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Foulongne-Oriol, M., Spataro, C., Savoie, J.-M.

Novel microsatellite markers suitable for genetic studies in the white button mushroom *Agaricus bisporus*

(2009) *Applied Microbiology and Biotechnology*, pp. 1-11. Article in Press.

UR1264 Mycologie et Sécurité des aliments, INRA, Centre de Recherche Bordeaux-Aquitaine, BP 81, Villenave d'Ornon cedex, 33883, France

Abstract

Co-dominant microsatellite molecular markers were obtained from the *Agaricus bisporus* cultivated mushroom. Their potential for both the molecular characterisation of commercial strains and the monitoring of the intraspecific genetic variation was demonstrated. The analysis of 673 unique sequences issued from public database and 59 from an enriched *A. bisporus* genomic library resulted in the development of a total of 33 single sequence repeat or microsatellite (SSR) markers. Their usefulness for genetic analysis was assessed on 28 strains, which include six cultivars representative of traditional lineage, two hybrids and 20 strains originating from wild populations. *A. bisporus* SSR markers displayed each from two to ten alleles, with an average of 5.6 alleles per locus. The observed heterozygosity ranged from 0 to 0.88. Cluster analysis resulting from SSR fingerprintings was in agreement with published *A. bisporus* population structure. A combination of only three selected SSR markers was sufficient to discriminate unambiguously 27

out of 28 distinct genotypes. However, the two genetically related hybrids were not distinguishable. Multiplexing was tested, and up to seven loci could be genotyped simultaneously. We are therefore reporting the first development in *A. bisporus* of a set of microsatellite markers powerful and suitable for genetic analysis. © 2009 Springer-Verlag.

Author Keywords

Agaricus bisporus; Genetic diversity; SSR; Strain typing

Document Type: Article in Press

Source: Scopus

Heneghan, M.N.^a, Porta, C.^b, Zhang, C.^b, Burton, K.S.^b, Challen, M.P.^b, Bailey, A.M.^a, Foster, G.D.^{a c}

Characterization of serine proteinase expression in *Agaricus bisporus* and *Coprinopsis cinerea* by using green fluorescent protein and the *A. bisporus* SPR1 promoter

(2009) *Applied and Environmental Microbiology*, 75 (3), pp. 792-801.

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Abstract

The *Agaricus bisporus* serine proteinase 1 (SPR1) appears to

be significant in both mycelial nutrition and senescence of the fruiting body. We report on the construction of an SPR promoter::green fluorescent protein (GFP) fusion cassette, pGreen-hph1-SPR-GFP, for the investigation of temporal and developmental expression of SPR1 in homobasidiomycetes and to determine how expression is linked to physiological and environmental stimuli. Monitoring of *A. bisporus* pGreen-hph1-SPR-GFP transformants on media rich in ammonia or containing different nitrogen sources demonstrated that SPR1 is produced in response to available nitrogen. In *A. bisporus* fruiting bodies, GFP activity was localized to the stipe of postharvest senescing sporophores. pGreen-hph1-SPR-GFP was also transformed into the model basidiomycete *Coprinopsis cinerea*. Endogenous *C. cinerea* proteinase activity was profiled during liquid culture and fruiting body development. Maximum activity was observed in the mature cap, while activity dropped during autolysis. Analysis of the *C. cinerea* genome revealed seven genes showing significant homology to the *A. bisporus* SPR1 and SPR2 genes. These genes contain the aspartic acid, histidine, and serine residues common to serine proteinases. Analysis of the promoter regions revealed at least one CreA and several AreA regulatory motifs in all sequences. Fruiting was induced in *C. cinerea* dikaryons, and fluorescence was determined in different developmental stages. GFP expression was observed throughout the life cycle, demonstrating that serine proteinase can be active in all stages of *C. cinerea* fruiting body development. Serine proteinase expression (GFP fluorescence) was most concentrated during development of young tissue, which may be indicative of high protein turnover during cell differentiation. Copyright © 2009, American Society for Microbiology. All Rights Reserved.

Document Type: Article

Source: Scopus

Kavousi, H.R., Farsi, M., Shahriari, F.

Comparison of random amplified polymorphic DNA markers and morphological characters in identification of homokaryon isolates of white button mushroom (*Agaricus bisporus*)

(2008) *Pakistan Journal of Biological Sciences*, 11 (14), pp. 1771-1778.

Department of Biotechnology and Plant Breeding, College of Agriculture, Ferdowsi University of Mashhad, Iran

Abstract

The secondarily homothallic life cycle of the white button mushroom that results in scarcity of uninucleate basidiospores (homokaryons) in its progeny, is the most important impediment for genetic improvement of the commercial strains. Identification of homokaryons for breeding programs of *Agaricus bisporus* (button mushroom) is, therefore, crucial. Verifying homokaryons through fruiting trial is time consuming and unreliable. In this study, ability of RAPD markers, compared to morphological characters for identification of homokaryon isolates, was investigated. Based on morphological characters, 42 isolates were screened and exposed to RAPD markers. The results showed that RAPD markers could discriminate homokaryons from heterokaryons, based on number of bands generated. The numbers of band in homokaryons were significantly less than those of heterokaryons. Results also showed that cluster analysis, based on average of band number generated could separate homokaryon from heterokaryon isolates. It is suggested that RAPDs could be used to identify homokaryons from heterokaryons for breeding program of *A. bisporus*. © 2008 Asian Network for Scientific Information.

Author Keywords

Homokaryon; RAPD markers; Secondarily homothallic; White button mushroom

Document Type: Article

Source: Scopus

Adams, L.S.^a , Phung, S.^a , Wu, X.^a , Ki, L.^a , Chen, S.^{a b}

White button mushroom (*Agaricus bisporus*) exhibits antiproliferative and proapoptotic properties and inhibits prostate tumor growth in athymic mice
(2008) *Nutrition and Cancer*, 60 (6), pp. 744-756.

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Abstract

White button mushrooms are a widely consumed food containing phytochemicals beneficial to cancer prevention. The purpose of this research was to evaluate the effects of white button mushroom extract and its major component, conjugated linoleic acid (CLA) on prostate cancer cell lines in vitro and mushroom extract in vivo. In all cell lines tested, mushroom inhibited cell proliferation in a dose-dependent manner and induced apoptosis within 72 h of treatment. CLA inhibited proliferation in the prostate cancer cell lines in vitro. DU145 and PC3 prostate tumor size and tumor cell proliferation were decreased in nude mice treated with mushroom extract, whereas tumor cell apoptosis was increased compared to pair-fed controls. Microarray analysis of tumors identified significant changes in gene expression in

the mushroom-fed mice as compared to controls. Gene network analysis identified alterations in networks involved in cell death, growth and proliferation, lipid metabolism, the TCA cycle and immune response. The data provided by this study illustrate the anticancer potential of phytochemicals in mushroom extract both in vitro and in vivo and supports the recommendation of white button mushroom as a dietary component that may aid in the prevention of prostate cancer in men. Copyright © 2008, Taylor & Francis Group, LLC.

Document Type: Article

Source: Scopus

Eastwood, D.C.^a , Challen, M.P.^a , Zhang, C.^a , Jenkins, H.^b , Henderson, J.^{b c} , Burton, K.S.^a

Hairpin-mediated down-regulation of the urea cycle enzyme argininosuccinate lyase in *Agaricus bisporus*
(2008) *Mycological Research*, 112 (6), pp. 708-716. Cited 2 times.

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^c School of Science and Technology, Innovation and Virtual Reality Centre, University of Teesside, Middlesbrough, TS1 3BA, United Kingdom

Abstract

A double-stranded (ds) RNA hairpin-mediated down-regulation system was developed for the cultivated mushroom *Agaricus bisporus*, and the role of the urea cycle enzyme

argininosuccinate lyase (asl) in mushroom post-harvest development was investigated. Hairpin expression vectors were constructed to initiate down-regulation of asl and introduced into *A. bisporus* by *Agrobacterium tumefaciens*-mediated transformation. Transcripts of asl were significantly reduced (93.1 and 99.9 %) in two transformants and hairpin vector transgene sequences were maintained throughout sporophore development. Single and multiple hairpin integration events were observed in Southern analysis. Transformants with down-regulated asl exhibited reduced yield and cap expansion during post-harvest sporophore development. There were no detectable differences in urea levels between the hairpin-transformed and control strains. This is the first report of reduced gene expression resulting from the introduction of dsRNA hairpins in *A. bisporus* and the applications of this technology will facilitate functional studies in the mushroom. © 2008 The British Mycological Society.

Author Keywords

Agaricus bisporus; *Agrobacterium*-mediated transformation; Argininosuccinate lyase; Hairpin; Post-harvest; RNA interference (RNAi); Urea cycle

Document Type: Article

Source: Scopus

Teke, M.^b, Sezgintürk, M.K.^{a c}, Dinçkaya, E.^a, Telefoncu, A.^a

Two biosensors for phenolic compounds based on mushroom (*Agaricus bisporus*) homogenate: Comparison in terms of some important parameters of the biosensors

(2008) *Preparative Biochemistry and*

Biotechnology, 38 (1), pp. 51-60. Cited 1 time.

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Abstract

Interest in molecular imprinted polymer techniques has increased because they allows for the improvement of some stability characteristics of enzymes. The high stability of molecularly imprinted enzymes for a substrate can make them ideal alternatives as recognition elements for sensors. A bioimprinted mushroom tissue homogenate biosensor was constructed in a very simple way. For this purpose, sulfite was used. The enzyme, polyphenol oxidase, was first complexed by using a competitive inhibitor, sulfite, in aqueous medium and then the enzyme was immobilized on gelatin by crosslinking with glutaraldehyde on a glass electrode surface. Similarly, polyphenol oxidase uncomplexed with sulfite was also immobilized on a glass electrode in the same conditions. The aim of the study was to compare the two biosensors in terms of their repeatability and thermal, pH, and operational stability; also, the linear ranges of the two biosensors were compared with each other. Copyright © Taylor & Francis Group, LLC.

Author Keywords

Bioimprinted biosensors; Biosensor stability; Enzyme based biosensors; Polyphenol oxidase

Document Type: Article

Source: Scopus

Yadav, M.C.^a , Challen, M.P.^b , Singh, S.K.^a , Elliott, T.J.^b
DNA analysis reveals genomic homogeneity and single nucleotide polymorphism in 5.8S ribosomal RNA gene spacer region among commercial cultivars of the button mushroom *Agaricus bisporus* in India
(2007) *Current Science*, 93 (10), pp. 1383-1389.

^a Molecular Genetics Laboratory, National Research Centre for Mushroom, Solan 173 213, India

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Abstract

Molecular variation was studied among 22 white pileus cultivars of *Agaricus bisporus* using random amplified polymorphic DNA markers and by sequence analysis of 5.8S rRNA gene along with ITS regions. Twentyfour primers amplified 175 RAPD markers, of which 53.7% were polymorphic. Genetic similarity index varied from 0.64 to 0.99, with an average of 0.81. The varieties exhibited 92.7% genetic similarity, while the hybrids showed 84.3% similarity amongst them. Both the UPGMA dendrogram and PCO plot grouped all the varieties into a single cluster, while the hybrids formed a separate cluster exhibiting DNA polymorphism. The length of ITS1, 5.8S rRNA gene and ITS2 was 290, 154 and 208 bases respectively, in all the genotypes of *A. bisporus* studied. We report two single nucleotide polymorphisms (SNPs) at 522 and 563 nucleotide positions in the ITS2 region which distinguished different strains within the species. This study demonstrates that the RAPD markers are useful and robust tools for identification of hybrids in the

germ plasm and for detection of intraspecific molecular variation in the white button mushroom cultivars. In the present study we report the identification of SNPs in the ITS2 region of the 5.8S ribosomal RNA gene which could differentiate cultivars of *A. bisporus*.

Author Keywords

Agaricus bisporus; Commercial cultivars; DNA polymorphism; ITS sequencing

Document Type: Article

Source: Scopus

Romaine, C.P., Schlagnhauser, C.

Mushroom (*Agaricus bisporus*).

(2006) *Methods in molecular biology* (Clifton, N.J.), 344, pp. 453-463.

Department of Plant Pathology, The Pennsylvania State University, University Park, PA, USA.

Abstract

We have devised an easy and effective genetic transformation method for the preeminent edible mushroom, *Agaricus bisporus*. Our method exploits the T-DNA transfer mechanism in *Agrobacterium tumefaciens* and relies on the reproductive fruiting body as the recipient tissue. The use of fruiting body explants, particularly the gill, provided high-frequency transformation, overcoming the inefficacy of *Agrobacterium*-based methods targeting fungal spores or vegetative mycelium. The protocol entails incubation of *A. tumefaciens* for 3 h with acetosyringone, a signaling molecule that launches the gene transfer mechanism, co-cultivation of the induced bacterium and gill explants for 3 d, and selection for

transformants based on an inherited resistance to the antibiotic hygromycin. Between 7 and 28 d on the selection medium, upwards of 95% of the gill explants generate hygromycin-resistant colonies. About 75% of the mushroom transformants show a single-copy of the hygromycin-resistant gene integrated at random sites in the genome.

Document Type: Article

Source: Scopus

Wagemaker, M.J.M.^a, Eastwood, D.C.^b, Van Der Drift, C.^a, Jetten, M.S.M.^a, Burton, K.^b, Van Griensven, L.J.L.D.^c, Op Den Camp, H.J.M.^a

Expression of the urease gene of *Agaricus bisporus*: A tool for studying fruit body formation and post-harvest development

(2006) *Applied Microbiology and Biotechnology*, 71 (4), pp. 486-492. Cited 5 times.

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^b Fungal Biotechnology Group, Warwick HRI, Wellesbourne, Warwickshire, United Kingdom

^c Wageningen UR, Plant Research International B.V., Wageningen, Netherlands

Abstract

Fruit body initials of *Agaricus bisporus* contain high levels of urea, which decrease in the following developmental stages until stage 4 (harvest) when urea levels increase again. At storage, the high urea content may affect the quality of the mushroom, i.e. by the formation of ammonia from urea

through the action of urease (EC 3.5.1.5). Despite the abundance of urea in the edible mushroom *A. bisporus*, little is known about its physiological role. The urease gene of *A. bisporus* and its promoter region were identified and cloned. The coding part of the genomic DNA was interrupted by nine introns as confirmed by cDNA analysis. The first full homobasidiomycete urease protein sequence obtained comprised 838 amino acids (molecular mass 90,694 Da, pI 5.8). An alignment with fungal, plant and bacterial ureases revealed a high conservation. The expression of the urease gene, measured by Northern analyses, was studied both during normal development of fruit bodies and during post-harvest senescence. Expression in normal development was significantly up-regulated in developmental stages 5 and 6. During post-harvest senescence, the expression of urease was mainly observed in the stipe tissue; expression decreased on the first day and remained at a basal level through the remaining sampling period. © Springer-Verlag 2005.

Document Type: Article

Source: Scopus

Sreenivasaprasad, S., Eastwood, D.C., Browning, N., Lewis, S.M.J., Burton, K.S.

Differential expression of a putative riboflavin-aldehyde-forming enzyme (raf) gene during development and post-harvest storage and in different tissue of the sporophore in *Agaricus bisporus*

(2006) *Applied Microbiology and Biotechnology*, 70 (4), pp. 470-476. Cited 3 times.

Warwick HRI, University of Warwick, Wellesbourne, Warwickshire, CV35 9EF, United Kingdom

Abstract

Cloning and characterisation of a putative riboflavin-aldehyde-forming enzyme gene (raf) from the cultivated mushroom *Agaricus bisporus* and its expression during morphogenesis are described. Three cDNA clones were isolated following differential screening of cDNA libraries from rapidly expanding sporophores and post-harvest stored sporophores. The cDNA sequence and predicted translation analysis revealed an open reading frame (ORF) of 348 nucleotides encoding a polypeptide of 115 amino acids, with three introns (56-66 bases) interrupting the genomic ORF. Blast X searches of the databases with the gene sequence showed homology (40% identity and 56% similarity) to the riboflavin-aldehyde-forming enzyme gene from *Schizophyllum commune*. In *A. bisporus*, the raf gene sequence upstream of the ORF contained a large CT-rich putative regulatory element (-64 to -24 bases) found in highly expressed genes in various mushrooms, and a 6-base motif present in the 3' end of the genomic sequence, but not in the corresponding 3' non-coding part of the cDNA, was identified. The raf gene transcripts increased abundantly in rapidly developing sporophores as well in post-harvest stored sporophores. Differential expression of the raf gene transcripts in different tissues of the sporophore was also observed, with higher levels in the stipe compared with the cap and gills. The temporal and spatial expression patterns observed suggest transcriptional regulation of the raf gene during *A. bisporus* morphogenesis. © Springer-Verlag 2005.

Document Type: Article

Source: Scopus

Callac, P., Spataro, C., Caille, A., Imbernon, M.

Evidence for outcrossing via the buller phenomenon in a substrate simultaneously inoculated with spores and mycelium of *Agaricus bisporus*

(2006) *Applied and Environmental Microbiology*, 72 (4), pp. 2366-2372.

Mycologie et Sécurité des Aliments, INRA, B.P. 81, F-33883 Villenave d'Ornon Cedex, France

Abstract

In *Agaricus bisporus*, traditional cultivars and most of the wild populations belong to *A. bisporus* var. *bisporus*, which has a predominantly pseudohomothallic life cycle in which most meiospores are heterokaryons ($n + n$). A lower proportion of homokaryotic (n) meiospores, which typify the heterothallic life cycle, also are produced. In wild populations, pseudohomothallism was thought previously to play a major role, but recent analyses have found that significant outcrossing also may occur. We inoculated a standard substrate for *A. bisporus* cultivation simultaneously with homokaryotic mycelium from one parent and spores from a second parent. Culture trays produced numerous sporocarps that could theoretically have resulted from five different reproductive modes (pseudohomothallism, selfing or outcrossing via heterothallism, and selfing or outcrossing via the Buller phenomenon [i.e., between a homokaryon and a heterokaryon]). Most or all of the sporocarps resulted from outcrossing between the inoculated homokaryon and the inoculated heterokaryotic spores (or mycelia that grew from them). These data broaden our understanding of population dynamics under field conditions and provide an outcrossing method that could be used in commercial breeding programs. Copyright © 2006. American Society for Microbiology. All Rights Reserved.

Document Type: Article

Source: Scopus

Burns, C.^a , Leach, K.M.^b , Elliott, T.J.^b , Challen, M.P.^b , Foster, G.D.^a , Bailey, A.^a

Evaluation of agrobacterium-mediated transformation of *Agaricus bisporus* using a range of promoters linked to hygromycin resistance

(2006) *Molecular Biotechnology*, 32 (2), pp. 129-138. Cited 4 times.

^a School of Biological Sciences, University of Bristol, Bristol BS8 1UG, United Kingdom

^b Warwick HRI, University of Warwick, Wellesbourne, Warwick CV35 9EF, United Kingdom

Abstract

There is interest in establishing genetic modification technologies for the cultivated mushroom *Agaricus bisporus*, both for improved crop characteristics and for molecular pharming. For these methods to be successful, it is necessary to establish a set of transformation systems that include robust and reliable vectors for gene manipulation. In this article, we report the evaluation of a series of promoters for driving expression of the *Escherichia coli* hph gene encoding hygromycin phosphotransferase. This was achieved using the *Aspergillus nidulans* gpdA and the *A. bisporus* gpdII and trp2 promoters. The *Coprinus cinereus* β -tubulin promoter gave contrasting results depending on the size of promoter used, with a 393-bp region being effective, whereas the longer 453-bp fragment failed to yield any hygromycin-resistant transformants. The *C. cinereus* trp1 and the *A. bisporus* lcc1

promoters both failed to yield transformants. We also show that transformation efficiency may be improved by careful selection of both appropriate *Agrobacterium* strains, with AGL-1 yielding more than LBA1126 and by the choice of the binary vectors used to mobilize the DNA, with pCAMBIA vectors appearing to be more efficient than either pBIN19- or pGREEN-based systems. © 2006 Humana Press Inc. All rights of any nature whatsoever reserved.

Author Keywords

Molecular pharming; Mushroom transformation

Document Type: Article

Source: Scopus

Singh, S.K., Vijay, B., Mediratta, V., Ahlawat, O.P., Kamal, S.
Molecular characterization of *Humicola grisea* isolates associated with *Agaricus bisporus* compost
(2005) *Current Science*, 89 (10), pp. 1745-1749.

National Research Centre for Mushroom, Chambaghat, Solan
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Abstract

Composite compost samples were collected from Solan, Sonapat, Gangtok, Kaithal, Phagwara and Ooty, at different stages of *Agaricus bisporus* compost preparation by long and short methods using a variety of agrowaste substrates. Eight isolates of *Humicola grisea* were retrieved on yeast agar medium at 45 and 52° C. PCR amplification of internal transcribed spacer (ITS) region of 5.8S ribosomal RNA (rRNA) gene was done using ITS-1 and ITS-4 primers. An ITS fragment of approximately 550 bp was amplified from all the eight *H. grisea* isolates with no intra-specific diversity in the

ITS region of 5.8S rRNA gene. RAPD genotyping was performed using five decamer primers. Combined phylogenetic analysis of RAPD profiles of *H. grisea* by five primers depicted intra-specific variation amongst the eight isolates and divided these into five distinct sub-clades. Molecular analysis carried out in the present study would suggest that isolates within this species exhibit genetic differences, which correlates well with morphological variations.

Author Keywords

Agaricus bisporus; Compost microflora; Genetic diversity; *Humicola grisea*; RAPD

Document Type: Article

Source: Scopus

Wang, H.X.^a, Wang, Z.S.^b

A brief review of the breeding and cultivation of button mushroom *Agaricus bisporus* (J. Lge) imbach in China with the promotion of Professor Shu-Ting Chang

(2005) *International Journal of Medicinal Mushrooms*, 7 (1-2), pp. 15-21.

^a Fujian Research Institute of Light Industry (FRILI), Fuzhou, 350005, China

^b Fujian Mushroom Research and Development Station (FMRDS), Fuzhou, 350005, China

Document Type: Short Survey

Source: Scopus

Lankinen, P.^a , Hildén, K.^a , Aro, N.^b , Salkinoja-Salonen, M.^a , Hatakka, A.^a

Manganese peroxidase of *Agaricus bisporus*: Grain bran-promoted production and gene characterization

(2005) *Applied Microbiology and Biotechnology*, 66 (4), pp. 401-407. Cited 9 times.

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^b VTT Biotechnology, P.O. Box 1500, 02044 VTT Espoo, Finland

Abstract

The main manganese peroxidase (MnP) isoenzyme of *Agaricus bisporus* ATCC 62459 produced in lignocellulose-containing cultures was isolated, cloned and sequenced. In liquid medium, where MnP was previously detected only in trace amounts, the production of MnP was enhanced by rye and wheat bran supplements. The pI (3.25) and N-terminal amino acid sequence (25 aa) of the enzyme from bran-containing cultures were identical to those reported from compost-isolated MnP1. MnP1 is a 328-aa long polypeptide preceded by a 26-aa leader peptide. The nucleotide sequence and putative amino acid sequence of MnP1 reveal its similarity to *Pleurotus ostreatus* MnP3 (62.5%), *Lepista irina* versatile peroxidase (VP) (61.8%) and *Pleurotus eryngii* VPs VPL2 and VPL1 (61.9% and 61.2%, respectively). The intron-exon structure resembles that of *P. ostreatus* MnP1 and *P. eryngii* VPL1. Despite the sequence similarity to VPs, in the *A. bisporus* MnP1 sequence, alanine (A163) is present instead of tryptophane (W164), distinguishing it from the veratryl alcohol oxidising *P. eryngii* VPLs. The MnP sequence can be used as a tool to examine the pattern of ligninolytic gene

expression during the growth and fruiting of *A. bisporus* to optimise compost composition, fungal growth and mushroom production. © Springer-Verlag 2004.

Document Type: Article

Source: Scopus

Chen, R., Chen, L., Song, S.

Identification of Two Thermotolerance-Related Genes in *Agaricus bisporus*

(2003) *Food Technology and Biotechnology*, 41 (4), pp. 339-344. Cited 2 times.

Key Laboratory, Xiamen University, Min. Educ. Cell Biol./Tum. Cell Eng., Xiamen, Fujian 361005, China

Abstract

To characterize thermotolerance-related genes in *Agaricus bisporus* strain 02, we employed differential display PCR (DD-PCR) to analyze total RNA samples extracted from the mycelia grown at different temperatures. Two partial DNA fragments (023-11A and 023-11B) were cloned thus far, the expression of which was correlated with the culturing temperature. The sequences of the two DNA fragments were determined and the results showed that the nucleotide sequence of 023-11A was unknown, and 023-11B was highly similar in nucleotide sequence (identities 24 %, positives 45 %) to a gene coding for the karyopherin docking complex of the nuclear pore complex of *Saccharomyces cerevisiae*. It is possible to use the two fragments for further characterization of full-length coding sequences, which can potentially be used for generating new thermotolerant mushroom strains by transgenic technique.

Author Keywords

Agaricus bisporus; Fluoro differential display polymerase chain reaction; Thermotolerance-related gene

Document Type: Article

Source: Scopus

Amey, R.C.^a, Mills, P.R.^b, Bailey, A.^a, Foster, G.D.^a

Investigating the role of a *Verticillium fungicola* β -1,6-glucanase during infection of *Agaricus bisporus* using targeted gene disruption

(2003) *Fungal Genetics and Biology*, 39 (3), pp. 264-275. Cited 16 times.

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^b Horticulture Research International, Wellesbourne, Warwick CV35 9EF, United Kingdom

Abstract

Studies on the mycopathogen *Verticillium fungicola* have shown the up-regulation of β -1,6-glucanases when grown in the presence of host cell walls and host cell wall components including chitin. These cell-wall-degrading enzymes are hypothesized to contribute to the pathogenic ability of mycopathogens. A β -1,6-glucanase gene, VfGlu1, showing high similarity to β -1,6-glucanase genes from *Hypocrea virens*, *Neotyphodium* sp., and *Trichoderma harzianum*, was isolated using degenerate PCR from *V. fungicola*, a serious mycopathogen of the cultivated mushroom *Agaricus bisporus*. *Agrobacterium*-mediated transformation of *V. fungicola* using homologous DNA from VfGlu1 resulted in homologous integration at the VfGlu1 locus in 75% of transformants,

generating mutants disrupted in the VfGlu1 gene. VfGlu1 mutants displayed reduced virulence and diminished ability to utilize chitin as a carbon source, implicating VfGlu1 in the disease process. Agrobacterium-mediated transformation affords an efficient technique for the disruption of genes associated with disease symptom development in the complex *V. fungicola*-*A. bisporus* interaction. © 2003 Elsevier Science (USA). All rights reserved.

Author Keywords

Agaricus bisporus; *Agrobacterium*; Enzyme; Glucanase; Targeted gene disruption; *Verticillium fungicola*

Document Type: Article

Source: Scopus

Wichers, H.J., Recourt, K., Hendriks, M., Ebbelaar, C.E.M., Biancone, G., Hoeberichts, F.A., Mooibroek, H., Soler-Rivas, C.

Cloning, expression and characterisation of two tyrosinase cDNAs from *Agaricus bisporus*

(2003) *Applied Microbiology and Biotechnology*, 61 (4), pp. 336-341. Cited 17 times.

Agrotechnological Research Institute, Bornsesteeg 59, 6708 PD Wageningen, Netherlands

Abstract

Using primers designed on the basis of sequence homologies in the copper-binding domains for a number of plant and fungal tyrosinases, two tyrosinase encoding cDNAs were cloned from an *Agaricus bisporus* U1 cDNA-library. The sequences AbPPO1 and AbPPO2 were, respectively, 1.9 and 1.8 kb in size and encoded proteins of approximately 64 kDa.

The cDNAs represent different loci. Both AbPPO1 and AbPPO2 occur as single copies on the genomes of the U1 parental strains H39 and H97. The genomic size of AbPPO1 and AbPPO2 is minimally 2.3 and 2.2 kb, respectively. Alignment and phylogenetic analysis of 35 tyrosinase and polyphenol oxidase sequences of animal, plant, fungal, and bacterial origin indicated conserved copper-binding domains, and stronger conservation within genera than between them. The translation products of AbPPO1 and AbPPO2 possess putative N-glycosylation and phosphorylation sites and are recognised by antibodies directed against a 43-kDa tyrosinase. The observations are consistent with previously proposed maturation and activation models for plant and fungal tyrosinases.

Document Type: Article

Source: Scopus

De La Bastide, P.Y.^{a b}, Horgen, P.A.^a

Mitochondrial inheritance and the detection of non-parental mitochondrial DNA haplotypes in crosses of *Agaricus bisporus* homokaryons

(2003) *Fungal Genetics and Biology*, 38 (3), pp. 333-342. Cited 7 times.

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^b Department of Biology, University of Victoria, P.O. Box 3020, Victoria, BC V8W 3N5, Canada

Abstract

This study evaluates mtDNA transmission in *Agaricus bisporus*, as well as the occurrence of non-parental

haplotypes in heterokaryons produced by controlled crosses. Sixteen crosses were performed with blended liquid cultures, using different combinations of 13 homokaryotic strains. For each cross, different mtDNA haplotypes were present in each homokaryon. Heterokaryons generated from these crosses were subject to genetic analysis with RFLP markers to identify (i) karyotic status, (ii) mtDNA haplotype, and (iii) the occurrence of non-parental mtDNA haplotypes. These analyses generally supported the occurrence of uniparental mitochondrial (mt) inheritance in *A. bisporus*, with one mtDNA haplotype usually favoured in the new heterokaryon. The preponderance of one mtDNA haplotype in a new heterokaryon did not necessarily show a correlation with a greater mycelial growth rate for the parent homokaryon possessing that haplotype. Mixed mtDNA haplotypes and non-parental haplotypes were also identified in the heterokaryons from some crosses. Evidence for the occurrence of two mtDNA haplotypes in one heterokaryotic mycelium was observed in 8 of 16 crosses, suggesting the maintenance of true heteroplasmons after three successive subculturing steps. Non-parental mtDNA haplotypes were seen in heterokaryons produced from 7 of 16 crosses. The mating protocol described can be utilized to generate novel mtDNA haplotypes for strain improvement and the development of strain-specific markers. Mechanisms of mt selection and inheritance are discussed. © 2003 Elsevier Science (USA). All rights reserved.

Author Keywords

Agaricus; Biased inheritance; Heteroplasmon; Mitochondrial inheritance; Mitochondrial recombination

Document Type: Article

Source: Scopus

Callac, P.^a , De Haut, I.J.^a , Imbernon, M.^a , Guinberteau, J.^a , Desmerger, C.^b , Theochari, I.^c

A novel homothallic variety of *Agaricus bisporus* comprises rare tetrasporic isolates from Europe

(2003) *Mycologia*, 95 (2), pp. 222-231. Cited 9 times.

^a INRA, U. de Recherches sur les Champignons, BP 81, 33883 Villenave d'Ornon Cedex, France

^b Centre Technique du Champignon, Munet, 49400 Distré, France

^c NAGREF, Laboratory of Edible Fungi, 41110 Larissa, Greece

Abstract

Among 400 wild specimens of *A. bisporus* collected in Europe, only three were tetrasporic. In the case of two of them from France, a previous study showed that one was homokaryotic and hypothetically belonged to a homothallic entity while the other was heterokaryotic and possibly resulted from hybridization between a member of this entity and a classical bisporic strain. A third tetrasporic specimen recently was discovered in Greece. Morphological and genetic comparisons, using alloenzymatic markers, molecular markers and ITS polymorphisms, reveal that this third specimen is homokaryotic and belongs, with the homokaryotic specimen from France, to the same entity. Dissimilarity analysis confirms the hybrid origin of the heterokaryotic specimen. Varietal status is proposed for this homothallic, highly homogeneous entity, and *A. bisporus* var. *eurotetrasporus* is described. This novel variety clearly differs from var. *bisporus* by its tetrasporic basidia and from var. *burnettii* by its longer spores. It has a complex story because it can interbreed with var. *bisporus* and shares the same habitat; however, because

of its homothallic life cycle and its partial intersterility, it is probably in the process of speciation.

Author Keywords

Agaricus bisporus var. eurotetrasporus; Basidial spore number variation; Cultivated mushroom; Evolution; Homothallism; ITS polymorphism

Document Type: Article

Source: Scopus

Staniaszek, M.^{a d}, Marczewski, W.^b, Szudyga, K.^a,
Maszkiewicz, J.^a, Czaplicki, A.^c, Qian, G.^e

Genetic relationship between Polish and Chinese strains of the mushroom *Agaricus bisporus* (Lange) Sing., determined by the RAPD method

(2002) *Journal of Applied Genetics*, 43 (1), pp. 43-47.

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^b Plant Breed. Acclimatization Inst., Młochów, Poland

^c Plant Breed. Acclimatization Inst., Radzików, Poland

^d Res. Institute of Vegetable Crops, ul. Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

Abstract

The genetic relationship between twenty-six strains of *Agaricus bisporus* were analysed by the RAPD (random amplified polymorphic DNA) method. DNA amplification was performed with the use of twelve arbitrary 10-mer primers. Four primers, which gave polymorphic band patterns were chosen for RAPD analysis. In total, they gave 24 distinguishable bands, of which nine were polymorphic. The

conducted research showed that there is a great genetic similarity among the examined strains. Low polymorphism of the strains may be a proof of a limited genetic pool used in the cultivation of those strains.

Author Keywords

Genetic diversity; Mushroom strains; RAPD

Document Type: Article

Source: Scopus

Juarez del Carmen, S.^a , Largeteau-Mamoun, M.L.^a ,
Rousseau, T.^b , Regnault-Roger, C.^c , Savoie, J.-M.^a

**Genetic and physiological variation in isolates of
Verticillium fungicola causing dry bubble disease of the
cultivated button mushroom, Agaricus bisporus**
(2002) *Mycological Research*, 106 (10), pp. 1163-
1170. Cited 4 times.

^a Inst. Natl. de la Rech. Agronom., U. de Recherche sur les
Champignons, B.P. 81, F-33883 Villenave d'Ornon, France

^b Centre Technique du Champignon, 22 rue Bizard, F-49400
Distré, France

^c Universite Pau et Pays de l'Adour, Laboratoire d'Ecologie
Moleculaire, Avenue de l'Université, F-64000 Pau, France

Abstract

The objectives of this study were to examine genetic variation, physiological dissimilarities and diversity in pathogenicity between *Verticillium fungicola* var. *aleophilum* and var. *fungicola* and within a population of six *V. fungicola* var. *fungicola* strains responsible for dry bubble outbreaks on

mushroom farms. Genetic variability was investigated using random amplification of polymorphic DNA (RAPD). Mycelial growth rate, extracellular enzyme production and susceptibility to hydrogen peroxide were used to examine physiological dissimilarities. Variation in pathogenicity was studied both in vitro and during mushroom cultivation. All the physiological properties studied indicated that var. *aleophilum* isolates were potentially more efficient than var. *fungicola* isolates for rapid colonisation of the mushroom cultivation medium. They could then interact more efficiently with *Agaricus bisporus* to produce dry bubble disease. RAPD analysis confirmed that all the French isolates belonged to var. *fungicola*, and two isolates were distinguishable from the homogeneous group constituted by the others. These isolates had a higher mycelial growth rate and lower extracellular enzyme activities in liquid media, except for chitinases. Their spores were more susceptible to germination inhibition by hydrogen peroxide, and they were responsible for higher levels of affected mushrooms. The two varieties might be regarded as pathotypes that are geographically isolated, and variation in isolates of var. *fungicola* might have consequence for mushroom growers.

Document Type: Article

Source: Scopus

Xu, J., Desmerger, C., Callac, P.

Fine-scale genetic analyses reveal unexpected spatial-temporal heterogeneity in two natural populations of the commercial mushroom *Agaricus bisporus*

(2002) *Microbiology*, 148 (5), pp. 1253-1262. Cited 7 times.

Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ont. L8S 4K1, Canada

Abstract

This study examined the fine-scale genetic variation of the commercial mushroom, *Agaricus bisporus*, over 2 years at two sites in France. One site was a meadow fertilized with horse manure and disturbed regularly by humans; the other was a Monterey cypress forest free of human disturbance. Altogether, 50 mushrooms were collected and analysed for mitochondrial and nuclear genetic variation marked by RFLPs and multilocus enzyme electrophoretic polymorphisms. Population samples from these two sites were genetically different and both sites contained high levels of genetic diversity. No identical genotypes were found at either site between the 2 years and there was little evidence for extensive vegetative clonality for this species. Contrary to expectations, very limited evidence of pseudohomothallic reproduction was found. Results from tests of Hardy-Weinberg equilibrium and genotypic equilibrium showed that outcrossing and recombination have played significant roles in both populations. The results demonstrated spatial-temporal genetic heterogeneity of *A. bisporus* in natural populations.

Author Keywords

Fungi; Modes of reproduction; Molecular markers; Population structure

Document Type: Article

Source: Scopus

Challen, M.P., Zhang, C., Elliott, T.J.

***Agaricus bisporus* and *Coprinus bilanatus* TRP2 genes are tri-functional with conserved intron and domain organisations**

(2002) *FEMS Microbiology Letters*, 208 (2), pp. 269-

274. Cited 4 times.

Horticulture Research International, Wellesbourne
Warwickshire CV35 9EF, United Kingdom

Abstract

Cloned homobasidiomycete TRP2 genes for *Agaricus bisporus* and *Coprinus bilanatus* were sequence-characterised. Both genes encode tri-functional proteins with activity domains for glutamine amidotransferase (GAT; G domain), indole glycerol phosphate synthase (InGP; C domain) and phosphoribosyl anthranilate isomerase (F domain). A conserved intron disrupts the GAT-coding sequence in both genes. Consensus amino acid (aa) signatures were identified for GAT and InGP, but in the latter 15-aa signature, one residue did not fit the previously defined consensus. Protein architecture and parsimony analysis with analogous proteins indicate domain organisation (NH₂-G-C-F-COOH) was as for other filamentous fungi. The data do not support earlier suggestions that the three activity domains are detached in *A. bisporus*. © 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Author Keywords

Agaricus bisporus; *Coprinus bilanatus*; Tryptophan biosynthesis

Document Type: Article

Source: Scopus

Diamantopoulou, P., Philippoussis, A.

Production attributes of *Agaricus bisporus* white and off-white strains and the effect of calcium chloride irrigation on productivity and quality

(2001) *Scientia Horticulturae*, 91 (3-4), pp. 379-391. Cited 3 times.

Natl. Agricultural Res. Foundation, Inst. of Agricultural Engineering, Edible Fungi Research Laboratory, 61 Demokratias Street, 13561 Ag. Anargyri, Athens, Greece

Abstract

Five *Agaricus bisporus* commercial cultivars (one off-white, two white, and two pure-white) were evaluated as regards their colonization rates, production characters, and quality attributes. Although the mycelia growth rates on sterile compost did not differ significantly, an important difference was detected between the lowest (off white strain) and the highest (white strain) yield value. Average mushroom weight showed the tendency to decrease as yield increased. Irrigation of the white strain 203 (selected among tested strains for its good productivity and quality) with 0.05-0.25% (w/v) calcium chloride did not result in reduced yields, on the contrary increased yields were calculated for 0.10 and 0.15% treatments. Average mushroom weight was positively affected, while firmer mushrooms were produced when crops were treated with 0.05 and 0.10% calcium chloride. Also, color improvement was detected at the dosage of 0.25% calcium chloride. © 2001 Elsevier Science B.V. All rights reserved.

Author Keywords

Calcium chloride irrigation; Color; Firmness; Mushroom; Productivity

Document Type: Article

Source: Scopus

Godfrey Crop, S.A.C.^{a c}, Marshall, J.W.^b, Klena, J.D.^a
Genetic characterization of *Pseudomonas* 'NZI7' - A novel pathogen that results in a brown blotch disease of *Agaricus bisporus*

(2001) *Journal of Applied Microbiology*, 91 (3), pp. 412-420. Cited 8 times.

^a University of Canterbury, Christchurch, New Zealand

^b New Zealand Institute of Crop and Food Research Ltd., Christchurch, New Zealand

^c New Zealand Institute of Crop and Food Research Ltd., Private Bag 4704, Christchurch, New Zealand

Abstract

Aims: To characterize a novel pseudomonad isolate capable of causing brown blotch disease of *Agaricus bisporus*. **Methods and Results:** Using the white-line-in-agar (WLA) assay, fluorescent pseudomonads isolated from a New Zealand mushroom farm were screened for the lipodepsipeptide tolaasin, a characteristic marker of *Pseudomonas tolaasii*. One isolate, NZI7, produced a positive WLA assay and caused brown lesions of *A. bisporus* comparable with those produced by *Ps. tolaasii*. However, genetic analysis suggested that *Ps. tolaasii* and NZI7 were genetically dissimilar, and that NZI7 is closely related to *Pseudomonas syringae*. Nucleotide sequence analyses of a gene involved in tolaasin production indicated that similar genes are present in both NZI7 and *Ps. tolaasii*. **Conclusions:** NZI7 represents a novel *Pseudomonas* species capable of causing brown blotch disease of *A. bisporus*. **Significance and Impact of the Study:** Phenotypic identification of *Ps. tolaasii* based on *A. bisporus* browning and positive WLA may have limited specificity.

Document Type: Article

Source: Scopus

Kingsnorth, C.S., Eastwood, D.C., Burton, K.S.

Cloning and postharvest expression of serine proteinase transcripts in the cultivated mushroom *Agaricus bisporus*

(2001) *Fungal Genetics and Biology*, 32 (3), pp. 135-144. Cited 12 times.

Department of Plant Pathology and Microbiology, Horticulture Research International, Wellesbourne, Warwickshire, CV35 9EF, United Kingdom

Abstract

Increases in both the levels and the activity of serine proteinase have been previously described in the senescing mushroom *Agaricus bisporus*. cDNA encoding serine proteinase was amplified by reverse transcriptase-polymerase chain reaction using a degenerate primer based on the N-terminal sequence of a previously isolated *A. bisporus* serine proteinase and then cloned. The cDNA was sequenced and shown to be homologous to those of other fungal serine proteinases. Northern analysis showed that this serine proteinase gene (Spr1) was not expressed in freshly harvested sporophores but was strongly up-regulated postharvest and found almost entirely in the stipe of the sporophore (approximately 0.08% of mRNAs 2 days after harvest). Low-level expression was detectable in the flesh (pileus trama) and gill (lamellae) tissues of the cap, but none was detected in the skin (pilei pellis). In three of the cloned cDNAs, sequence analysis showed that the poly(A) tail starts at different positions. Expression of Spr1 in *Escherichia coli* caused restricted colony growth. © 2001 Academic Press.

Author Keywords

Agaricus bisporus; cDNA; Gene expression; Polyadenylation; Proteolysis; RT-PCR; Senescence; Serine proteinase; Sporophore

Document Type: Article

Source: Scopus

Moore, A.J.^a, Challen, M.P.^a, Warner, P.J.^b, Elliott, T.J.^a

RAPD discrimination of Agaricus bisporus mushroom cultivars

(2001) *Applied Microbiology and Biotechnology*, 55 (6), pp. 742-749. Cited 14 times.

^a Horticulture Research International, Wellesbourne, Warwickshire, CV35 9EF, United Kingdom

^b Cranfield University, Silsoe, Bedfordshire, MK45 4DT, United Kingdom

Abstract

Cultivars of the white button mushroom *Agaricus bisporus* are difficult to differentiate, which has made strain protection problematic for this crop species. We have used RAPDs to discriminate between 26 strains of *A. bisporus*, 24 of which were commercial cultivars, and to characterise the genetic relatedness of these strains. Using 20 primers, 211 RAPD markers were identified and used in hierarchical cluster, patristic distance and parsimony analyses. All strains could be differentiated using the aggregated primer data. Although no one primer could differentiate all 26 strains, several individual primers yielded unique fingerprints for a variety of strains. The greatest differences (up to 28% variation) were observed

in comparisons with or between two wild collections of *A. bisporus*. Quondam cultivars, commercial brown and off-white varieties proved more variable than the widely grown 'hybrid' types. Of the 15 hybrid varieties analysed, only one differed substantially (20% or more variable). The patristic and parsimony analyses both demonstrated the gross similarity of the hybrids, many of which appear to be essentially derived varieties from two original hybrid cultivars. RAPD analyses can assist mushroom strain identification and could play a role in the protection of novel cultivars.

Document Type: Article

Source: Scopus

Tang, C.M.^a, Waterman, L.D.^{a c}, Smith, M.H.^{a d}, Thurston, C.F.^{a b}

The *cel4* Gene of *Agaricus bisporus* Encodes a β -Mannanase

(2001) *Applied and Environmental*

Microbiology, 67 (5), pp. 2298-2303. Cited 10 times.

^a Microbiology Section, Division of Life Sciences, King's College, London, London SE1 8WA, United Kingdom

^b Division of Life Sciences, King's College, London, 150 Stamford St., London SE1 8WA, United Kingdom

^c Biology Department, University of the West Indies, Cave Hill Campus, Bridgetown, Barbados

^d Pub. Hlth. Lab. and Med. Microbiol., Public Health Laboratory Service, King's College Hospital (Dulwich), East Dulwich Grove, London SE22 8QF, United Kingdom

Abstract

Mannases have industrial uses in food and pulp industries, and their regulation may influence development of the mushrooms of commercially important basidiomycetes. We expressed an *Agaricus bisporus* cel4 cDNA, which encodes a mannanase, in *Saccharomyces cerevisiae* and *Pichia pastoris*. CEL4 had no detectable activity on cellulose or xylan. This gene is the first isolated from this economically important fungus to encode a mannanase. *P. pastoris* secreted about three times more CEL4 than *S. cerevisiae*. The removal of the cellulose-binding domain of CEL4 lowered the secreted specific activity by *P. pastoris* by approximately 97%. The genomic sequence of cel4 was isolated by screening a cosmid library of *A. bisporus* C54-carb8. The open reading frame was interrupted by 12 introns. The level of extracellular CEL4 increases dramatically at the postharvest stage in compost extracts of *A. bisporus* fruiting cultures. In laboratory liquid cultures of *A. bisporus*, the activity of CEL4 detected in the culture filtrate reached a maximum after 21 days. The levels of CEL4 broadly mirrored the levels of enzyme activity. In the Solka floc-bound mycelium, CEL4 protein showed a maximum after 2 to 3 weeks of culture and then declined. Changes in CEL4 activity during fruiting-body development suggest that hemicellulose utilization plays an important role in sporophore formation. The availability of the cloned gene will further studies of compost decomposition and the extracellular enzymes that fungi deploy in this process.

Document Type: Article

Source: Scopus

Ramírez, L.^a , Muez, V.^{a b} , Alfonso, M.^a , García

Barrenechea, A.^a , Alfonso, L.^a , Pisabarro, A.G.^a

Use of molecular markers to differentiate between

commercial strains of the button mushroom *Agaricus bisporus*

(2001) *FEMS Microbiology Letters*, 198 (1), pp. 45-48. Cited 8 times.

^a Depto. de Producción Agraria, Univ. Pub. de Navarra, 31006, Pamplona, Spain

^b Gurelan, Huarte-Pamplona, Spain

Abstract

Agaricus bisporus is an edible basidiomycete cultivated industrially for food production. Different spawn and mushroom producers use genetically related *A. bisporus* strains frequently marketed as different products. In this paper we show that the use of suitable molecular markers reveals the high level of genetic homology of commercial strains of *A. bisporus*, and allows, at the same time, to distinguish between them. In the course of this work, a molecular marker potentially linked to the agronomic character 'mushroom weight' has been identified by bulked segregant analysis. © 2001 Federation of European Microbiological Societies.

Author Keywords

Agaricus bisporus; Bulked segregant analysis; Button mushroom; Molecular marker; Strain typing

Document Type: Article

Source: Scopus

Mikosch, T.S.P., Lavrijssen, B., Sonnenberg, A.S.M., van Griensven, L.J.L.D.

Transformation of the cultivated mushroom *Agaricus*

bisporus (Lange) using T-DNA from Agrobacterium tumefaciens

(2001) *Current Genetics*, 39 (1), pp. 35-39. Cited 27 times.

Department of Genetics and Breeding, Mushroom
Experimental Station, P.O. Box 6042, 5960 AA Horst,
Netherlands

Abstract

Agrobacterium tumefaciens is known to transfer parts of its tumor-inducing plasmid, the T-DNA, to plants, yeasts and filamentous fungi. We have used this system to transform germinating basidiospores and vegetative mycelium of a commercial strain of the cultivated basidiomycete *Agaricus bisporus*. Analysis of transformants shows that the T-DNA integrates at random sites into the host genome and that the selection marker is stable during mitosis and meiosis. The *Agrobacterium* system allows the transformation of both homokaryons and heterokaryons of *A. bisporus*. Also, both karyotypes of an heterokaryon can be transformed simultaneously. Furthermore, this is the first report on the transformation of vegetative mycelium of a commercial strain of *A. bisporus*.

Author Keywords

Agaricus; Fungi; Hygromycin B; T-DNA

Document Type: Article

Source: Scopus

Eastwood, D.C., Kingsnorth, C.S., Jones, H.E., Burton, K.S.
**Genes with increased transcript levels following
harvest of the sporophore of *Agaricus bisporus* have
multiple physiological roles**

(2001) *Mycological Research*, 105 (10), pp. 1223-1230. Cited 13 times.

Horticulture Research International, Wellesbourne,
Warwickshire CV35 9EF, United Kingdom

Abstract

We screened a cDNA library generated from harvested and stored sporophores of *Agaricus bisporus* and identified 19 genes with higher transcript levels than at the time of harvest. Five of these genes had no detectable mRNA levels prior to detachment from the mycelium. Sequence analysis of ten clones revealed significant similarities to known genes, these code for proteins involved in polymer breakdown and metabolism, cell wall synthesis, stress tolerance, cytochrome P450 activity and DNA binding. The diversity of functions of these genes suggests the changes in the sporophore after harvest involve several different physiological processes.

Document Type: Article

Source: Scopus

Collopy, P.D., Largeau-Mamoun, M.L., Romaine, C.P.,
Royse, D.J.

Molecular phylogenetic analyses of *Verticillium fungicola* and related species causing dry bubble disease of the cultivated button mushroom, *agaricus bisporus*

(2001) *Phytopathology*, 91 (9), pp. 905-912. Cited 12 times.

Department of Plant Pathology, Pennsylvania State University,
University Park, PA 16802, United States

Abstract

Molecular phylogenetic analyses were performed on 40 isolates of *Verticillium fungicola* collected from various Pennsylvania mushroom farms in 1999 and 28 isolates of *Verticillium* spp. collected during the last 50 years from various geographic locations. Sequence analysis of internal transcribed spacers 1 and 2 (ITS1 and ITS2) and 5.8S regions of the nuclear ribosomal DNA (rDNA) transcriptional unit and analysis of random amplified polymorphic DNA (RAPD) data were performed for the 68 isolates of *Verticillium* spp. Identical rDNA sequences were obtained for all 40 Pennsylvania isolates collected during 1999, 13 North American isolates collected during the last 50 years, and the ex-type strain of *V. fungicola* var. *aleophilum*. Sequence analysis of European isolates revealed a close relationship to the ex-type strain *V. fungicola* var. *fungicola*. No European-like isolates of *V. fungicola* var. *fungicola* were detected in the collection of North American isolates examined. Results from six decamer RAPD primers strongly indicate the presence of a clonal population of *V. fungicola* among Pennsylvania isolates. In addition, RAPD data delineated a Korean isolate (DC130) and ex-type strain *V. fungicola* var. *aleophilum* from the North American group. Virulence assays, based on spore inoculation of mushroom *pilei*, revealed variation corresponding to each neighbor-joining and RAPD grouping. All isolates with rDNA sequence and RAPD grouping similarity to ex-type strains *V. fungicola* var. *aleophilum* and *V. fungicola* var. *fungicola* displayed the highest level of virulence. Based on rDNA sequence and RAPD analyses, isolates displaying reduced or no virulence were distantly related to these two varieties. All results obtained for the analyses of ex-type strain *U. fungicola* var. *flavidum* suggested that this fungal isolate should not be considered a variety of *V. fungicola*, but rather a distinct species.

Author Keywords

Acremonium strictum; Helicoon sessile; Nectria inventa;
Orbilia luteorubella; Tolypocladium parasiticum; V. tenerum

Document Type: Article

Source: Scopus

Barroso, G.^a, Sonnenberg, A.S.M.^b, Van Griensven,
L.J.L.D.^b, Labarère, J.^a

**Molecular cloning of a widely distributed microsatellite
core sequence from the cultivated mushroom *Agaricus
bisporus***

(2000) *Fungal Genetics and Biology*, 31 (2), pp. 115-
123. Cited 5 times.

^a Laboratory of Molecular Genetics and Breeding of Cultivated
Mushrooms, INRA, University Victor Segalen Bordeaux 2, B.P.
81, 33883, Villenave d'Ornon Cedex, France

^b Mushroom Experimental Station, P.O. Box 6042, 5960 AA,
Horst, Netherlands

Abstract

An *Agaricus bisporus* microsatellite with the tetranucleotide
motif TATG tandemly repeated was isolated from an *A.*
bisporus library enriched in repeated sequences. The use of
the 16-mer oligonucleotide (TATG)₄ indicates that many loci
contain nearby copies of the microsatellite in opposite
orientations. The wide distribution of the microsatellite in the
A. bisporus genome was assessed (i) by polyacrylamide gel
electrophoresis of the products generated by directed
amplification of microsatellite-region DNA (DAMD) and (ii) by
hybridization of these products with *A. bisporus* chromosomes
separated by pulsed-field gel electrophoresis. This is, to our

knowledge, the first microsatellite reported in the cultivated edible mushrooms. DAMD-PCR products were generated using DNA of three *Pleurotus* species (*P. pulmonarius*, *P. sajor-caju*, and *P. florida*), indicating that (TATG)₄ repeats are also present in these cultivated species. The variability found within closely related strains indicates that such microsatellites are useful in fingerprinting and studying genetic variability in wild and commercial mushrooms. © 2000 Academic Press.

Author Keywords

Agaricus bisporus; Basidiomycota; Directed amplification of microsatellite-region DNA (DAMD); Microsatellite; Mushroom; *Pleurotus*

Document Type: Article

Source: Scopus

Inglis, P.W., Peberdy, J.F., Sockett, R.E.

Cloning of a chitinase gene from *Ewingella americana*, a pathogen of the cultivated mushroom, *Agaricus bisporus*

(2000) *Genetics and Molecular Biology*, 23 (3), pp. 685-688. Cited 1 time.

Lab. de Gen. Mol. de Microrganismos, EMBRAPA Recursos Gen. e Biotecnol., Caixa Postal 02372, 70849-970 Brasília, DF, Brazil

Abstract

We have isolated a gene encoding a chitinase (EC 3.2.1.14) from *Ewingella americana*, a recently described pathogen of the mushroom *Agaricus bisporus*. This gene, designated chiA (EMBL/Genbank/DDBJ accession number X90562), was

cloned by expression screening of a plasmid-based *E. americana* HindIII genomic library in *Escherichia coli* using remazol brilliant violet-stained carboxymethylated chitin incorporated into selective medium. The *chiA* gene has a 918-bp ORF, terminated by a TAA codon, with a calculated polypeptide size of 33.2 kDa, likely corresponding to a previously purified and characterised 33-kDa endochitinase from *E. americana*. The deduced amino acid sequence shares 33% identity with chitinase II from *Aeromonas* sp. No. 10S-24 and 7.8% identity with a chitinase from *Saccharopolyspora erythraeus*. Homology to other chitinase sequences was otherwise low. The peptide sequence deduced from *chiA* lacks a typical N-terminal signal sequence and also lacks the chitin binding and type III fibronectin homology units common to many bacterial chitinases. The possibility that this chitinase is not primarily adapted for the environmental mineralisation of pre-formed chitin, but rather for the breakdown of nascent chitin, is discussed in the context of mushroom disease.

Document Type: Article

Source: Scopus

Sreenivasaprasad, S., Burton, K.S., Wood, D.A.

Cloning and characterisation of a chitin synthase gene cDNA from the cultivated mushroom *Agaricus bisporus* and its expression during morphogenesis¹

(2000) *FEMS Microbiology Letters*, 189 (1), pp. 73-77. Cited 6 times.

Dept. Plant Pathol. and Microbiol., Hort. Res. Intl.,
Wellesbourne, C., Warwick, United Kingdom

Abstract

Full-length cDNA of a chitin synthase gene (*chs1*) was cloned

from *Agaricus bisporus* by screening a cDNA library with a PCR amplified fragment of the chitin synthase gene. The chs1 contains an open reading frame of 2727 bp encoding a polypeptide of 909 amino acids and deduced molecular mass 102.3 kDa and pI 8.23. The central region of chs1 showed strong homology to other fungal chitin synthase genes with seven conserved domains. It belongs to the chitin synthase class III, analogous to chsB from *Aspergillus nidulans*, chsC and chsG from *A. fumigatus* and chs-1 from *Neurospora crassa*. It appears to be fruit body induced as the transcripts were higher in the developing mushroom compared to any mycelial stage. Copyright (C) 2000 Federation of European Microbiological Societies.

Author Keywords

Agaricus bisporus; Chitin synthase gene; Cloning; Expression; Morphogenesis

Document Type: Article

Source: Scopus

Chen, X., Stone, M., Schlagnhauer, C., Romaine, C.P.

**A fruiting body tissue method for efficient
Agrobacterium-mediated transformation of *Agaricus bisporus***

(2000) *Applied and Environmental*

Microbiology, 66 (10), pp. 4510-4513. Cited 62 times.

Department of Plant Pathology, 209 Buckhout Laboratory,
Pennsylvania State University, University Park, PA 16802,
United States

Abstract

We describe a modified *Agrobacterium*-mediated method for

the efficient transformation of *Agaricus bisporus*. Salient features of this procedure include cocultivation of *Agrobacterium* and fruiting body gill tissue and use of a vector with a homologous promoter. This method offers new prospects for the genetic manipulation of this commercially important mushroom species.

Document Type: Article

Source: Scopus

Mendoza, C.G.

Some structural and functional aspects on *Agaricus bisporus* cell wall and their more immediate applications [Algunos aspectos estructurales y funcionales de la pared celular de *Agaricus bisporus* y sus aplicaciones mas inmediatas]

(2000) *Anales de la Real Academia de Farmacia*, 66 (1), pp. 5-22.

Ctro. de Invest. Biológicas, CSIC, Madrid

Abstract

After an introduction describing the scientific experience of the author, before her presentation in this Academy, different aspects on the cell wall structure and function of *Agaricus bisporus* were approached. This fungus, being a higher Basidiomycete cultivated for human nutrition, is better known as the common mushroom. The importance of some structural components of this outer cellular envelope is stressed for the legal protection of those strains with industrial interest, and as a barrier to be overcome before the genetic improvement, as well as its structural and functional role in the verticillium disease control which is the most injurious plague of the commercial mushroom cultures.

Author Keywords

Agaricus bisporus; Biochemical markers; Cell wall; Genetic improvement; Industrial Culture; Verticillium disease

Document Type: Article

Source: Scopus

Umar, M.H., Van Griensven, L.J.L.D.

**Studies on the morphogenesis of Agaricus bisporus:
The dilemma of normal versus abnormal fruit body
development**

(1999) *Mycological Research*, 103 (10), pp. 1235-1244. Cited 4 times.

Mushroom Experimental Station, P.O. Box 6042, 5960 AA
Horst, Netherlands

Abstract

Development of mushrooms is driven by genetic and epigenetic factors in a continuous interaction with the environment. It is assumed that each successive stage of morphogenesis depends on specific sets of signals arising at the appropriate time and place during the growth process. Morphogenetic dynamism proceeds in a time dimension through a cascade of signal-effect associations. Developmental errors may occur when such signals originate in the wrong place and/or at the wrong time. As a result various abnormalities such as ectopic tissues can develop and morphogenesis can be severely disturbed. Both endogenous genetic disturbances and exogenous factors can cause developmental errors. Lamellar dysplasia, which is a pore-like proliferation of the gills, forms an example; it may be induced experimentally. Both lamellar dysplasia and rosecomb disease

of *Agaricus bisporus* result from endogenous genetic instability, whereas the developmental errors observed in wet bubble disease, which is caused by the infection of *Mycogone perniciosus*, originate from an exogenous factor.

Morphogenesis normally leads to a symmetrical form of primordia of *A. bisporus*. Asymmetry is very frequently associated with an underlying pathological situation. Defining exact criteria of and sharp borderlines between normal and abnormal development seems infeasible. Fungi may readily tolerate morphogenetic imprecision. In this report, various macro- and microscopic features of normal and pathological development are illustrated; the dilemma of normal versus abnormal fruit body development has been discussed.

Document Type: Article

Source: Scopus

Moquet, F.^{a b}, Desmerger, C.^b, Mamoun, M.^a, Ramos-Guedes-Lafargue, M.^b, Olivier, J.-M.^a

A quantitative trait locus of *Agaricus bisporus* resistance to *Pseudomonas tolaasii* is closely linked to natural cap color

(1999) *Fungal Genetics and Biology*, 28 (1), pp. 34-42. Cited 8 times.

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^b Centre Technique du Champignon, 22 rue Bizard Munet, 49400, Distre, France

Abstract

A quantitative trait locus (QTL) of resistance to *Pseudomonas tolaasii* was detected in *Agaricus bisporus* using a cross between a wild strain from the Sonoran desert and a cultivated strain. The resistance QTL was strongly linked with the brown color allele of PPC1. This QTL explained about 30% of the variation observed for living bacteria-induced symptoms. The use of bacterial toxin did not reproduce living bacteria symptoms but revealed the same QTL. The latter QTL was not affected by environmental variation. No relation was found between the resistance QTL and the tyrosinase gene, which is involved in the browning process.

Author Keywords

Agaricus bisporus; Bacterial blotch; Cap color; *Pseudomonas tolaasii*; Quantitative trait locus

Document Type: Article

Source: Scopus

Friel, M.T., McLoughlin, A.J.

Immobilisation as a strategy to increase the ecological competence of liquid cultures of *Agaricus bisporus* in pasteurised compost

(1999) *FEMS Microbiology Ecology*, 30 (1), pp. 39-46. Cited 1 time.

Dept. of Industrial Microbiology, Univ. College Dublin, Belfield, 4, Dublin, Ireland

Abstract

This study investigated the applicability of an immobilisation technique based on cell entrapment in a polymer gel (sodium alginate) as a strategy to increase the ecological competence of liquid cultures (liquid spawn) of *Agaricus bisporus* in

pasteurised compost. This delivery system had a shorter adaptation (lag) period and a higher growth rate in pasteurised compost than both liquid spawn and the conventional grain spawn. The ecological competence of this delivery system was attributed to the high biomass loading capacity of these beads, mycelial protection in the bead microenvironment and the spatial distribution of the beads in the macroenvironment. Exploitation of this delivery system depends on both commercial product parameters and on the genetic stability of the inoculum. Copyright (C) 1999 Federation of European Microbiological Societies.

Author Keywords

Agaricus bisporus; Alginate; Ecological competence; Immobilisation; Microenvironmental control; Spatial distribution

Document Type: Article

Source: Scopus

Chen, X.^a, Romaine, C.P.^{a b}, Tan, Q.^{a c}, Schlagnhauser, B.^{a d}, Ospina-Giraldo, M.D.^a, Royse, D.J.^a, Huff, D.R.^{a e}

PCR-based genotyping of epidemic and preepidemic Trichoderma isolates associated with green mold of Agaricus bisporus

(1999) *Applied and Environmental*

Microbiology, 65 (6), pp. 2674-2678. Cited 12 times.

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Abstract

We used randomly amplified polymorphic DNA (RAPD)-PCR to estimate genetic variation among isolates of *Trichoderma* associated with green mold on the cultivated mushroom *Agaricus bisporus*. Of 83 isolates examined, 66 were sampled during the recent green mold epidemic, while the remaining 17 isolates were collected just prior to the epidemic and date back to the 1950s. *Trichoderma harzianum* biotype 4 was identified by RAPD analysis as the cause of almost 90% of the epidemic-related episodes of green mold occurring in the major commercial mushroom-growing region in North America. Biotype 4 was more closely allied to *T. harzianum* biotype 2, the predominant pathogenic genotype in Europe, than to the less pathogenic biotype 1 and *Trichoderma atroviride* (formerly *T. harzianum* biotype 3). No variation in the RAPD patterns was observed among the isolates within biotype 2 or 4, suggesting that the two pathogenic biotypes were populations containing single clones. Considerable genetic variation, however, was noted among isolates of biotype 1 and *T. atroviride* from Europe. Biotype 4 was not represented by the preepidemic isolates of *Trichoderma* as determined by RAPD markers and PCR amplification of an arbitrary DNA sequence unique to the genomes of biotypes 2 and 4. Our findings suggest that the onset of the green mold epidemic in North America resulted from the recent introduction of a highly virulent genotype of the pathogen into

cultivated mushrooms.

Document Type: Article

Source: Scopus

De Groot, P.W.J.^{a c}, Roeven, R.T.P.^b, Van Griensven, L.J.L.D.^b, Visser, J.^a, Schaap, P.J.^a

Different temporal and spatial expression of two hydrophobin-encoding genes of the edible mushroom *Agaricus bisporus*

(1999) *Microbiology*, 145 (5), pp. 1105-1113. Cited 18 times.

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^c Institute for Molecular Cell Biology, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, Netherlands

Abstract

In a search for genes that are only expressed in fruit bodies of the basidiomycete *Agaricus bisporus*, two cDNAs, hypA and hypB that encode hydrophobins have been isolated previously. In this study, the structure of hypB is resolved and it is shown that the two genes are differentially expressed, indicating that the encoded hydrophobins serve different functions in *A. bisporus* mushrooms. hypB encodes a polypeptide (HYPB) of 119 aa that shows little sequence identity with HYPA apart from the characteristic arrangement of eight cysteines found exclusively in hydrophobins. The temporal and spatial expression of the two hydrophobin-

encoding genes during fruit body development was compared using Northern analysis and in situ hybridization. Accumulation of hypA mRNA was found in tissue fractions consisting of undifferentiated white hyphae. In situ hybridization showed that the highest hypA mRNA levels are not found in the outermost cell layers of the pileipellis but in the cell layers adjacent to that. The highest level of expression of hypB occurs early in development when the primordium differentiates into densely packed, randomly oriented cap hyphae and loosely packed, vertically oriented stipe hyphae. In mature mushrooms, a strong accumulation of hypB transcripts was found only in the transitional zone between cap and stipe tissue, demonstrating that transcription regulation of hypB is clearly distinct from hypA.

Author Keywords

Agaricus bisporus; Fruit body development; Hydrophobin; hypA; hypB

Document Type: Article

Source: Scopus

Stoop, J.M.H.^{a b}, Mooibroek, H.^a

Advances in genetic analysis and biotechnology of the cultivated button mushroom, Agaricus bisporus

(1999) *Applied Microbiology and Biotechnology*, 52 (4), pp. 474-483. Cited 7 times.

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Abstract

During the last decade several major breakthroughs have been achieved in mushroom biotechnology, which greatly enhanced classical mushroom breeding. DNA-based technologies such as restriction fragment length polymorphisms and randomly amplified polydisperse DNA sequences have allowed for a measure of genetic diversity, for the isolation of homokaryons, for the determination of inheritance of nuclear and mitochondrial markers, and for the production of a genetic linkage map. The recent availability of ready-to-use and affordable DNA technologies has resulted in a substantial increase in the number of *Agaricus bisporus* genes that have been identified and characterized. A major breakthrough was achieved in 1996 when the first successful and stable transformation system of *A. bisporus* was reported. Together, the availability of an increasing number of known genes and the possibility to produce transgenic mushrooms will result in a better understanding of the molecular, physiological and biochemical processes that are essential for mushroom production, shelf life and quality aspects such as flavor, texture and disease resistance. Some potential targets for strain improvement are discussed, such as the genes involved in brown discoloration, substrate utilization, carbon and nitrogen metabolism, and fruit body development.

Document Type: Short Survey

Source: Scopus

Jolivet, S.^a, Arpin, N.^a, Wichers, H.J.^b, Pellon, G.^c

***Agaricus bisporus* browning: A review**

(1998) *Mycological Research*, 102 (12), pp. 1459-

1483. Cited 24 times.

^a Laboratoire de Mycochimie, U. Form. de Rech. de Chim.-Biochim., Univ. Claude Bernard-Lyon I, 43, boulevard du 11 novembre 1918, 69622 Villeurbanne Cedex, France

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Abstract

Agaricus bisporus browning is a common and economically detrimental phenomenon, in which melanogenic phenols are enzymically processed into quinones, which evolve eventually to melanins. This review deals with the two fundamental sides of this process, enzyme(s) and phenolic substrates.

Mushroom tyrosinase, the main polyphenol oxidase encountered in the *A. bisporus* sporophore, is treated in the first part. Its overall molecular architecture, isoforms, primary sequence and genetic background are considered. The presentation of tyrosinase catalytic features, including enzyme assays, kinetic properties, substrates and inhibitors, is followed by a comprehensive description of the active site and reaction mechanisms. Because of their relevance for studies of mushroom browning during development and post-harvest storage, the occurrence and properties of latent enzyme forms, as well as the location of tyrosinase and variations of its activity during the *A. bisporus* life cycle, are also reviewed. The second part deals with the substrates, particularly γ -L-glutaminy-4-hydroxybenzene (GHB) and its derivatives. Main data concerning the nature, obtention (by extraction or synthesis), spectrometric and chromatographic characteristics, chemical stabilities and biological properties of these typical Agaricaceae compounds are presented. Their distribution and levels according to the strains and flushes are described, as well as their variations during storage. Thirdly,

the relationship between browning and the natural or pathogenic discolouration intensity is developed.

Document Type: Review

Source: Scopus

De Groot, P.W.J.^a , Visser, J.^a , Van Griensven, L.J.L.D.^b , Schaap, P.J.^a

Biochemical and molecular aspects of growth and fruiting of the edible mushroom *Agaricus bisporus*

(1998) *Mycological Research*, 102 (11), pp. 1297-1308. Cited 8 times.

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^b Mushroom Experimental Station, P.O. box 6042, NL-5960 AA, Horst, Netherlands

Abstract

The introduction of recombinant DNA technology in the field of mushroom research has resulted in the cloning and characterization of a large number of genes. In order to study the genetics of compost colonization of *A. bisporus*, genes encoding enzymes involved in utilization of this substrate have been isolated. In addition, a number of genes which are induced in fruit bodies during fruit body development have been cloned and they will provide more insight in the genetics of this economically important aspect of the life cycle. Other genes that were cloned encode proteins of basic biochemical routes. They provide knowledge on the importance and regulation of these routes in the life cycle of *A. bisporus* and

add to knowledge on the general architecture of *A. bisporus* genes. Here we present an overview of the currently available biochemical and molecular data of *A. bisporus* and we discuss the importance of the available genes as genetic markers for breeding purposes.

Document Type: Article

Source: Scopus

Moquet, F.^a, Guedes-Lafargue, M.R.^b, Mamoun, M.^a,
Olivier, J.-M.^a

Selfreproduction induced variability in agronomic traits for a wild *Agaricus bisporus*

(1998) *Mycologia*, 90 (5), pp. 806-812. Cited 1 time.

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Abstract

Consequences of selfreproduction (intramictic reproduction) were studied on a wild *Agaricus bisporus* var. *bisporus* strain. Individual variations were observed on several agronomic traits, natural cap color, susceptibility to bacterial blotch, mycelial growth and yield, for each of the two studied intramictic generations. RAPD markers showed a high level of recombination between intramictic generations. With all traits, the same level of transgression was obtained at each generation, with the exception of mycelial growth which dramatically increased in the first generation. Bacterial blotch susceptibility decreased, reaching a threshold, therefore

resistance was not reached. Variability induced by intramictic reproduction in *A. bisporus* could be integrated favorably in breeding programs.

Author Keywords

Bacterial blotch; Color; Intramictic reproduction; Recombination

Document Type: Article

Source: Scopus

Lugones, L.G., Wösten, H.A.B., Wessels, J.G.H.

A hydrophobin (ABH3) specifically secreted by vegetatively growing hyphae of *Agaricus bisporus* (common white button mushroom)

(1998) *Microbiology*, 144 (8), pp. 2345-2353. Cited 57 times.

Molecular Plant Biology Laboratory, Groningen Biomol. Sci. Biotech. I., University of Groningen, Kerklaan 30, 9751 NN Haren, Netherlands

Abstract

Aerial mycelium and hyphal strands of *Agaricus bisporus*, strain U1, exhibited a rodlet pattern at their surfaces characteristic for assembled class I hydrophobins. An SDS-insoluble/trifluoroacetic-acid-soluble fraction from strands was found to contain one abundant protein with an apparent molecular mass on gel of 19 kDa. Two sequences for this protein (ABH3), typical of class I hydrophobins, could be deduced by sequencing cDNA clones obtained by RT-PCR. The two forms of the protein could be assigned to different alleles present in the two homokaryons that constitute the heterokaryotic U1 strain. ABH3 displays all the in vitro properties of a typical class I hydrophobin such as SC3 from

Schizophyllum commune but is not glycosylated or otherwise post-translationally modified because the molecular mass values deduced from the amino acid sequence (9228 and 9271 Da) and derived from mass spectrometry were in good agreement. The ABH3 transcript was found to be present in the vegetative mycelium of both primary and secondary mycelium but not in the fruiting bodies, whereas the reverse was found for the ABH1 hydrophobin. Using an *S. commune* mutant with a disrupted SC3 gene it was found that ABH3 can substitute for SC3 in inducing formation of aerial hyphae, suggesting a role of ABH3 in the emergence of aerial hyphae and strands in *A. bisporus*.

Author Keywords

Agaricus bisporus; Hydrophobin; Substrate mycelium; Wall protein

Document Type: Article

Source: Scopus

Smith, M.^a, Shnyreva, A.^{a b}, Wood, D.A.^a, Thurston, C.F.^a

Tandem organization and highly disparate expression of the two laccase genes lcc1 and lcc2 in the cultivated mushroom Agaricus bisporus

(1998) *Microbiology*, 144 (4), pp. 1063-1069. Cited 29 times.

^a Division of Life Sciences, King's College London, London W8 7AH, United Kingdom

^b Department of Mycology and Algology, Moscow Lomonosov University, Moscow 1198799, Russian Federation

Abstract

Two non-allelic laccase genes (lcc1 and lcc2) in *Agaricus*

bisporus have been mapped to the same cosmid clone and are close together, in tandem. The intergenic region consists of 1562 bp between the stop codon of lcc1 and the start codon of lcc2. Differences between the 5' non-coding regions of the two genes suggest the potential for their differential regulation. By employing competitive RT-PCR and specific primer pairs that discriminate between lcc1 and lcc2, it has been shown that the level of lcc2 mRNA is approximately 300 times higher than that of lcc1 mRNA in malt extract liquid cultures; in compost cultures lcc2 mRNA is almost 7000 times more abundant than lcc1 mRNA.

Author Keywords

Agaricus bisporus; Differential regulation; Laccase genes; Polyphenol oxidase

Document Type: Article

Source: Scopus

Callac, P.^a , Moquet, F.^a , Imbernon, M.^a , Guedes-Lafargue, M.R.^b , Mamoun, M.^a , Olivier, J.-M.^a

Evidence for PPC1, a determinant of the pilei-pellis color of Agaricus bisporus fruitbodies

(1998) *Fungal Genetics and Biology*, 23 (2), pp. 181-188. Cited 9 times.

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^b Centre Technique du Champignon, 37370 St. Paterne, France

Abstract

In the present study, we investigated the genetic basis of mushroom cap color. In first generation hybrids between a brown isolate and the white commercial hybrid U1, the white trait was recessive. Color was determined using color meter technology in second generation hybrids obtained by crossing the homokaryotic progeny of a first generation hybrid with a homokaryon from U1. Statistical analysis revealed a bimodal distribution describing two classes of white and not-white hybrids. We postulate that a recessive allele at a single locus (PPC1) encodes the white pilei-pellis color. Joint segregation analyses indicated that PPC1 was linked to the ADH (alcohol dehydrogenase) locus. Through the analysis of the heterokaryotic progeny of the first generation hybrid, a recombination model is proposed in which PPC1 is located between the centromere and the ADH locus.

Author Keywords

Agaricus bisporus; Alcohol dehydrogenase; Likelihood analyses; Mixed mating system; Pilei-pellis color locus; White color

Document Type: Article

Source: Scopus

Xu, J.^{a e}, Kerrigan, R.W.^b, Sonnenberg, A.S.^c, Callac, P.^d, Horgen, P.A.^a, Anderson, J.B.^a

Mitochondrial DNA variation in natural populations of the mushroom *Agaricus bisporus*

(1998) *Molecular Ecology*, 7 (1), pp. 19-33. Cited 11 times.

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^e Department of Botany, Duke University, Durham, NC 27708, United States

Abstract

We investigated the patterns of mitochondrial DNA variation in the global population of the commercial mushroom *Agaricus bisporus*. Through the analysis of RFLP's among 441 isolates from nine countries in North America and Eurasia, we found a total of 140 mtDNA haplotypes. Based on population genetic analysis, there are four genetically distinct natural populations in this species, found in coastal California, desert California, France and Alberta (Canada). While 134 of the 140 mtDNA haplotypes were unique to single geographical regions, two mtDNA haplotypes, mt001 and mt002, were found in almost every population surveyed. These two mtDNA haplotypes also predominate among cultivars used throughout the world for at least the last two decades. These two mtDNA haplotypes are more similar to the cosmopolitan groups of mtDNA haplotypes than to the indigeneous clusters of mtDNA haplotypes from the two Californian regions.

Author Keywords

Artificial dispersal; Genetic polymorphism; Germ plasm; Population structure

Document Type: Article

Source: Scopus

Kersten, M.A.S.H.^a , Müller, Y.^b , Op den Camp, H.J.M.^a ,
Vogels, G.D.^a , Van Griensven, L.J.L.D.^c , Visser, J.^b , Schaap,
P.J.^b

**Molecular characterization of the glnA gene encoding
glutamine synthetase from the edible mushroom**

Agaricus bisporus

(1997) *Molecular and General Genetics*, 256 (2), pp. 179-
186. Cited 15 times.

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of Nijmegen, Toernooiveld 1, NL-6525 ED Nijmegen,
Netherlands

^b Sect. Molec. Genet. Indust. M., Wageningen Agricultural
University, Dreijenlaan 2, NL-6703 HA Wageningen,
Netherlands

^c Mushroom Experimental Station, P.O. Box 6042, NL-5960
AA Horst, Netherlands

Abstract

The gene encoding glutamine synthetase (glnA) was isolated
from an *Agaricus bisporus* H39 recombinant λ phage library.
The deduced *A. bisporus* glutamine synthetase amino acid
sequence contains 354 residues. The amino acid sequence is
very similar to that derived from the gene coding for
glutamine synthetase of the yeast *Saccharomyces cerevisiae*.
The open reading frame is interrupted by four introns.
Northern analysis revealed that transcription of the gene is
repressed upon addition of ammonium to the culture but the
repression was not as strong as that of the gene encoding
NADP⁺-dependent glutamate dehydrogenase (gdhA). Enzyme
activities are low in the presence of ammonium, glutamine

and albumin and do not correlate with the mRNA levels revealed by Northern analysis. This suggests that glutamine synthetase expression in *A. bisporus* is also post-transcriptionally regulated by the nitrogen source.

Author Keywords

Agaricus bisporus; Gene structure; Glutamine synthetase; Molecular cloning; Mushroom

Document Type: Article

Source: Scopus

Xu, J.^{a d}, Kerrigan, R.W.^b, Callac, P.^c, Horgen, P.A.^a, Anderson, J.B.^a

Genetic structure of natural populations of *Agaricus bisporus*, the commercial button mushroom

(1997) *Journal of Heredity*, 88 (6), pp. 482-488. Cited 25 times.

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^c INRA-CTC, Stn. de Rech. sur les Champignons, 33883 Villenave d'Ornon, France

^d Department of Microbiology, Duke University, Medical Center, Durham, NC 27710, United States

Abstract

Agaricus bisporus (Lange) Imbach, the familiar button mushroom of commerce, is a major vegetable crop around the world. In the past 10 years a significant world-wide effort

has been made to collect *Agaricus* germ plasm from the wild. Here we report the genetic analysis of a collection of 342 isolates from 12 locations. For 10 nuclear loci marked by RFLPs, we found high genetic diversity in all geographic populations. Among the 342 isolates, three different analyses of genetic diversity were carried out: the first on the total sample of 342 isolates, the second on a subset of 108 'cultivar-like' isolates shown previously to carry either of the two mitochondrial DNA haplotypes found in cultivated strains, and the third on the other 234 isolates that carry a diversity of mtDNA haplotypes not found in cultivated strains. We found that the samples of cultivar-like isolates from various locations were genetically more similar among themselves and to the cultivars than to the samples of other isolates from the same locations. Furthermore, the cultivar-like samples showed no evidence of genetic differentiation between continents and between regions within a continent. In contrast, samples of other isolates showed significant differentiation at the same levels of the geographic hierarchy. A comparison of gene frequencies was consistent with the occurrence of hybridization between the cultivar-like and the other strains in the coastal California population. Analyses of genetic diversity and genetic distance were all consistent with the historical record that cultivars and cultivar-like strains in the wild originated from Western Europe.

Document Type: Article

Source: Scopus

De La Bastide, P.Y.^{a c}, Sonnenberg, A.S.M.^b, Van Griensven, L.J.L.D.^b, Anderson, J.B.^a, Horgen, P.A.^a

Mitochondrial haplotype influences mycelial growth of *Agaricus bisporus* heterokaryons

(1997) *Applied and Environmental Microbiology*, 63 (9), pp. 3426-3431. Cited 5 times.

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^b Mushroom Experimental Station, NL-5960 AA Horst, Netherlands

^c Biology Group, University of Toronto at Mississauga, 3359 Mississauga Road North, Mississauga, Ont. L5L 1C6, Canada

Abstract

We evaluated the influence of mitochondrial haplotype on growth of the common button mushroom *Agaricus bisporus*. Ten pairs of heterokaryon strains, each pair having the same nuclear genome but different mitochondrial genomes, were produced by controlled crosses among a group of homokaryons of both wild and commercial origins. Seven genetically distinct mitochondrial DNA (mtDNA) haplotypes were evaluated in different nuclear backgrounds. The growth of heterokaryon pairs differing only in their mtDNA haplotypes was compared by measuring mycelial radial growth rate on solid complete yeast medium (CYM) and compost extract medium and by measuring mycelial dry weight accumulation in liquid CYM. All *A. bisporus* strains were incubated at temperatures similar to those utilized in commercial production facilities (18, 22, and 26° C). Statistically significant differences were detected in 8 of the 10 heterokaryon pairs evaluated for one or two of the three growth parameters measured. Some heterokaryon pairs showed differences in a single growth parameter at all three temperatures of incubation, suggesting a temperature-independent difference. Others showed differences at only a single temperature, suggesting a temperature-dependent difference. The influence of some mtDNA haplotypes on

growth was dependent on the nuclear genetic background. Our results show that mtDNA haplotype can influence growth of *A. bisporus* heterokaryons in some nuclear backgrounds. These observations demonstrate the importance of including a number of mitochondrial genotypes and evaluating different nuclear-mitochondrial combinations of *A. bisporus* in strain improvement programs.

Document Type: Article

Source: Scopus

Callac, P.^a , Desmerger, C.^b , Kerrigan, R.W.^c , Imbernon, M.^a

Conservation of genetic linkage with map expansion in distantly related crosses of *Agaricus bisporus*

(1997) *FEMS Microbiology Letters*, 146 (2), pp. 235-240. Cited 11 times.

^a Inst. Natl. de la Rech. Agronomique, Stn. Rech. Sur Les Champ., B.P. 81, 33883 Villenave d'Ornon, France

^b Centre Technique du Champignon, 37370 St Paterne, France

^c Research Department, Sylvan, Inc., W. Hills Indust. Park, Kittanning, PA 16201, United States

Abstract

A previous map of the genome of a hybrid strain which had European parents belonging to the secondarily homothallic fungus *Agaricus bisporus* var. *bisporus* appeared to be unusually compact, with a particularly recombophobic segment in the central part of chromosome I. A new map of this segment was constructed based on allelic segregations among 103 homokaryotic offspring of an *A. bisporus* hybrid

between a European parent of the var. bisporus and a Californian parent of the heterothallic var. burnettii. Markers completely linked on the previous map were distributed along 28 cM in the new map. These results suggest that the greater recombination rate could be correlated with the outbreeding behaviour of the var. burnettii.

Author Keywords

Agaricus bisporus; breeding system; genetic linkage; map expansion

Document Type: Article

Source: Scopus

Revill, P.A., Wright, P.J.

RT-PCR detection of dsRNAs associated with La France disease of the cultivated mushroom Agaricus bisporus (Lange) Imbach

(1997) *Journal of Virological Methods*, 63 (1-2), pp. 17-26. Cited 7 times.

Department of Microbiology, Monash University, Clayton, Vic. 3168, Australia

Abstract

A reverse transcription-polymerase chain reaction assay (RT-PCR) is described for the detection of double-stranded RNA (dsRNA) molecules (M1, M2, and L3) associated with La France disease of the cultivated mushroom *Agaricus bisporus*. RT-PCR was faster and more sensitive than current methods used to detect dsRNA, such as dsRNA extraction and analysis by electrophoresis. Another major advantage of RT-PCR was the detection of M1 dsRNA in rapidly prepared homogenates of sporophores and spawn, and in compost before sporophore

production. The early detection of La France disease by RT-PCR will enable implementation of control measures by growers that may reduce losses in production time associated with a disease outbreak. Sequence analysis of dsRNA molecules in two Australian isolates showed that M1 was more conserved than M2 or L3 dsRNA.

Author Keywords

Agaricus bisporus; dsRNA; La France disease; mushroom; RT-PCR

Document Type: Article

Source: Scopus

Sonnenberg, A.S.M.^{a c}, De Groot, P.W.J.^{a b}, Schaap, P.J.^b, Baars, J.J.P.^a, Visser, J.^b, Van Griensven, L.J.L.D.^a

Isolation of expressed sequence tags of Agaricus bisporus and their assignment to chromosomes

(1996) *Applied and Environmental*

Microbiology, 62 (12), pp. 4542-4547. Cited 24 times.

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Abstract

The genome of the cultivated basidiomycete Agaricus bisporus Horst U1 and of its homokaryotic parents has been characterized by using an optimized method of pulsed-field

gel electrophoresis. Expressed sequence tags obtained as expressed cDNAs from a primordial tissue-derived cDNA library and a number of previously isolated genes were used to identify the individual chromosomes of the parental lines of Horst U1. The genome consists of 13 chromosomes, and its total size is 31 Mb. For those chromosomes that could not be resolved by contour-clamped homogeneous electric field electrophoresis, the segregation of marker genes was studied in a set of 86 homokaryotic offspring of Horst U1. At least two markers were assigned to each individual chromosome. In this way all individual chromosomes were unequivocally identified. The large size difference observed between the homologous chromosomes IX, harboring the rDNA repeat, was shown to be largely due to a higher copy number of rDNA in parental strain H97 than in parental strain H39.

Document Type: Article

Source: Scopus

Horgen, P.A.^a, Carvalho, D.^a, Sonnenberg, A.^b, Li, A.^a, Van Griensven, L.J.L.D.^b

Chromosomal abnormalities associated with strain degeneration in the cultivated mushroom, *Agaricus bisporus*

(1996) *Fungal Genetics and Biology*, 20 (3), pp. 229-241. Cited 12 times.

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Abstract

Commercially grown strains of the button mushroom, *Agaricus bisporus*, on occasion produce sectors that display undesirable phenotypes. Such sectors show altered compost colonization, have reduced yield, and produce inferior quality mushrooms. The current study compared isolates of eight irreversible sectors to the original cultivator (U1) from which they arose. Gene-specific and anonymous DNA probes were used to identify RFLPs and chromosomal differences (CHEF analyses) between the U1 sectors and the normal U1 cultivar. A number of differences were noted including loss of heterozygosity at specific loci, deheterokaryotization, somatic recombination, chromosomal loss, chromosomal length polymorphisms, possible chromosomal translocations, and changes in copy number of the ribosomal DNA repeat.

Author Keywords

Chromosome length polymorphisms; Chromosome loss; Mitotic instability; Parasexuality; Sectoring; Somatic recombination

Document Type: Article

Source: Scopus

Imbernon, M.^a, Callac, P.^a, Gasqui, P.^b, Kerrigan, R.W.^c, Velcko Jr., A.J.^c

BSN, the primary determinant of basidial spore number and reproductive mode in *Agaricus bisporus*, maps to chromosome I

(1996) *Mycologia*, 88 (5), pp. 749-761. Cited 14 times.

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33883, Villenave d'Ornon, France

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^c Research Department, Sylvan, Inc., West Hills Industrial Park, Kittanning, PA 16201, United States

Abstract

In the mushroom species *Agaricus bisporus*, heterokaryotic individuals of the geographically isolated varieties *bisporus* and *burnettii* respectively appear to rely primarily upon inbreeding or outbreeding reproductive strategies. These two divergent syndromes depend upon the 'ploidy level' (n vs $n+n$) of offspring. This in turn is determined by the number of spores produced upon the basidia, which are respectively predominantly bisporic or tetrasporic. This study investigated the genetic basis of control over the reproductive syndrome by analyzing transmission of basidial spore number traits in two intervarietal hybrid pedigrees. For two different pedigrees, 103 or 71 homokaryotic offspring of a first-generation intervarietal hybrid were all crossed with a single homokaryon, from a bisporic parent, to produce a second generation of sibling heterokaryons. In each pedigree, the average basidial spore number, or ASN, had a bimodal frequency distribution and was the most useful discriminant variable for resolving the two classes of offspring. Our results indicate that basidial spore number is primarily determined by a single genetic locus (BSN). Statistical analyses of joint segregants indicate that the locus is linked to the mating type locus (MAT) and other loci on chromosome I. Using fruiting tests, mating tests, and genotype analysis, it was shown that the offspring of preponderantly bisporic or tetrasporic second generation hybrids were respectively preponderantly heterokaryotic ($n+n$) or homokaryotic (n). Homokaryons were

capable of normal mating behavior, unlike most of their heterokaryotic siblings. This is consistent with earlier observations on this and other species. We propose that BSN is the primary locus regulating the two alternative reproductive modes.

Author Keywords

Agaricus bisporus var. *burnettii*; amphithallism; basidial spore number locus; inbreeding; mixed mating systems; outbreeding; ploidy level

Document Type: Article

Source: Scopus

Van De Rhee, M.D., Mendes, O., Werten, M.W.T., Huizing, H.J., Mooibroek, H.

Highly efficient homologous integration via tandem $\text{exo-}\beta\text{-1,3-glucanase}$ genes in the common mushroom, *Agaricus bisporus*

(1996) *Current Genetics*, 30 (2), pp. 166-173. Cited 26 times.

DLO-Inst. for Agrotechnological Res., P.O. Box 17, 6700 AA Wageningen, Netherlands

Abstract

Homologous integration was studied in the common mushroom, *Agaricus bisporus*, using a plasmid (pHAG3-1) carrying the hygromycin-resistance gene and a 3.2-kb genomic fragment from *A. bisporus*. Homologous integration was found in 30-60% of the transformants obtained with pHAG3-1 linearized at three different positions within the homologous sequence, generating either blunt, 5'- or 3'-protruding ends. The genomic fragment was found to contain two homologous open reading frames in tandem, which

showed 60% similarity to exo- β -1,3-glucanases from *Saccharomyces cerevisiae* and *Candida albicans*. The level of the corresponding mRNA is low in the vegetative mycelium and relatively high in fruiting bodies. In the vegetative mycelium of a transformant with tandemly integrated pHAG3-1 plasmids at the homologous position, exoglucanase mRNA was strongly increased without any apparent effect on growth rate or morphology.

Author Keywords

Agaricus bisporus; Exo- β -1,3-glucanase; Homologous recombination; Transformation

Document Type: Article

Source: Scopus

Lugones, L.G., Bosscher, J.S., Scholtmeyer, K., De Vries, O.M.H., Wessels, J.G.H.

An abundant hydrophobin (ABH1) forms hydrophobic rodlet layers in *Agaricus bisporus* fruiting bodies

(1996) *Microbiology*, 142 (5), pp. 1321-1329. Cited 71 times.

Department of Plant Biology, Groningen Biomol. Sci. Biotech. I., University of Groningen, Kerklaan 30, 9751 NN Haren, Netherlands

Abstract

The SDS-insoluble protein fraction of *Agaricus bisporus* fruiting bodies was solubilized with trifluoroacetic acid. On SDS-PAGE this fraction was found to contain one abundant protein with an apparent $M(r)$ of 16 kDa. The N-terminal amino acid sequence of this protein was determined and RT-PCR used to isolate a cDNA clone which upon sequencing identified the protein as a typical class I hydrophobin (ABH1).

The gene (ABH1) was isolated and sequenced, and a second hydrophobin gene (ABH2) was found about 2.5 kbp downstream of ABH1. Purified ABH1 self-assembled at hydrophobic-hydrophilic interfaces, producing the typical rodlet layer known from other hydrophobins. Similar rodlets were observed on the surface of the fruiting body, while immunological localization showed the hydrophobin to be particularly abundant at the outer surface of fruiting bodies, in the veil and in the core tissue of the stipe. Transcripts of ABH1 were found only in fruiting-body hyphae. The ABH1 hydrophobin is probably solely responsible for the hydrophobicity of the fruiting-body surface but may also line air channels within fruiting bodies.

Author Keywords

Agaricus bisporus; Fruiting body; Hydrophobin; Mushroom; Wall protein

Document Type: Article

Source: Scopus

De Groot, P.W.J.^{a b}, Schaap, P.J.^a, Sonnenberg, A.S.M.^b, Visser, J.^a, Van Griensven, L.J.L.D.^b

The *Agaricus bisporus* hypA gene encodes a hydrophobin and specifically accumulates in peel tissue of mushroom caps during fruit body development

(1996) *Journal of Molecular Biology*, 257 (5), pp. 1008-1018. Cited 57 times.

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^b Mushroom Experimental Station, P.O. Box 6042, NL-5960 AA, Horst, Netherlands

Abstract

Differential screening of a cDNA library was used to clone genes that are specifically expressed during mushroom development in the basidiomycete *Agaricus bisporus*. One of the isolated genes encodes a polypeptide of 112 amino acid residues and belongs to the fungal gene family encoding hydrophobins. This gene, *hypA*, has the characteristic pattern of eight cysteine residues at conserved positions and a hydrophobicity pattern that is very similar to class I hydrophobins. Elucidation of the genomic structure of *hypA* led to the identification of a second copy, *hypC*, located downstream of *hypA*. Although at a much lower level, *hypC* is like *hypA* specifically expressed in fruit bodies. The *hypA* mRNA level is transiently increased ten days after fruit body induction and expression appears to be associated with rapid expansion of the mushroom caps. In mushroom caps, very high concentrations of *hypA* messengers were found in the (outer) peel tissue, where they accumulate to more than 60% of the total mRNA mass. The corresponding protein with a molecular mass of 8 to 9 kDa was purified from this peel tissue and was identified by N-terminal sequencing. Our results suggest that HYPA forms a protective hydrophobic layer instrumental in cap formation.

Author Keywords

Agaricus bisporus; Fruit body; Gene duplication; Hydrophobin; *hypA* gene

Document Type: Article

Source: Scopus

Van De Rhee, M.D., Graça, P.M.A., Huizing, H.J., Mooibroek, H.

Transformation of the cultivated mushroom, *Agaricus bisporus*, to hygromycin B resistance

(1996) *Molecular and General Genetics*, 250 (3), pp. 252-258. Cited 51 times.

DLO Inst. for Agrotechnological Res., P.O. Box 17, 6700 AA Wageningen, Netherlands

Abstract

Application of biotechnology to the cultivated mushroom, *Agaricus bisporus*, has been hampered thus far by the lack of a transformation system. Here, transformation of both a home- and a heterokaryotic strain of *A. bisporus* to hygromycin B resistance is described. Transforming DNA was integrated into the *A. bisporus* genome and stably maintained throughout vegetative growth. Transformants of the heterokaryotic strain formed transgenic fruiting bodies. Promoters derived from the unrelated ascomycete *Aspergillus nidulans* and from *A. bisporus* itself, were able to drive expression of the hygromycin B resistance gene. Expression controlled by a fragment of 265 bp from the *A. bisporus* GPD promoter was sufficient to generate transformants. However, transformation efficiency was not enhanced by using this homologous promoter.

Author Keywords

Agaricus bisporus; Edible mushrooms;
Homobasidiomycetes; Hygromycin B phosphotransferase;
Transformation

Document Type: Article

Source: Scopus

Van Der Lende, T.R., Duitman, E.H., Gunnewijk, M.G.W., Yu, L., Wessels, J.G.H.

Functional analysis of dsRNAs (L1, L3, L5, and M2) associated with isometric 34-nm virions of *Agaricus bisporus* (white button mushroom)

(1996) *Virology*, 217 (1), pp. 88-96. Cited 8 times.

Department of Plant Biology, Groningen Biomol. and Biotech. Inst., University of Groningen, Kerklaan 30, 9751 NN Haren, Netherlands

Abstract

cDNA clones of dsRNAs associated with La France disease of *Agaricus bisporus* were isolated. Clones corresponding to L1 and L5 dsRNAs were sequenced. The deduced amino acid sequence of L1 dsRNA (1078 amino acids, M(r) 121K) showed significant homology with RNA-dependent RNA polymerases of other dsRNA viruses. The deduced amino acid sequence of L5 dsRNA (724 amino acids, M(r) 82K) showed no homology with known proteins. Amino acid sequences of tryptic digests of three virion-associated proteins were determined. The 34-nm virion-associated protein of M(r) 115K was encoded by the L1 dsRNA, thus identifying this protein as the RNA-dependent RNA polymerase. The virion-associated protein of M(r) 90K was encoded by the previously sequenced L3 dsRNA. A cDNA clone of the previously sequenced M2 dsRNA was expressed in *Escherichia coli* and antibodies raised against this protein reacted only with a protein present in the cytoplasm of diseased *A. bisporus* fruit bodies but not in the 34-nm virions.

Document Type: Article

Source: Scopus

Xu, J., Horgen, P.A., Anderson, J.B.

**Somatic recombination in the cultivated mushroom
*Agaricus bisporus***

(1996) *Mycological Research*, 100 (2), pp. 188-192. Cited 7 times.

Department of Botany, University of Toronto, Mississauga,
Ont. L5L 1C6, Canada

Abstract

Agaricus bisporus, the cultivated button mushroom, has a mostly secondarily homothallic life cycle. This mode of sexual reproduction could limit outcrossing and recombination among homokaryons in natural populations and also creates difficulties in mushroom breeding. An alternative source of recombinant genotypes is from somatic pairings of heterokaryons. In this study, two homokaryon \times heterokaryon and three heterokaryon \times heterokaryon pairings were made to examine the possibility of somatic recombination. Four subcultures from the confrontation zone of each pairing were taken for analysis of restriction fragment length polymorphisms at 18 nuclear loci representing seven linkage groups and two regions of mitochondrial DNA. A strikingly high frequency of somatic recombination was observed. Five of the eight subcultures from the two homokaryon \times heterokaryon pairings and five of the 12 subcultures from the three heterokaryon \times heterokaryon pairings were recombinant. No loss of marker alleles was detected in any of the self-self pairings of the six original strains. No recombination was observed between the two regions of mtDNA examined in this study. The recombination among nuclear loci involves nuclear reassortment, exchange of genetic material between nuclei, and, in one case, crossing

over between markers located on the same chromosome. While the mechanism of somatic recombination in *A. bisporus* is not known, heterokaryons might be used in pairings with other heterokaryons or with homokaryons to produce abundant recombinant genotypes for mushroom breeding.

Document Type: Article

Source: Scopus

Kerrigan, R.W., Carvalho, D.B., Horgen, P.A., Anderson, J.B.
Indigenous and introduced populations of *Agaricus bisporus*, the cultivated button mushroom, in eastern and western Canada: Implications for population biology, resource management, and conservation of genetic diversity

(1995) *Canadian Journal of Botany*, 73 (12), pp. 1925-1938. Cited 17 times.

Sylvan Spawn Laboratory Inc., Dept Res., 1163 Winfield Rd,
Cabot, PA 16023, United States

Document Type: Article

Source: Scopus

Xu, J.

Analysis of inbreeding depression in *Agaricus bisporus*

(1995) *Genetics*, 141 (1), pp. 137-145. Cited 15 times.

Department of Botany, Erindale College, University of
Toronto, Mississauga, Ont. L5L 1C6, Canada

Abstract

Inbreeding depression was observed in the commercial button

mushroom, *Agaricus bisporus*, by examining two laboratory populations. The outbred population consisted of 20 compatible pairings, 10 homokaryons with each of the homokaryons Ag1-1 and Ag89-65. The inbred population consisted of 104 backcrosses (among which 52 were expected to be sexually compatible) obtained from the pairings of two progenitor homokaryons, Ag1-1 and Ag89-65, with 52 progeny homokaryons derived from the mating between Ag1-1 and Ag89-65. The eight fitness components examined for these two populations were successful matings as identified by the analysis of restriction fragment length polymorphisms, positive mycelial interaction in these successful matings, heterokaryon growth rate, primordium formation by the successful matings, fertile fruiting body formation, time to first break, average number of fruiting bodies per square foot, and average weight per fruiting body. The outcrossed population showed a significant advantage over the inbred population in three of eight fitness components. Two pairs of traits were significantly correlated. The multiplicative illness ratio of the inbred to the outcrossed population was 0.18. The relevance of inbreeding depression to the evolution of fungal mating systems and to mushroom breeding is discussed.

Document Type: Article

Source: Scopus

Grewal, S.I.S., Han, B., Johnstone, K.

Identification and characterization of a locus which regulates multiple functions in *Pseudomonas tolaasii*, the cause of brown blotch disease of *Agaricus bisporus* (1995) *Journal of Bacteriology*, 177 (16), pp. 4658-4668. Cited 41 times.

Department of Plant Sciences, University of Cambridge,

Downing Street, Cambridge CB2 3EA, United Kingdom

Abstract

Pseudomonas tolaasii, the causal agent of brown blotch disease of *Agaricus bisporus*, spontaneously gives rise to morphologically distinct stable sectors, referred to as the phenotypic variant form, at the margins of the wild-type colonies. The phenotypic variant form is nonpathogenic and differs from the wild type in a range of biochemical and physiological characteristics. A genomic cosmid clone (pSISG29) from a wild-type *P. tolaasii* library was shown to be capable of restoring a range of characteristics of the phenotypic variant to those of the wild-type form, when present in trans. Subcloning and saturation mutagenesis analysis with Tn5lacZ localized a 3.0-kb region from pSISG29, designated the pheN locus, required for complementation of the phenotypic variant to the wild-type form. Marker exchange of the Tn5lacZ-mutagenized copy of the pheN locus into the wild-type strain demonstrated that a functional copy of the pheN gene is required to maintain the wild-type pathogenic phenotype and that loss of the pheN gene or its function results in conversion of the wild-type form to the phenotypic variant form. The pheN locus contained a 2,727-bp open reading frame encoding an 83-kDa protein. The predicted amino acid sequence of the PheN protein showed homology to the sensor and regulator domains of the conserved family of two component bacterial sensor regulator proteins. Southern hybridization analysis of pheN genes from the wild type and the phenotypic variant form revealed that DNA rearrangement occurs within the pheN locus during phenotypic variation. Analysis of pheN expression with a pheN::lacZ fusion demonstrated that expression is regulated by environmental factors. These results are related to a model for control for phenotypic variation in *P. tolaasii*.

Document Type: Article

Source: Scopus

Yague, E., Wood, D.A., Thurston, C.F.

Regulation of transcription of the cel1 gene in Agaricus bisporus

(1994) *Molecular Microbiology*, 12 (1), pp. 41-47. Cited 22 times.

Division of Life Sciences, King's College London, Camden Hill Road, London W8 7AH, United Kingdom

Abstract

Northern analysis showed that accumulation of *Agaricus bisporus* cel1 mRNA was regulated by two independent mechanisms: (i) induction by cellulose; and (ii) repression by glucose and other sugars. Isolated *A. bisporus* nuclei were transcriptionally active. Nuclei isolated from cellulose-grown mycelium synthesized six times more cel1 mRNA than nuclei from glucose-grown mycelium. The start point of transcription (tsp) was identified by primer extension and S1 nuclease analysis. Putative glucose-, and cAMP-responsive elements as well as regions with homology to promoter regions of other fungal cellulase genes were detected both upstream and downstream from the tsp of the cel1 gene.

Document Type: Article

Source: Scopus

Armesilla, A.L., Thurston, C.F., Yaguc, E.

CEL1: A novel cellulose binding protein secreted by Agaricus bisporus during growth on crystalline cellulose

(1994) *FEMS Microbiology Letters*, 116 (3), pp. 293-300. Cited 9 times.

Microbiology Group, Division of Life Sciences, King's College London, Campden Hill Rd., London W8 7AH, United Kingdom

Abstract

The cell gene of *Agaricus bisporus* encodes a protein (CEL1) that has an architecture resembling the multi-domain fungal cellulases, although the sequence of its putative catalytic core is not matched by any other in the protein and nucleic acid data bases. The N-terminal half of the putative catalytic domain of CEL1 was expressed in *Escherichia coli* as a fusion protein with glutathione-S-transferase. The fusion protein was used to raise a CEL1-specific antibody. CEL1 was detected as an extracellular 49.8 kDa protein in *A. bisporus* cellulose-grown cultures, where it bound strongly to cellulose. CEL1 was neither an endoglucanase, a cellobiohydrolase able to hydrolyze fluorogenic cellobiosides, a β -glucosidase, a xylanase, nor a cellobiose:quinone oxidoreductase. CEL1 was present in some fractions of culture fluid separated by electrophoresis which released soluble sugars from crystalline cellulose.

Author Keywords

Agaricus bisporus; Antibody; CEL1; Cellulase; Cellulose binding; Fusion protein

Document Type: Article

Source: Scopus

Romaine, C.P., Schlagnhauser, B., Goodin, M.M.

Vesicle-associated double-stranded ribonucleic acid gellitic elements in *Agaricus bisporus*

(1994) *Current Genetics*, 25 (2), pp. 128-134. Cited 7 times.

Department of Plant Pathology, 210 Buckhout Laboratory, The Pennsylvania State University, University Park, PA 16802, United States

Abstract

Double-stranded ribonucleic acids (dsRNAs) were isolated from fruit bodies of commercial strains of the cultivated mushroom (*Agaricus bisporus*) by polyethylene glycol-NaCl precipitation, differential centrifugation, rate-zonal centrifugation in sucrose, and equilibrium centrifugation in cesium sulphate. In all seven of the mushroom isolates examined, three dsRNAs were identified: two major dsRNA segments of > 13.1-kb (L-RNA) and 2.4-kb (S-RNA) and a minor segment of 5.2-kb (M-RNA). L-, M-, and S-RNAs co-purified with spherical fungal vesicles measuring approximately 75 nm in diameter. The three dsRNAs were intimately associated with the vesicles as suggested by their lower buoyant density in cesium sulphate (1.27 g/cc) compared to that of phenol-extracted dsRNAs (1.42 g/cc) and by their resistance to hydrolysis by ribonuclease at low ionic strength. Using a variety of conditions during purification, no virus-like particles were found to be associated with the dsRNAs. In Northern analysis, L-, M-, and S-RNAs failed to cross-hybridize with the genomic dsRNAs of La France isometric virus. We report here the first description of non-encapsidated, vesicle-associated, dsRNA genetic elements in the common cultivated mushroom.

Author Keywords

Agaricus bisporus; dsRNA; dsRNA genetic elements; Fungal vesicles

Document Type: Article

Source: Scopus

Li, A., Begin, M., Kokurewicz, K., Bowden, C., Horgen, P.A.
Inheritance of strain instability (sectoring) in the commercial button mushroom, *Agaricus bisporus*
(1994) *Applied and Environmental Microbiology*, 60 (7), pp. 2384-2388. Cited 11 times.

Center for Plant Biotechnology, University of Toronto, Erindale Campus, Mississauga, Ont. M9R 1S9, Canada

Abstract

The button mushroom, *Agaricus bisporus*, is a commercially important cultivated filamentous fungus. During the last decade, the button mushroom industry has depended mainly on two strains (or derivatives of these two strains). Using one of these highly successful strains (strain U1) we examined the phenomenon of strain instability, specifically, the production of irreversible sectors. Three 'stromatal' and three 'fluffy' sectors were compared with a healthy type U1 strain and with a wild-collected isolate. Compost colonization and fruit body morphology were examined. The main objective of this study, however, was to examine the meiotic stability of the sectorized phenotype. Single basidiospores were isolated and subjected to a grain bioassay in which the ability to produce sectors was measured. Our results were as follows: (i) basidiospore cultures obtained from a wild-collected isolate showed no tendency to produce sectors; (ii) approximately 5% of the basidiospore cultures obtained from healthy type U1 strains produced irreversible sectors in the grain bioassay; (iii) the five primary sectors examined produced basidiospore cultures, half of which produced normal-looking growth in the grain bioassay and half of which produced some degree of sectoring; and (iv) the one sectorized isolate that represented

the F2 generation gave ratios similar to the 1:1 ratio observed for the F1 cultures.

Document Type: Article

Source: Scopus

Chow, C.-M., Yague, E., Raguz, S., Wood, D.A., Thurston, C.F.

The cel3 gene of *Agaricus bisporus* codes for a modular cellulase and is transcriptionally regulated by the carbon source

(1994) *Applied and Environmental Microbiology*, 60 (8), pp. 2779-2785. Cited 37 times.

Division of Life Sciences, King's College London, Campden Hill Rd., London W8 7AH, United Kingdom

Abstract

A 52-kDa protein, CEL3, has been separated from the culture filtrate of *Agaricus bisporus* during growth on cellulose. A PCR-derived probe was made, with a degenerate oligodeoxynucleotide derived from the amino acid sequence of a CEL3 CNBr cleavage product and was used to select cel3 cDNA clones from an *A. bisporus* cDNA library. Two allelic cDNAs were isolated. They showed 98.8% identity of their nucleotide sequences. The deduced amino acid sequence and domain architecture of CEL3 showed a high degree of similarity to those of cellobiohydrolase II of *Trichoderma reesei*. Functional expression of cel3 cDNA in *Saccharomyces cerevisiae* was achieved by placing it under the control of a constitutive promoter and fusing it to the yeast invertase signal sequence. Recombinant CEL3 secreted by yeast showed enzymatic activity towards crystalline cellulose. At long reaction times, CEL3 was also able to degrade

carboxymethyl cellulose. Northern (RNA) analysis showed that cel3 gene expression was induced by cellulose and repressed by glucose, fructose, 2-deoxyglucose, and lactose. Glycerol, mannitol, sorbitol, and maltose were neutral carbon sources. Nuclear run-on analysis showed that the rate of synthesis of cel3 mRNA in cellulose-grown cultures was 13 times higher than that in glucose-grown cultures. A low basal rate of cel3 mRNA synthesis was observed in the nuclei isolated from glucose-grown mycelia.

Document Type: Article

Source: Scopus

Callac, P., Billette, C., Imbernon, M., Kerrigan, R.W.

Morphological, genetic, and interfertility analyses reveal a novel, tetrasporic variety of *Agaricus bisporus* from the Sonoran desert of California

(1993) *Mycologia*, 85 (5), pp. 835-851. Cited 40 times.

Sylvan Spawn Laboratory Inc., Dept Res., West Hills Industrial Pk, Kittanning, PA 16201, United States

Document Type: Article

Source: Scopus

Kerrigan, R.W., Royer, J.C., Baller, L.M., Kohli, Y., Horgen, P.A., Anderson, J.B.

Meiotic behavior and linkage relationships in the secondarily homothallic fungus *Agaricus bisporus*

(1993) *Genetics*, 133 (2), pp. 225-236. Cited 67 times.

Research Department, Sylvan Spawn Laboratory, Inc., West Hills Industrial Park, Kittanning, PA 16201, United States

Abstract

This study followed the transmission of 64 segregating genetic markers to 52 haploid offspring, obtained from both homokaryotic and heterokaryotic meiospores, of a cross (AG 93b) of *Agaricus bisporus*, the commonly cultivated 'button mushroom.' The electrophoretic karyotypes of the AG 93b component nuclei were determined concurrently ($n = 13$). Eleven distinct linkage groups were identified by two-point analysis. DNA-DNA hybridization showed that nine of these corresponded to unique chromosome-sized DNAs. Two other chromosomal DNAs were marked with nonsegregating markers, including the rDNA repeat. Two remaining chromosomes remained unmarked but hybridized to repeated-sequence probes. Cross 93b had an essentially conventional meiosis in which both independent assortment and joint segregation of markers occurred, but in which crossing over was infrequent over much of the mapped genome. The 48 homokaryotic spore-offspring had overall crossover frequencies that were similar to, but possibly slightly less than, those of three homokaryon constituents of heterokaryotic spore-offspring. These data provide support for our earlier cytogenetic model of sporogenesis in *A. bisporus*, that explains why heterokaryotic spore-offspring usually appear to exhibit no recombination. No evidence favoring an alternative, mitotic model of sporogenesis was found. The resulting genetic map appears to survey the genome extensively and for the first time permits localization of loci determining economically important traits in this fungal crop species. Large differences in the vigor of homokaryotic offspring were correlated with the inheritance of certain chromosome segments and were also often associated with significant departures from Mendelian segregation ratios.

Document Type: Article

Source: Scopus

Iracabal, B., Labarere, J.

Comparison of polymorphism and phenetic variability as determined by the study of hydrolases and oxidoreductases in two cultivated mushrooms, *Agaricus bisporus* and *Pleurotus cornucopiae*
(1993) *Experimental Mycology*, 17 (2), pp. 90-102.

Laboratoire de Genetique Moleculaire, C.R.A. de Bordeaux,
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Ferrade, 33883 Villenave d'Ornon Cedex, France

Abstract

The isozyme polymorphism and phenetic variability of two cultivated mushrooms *Agaricus bisporus* and *Pleurotus cornucopiae* were compared by studying the activity of five enzymes belonging to two different functional groups: (i) oxidoreductases: peroxidases (POD), tolidine-reacting enzyme phenoloxidases (TRE), and dopa-reacting enzyme phenoloxidases (DRE); and (ii) hydrolases: butyrate esterases and acetate esterases. Reproducibility studies showed that few variations were observed in the isozyme patterns and that the enzyme activities were conserved during at least 6 months of storage at - 20° C, except for POD activity which was stable for only 2 months. The same level of relative polymorphism was observed for each enzyme activity in the two species, although they present differences in their life cycle and their genetic background. The most polymorphic activities were found for the hydrolases, whereas the most conserved were POD and TRE, DRE having an intermediate level of conservation. These results indicated that enzyme polymorphism and phenetic variability seem to be correlated with the functional group of the enzyme rather than with the

genus. The phenetic distances, as determined by numerical analysis, allowed discrimination among the 12 *A. bisporus* commercial strains in three cluster groups. In the case of *P. cornucopiae*, results showed a large variability between wild strains in accordance with their geographical origin, and that the two cultivars studied corresponded to two replicas of the same strain.

Author Keywords

Cultivated mushrooms; enzyme polymorphism; hydrolases; isozymes markers; oxidoreductases; phenetic variability

Document Type: Article

Source: Scopus

Jin, T., Horgen, P.A.

Further characterization of a large inverted repeat in the mitochondrial genomes of *Agaricus bisporus* (= *A. brunnescens*) and related species

(1993) *Current Genetics*, 23 (3), pp. 228-233. Cited 8 times.

Centre for Plant Biotechnology, Department of Botany,
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Abstract

The mitochondrial (mt) genome of *Agaricus bisporus* Ag50 (a heterokaryon) is a 136-kilobase (kb) circular molecule which contains a pair of large inverted repeats (IRs). Two large BAMHI fragments (B1 and B2) which contain the IR regions were further mapped. The repeated regions were determined to be approximately 7.7 kb in length. The mt small ribosomal RNA (S rRNA) gene is located adjacent to one of the repeated regions. Orientational isomers, generated by homologous

recombination between the repeated regions, were not observed in mtDNA extractions from Ag50 mycelium (liquid culture) or from Ag50 fruit bodies. We also did not observe any orientational isomers in Ag50HA or Ag50HB, two homokaryons somatically isolated from Ag50. DNA homologous to the Ag50 mt repeated regions was observed in ten other isolates of *Agaricus* including four isolates of *A. bisporus*, two isolates of *A. subperonatus*, two isolates of *A. subfloccosus*, one isolate of *A. bitorquis*, and one isolate of *A. pattersonae*. The repeated regions and the small unique regions in two other heterokaryotic strains of *A. bisporus*, Ag2 and Ag85, were physically mapped. The repeated regions in these two strains are also in the inverted forms. Restriction endonuclease mapping indicated that the two copies of the IR in Ag85 were not identical.

Author Keywords

Agaricus; Inverted repeat; Mitochondria; MtDNA; Orientational isomer; Recombination

Document Type: Article

Source: Scopus

Perry, C.R., Matcham, S.E., Wood, D.A., Thurston, C.F.

The structure of laccase protein and its synthesis by the commercial mushroom *Agaricus bisporus*

(1993) *Journal of General Microbiology*, 139 (1), pp. 171-178. Cited 43 times.

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Abstract

Agaricus bisporus secretes abundant laccase activity into the medium during mycelial growth. SDS-PAGE analysis of extracellular laccase protein, purified from compost extract, showed a predominant band of 65 kDa molecular mass, together with lesser amounts of smaller polypeptides. The main polypeptide was purified electrophoretically. Amino acid sequence analysis of the N-terminal region of the main polypeptide was used to specify the sequence of a 15-residue chemically synthesized peptide (N-terminal peptide). Rabbit antibodies were raised against pure laccase, electrophoretically purified main polypeptide and the synthetic N-terminal peptide. Electrophoretically purified main polypeptide antibody was further purified by affinity chromatography on laccase-CNBr-Sepharose. Western blot analysis showed that the antigenic behaviour of laccase in compost extract, culture filtrate from malt-extract culture, and the purified enzyme from both sources, differed. The patterns of bands revealed are most simply explained by generation of (proteolytically) partially cleaved enzyme molecules in the culture medium, possibly combined with differences in extent of glycosylation. [³⁵S]Methionine incorporation and immunoprecipitation were used to follow laccase synthesis in cultures grown on malt extract. After short-term labelling, a single polypeptide of 68 kDa apparent molecular mass was immunoprecipitated from both mycelial extracts and the culture medium. When poly(A)-containing RNA from malt-extract-grown mycelium was translated in vitro in rabbit reticulocyte lysate, a single polypeptide of about 57 kDa molecular mass was immunoprecipitated, consistent with the previously measured carbohydrate content of 15% for the pure enzyme. After treatment with N-glycanase, the polypeptide showed an increase in mobility during SDS-PAGE consistent with a reduction in molecular mass of about 5 kDa, indicating about equal amounts of N- and O-linked carbohydrate. C-terminal labelling of pure

laccase was attempted by transpeptidation with carboxypeptidase Y. Although some minor bands were labelled, the main polypeptide was not, indicating that the C-terminus of the enzyme may be blocked.

Document Type: Article

Source: Scopus

Harmsen, M.C., Schuren, F.H.J., Moukha, S.M., Van Zuilen, C.M., Punt, P.J., Wessels, J.G.H.

Sequence analysis of the glyceraldehyde-3-phosphate dehydrogenase genes from the basidiomycetes *Schizophyllum commune*, *Phanerochaete chrysosporium* and *Agaricus bisporus*

(1992) *Current Genetics*, 22 (6), pp. 447-454. Cited 55 times.

Department of Plant Biology, University of Groningen,
Kerklaan 30, NL-9751 NN Haren, Netherlands

Abstract

GPD genes encoding glyceraldehyde-3-phosphate dehydrogenase were isolated from the homobasidiomycetes *Schizophyllum commune*, *Phanerochaete chrysosporium* and *Agaricus bisporus*. All three species contain one transcriptionally active GPD gene, but *A. bisporus* also contains an inactive GPD gene (tandemly linked to the active gene). These genes contain 5-9 introns located at conserved positions, differing (except in one case) from intron positions in ascomycetous GPD genes. The predicted amino-acid sequences of the proteins encoded by the three active GPD genes are highly homologous. A comparison with protein sequences from filamentous ascomycetes shows a clear distinction, whereas the GPD genes from ascomycetous yeasts are quite distinct from both the filamentous

ascomycetes and basidiomycetes. Promoter regions of ascomycetous GPD genes do not correspond to those of the GPD genes of basidiomycetes which may (partly) explain poor expression in basidiomycetes of introduced genes driven by an ascomycete GPD promoter.

Author Keywords

Basidiomycete; Evolution; Glyceraldehyde-3-phosphate dehydrogenase (GPD); Sequence

Document Type: Article

Source: Scopus

Raguz, S., Yague, E., Wood, D.A., Thurston, C.F.

Isolation and characterization of a cellulose-growth-specific gene from *Agaricus bisporus*

(1992) *Gene*, 119 (2), pp. 183-190. Cited 28 times.

Division of Biosphere Sciences, King's College, Campden Hill Rd., London W8 7AH, United Kingdom

Abstract

The edible basidiomycete, *Agaricus bisporus*, produces extracellular endoglucanase. Endoglucanase production is induced by cellulose and repressed by fructose in *A. bisporus* grown on minimal medium, and is regulated in activity during fruiting body development. An anti-endoglucanase antibody was used to isolate cellulase-related genes. Three main polypeptides of 38, 58, and 60 kDa were immunoprecipitated by the antibody from products of in vitro cell-free translation of mRNAs isolated from cellulose-grown mycelium. No cross-reaction was detected with the translated products from fructose-grown mycelium. This antibody was used to immunoscreen a λ ZAPII-cDNA expression library made from

mRNA isolated from cellulose-grown mycelium. Two cDNA cross-reacting clones, pSRc110 and pSRc200, were isolated. Clones pSRc110 and pSRc200 cross-hybridized and had the same restriction map. Clone pSRc200 hybrid selected an mRNA that on cell-free translation produced a 38-kDa polypeptide. The cDNA fragment from pSRc200 hybridized to a 1.3-kb mRNA from cellulose-grown mycelium. No hybridization was observed when using fructose-grown mycelium mRNA. Thus, the gene (*cel1*) expressing the 1.3-kb mRNA, is differentially regulated by the carbon source of the culture medium. The *cel1* gene was isolated in a 8.9-kb *EcoRI* genomic fragment after hybridization to pSRc200. Sequences similar to those in the *egl1* and *cbh2* genes from *Trichoderma reesei* were found upstream from the ATG start codon in *cel1*. Nine short intervening sequences disrupt the *cel1* coding sequence, and a strong bias against codons ending with G and A was observed. CEL1 (protein encoded by *cel1*) showed a primary structure in which four putative functional domains were recognized: a predicted 29-amino-acid (aa) signal peptide, a core of 233 aa, a Pro-Ser-Thr-rich domain of 22 aa, and a C-terminal 36-aa cellulose-binding domain similar to those found in other fungal cellulolytic enzymes. No homology was observed between the CEL1 core and any β -glycanase sequences described to date.

Author Keywords

basidiomycete; cellulase; cellulose-binding domain; codon bias; introns; multifunctional domains; mushroom; recombinant DNA

Document Type: Article

Source: Scopus

Jin, T., Sonnenberg, A.S.M., Van Griensven, L.J.L.D., Horgen,

P.A.

Investigation of mitochondrial transmission in selected matings between homokaryons from commercial and wild-collected isolates of *Agaricus bisporus* (= *Agaricus brunnescens*)

(1992) *Applied and Environmental*

Microbiology, 58 (11), pp. 3553-3560. Cited 12 times.

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Abstract

Ten heterokaryons of *Agaricus bisporus* (= *Agaricus brunnescens*) were shown to carry four different mitochondrial (mt) genotypes by analysis of mt restriction fragment length polymorphisms (RFLPs). Fifteen homokaryons derived from these strains were used to investigate mt inheritance in *A. bisporus*. One hundred eighty-nine pairings were performed in 25 different combinations. Pairings in 15 different combinations produced heterokaryons on the basis of nuclear RFLP analyses and/or fruiting trials. The mt genotype of each new intraspecies hybrid was examined by using mt RFLPs as genetic markers. Our results suggest the following. (i) Recombination between the mt genomes was not a common event. (ii) From most individual pairings, all heterokaryons carried the same mt genotype. (iii) Heterokaryons carrying either of the two possible mt genotypes were observed in certain crosses after modification of the pairing procedure. A biparental transmission pattern was demonstrated for some crosses, but there appears to be a preference for one of the mt genotypes to predominate in any specific pairing.

Document Type: Article

Source: Scopus

Khush, R.S., Becker, E., Wach, M.

DNA amplification polymorphisms of the cultivated mushroom *Agaricus bisporus*

(1992) *Applied and Environmental*

Microbiology, 58 (9), pp. 2971-2977. Cited 24 times.

Monterey Laboratories, P.O. Box 189, Watsonville, CA 95076, United States

Abstract

Single 10-bp primers were used to generate random amplified polymorphic DNA (RAPD) markers from commercial and wild strains of the cultivated mushroom *Agaricus bisporus* via the polymerase chain reaction. Of 20 primers tested, 19 amplified *A. bisporus* DNA, each producing 5 to 15 scorable markers ranging from 0.5 to 3.0 kbp. RAPD markers identified seven distinct genotypes among eight heterokaryotic strains; two of the commercial strains were shown to be related to each other through single-spore descent. Homokaryons recovered from protoplast regenerants of heterokaryotic strains carried a subset of the RAPD markers found in the heterokaryon, and both of the haploid nuclei from two heterokaryons were distinguishable. RAPD markers also served to verify the creation of a hybrid heterokaryon and to analyze meiotic progeny from this new strain: most of the basidiospores displayed RAPD fingerprints identical to that of the parental heterokaryon, although a few selected slow growers were homoallelic at a number of loci that were heteroallelic in the parent, suggesting that they represented rare homokaryotic basidiospores; crossover events between a RAPD marker locus and its respective centromere appeared to be infrequent. These results demonstrate that RAPD markers provide an efficient alternative for strain fingerprinting and a

versatile tool for genetic studies and manipulations of *A. bisporus*.

Document Type: Article

Source: Scopus

Loftus, M.G.^a , Moore, D.^a , Elliott, T.J.^b

DNA polymorphisms in commercial and wild strains of the cultivated mushroom, *Agaricus bisporus*

(1988) *Theoretical and Applied Genetics*, 76 (5), pp. 712-718. Cited 19 times.

^a Microbiology Research Group, Department of Cell and Structural Biology, The University, Stopford Building, Manchester, M13 9PT, United Kingdom

^b Department of Microbiology and Plant Pathology, AFRC Institute of Horticultural Research, Littlehampton, BN17 6LP, Sussex, United Kingdom

Abstract

DNA from the cultivated mushroom, *Agaricus bisporus*, was cloned into the bacteriophage lambda vector EMBL3 creating a partial genomic library. Ten random clones from the library were used to probe for restriction fragment length polymorphisms (RFLPs). Six of the ten probes detected polymorphisms and were used to demonstrate variation in wild and cultivated strains of the mushroom. These results suggest that RFLPs could form a basis for genetic fingerprinting and subsequent strain protection in *A. bisporus*. In single spore progeny, RFLPs were used to demonstrate normal meiotic segregation and to differentiate between homokaryons and heterokaryons. RFLPs therefore have great potential in the development of the genetics and breeding of

this commercially important species. © 1988 Springer-Verlag.

Author Keywords

Agaricus bisporus; DNA polymorphisms; Mushroom; RFLPs

Document Type: Article

Source: Scopus

Elliott, T.J., Langton, F.A.

Strain improvement in the cultivated mushroom

Agaricus bisporus

(1981) *Euphytica*, 30 (1), pp. 175-182.

Glasshouse Crops Research Institute, Worthing Road,
Littlehampton, BN16 3PU, West Sussex, United Kingdom

Abstract

Early attempts at genetic improvement in the cultivated mushroom *Agaricus bisporus*(Lange) Imbach were empirical, for little was understood of its natural breeding system. The mushroom is now known to be a 'secondarily homothallic' species with a single multiallelic mating-type factor. This better understanding makes it possible to evaluate those breeding methods previously used and to suggest alternatives. Strain selection alone based on single spores, multispores or tissue culture may give improvement in the short term but it is unlikely to be as effective as methods involving controlled crossing. Mixing fertile strains may produce hybrids but it is difficult to identify them. It is better to use non-fertile isolates because only hybrids fruit. The earlier recognition of hybrids can be achieved using markers which are expressed in culture and genetic resistances may be especially useful in this respect. There is also a possible role for other *Agaricus* species which may be grown

commercially and are more amenable to genetic manipulation than is *A. bisporus*. © 1981 Veeman B.V., Wageningen.

Author Keywords

Agaricus bisporus; breeding methods; Cultivated mushroom; strain improvement

Document Type: Article

Source: Scopus

Vaisius, A.C., Horgen, P.A.

Purification and characterization of RNA polymerase II resistant to α -Amanitin from the mushroom *Agaricus bisporus*

(1979) *Biochemistry*, 18 (5), pp. 795-803.

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Abstract

The DNA-dependent RNA polymerase II or B (ribonucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6) from the mushroom *Agaricus bisporus* has been purified to apparent homogeneity. The purification procedures involve precipitation with polyethylenimine, selective elution of RNA polymerase II from the polyethylenimine precipitate, ammonium sulfate fractionation, DEAE-cellulose chromatography, CM-cellulose chromatography, and exclusion chromatography on Bio-Gel A-1.5M. With this procedure 11 mg of RNA polymerase II is recovered from 1.5 kg of mushroom tissue. RNA polymerase II from *Agaricus bisporus* has 12 subunits with the following molecular weights: 182 000, 140 000, 89 000, 69 000, 53 000, 41 000, 37 000, 31 000, 29 000, 25 000, 19 000, and 16 500. Purified RNA polymerase II

from *Agaricus bisporus* was half-maximally inhibited by the mushroom toxin α -amanitin at a concentration of 6.5 $\mu\text{g/mL}$ (7×10^{-6} M), which is 650-fold more resistant than mammalian RNA polymerases II. The apparent K , for the α -amanitin-RNA polymerase complex was estimated to be 12×10^{-6} M. The activity of purified RNA polymerase II from the mushroom was quite typical of other eukaryotic RNA polymerases II with regard to template preference, salt optima, and divalent metal cation optima.

Document Type: Article

Source: Scopus

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