

Advances in Mushroom Research in the Last Decade

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Summary

There has been a lot of progress in mushroom science and biotechnology in the last decade. The optimization of PFGE separation of fungal chromosomes allowed the study of the molecular karyotype of mushrooms and the assignment of genes to chromosomes. There are 115 genes encoded from different species of mushrooms. Cross breeding continues to be the principal method, but it is accompanied by the analyses of RAPD or RFLPs methods. The genetic markers are used and introduced into commercial large hybrids *via* introgression breeding. The complex traits such as yield, resistance to disease and quality characteristics, and quantitative traits more than one quantitative trait locus (QTL) are found and used in practice. The transformants or transgenic mutant strains were obtained by *Agrobacterium* system or particle bombardment. At least 651 species representing 182 genera of hetero- and homobasidiomycetes mushrooms were researched containing anti-tumor or immunostimulating polysaccharides. Ergosterol in the lipid fraction was identified as one of the most active constituents. New sesquiterpenoid hydroquinones, steroids, oxalic acid, triterpenes, water-soluble lignins, sulfated polysaccharides, protein-bound polysaccharides are researched intensively as antimicrobial or antiviral agents. Many small molecular mass compounds exhibit cytotoxic activities, such as illudins, leaianafulvene, triterpenes (ganoderic acids), acetoxyscirpenediol, ergosterol peroxide, sterols. There are many other compounds or activities found in the mushrooms, such as antioxidative, hypoglycemic action, anti-inflammatory effect, hepatoprotective compounds, psychoactive compounds and activities.

Key words: cytogenetics, genetic breeding, antimicrobial, antitumor, mushrooms, dietary supplement

Introduction

For millennia, mushrooms have been valued as edible and medical provisions for humankind. With the popularization of mushroom farming and/or industrialization, mushroom production worldwide continues to increase. It is estimated that more than 10 million metric

tonnes of edible and medicinal mushrooms were produced last year in various countries (1). Mushroom production can convert the huge lignocellulosic waste materials into a wide diversity of products (edible or medicinal food, feed and fertilizers), protecting and regenerating the environment. In addition, the mushroom production can generate equitable economic growth that

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has already had an impact at national and regional levels. This impact is expected to continue increasing and expanding in the future, because more than 70 % of agricultural and forest materials are nonproductive and have been wasted in the processing. The mushroom conversion has been named the »non-green revolution« (2,3). However, the mushroom science is a relatively new applied science and the mushroom industry is still small compared to many plant crops, so the investment is limited. As a consequence, scientific research on mushrooms generally lags behind that of plant and animal (4). This article deals with the advances of mushroom science and biotechnology in the last decade.

Genetics and Breeding of Mushrooms

Cytogenetic study

The molecular karyotype of mushrooms has not been fully clarified yet because of small size of the chromosomes and the occurrence of endomitosis (5). Different chromosome numbers and genome sizes have been reported by using various analytical techniques, including microscopy, restriction fragment length polymorphism (RFLP) analysis, and PFGE. However, the results are sometimes contradictory, for example, the number of chromosomes in *Pleurotus ostreatus* ranges from 6 to 10 (5–7). In the last decade, the optimization of PFGE separation of fungal chromosomes (7) allowed the study of molecular karyotype of mushrooms and the assignment of genes to chromosomes. The genome of *Agaricus bisporus* consists of 13 chromosomes, and its total size is 31 Mbp (8). *Pleurotus ostreatus* contains 11 pairs of chromosomes with sizes ranging from 1.4 to 4.7 Mbp (9). There is no report for other mushrooms in this respect.

Encoded genes of the principal mushrooms

Gene cloning is one of the most active areas in mushroom science. Along with the PCR-based techniques and equipments popularized in the laboratory, the genes have been cloned rapidly and encoded with complete cds, other than partial cds before. According to the National Center of Biotechnology Information (NCBI), the encoded genes of the principal mushroom species (edible and medicinal mushrooms) were included in this respect. Here are counted just the genes with complete cds, excluding the genes with partial cds (or regions), ribosomal RNA genes, mitochondrial complete genome or genes for mitochondrial product, ITSs. The largest group is from *Coprinus cinereus*, which includes 115 encoded genes, such as laccase precursor genes, mating-type protein $\beta 1$ (b1) genes and alleles, pheromone receptor and precursor genes. There are 87 genes encoded in *Schizophyllum*, 46 in *Pleurotus*, including *P. ostreatus*, *P. sajor-caju*, *P. cornucopiae*, *P. eryngii* and *P. djamor*. There are 25 genes encoded in *Agrocybe* and in *Lentinula*, 23 genes encoded in *Agaricus bisporus*, 12 genes in *Genoderma*, 7 in *Flammulina*, 6 in *Polyporus* and *Pholiota*, 3 in *Hericium* and *Grifora*. There are 2 genes encoded in *Volvariella volcaeae*, and 1 in *Auricularia polytricha*. The genes detailed can be checked in the GenBank (10).

Breeding strategies

High production and good quality are always the principal goals for agriculturally important crops, without the exception of mushrooms. There are a lot of breeding methods, such as mass selection based on the natural chance mutation and programmed mutation, including ionizing radiation (X-rays, neutrons, γ -rays, laser beams, ultraviolet light) and chemicals, cross breeding and transgenic breeding. However, the cross and transgenic breeding are more objective and promising, and have made a lot of progress from the theory to the practice in the last decade.

Cross breeding

Since the first cross breeding, which happened in 1983, generating two *Agaricus bisporus* hybrids Horst U1 and Horst U3 (11), a lot of hybrids have been created, including *Lentinula edodes* (12,13), *Pleurotus* and other mushroom species (12). The breeding of strains has traditionally been accomplished by trial and error, and large numbers of hybrids, obtained by pairing monosporic cultures, need to be cultivated to evaluate the production characteristics, accompanied by analyses of RAPD or RFLPs methods (14–16).

However, there has been an enormous increase during the last decade in the efficiency of generating DNA markers by PCR based techniques. Repetitive DNA sequences are in this respect very useful because a number of markers can be generated in each individual PCR reaction. Advanced computer software is available that can use the experimental data to generate genetic maps. Thus comes the era of the genetic maps that guide the cross breeding objectively and accurately (17–19).

The use of genetic makers is straightforward for monogenic traits that segregate in distinct phenotypes such as cap colour in mushrooms (20,21). Previously, genetic analyses have shown that the cap colour of *Agaricus bisporus* is mainly determined by one single locus on chromosome 8 and that the brown allele is dominant (22,23). An isoenzyme marker linked to the trait was found and the molecular markers based on repetitive elements that are very tightly linked to the cap colour were generated. These markers have been used to introduce the brown cap colour of a wild line into a commercial large hybrid *via* introgression breeding (22). Another example is the introduction of sporeless trait to the commercial strain of *Pleurotus ostreatus*, because most people that handle and harvest this crop develop respiratory disease (4,21,22).

However, complex traits such as yield, resistance to disease and quality characteristics are usually inherited quantitatively. For quantitative traits, more than one quantitative trait locus (QTL) is normally found. Larraya *et al.* (24) found 1 to 4 QTL for each of several diverse agronomic traits in the oyster mushroom *P. ostreatus*. Moquet *et al.* (25) found only one QTL for sensitivity to bacterial blotch caused by *Pseudomonas tolaasii* in *A. bisporus*. Sonnenberg *et al.* (4) found two significant QTL in one homokaryon of the wild line and five QTL in the other wild homokaryon of *A. bisporus* resistant to *Verticillium fungicola*. After some intercross single spore iso-

lates (SSIs) of the backcross, some hybrids proved to be less sensitive to infection.

Transgenic breeding

The advent of a tractable method for DNA transfer in mushroom has created new vistas for the genetic enhancement. The first transformant was of *Agaricus bisporus* by the end of 1993 (26–28). The recipient strain used initially was the adenin-auxotrophic strain (ATCC 24663), which appeared to form protoplasts very efficiently and responded well to the selection with hygromycin. Later on, the protocol also appeared applicable to a derivative of commercial strain Horst U1 (ATCC 62462). Fertile crosses between the transgenic mutant strain and a U1 homokaryon were easily obtainable. After filing a PCT patent (28) and the first communication at the Vancouver Congress in 1994 (29), this work was published as well as the results on highly efficient homologous, site-directed integration of the transformation plasmid that contained the *A. bisporus* β -1,3-glucanase gene (30,31). The first target was to study mushroom browning, for which *A. bisporus* tyrosinase genes were isolated and one of them was introduced in the antisense orientation. Either approach using site-directed integration or antisense inhibition was supposed to provide straightforward tools for gene silencing in *A. bisporus*. However, the multinuclear nature of fertile *A. bisporus* mycelia presented an additional problem (32,33). Another challenge was to isolate and identify the *A. bisporus* mannitol dehydrogenase (MtDH) gene. With mannitol being the most abundant metabolite (up to about 50 % dry mass and excluding water), white button mushrooms might as well be considered mannitol-rooms. In collaboration with a Swiss group (J. Sassoon and U. Baumann, University of Bern), the MtDH 3-dimensional structure has become available (34,35), which would now allow, in principle, the generation of transgenic mushrooms containing altered mannitol profiles or altered MtDH enzymes and, thus, would allow the study of the function(s) of mannitol. Ultimately, such activities might also yield new commercial strains with, for example, a higher dry matter content or better pathogen resistance.

Other transformation protocols

More recently, a novel transformation protocol for different fungal species, including commercial heterokaryotic *A. bisporus* strain Horst U1, has been described (27,35), which is based on infection with the plant-pathogenic bacterium *Agrobacterium tumefaciens* and induction of its virulence (*vir*) gene with the plant hormone acetosyringone. Chen *et al.* (36) have 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 mushroom species. The *Agrobacterium* system allows the transformation of both homokaryons and heterokaryons of *A. bisporus*. Also, both karyotypes of a heterokaryon can be transformed simultaneously. Furthermore, the first report on the transformation of vegetative mycelium of a commercial strain of *A. bisporus* appeared (37).

An alternative way to introduce donor DNA into a vast number of organisms, including many plant and fungal species, and even intact tissues, is particle bombardment (38). This technique is based on gas-driven bombardment with tungsten or gold particles coated with the donor DNA, thus penetrating the recipient tissue. A number of reports describe its use for transformation of intact plants and for a number of fungi (38). Guo *et al.* (39) reported that the thermal hysteresis protein gene was integrated into *Volvariella volvacea* genome through particle bombardment. The transformants showed stronger cold tolerance than the host strain.

Active Compounds of Mushrooms

Antitumor polysaccharides or peptidoglycan

Polysaccharides or peptidoglycan, pharmaceutically active mushroom compounds, continue to be the subject of most researches, including isolation, chemical structures and experiments *in vitro* or *in vivo*. Ten years ago the researches were concentrated on the four mushrooms, *Lentinus* (*Lentinula*) *edodes*, *Schizophyllum commune*, *Grifola frondosa*, and *Sclerotinia sclerotiorum*, particularly their respective β -glucans, lentinan, schizophyllan (also called SPG, sonifilan, or sizofiran), grifolan, and SSG. Most of them, β -(1-6)-branched β -(1-3)-linked glucans, were found to exhibit significant antitumor activity (40). In recent years, little additional research has been conducted with these four mushrooms, but a host of other species has been investigated and a variety of species has been explored (41,42). At least 651 species representing 182 genera of hetero- and homobasidiomycetes mushrooms contain antitumor or immunostimulating polysaccharides (43–46).

There are also several reports of mushrooms containing more than one polysaccharide with antitumor activity (47). An interesting example is *A. blazei*. It contains an antitumor glucan with a β -1,6 backbone (48–50), which differs from the β -1,3 backbone with β -1,6 branches shared by many other antitumor glucans. In addition, a glucomannan with the main chain of β -1,2-linked D-mannopyranosyl residues has been isolated from this mushroom and found to inhibit tumorigenesis (50).

There is evidence that β -D-glucans induce a biological response by binding to membrane complement receptor type 3 (CR3, alpha Mb2 integrin or CD11b/CD18) on immune effector cells. The ligand-receptor complex can be internalized. The intercellular events that occur after glucan-receptor binding have not been fully determined until now (51). In a recent experimental approach it has been shown that schizophyllan produced by *S. commune* is able to bind the mRNA poly(A) tail (52). Molecular mass, degree of branching, number of substituents, as well as ultrastructure, including the presence of single and triple helices, significantly affect the biological activities of β -glucans (53–55). Higher antitumor activity seems to be correlated with higher molecular mass, lower level of branching and greater water solubility of β -glucans (40). However, the high branched MD-fraction from *G. frondosa* ($M_r = 1\ 000\ 000$ – $1\ 200\ 000$ Da) exerts a high antitumor activity (56).

Other antitumor compounds

The lipid fraction of *A. blazei* was found to contain a compound with antitumor activity, subsequently identified as ergosterol (57). The lipid fraction of *Grifola frondosa* exhibited antioxidant activity and inhibited the cyclooxygenase enzymes, COX-1 and COX-2 (56,58). Ergosterol was again identified as one of the most active constituents. Oxidative damage is strongly implicated in the development of many chronic diseases, including cancer. The inducible form of COX, COX-2, also appears to play an important role in certain cancers. Its inhibition can result in the inhibition of tumor development, and it appears to be beneficial even in some established tumors (59). Other mushroom constituents may inhibit promotion or progression by exerting direct cytotoxicity against tumor cells (60), interfering with tumor angiogenesis, or upregulating other nonimmune tumor-suppressive mechanisms.

Antibacterial compounds

New sesquiterpenoid hydroquinones, produced by the European *Ganoderma* species *Ganoderma pfeifferi* Bres. and named ganomycins, inhibit the growth of methicillin-resistant *Staphylococcus aureus* and other bacteria (61). Besides, whole extracts of this mushroom inhibit the growth of microorganisms responsible for skin problems (*Pityrosporum ovale*, *Staphylococcus epidermidis*, *Propionibacterium acnes*, unpublished results). Steroids like 5 α -ergosta-7,22-dien-3 β -ol (3) or 5,8-epidioxy-5 α ,8 α -ergosta-6,22-dien-3 β -ol (62,63), isolated from *Ganoderma applanatum* (Pers.) Pat., proved to be weakly active against a number of Gram-positive and Gram-negative microorganisms (63). Oxalic acid is an agent responsible for the antimicrobial effect of *Lentinula edodes* (Berk.) Pegler against *S. aureus* and other bacteria (64). Ethanolic mycelial extracts from *L. edodes* possess antiprotozoal activity against *Paramecium caudatum* (64). The antimicrobial activity of *Podaxis pistillaris* (L.: Pers.) Morse, used in some parts of Yemen for the treatment of nappy rash of babies and in South Africa against sun burn (65), is caused by epicoazines. These substances belong to the group of epipolythiopiperazine-2,5-diones, an important class of biologically active fungal metabolites (65). Other antimicrobial compounds from the Aphyllphorales were summarized by Zjawiony (54).

Antiviral compounds

Several triterpenes from *Ganoderma lucidum* (M.A. Curtis: Fr.) P. Karst. (*i.e.* ganoderiol F, ganodermanontriol, and ganoderic acid B) are active as antiviral agents against human immunodeficiency virus type 1 (HIV-1). Ganoderic acid B inhibits HIV-1 protease with an IC 50 value of 0.17 mM (66,67). Ganodermediol, lucidadiol and applanoxidic acid G, isolated from *G. pfeifferi*, but also known from other *Ganoderma* species, possess *in vitro* antiviral activity against influenza virus type A. Further, ganodermediol is active against herpes simplex virus type 1, causing lip exanthema and other symptoms (68). *In vitro* antiviral activity against influenza viruses type A and B was demonstrated for mycelial extracts of *Kuehneromyces mutabilis* (Schaeff.: Fr.) (69), two isolated phenolic compounds from *Inonotus hispidus* (Bull.: Fr.) P.

Karst (70) and ergosterol peroxide, present in several mushrooms (67,70–72).

Water-soluble lignins isolated from *Inonotus obliquus* (Pers.: Fr.) inhibited HIV protease with an IC 50 value of 2.5 mg/mL (70). Anti-HIV activities were reported for mycelial culture medium of *L. edodes* (LEM) and water-soluble lignin in LEM (71,72). Sulfated lentinan from *L. edodes* completely prevented HIV-induced cytopathic effect (72). The protein-bound polysaccharides PSK and PSP from *Trametes versicolor* (L.: Fr.) were also found to have an antiviral effect on HIV and cytomegalovirus *in vitro* (71). Besides immunostimulation, other effects of the polysaccharide–protein complexes contribute to the antiviral activity, *e.g.* inhibition of binding of HIV-1 gp120 to immobilized CD4 receptor and of reverse transcriptase activity of viruses (72). Inhibition of HIV-1 reverse transcriptase was caused by velutin, a ribosome inactivating protein from *Flammulina velutipes* (M.A. Curtis: Fr.) P. Karst (73). The maitake D-fraction (MD-fraction) from *Grifola frondosa* (Dicks: F) S.F. Gray was tested in a long-term trial with 35 HIV patients. A total of 85 % of responders reported an increased sense of well-being with regard to various symptoms and secondary diseases caused by HIV. Twenty patients showed an increase in CD4+ cell counts to 1.4–1.8 times and eight patients a decrease to 0.8–0.5 times (74).

Cytostatic compounds

Many small molecular mass compounds exhibit cytotoxic activities against tumor cells. To them belong the illudins, tricyclic sesquiterpenes from *Omphalotus olearius* (DC.: Fr.) Singer and *Lampteromyces japonicus* (Kawam.) Singer and their derivatives (75), the terpenoid leaianafulvene (10) from *Mycena leaiana* (Berk.) Sacc. (76), triterpenes (ganoderic acids Z, Y, X, W, V, T; lucialdehydes A, B, C and australic acid) from *G. lucidum* (77–80) or *Ganoderma australe* (Fr.) Pat., acetoxyscirpenediol, ergosterol peroxide (4) from *P. tenuipes* and sterols from the mycelia of *C. sinensis* (Berk.) Sacc. (77–79). Acetoxyscirpenediol exerts its activity by inducing apoptosis in leukemia cell lines *in vitro* (80). Apoptosis in HL-60 cells could be induced by triterpenes from *Ganoderma concinnum* Ryvarden (81). The triterpenes applanoxidic acids A–H, isolated first from *G. applanatum*, were effective against mouse skin tumor promoters, applanoxidic acid B being the most potent of the acids. The activities were shown in the short-term *in vitro* assay of Epstein–Barr virus early antigen activation in Raji cells induced by 12-O-tetradecanoylphorbol-13-acetate (82,83). A derivative of illudin S has progressed to phase II in human clinical trials (84). The specificity of the cytotoxic action against tumor cells remains to be determined.

Phellinus linteus (Berk. & M. A. Curtis) Teng was found to contain antiangiogenic activity in the chick embryo chorioallantoic membrane assay (85) and to inactivate cancer-related kinases (86). Antiangiogenic activity was also reported for polysaccharides from *A. brasiliensis* (87). The sesquiterpenoid cryptoporin acids A–G from *Cryptoporus volvatus* (Peck) Murrill inhibit the tumor promotion activity of okadaic acid in two-stage carcinogenesis experiments. The effect is possibly related to their strong radical scavenging activity (88). Antimutagenic effects were found for methanolic extracts of *P. ostreatus*,

Lactarius vellereus (Fr.) Fr., and for water extracts of *A. bisporus* and *G. lucidum*. The genoprotective effect of *A. bisporus* has been related to the enzyme tyrosinase (89–91). **A dried oyster mushroom (*P. ostreatus*) diet (5 %) reduced pathological changes in dimethylhydrazine-induced colon cancer in rats but did not influence significantly the incidence of tumors. This effect is explained by the antioxidant properties of this mushroom and by its fiber content (92).**

Antiallergic compounds

Ethanolic extracts of the edible Japanese basidiomycetes *H. marmoreus*, *F. velutipes*, *Pholiota nameko* (T. Ito) S. Ito and *Pleurotus eryngii* (DC.: Fr.) Quél. show significant antiallergic effects in mice (oxazolone-induced type IV allergy) also after *per os* application (93). Some compounds from *G. lucidum* (ganoderic acids C and D, cyclooctasulfur) inhibit the histamine release from rat mast cells (94,95). Eating of *Tricholoma populinum* J. E. Lange led to the regression of severe allergic symptoms in a patient with thromboangitis obliterans and in another patient with urticaria. The effects could be confirmed in animal models, as one responsible compound ergosterol peroxide was identified (96,97). Hispolon and hispidin, isolated from fruit bodies of *Indocalamus hispidus*, inhibit the chemiluminescence response of human mononuclear blood cells and the mitogeninduced proliferation of spleen lymphocytes of mice (98). There are many other compounds or activities found in the mushroom, such as antioxidative, hypoglycemic action, anti-inflammatory effect, hepatoprotective compounds, psychoactive compounds and activities (41).

Mushrooms and/or Mushroom Extracts as Dietary Supplements

There is significant interest in the use of mushrooms and/or mushroom extracts as dietary supplements based on the facts that they have a lot of active compounds. However, there are few experiments about epidemiologic and experimental studies that address the biological activities of whole mushrooms or crude extracts by oral administration to humans. There has only been one epidemiological study assessing the association between mushroom intake and cancer, more specifically gastric cancer (99). An inverse correlation between mushroom intake and the risk of gastric cancer was found. Using the risk of gastric cancer in relation to low mushroom intake as the reference value, the probability ratio was 0.38 (95 % confidence interval, CI=0.21–0.66) for medium intake and 0.30 (CI=0.15–0.62) for high intake.

An aqueous extract of *Agaricus blazei* Murill in the drinking water given to rats and mice before chemical cancer induction exhibited antimutagenic effects but was ineffective when administered in the postinduction period (100–102). Interestingly, the same strain of *A. blazei* fed in dry powdered form to rats at 10 % of the diet exhibited significant antimutagenic activity even when given in the postinitiation period, suggesting that some active constituent was lost during the aqueous extraction (103). Similar antimutagenic effects were reported for diets containing powdered *L. edodes* (shiitake) (104,

105). These results suggest they could potentially provide additive, or even synergistic effects in the prevention and treatment of cancer.

Oral administration of whole mushroom or extracts to human cancer patients is the most important. A study was conducted with orally administered polysaccharide peptides (PSP) isolated from *Coriolus versicolor* in a total of 68 patients with advanced (stages III or IV) non-small cell lung cancer (106). The results show that leukocyte and neutrophil counts rose significantly after PSP treatment, whereas they decreased in the control group. Total IgG and IgM levels were significantly increased in the PSP group but not in the control group, with the difference between the groups being statistically significant. A phase I/II study was conducted with GanoPoly, crude polysaccharide fraction of *G. lucidum* (Curt.: Fr.) P. Karst, 600 mg given orally three times a day to patients with advanced cancer (107). In a case series of eight patients with various cancers (mostly stage III, 1 stage II, 1 stage IV), who were given 100 mg of D-fraction, a polysaccharide isolated from *G. frondosa*, daily for up to 34 months, there was an, at times marked, increase in natural killer cell activity (108). Based on a list of reduced or enhanced parameters, the same group reported that a positive response occurred in 23 of 36 cancer patients who took a combination of MD-fraction (which appears to be identical to the D-fraction) and maitake (109).

As can be seen, only a few mushroom-derived substances are classified as drugs (lentinan, krestin and schizophyllan), the majority have been developed as dietary supplements. The principal mushroom products or derivatives are in the form of powders, capsules or tablets, made of dried fruiting bodies, extracts of fruiting bodies or mixtures; or extract of mycelium with substrate, biomass or extract from liquid fermentation (110). The US dietary supplement sales were estimated to be US\$14.0 billion in 2000 (111). It is believed that the mushroom dietary supplements will be recognized and marketed extensively.

Conclusion

There has been a lot of progress in mushroom science and biotechnology in the last decade. The optimization of PFGE separation of fungal chromosomes allowed the study of the molecular karyotype of mushrooms and the assignment of genes to chromosomes, such as with 13 chromosomes of *A. bisporus* (31 Mbp) and 11 pairs of chromosomes of *P. ostreatus*. According to the GenBank, there are 115 genes encoded from *Coprinus cinereus*, 87 genes in *Schizophyllum*, 46 in *Pleurotus*, 25 genes encoded in *Agrocybe* and *Lentinula*, 23 genes encoded in *Agaricus bisporus*, 12 genes in *Genoderma*, 7 in *Flammulina*, 6 in *Polyporus* and *Pholiota*, 3 in *Hericium* and *Grifora*. There are 2 genes encoded in *Volvariella volcaeae*, and 1 in *Auricularia polytricha*.

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The transformants or transgenic mutant strains were obtained by *Agrobacterium* system or particle bombardment.

Pharmaceutically active mushroom compounds continue to be the main subject of most researches, including isolation, chemical structures and experiments *in vitro* or *in vivo*. At least 651 species representing 182 genera of hetero- and homobasidiomycetes mushrooms contain antitumor or immunostimulating polysaccharides. The lipid fraction was found to contain a compound with antitumor activity, subsequently identified as ergosterol. The lipid fraction exhibited antioxidant activity and inhibited the cyclooxygenase enzymes. Ergosterol was again identified as one of the most active constituents. Its inhibition can result in the inhibition of tumor development, and it appears to be beneficial even in some established tumors. Other mushroom constituents may inhibit promotion or progression by exerting direct cytotoxicity against tumor cells, interfering with tumor angiogenesis, or upregulating other nonimmune tumor-suppressive mechanisms.

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