides a large bank of data that can be interrogated regarding how

genes influence mushroom yield. Cluster 26 contained many genes known to be involved in lignocellulose degradation. These data have now generated a transcriptomic atlas of Agaricus bisporus during mushroom substrate utilisation which can be referred to with a high degree of confidence.

Secondly, there was a remarkable similarity in the gene expression profiles of three selected genes of interest between two commercial strains with different productivity attributes (a high yielding white strain and a lower yielding brown strain), suggesting that yield differences may not reflect the ability to utilise the substrate. The results mean that the gene expression profiles and clusters of genes with similar responses can now be interpreted with a high degree of confidence and used as an atlas for future research by both industry researchers and academia. The remarkable similarity of gene expression in the substrate between strains with different yielding characteristics will inform industry in strain selection and breeding.

The bioinformatic analysis of over 500 yeast, ascomycete and basidiomycete Transcription Factor Binding Sites (TFBS) showed the presence of known cellulase and hemicellulase TFBS present within selected candidate genes. A TFBS database for A. bisporus was constructed, providing a valuable resource for other researchers.

Proteomic analysis identified 558 A. bisporus proteins in the period spanning before, during and after the 1st flush. It was found that the total number of proteins changed similarly to the transcriptome data, but when the gene expression patterns were compared to the proteome data, the proteins took longer to reach their peak.

Section 2 - Research Outputs

Summary of Benefits / Improvements of Project Findings

One of the main objectives of the AgGenes project was to characterise the biological activity of the mushroom organism, Agaricus bisporus, as the crop progressed, identifying the genes that were actively involved during the mushroom production phase and the promotors that control them. This gene activity ultimately results in a crop of mushrooms from the substrate, and the yield of the crop directly relates to the activity of the mushroom mycelium in the substrate. AgGenes identified 40 clusters of genes that were active during this phase, with each cluster having a unique activity profile, suggesting that genes in a cluster may be involved in the same biological process. This large bioinformatics dataset is now available to the mushroom scientific community to interrogate and shed light on mushroom substrate degradation and other biological processes. It can be used by mushroom geneticists involved in breeding new strains of mushrooms and may help to identify important genes that act together in important biological processes. A draft manuscript for peer review is in an advanced stage of preparation and will be publically available in due course. Future research in mushroom science will need to look for genes that confer beneficial traits such as substrate- and water-use efficiency. Further work is needed to characterise the genes in each cluster and this could form the basis of many PhD studies into the future, developing scientific expertise and training the next generation of scientists.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
Post Doctorates	1	0	1
PhD Students	1	0	1
Other / Temporary	1	0	1

Summary of Academic Outputs

Research Outputs	Total Number	Details
PhD Theses	1	Keith Dunne. PhD thesis entitled 'A genomic approach to understanding and improving mushroom compost utilisation', Submitted August 2021. Thesis was successfully defended in February 2021 and PhD will be awarded in November 2021.
Training Courses	1	Undergraduate training at IT Sligo. An undergraduate at IT Sligo undertook work related to the project in 2019, as training in research. Poster Presentation – First Place: 'Employment of a Rapid Fungal Detection System for High Throughput Screening of Basidiomycete Transformants' by Christopher Golden (Institute of Technology, Sligo), Mary Heneghan http://sure-network.ie/conference/sligo/

Intellectual Property

The main results from the project are at an advanced stage of preparation for peer review. The authors and their institutes will be the owners of the IP, which will be accessible to the scientific community.

Summary of other Project Outputs

Project Outputs	Details	Total No.
New Processes	1. Dr Mary Heneghan's group at IT Sligo have developed new laboratory protocols for the induction of of selected promotors in basidiomycete transformants, as a tool to verify gene activity. This will facilitate future research into gene regulation in basidiomycetes.	1

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Industry	Fundamental applied research such as that conducted by AgGenes is needed to understand how mushroom mycelium functions in the substrate to efficiently biologically convert the substrate into a crop of mushrooms and maximise productivity and hence, sustainability. If industry cannot maintain high levels of productivity, the business will be less profitable and eventually, unsustainable The absence of such data and research will slow down progress and advancement of the sector.
Other	Expertise in mushroom science and fungal bioscience is being increased in Irish third level institutes with permanent researchers active in mushroom science in Teagasc, IT Sligo and Maynooth University. This expertise is essential if we are to address industry issues such as biological control of pathogens, pesticide resistance and resitant cultuvars, peat replacement and microbiome influences on sustainable production. In addition, 'fungal bio-products' represent a new research area for more sustainable products such as insulation, leather and packaging, and fungal expertise is critical to exploit such new opportunities.

Dissemination Activities

Activity	Details
Workshops at which results were presented	16 October 2018. The 4th AgGenes Stakeholder Meeting, IT Sligo. This was well attended by Stakeholders who were given a detailed presentation of the key results of the work.
Other	A short YouTube video was produced to describe the project. Link: https://youtu.be/Btqck0gMDdo

Knowledge Transfer Activities

Identify knowledge outputs	The key outputs are one PhD thesis and a peer review scientific publication that is in progress (submission planned for 2021/22).
Identify any knowledge transfer activities executed within the project.	Technical articles for the scientific and industry communities have been written for Teagasc's TResearch magazine and Technical Updates publications. An article has also been published in the International industry magazine 'Mushroom Business', which is published by Global Roel Media in the Netherlands
List any impacts resulting from the knowledge transferred during the project.	Increased awareness of the mushroom industry of genomic research underpinning substrate utilisation. Contact made with research group at Utrecht University, the Netherlands.

Section 3 - Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Exchequer National Funding	€17,000.00	The AgGenes project leader at IT Sligo was successful in an application to the IT Sligo Research Capacity Fund for €17,000 for equipment and student funding.

Future Strategies

Our ambition is to build a multidisciplinary mushroom research network across Ireland that includes Teagasc, Maynooth University, IT Sligo, IT Carlow, UCD and AFBI, Northern Ireland, with links to other mushroom research groups and research organisations around the world. Sustainability will dominate the global agricultural food production agenda for the foreseeable future and Teagasc's ambition is to make sustainability 'front and centre' of all of our activities. This will require innovative research that utilises the power of modern technologies, such as genomics, proteomics and bioinformatics to deliver sustainable solutions to challenging problems. AgGenes researchers are continually looking for research funding opportunities that build on the advanced genomic technologies developed in the AgGenes project to advance mushroom science for sustainability objectives.

(a) Irish Research Council (Post Doctoral Fellowship 2018-2020)
(b) Teagasc-EU Research Leaders 2025 (Fellowship 2021-2024)
(c) EU H2020 (SoftGrip 2021-2023)
(d) US-Ireland R&D Partnership Programme 2020 (unsuccessful)
(e) DAFM Stimulus 2020 (Peat Replace 2021-2026 - under negotiation)
(f) Teagasc Walsh Scholarships (3 successful), 2017, 2020, 2021)
(g) Excelomics: state-of-the-art genomics, transcriptomics, proteomics The AgGenes project team at Maynooth University have applied for SFI funding through the "Research Infrastructure Programme" for a new proteomics suite and DNA sequencer. Awaiting outcome.

Project Publications

1. Dunne, K. A genomic approach to understanding and improving mushroom compost utilisation. A thesis submitted to Institute of Technology Sligo for the degree of Doctor of Philosophy. August 2020 (Awarded November 2021).

2. Grogan H, O`Donoghue M, Dunne K, Heneghan M, O'Connor E, Fitzpatrick D. Understanding the genetics behind mushroom production. TResearch, Spring 2021 https://www.teagasc.ie/media/website/publications/2021/TResearch_MushroomGenetics_Spring-2021_Web.pdf

3. Grogan H, O`Donoghue M, Dunne K, Heneghan M, O'Connor E, Fitzpatrick D. Understanding the genetics behind mushroom production. Mushroom Business 107, July 2021 https://www.mushroombusiness.com/107-%E2%80%93-july-2021

Posters:

4. Optimisation of Compost Utilisation by Agaricus Bisporus through Genomic Analysis. Environ 2016, Limerick, Ireland, 22/24 March 2016. Dunne, K. O`Donoghue, M. Grogan, H. Burton, K. Heneghan, M.

5. A Genomic Approach to Understanding and Improving Mushroom Compost Utilisation. ECFG13, 13th European Conference on FungalGenetics, Paris, France, 4-7 April 2016. Dunne, K. O`Donoghue, M. Grogan, H. Burton, K. Heneghan, M.

6. A Genomic Approach to Understanding and Improving Mushroom Compost Utilisation. ISMS Congress, International Society forMushroom Science, Amsterdam, Netherlands, 29 May-02 June 2016. Dunne, K. O`Donoghue, M. Grogan, H. Burton, K. Heneghan, M.

7. Understanding and Improving Mushroom Compost Utilisation through a Genomic Approach. Institute of Technology Sligo PostGraduate showcase, Sligo, Ireland, 13 June 2017 *. Dunne, K. O`Donoghue, M. Grogan, H. Burton, K. Heneghan, M. * Won best poster presentation award.

8. Understanding and Improving Mushroom Compost Utilisation Through a Genomic Approach. IFS, Irish Fungal Society Annual Scientific Meeting, Limerick, Ireland, 15/16 June 2017. Dunne, K. O`Donoghue, M. Grogan, H. Burton, K. Heneghan, M.

9. Differential screening of Agaricus bisporus to identify genes involved in yield control. IFS, Irish Fungal Society Annual Scientific Meeting, Limerick, Ireland, 15/16 June 2017. O`Donoghue, Dunne, K. M. Grogan, H. Burton, K. Heneghan, M.

10 The genetic regulation of mushroom production and its relevance to fungal plant decomposition and carbon sequestration. Irish Fungal Society Conference, Maynooth University, Ireland, 18/19 June 2018. O`Donoghue, Dunne, K. M. Grogan, H. Burton, K. Heneghan, M.

11. Transcriptomic screening of Agaricus bisporus to identify genes involved in yield control and compost utilisation. State of the Worlds Fungi Symposium, Kew Gardens, London, UK, 14-15 September 2018 O`Donoghue, Dunne, K. M. Grogan, H. Burton, K. Heneghan, M.