Breeding for the Future: New Technologies and New Targets

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Abstract

Mushroom breeding is still a rather applied science with traditional breeding targets mainly directed towards growers (yield, quality, shelf life). The unique combination of traits in mushrooms such as nutritional value and bioactive compounds offers new breeding targets directed to the needs of consumers and industrial users. In addition, mushrooms can degrade a wide range of complex organic substrates, while producing a vast amount of biomass in a relatively short time. This characteristic attracts attention of industries interested in upgrading organic waste streams. Variation within mushroom species with respect to these characteristics has hardly been addressed so far. These new breeding targets and the rapidly evolving breeding techniques offer an enormous opportunity for mushrooms to change from a minor crop or food ingredient to an prominent product comparable to many plant crops.

Keywords. Mushroom Breeding, Breeding Technologies, Genetic Variety, Health Food, Biobased Economy

Introduction

Mushrooms represent a small crop compared to the staple crops such as Maize, potatoes and barley but the production of mushrooms is worldwide increasing steadily and compares now in annual production to crops as Citrus and Asparagus (http://faostat. fao. org/; Sonnenberg et al. , 2011). In Europe, however, growth in consumption of edible mushrooms is stagnating leading in some countries to overproduction of low prizes (Van Griensven, 2009). An increase in consumer awareness of nutritional values and mushrooms as a rich source of bioactive compounds (Wani et al. , 2010) would certainly help to stimulate mushroom consumption. For this, research is needed that underpins the beneficial effects of mushrooms after consumption. Next to that, breeding can also help to improve the quality and quantity of nutritional and other beneficial compounds in mushrooms. So far, breeding has played a minor or no role at all in improving these traits and has been mainly directed towards the need of mushroom growers with yield, shelf life and disease resistance as the main characteristics. In order for breeding to play a role, genetic variation between varieties within species must exist. That issue has hardly been addressed but a few examples that will be discussed in this article show that variation in quantity and quality of bioactive compounds is likely to exist and that collections of varieties may harbor large genetic resources for many interesting traits. Mushroom breeding is still not a very advanced science and it is practiced by a limited number of research groups and companies. Plant breeding, on the other hand, is an advance science and quick progress is being made in breeding techniques to satisfy the increasing demand for high quality of food. Many of the newly developed plant breeding techniques can be used by mushroom breeders and will certainly help in generating new products directed to consumer demands and processors of mushrooms.

New Breeding Techniques

The world population increases to approximately 9 billion people in 2050. Next to change in consumers behavior and climate changes this would require raising overall food production by some 70% between 2005/07 and 2050 according to the FAO (FAO report: http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper). In order to reach this goal, new plant breeding techniques beyond the traditional genetic modification are needed (Lusser et al., 2012). Some are experimental, others are in use and the first crops obtained through new plant breeding techniques are close to commercialization. Whole genome sequences available for many crop species facilitate the use of these new breeding techniques. For a number of edible fungi whole genome sequences are now also available in publically accessible databases (http://genome.jgi-psf.org/). A few of the new breeding techniques are relevant and useful for mushroom breeding too and are not considered as GM techniques and thus acceptable for most consumers.

One of these techniques is TILLING (Targeting Induced Local Lesions IN Genomes), a technique where traditional chemical mutagenesis is followed by high-throughput screening for point mutations (Slade and Knauf, 2005). Strains with the desired mutation are subsequently used in repeated back crossings to the original non-mutated variety to restore all mutations except for the target gene. New strategies have been designed recently that make the usually complex and time consuming mapping and identification of mutations easier (Pomraning et al., 2011; Zuryn et al., 2010). The technique has already been used successfully in fungi (Liu et al., 2011; Pomraning et al., 2011).

Another technique is RNA-dependent DNA methylation (RdDM). By introducing a short RNA sequence that has the same sequence as the promotor of a gene, the gene is silenced by methylation of some of its nucleotides in the promotor (Mette et al., 2000). This is one of a number of mechanisms that most organisms have to regulate gene activity. RdDM allows breeders to produce plants that do not contain foreign DNA sequences and in which no changes or mutations are made in the nucleotide sequence but in which gene expression is modified epigenetically. Previous research has shown that the genes involved in methylation are present and active in most fungi that produce edible mushrooms. Although not used yet, this indicates that this technique will work and might be used soon for breeding in edible mushrooms too.

A third interesting plant breeding technique is grafting. Grafting is a horticultural technique whereby tissue of one plant is inserted into that of another plant so that the two sets of vascular tissues may join together. An example is stem grafting in cherry cultivation where a shoot of one variety is grafted onto a stem of another variety. One of the advantages of this system is that varieties can be used that are optimized for different purposes. In case of cherry grafting, the stem can be adapted to certain soils and diseases whereas the shoot is optimized for fruiting. The cultivation system of the button mushroom *Agaricus bisporus* is in principle suitable for grafting-like cultivation techniques. Button mushrooms are cultivated in a two layer system, i.e. the bottom layer consists of substrate and a top layer of a nutrient poor medium that consists of peat and lime stone (casing soil). The latter layer induces the aggregation of mycelium and the formation of fruiting bodies when temperature is decreased and fresh air is introduced in the growing room (venting). In some countries both layers are inoculated with different spawn types of the same variety. Woolston et al. (Woolston et al., 2011) elegantly show that as with grafting in plants, in a crop of button mushrooms also two different strains/inocula can be used. They used a genetically modified strain in the substrate that produces a fluorescent protein. The same, non-modified strain was used to inoculate the casing layer. The fluorescent protein produced by the GMO strain in the substrate was translocated to the fruiting bodies that did not contain any of the genetic material that produces the fluorescent protein (neither DNA nor RNA). This dual system demonstrated nicely that fruiting bodies were solely constructed from mycelium inoculated in the casing layer. That offers opportunities for breeding strains directed to substrate degradations on one hand and strains optimized for quality of fruiting bodies on the other hand. Prerequisite is that both varieties are vegetative compatible.

The button mushroom *A. bisporus* offers also unique breeding techniques due to its typical life cycle. It represents a species with two different types of sexual propagation. The majority of strains found in nature and all commercially cultivated varieties have a secondary homothallic life cycle. Most basidia produce two spores each receiving two non-sister haploid nuclei. This usually leads to pairing of opposite mating types and spores germinate into fertile mycelia (heterokaryons). Only a minor number of basidia produce three or four spores, with spores receiving one haploid nucleus (homokaryons). In the other type of sexual propagation, most basidia produce four spores, each receiving one haploid nucleus and germinate into infertile mycelia (Callac et al., 1993). Using SNP and other markers derived from the whole genome sequence, meiosis was studied in both types. This showed that, next to the different number of spores produced per basidium, both types also show a remarkable difference in recombination frequency between homologous chromosomes in meiosis I (Sonnenberg et al., 2011). Whereas the four-spored type shows an apparently normal recombination frequency, recombination is almost absent in the two-spored variety. Both nuclear types in the heterokaryotic spores from the bisporic variety were recovered by protoplasting and were shown to have a complementary set of parental chromosomes. Previous cytological analysis of the bisporic variety has shown that pairing of homologous chromosomes in synaptonemal complexes is rare in the two spored variety (Mazheika et al., 2006) and may thus explain the almost absence of meiotic recombination. This generates heterokaryotic spores with the same allelic constitution as the parental line but with a different distribution of chromosomes over the constituent nuclei. This causes a phenotypic effect and has been used frequently to generate new, derived varieties from the first commercial hybrid released in 1980. The absence of recombination also generates chromosome substitutions in the rare haploid spores of the two-spored variety and can thus be used to study phenotypic effect per chromosome and breeding per chromosome.

New Breeding Targets

Breeding projects are now primarily directed to the needs of mushroom growers. Characters such as yield, shelf life and disease resistances are main breeding issues. Consumers and food processors, however, have different interests such as taste, nutritional values, processing quality and bioactive compounds. There is a vast amount of literature available on these issues in edible and medicinal mushrooms (Chatterjee et al., 2011; Cheung, 2010; Guillamón et al., 2011; Mau, 2005; Wani et al., 2010). However, knowledge on the variation in quantity and quality of these compounds with respect to genetic variation within species is almost nonexistent. A literature search on genetic variation within mushroom species and nutritional value or other compounds results in a very limited number of papers, with *Agaricus brasiliensis* as an exception (Largeteau et al., 2011). Without this knowledge, data published on nutritional values and other characteristics of mushroom species cannot be extrapolated to all varieties within each species as is often done now. Knowledge of this variation is also a prerequisite for breeders. Since breeding of mushrooms is still a rather applied science and done on a very limited scale, it is not surprising that genetic diversity with respect to quality and nutritional components has hardly been addressed so far. Nevertheless, a few examples show that variation exists and is strongly related to the genetic variation within species. Thomassen et al. (2011) used lentinan extracts of different *Lentinula edodes* strains to study effects of these extracts on cells of the immune system. This was done by assessing the production of nitric oxide (NO) by challenged macrophages. Next to differences due to storage of lentinan, large differences were found on the inhibition of NO production by lentinan isolated from different strains. The difference in inhibition of NO production measured between the best and the worst strain amounts up to fifteen times. Van de Velde et al. (unpublished results) measured the effect of β-glucan extracted from different varieties of the button mushroom *Agaricus bisporus* on the production of the anti-inflammatory cytokine interleukine 10 (IL 10) and pro-inflammatory cytokine interleukine 6. Large variations were seen in the production of both cytokines between the varieties tested. The extracts of commercial lines hardly induced the production of IL 10 and IL 6 but some wild isolates have a considerable effect (Fig. 1). It is expected that variations also exists for nutritional values and other health beneficial compounds. This variation within species is an excellent source for breeders to improve quality and quantity of bioactive compounds in mushroom varieties and can generate many new products.

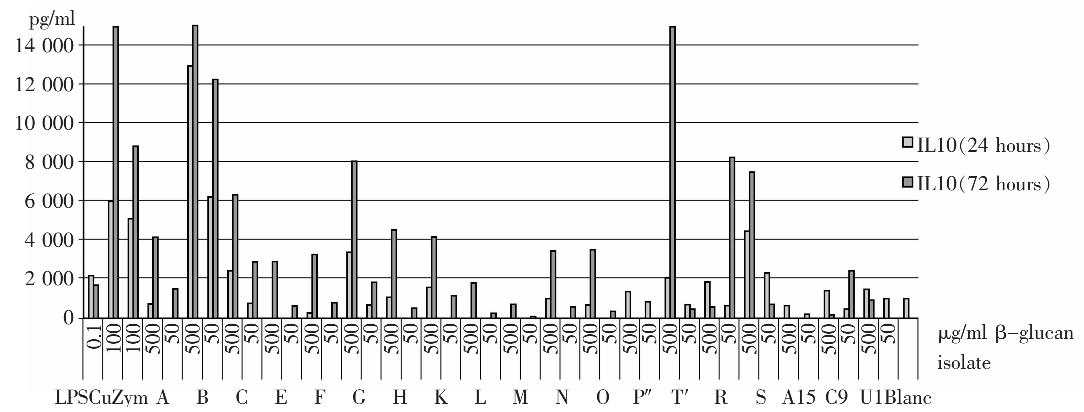

Fig. 1 Effects of β-glucan extracts from different button mushroom varieties on the production of anti-inflammatory interleukine 10 in a immune cell line. Production of IL 10 were measured 24 and 72 hours after induction. Extracts of wild lines (A-S) and commercial lines (A15, C9 and U1) varied enormously in the induction of IL 10.

Mushroom producing fungi have evolved in millions years to become specialists in degradation of complex organic matter. For this they produce a number of oxidative enzymes that can be used either for bioremediation of hazardous chemicals (Harms et al., 2011) or to valorize organic waste products (Lomascolo et al., 2011; Salvachúa et al., 2011; Tuyen et al., 2012). These fungi especially degrade lignin during vegetative growth to gain access to cellulose and hemicellulose that is subsequently used for the production of mushrooms. Incubation of different organic waste products with these fungi can thus replace the chemical pretreatment, thereby reducing costs and preventing the production of chemical wastes. Literature on this subject is mainly based on the use of different species and hardly any data are available on the variation in efficiency related to genetic variations within a species. One of the few exceptions found in literature shows that classical breeding is a promising tool to improve fermentation efficiency in fungi. Lettera, et al. (2011) made crosses between *Pleurotus ostreatus* var. *florida* and *P. ostreatus* var. *ostreatus* and the excreted laccase activity of some of the newly formed hybrids were 3 – 5 times higher than those of the parental lines. This offers also new targets for breeding, either towards the production of enzymes for different biotechnological purposes or towards improved strains for in situ upgrading of organic waste streams.

Conclusions

Mushrooms have a unique combination of a number of traits related to nutritional values and bioactive compounds. In addition, mushrooms represent fungi that are able to upgrade organic waste to valuable products. The genetic variation within each species with respect to these traits has hardly been addressed so far. With the availability of new breeding techniques it is possible to create new mushroom varieties that have much larger share in the food (or feed) market than at present.

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