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专论与综述

鱼类病原真菌水霉及其防控研究进展

程万[1,2]，肖丕清[3]，王素钦[2]，王伯华[2]，寻晴[2]，罗玉双*[1,2]

1 湖南科技大学 生命科学与健康学院，湖南 湘潭 411201
2 湖南文理学院 水生动物重要疫病分子免疫技术湖南省重点实验室，湖南 常德 415000
3 大湖水殖股份有限公司，湖南 常德 415000

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摘　要：由病原真菌水霉(*Saprolegnia* spp.)引起的鱼病在全球范围内广泛流行，因水霉病防治特效药孔雀石绿的禁用和水霉对寄主无种属特异性，并且能产生生物膜抵抗药物等因素，增加了其防治难度，严重影响淡水养殖业的健康发展。本文对水霉的繁殖方式、分类鉴定方法和防治方法等进行了综述，并以其他水产致病菌的耐药性产生机制为参考，提出了改进水霉鉴定方法、丰富水霉菌种库、阐明其生物膜耐药机制、建立其感染疾病模型、研发高效的复方中草药剂和优化其抗菌活性产物的提取工艺，以及开展活性物质的有效性和安全性评估等综合防治策略，以期为开发水霉病防治特效药提供思路。

关键词：水霉；分类鉴定；耐药机制；防治方法

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*Corresponding author. E-mail: luofish@huas.edu.cn
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Pathogenic fungi of *Saprolegnia* in fish and prevention measures: a review

CHENG Wan[1,2], XIAO Piqing[3], WANG Suqin[2], WANG Bohua[2], XUN Qing[2], LUO Yushuang[*1,2]

1 School of Life and Health Sciences, Hunan University of Science and Technology, Xiangtan 411201, Hunan, China
2 Hunan Provincial Key Laboratory for Molecular Immunity Technology of Aquatic Animal Diseases, Hunan University of Arts and Science, Changde 415000, Hunan, China
3 Dahu Aquaculture Limited Company, Changde 415000, Hunan, China

Abstract: Saprolegniasis caused by the pathogenic fungi of *Saprolegnia* spp. is widely prevalent worldwide. The prohibition of the effective and specific drug malachite green for the prevention and control of *Saprolegnia*, as well as the non-specific hosts and drug resistance development via biofilm formation of *Saprolegnia*, increases the difficulty in the prevention and control of this disease, which seriously affects the healthy development of freshwater aquaculture. This paper reviewed the reproductive patterns, identification methods, and prevention and control measures of *Saprolegnia*. With the mechanism of drug resistance of other related pathogens in aquaculture as a reference, we proposed the measures including improving the identification methods of *Saprolegnia*, enriching the species of *Saprolegnia* in the culture collection, elucidating the mechanism of *Saprolegnia* in developing drug resistance via biofilm formation, establishing the disease models of *Saprolegnia* infection, developing efficient compound Chinese herbal medicines, optimizing the extraction process of antifungal compounds from Chinese herbal medicines, and evaluating the effectiveness and safety of antifungal compounds for the comprehensive prevention and control of *Saprolegnia*. The review provided ideas for the development of effective agents for the prevention and control of saprolegniasis in fish.
Keywords: *Saprolegnia* spp.; identification; drug resistance mechanism; prevention and control measures

水霉病(saprolegniasis)是淡水鱼类养殖中常见的真菌性疾病，具有强致病性和传播性[1]。疾病发生早期很难被肉眼所察觉，一旦暴发便可造成鱼类大面积感染或死亡[2]。自水霉病防治特效药孔雀石绿因“致畸、致癌和致突变”三致作用被列为禁用渔药后[3]，建立了化学药物、中草药、微生物和免疫等一系列水霉病防治体系[4]，但防治效果皆不如孔雀石绿。Ali 等[5]于2013 年首次报道水霉(*Saprolegnia* spp.)可以形成生物膜。袁海兰等[6]研究表明水霉生物膜可以有效阻碍药物进入膜内杀死细胞，生物膜的形成成为水霉病防治不佳的重要原因之一。病害防治困难的另一个较为重要的原因是对水霉的分类研究不足，对其缺乏系统的认识[7]。魏冬梅[8]指出不同地区、不同水体和不同寄主分离的水霉病原菌有着种类的多样性，且致病优势菌及其致病力差异显著。欧仁建[2]在探究不同药物作用于异丝绵霉(*Achlya klebsiana*)的有

效性研究中指出，病原菌的种类及生存环境可导致其药敏特性不同。然而目前分离的致病性水霉中大部分无法判定其具体种类。因此阐明水霉生物膜耐药机制和改进其分类鉴定方法，有助于提升对水霉的系统认识，为开发孔雀石绿替代药提供理论支持。

1 鱼类病原真菌水霉概述

1.1 水霉简介

水霉可感染罗非鱼[9]、鳟鱼[10]和草鱼[11]等几乎所有淡水鱼类，其隶属于茸鞭生物界(*Kingdom Stramenopilia*)鞭毛菌门(*Mastigomycotina*)卵菌纲(*Oomycetes*)水霉目(*Saprolegniales*)水霉科(*Saprolegniaceae*)[12]。引起水霉病的有水霉属(*Saprolegnia*)、绵霉属(*Achlya*)、丝囊霉属(*Aphanomyces*)、隐囊霉属(*Calyptralegnia*)、网囊霉属(*Dictyuchus*)和破囊霉属(*Thraustotheca*)等，主要致病原为水霉属[13]，部分水霉科菌株的菌落形态如图1所示。水霉科多达17属122种，但是对淡水水生动物危害较大的种类并不多，目前报道的可对鱼类产生致病性的水霉有多子水霉(*S. ferax*)[14]、寄生水霉(*S. parasitica*)[15]、同丝水霉(*S. monoica*)[16]和结节水霉(*S. torulosa*)[17]等，部分水霉属菌株的菌落形态如图2所示。

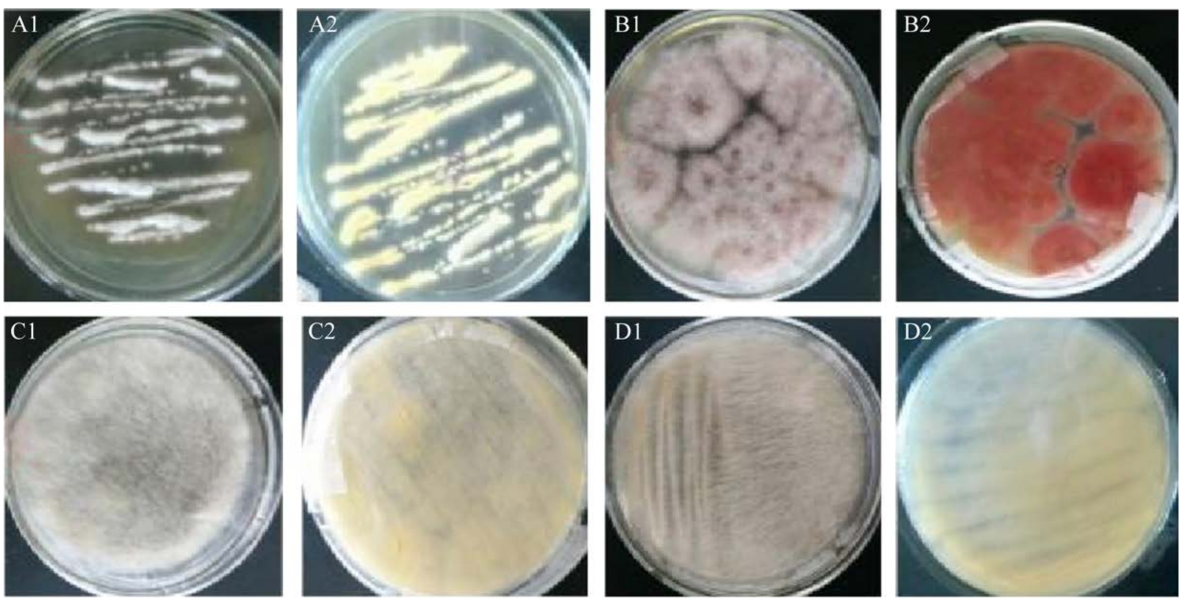

图1 水霉科不同属部分菌株菌落形态[8] 依据菌株形态特征初步分类. A：水霉属，不规则、小、干燥致密、不整齐、正面白，背面浅黄. B：绵霉属，不规则、大、湿润疏松、不整齐、正面白，背面紫红. C：网囊霉属，圆形无凸起、大、湿润疏松、整齐、正面灰白，背面浅黄. D：丝囊霉属，圆形无凸起、大、干燥稀疏、不整齐、正面浅白，背面乳黄. 1：正面照；2：背面照

Figure 1 Colony morphology of some strains of different genera of *Saprolegniaceae*[8]. Preliminary classification based on the morphological characteristics. A: *Saprolegnia*, irregular, small, dry and dense, uneven, front white, back light yellow. B: *Achlya*, irregular, large, moist and loose, uneven, front white, back purple red. C: *Dictyuchus*, circular without protrusions, large, moist and loose, neat, with a gray white front and a light yellow back. D: *Aphanomyces*, circular without protrusions, large, dry and sparse, uneven, with a light white front and a milky yellow back. 1: Observed view; 2: Reverse view.

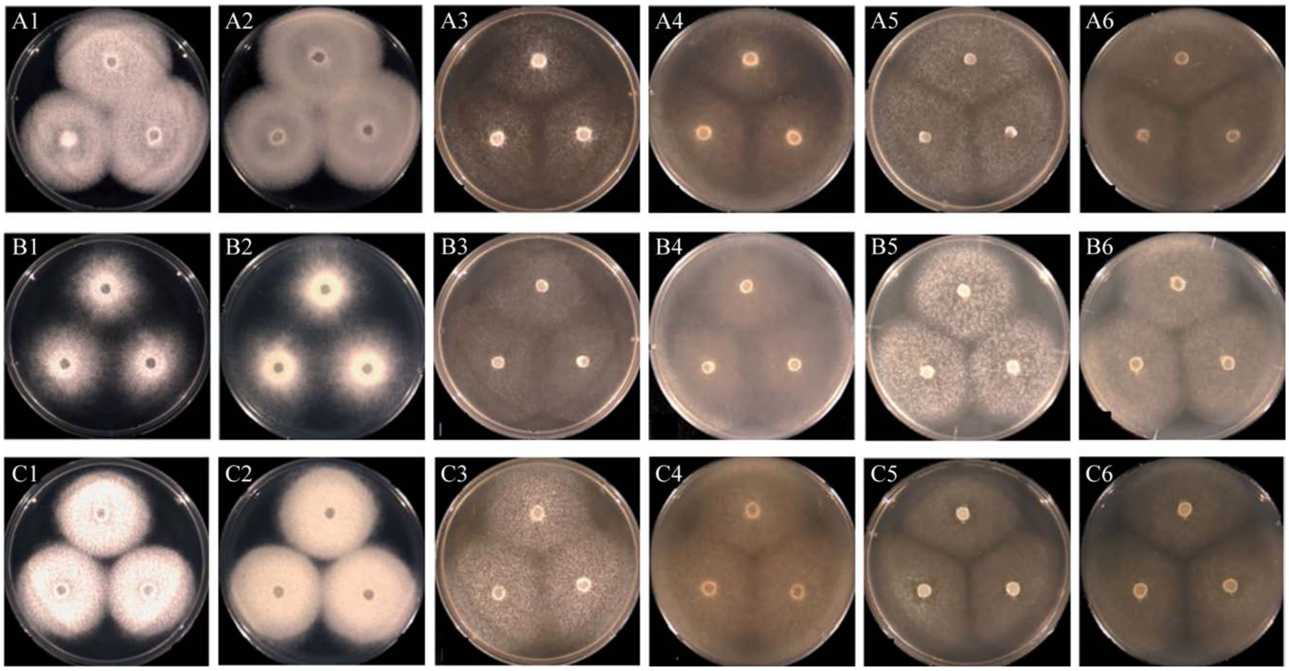

图 2 水霉属部分种类菌株菌落形态[7] A：难解水霉 W1247. B：异丝水霉 W724. C：多子水霉 W956. 1、2：马铃薯葡萄糖琼脂培养基；3、4：V8 汁琼脂培养基；5、6：玉米粉琼脂培养基. 1、3、5：正面照；2、4、6：背面照

Figure 2 Colony morphology of some strains of *Saprolegnia*[7]. A: *Saprolegnia aenigmatica* W1247. B: *S. diclina* W724. C: *Saprolegnia ferax* W956. 1 and 2: Potato dextrose agar medium; 3 and 4: V8 agar medium; 5 and 6: Corn meal agar medium; 1, 3, and 5: Observed view; 2, 4, and 6: Reverse view.

20 世纪 60 年代，有学者将卵菌纲单独地分类于真菌界中的一个鞭毛菌亚门，但随着研究的深入，发现卵菌纲与真菌纲在形态学特征、细胞壁组分、减数分裂位置、超微结构和 DNA 的 G+C 含量等方面存在明显差异，此外系统发育分析也证实两者分属不同，卵菌纲最终被分类于茸鞭生物界，而不是真菌界[8]。

1.2 水霉的繁殖方式

水霉可以通过有性生殖和无性生殖方式进行繁殖。当条件有利有性生殖发生时，其营养菌丝上生出短侧枝并发育为较大的包含卵球的藏卵器，藏卵器呈球形或梨形，多数顶生，少数侧生，卵壁光滑。同时，雄器也由菌丝短侧枝生成并缠绕在藏卵器上，雄器短而粗，与藏卵器同枝，包括同体或异枝[18-19] (图 3A–3D)。受精发生时雄器芽管穿过藏卵器壁将雄核移至卵球核处并与卵核结合形成卵孢子，卵孢子数量不等，分化呈中央型或亚中央型，萌发后可产生动孢子囊或菌丝完成整个繁殖过程[20]。无性生殖最为常见，包括出芽生殖和孢子生殖，出芽生殖即在菌丝上形成芽体并发育成新菌丝，该方式在外菌丝中极为普遍；孢子生殖即菌丝发育并产生厚垣孢子和动孢子囊(图 3E–3H)，动孢子囊侧生或顶生于菌丝，一般比菌丝粗壮，其内可形成游动孢子，游动孢子的形态、逸出方式和动孢子囊形态等因不同属而异[8] (图 4)，新孢子囊则以内层出或从空孢子囊基部侧生，孢子生殖的完整发展过程如图 5 所示[21-22]。

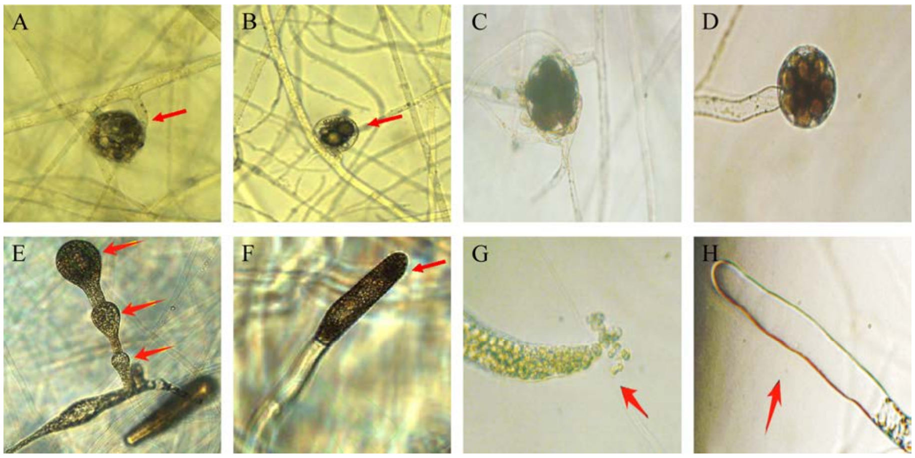

图 3　水霉部分有性和无性生殖细胞及结构[18-19]　倒置显微镜观察结果. A：藏卵器与雄器同枝. B：藏卵器与雄器异枝. C：有雄器围绕的包含多个卵孢子的藏卵器. D：成熟的顶生藏卵器. E：链状孢芽. F：棒状动孢子囊. G：游动孢子释放. H：释放完孢子的空孢子囊

Figure 3　Some sexual and asexual germ cells and structures of *Saprolegnia*[18-19]. Observation results of Inverted microscope. A: Oogonium and antheridium exist in the same branching manner. B: Oogonium and antheridium exist in different branching manner. C: An oogonium containing multiple oospores surrounded by antheridium. D: Mature oogonium. E: Catenulate gemmae. F: Rod-shaped sporangium. G: Release of zoospores. H: Empty sporangium after releasing spores.

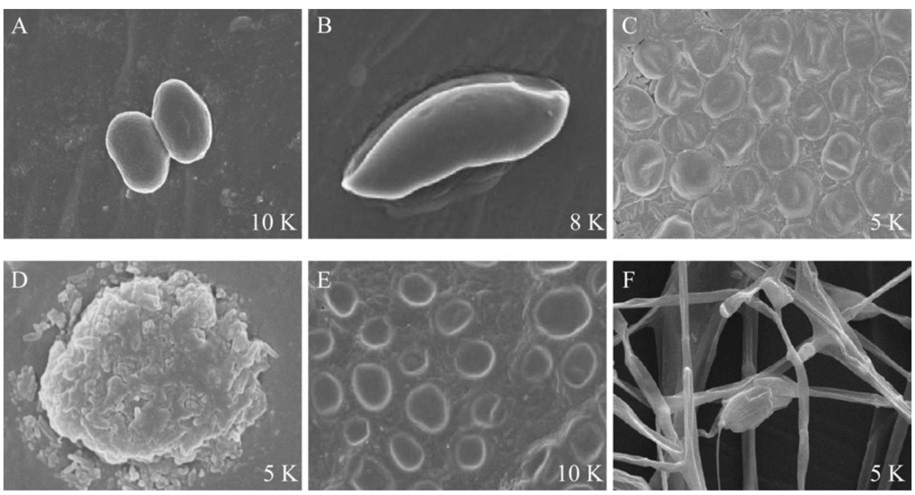

图 4　扫描电子显微镜下水霉科不同属菌株的动孢子形态[8]　A：水霉属，肾形，表面较光滑有褶皱. B：绵霉属，梭形，表面光滑有凹陷. C：网囊霉属，圆形，表面光滑有凹陷. D：破囊霉属，梨形，不光滑，有凸起. E：丝囊霉属，饱满圆形，光滑无凸起. F：隐囊霉属，圆形，光滑无凸起

Figure 4　Morphology of zoospores of different genera of *Saprolegniaceae* under scanning electron microscopy[8]. A: *Saprolegnia*, kidney shaped, with a smooth and wrinkled surface. B: *Achlya*, shuttle shaped, smooth surface with indentations. C: *Dictyuchus*, circular, with a smooth surface and indentations. D: *Thraustotheca*, pear shaped, not smooth, with protrusions. E: *Aphanomyces*, full and round, smooth without protrusions. F: *Calyptralegnia*, circular, smooth without protrusions.

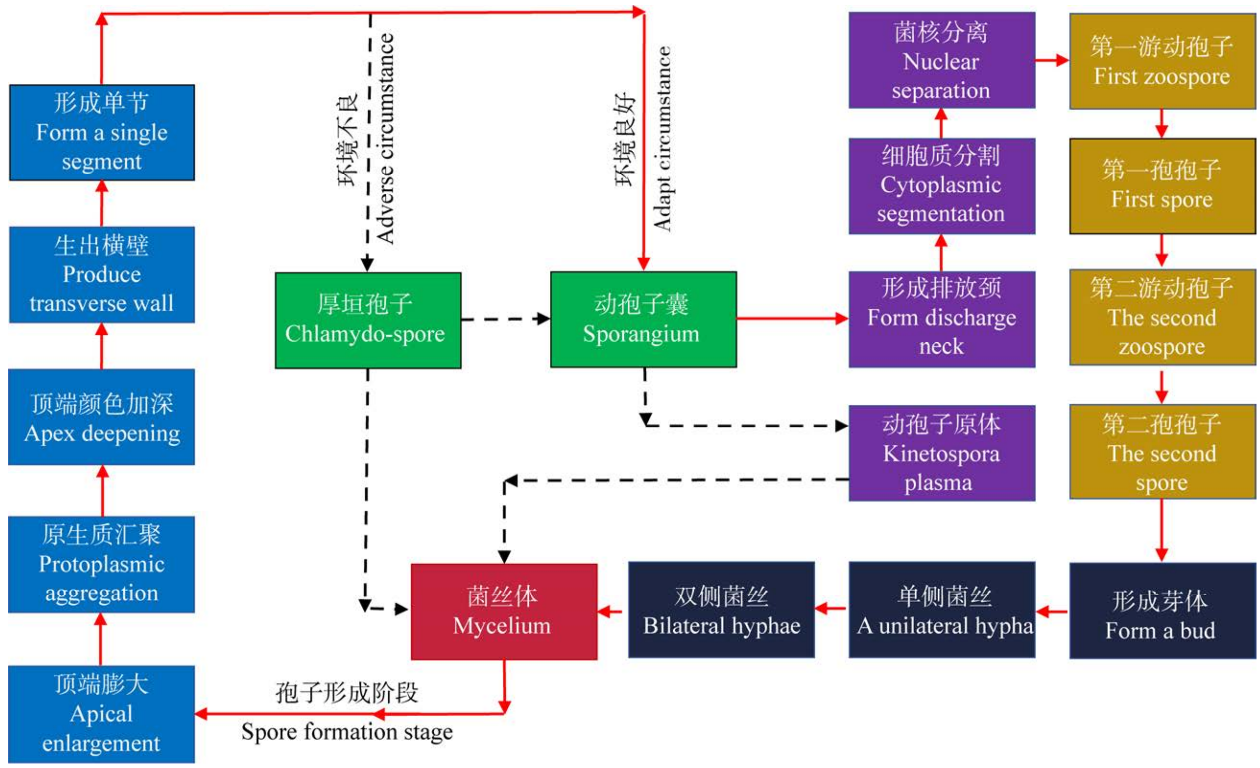

图 5　水霉的无性孢子生殖流程图　不同区域颜色表示水霉菌丝或孢子进入不同的发育时期，环境良好时，水霉菌丝可形成动孢子囊并产生游动孢子或动孢子原体实现大量繁殖；环境不良时，菌丝产生的厚垣孢子进入休眠状态以抗逆或极少量发育成菌丝体，并在环境适宜下重新发育为动孢子囊完成大量繁殖

Figure 5　Flow chart of asexual spore reproduction of *Saprolegnia*. Different colors indicate that *Saprolegnia* hyphae and spores are in different developmental stages. When environmental conditions are good, *Saprolegnia* hyphae can form sporangium and produce a large number of zoospores or kinetospora plasma to achieve large-scale reproduction; When environmental conditions are poor, the chlamydospores produced by the mycelium directly enter a dormant state to resist stress or develop into mycelium in a very small amount, and under suitable conditions, they redevelop into sporangium to complete large-scale reproduction.

2　水霉的分类鉴定方法

水霉的分类鉴定方法包括传统形态学方法、分子生物学方法和其他辅助性方法等(图 6)。传统形态学方法即依据水霉的形态特征包括无性生殖阶段的孢子囊形态和孢子囊再生方式等确定属，依据有性生殖器官的特征确定属内具体种类[23-25]。分子生物学方法包括 DNA 指纹技术[26]、随机扩增多态性 DNA 技术[27]及基于 rDNA ITS 序列分析技术[28]。可小丽等[29]建立了水霉基因组 DNA 的提取及 ITS 序列 PCR 鉴定的方法，被许多学者借鉴应用于水霉的分类鉴定，其他分子生物学方法如限制性片段长度多态性(restriction fragment length polymorphism, RFLP)分析法等也被应用于水霉的分类鉴定[19]。研究发现水霉间的特征性差异也可作为其鉴定的依

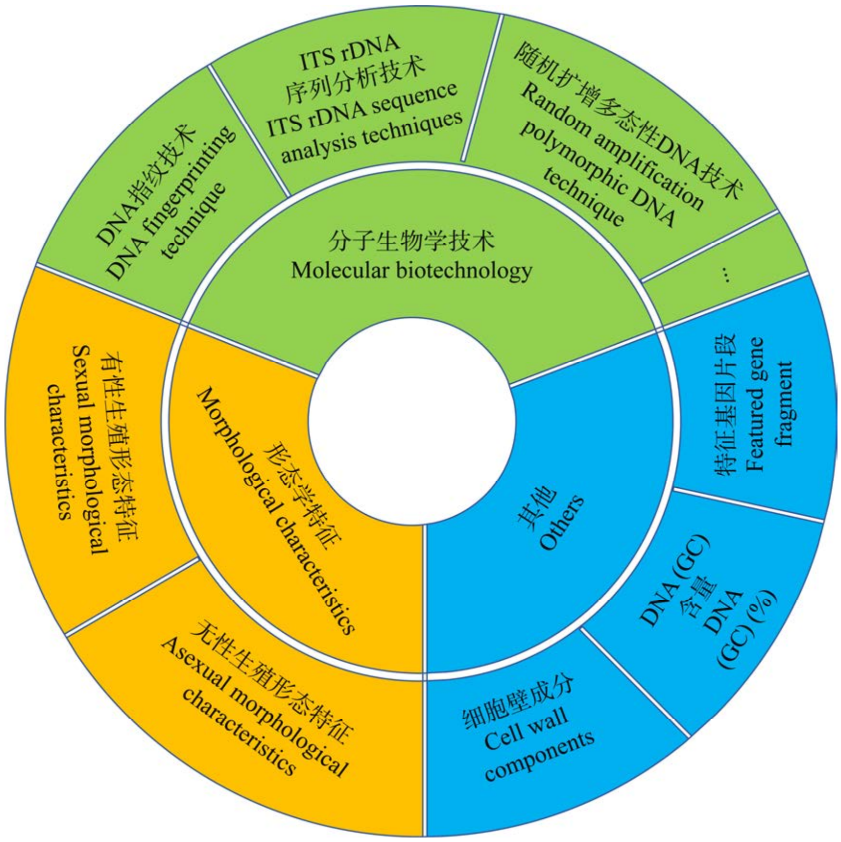

图 6　水霉的鉴定方法　形态学方法应用较早但可靠性低；rDNA ITS 序列片段小、易分析且种间差异明显但序列保守性较强，在种的水平上区分真菌仍有局限性；随机扩增多态性 DNA 技术(RAPD)分析用量少、鉴定迅速且具有可重复性，但研究种间及近缘属之间的亲缘关系有一定的局限性；DNA (G+C) (%)含量分析稳定性高于形态学方法，但鉴定目以下菌株时，只能作为辅助手段；...：其他分子生物学方法如扩增片段长度多态性分析等

Figure 6　Methods for identification of *Saprolegnia*. Morphological methods were applied earlier but with low reliability; rDNA ITS sequence has small fragments, is easy to analyze, and significant inter-species differences, but strong sequence conservation, there are still limitations in distinguishing fungi at the species level; The amount of random amplification polymorphic DNA technique (RAPD) analysis is small, the identification is rapid, and it has repeatability, but there are certain limitations in studying the phylogenetic relationships between species and related genera; The stability of DNA (G+C) (%) content analysis is higher than that of morphological classification, but it can only be used as an auxiliary method for identifying strains below the order; ...: Other methods, such as amplification fragment length polymorphism analysis (AFLP), etc.

据，魏冬梅[8]发现不同种类的水霉间其全基因组 G+C 的含量有差异，并表明将 18S rRNA 基因序列中的 G+C 含量辅助于水霉的分类鉴定具有一定的可行性。叶鑫[30]证实了 *sphtp1* 基因在寄生水霉中存在特异性，并认为可将该基因作为鉴定寄生水霉的一种 DNA 条形码。

张书俊等[25]依据传统形态学方法将从患病施氏鲟幼鱼肌肉组织中分离的菌株 XJ001 鉴定为寄生水霉。然而该方法在实际使用时有很大局限性，这是因为鉴定水霉所依据的无性特征

有一定的可塑性和重叠性。研究发现，不同培养基或相同培养基在不同温度、pH 等条件下培养水霉时，水霉的无性形态特征存在差异，而且水霉科不同属之间或与毛霉科(*Mucoraceae*)、半知菌类(*Deuteromycetes*)的一些属的属特征存在交叉[2,8,23-24]。同时水霉的有性特征在实验室条件下通常不能或很难形成[23,25]，寄生水霉在 15–20 ℃条件下培养不产生藏卵器[24]。因此，学者们更多的是采用组合法来完成从不同寄主分离的水霉的鉴定(表 1)。张楠[19]采用 ITS-PCR-RFLP 技术从我国沪、浙、苏、冀、鄂、赣、川七地淡水养殖区的不同患水霉病鱼体中分离得到多株多子水霉和寄生水霉等。Liu 等[39]结合形态学方法和 ITS 序列分析技术从我国粤沪鄂等地的患病鱼体和养殖水体中分离得到根形水霉(*S. bulbosa*)和衰退水霉(*S. hypogyna*)等菌株。

3 水霉生物膜耐药机制

生物膜是指微生物在生长过程中附着于物体表面形成的由微生物的细胞及其分泌的胞外基质所组成的多细胞复合体[40]。已证实生物膜的形成是真菌产生耐药性的重要因素之一，也是许多感染性疾病反复发作和难以治疗的主要原因[41]，这也是施加药物后水霉病仍不定期暴发的原因。目前关于水霉形成生物膜及产生耐药机制的研究报道相对较少，已有的研究表明，水产养殖动物强致病菌[如哈维氏弧菌(*Vibrio harveyi*)[42]和铜绿假单胞菌(*Pseudomonas aeruginosa*)[43]等]生物膜的形成和耐药机制的产生主要受基因调控和环境条件等因素的影响[44]。

基因调控系统包含多种基因及下游元件的参与及表达，涉及毒力因子、基因转移和群体效应信号分子等。其中毒力是致病性的一个属性，毒力因子主要包括黏附素、脂多糖、荚膜多糖、鞭毛、铁载体和分泌系统等[45]，其中较常见的 VI 型分泌系统是一个跨膜蛋白分泌系统，可将效应蛋白输送到宿主细胞内，对宿主产生毒害作用。蒋魁[42]研究发现参与哈维氏弧菌 T6SS 的毒力基因主要有 *vgrG* 和 *rbsB*，分别负责编码转运蛋白和效应蛋白，敲除 *vgrG* 基因后哈维氏弧菌对斑马鱼的致死率下降了 40%。基因转移是导致病原菌出现耐药性的一个重要原因，生物膜的形成增加了细胞密度，为 DNA 交换提供了最佳的环境条件，水平基因转移明显更高[44]。Igbinosa 等[46]在气单胞菌(*Aeromonas* spp.)中检测到I类整合子，它是具有获得许多抗生素抗性基因的可移动遗传元件，耐药基因在致病菌群体之间的水平转移导致感染难以治疗。群体感应是一种基于微生物群体密度以维持生物膜体系中的细胞数量处于动态平衡的信号调节机制[47]，在生物膜的形成中发挥重要作用[48]。Wang 等[49]将荧光假单胞菌(*P. fluorescens*)与金黄色葡萄球菌(*Staphylococcus aureus*)共培养时，发现信号分子群体感应自诱导剂-2 在种间相互作用中发挥了重要作用，刺激了双重培养中荧光假单胞菌的生物膜形成和胞外基质的产生，使形成的双重生物膜更厚、异质性更低。

环境条件也可对病原菌生物膜的形成和耐药机制产生影响。李翠苹[50]实验发现水环境中添加氮磷营养盐可诱发副溶血弧菌(*V. parahaemolyticus*)对头孢他啶和恩诺沙星等多种抗生素耐药，并且耐药率与氮磷营养盐的添加浓度有关。尹清干[44]发现培养时间、温度、初始菌浓度、pH、盐度、金属离子，以及鱼体黏液和组织等各种环境因素均能显著影响鳗弧菌(*V. anguillarum*)生物膜的形成，在含有 0.06–0.50 mmol/L 的 $MgCl_2$ 培养基中，随着浓度的增高，鳗弧菌生物膜的形成量越大，在 $CaCl_2$ 培养基中，随着浓度的增大，生物膜形成量不显著。袁海兰等[51]发现在培养基中加入

表 1 已报道的水霉种类、易感对象和鉴定方法

Table 1 Some reported species, susceptible objects and identification methods of *Saprolegnia* in fish

菌株编号 Strain number	病原菌种类 Pathogenic specie	感染对象 Infectious object	养殖环境 Aquaculture environment	鉴定方法 Identification method	参考文献 Reference
YC	异丝绵霉 *Achlya klebsiana*	黄颡鱼卵 *Pelteobagrus fulvidraco* eggs	孵化场 Hatchery	MC+MB III	[2]
CCF1301	多子水霉 *Saprolegnia ferax*	草鱼 *Ctenopharyngodon idella*	校养殖基地 School breeding base	MC+MB III	[4]
YL2	寄生水霉 *S. parasitica*	七彩神仙鱼卵 *Symphysodon aequfasciatus* eggs	上海海洋大学观赏水族实验室 Ornamental aquarium laboratory of Shanghai Ocean University	MC+MB III+MB IV	[13]
CNUaq1	*S. parasitica*	虹鳟 *Oncorhynchus mykiss*	渔场 Farm in Korea	MC+MB III	[15]
NM	水霉属 *Saprolegnia*	斑点叉尾鮰 *Ictalurus punctatus*	养殖场 Aquiculture area	MB II	[17]
GLUD2110	*S. ferax*	加州鲈鱼苗 *Micropterus salmoides*	加州鲈苗场 *Micropterus salmoides* fry farm	MC+MB III	[18]
HSY	*S. ferax*	黄颡鱼 *Pelteobagrus fulvidraco*	武汉大学水利水电学院集贸市场 Traditional market of School of Water Resources and Hydropower Engineering	MC+MB III	[23]
JL1	*Saprolegnia* sp.	彭泽鲫卵 *Carassius auratus* var. eggs	湖北仙桃沙湖水产技术推广站 Shahu aquatic technology promotion station in Xiantao City, Hubei, China	MC+MB III	[24]
XJ001	*S. parasitica*	施氏鲟 *Acipenser schrencki*	东海渔场 East China Sea fishing ground	MC	[25]
ManS22	*S. parasitica*	尼罗罗非鱼 *Oreochromis niloticus*	私人渔场 Private fish farm	MC+MB III	[31]
HP	*S. ferax*	黄颡鱼卵 *Pelteobagrus fulvidraco* eggs	湖北仙桃沙湖水产技术推广站 Shahu aquatic technology promotion station in Xiantao City, Hubei, China	MC+MB III	[32]
SN	鲑水霉 *S. salmonis*	山女鳟 *Oncorhynchus masou*	渤海冷水鱼类实验站 Cold water fish experimental station in the Bohai Sea	MC+MB III	[33]
LF04	*Saprolegnia* sp.	尼罗罗非鱼 *Oreochromis niloticus*	人工养殖池 Artificial breeding ponds	MC+MB III	[34]
NM	*S. aenigmatica*	尼罗罗非鱼 *Oreochromis niloticus*	养殖场 Aquiculture area	MC+MB III	[35]
ML18040	两性绵霉 *A. bisexualis*	孔雀慈鲷 *Peacock Cichlid*	观赏鱼场 Ornamental fish farm	MC+MB III	[36]
LY01	澳大利亚水霉 *S. australis*	拉萨裂腹鱼 *Schizothorax waltoni*	雅鲁藏布江日喀则段 Shigatse section of the Yarlung Zangbo River	MC+MB III	[37]
6b 1b 2	*S. salmonis* *S. parasitica* *S. anomalies*	巨须裂腹鱼 *Schizothorax macropogon*	研究所冷水鱼养殖基地 Institute of cold water fish breeding base	MC+MB III	[38]

NM：未提及；MC：形态特征；MB：分子生物学技术；MB I：DNA 指纹技术；MB II：随机扩增多态性 DNA 技术；MB III：rDNA ITS 序列分析技术；MB IV：限制性片段长度多态性分析法

NM: Not mentioned; MC: Morphological characteristics; MB: Molecular biotechnology; MB I: DNA fingerprinting technique; MB II: Random amplification polymorphic DNA technique; MB III: rDNA ITS sequence analysis techniques; MB IV: Restriction fragment length polymorphism analysis.

0.12 mmol/L 以上 $CaCl_2$，能促进水霉生物膜形成；添加 0.03–2.00 mmol/L $MgCl_2$ 对生物膜形成无影响；添加 0.5 mmol/L 以上 Cu^{2+} 几乎不形成生物膜。这些研究表明不同环境因子对不同致病菌生物膜形成的影响不同。

4 水霉的防治方法

自孔雀石绿被列为禁用渔药以来，各国学者转而探索水霉防治的新方法，目前，已报道的水霉防控方法有：化学药物、中草药、微生物拮抗菌和免疫调节等，其具体防治措施如图 7 所示。

4.1 化学防治

化学药物防治是目前水霉病防治的主要手段，福尔马林[52]等防腐剂、臭氧[53]等氧化剂都曾被用于水霉病的防治，但这些药物因安全性和实用性等原因难以得到推广。广大学者进而致力于其他安全有效的化学药物的开发利用(表 2)。Saha 等[62]表明饲料中掺杂微量氟康唑(吡咯类)

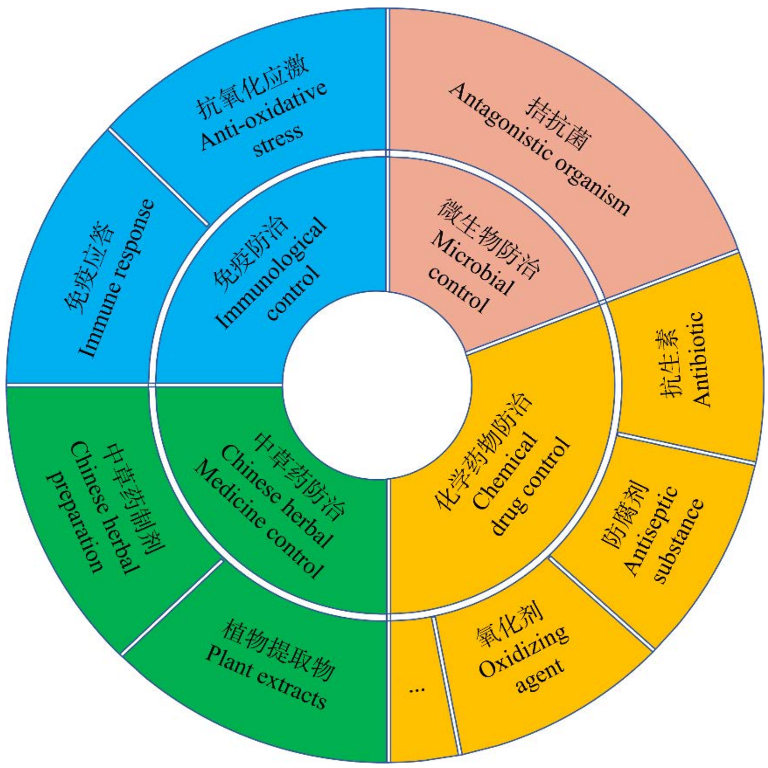

图 7 水霉的防治方法 化学防治抗菌谱广、见效快但易使致病菌产耐药性和污染环境；...：其他化学药物如盐类化合物硫酸铜等、酸类硼酸等和化学衍生物等；中草药防治原材料来源广、环境友好等但成分复杂且药理作用不明了；微生物防治绿色环保但活性物质分离困难；免疫防治安全但周期长

Figure 7 Prevention and control methods of *Saprolegnia*. Chemical control has a wide spectrum of antibacterial properties and quick effectiveness, but it can easily cause pathogenic fungi to develop resistance and pollute the environment; ...: Other chemical drugs, such as salt compounds: copper sulfate, acid compounds: boric acid, and chemical derivatives; Chinese herbal medicine has a wide range of raw materials for prevention and control, is environmentally friendly, but its ingredients are complex and its pharmacological effects are unclear; Microbial prevention and control is green and environmentally friendly, but it is difficult to separate active substances; Immune prevention and treatment are safe but have a long cycle.

表 2 已报道的抑制水霉的化学药物

Table 2 Some chemical agents that have been reported to inhibit *Saprolegnia*

药物名称 Drug name	抑制水霉形式 Inhibited *Saprolegnia* form	有效浓度 Effective concentration (mg/L)	参考文献 Reference
防霉剂 X Fungicide X	菌丝 Hypha	0.50	[2]
卡松 Casson	菌丝/孢子 Hypha/Spore	16.00	[13]
苯氧菌酯 Kresoxim-mehtyl	菌丝 Hypha	1.00	[54]
嘧菌酯 Azoxystrobin	菌丝 Hypha	0.50	[54]
硫酸铜 Cupric sulfate	菌丝/孢子 Hypha/Spore	0.50	[55]
克霉唑 Clotrimazole	菌丝/孢子 Hypha/Spore	1.00–2.00	[56]
噻唑啉酮 Thiazolinone	菌丝 Hypha	8.00	[57]
尼泊金乙酯 Ethylparaben	菌丝 Hypha	32.00	[57]
立达霉 Metalaxyl	菌丝 Hypha	8.00	[58]
卫可 Virkon-S	菌丝/孢子 Hypha/Spore	40.00	[59]
葡萄糖氯已定 Chlorhexidine gluconate	菌丝 Hypha	50.00	[60]
苯扎溴铵碘 Benzalkonium bromide iodine	孢子 Spore	3.84	[61]

可降低露斯塔野鲮的水霉感染率；Werner 等[63]通过实验发现 2′,4′-二羟基二氢查尔酮的衍生物 9 有较好的抑制水霉效果，其对寄生水霉、澳大利亚水霉(*S. australis*)和异丝水霉的最小抑菌浓度(minimum inhibitory concentration, MIC)分别为 175、150 和 100 μg/mL；Ali 等[64]改进了一种基于使用 FUN-1 染料和钙荧光白 M2R 的荧光测定体系用于体外水霉抑制剂的筛选，该团队发现硼酸可干扰水霉的正常代谢并造成线粒体功能缺陷[65]，丙酸处理后的水霉生物膜菌丝活性显著降低[66]，并表明硼酸和丙酸皆可对游离或生物膜状态下的水霉孢子和菌丝产生抑制作用。许佳露等[32]构建了鱼类水霉疾病模型，并应用该模型进行了广泛的抗水霉药物筛选。杨先乐等[67]筛选出一种孔雀石绿替代药物“美婷” (主要成分为类似于生化黄腐酸的立达霉)，在全国主要水产养殖区进行了中试推广验证，并建立了该药在水产品中的残留检测方法[68]，但由于制造原料涨价原因，使得药物的推广应用受限。

4.2 中草药防治

现已经发现 300 多种中草药对抑制真菌有效果[69]，川楝子[37]、五味子[70]和山苍子[71]等已用于实际生产中水霉的防治。同时，其他能有效抑制水霉的中草药如洋葱[72]、接骨木[73]和银斑百里香[74]等也被陆续报道(表 3)。由于中草药活性成分种类繁多，部分中草药在单方使用时抗菌效果不明显，因此研发复方中草药也成为了水霉病防治研究的热点。刘云鹏等[69]通过实验发现苦参、大黄和五倍子复合水煎剂对水霉抑菌圈直径比单方大黄组和五味子组分别多 5.5 mm 和 7.5 mm；马国红等[83]在乌鳢水霉病暴发期间，连续使用五倍子、大黄和板蓝根水煎剂泼洒 5 d，治愈率达 96.01%；李绍戊等[84-85]通过用复方 A (苦参、野菊花等)的水煎剂和复方 B (丁香、黄连和土槿皮等)的水煎醇提剂分别处理山女鳟和哲罗鲑的受精卵，相较于对照组，山女鳟和哲罗鲑受精卵的发眼率分别提高了 29.50% 和 31.67%，而其水霉感染率分别下降了 35.29% 和 21.70% (P<0.05)。Meneses 等[86]用 4.33 mg/L

表 3 已报道的抑制水霉的中草药和植物提取物

Table 3 Some of the Chinese herbal medicines and plant extracts that have been reported to inhibit *Saprolegnia*

抑菌植物 Bacteriostatic plant	有效组分 Active principle	有效浓度 Effective concentration	受试水霉 *Saprolegnia* spp. under test	参考文献 Reference
黄连 *Goldthread*	生物碱 Alkaloid	256.00 mg/L	*Achlya klebsiana*	[2]
散沫花 *Lawsonia inermis*	萘醌类 Lawsone	200.00 μg/mL	*Saprolegnia parasitica*	[15]
刺芹 *Eryngium foetidum*	刺芹精油 Essential oil of parsley	0.50 μg/mL	*S. parasitica*	[75]
地衣 *Lichens*	松萝酸 Usnic acid	2.50 mg/L	*S. parasitica*	[76]
厚朴 *Mangnolia officinalis*	和厚朴酚 Honokiol	8.00 mg/L	*S. parasitica*	[77]
叉蕊薯蓣 *Dioscorea collettii*	皂苷类 Saponin	2.00 mg/L	*S. parasitica*	[78]
百里香 *Thymus mongolicus*	香芹酚 Carvacrol	50.00 μL/mL	*S. parasitica*	[79]
土槿皮 *Cortex Pseudolaricis*	土槿皮甲酸/乙酸 Pseudolaric acid a/acid b	15.63 mg/mL	*Saprolegnia*	[80]
山柰 *Kaempferia galanga* L.	多酚类化合物 Polyphenolic compounds	3.90 mg/mL	*Saprolegnia*	[81]
掌叶大黄 *Rheum palmatum*	大黄酸 Rhein	16.00 mg/L	*Saprolegnia*	[82]

的纳米复合材料(银纳米颗粒+榄仁叶水提物)药浴人工感染寄生水霉的点鳍红眼脂鲤 4 d，药浴组点鲤的感染面积和存活率较对照组差异显著，分别下降 98.7%和提高 100.0%，并通过预防实验表明该复合材料可作为鱼类长途运输时水霉病防治的候选药物。

植物提取物如植物精油等广泛存在于芳香植物中，具有高效的抗菌作用，被视为潜在的饲用抗生素替代品，在畜禽和反刍动物中研究较多，近年来也被应用于水霉的防治[75]。Montenegro 等[87]从蒿科植物中分离出多种有效抑制寄生水霉和澳大利亚水霉的化合物；Tang 等[88]通过实验发现芳樟醇可表现出良好地抑制多子水霉菌丝生长和孢子萌发活性，其 MIC 分别为 0.1%和 0.025%，转录组学分析表明其通过抑制水霉的能量供应和蛋白质合成等代谢活动发挥抑菌作用。

4.3 微生物防治

自然界微生物资源极其丰富，目前已报道对水霉有拮抗作用的菌属有：假单胞菌属(*Pseudomonas*)[89]、芽孢杆菌属(*Bacillus*)[90]和链霉菌属(*Streptomyces*)[91]等。刘韵怡等[92]发现由铜绿假单胞菌产生的生物表面活性剂鼠李糖脂可有效抑制寄生水霉的菌丝生长和孢子萌发，其 MIC 分别为 125 mg/L 和 15. 625 mg/L；华亚南等[93]发现溶壁酶有很好地抑制同丝水霉菌丝生长和孢子萌发活性，且抑制效果与水浴条件相关；Anggani 等[94]发现地衣芽孢杆菌(*B. licheniformis*)和橄榄绿链霉菌(*S. olivaceoviridis*)可产生几丁质酶破坏水霉细胞壁结构从而影响水霉的正常生长；Deutsch 等[95]从海洋羽藻细菌内生菌中分离得到一株弗拉瓦库克菌(*Kocuria flava*)，其能产生一种具有挥发性的次级代谢活性物质—“8-壬烯酸”，实验发现 5 mg/L 8-壬烯酸处理组的感染寄生水霉的罗非鱼孵化卵存活率较对照组增加了 54.5%，并且 98%的存活卵正常孵化且发育良好，当提升 8-壬烯酸的作用浓度时，孵化卵未出现中毒和发育不良等异常现象，表明 8-壬烯酸可作为潜在的抗水霉活性物质。国内学者也在筛选水霉拮抗菌方面收获颇多(表 4)，这些拮抗菌都表现出良好的抑制水霉菌丝生长和孢子萌发效果，具有很好的开发应用前景。罗玉双

表 4　水霉拮抗菌及其活性物质的性质

Table 4　Some reported *Saprolegnia* antagonism and the properties of their active substances

菌株编号 Strain number	拮抗菌种属 Species of antagonistic bacteria	拮抗菌来源 Source of antagonistic bacteria	热稳定性 Heat stability	pH 稳定性 pH stability	蛋白酶稳定性 Stability of protease	遗传稳定性 Hereditary stability	安全性 Security	参考文献 Reference
QHV2	山丘链霉菌 *Streptomyces collinus*	池塘底泥 Sediment in the pond	+			+		[91]
JL04	枯草芽孢杆菌 *Bacillus subtilis*	鲤鱼体表 Body surface of *Cyprinidae*	–	+	–	+	+	[34]
JL50	短小芽孢杆菌 *B. pumilus*	鲤鱼体表 Body surface of *Cyprinidae*				+	+	[34]
YB5	特基拉芽孢杆菌 *B. tequilensis*	池塘水体及底泥 Pond water and sediment	+	+		+		[90]
LD038	黏质沙雷氏菌 *Serratia marcescens*	东海渔场 East China Sea fishing ground						[96]
Sh1	解淀粉芽孢杆菌 *B. amyloliquefaciens*	养殖水体附近土壤 Soil near the aquatic water	+		+		+	[97]
DTJ-24	淡紫灰链霉菌 *S. lavendulae*	土壤 Soil						[98]
$7^{\#}$	特基拉芽孢杆菌 *B. tequilensis*	土壤 Soil						[98]
JD	荧光假单胞菌 *Pseudomonas fluorescens*	池塘水样 Aqueous sample						[99]
FX11	链霉菌属 *Streptomyces*	池塘周围土壤 Soil around the pond	+	+		+	+	[100]
FX17	*Streptomyces*	池塘周围土壤 Soil around the pond	+	+		+	+	[101]
HJ010		河鲈肌肉 Muscle of the *Perca fluviatilis*	+	+	–			[102]
S26	紫色链霉菌 *S. violaceorectus*	海底沉积物 Seafloor sediment	+	+	+/–			[103]
BA1	蜡样芽胞杆菌 *B. cereous*	国家水生动物病原菌菌库 National aquatic animal pathogen bank						[104]
SZK15		底泥 Sediment	+		+/–			[105]
TCCC11322	芽孢杆菌属 *Bacillus*	惠赠菌株 Gift strain						[106]
HD05	伯克霍尔德菌属 *Burkholderia*	底泥 Sediment	+					[107]
XL03	防御假单胞菌 *Pseudomonas protegens*	底泥 Sediment	+	+	–			[108]

空白：未提及；+：拮抗菌代谢物满足该条件；–：不满足该条件；+/–：此条件下活性适中；热稳定性：*T*>70 ℃；pH 稳定性：pH 5.0–9.0；遗传稳定性：传代 10 代

Blank: Not mentioned; +: Satisfy the condition; –: Do not satisfy the condition; +/–: Moderate satisfaction; Heat stability: *T*>70 ℃; pH stability: pH 5.0–9.0; Hereditary stability: For 10 generations.

等[91]也筛选得到一株对寄生水霉有拮抗作用的链霉菌 QHV2，连续传代 10 代的 QHV2 菌株发酵 5 d 的产物对黄颡鱼卵水霉的抑菌率高达 71%，80 ℃水浴处理的发酵液仍然保持 62%的抑菌率，说明其抑水霉活性物质具有热稳定性与遗传稳定性，有开发为水霉生物防控剂的潜能。

4.4 免疫防治

鱼类在长途运输和拉网捞捞等特殊环境下导致的体表损伤都会增加患水霉病的风险率。在水霉感染泥鳅的实验中，罗雁支[109]发现组成 T 细胞受体的 γ 亚基和 δ 亚基，其相关基因表达量随着感染时间的延长而增加。陈晓瑶[110]观察到在先天性免疫和适应性免疫中发挥重要作用的白细胞介素 15 和白细胞介素 15 受体 α 链在泥鳅肾、脾、皮肤和鳃中都高效表达，表明水霉感染可引发鱼类发生免疫应答。研究发现，一些防治药物可通过提升鱼类免疫能力来降低水霉感染率。冯东岳[111]指出在饲料中添加抗菌肽制剂能显著提高鱼类抗病能力；Saha 等[112]发现用含 0.02%的食用吡哆醇持续投喂感染寄生水霉的露斯塔野鲮后，其存活率较对照组提升约 50%，并且投喂组露斯塔野鲮体内的白蛋白、球蛋白、溶菌酶含量和吞噬细胞活性均较对照组显著提升，表明投喂吡哆醇可增强露斯塔野鲮先天性免疫能力以抵抗水霉病的侵扰；同时，研发鱼体疫苗也是一种有效的防治选择。Choudhury 等[113]以寄生水霉的 3 组典型致病性蛋白 Sphtp1、Sphtp3 和 Sprg 19320 序列为靶标，筛选出 B 淋巴细胞的线性表位及主要组织相容性复合体(major histocompatibility complex, MHC)家族I和 MHC 家族II的表位，然后将筛选的抗原肽及 50S 核糖体蛋白连接组装成多位点多靶标候选疫苗，通过免疫刺激动力学实验发现，该候选疫苗可有效刺激免疫系统产生多种免疫细胞和抗体并生成记忆细胞，多结构多系统评估表明其具有潜在稳定性、实用性和可行性，表明该候选疫苗极具开发前景。

另外，有研究报道水霉感染可诱发鱼类氧化应激而造成组织损伤。研究发现感染寄生水霉的尼罗罗非鱼与健康组相比，其体内的氧化参数如丙二醛水平显著提高，但抗氧化参数如过氧化氢酶、超氧化物歧化酶和还原性谷胱甘肽水平显著降低[114]；Baldissera 等[115-116]发现被寄生水霉感染的草鱼肝脏内活性氧和脂质过氧化水平较未感染组显著提升，但抗过氧自由基的超氧化物歧化酶、谷胱甘肽过氧化物酶和谷胱甘肽 S-转移酶水平显著下降，并表示机体内活性氧蓄积可抑制肌酸激酶的活性，从而影响组织能量供应。Ibrahim 等[117]用 0.6 mg/L 的辣木银纳米颗粒药浴感染水霉的罗非鱼后存活率较对照组提升了 31%，并且其体内的超氧化物歧化酶和过氧化氢酶等表达量显著提升，表明该复合材料可能作为抗氧化剂来增强鱼类自身抗氧化应激能力，以降低水霉感染给机体带来的氧化损害。

4.5 水霉生物膜综合防治

基因调控和环境因素对水产动物病原菌生物膜的形成和耐药性的产生有重要影响，鱼类水霉生物膜的综合防治可以从以下几方面着手：(1) 基因角度：开展不同种类水霉的全基因组测序，预测与生物膜形成及耐药机制产生的相关基因及功能。(2) 抗生素角度：应根据致病菌的药敏差异性合理选择用药，切不可盲目用药。(3) 生物膜角度：通过群体感应抑制剂干扰自诱导分子检测和使信号分子的失活来抑制生物膜形成；此外，生物膜的胞外基质是生物膜结构的重要组成成分，主要成分为胞外多糖、蛋白质、脂质和 eDNA 等，可以尝试通过糖苷酶、蛋白酶和DNA 酶等降解胞外基质来破坏生物膜，同时也可将酶与抗生素联用进而充分杀灭膜内

致病菌[118]。(4) 环境角度：定期给养殖水体消毒，研究发现当致病菌的菌浓度在 10^7 CFU/mL 以上时，才更容易形成大量的生物膜[44]，张楠[19]研究表明养殖水体中水霉孢子浓度达到 10^4 个/mL 时可引起草鱼暴发水霉病，当水霉孢子浓度超过 10^6 个/mL 时，可以引起草鱼出现死亡。因此，可筛选养殖水体中水霉的快速监测方法以便控制其孢子浓度，实时定量 PCR[119]、多重 PCR[120]和微滴式数字 PCR[121]等均可用于水环境中寄生水霉的监测。同时也要防止污染，农场径流、城市污水和抗生素制造的工业废物等都可能严重影响致病菌群落的系统发育组成，进而导致水生环境中抗生素耐药基因发生转移或进化[122]。(5) 开发安全和不易产生耐药性的新药物：如分离中草药和拮抗菌的活性天然产物等。展文豪[123]发现五味子、乌梅和百部等中草药的水提物可通过破坏水产致病弧菌的细胞壁、细胞膜结构和抑制生物膜的形成等抑制其生长；Raissa 等[124]从海洋中分离得到 16 株放线菌且其活性代谢产物皆可能作为群体感应抑制剂来抑制哈维氏弧菌、嗜水气单胞菌(*Aeromonas hydrophila*)和无乳链球菌(*Streptococcus agalactiae*)的生物膜形成。

5 问题与展望

鱼类病原真菌水霉广泛存在于水体中，不仅对寄主无选择性，还能在水中形成生物膜以抵抗恶劣的水环境条件，其防治已成为目前鱼类病害防治中最棘手的难题之一。由于其显著的种间差异导致药敏性不同，盲目防治往往事倍功半，因此开展其分类鉴定及多样性研究是系统认识水霉和后续精准防控水霉病的关键一步[125]，此举的意义有：(1) 完成对水霉的鉴定并详细描述形态学特征，丰富了其种类。(2) 为确定水霉未来研究方向提供思路[126]。(3) 有助于根据鱼类生长周期选择合适的防治模式。Engblom 等[127]发现从鲑鱼鱼卵和成鱼中分离得到的寄生水霉间存在明显的基因差异，并且孵化时期发生的水霉病通常不会传染给成鱼，表明寄生水霉对鲑鱼的感染或鲑鱼对寄生水霉的耐受与其发育阶段有关。(4) 开展对特定种类水霉的研究，为系统认识该致病菌和开发防治特效药提供理论基础，如寄生水霉因其强致病性、高致死率而备受关注，与之相关的研究报道也较多，Srivastava 等[128]系统描述了其体外发育的 4 个阶段及对应的代谢特征；Wang 等[129]通过转录组技术鉴别出寄生水霉的 3 组蛋白质相关基因(*sprg_08456*、*sprg_03679* 和 *sprg_10775*)可能作为抗寄生水霉药物的靶标基因。Engblom 等[127]发现从野外或养殖鲑鱼中分离得到的大部分寄生水霉菌株中都存在一个包含 4 个主要序列类型(sequence type, ST1–ST4)和 13 个独特的序列类型的主克隆；Shreves 等[130]表明从不同鲑鱼养殖场处分离得到的 91.1%的寄生水霉中都存在种系型 S2，上述研究结果表明该序列片段可能与鲑鱼水霉病的暴发有关，未来可进一步研究其来源或有利的生命周期和环境条件。Biswas 等[131]通过模拟实验开展对寄生水霉几丁质合成酶 5 的结构、动力学和功能特征的综合研究，可为未来开发安全有效的酶抑制剂提供思路。然而，目前依据分子生物学方法完成对水霉的分类鉴定还存在以下问题：(1) 国内外相关研究较少，搜索到的同源序列大多为已报道的其他种属序列，或基因序列存在较高错误率，导致在分类分析时出现偏差；(2) 测定其 rDNA ITS 序列时所用引物均为真菌通用引物，而水霉属于类真菌[20]；(3) 使用单一方法鉴定水霉的准确性不高。

尽管化学药物、中草药、微生物拮抗菌和免疫调节等方法对水霉防治都有一定的效果，

但是也存在许多问题亟待解决:(1) 化学药物的大量使用带来的环境污染、食品安全和菌株耐药性等问题; (2) 中草药成分复杂, 对其药用成分及抑菌机理的研究还不够透彻, 导致抗菌有效成分的得率低、最佳药浴浓度和药浴时间难以掌控, 而且高效速效的中草药制剂少有报道; (3) 拮抗菌产生的活性物质大多属于蛋白类、肽类等化合物, 稳定性差, 需要更完善的分离纯化体系, 同时, 微生物拮抗机制的研究大多是在实验室条件下进行的, 未来投入使用时还需考虑实际条件如温度、pH 和硬度等理化指标对抑菌效果的影响, 并还需重视鱼类活体实验, 缺少对活性物质的有效性和安全性评估; (4) 通过提升鱼类免疫和抗氧化应激能力来降低水霉感染率是一种有效的防治方法, 但目前关于如何提升鱼体由水霉感染引起的抗氧化应激能力及相应的抗氧化调控机制的报道相对较少; (5) 水霉生物膜的存在使水霉病害反复发生, 突破生物膜防线有望降低其病害风险, 但目前关于水霉生物膜的研究鲜有报道, 对其生物膜形成和耐药机制产生机理仍缺乏清晰的认识。

因此, 为进一步解决鱼类病原真菌水霉的防控问题, 未来还需考虑以下问题: (1) 建立并丰富鱼类水霉菌种库, 对致病性病原菌进行基因组测序, 并提高其序列的准确率, 改进水霉的鉴定方法或通过组合方法降低菌株分类鉴定的错误率, 解析水霉生物膜耐药机制; (2) 研究中草药活性组分抗水霉的作用机理, 优化其提取工艺, 精确药浴时间和药浴浓度, 研发复方制剂; (3) 改进水霉拮抗菌天然活性物质的分离纯化方法, 系统考虑实际条件对其功能的影响, 开展活体试验评估其有效性和安全性; (4) 建立鱼类水霉感染疾病模型, 筛选更为安全有效的孔雀石绿替代药。

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