Edible and medicinal fungi breeding techniques, a review: Current status and future prospects

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\textbf{ABSTRACT}

Mushrooms of the edible and medicinal which are highly nutritious and environmentally friendly crops carry numerous medicinal benefits. For the abundant and high diversity of bioactive metabolites they possess, which are considered to be an important pool of bioresources. The efficient breeding technique is always a challenging task in mushrooms for obtaining better character strains, which are essential for developing healthy products and even consumption. This review comprehensively summarizes the breeding techniques applied to the edible and medicinal mushrooms. Including the traditional mutagenesis method, and even modern gene-editing breeding techniques, the effects of each method, and the comparison of each breeding technique are systematic illustrations. Strategies for mushroom breeding techniques in the future are also discussed in this review paper. With the ongoing sequencing of the mushroom genome, knowledge of the gene background of the strains and functions can be available for developing better markers for gene-editing breeding as CRISPR/Cas9 systems. Combine the metabolism engineering and in-silico tools analysis was the rational design of the novel strains. Modern physical mutagenesis techniques such as the ARTP and the combination of the other physical, and chemical breeding mutagens with cross-breeding techniques or the protoplasts fusion will also lead to superior strains for cultivation and pave the way for higher quality and yield.

1. Introduction

Mushrooms have achieved significant importance due to their nutritive and medicinal values and as an income generating industry in the world. Especially for the wild edible and medicinal mushrooms (WEMM) with high nutritional and economic value (Khaund and Joshi, 2015). They have become an attractive functional food in the human diet in both developing and developed countries (S. Nan Zhao et al., 2020), (Carrasco and Preston, 2020), (Cohen et al., 2014). Besides the nutritional value, about 130 therapeutic actions are thought to exist in edible and medicinal mushrooms and fungi (Chang, 1996). Including antitumor, antidiabetic, immunomodulating, antiviral, antioxidant, detoxivating, hepatoprotective effects, etc. (Wasser and Didukh, 2003). China is the earliest country in the world to recognize and use edible fungi, and is the largest mushroom producer over the world. The annual mushroom production in China accounts for 80% of the world. China also has the largest number of mushroom researchers on both fundamental science and applied research (J. Zhang, 2014). Through the support of science and technology, edible and medicinal mushrooms industry can achieve the integrated development of primary, secondary and tertiary industries.

The development of cultivation and breeding techniques which contribute to “Relief and Safety” and “Delicacy” of mushrooms and research which shows clearly that it is healthy to eat mushrooms regularly are expected to lead to new growth of mushroom production in the future (TAKABATAKE, 2015). Efficient breeding techniques has to play an important role for an enlarge production of desirable strains as higher yield and more biocompounds stains for commercial use or on a large scale, for it can use the available genetic resources to adapt strains to these demands. In addition, increasing the quality of the mushrooms

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that resistance to diseases are vital for the mushroom research and breeding. Microbial breeding by altering the genomes is of great importance for biotechnology research and the bio-industry (X. Zhang et al., 2014). Artificial breeding as traditional mutagenesis (chemical: EMS mutagenesis (Z. Liu, Zhang, Lin and Guo, 2011) and physical: Laser (Zhu et al., 2020), ARTP (H. Zhang et al., 2019), etc.) and even modern molecule breeding have been applied to obtain desirable strains which could be potential for industrial-scale use. For the traditional breeding techniques often time consuming to obtain desired strains and low mutagenesis rate, and random, with many mutagenesis sites. Thus the trends of the breeding focus on the modern breeding techniques, with the characters of direct and relative short time to get desired strains, and function of the genes are clearly studied and for further mushroom breeding.

The breeding methods are according to the type of the strains, and the background information of the strains. In addition, rational screening is equally important to screen out better character strains that accompany with the mushroom breeding. Traditional screening method as the antagonism test (Gloer, 1995), isoenzyme electrophoresis (MCMANUS, 1990), clamp connection (Furtado, 1966), fluorescent staining, drug resistance marker (Kim et al., 1998), temperature sensitive labeling. Recently, molecular markers (A. V. Shnyreva et al., 2003) act as screening marker have been applied widely used to differentiate groups of genetically similar and distant strains.

Mushroom is the fifth in agricultural after grain, vegetables, fruits and oil, that have the great potential for development. Mushroom industry is still small compared to other crops. To boost the mushroom industry especial the mushroom breeding, this paper comprehensive review the breeding techniques and screening methods applied in the edible and medicinal mushroom breeding, which will give the knowledge of the proper breeding techniques used in the different strains, and the future breeding strategies for the mushrooms with higher quality and yield were also discussed in this review.

2. Breeding techniques

In general, mushroom breeding techniques include various methods like traditional mutagenesis, protoplast fusion, and modern molecule breeding techniques, that have been widely applied. Fig. 1 (a) showed the mushrooms breeding techniques, and the data of published research papers from the web of science were shown in Fig. 1 (b). The part research about the breeding techniques application in the mushrooms was shown in the Table 1.

![Flow chart of the mushroom breeding to obtain better strains](a)

![The papers have been published search on the web of science 5.3.2022](b)
Table 1
Breeding techniques and application in the edible and medicinal mushrooms.

| Breeding techniques and their characters | Test Strains | Main results | ref |
|-----------------------------------------|--------------|--------------|-----|
| Modern breeding techniques CRISPR/Cas9 | Pleurotus ostreatus | Marker-free genome editing; 5-FC resistance and Hyg sensitivity strains were isolated; CRISPR/Cas9 techniques; Accurate regulation one or more genes, the characteristics are the same as the traditional mutagenesis; More efficient, accurate and predictable than traditional mutagenesis; | Koshi et al. (2022) |
| Pleurotus ostreatus | A mutation in eyI via homology-directed repair using this CRISPR/Cas9 system was efficiently introduced | Boontawan et al. (2021) |
| Lentinula edodes | Transcription of critical mating-related genes was impaired by the CRISPR/Cas9-mediated deletion mutation in the HD1 gene. | Moon et al. (2021) |
| Ganoderma species | Disruption of ura3 gene of both *G. lucidum* and *G. lingzhi* was successfully demonstrated | Qin et al. (2017) |
| Pleurotus eryngii | Cloned and introduced a point mutation in an endogenous gene cbx; a highly efficient pyrG gene editing system has been established in *P. eryngii*; | Wang et al. (2021) |
| G. lucidum | Significant decrease in the titer of four identified GAs was found in the mutant compared to WT. The use of pre-assembled Cas9 RNPs in a mushroom-forming basidiomycete and may also improve the genetic accessibility of non-model species; | Wang et al. (2020) |
| Schizopyllum commune | The identification of key genes regulating processes such as fruiting body development and the generation of useful metabolites | Sugano et al. (2017) |
| Coprinopsis cinerea | Increase the production of bioactive strain degeneration delay | Chen et al. (2018) |
| Cordyceps militaris | Block hypocrellin biosynthesis will | Deng et al. (2017) |
| Shiraia bambusicola | Enhance mycelial growth and slower spawn run and creamish white sporophores; | Boontawan et al. (2021) |
| Pleurotus ostreatus | CRISPR/Cas9 techniques; More efficient, accurate and predictable than traditional mutagenesis; | Boontawan et al. (2021) |
| Pleurotus ostreatus | Enhance the production of polysaccharide; | Wang et al. (2018) |
| Phellinus igniarius | Improvement of the polysaccharide production; | Zhang et al. (2018) |
| Phellinus igniarius protoplasts | Improvement of the polysaccharide production; | Zhang et al. (2008) |
| Pleurotus ostreatus | New selected 214A mutant shows a significant increase in stability at acidic pH; | Miele et al. (2010) |
| Pleurotus Ostreatus | Appressed mycelial growth and slower spawn run and creamish white sporophores; | Rishe & Sharma (2014) |
| Pleurotus ostreatus | Improvement of the polysaccharide production; | Chen et al. (2016) |
| Pleurotus ostreatus | Improvement of the polysaccharide production; | Festa et al. (2008) |
| Pleurotus ostreatus | Improvement of the polysaccharide production; | Zhu et al. (2020) |
| Pleurotus ostreatus | Improvement of the polysaccharide production; | Zhang et al. (2019) |

(continued on next page)
Table 1 (continued)

| Breeding techniques and their characters | Test Strains | Main results | ref |
|----------------------------------------|--------------|--------------|-----|
| Physical mutagenesis: ARTP              | Phellinus baumii protoplasts | production and flavonoid production; | - |
| Traditional breeding techniques        | Phellinus igniarius protoplasts | Enhance the fermentation mycelia; | Dong et al. (2021) |
| Physical mutagenesis: He-Ne laser combine pulsed light | Sparassis crispa | Shortened the cultivation time and with high β-glucan content; | Kim et al. (2013) |
| Traditional breeding techniques        | F. velutipes | High-temperature-tolerant strains were obtained; | Lin et al. (2013) |
| Traditional breeding techniques        | P. ostreatus MTCC 142 | Obtain a highly mutated stable fungal strain for the hyper-production of Mn²⁺; | Arunkumar and Sheik Abdulla (2014) |
| Random mutagenesis by UV and chemical mutagens EiBr | ▶ Physical mutagenesis: ARTP The high energy released during the formation of plasma gives rise to DNA mutations | | |
| Physical mutagenesis: He-Ne laser combine pulsed light | | | |
| Traditional breeding techniques        | | | |

2.1. Traditional breeding techniques

Traditional techniques for strain improvement via sequential random mutagenesis and screening is the leading method for industrial microorganisms. It was usually achieved by recombination and using mutation, recombination, and selection under controlled conditions (Esser).

2.2. Mutations, mechanism

Mutations are defined as stable and heritable changes in the DNA base (McGregor, 1999). Mutations can be random or directly performed by physical, chemical, or combination for enhancement of their genotypic and phenotypic performances. The capacity to undergo mutation is an inherent property of genetic material, including both the genes and the chromosones on which the genes are arranged linearly in a certain order (Obe et al., 1971). Various traditional mutagenic sources, such as chemical mutagens, physical mutagens as UV-rays (Rachmayati et al., 2020), γ-rays (Hidayati et al., 2021), and the N⁺ ion beam (C. Wang, Zhang and Xu, 2012), have been developed and successfully applied to acquire valuable strains.

2.2.1. Chemical mutagenesis

Chemical mutagens are potent as effective radiations in bringing about genetic changes (Hussain et al., 2020). Chemical mutagenesis generates a broad spectrum of mutant alleles (Sarin, Prabhu, O’Meara, Pe’er, & Hobert, 2008), (Haeltnerman et al., 2014) such as gain-of-function, temperature-sensitive, and null alleles (Talih et al., 2020). Chemical agents could mainly cause cell damage, lethality, and genetic injury; different concentrations between the test agent in the medium and around the genome; complex induction or suppression by chemical agents in cellular repair systems, and the accessibility of different regions of the genome were different for the sequestration effects etc. (J. M. Van Tuyl and De Jeur, 1997). Shortened cultivation time and high β-glucan content characters mutant strains of Sparassis crispa were obtained through the chemical mutagenesis method (S. R. Kim, Kang and Ro, 2013). For expanding the cultivation region and season, two cold-tolerant mutant strains (Em-16 and Em-18) of V. volvacea were successfully obtained from EMS mutagenesis (Z. Liu et al., 2011). Chemical mutagens lithium chloride or the combination of lithium chloride with Triton X-100 have treated the protoplasts of the G. lucidum for enhancement the production of polysaccharides and triterpenoid (Peng et al., 2016). For the multiple genes induced by the chemical mutagens and also dangerous for users who usually exposed to high (methyl)nitrotritosuganidine and ethyl methanesulfonate) or very high toxicity (sodium azide and ethidium bromide).

2.2.2. Physical mutagenesis

Compare with the chemical mutagens, physical method with the green and safe characters. As traditional method, physical mutagenesis have been widely used for the strain improvement for the strains with or without clear background.

2.2.2.1. UV. Ultraviolet radiation is one of the effective physical methods for strain improvement has strong genotoxic effects to produce DNA damage, and induce mutations. As a ubiquitous mutagen, the error-prone pathway is responsible for the most UV-induced mutations (McGregor, 1999). Higher kojic acid production of A. terreus (Shakibaie et al., 2018), Pleurotus sajorcaju (Adununji and Adejumo, 2017) and the white button mushroom (Agaricus bisporus) (A. Motalebibazaz et al., 2017) were obtain a suitable mutant through the UV irradiation. In addition, high-yield straw mushroom mutant strains (Bangreekhun et al., 2020), mycelia ramification rate and sclerotia yield mutant strains of P. tuberregium (Ramigboye et al., 2019) were obtained by the UV radiation by irradiating the mycelia, spore suspension, and sterile spore suspension.

2.2.2.2. Laser. Low-power laser as one kind of effective physical mutagen has been applied used to mutagenesis the plants, mammalian cells, bacterial and fungi as well. Laser irradiation technology have arouse much attention, especially in the past years. The main mechanism of low-power laser affecting on the organism and producing variation lies on the flight effect and the magnetic field of the laser, and also laser of any wavelength can produce biological effect (Alam et al., 2018).

He-Ne laser at 632.8 nm wavelength as one kind of mutagens was widely applied in the mushrooms mutagenesis. A 40 mW He-Ne laser have been used for irradiation the P. igniarius protoplasts, combine with the guaiacol plate screening and passing by generations,with a higher laccase activity strain SJZ2 (increased by 36.84%) was obtained (Zhu et al., 2020). The same laser machine was applied to obtain a higher fermentation mycelia(20.715 g/L) and polysaccharide (1.428 g/L) of the screened mutant (JZx) from the low power He–Ne laser combine with ultraviolet induction, which were increased 40.31% and 56.58% than those of the wild strain (H.-N. Zhang et al., 2018). Besides, a higher mycelia production mutant strain JQ9 (17.685 ± 0.309 g/L) was obtained through the pulsed light combine with the He–Ne laser treatment, compared with the wild strain (12.062 ± 1.119 g/L) (Dong et al., 2021). Besides the He–Ne laser, FIRL (far infrared ray laser) may produce the mutagenesis by the resonance excitation effect thus breed new species depend on the higher laser intensity and longer irradiation time (Zhou et al., 2005).

2.2.2.3. ARTP. ARTP (Atmospheric and Room Temperature Plasma) mutagenesis techniques as one kind of modern physical mutagens has been widely used as an effective tool to produce stable and high-yield mutant strains applied in bacteria, fungi, and microalgae (X. Zhang et al., 2014), (Ottenheim et al., 2018). The plasma from ARTP has far-reaching impacts on microorganisms, including ultraviolet radiation, heat, electromagnetic fields, charged particles, and even reactive
oxygen species (Cheng et al., 2016), (Maisch et al., 2012). The thermal damage to the microorganisms can be avoided for the plasma temperature. In addition, the high activity and the evenly distributed particles from the ARTP can instantaneously act on the strands of DNA, and an incomplete gene repair process can cause gene mutation (C. Zhang, Qin, Dai, Mu and Zhang, 2019).

ARTP acted as an efficient mutagenesis method, it has been used in the mushrooms breeding for strains especial for higher basidiomyces mushrooms. A P. baumii mutants A67 with higher flavonoid and polyphenols content were significantly increased by 1.87 and 1.33 folds respectively using the ARTP mutagenesis strategy (H. Zhang et al., 2019). Volvariella volvacea protoplast mutation by ARTP obtained three mutant strains which possess higher mycelia growth speed and stronger low-temperature resistant (increased 17%–57% under 20 °C) (He et al., 2014). A superior strain (AR0) white Flammulina velutipes from the industrialized cultivation was treated by the ARTP radiation, two mutants strains among six strains with higher resistivity and lower cellulose content were obtained ((AR 12) improved 15.49% and reduced 15.82%; (AR 17) improved 1.90% and reduced 36.31%). (Yang et al., 2017). 67 regenerative strains of Agrocybe cylindracea with excellent decomposition ability and higher mycelial growth rate were obtained through mutagenesis by using the ARTP method. Among them, AL20 was found to be excellent with higher mycelial growth rate(3.13 mm/d) and higher biological efficiency (Wang et al., 2017).

2.2.2.4. Others. Low-energy ion beam, an useful mutagenesis method for its broad mutation spectrum, limited physiological damage, and even high mutation frequency (Feng et al., 2007; Ichida et al., 2008), which has been widely applying in plants (L. Zhang, Qi, Xu, Wang and Jiao, 2016), bacteria (Mahadtanapuk et al., 2007) and fungi to induce phenotypic changes (C. Chao Wang et al., 2012). The effects of the ion-beam irradiation depend on the implantation dose and ion energy level (Xie et al., 2018). Though the essential mechanisms of the low-energy beams are still unclear, the pathway formation by the low-energy is the prerequisite and basis for the gene transfer into the receptor cells (Gu et al., 2008). Ar 5002 and Ar 5012 two defective in β-1,4-endoglucanase activity mutants of T. matsutake NBRC 33136 were generated by the argon-ion beam irradiation (Murata et al., 2021).

The intense pulses of the pulsed light which have broad-spectrum electromagnetic radiation from 200 nm to 1000 nm including UV, visible and infrared (IR) radiation. Besides the obvious microorganisms inactivation, PL have been applied to mutation breeding of high-producing strains, as Phellinus igniarius (Agaricomycetes), the mycelium biomass and the flavonoid production of the screened mutant strain QB72 were increased 20.87% and 53.51%, respectively. The total amount of the accumulated extracellular laccase of the QB72 in the first 6 and 8 days increased 23.38% and 22.37% respectively (Dong et al., 2022).

2.3. Cross breeding

Hybridization can only take place within strains of the same species
or different varieties and between relatively close species of the same genus, but not across species. By selecting varieties, lines and individuals with excellent characters to cross, combine two or more good traits from the same species into a new variety, and possible to produce heterosis, resulting in a new variety that is stronger or performs better than its parents. Cross breeding make the character separation in hybrid progeny, but the breeding process is slow and complicated. The general process of the cross breeding are shown in the Fig. 2 (a).

Hybridization between the two strains of C. militaris could highly improve the production of carotenoid and cordycepin (Lin et al., 2021). A longer shelf-life hybrid strain CS of P. eryngii was developed by mono-mono crossing between monokaryotic strains derived from KNR2594 and KNR2610 (M. K. Kim, Ryu, Lee and Kim, 2013). Interspecific crossing of monokaryons between P. taiensis and P. eryngii obtained a white-colored fruit body hybrids, with improved biological efficiency (62.5%-64.2%) compared to the parent strains P. t.aiensis (41.8%) and P. eryngii (52.1%) (X.-R. Liu, Liu et al., 2020). The hybrid of the oyster mushroom (Pleurotus ostreatus, P. eryngii and P. comteupica) were conducted to aggregate benefit special characters strains as cap diameter, yield and biomass, dry and fresh weight of the fruiting body (Tagavi et al., 2016). Interspecific hybridization among more species were developed and the screened cultivated strains were detected with different genotypes as new cultivars (Du et al., 2019).

2.4. Protoplast fusion and genome shuffling technique

Fungal protoplasts are an important tool for the genetic research (Peberdy, 1989), (Hamari et al., 1997). Protoplast fusion has been used to modify the phenotypic traits since the late 1970s (Scheinbach, 1983). As one kind of cross breeding techniques, protoplast fusion was a broad applicability method used in cell engineering, which have been used to creat mushroom hybrids, and performed interspecifically, intraspecifically, and interhetero generically (Dhitaphichit and Pornsuriya, 2005). Protoplasts fusion in filamentous fungi can successfully realize the genetic manipulation, especial for the fungi lacking the sexual reproduction capacity. The protoplast fusion techniques have been widely used in the edible and medicinal mushrooms breeding for better characters strains with higher bio-efficiency and nutritional properties, and also for valuable metabolites. The process of the protoplast fusion are shown in the Fig. 2 (b).

Inter-specific hybridization strains were obtained between the Pleurotus species, with high amount of nutritional properties (Selvakumar et al., 2015), (A. Porsevi and Vijayakumar, 2020). Highly productive and longer storage life mushroom strains were obtained by the protoplast fusion between the white and brown oyster mushrooms (Dijanjega and Masduki, 2010). Hybridization could be conducted between two edible mushrooms Calocybe indica and Pleurotus sojor-caju (Das et al., 2021) (Chakraborty and Sidkar, 2009) for higher bio-efficiency and ε-linoleic acid content, and two low-temperature tolerance ability strains (VP1 and VP2) for longer cultivation region, season and biological efficiency of the macrofungus (He et al., 2018).

Intra-strain protoplast fusion of the T. reesii have been conducted for the extracellular carboxymethyl cellulase (CMCase) enhancement in the fusant progenies (Prabavathy et al., 2006).Fusion technique can successfully applied in the interspecific hybridize the strains Ganoderma lingzhi and G. applanatum (Raman et al., 2021), between the strains M. importuna and M. sextelata (P. He et al., 2020), and also the L. sulphureus and culinary basidioomsycetes (Okamura et al., 2000).

Besides the cross breeding and protoplast fusion breeding techniques, genome shuffling is a technology for strain improvement based on protoplast fusion, but with a difference, recombination could be conducted between multiple parents at each generations, and several rounds of the recursive genome fusion increase the genetic diversity (Gong et al., 2009). In addition, genetic breeding technique of the genome shuffling can be performed on the tested strains with unclear genetic background (PU Li-zhang et al., ), (Wang et al., 2014).

Phenotypic improvement by genome shuffling is important in cell improvement not only for industrial, but also medical applications (Stephanopoulos, 2002). At the condition of the protoplasts treated with 1% (v/v) ethylmethylsulfonate (EMS), 40 s of ultraviolet irradiation (UV), 600 Gy electron beam implantation and 750 Gy 60Co-γ irradiation. Genome shuffling greatly improved the low-temperature resistance of Volvariella. Volvacea, and shortened the course of screening required to generate desirable strains(Zhu et al., 2016). DNA shuffling of pox and poxA1b cDNAs was performed and the P. ostreatus laccases were successfully expressed in yeast (Festa et al., 2008).

2.5. Combine mutagenesis

Using different mutagenesis method combined can improve the efficiency of mutation (Zhang et al., 2017). The combine method usually happen in the two physical methods, physical method combine with the chemical method, and even Protoplast fusion can be combined with other mutagenesis methods. Three methods also could combine together.

Higher glucoamylase activity strain of Aspergillus niger (An-1) was obtained through the UV and 60Co-γ ray combined mutagenesis by (Zhao et al., 2014). The average enzymatic activity of strains isolated from the magnetic field + UV treatment, which have an evident increase of 17.2% when compared with those treated only with UV treatment (Yang et al., 2003). Two physical mutagenesis method 60Co-γ irradiation and microwave treatment were combine together to treated the Gluconobacter oxydans, and a stable mutant H-8 with high mSLDH activity and higher cell biomass was obtained (D. Liu, Hu, Ke and Zheng, 2020). Another two physical mutagenesis treatment method as UV-ARTP was treated the Cordyceps militum CICCI4014, the yield of the mutant was 146.1% higher than that of the initial strain (2.43 g/L) (Zhao et al., 2020).

A hyper-production of MnP strains of P. ostreatus MTCC 142 was obtained through the random mutagenesis by using UV radiation combine with the chemical EtBr mutagens treatment (Arunkumar et al., 2014). Physical (ultraviolet rays) and chemical mutagens (ethidium bromide and ethyl methanesulfonate) were applied to treat the Cellulasimicrobium sp. CKMX1 for xylanase improvement (35.89%) in mutant E5 over wild strain in solid state fermentation (Guleria et al., 2013). Low sporining strains of P. Ostreatus through mutagenesis (Rishu and Sharma, 2014), (T., 2009) and a mutant strain (mSM-105) Cathalactes verticosum with higher yield of β-glucan (85.3%) were obtained by using the physical mutagen (UV light) combine with chemical mutagen (ethyl methyl sulfate, EMS) (Y. Ke Liu et al., 2019).

Protoplast fusion can be combined with other mutagenesis methods (such as the formal mutagenesis or the protoplast mutagenesis). Fast-growing mycelia of Hypsizygus marmoreus were selected after firstly treated with methanesulfonate methylesterantandwyty and then mated each other by hyphal fusion (Lee et al., 2011). Mutagenesis (UV and EMS) of P. tannophilus MTCC 1077 and protoplast fusion between Saccharomycices cerevisiae G can be applied for obtaining variants and fusants with ethanol production capacity from xylose and xylose, glucose mixtures (sushma Gurumayum et al., 2017). Three mutagens (UV + LiCl + EMS) were used to treated the F. velutipes mycelial fragments, three high-temperature-tolerant mutants FIU4, F16e, and F1C4 were successfully obtained (Kang et al., 2013).

2.6. Homologous recombination (HR)

Homologous recombination (HR) is a DNA metabolic process occurs in all forms of life, which is a key pathway to maintain genomic integrity between generations and during ontogenic development in a single organism (DNA repair) (Heyer et al., 2010). HR plays the roal in preserving genome, also act as a prominent role in faithfully duplicating the genome through providing critical support for telomere maintenance and DNA replication (X. Li and Heyer, 2008). HR with the character of high-fidelity, template-dependent repair or tolerance of complex DNA
 damages. However, recombination can lead to potentially lethal intermediates and gross chromosomal rearrangements (Kolodner et al., 2002). The disadvantage of this method is low efficiency and high error rate.

The functional gene analysis using the traditional homologous recombination based gene disruption technology can apply in a few basidiomycete mushrooms Schizophyllum commune (Ohm et al., 2010) and Coprinopsis cinerea (Nakazawa et al., 2011). Efficient homologous integration was also conducted in the mushroom Agaricus bisporus, using a plasmid (pHAG3-1) carrying the hygromycin-resistance gene and a 3.2 kb genomic fragment from A. bisporus (van de Rhee et al., 1996).

2.7. Modern breeding techniques

2.7.1. Omics technology

Mutagenesis methods as effective approaches have been applied in exploring the unknown molecular functions of microbes by combining modern omics analysis (Huang et al., 2021). DNA sequencing is a gold standard for determining the exact nature of a mutation and has been widely used for DNA diagnostics and functional studies of genes of interest. Genome sequencing and functional annotation will provide valuable information for establishing key molecular genetic markers that can be used to improve the quality and usage of the mushroom. Genome sequencing could provide fundamental information for the mechanical study of biological processes, and facilitates the genetic breeding of the mushrooms.

Recently, genome sequencing have been applied in world popular cultivated edible mushroom, Lentinula edodes monokaryon B17 (Shim et al., 2016). A high quality whole-genome sequence of Sanghuang, P. gilvus strain S12 was reported and The related candidate genes of phenylpropanoids’ synthesis pathway was screened by molecular docking analysis (Huo et al., 2020). Genome sequence was conducted in the S. rugosoannulata monokaryotic strain (A15) (S. Li, et al., 2022). Genome sequence provides important insights into the biology of medicinal mushroom R. gibescarnosa (F. Yu, Song, Liang, Wang and Lu, 2020). In addition, complementary genome sequencing has proved useful for identifying single-point mutations (Timoshenko et al., 2009). Genome and transcriptome sequencing of P. portentosus was performed during the different growing stages, provide a new perspective for understanding the key pathways and hub genes involved in P. portentosus development (Wan et al., 2021). Transcriptomes analysed four developmental stages of S. japonica. indicated out that S. japonica sporophytes persistently respond possible pathogen and environment stresses (Ding et al., 2018). Using transcriptomics and metabolomic analysed the mechanism of the browning, the expression of AbPPO expression play an important role in the browning of A. bisporus and multiple PPO family members are involved in the regulation of browning (Cai et al., 2022).

The genomic sequence of S. commune could unravel related mechanisms by which mushroom-forming fungi degrade their natural substrates and form fruiting bodies (R. A. Ohm et al., 2010). Comparative transcriptome analyses of the fruiting bodies of three morphologically distinguishable A. auricula-judae, the cultivated varieties among major cultivars could provide molecular guidance for breeding and cultivation (Y. Zhao et al., 2019). Comparative transcriptomic analysis was conducted in the Bailinggu’s mycelia, primordia, and fruiting body at different stages, research provide new genes regulating fruiting body development, SSR markers, and germlasm that enhance the breeding of commercially cultivated Bailinggu(Fu et al., 2017). Comparative transcriptomes of fruiting bodies of complex multicellular Agaricomycetes (mushroom-forming fungi) combine with comparisons of whole genomes conserving the developmental functions and complex multicularity in fruiting bodies(Grzian et al., 2019). Complementary Genome Sequencing has proved useful for identifying single-point mutations in a temperature-sensitive mutant, however CGS failed to recognize some SNPs and false positive SNPs were also identified out(Timoshenko et al., 2009).

2.7.2. In-silico tools

As important as Omics technology, a number of in silico tools (e.g. Mutation Surveyor, SeqScape, VarDetect, PolyPhred and Sequencher) have been developed to assist in SNP and Indel analysis for detection of heterozygous and homozygous mutations (C. D. a. B. Yu). Combined the metabolic pathway analysis and flux balance analysis could interpret shifts in metabolic routing that may occur in response to environmental and internal/genetic challenges(Christophe H. Schilling et al., 2000).

FBA(flux balance analysis) to estimate intracellular fluxes also provides flux values that can be used as a starting point for rational engineering of Chlamydomonas reinhardtii (Boyle and Morgan, 2009).

In-silico tools could also used for drug design. FXR represents an attractive target for computer-aided drug design have been applied in the mushrooms Ganoderma lucidum(Curtis) P. Karst (Ganodermaeae) by virtual screening of chinese herbal medicine database (Grienke et al., 2011). By using an in silico approach, possible lanostanoids were found from an extensive library of lanostanoids Ganoderma mushrooms, which interact with the VDR ligand-binding pocket in the same way as calcitriol(Suarez-Medellin et al., 2016).

2.7.3. CRISPR/Cas9 gene editing breeding

As a new third generation of genome editing technology, clustered regularly interspersed short palindromic repeat sequences CRISPR-Cas9 based gene editing has been well applied to various species, as plant cells, bacteria, animals and even some fungus (Liu et al., 2019). The CRISPR/Cas9 gene editing system including three basic components, that are the CRISPR RNAs (crRNAs), CRISPR associated protein (Cas) and transactivating crRNA (tracrRNA).

CRISPR/Cas9 system was firstly successfully adapted for the agaricomycete C. cinerea in 2017 (Sugano et al., 2017), and also applied in the edible mushrooms P. ostreatus (Boontawon et al., 2021). Marker-free genome editing in Pleurotus ostreatus was conducted using transient expression of genes using the CRISPR/Cas9 system (Koshi et al., 2022). The constructed ku70 mutant using the CRISPR/Cas9 system was suitable for target gene insertion and replacement in G. lucidum (Jun-Liang Tu). Functional genes of pc1 and clp1 from the Pleurotus ostreatus were also studied by the CRISPR/Cas9 knockout system (Boontawon et al., 2021). The Ca9 system was successfully constructed in the Lentinula edodes for expression of guide RNAs (gRNAs) which could target the mating-type gene HD1 (LeA1 (Moon et al., 2021). The ura3 gene of the two G. lucidum (G. lucidum 260125 and G. lingzhi) were successfully disrupted by codon-optimized Cas9 and transcribed gRNA (Qin et al., 2017). A highly efficient pyrG Cas9 system gene editing system was established in P. eryngii, which was based on the varied insertions and deletions (indels) by homology-directed repair (HDR) and non-homologous end joining (NHEJ) (T. Wang, Yue, Jin, Wei and Lu, 2021).

A CRISPR-Cas9 system was successfully developed to disrupt a marker gene (ura3) and a functional gene cyp5150l8 in G. lucidum (P. A. Wang, Xiao and Zhong, 2020). The homodepsiation transcription factor gene hom2 was efficiently deleted using the Pre-assembled Cas9-sgRNA ribonucleoproteins (RPNPs) in mushroom-forming basidiomycete Schizophyllum, which may improve the genetic accessibility of non-model species (Jan Vonk, Escobar, Wosten, Lugones and Ohm, 2019). A CRISPR system was successful constructed in C. militaris (Chen et al., 2018), and Shiraia sp. SUPER-H168 for study the a targeted polyketide synthase (SbaPKS) gene (Deng et al., 2017).

3. Screening strategies in the mushroom breeding

After effective irradiation treatment, proper screening process are vital for strains screening. The flow chart of general breeding process was shown in the Fig. 2 (c). Screening plate as the guaiacol laccase screening plate (Zhu et al., 2020), and effective selection markers as fluorescent staining, drug resistance marker (Kim et al., 1998), molecular markers (A. V. Shnyreva et al., 2003), temperature sensitive
labeling, and clamp connection (Furtado, 1966), and the antagonism test (Gloer, 1995) have been widely used to differentiate groups of genetically similar and distant strains. After passing generation experiments, the genetic polymorphism were conducted between the target strains and the wild strains. There are three levels of variants could showed the Genetic variants, that are the population level, individual level, cellular level, and molecular level (Ayala, 1992). The three levels of variants usually focus on the study of the chromosome polymorphism, protein polymorphism and DNA polymorphism.

The use of genetic characterization approaches such as isoenzyme electrophoresis analysis (MCMANUS, 1990) has the significant benefit of allowing for the examination of a very large number of structural genes (Thompson, 1991). Isoenzyme electrophoresis spectrogram analysis results between the wild strain and the screened mutants indicated that the genetic materials were altered (H.-N. Zhang et al., 2018).

On the DNA polymorphism aspects, several molecular genotyping methods such as RFLP, PFGE, AFLP, MLST, MLEE and a number of PCR based approaches have proved to be valuable for typing of a number of microbial systems (Cebula et al., 2005), (Holmes et al.). Hybrids obtained by pairing monosporic cultures are cultivated to evaluate the production characteristics accompanied by RAPD and RFLP analysis (Bipasha Chakravarty, 2011). Genetics and hybrid breeding systematic scheme was established for P. pulmonarius and identified by both morphological and molecular fingerprinting (PCR-RFLP) (Avin et al., 2016). The mutagenic effects of ion implantation on arabidopsis thaliana were analysed using an amplified fragment length polymorphism (AFLP) fingerprinting (Li et al., 2007). Combined randomly amplified polymorphic DNA (RAPD)/inter-simple sequence repeat (ISSR) could used to assess the genetic diversity of P. eryngii strain (S. Wang, Yin, Liu and Xu, 2012).

A novel selection marker gene for transformation of the white-rot basidiomycete Pleurotus ostreatus was developed by introducing a point mutation in a gene which encodes the iron-sulfur protein (Ip) subunit of succinate dehydrogenase (Y. Honda et al., 2000).

The growth characters could act as screening index for the screened strains and the hybridization.

Fruiting body and colony morphology, and the cultural characteristics (Selvakumar et al., 2015), biological activity and nutritional properties (A. Porselvi and Vijayakumar, 2020) (Das et al., 2021), longer storage life (Masduki, 2010), higher bio-efficiency and c-linoleic acid strains and the hybridization. (AFLP) fingerprinting (Li et al., 2007). Combined randomly amplified polymorphic DNA (RAPD)/inter-simple sequence repeat (ISSR) could used to assess the genetic diversity of P. eryngii strain (S. Wang, Yin, Liu and Xu, 2012).

The growth characters could act as screening index for the screened strains and the hybridization.

Both the traditional and modern breeding techniques applied for the mushroom improvement for cultivation application. Modern techniques are better for the mushroom research for study the gene functional. Omics technology are used for the gene mining, combine the metabolism engineering and in-silico tools analysis could be better interpret shifts in metabolic routing that may occur in response to environmental and internal/genetic challenges.

Gene (genome) editing breeding techniques are applied for the edible and medicinal with clear background. Physical and chemical mutagens using spores or the protoplast as treatment materials. Further more, crossbreeding, protoplast fusion and genome shuffling breeding method could be obtain better character strains for cultivation, and consumption. For the mushrooms without clear gene background, mutagenesis mechanism are relative hard to be analysis by the CRISPR/Cas9 gene editing techniques. As for the Basidiomycetes the breeding techniques are focus on the protoplast fusion, cross breeding and genome shuffling techniques.

Besides the proper breeding techniques, there are still other specific challenges for the mushrooms breeding. Selective marker genes are quite insufficient. Different kind of strains complicated mushroom genetics, for example, heterokaryosis, sysergism of dikaryon and dominant nucleus phenomena. Before mushroom breeding, make sure the breeding material are the haploid, which could be realized by collecting the haploid spores, or make the protoplast, then using the proper breeding techniques.

5. Conclusions

Comparison to the conventional genetic methods, CRISPR/Cas 9 as a new third generation of genome editing technology which will provide more rapidly executable tools to carry out functional genomics in basidiomycetes and ascomycetes species that obtain target desired traits. For the most of the strains, with unclear gene background, cross-breeding, protoplast fusion and genome shuffling, are often choose as breeding techniques to obtain target strains. Modern physical mutagenesis as ARTP are effective applied for the strain improvement, He–Ne laser, UV and even low-energy ion beam could be used as physical mutagen. Combine breeding method like using mutants from physical and chemical method, and then conduct the crossbreeding or protoplast fusion for obtaining strains for cultivation.
CRediT authorship contribution statement

Yating Dong: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. Renyun Miao: Supervision, Methodology. Rencai Feng: Supervision, Methodology. Tao Wang: Writing – review & editing. Junanye Yan: Funding acquisition. Xu Zhao: Funding acquisition. Xing Han: Supervision, Methodology. Ying Gan: Supervision, Methodology, Writing – review & editing. Junbin Lin: Supervision, Methodology. Yujia Li: Supervision, Methodology. Bingcheng Gan: Conceptualization, Investigation, Funding acquisition. Jin Zhao: Supervision, Methodology, collecting the papers.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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