

灵芝真菌液体发酵及其产物应用的研究进展

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摘 要: 灵芝作为一种白腐真菌, 同时也是珍稀的食药两用真菌, 富含多种生物活性成分。液体发酵技术生产周期短、效率高、产量大、品质稳定, 是开发利用灵芝资源的重要途径。近年来, 灵芝属真菌菌丝体液体发酵技术的开发与应用取得了较大进展。本文对灵芝属真菌液体发酵产物的主要活性成分及其药用效果、液体发酵工艺优化和发酵产物的应用进行综述, 并对本领域的未来进行展望。

关键词: 灵芝属; 白腐真菌; 液体发酵; 活性成分; 发酵产物

Liquid fermentation of *Ganoderma* and application of its products

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Abstract: A white-rot basidiomycete *Ganoderma* spp. is a rare edible and medicinal fungus, which is rich in a variety of bioactive components. Liquid fermentation technology has the advantages of short production cycle, high efficiency, high yield and stable quality, which is an important way to develop and utilize *Ganoderma* resources. In recent years, great progress has been made in the development and application of liquid fermentation of mycelia of *Ganoderma* species. In this paper, the main active components and medicinal effects of liquid fermentation products of *Ganoderma* spp., the optimization

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of liquid fermentation process and the application of fermentation products were reviewed, and the future of this field was prospected.

Keywords: *Ganoderma*; white rot fungi; liquid fermentation; active components; fermentation products

本文所述灵芝(*Ganoderma lucidum*)是指隶属于担子菌门(*Basidiomycota*)伞菌纲(*Agaricomycetes*)多孔菌目(*Polyporales*)灵芝科(*Ganodermataceae*)寄生于腐木中的灵芝属(*Ganoderma*)^[1]真菌。灵芝具有独特的疗效,在我国被用作名贵中药材已有1 000多年。《本草纲目》中记载“灵芝性平, 味苦, 无毒, 主胸中结, 益心气, 补中, 增智慧, 不忘, 久服轻身不老, 延年神仙”。现代药理学研究证实, 灵芝含有灵芝多糖、灵芝三萜类化合物、甾醇等多种生物活性成分, 具有调节免疫^[2]、抗病毒^[3]、抗肿瘤^[4]、抗衰老^[5]、保肝护肝、治疗心脑血管疾病^[6]等药理作用。

野生灵芝由于生长环境要求苛刻, 造成产量严重不足。因此, 人工栽培便成为灵芝增产

的一条途径, 其在一定程度上提高了灵芝的产量^[7]。人工栽培虽能扩大生产规模, 但存在产量不稳定、品质参差不齐、生产周期长、劳动强度大、占地面积广、受气候影响等问题, 其中生产周期长与成本高是主要问题。可见, 开发生产周期短、产量高、品质稳定且能工业化生产的技术途径显得尤为重要。研究表明, 通过液体深层发酵生产的灵芝菌丝体, 不但苦味淡, 而且灵芝多糖等主要有效成分不低于甚至有些指标还高于子实体; 不同灵芝的菌丝体所含蛋白、脂肪、糖和灰分等主要营养成分均比子实体高(表1); 同时也有研究表明, 菌丝体的生物活性成分易于分离利用, 人体对其吸收的效果比子实体和孢子粉要好, 而且粗纤维比子实体少, 可以直接食用或用于食品加工^[8-11]。近年来,

表1 3种灵芝子实体和菌丝体的主要营养成分对比(占干重的百分比, %)^[8]
Table 1 Comparison of main nutritional components of 3 kinds of *Ganoderma* fruiting body and mycelium (% of dry weight)^[8]

品种 Variety	来源 Source	水分 Moisture	粗蛋白 Crude protein	纯蛋白 Pure protein	粗脂肪 Crude fat	粗纤维 Crude fiber	灰分 Ash content	总糖 Total sugar
野生灵芝 Wild <i>Ganoderma lucidum</i>	子实体 Fruiting body	10.20	8.88	6.22	6.60	18.10	5.54	22.34
	菌丝体 Mycelium	9.81	27.42	19.22	8.12	1.06	6.98	30.54
紫芝 <i>Ganoderma sinense</i>	子实体 Fruiting body	13.58	16.39	11.48	7.80	14.6	3.70	17.00
	菌丝体 Mycelium	10.44	30.02	21.04	8.34	1.94	4.26	29.46
川芝6号 Sichuan <i>Ganoderma</i> of six	子实体 Fruiting body	16.31	15.66	10.97	8.60	13.70	3.09	19.60
	菌丝体 Mycelium	11.10	29.95	20.99	9.77	1.22	11.44	22.60

注: 原表中的总计及平均值项目未列入本表格
Note: The total and average items in the original table are not included in this table.

灵芝菌丝体液体发酵技术的开发与应用取得了较大进展。本文主要从灵芝液体发酵产物(菌丝体与发酵液)的生物活性成分及其药用效果、液体发酵培养工艺及其开发应用等方面的进展进行综述,并对其中存在的问题加以分析讨论,以期对灵芝液体发酵的深入研究和广泛应用提供参考。

1 灵芝真菌液体发酵的菌株与优势

1.1 液体发酵常用菌株

利用液体深层发酵生产灵芝菌丝体的技术途径具有生产周期短、产量高、产品质量稳定、

不受季节影响等优点,并可实现工业化生产。液体发酵技术改变了人工栽培生产周期长与成本高的生产现状,极大地提高了生产效率。目前已知可用于人工栽培的灵芝品种多达 250 种,国内常见的有赤芝(*G. lucidum*)、紫芝(*G. sinense*)和黑芝(*G. atrum*)等种类,而在液体发酵领域常用到的灵芝菌株见表 2。

1.2 灵芝真菌液体发酵优势

传统的灵芝产品主要是子实体、孢子粉和孢子油。子实体纤维化、木质化程度高,味苦,一般不直接食用,而是辅以熬制入药;孢子粉细胞壁厚,不易被人体利用,不太适宜直接食

表 2 用以液体发酵的主要灵芝菌株

Table 2 Main strains of *Ganoderma lucidum* for liquid fermentation

菌株学名 Scientific name of strain	主要产地 Major origin	液体发酵用途 Application of liquid fermentation	参考文献 References
赤芝 <i>Ganoderma lucidum</i>	中国华东、西南及吉林、河北等地 East China, Southwest and Jilin, Hebei and other places in China	生产灵芝多糖、三萜 Production of <i>Ganoderma lucidum</i> polysaccharide, triterpene	[12]
紫芝 <i>Ganoderma sinense</i>	欧洲、美洲、非洲、亚洲东部,中国东部、南部 Europe, America, Africa, eastern Asia, eastern and southern China	生产灵芝多糖、三萜、菌丝体 Production of <i>Ganoderma lucidum</i> polysaccharide, triterpene and mycelia	[13]
黑芝 <i>Ganoderma atrum</i>	主要分布在中国南部沿海地区 Mainly distributed in the coastal areas of southern China	生产灵芝多糖 Production of <i>Ganoderma lucidum</i> polysaccharide	[14]
四川灵芝 <i>Ganoderma sichuanense</i>	中国四川省 Sichuan province in China	—	[15]
韦伯灵芝 <i>Ganoderma weberianum</i>	中国台湾省、云南省等地 Taiwan, Yunnan and other places in China	生产漆酶、多糖 Production of laccase and polysaccharide	[16]
松杉灵芝 <i>Ganoderma tsugae</i>	中国黑龙江、吉林、甘肃等地 Heilongjiang, Jilin, Gansu, etc. in China	生产灵芝多糖 Production of <i>Ganoderma lucidum</i> polysaccharide	[17]
树舌灵芝 <i>Ganoderma applanatum</i>	中国东南部与西北部,东多西少 In the southeast and northwest of China, there are more in the east and less in the west	生产漆酶、三萜 Production of laccase and triterpene	[18]
血芝 <i>Amauroderma rude</i>	中国大部分地区 Most of China	生产灵芝多糖 Production of <i>Ganoderma lucidum</i> polysaccharide	[19]

注:—:用途不明确

Note: —: The purpose is not clear.

用,一般对其进行破壁处理后泡水饮用;孢子油成本高,价格昂贵,应用范围窄。相对于传统野生型灵芝与人工栽培灵芝而言,灵芝真菌的液体发酵具有非常大的优势。由于液体发酵技术的优势所在,在发酵的过程中可有效地人为控制,使得发酵向着所需方向进行。

液体发酵技术的主要优势在于生产周期短、成本低、产量高。相对于传统的仿野生型灵芝生长环境的人工栽培技术而言,使用液体发酵技术可大大提高灵芝真菌某种活性物质的产量,从而定向富集目的产物(表 3)。目前,关于灵芝液体发酵富集的目的产物主要集中于某类化合物的大类上。

2 灵芝发酵产物的主要活性成分及其药用效果

2.1 主要活性成分

已有的研究表明,从灵芝中可分离出的活性成分多达 400 余种,主要分为多糖、三萜、蛋白、生物碱、甾醇等^[26],这些活性物质具有抗肿瘤、免疫调节、抗衰老及降血糖血脂等功

效^[27]。灵芝液体发酵产物(菌丝体和发酵液)不但能产生灵芝的主要活性成分,而且具有连续大量生产、周期短、易于干预控制等优点,便于灵芝活性成分的大规模制备和工业化生产^[28-29](表 4)。

2.2 主要药用效果

尽管菌丝体与发酵液的活性成分不尽相同,但两者有着相同或相似的药用效果(表 4),现主要对灵芝液体发酵产物(菌丝体和发酵液)的药用效果进行归纳总结。因为具体活性成分的药理作用与子实体的基本一致,在此不赘述。

2.2.1 抗肿瘤

灵芝三萜类作为灵芝的主要活性成分之一,存在于菌丝体及发酵液中,具有多种药用效果,其中抗肿瘤效果尤为显著。从灵芝菌丝体中分离得到的 4 个三萜类化合物能抑制肿瘤细胞 L1210 及 K562 的增殖^[46];Sun 等^[47]发现菌丝体多糖是一种典型多糖,并且具有较好的抗肿瘤活性;同时,研究发现松杉灵芝(*G. tsugae*)发酵产物对 H22 肝腹水瘤细胞的增殖具有抑制作用^[48]。

表 3 灵芝液体发酵富集子实体中的化合物成分

Table 3 Enrichment of compounds in fruiting bodies by liquid fermentation of *G. lucidum*

子实体中的化合物成分 Compounds in the fruiting body	是否有药理活性 Have pharmacological activity or not	是否可通过液体发酵富集 Enriched by liquid fermentation or not	参考文献 References
三萜类化合物 Triterpenes	Yes	Yes	[20]
多糖类化合物 Polysaccharides	Yes	Yes	[21]
核苷类化合物 Nucleoside compound	—	—	—
氨基酸、蛋白质类化合物 Amino acid and protein compounds	Yes	Yes	[22]
甾醇类化合物 Sterol compounds	Yes	Yes	[23]
生物碱类化合物 Alkaloid compound	Yes	Yes	[24]
无机元素 Inorganic elements	Yes	Yes	[25]
其他 Others	—	—	—

注:—: 无相关文献

Note: —: No relevant literature.

表 4 灵芝液体发酵产物的活性成分

Table 4 Active ingredients of *Ganoderma* liquid fermentation product

活性成分类型及来源 Types and sources of active components	种类 Species	药用效果 Medicinal effect	参考文献 References
糖类 Sugars	发酵液 Fermented liquid	灵芝多糖 GLP-1、GLP-2 等 <i>Ganoderma lucidum</i> polysaccharide GLP-1, GLP-2	抗肿瘤、抗氧化、降血糖、降血脂、抑菌 Antitumor, antioxidant, lower blood sugar, reduce blood lipid, [30-31]
	菌丝体 Mycelium	灵芝多糖 GLP <i>Ganoderma lucidum</i> polysaccharide GLP	抗肿瘤、缓解炎症、增强机体免疫力、抗病毒 Antitumor, relieve inflammation, enhance the body's immunity, antiviral [32-33]
三萜类 Triterpenes	发酵液 Fermented liquid	灵芝酸 T、S、Me、Mk 等 Ganoderic acid T, S, Me, Mk	抗肿瘤、缓解炎症、增强机体免疫力、抗病毒 Antitumor, relieve inflammation, enhance the body's immunity, antiviral [26,34]
	菌丝体 Mycelium	灵芝酸 O、灵芝酸甲酯、灵芝醇、灵芝醛等 Ganoderic acid O, methyl ganoderate, <i>Ganoderma lucidum</i> alcohol, <i>Ganoderma lucidum</i> aldehyde	抗肿瘤、缓解炎症、增强机体免疫力、抗病毒 Antitumor, relieve inflammation, enhance the body's immunity, antiviral [26,34-36]
蛋白类 Proteins	发酵液 Fermented liquid	糖蛋白、多肽类等 Glycoproteins, peptides	免疫调节、抗肿瘤、抗氧化、抑菌、抗病毒、降血压 Immune regulation, antitumor, antioxidant, antibacterial, antiviral, reduce blood pressure [37]
	菌丝体 Mycelium	真菌免疫调节蛋白、凝集素、糖蛋白、灵芝素、降压肽等 Fungal immunomodulatory proteins, lectins, glycoproteins, <i>Ganoderma lucidum</i> enzyme, antihypertensive peptides	免疫调节、抗肿瘤、抗氧化、抑菌、抗病毒、降血压 Immune regulation, antitumor, antioxidant, antibacterial, antiviral, reduce blood pressure [38-40]
	菌丝体 Mycelium	胆碱、烟酸、灵芝胺、甜菜碱等 Choline, niacin, <i>Ganoderma lucidum</i> amine, betaine	降低胆固醇、改善冠状动脉血流量、降低心肌耐氧量、增强心肌和肌体对缺氧的耐受性 Reduce cholesterol, improve coronary blood flow, reduce myocardial oxygen-resistant, enhance myocardial and body to hypoxia tolerance [24,41-42]
甾醇 Sterol	发酵液 Fermented liquid	灵芝甾酮、麦角甾醇及其衍生物、羊毛甾醇类化合物、胆甾醇类化合物等 <i>Ganoderma lucidum</i> sterone, ergosterol and its derivative, lanosterol compound, cholesteric compound	降低胆固醇、改善冠状动脉血流量、降低心肌耐氧量、增强心肌和肌体对缺氧的耐受性 Reduce cholesterol, improve coronary blood flow, reduce myocardial oxygen-resistant, enhance myocardial and body to hypoxia tolerance [43]
	菌丝体 Mycelium	灵芝甾酮、胆甾醇类、麦角固醇、羊毛甾醇、 β -谷甾醇等 <i>Ganoderma lucidum</i> sterone, cholesterol, wheat alcohol, lanosterol, β -grains	降低胆固醇、改善冠状动脉血流量、降低心肌耐氧量、增强心肌和肌体对缺氧的耐受性 Reduce cholesterol, improve coronary blood flow, reduce myocardial oxygen-resistant, enhance myocardial and body to hypoxia tolerance [44-45]

注：—：种类不明确

Note: —: The type is not clear.

灵芝三萜类化合物对不同类型肿瘤有着同样的抑制效果，后续可利用灵芝发酵产物开发有关的抗肿瘤药物。

2.2.2 增强免疫力

最近有研究报道发现，灵芝液体发酵产物

可提高断奶仔猪对猪圆环病毒-2 的免疫力并促进其生长^[49]；类似的研究发现肉鸡在喂食灵芝液体发酵物后，其免疫功能、生产性能和抗氧化性能均得到显著提高^[50]。可见，灵芝发酵产物在提高动物机体免疫力上有着良好的效应。

尽管目前灵芝发酵产物在增强免疫力上的临床研究大部分体现于家禽牲畜, 但其效果已非常显著, 相信其在灵长类动物中可能也具有较好的效果, 但需要进一步的研究验证。

2.2.3 抗氧化

现已发现, 从发酵液中提取得到的灵芝多糖具有抗氧化作用, 而且抗氧化活性已被证实^[51], 对于其抗氧化能力强弱的问题, 有研究表明灵芝多糖的抗氧化能力与其分子量大小和浓度成正相关。Kan 等^[52]研究了不同分子量灵芝多糖的抗氧化作用, 发现分子量最大的 GLP80 的抗氧化效果最佳, 而分子量最小的 GLP40 的效果最差。此外, 不同品种灵芝的抗氧化能力不同, 与发酵产物中黄酮、多糖、三萜和多酚的含量有关^[53]。根据这一特性, 后续可利用发酵产物开发相关的美白抗衰产品。

2.2.4 抗菌及抗病毒

真菌是天然抗生素最丰富的来源之一, 灵芝属于多孔真菌, 其液体发酵产物中含有抑制微生物生长的活性物质^[54]。研究发现, 灵芝活性成分可抑制多达 15 种细菌的生长与繁殖^[55], 包括革兰氏阴性菌和革兰氏阳性菌, 常见的如大肠杆菌和金黄色葡萄球菌^[56], 罕见的有幽门螺旋杆菌^[57]; 而在抗病毒方面, 已有研究发现提取的灵芝酸能阻止一些特殊病毒的侵蚀作用, 如 EV71 病毒^[58]。最近有报道^[59]称从灵芝菌丝体中提取的有效抗菌物质可能是一些以前未知的次级代谢产物, 这为灵芝液体发酵生产抗菌物质提供了新的思路。

2.2.5 降血糖和血脂

近年来, 糖尿病的患病率与发病率逐年升高, 而血糖水平过高是糖尿病患者的主要特征。已有研究表明, 灵芝发酵产物具有降低血糖^[60]和血脂^[61]水平的作用, 从灵芝发酵产物中提取到的灵芝多糖可有效降低处于妊娠期的患有糖

尿病大鼠的血糖水平^[62], 灵芝菌丝体多糖 (Polysaccharides of *Ganoderma lucidum* strain S₃, GLPS₃) 对慢性胰腺炎有较好的疗效, 可降低慢性胰腺炎发展为糖尿病的几率^[63], 灵芝真菌液体发酵产物在降血糖和血脂方面具有潜在的应用价值。

3 灵芝真菌的液体深层发酵培养

液体发酵是一种将菌丝体生长所必需的营养制成液体培养基, 再进行摇瓶发酵或发酵罐发酵以达到扩大化培养菌丝球的技术。摇瓶发酵和发酵罐发酵的区别在于其发酵规模与用途, 前者主要用于菌种选育与工艺分析研究, 后者则主要用于工业化生产。灵芝真菌液体发酵可产生大量的菌丝体, 其产生的生物活性物质的含量和种类与天然采集的灵芝子实体的基本一致, 甚至某些重要活性物质的含量还高于子实体^[10]。相较于传统栽培, 液体发酵技术具有生产周期短、成本低、产量大、产品质量稳定且能自动化工业生产等特点, 正在成为灵芝培养的新模式, 同时也成为关于灵芝属真菌生产利用研究的热点。

3.1 摇瓶发酵培养

灵芝真菌液体摇瓶发酵的探索主要集中于发酵培养基条件的筛选与优化以及生产菌种的选育上, 通过初步的摇瓶发酵实验可筛选出高产目的产物的菌株及其最优培养条件。近来有报道称在灵芝真菌液体发酵过程中使用超声波处理后可促进灵芝菌丝体的生长和代谢^[64]; 武梅等^[65]对灵芝摇瓶发酵培养产灵芝多糖的过程进行了动态研究, 通过研究发酵过程中 pH、总糖、还原糖等的变化, 初步优化确定了高产灵芝多糖的摇瓶培养条件。然而针对培养基最适碳源的选择, Cui 等^[66]发现来源丰富、价格低廉的麦

芽汁可取代常用的葡萄糖作为唯一碳源。

通过摇瓶发酵培养筛选,研究者选育出在最优发酵工艺下能高产某种生物活性代谢产物(如漆酶、灵芝多糖、灵芝三萜等)的菌株^[67-71],为灵芝发酵工业化生产提供了多元化选择。

3.2 小试、中试发酵

小试、中试发酵生产主要是探索经摇瓶发酵优化后的培养基及发酵条件的可行性,为进一步开展大型发酵罐工业化生产提供发酵工艺与试验数据的支持。灵芝菌丝体中含有丰富的三萜、甾醇等活性物质,有研究报道灵芝新菌株 G0017 菌丝体在一种有效的三萜和甾醇发酵生产多级曝气速率控制工艺下的产率比固定曝气速率每体积液体每分钟 1.50 体积空气量提高了 69.54% 和 75.63%,并在 3 L 和 50 L 发酵罐中得到验证^[23]。王艳对灵芝小试发酵生产多糖的工艺条件进行研究,优化出一个最佳液体培养基配方,为扩大规模生产提供了参考依据^[72]。陈琼华等以韦伯灵芝(*G. weberianum*) TZC-1 作为生产菌株,在确定摇瓶发酵培养^[73]及 5 L 小型发酵罐最优生产工艺的基础上进行中试放大发酵工艺的优化,结果显示在最优的中试放大发酵工艺下获得的漆酶活力是摇瓶发酵水平的 2.5 倍^[74]。Wei 等^[75]对筛选出的一株高产多糖与灵芝酸的灵芝菌株进行发酵,在摇瓶发酵培养条件下确定最优的培养基组成,并在 10 L 的生物反应器中得到了验证,同时进一步扩大中试发酵培养,使得生物量、多糖、灵芝酸等产量均得到提高。

3.3 工业化发酵

近年来,采用液体发酵技术来获取灵芝中的有效成分受到了人们的广泛关注,并且也逐渐成为目前国内外工业化应用的重要手段之一。Lee 等^[76]在气升式发酵罐中引入双阶段 pH 控制技术生产灵芝多糖,发现在菌丝体生长对

数初期将 pH 值从 3.0 调节到 6.0,可改善菌丝的生长与多糖的产量;Tang 等^[77]在搅拌型生物反应器发酵生产灵芝多糖与灵芝酸的过程中通过控制溶解氧含量与分批补料的方式逐渐提高发酵液含量,提高了多糖与灵芝酸的产量。已有的研究成果均表明,灵芝真菌液体发酵的工业化是具有可行性与可实践性。截至目前已经开发出多个灵芝液体深层发酵工业产品,如“灵芝酸枣仁胶囊”(浙江方格药业有限公司)、“中祥灵芝菌丝体胶囊”(上海中祥生物制品有限公司)、灵芝银耳保健口服液^[78]和灵芝酸奶^[79]等。

4 灵芝真菌液体发酵技术及其产物的应用

通过液体发酵获得灵芝菌丝体的时间远少于传统栽培获得灵芝子实体的时间,从液体发酵产物中提取主要活性成分的过程相较于子实体也简单容易得多。目前越来越多的研究者更倾向于开发以灵芝液体发酵菌丝体和发酵液作为原料的产品。

4.1 食品开发

4.1.1 菌丝体与发酵液共用

灵芝真菌在液体发酵的过程中自身会分泌某些活性物质,这些活性物质存在于发酵液中。因此可间接使用灵芝发酵液生产饮料与保健酒,如:以牛蒡为培养基,加入灵芝、杏鲍菇进行混合液体发酵,再取发酵液加入各种配料调制成营养、美味的复合保健茶饮料^[80];张帅等^[81]向灵芝发酵液中加入糯米与酒曲,通过再发酵制备了一种香气浓郁的灵芝糯米酒;邓功成等^[82]优化确定了一种凝固型灵芝酸乳发酵工艺条件:灵芝菌液(含菌丝体) 25%,鲜牛乳 75%,蔗糖 8%,接种量 5%,42 °C 下发酵 2 h,直接可获得凝固型灵芝酸乳特色食品;黄清铎等^[83]研制出一种以甘蔗汁进行灵芝液体发

醇的功能性饮料, 并对饮料配方进行了优化。

4.1.2 菌丝体单用

利用液体深层发酵产物开发灵芝相关的功能性食品具有广阔的发展前景。早在 20 世纪 60 年代, 美国、英国和法国等^[84]就已经开始通过液体发酵大规模生产灵芝菌丝体来制作调味品; 而将发酵后的菌丝体经过干燥粉碎后加入面粉、木薯淀粉, 可生产出具有保健功能的膳食方便食品^[85]。灵芝发酵获得的菌丝体具有很大的应用价值, 后续可将菌丝体用作辅料添加到食品中制作成各种具有保健和营养功能的食品, 如添加到饼干中^[86]。

4.2 环保应用

4.2.1 灵芝菌丝球在污水处理中的应用

灵芝液体发酵获得的菌丝体, 其呈球状并附有星射状的菌丝形态, 使得其具有一定的吸附性。根据灵芝菌丝体这一特点, Mohd Hanafiah 等^[87]研究发现塞尔维亚野生灵芝(*wild-Serbian Ganoderma*)菌丝体可有效清除合成污水中的污染物; Torres-Farradá 等^[16]发现韦伯灵芝(*G. weberianum*) B-18 菌丝体在半连续条件下对工业废水能起到有效的脱色与解毒作用; Ma 等^[88]研究发现, 白腐真菌灵芝对纺织废水中活性橙 16 染料具有很好的脱色脱毒作用; Zhou 等研究发现, 韦伯灵芝(*G. weberianum*) TZC-1 的菌丝体可使靛蓝胭脂红脱色^[89]。

尽管不同品种的灵芝菌丝体对不同颜料有着不同的脱色效果, 但依旧可以看出灵芝属真菌菌丝体在处理各种污水方面具有较好的潜力。

4.2.2 灵芝漆酶的应用

人们熟知的真菌类漆酶是一种含铜的多酚氧化酶, 其特有的漆酶化学基团及其氧化能力使得真菌类漆酶在纸浆漂白、污水处理和染料脱色等环保领域有较大的应用价值^[90-91]。王艳就韦伯灵芝(*G. weberianum*) TZC-1 液体发酵的

漆酶粗提液对多种染料的脱色进行了优化分析, 发现在 pH 3.0、50 °C 条件下反应 120 min, 对靛蓝的脱色率为 92.6%^[72]; 在 pH 4.5、20 °C 条件下反应 30 min, 对阳离子红 2GL (cationic red 2GL)的脱色率为 90.3%^[92]; 在 pH 3.0、40 °C 条件下反应 70 min, 对直接耐晒翠蓝 GL 的脱色率为 94.3%^[93]。除此之外, 在生物膜方面的应用上, 已有研究利用灵芝废菌丝体一步固定化生产漆酶形成生物膜, 经实验分析测定, 形成的菌丝膜具有良好的耐热性, 可使甲基紫和孔雀石绿脱色, MTT 比色法(一种检测细胞存活和生长的方法)表明该膜还具有良好的生物相容性^[94]。

灵芝漆酶高效脱色的效果预示着灵芝真菌液体发酵产物在环境保护方面有着广阔的应用前景。

4.3 基因工程应用

灵芝真菌液体发酵产品的应用前景十分广阔, 而且早在 2012 年我国科学家就已公布灵芝的全基因组序列^[95], 推动灵芝成为天然药物合成研究的模式真菌^[96], 这将有利于整合现代生命科学技术和深入研究次生代谢的机制及规律, 为高效、可控的天然药物合成平台的建设奠定基础。

4.3.1 灵芝遗传转化体系的建立

Zhou 等利用农杆菌介导法建立了一种包括表达载体和转基因方法等在内的简便高效的韦伯灵芝(*G. weberianum*)遗传转化体系^[97], 同样的方法在其他品种的灵芝上也有应用^[98]; 而 Kim 等^[99]则是利用限制性内切酶介导建立了另一种灵芝转化体系。

4.3.2 外源基因在灵芝中的表达

利用转基因方法提高灵芝属真菌液体发酵高产活性物质是目前研究的热点之一, 其目的是进一步提高灵芝液体发酵的效益。Li 等^[100]

利用基因工程技术将透明颤菌血红蛋白(*Vitreoscilla hemoglobin*)基因 *vgd* 导入灵芝菌丝细胞中,成功构建一株在液体发酵中能高产胞外多糖的灵芝基因工程菌株;倪挺等^[101]将人胰岛素基因导入灵芝菌丝细胞中,通过液体发酵生产出人胰岛素。

4.3.3 灵芝基因克隆与异源表达

将灵芝中编码合成某种活性物质的基因片段导入其他细胞内表达,有助于寻找一条新的液体发酵生产路线。肖建勇等^[102]利用基因工程技术将灵芝中的 LZ-8 基因导入毕赤酵母(*Pichia pastoris*)中,并成功在毕赤酵母(*P. pastoris*)的表达系统中诱导表达 LZ-8 蛋白;Zhou 等从韦伯灵芝(*G. weberianum*) TZC-1 中克隆出一个新的漆酶基因,并成功转入毕赤酵母(*P. pastoris*)中表达^[103];Wang 等^[104]将灵芝中的细胞色素 P450 (cytochrome P450)基因转入酿酒酵母(*Saccharomyces cerevisiae*)中并成功表达产出抗肿瘤的灵芝酸。

4.4 医药应用

灵芝凭借着其独有的药理功效,自古便是一种名贵中药材,这也使得灵芝真菌成为研究最为深入的药用真菌之一^[105]。正如本文第 2 章节介绍,灵芝真菌液体发酵产物(发酵液和菌丝体)含有多种具有药用效果的活性成分。关于灵芝真菌液体深层发酵产物在医学领域的研究也正在逐渐增多,最近有研究表明,灵芝液体发酵产物对人体内肠道菌群有着积极的影响,并间接性增强人体成骨细胞的活性,对骨骼生理产生有益的影响^[106];另有报道发现灵芝发酵液能有效抑制胰腺癌细胞和前列腺癌细胞生长^[107]。灵芝三萜作为一种具有广泛药理活性的物质,是目前深入研究的灵芝主要活性成分之一。李娜等^[108]对液体发酵生产灵芝三萜的进展进行了综述,认为具有医药用价值的灵芝三萜类化

合物可通过液体发酵技术的探索、优化和改良而进一步提高产量,为后续进一步研究灵芝液体发酵生产药用三萜类物质提供理论参考。

5 展望

2019 年,由北京大学基础医学院林志彬与杨宝学共同主编的 *Ganoderma and Health*^[109]一书系统地介绍了灵芝的分类学、生物学特性、人工栽培、化学成分、产业研究进展、灵芝及其提取物的药理学研究进展和临床应用等,但关于灵芝液体发酵方面的介绍比较欠缺。

目前,灵芝真菌液体发酵主要集中于液体发酵培养液及培养条件的优化、发酵产物有效成分的分析研究、液体发酵制作健康风味的饮料产品以及灵芝基因工程菌株相关产品的开发等领域。同时,关于灵芝的活性成分、药理作用与临床应用的分析研究也在不断深入,这些研究有助于人们更清楚灵芝中哪些成分最有效、性价比最大、疗效最好,也让灵芝液体发酵生产目的产物的目标更加明确,更有利于灵芝液体发酵的工业化发展。

灵芝真菌液体发酵技术及其产品在食品、医药保健品、饲料、化妆品等领域均有应用。关于灵芝真菌液体发酵的未来研究则应承前继后。

(1) 根据目的产物的不同,进一步优化相应液体发酵培养基的组成及培养条件,在最优的培养方式下最大限度地获得相关的发酵产品,寻求一种既可以满足灵芝发酵生长需求,又满足原料来源广、价格低廉、适合大规模生产的特色发酵原料替代品。

(2) 继续深入研究灵芝液体发酵产物中的活性成分及其功效。目前对于灵芝活性成分的分析主要体现在灵芝子实体上,有关灵芝液体发酵菌丝体及其发酵液活性成分的分析还应进一步深入。

(3) 随着现代生物科技的发展, 进一步补充、完善灵芝真菌在微生物学、代谢组学、基因组学、发酵工程和食药真菌学等领域的学科知识, 有助于加快灵芝真菌液体发酵技术及其相关产业的发展。

REFERENCES

- [1] Du Z, Dong CH, Wang K, Yao YJ. Classification, biological characteristics and cultivations of *Ganoderma*[J]. *Advances in Experimental Medicine and Biology*, 2019, 1181: 15-58
- [2] Wang PY, Zhu XL, Lin ZB. Antitumor and immunomodulatory effects of polysaccharides from broken-spore of *Ganoderma lucidum*[J]. *Frontiers in Pharmacology*, 2012, 3: 135
- [3] Ngoc TM, Phuong NTT, Khoi NM, Park S, Kwak HJ, Nhiem NX, Trang BTT, Tai BH, Song JH, Ko HJ, et al. A new naphthoquinone analogue and antiviral constituents from the root of *Rhinacanthus nasutus*[J]. *Natural Product Research*, 2019, 33(3): 360-366
- [4] 闫征, 王宏旭, 刘莉莹, 杜国华, 陈若芸. 灵芝三萜类化合物的体外抗肿瘤活性研究[J]. *国际检验医学杂志*, 2017, 38(5): 633-634, 637
Yan Z, Wang HX, Liu LY, Du GH, Chen RY. Study on *in vitro* anti-tumor activity of triterpenoids from *Ganoderma lucidum*[J]. *International Journal of Laboratory Medicine*, 2017, 38(5): 633-634, 637 (in Chinese)
- [5] Dai CL, He LZ, Ma B, Chen TF. Facile nanolization strategy for therapeutic *Ganoderma lucidum* spore oil to achieve enhanced protection against radiation-induced heart disease[J]. *Small*, 2019, 15(36): e1902642
- [6] 陈玉胜, 陈全战. 灵芝多糖对 CCl_4 诱导的急性肝损伤小鼠的抗炎和保肝活性[J]. *食品科学*, 2017, 38(17): 210-215
Chen YS, Chen QZ. Anti-inflammatory and hepatoprotective effects of *Ganoderma lucidum* polysaccharides on carbon tetrachloride-induced acute liver injury in mice[J]. *Food Science*, 2017, 38(17): 210-215 (in Chinese)
- [7] 石凤敏, 丁自勉, 陈士林, 佟曦然. 灵芝资源及其鉴别研究进展[J]. *世界科学技术: 中医药现代化*, 2012, 14(2): 1473-1480
Shi FM, Ding ZM, Chen SL, Tong XR. Research progress on resource and identification of *Ganoderma*[J]. *World Science and Technology: Modernization of Traditional Chinese Medicine and Materia Medica*, 2012, 14(2): 1473-1480 (in Chinese)
- [8] 周选围, 林娟, 周良. 灵芝主要营养成分的测定分析[J]. *陕西师范大学学报(自然科学版)*, 1998, 26(S1): 4
Zhou XW, Lin J, Zhou L. Determine and analysis on primary nutritional components of *Ganoderma* spp.[J]. *Journal of Shaanxi Normal University: Natural Science Edition*, 1998, 26(S1): 4 (in Chinese)
- [9] 于华峰, 刘艳芳, 周帅, 颜梦秋, 薛令坤, 唐庆九, 张劲松. 灵芝子实体、菌丝体及孢子粉中多糖成分差异比较研究[J]. *菌物学报*, 2016, 35(2): 170-177
Yu HZ, Liu YF, Zhou S, Yan MQ, Xue LK, Tang QJ, Zhang JS. Comparison of the polysaccharides from fruiting bodies, mycelia and spore powder of *Ganoderma lingzhi*[J]. *Mycosystema*, 2016, 35(2): 170-177 (in Chinese)
- [10] 陆正清. 灵芝液体深层发酵技术[J]. *江苏食品与发酵*, 2007(3): 38-40
Lu ZQ. *Ganoderma lucidum* liquid deep fermentation technology[J]. *Jiangsu Food & Fermentation*, 2007(3): 38-40 (in Chinese)
- [11] 李静静, 胡晓琴, 张新风, 刘京晶, 曹隆枢. 赤芝孢子粉和子实体主要化学成分变异规律研究[J]. *中国中药杂志*, 2014, 39(21): 4246-4251
Li JJ, Hu XQ, Zhang XF, Liu JJ, Cao LS. Study on variation of main ingredients from spores and fruiting bodies of *Ganoderma lucidum*[J]. *China Journal of Chinese Materia Medica*, 2014, 39(21): 4246-4251 (in Chinese)
- [12] 臧晋, 罗建成, 黄开勋. 灵芝液体培养菌株的选择及其发酵条件研究[J]. *四川食品与发酵*, 2007, 43(5): 28-31
Zang J, Luo JC, Huang KX. Study on selecting the strains and optimizing the culture conditions for production of *Ganoderma lucidum* polysaccharide by submerged fermentation[J]. *Sichuan Food and Fermentation*, 2007, 43(5): 28-31 (in Chinese)
- [13] 付丽红, 王金菊, 郝利民, 王艳萍. 紫芝液体深层发酵条件的初步研究[J]. *天津科技大学学报*, 2013, 28(5): 19-22, 27
Fu LH, Wang JJ, Hao LM, Wang YP. Preliminary study of liquid submerged fermentation of *Ganoderma sinensis*[J]. *Journal of Tianjin University of Science & Technology*, 2013, 28(5): 19-22, 27 (in Chinese)
- [14] 廖婷婷, 邓功成, 夏进梅, 况明燕, 王锡高, 王登榜, 何丽菊, 黎红杏. 黑芝菌丝体液体发酵条件研究[J]. *现代农业科技*, 2015(3): 71-72

- Liao TT, Deng GC, Xia JM, Kuang MY, Wang XG, Wang DB, He LJ, Li HX. Study on liquid fermentation conditions of *B Ganoderma atrum* mycelium[J]. Modern Agricultural Science and Technology, 2015(3): 71-72 (in Chinese)
- [15] 梁艳华, 孙勇, 吴龙龙, 张刘强, 李医明. 四川灵芝液体发酵样品两种异黄酮的结构鉴定和生源初探[J]. 天然产物研究与开发, 2020, 32(3): 398-402
- Liang YH, Sun Y, Wu LL, Zhang LQ, Li YM. Structural identification and preliminary biogenic study of two isoflavones from *Ganoderma sichuanense* liquid fermentation samples[J]. Natural Product Research and Development, 2020, 32(3): 398-402 (in Chinese)
- [16] Torres-Farrad G, Manzano AM, Ramos-Leal M, Domnguez O, Snchez MI, Vangronsveld J, Guerra G. Biodegradation and detoxification of dyes and industrial effluents by *Ganoderma weberianum* B-18 immobilized in a lab-scale packed-bed bioreactor[J]. Bioremediation Journal, 2018, 22(1/2): 20-27
- [17] 陈国梁, 高瑜, 张昊, 李旺莉, 李旭, 南佳盟. 松杉灵芝液体菌种发酵过程相关指标变化规律探究[J]. 榆林学院学报, 2019, 29(6): 53-55
- Chen GL, Gao Y, Zhang H, Li WL, Li X, Nan JM. A study on the variation rules of relative indexes during *Ganoderma tsugae* liquid fermentation process[J]. Journal of Yulin University, 2019, 29(6): 53-55 (in Chinese)
- [18] 潘志恒, 岳鹏, 孙勇民, 刘鹏. 树舌灵芝发酵产漆酶培养基优化[J]. 保鲜与加工, 2016, 16(4): 107-111
- Pan ZH, Yue K, Sun YM, Liu P. Optimization of culture medium for laccase production from *Ganoderma applanatum*[J]. Storage and Process, 2016, 16(4): 107-111 (in Chinese)
- [19] 杨丽梅, 苏平, 涂俊铭. 血芝液体深层发酵合成三萜类化合物的条件优化[J]. 安徽农业科学, 2016, 44(2): 158-161, 165
- Yang LM, Su P, Tu JM. Optimization of triterpenoids production by liquid submerged fermentation of *Amauroderma rude*(berk.) torrend[J]. Journal of Anhui Agricultural Sciences, 2016, 44(2): 158-161, 165 (in Chinese)
- [20] 李丽, 杨云丽, 杨小凡, 何伟, 袁恺, 朱威宇, 彭超, 何一凡, 董银卯, 周卫强. 液体发酵生产灵芝三萜酸的过程调控研究进展[J]. 食品与发酵工业, 2021, 47(8): 304-312
- Li L, Yang YL, Yang XF, He W, Yuan K, Zhu WY, Peng C, He YF, Dong YM, Zhou WQ. Advances on process regulation of *Ganoderma* triterpene acids production by liquid fermentation[J]. Food and Fermentation Industries, 2021, 47(8): 304-312 (in Chinese)
- [21] Feng J, Feng N, Tang QJ, Liu YF, Yang Y, Liu F, Zhang JS, Lin CC. Optimization of *Ganoderma lucidum* polysaccharides fermentation process for large-scale production[J]. Applied Biochemistry and Biotechnology, 2019, 189(3): 972-986
- [22] 秦卫东, 高明侠, 吕兆启. 灵芝深层发酵制备多糖蛋白生物活性钙的研究[J]. 中国食品添加剂, 2002(5): 19-22
- Qin WD, Gao MX, Lu ZQ. Study on getting polysacchroprotein bioactive calcium by cultivating in the *Ganoderma lucidum* deep fermentation[J]. China Food Additives, 2002(5): 19-22 (in Chinese)
- [23] Feng J, Feng N, Tang QJ, Liu YF, Tang CH, Zhou S, Wang JY, Tan Y, Zhang JS, Lin CC. Development and optimization of the triterpenoid and sterol production process with Lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* strain G0017 (agaricomycetes), in liquid submerged fermentation at large scale[J]. International Journal of Medicinal Mushrooms, 2021, 23(3): 43-53
- [24] Chen YS, Lan P. Total syntheses and biological evaluation of the *Ganoderma lucidum* alkaloids lucidimines B and C[J]. ACS Omega, 2018, 3(3): 3471-3481
- [25] 杨洋, 吴小勇, 张湛, 苏政权. 富硒灵芝发酵培养工艺及产物抗氧化能力研究[J]. 现代食品科技, 2010, 26(12): 1349-1353
- Yang Y, Wu XY, Zhang Z, Su ZQ. Fermentation of *Ganoderma lucidum* for selenium enrichment and the antioxidant capability of the protein product[J]. Modern Food Science and Technology, 2010, 26(12): 1349-1353 (in Chinese)
- [26] Baby S, Johnson AJ, Govindan B. Secondary metabolites from *Ganoderma*[J]. Phytochemistry, 2015, 114: 66-101
- [27] Bishop KS, Kao CHJ, Xu YY, Glucina MP, Paterson RRM, Ferguson LR. From 2000 years of *Ganoderma lucidum* to recent developments in nutraceuticals[J]. Phytochemistry, 2015, 114: 56-65
- [28] Wei ZH, Liu LL, Guo XF, Li YJ, Hou BC, Fan QL, Wang KX, Luo YD, Zhong JJ. Sucrose fed-batch strategy enhanced biomass, polysaccharide, and ganoderic acids production in fermentation of *Ganoderma lucidum* 5.26[J]. Bioprocess and Biosystems Engineering, 2016, 39(1): 37-44
- [29] Feng J, Zhang JS, Feng N, Yan MQ, Yang Y, Jia W, Lin

- CC. A novel *Ganoderma lucidum* G0119 fermentation strategy for enhanced triterpenes production by statistical process optimization and addition of oleic acid[J]. *Engineering in Life Sciences*, 2017, 17(4): 430-439
- [30] Zhao LY, Dong YH, Chen GT, Hu QH. Extraction, purification, characterization and antitumor activity of polysaccharides from *Ganoderma lucidum*[J]. *Carbohydrate Polymers*, 2010, 80(3): 783-789
- [31] Shi M, Zhang ZY, Yang YN. Antioxidant and immunoregulatory activity of *Ganoderma lucidum* polysaccharide (GLP)[J]. *Carbohydrate Polymers*, 2013, 95(1): 200-206
- [32] Shen J, Park HS, Xia YM, Kim GS, Cui SW. The polysaccharides from fermented *Ganoderma lucidum* mycelia induced miRNAs regulation in suppressed HepG2 cells[J]. *Carbohydrate Polymers*, 2014, 103: 319-324
- [33] Subramaniam S, Sabaratnam V, Kuppusamy UR, Tan YS. Solid-substrate fermentation of wheat grains by mycelia of indigenous species of the genus *Ganoderma* (higher Basidiomycetes) to enhance the antioxidant activities[J]. *International Journal of Medicinal Mushrooms*, 2014, 16(3): 259-267
- [34] Li AM, Shuai X, Jia ZJ, Li HY, Liang XB, Su DM, Guo WH. *Ganoderma lucidum* polysaccharide extract inhibits hepatocellular carcinoma growth by downregulating regulatory T cells accumulation and function by inducing microRNA-125b[J]. *Journal of Translational Medicine*, 2015, 13: 100
- [35] Zolj S, Smith MP, Goines JC, Ali TS, Huff MO, Robinson DL, Lau JM. Antiproliferative effects of a triterpene-enriched extract from Lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (agaricomycetes), on human lung cancer cells[J]. *International Journal of Medicinal Mushrooms*, 2018, 20(12): 1173-1183
- [36] Feng N, Wei YT, Feng J, Tang QJ, Zhang Z, Zhang JS, Han W. Preparative isolation of ganoderic acid S, ganoderic acid T and ganoderol B from *Ganoderma lucidum* mycelia by high-speed counter-current chromatography[J]. *Biomedical Chromatography*, 2018, 32(10): e4283
- [37] Zhang JS, Tang QJ, Zimmerman-Kordmann M, Reutter W, Fan H. Activation of B lymphocytes by GLIS, a bioactive proteoglycan from *Ganoderma lucidum*[J]. *Life Sciences*, 2002, 71(6): 623-638
- [38] Hsin IL, Chiu LY, Ou CC, Wu WJ, Sheu GT, Ko JL. CD133 inhibition via autophagic degradation in pemetrexed-resistant lung cancer cells by GMI, a fungal immunomodulatory protein from *Ganoderma microsporum*[J]. *British Journal of Cancer*, 2020, 123(3): 449-458
- [39] U Girjal V, Neelagund S, Krishnappa M. *Ganoderma lucidum*: a source for novel bioactive lectin[J]. *Protein and Peptide Letters*, 2011, 18(11): 1150-1157
- [40] Wu Q, Li Y, Peng K, Wang XL, Ding ZY, Liu LM, Xu P, Liu GQ. Isolation and characterization of three antihypertension peptides from the mycelia of *Ganoderma lucidum* (agaricomycetes)[J]. *Journal of Agricultural and Food Chemistry*, 2019, 67(29): 8149-8159
- [41] Zhang JJ, Dong Y, Qin FY, Yan YM, Cheng YX. Meroterpenoids and alkaloids from *Ganoderma australe*[J]. *Natural Product Research*, 2019: 1-7
- [42] Zhao ZZ, Chen HP, Feng T, Li ZH, Dong ZJ, Liu JK. Lucidimine A-D, four new alkaloids from the fruiting bodies of *Ganoderma lucidum*[J]. *Journal of Asian Natural Products Research*, 2015, 17(12): 1160-1165
- [43] Jeong YU, Park YJ. Ergosterol peroxide from the medicinal mushroom *Ganoderma lucidum* inhibits differentiation and lipid accumulation of 3T3-L1 adipocytes[J]. *International Journal of Molecular Sciences*, 2020, 21(2): 460
- [44] 孙金旭, 魏连秋, 朱会霞. 灵芝深层发酵中麦角甾醇含量的测定[J]. *现代食品科技*, 2013, 29(9): 2267-2270
Sun JX, Wei LQ, Zhu HX. Determination of ergosterol by HPLC in the fermentation of *Ganoderma lucidum*[J]. *Modern Food Science and Technology*, 2013, 29(9): 2267-2270 (in Chinese)
- [45] Shiao MS. Triterpenoid natural products in the fungus *Ganoderma lucidum*[J]. *Journal of the Chinese Chemical Society*, 1992, 39(6): 669-674
- [46] 朱晓璐, 岳亚文, 张劲松, 冯杰, 唐庆九, 冯娜, 韩伟. 灵芝菌丝体中一个三萜类化合物的核磁归属及活性初探[J]. *菌物学报*, 2020, 39(8): 1551-1558
Zhu XL, Yue YW, Zhang JS, Feng J, Tang QJ, Feng N, Han W. NMR attribution and bioactivity evaluation of a triterpene in mycelia of *Ganoderma lingzhi*[J]. *Mycosystema*, 2020, 39(8): 1551-1558 (in Chinese)
- [47] Sun XB, Zhao C, Pan W, Wang JP, Wang WJ. Carboxylate groups play a major role in antitumor activity of *Ganoderma applanatum* polysaccharide[J]. *Carbohydrate Polymers*, 2015, 123: 283-287
- [48] 黄静, 李爱欣, 周贤, 王淑敏. 松杉灵芝发酵产物对 H22 肝腹水瘤小鼠免疫功能影响及抗肿瘤作用[J]. *药学研究*, 2016, 35(10): 575-578

- Huang J, Li AX, Zhou X, Wang SM. The immune function and anti-tumor effects of *Ganoderma tsugae* fermentation product on H22 liver ascites tumor mice[J]. Journal of Pharmaceutical Research, 2016, 35(10): 575-578 (in Chinese)
- [49] 张洪文. 灵芝发酵产物对感染 PCV-2 病毒断奶仔猪生长性能和免疫功能的影响[J]. 中国饲料, 2021(2): 29-32
- Zhang HW. Effects of *Ganoderma lucidum* fermentation products on growth performance and immune function of weaned piglets infected with PCV-2 virus[J]. China Feed, 2021(2): 29-32 (in Chinese)
- [50] 彭彦淳, 范思远, 李晓慧, 魏峰. 灵芝液体发酵物对肉鸡生产性能、免疫功能和抗氧化性能的影响[J]. 黑龙江畜牧兽医, 2021(2): 107-112
- Peng YC, Fan SY, Li XH, Wei F. Effect of liquid fermentation products of *Ganoderma lucidum* on production performance, immune function and antioxidant capacity of broilers[J]. Heilongjiang Animal Science and Veterinary Medicine, 2021(2): 107-112 (in Chinese)
- [51] 孟歌, 崔宝凯, 李春道, 刘红霞, 司静. 药用真菌灵芝液体培养过程中的抗氧化活性研究[J]. 菌物学报, 2018, 37(4): 486-501
- Meng G, Cui BK, Li CD, Liu HX, Si J. Antioxidant activities of medicinal fungus *Ganoderma lingzhi* in the process of liquid cultivation[J]. Mycosystema, 2018, 37(4): 486-501 (in Chinese)
- [52] Kan YJ, Chen TQ, Wu YB, Wu JG, Wu JZ. Antioxidant activity of polysaccharide extracted from *Ganoderma lucidum* using response surface methodology[J]. International Journal of Biological Macromolecules, 2015, 72: 151-157
- [53] 谢丽源, 甘炳成, 彭卫红, 黄忠乾, 谭伟. 灵芝深层发酵产物抗氧化活性物质与抗氧化能力分析[J]. 食品工业科技, 2015, 36(2): 105-109
- Xie LY, Gan BC, Peng WH, Huang ZQ, Tan W. Analysis of antioxidant substances and antioxidant capacity of submerged fermentation product of *Ganoderma lucidum*[J]. Science and Technology of Food Industry, 2015, 36(2): 105-109 (in Chinese)
- [54] Vazirian M, Faramarzi MA, Ebrahimi SES, Esfahani HRM, Samadi N, Hosseini SA, Asghari A, Manayi A, Mousazadeh A, Asef MR, et al. Antimicrobial effect of the Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (higher Basidiomycetes) and its main compounds[J]. International Journal of Medicinal Mushrooms, 2014, 16(1): 77-84
- [55] Siwulski M, Sobieralski K, Golak-Siwulska I, Sokół S, Sękara A. *Ganoderma lucidum* (Curt.: Fr.) Karst. – health-promoting properties: a review[J]. Herba Polonica, 2015, 61(3): 105-118
- [56] 王晓玲, 刘高强, 周国英. 紫芝发酵菌体中三萜类化合物的抑菌活性研究[J]. 时珍国医国药, 2008, 19(11): 2636-2637
- Wang XL, Liu GQ, Zhou GY. *In vitro* bacteriostasis of the triterpenoids from the mycelium of *Ganoderma sinense*[J]. Lishizhen Medicine and Materia Medica Research, 2008, 19(11): 2636-2637 (in Chinese)
- [57] Naveenkumar C, Swathi S, Jayalakshmi G, Chidambaram R, Srikumar R. Screening of antifungal activity of *Ganoderma lucidum* extract against medically important fungi[J]. Indian Journal of Public Health Research & Development, 2018, 9(1): 269
- [58] Zhang WJ, Tao JY, Yang XP, Yang ZL, Zhang L, Liu HS, Wu KL, Wu JG. Antiviral effects of two *Ganoderma lucidum* triterpenoids against *Enterovirus 71* infection[J]. Biochemical and Biophysical Research Communications, 2014, 449(3): 307-312
- [59] Abdullah S, Jang SE, Kwak MK, Chong K. *Ganoderma boninense* mycelia for phytochemicals and secondary metabolites with antibacterial activity[J]. Journal of Microbiology, 2020, 58(12): 1054-1064
- [60] 黄林, 何莞嫣, 刘蔚, 黄康, 王艳, 王征. 灵芝菌发酵紫甘薯渣产物降血糖功效研究[J]. 中国食品学报, 2014, 14(3): 34-39
- Huang L, He WY, Liu W, Huang K, Wang Y, Wang Z. Hypoglycemic effect of purple sweet potato slag product fermented by *Ganoderma lucidum*[J]. Journal of Chinese Institute of Food Science and Technology, 2014, 14(3): 34-39 (in Chinese)
- [61] 唐占府, 李鲁扬, 王秀琴, 张继东. 灵芝菌液治疗原发性高脂血症 30 例[J]. 陕西中医, 2001, 22(8): 464-465
- Tang ZF, Li LY, Wang XQ, Zhang JD. Treatment of 30 cases of primary hyperlipidemia with *Ganoderma lucidum* liquid[J]. Shaanxi Journal of Traditional Chinese Medicine, 2001, 22(8): 464-465 (in Chinese)
- [62] 许梦丽, 徐琼, 崔翠, 匡金雄, 马丽珍. 灵芝多糖对妊娠期糖尿病大鼠肝脏损伤的保护作用[J]. 中国临床药理学杂志, 2020, 36(20): 3242-3245
- Xu ML, Xu Q, Cui C, Kuang JX, Ma LZ. Protective effect of *Ganoderma lucidum* polysaccharides on liver injury[J]. The Chinese Journal of Clinical Pharmacology, 2020, 36(20): 3242-3245 (in Chinese)
- [63] Li KK, Yu M, Hu Y, Ren GM, Zang TT, Xu XH, Qu JJ. Three kinds of *Ganoderma lucidum* polysaccharides

- attenuate DDC-induced chronic pancreatitis in mice[J]. *Chemico-Biological Interactions*, 2016, 247: 30-38
- [64] Sun L, Liu LP, Yang L, Wang YZ, Dabbour M, Mintah BK, He RH, Ma HL. Effects of low-intensity ultrasound on the biomass and metabolite of *Ganoderma lucidum* in liquid fermentation[J]. *Journal of Food Process Engineering*, 2021, 44(1): e13601
- [65] 武梅, 周应揆, 赵永昌, 李亚辉. 灵芝菌丝体液体发酵培养产灵芝多糖的动态研究[J]. *云南大学学报(自然科学版)*, 1999, 21(2): 165-166
- Wu M, Zhou YK, Zhao YC, Li YH. Studies on liquid fermentation of the polysaccharide produced by the mycelia of *Ganoderma lucidum*[J]. *Journal of Yunnan University: Natural Sciences*, 1999, 21(2): 165-166 (in Chinese)
- [66] Cui ML, Yang HY, He GQ. Submerged fermentation production and characterization of intracellular triterpenoids from *Ganoderma lucidum* using HPLC-ESI-MS[J]. *Journal of Zhejiang University-SCIENCE B*, 2015, 16(12): 998-1010
- [67] 雷德柱, 龙嘉欣, 田长恩. 产漆酶的草菇菌株的筛选[J]. *工业微生物*, 2008, 38(3): 51-55
- Lei DZ, Long JX, Tian CE. Screening for new resource of laccase producing strains from *Volvariella volvacea*[J]. *Industrial Microbiology*, 2008, 38(3): 51-55 (in Chinese)
- [68] 陈琼华, 周玉萍, 毕凡星, 程惠贞, 田长恩. 真菌漆酶高产菌株的筛选[J]. *广州大学学报(自然科学版)*, 2009, 8(5): 53-57
- Chen QH, Zhou YP, Bi FX, Cheng HZ, Tian CE. Screening for high-yield laccase-secreting fungal strains[J]. *Journal of Guangzhou University: Natural Science Edition*, 2009, 8(5): 53-57 (in Chinese)
- [69] 刘晓丹, 王俊玲, 王霞. 树舌灵芝复合诱变选育漆酶高产菌株[J]. *北方园艺*, 2019(14): 124-129
- Liu XD, Wang JL, Wang X. Breeding of laccase high yield strain for *Ganoderma applanatum* by compound mutation[J]. *Northern Horticulture*, 2019(14): 124-129 (in Chinese)
- [70] 董玉玮, 苗敬芝, 曹泽虹, 高明侠. 复合诱变灵芝原生质体选育高产胞外多糖菌株[J]. *食品科技*, 2011, 36(10): 12-16
- Dong YW, Miao JZ, Cao ZH, Gao MX. Breeding of high extracellular polysaccharide yield strains of *Ganoderma lucidum* by compound mutation of protoplasts[J]. *Food Science and Technology*, 2011, 36(10): 12-16 (in Chinese)
- [71] 董玉玮, 苗敬芝, 吕兆启, 曹泽虹, 高明侠. 氯化锂诱变赤灵芝原生质体选育高产三萜类菌株[J]. *食品科学*, 2011, 32(1): 120-123
- Dong YW, Miao JZ, Lu ZQ, Cao ZH, Gao MX. Screening and breeding of high-yield triterpenes-producing *Ganoderma lucidum* mutants induced by lithium chloride[J]. *Food Science*, 2011, 32(1): 120-123 (in Chinese)
- [72] 王艳. 灵芝小试发酵工艺研究[J]. *安徽农业科学*, 2010, 38(11): 5619-5620, 5775
- Wang Y. Small scale fermentation of *Ganoderma lucidum*[J]. *Journal of Anhui Agricultural Sciences*, 2010, 38(11): 5619-5620, 5775 (in Chinese)
- [73] 陈琼华, 周玉萍, 江桂杰, 李广宇, 程惠贞, 田长恩. 韦伯灵芝发酵产漆酶及其对靛蓝脱色作用的研究[J]. *食品与发酵工业*, 2010, 36(3): 25-30
- Chen QH, Zhou YP, Jiang GJ, Li GY, Cheng HZ, Tian CE. Laccase production by *Ganoderma weberianum* fermentation and its decolorization effect on indigo dye[J]. *Food and Fermentation Industries*, 2010, 36(3): 25-30 (in Chinese)
- [74] 陈琼华, 周玉萍, 桂林, 孙莉丽, 田长恩. 韦伯灵芝漆酶生产中试研究[J]. *食品与发酵工业*, 2016, 42(5): 44-49
- Chen QH, Zhou YP, Gui L, Sun LL, Tian CE. Study on pilot-scale fermentation of *Ganoderma weberianum* for laccase production[J]. *Food and Fermentation Industries*, 2016, 42(5): 44-49 (in Chinese)
- [75] Wei ZH, Duan YY, Qian YQ, Guo XF, Li YJ, Jin SH, Zhou ZX, Shan SY, Wang CR, Chen XJ, et al. Screening of *Ganoderma* strains with high polysaccharides and ganoderic acid contents and optimization of the fermentation medium by statistical methods[J]. *Bioprocess and Biosystems Engineering*, 2014, 37(9): 1789-1797
- [76] Lee KM, Lee SY, Lee HY. Bistage control of pH for improving exopolysaccharide production from mycelia of *Ganoderma lucidum* in an air-lift fermentor[J]. *Journal of Bioscience and Bioengineering*, 1999, 88(6): 646-650
- [77] Tang YJ, Zhong JJ. Fed-batch fermentation of *Ganoderma lucidum* for hyperproduction of polysaccharide and ganoderic acid[J]. *Enzyme and Microbial Technology*, 2002, 31(1/2): 20-28
- [78] 肖贵平. 灵芝银耳保健口服液的开发研制[J]. *食品与发酵工业*, 2002, 28(7): 54-58
- Xiao GP. The development of health liquid production from *Ganoderma lucidum* and *Tremella fuciformis*[J]. *Food and Fermentation Industries*, 2002, 28(7): 54-58 (in Chinese)

- [79] 李靖, 陈伟, 李小永, 程芳. 灵芝酸奶的研制[J]. 中国乳品工业, 2011, 39(5): 60-64
Li J, Chen W, Li XY, Cheng F. *Ganoderma* yogurt and changes in colonies, physical and chemical properties during storage[J]. China Dairy Industry, 2011, 39(5): 60-64 (in Chinese)
- [80] 王凡, 葛蒙蒙, 高忠辉, 王振宇, 苗敬芝, 董玉玮. 灵芝、杏鲍菇混合发酵牛蒡白果茶饮料的研制[J]. 食品工业, 2019, 40(7): 58-62
Wang F, Ge MM, Gao ZH, Wang ZY, Miao JZ, Dong YW. The preparation of tea beverage of *Arctium lappa* and *Ginkgo biloba* by mixing fermentation with *Ganoderma lucidum* and *Pleurotus eryngii*[J]. The Food Industry, 2019, 40(7): 58-62 (in Chinese)
- [81] 张帅, 王娟, 程昊. 发酵型灵芝糯米酒的研制[J]. 食品工业, 2018, 39(12): 92-95
Zhang S, Wang J, Cheng H. Development of fermented glutinous rice wine with *Ganoderma lucidum* mycelia[J]. The Food Industry, 2018, 39(12): 92-95 (in Chinese)
- [82] 邓功成, 赵洪, 高礼安, 李永波, 马媛, 黎娇凌, 李静. 灵芝酸乳特色食品研究与开发[J]. 黔南民族师范学院学报, 2014, 34(6): 96-98, 101
Deng GC, Zhao H, Gao LA, Li YB, Ma Y, Li JL, Li J. The research and exploitation of speciality food *Ganoderma lucidum* acid milk[J]. Journal of Qiannan Normal College for Nationalities, 2014, 34(6): 96-98, 101 (in Chinese)
- [83] 黄清铨, 王庆福, 梁磊, 张柳莲, 康佩姿, 班雯婷. 甘蔗汁灵芝发酵功能性饮料研制[J]. 食品与发酵工业, 2015, 41(9): 83-86
Huang QH, Wang QF, Liang L, Zhang LL, Kang PZ, Ban WT. Sugar cane juice functional beverage produced by submerged fermentation of *Ganoderma lucidum*[J]. Food and Fermentation Industries, 2015, 41(9): 83-86 (in Chinese)
- [84] Gilbert FA. The submerged culture of *Morchella*[J]. Mycologia, 1960, 52(2): 201-209
- [85] 韦玉芳, 王立升, 覃斐章. 灵芝型优良膳食纤维方便食品的研制[J]. 食品科学, 2009, 30(2): 275-278
Wei YF, Wang LS, Qin FZ. Development of *Ganoderma lucidum*-fermented instant food with high-quality dietary fiber[J]. Food Science, 2009, 30(2): 275-278 (in Chinese)
- [86] 杜国. 黑灵芝免疫功能饮料及其纤维饼干的研制[D]. 南昌: 南昌大学硕士学位论文, 2016
Du G. The development of immune functional *Ganoderma atrum* beverage and fiber biscuit[D]. Nanchang: Master's Thesis of Nanchang University, 2016 (in Chinese)
- [87] Mohd Hanafiah Z, Wan Mohtar WHM, Abu Hasan H, Jensen HS, Klaus A, Wan Abd Al Qadr Imad Wan-Mohtar. Performance of wild-Serbian *Ganoderma lucidum* mycelium in treating synthetic sewage loading using batch bioreactor[J]. Scientific Reports, 2019, 9: 16109
- [88] Ma L, Zhuo R, Liu HH, Yu D, Jiang ML, Zhang XY, Yang Y. Efficient decolorization and detoxification of the sulfonated azo dye Reactive Orange 16 and simulated textile wastewater containing Reactive Orange 16 by the white-rot fungus *Ganoderma* sp. En3 isolated from the forest of Tzu-chin Mountain in China[J]. Biochemical Engineering Journal, 2014, 82: 1-9
- [89] Zhou YP, Chen QH, Cheng HZ, Gui L, Sun LL, Lei DZ, Ke DS, Tian CE. Decolorization of indigo carmine by *Ganoderma weberianum*[J]. Advanced Materials Research, 2011, 183/184/185: 1035-1040
- [90] Bhattacharya SS, Banerjee R. Laccase mediated biodegradation of 2,4-dichlorophenol using response surface methodology[J]. Chemosphere, 2008, 73(1): 81-85
- [91] 闫世梁, 李培睿, 李宗义, 李宗伟, 秦广雍. 云芝 *Trametes versicolor* 1126 所产漆酶对靛蓝废水脱色的初步研究[J]. 菌物学报, 2008, 27(2): 309-315
Yan SL, Li PR, Li ZY, Li ZW, Qin GY. Preliminary study on decolorization of indigo dye wastewater with laccase from *Trametes versicolor* 1126[J]. Mycosystema, 2008, 27(2): 309-315 (in Chinese)
- [92] 乔治杰, 马斌, 陈琼华, 周玉萍, 程惠贞, 田长恩. 灵芝漆酶催化阳离子红 2GL 脱色的研究[J]. 菌物学报, 2010, 29(2): 261-266
Qiao ZJ, Ma B, Chen QH, Zhou YP, Cheng HZ, Tian CE. Decolorization of cationic red 2GL catalyzed by laccase of *Ganoderma lucidum*[J]. Mycosystema, 2010, 29(2): 261-266 (in Chinese)
- [93] 陈琼华, 周玉萍, 杨桃芳, 程惠贞, 田长恩. 灵芝漆酶催化直接耐晒翠蓝 GL 脱色条件的优化[J]. 微生物学通报, 2009, 36(12): 1812-1817
Chen QH, Zhou YP, Yang TF, Cheng HZ, Tian CE. Optimization of conditions in decolorization of direct fast turquoise blue GL catalyzed by laccase from *Ganoderma lucidum*[J]. Microbiology China, 2009, 36(12): 1812-1817 (in Chinese)
- [94] Li GH, Wang QQ, Lv P, Ding ZY, Huang FL, Wei QF, Lucia LA. Bioremediation of dyes using ultrafine membrane prepared from the waste culture of *Ganoderma lucidum* with *in situ* immobilization of laccase[J]. BioResources, 2016, 11(4): 9162-9174

- [95] Chen SL, Xu J, Liu C, Zhu YJ, Nelson DR, Zhou SG, Li CF, Wang LZ, Guo X, Sun YZ, et al. Genome sequence of the model medicinal mushroom *Ganoderma lucidum*[J]. Nature Communications, 2012, 3: 913
- [96] 孙超, 胡鸢雷, 徐江, 罗红梅, 李春芳, 宋经元, 郭红卫, 陈士林. 灵芝: 一种研究天然药物合成的模式真菌[J]. 中国科学: 生命科学, 2013, 43(6): 447-456
Sun C, Hu YL, Xu J, Luo HM, Li CF, Song JY, Guo HW, Chen SL. *Ganoderma lucidum*: an emerging medicinal model fungus for study of the biosynthesis of natural medicines[J]. Scientia Sinica: Vitae, 2013, 43(6): 447-456 (in Chinese)
- [97] Zhou YP, Chen MH, Lu JJ, Kang X, Chen QH, Huang XL, Tian CE. A simple and efficient genetic transformation method of *Ganoderma weberianum*[J]. Folia Microbiologica, 2015, 60(5): 417-423
- [98] Shi L, Fang X, Li MJ, Mu DS, Ren A, Tan Q, Zhao MW. Development of a simple and efficient transformation system for the basidiomycetous medicinal fungus *Ganoderma lucidum*[J]. World Journal of Microbiology and Biotechnology, 2012, 28(1): 283-291
- [99] Kim S, Song J, Choi HT. Genetic transformation and mutant isolation in *Ganoderma lucidum* by restriction enzyme-mediated integration[J]. FEMS Microbiology Letters, 2004, 233(2): 201-204
- [100] Li HJ, Zhang DH, Yue TH, Jiang LX, Yu XY, Zhao P, Li T, Xu JW. Improved polysaccharide production in a submerged culture of *Ganoderma lucidum* by the heterologous expression of *Vitreoscilla* hemoglobin gene[J]. Journal of Biotechnology, 2016, 217: 132-137
- [101] 倪挺, 胡鸢雷, 林忠平. 表达人胰岛素基因的转基因灵芝[A]//中国生物工程学会 2006 年学术年会暨全国生物反应器学术研讨会论文集[C]. 长春, 2006
Ni T, Hu YL, Lin ZP. Expression of designed human insulin gene in *Ganoderma lucidum*[A]//Abstracts of papers presented at the 2006 annual conference of Chinese Bioengineering Society and the national symposium on bioreactors[C]. Changchun, 2006 (in Chinese)
- [102] 肖建勇, 谭宇蕙, 李刚, 李宝健. LZ-8 基因克隆及其在毕赤酵母中的诱导表达[J]. 广州中医药大学学报, 2012, 29(1): 66-69
Xiao JY, Tan YH, Li G, Li BJ. LZ-8 gene cloning from *Ganoderma lucidum* and its inducible expression in *Pichia pastoris*[J]. Journal of Guangzhou University of Traditional Chinese Medicine, 2012, 29(1): 66-69 (in Chinese)
- [103] Zhou YP, Chen QH, Xiao YN, Ke DS, Tian CE. Gene cloning and characterization of a novel laccase from the tropical white-rot fungus *Ganoderma weberianum* TZC-1[J]. Applied Biochemistry and Microbiology, 2014, 50(5): 500-507
- [104] Wang WF, Xiao H, Zhong JJ. Biosynthesis of a ganoderic acid in *Saccharomyces cerevisiae* by expressing a cytochrome P450 gene from *Ganoderma lucidum*[J]. Biotechnology and Bioengineering, 2018, 115(7): 1842-1854
- [105] Boh B, Berovic M, Zhang JS, Zhi-Bin L. *Ganoderma lucidum* and its pharmaceutically active compounds[J]. Biotechnology Annual Review, 2007, 13: 265-301
- [106] Kerezoudi EN, Mitsou EK, Gioti K, Terzi E, Avgousti I, Panagiotou A, Koutrotsios G, Zervakis GI, Mountzouris KC, Tenta R, et al. Fermentation of *Pleurotus ostreatus* and *Ganoderma lucidum* mushrooms and their extracts by the gut microbiota of healthy and osteopenic women: potential prebiotic effect and impact of mushroom fermentation products on human osteoblasts[J]. Food & Function, 2021, 12(4): 1529-1546
- [107] Xie CL, Tang PP, Yan SW, Yang Q, Zhang ZM, Gong WB, Zhu ZH, Zhou YJ, Yan L, Hu ZX, et al. Comparative study on bioactivities from Lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (agaricomycetes), gives an insight into the fermentation broth showing greater antioxidative activities[J]. International Journal of Medicinal Mushrooms, 2020, 22(7): 627-639
- [108] 李娜, 冯杰, 冯娜, 刘艳芳, 张劲松. 灵芝液态深层发酵三萜类化合物研究进展[J]. 微生物学通报, 2020, 47(10): 3451-3469
Li N, Feng J, Feng N, Liu YF, Zhang JS. Research progress in submerged fermentation for triterpenes of *Ganoderma*[J]. Microbiology China, 2020, 47(10): 3451-3469 (in Chinese)
- [109] Lin ZB, Yang BX. *Ganoderma* and Health[M]. Singapore: Springer Singapore, 2019