2 Biological Basis of Spore Powder Production

2.1 Growth and development characteristics of *Ganoderma lucidum* and spore formation process

Ganoderma lucidum is a white-rot basidiomycete that naturally grows on wood or lignocellulosic substrates, and its life cycle proceeds from basidiospore germination to primary mycelium formation, substrate colonization, primordium differentiation, young fruiting body formation, maturation, and finally spore release (Ren et al., 2025). Basidiospores can germinate rapidly under suitable nutritional conditions; one cultivation study reported efficient germination in coconut water gelatin within 72 h, after which the sporoderm produced a single germ tube that elongated, septated, and branched into monokaryotic primary mycelia. During fruiting, visible morphogenesis follows a recognizable sequence including mycelial coat hardening, primordial initiation, antler-like development, basidiocarp maturation, and spore liberation, indicating that efficient spore production depends on precise coordination between vegetative growth and reproductive differentiation. Recent developmental transcriptome analyses further divide fruiting and sporulation into sequential stages from mycelium, primordium, and young fruiting body to mature and post-sporulation stages, with large transcriptomic shifts especially at the mycelium-to-primordium and young-to-mature fruiting transitions (Liu et al., 2022).

Spore formation itself occurs on the abaxial side of the pileus and is closely linked to structural differentiation of pores, tube tissues, basidia, and spore walls (Cai et al., 2021). Ultrastructural work showed that young tubes first appear as slight depressions in the tramal surface, accompanied by extensive hyphal death and disintegration, while surviving hyphae become coated with extracellular matrix; developing basidia then push through this matrix into the tube lumen, where basidiospores are formed. Nuclear migration into spores begins only after lipid droplets start to coalesce and the spore wall has begun to form, and the mature *G. lucidum* spore wall comprises an outer primary layer, inter-wall pillars, and an inner secondary wall, features that help explain the robust sporoderm of commercial spore powder materials. At the molecular level, sporulation is associated with differential expression of genes involved in meiosis, carbohydrate metabolism, and transcriptional regulation; meiosis-related genes such as *DMC1*, *MSH4*, *HOP1,* and *Mek1* are highly expressed during development, while genes for trehalose and malate synthesis are upregulated during spore morphogenesis, implying active energy provisioning and carbohydrate accumulation in mature spores (Liu et al., 2022).

2.2 Selection of elite strains and optimization of germplasm resources

Efficient production of *G. lucidum* spore powder begins with elite germplasm, because strain differences strongly affect growth rate, resistance, fruiting performance, and basidiospore yield. A major current limitation is that most cultivated varieties were bred for fruiting body production rather than spore powder, and "Longzhi No.1" has been one of the few varieties widely used for spores despite poor resistance to competing microorganisms, which has reduced its production suitability (Tang et al., 2023). Accordingly, recent breeding work has focused on selecting high-spore-yield, disease-resistant, and stable strains through mutation breeding, molecular identification, antagonism testing, and multi-site cultivation evaluation (Viceconte et al., 2021; Feng et al., 2024). This direction is consistent with broader industrial demand for fast-growing, high-yield strains capable of supporting the growing market for medicinal and nutraceutical *Ganoderma* products (Swallah et al., 2023).

Several studies now provide concrete breeding examples. UV mutagenesis of the high-spore-yield parent strain G0109 produced mutant UV119, which showed strong resistance to undesired microorganisms and increased fruiting body and basidiospore yields by 8.67% and 19.27% relative to the parent, with basidiospore yield 20.56% higher than "Longzhi No.1" (Tang et al., 2023). Another breeding study reported that strain XZ-2, obtained through tissue isolation and radiation treatment, delivered fruiting body yields 9.7%~30.1% higher and basidiospore yields 20.2%~45.7% higher than control strains across locations and years, while maintaining stable annual performance and elevated polysaccharide and triterpenoid levels. Atmospheric room-temperature plasma mutation combined with microbial microdroplet screening also yielded stable mutants YB05, YB09, and YB18 with faster growth, stronger antagonism, and higher biomass or triterpenoid production across five generations, showing that high-throughput breeding can accelerate germplasm improvement for industrial fermentation (Feng et al., 2024). Comparative local strain screening likewise identified G8 and Bendi as superior strains with faster