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鱼类原核和真核表达系统研究进展

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摘 要

构建功能蛋白高效表达系统，在鱼类人工养殖领域具有潜在的应用价值。重组蛋白获取是通过在宿主细胞中引入特定基因以表达目标蛋白来实现的，这一过程涉及基因克隆、载体构建、宿主细胞选择、蛋白表达和纯化等多个步骤。本文综述了常用的重组蛋白表达系统(如大肠杆菌、酵母、昆虫细胞和哺乳动物细胞)的潜力和局限性，以及不同表达系统在生产重组蛋白中的应用，旨在为鱼类重组蛋白的研究与应用提供重要参考。

关键词

原核表达系统，真核表达系统，重组蛋白

Research Progress on Prokaryotic and Eukaryotic Expression Systems in Fish

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Abstract

The construction of efficient expression systems for functional proteins has potential applications in the aquaculture field. Recombinant proteins are synthesized by introducing specific genes into host

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cells to achieve targeted protein expression, a process encompassing multiple steps such as gene cloning, vector construction, host cell selection, protein expression, and purification. This article reviews the strengths and limitations of commonly used recombinant protein expression systems in fish (e.g., *Escherichia coli*, yeast, insect cells, and mammalian cells), as well as the applications of different expression systems in producing recombinant proteins, aiming to provide critical insights for the research and application of recombinant proteins in fish.

Keywords

Prokaryotic Expression System, Eukaryotic Expression System, Recombinant Protein

1. 引言

DNA 重组技术是生产大量目标蛋白质的重要工具。重组蛋白表达是利用 DNA 重组技术，将目标基因导入到宿主细胞，通过宿主细胞的生物机制使其表达出特定蛋白的过程。鱼类重组蛋白通过原核和真核表达宿主系统进行生产。近几年，鱼类重组蛋白表达系统在宿主选择、表达载体优化及表达效率提升方面取得了显著进展，主要应用于鱼类疫苗开发、基因功能验证及环境毒理研究等方面。每个表达系统都有其优点和局限性，因此，研究几种表达系统的潜力和局限性来选择适合特定蛋白生产的表达系统非常重要。本文综述了鱼类重组蛋白表达系统的最新研究进展，重点探讨了不同宿主系统(如大肠杆菌、酵母、昆虫细胞和哺乳动物细胞)的优缺点及其在鱼类重组蛋白表达中的应用，为鱼类重组蛋白的研究与开发提供理论依据与技术参考。

2. 鱼类中常见的重组蛋白表达系统

2.1. 原核表达系统

原核表达系统是通过原核生物获得外源重组蛋白的系统，具有操作简便、生产成本低、表达周期短等优势。常用的原核表达系统主要包括：大肠杆菌(*Escherichia coli*)、枯草芽孢杆菌(*Bacillus subtilis*)和链霉菌(*Streptomyces*)表达系统，其中应用最广泛的是大肠杆菌表达系统。

2.1.1. 大肠杆菌表达系统

大肠杆菌是最早用于表达重组蛋白的宿主菌，至今仍在原核表达系统中占据核心地位。其具有基因组注释完善、生长速度快(倍增时间约 20 分钟)、转化效率高、发酵工艺成熟的优势[1] [2]。近几年，大肠杆菌表达系统在鱼类抗菌肽开发、代谢调控蛋白表达、抗体制备等方面的研究中取得了显著进展。例如，Guo 等人[3]从橙斑石斑鱼(*Epinephelus coioides*)肝脏中分离出 β 防御素，可以减少石斑鱼虹彩病毒(SGIV)和神经坏死病毒(NNV)的感染；大口黑鲈中的 Nesfatin-1 蛋白通过大肠杆菌系统成功表达，利用纯化后的重组蛋白制备了高效价和特异性多克隆抗体，并通过免疫荧光技术验证其在肝胰脏中的定位[4]。

在大肠杆菌中，重组蛋白在细胞质中产生，且表达效率高[5]。然而，高表达往往会导致不溶性蛋白的积累，形成包涵体。包涵体不仅存在于真核生物蛋白中，而且在一定程度上也存在于包括大肠杆菌在内的原核生物的过表达蛋白中。大肠杆菌中的翻译和折叠速率几乎是真核细胞中的 10 倍，这可能是真核蛋白容易产生包涵体的原因之一[6]。此外，该系统缺乏真核特异的翻译后修饰功能，导致糖基化依赖型

蛋白活性丧失。为了减少包涵体的形成，表达出可溶性、有适当折叠且活性高的蛋白质，目前的三个优化策略是：其一，将诱导表达温度从 37℃降至 15℃~30℃[7]，较低的温度降低了热激蛋白酶[8]的活性，减缓了转录、翻译和重折叠的速率，从而使重组蛋白能够正确折叠[9]；其二，将重组蛋白的 N 端或 C 端加到可溶性融合标签[10]上，可以促进蛋白质的可溶性。已经证明 GST、MBP [11]、SUMO 等标签通过增加蛋白表面亲水性，显著改善疏水蛋白的溶解性；其三，将分子伴侣与重组蛋白一起共表达于细胞质中，以促进蛋白的可溶性[12] [13]。尽管如此，包涵体复性效率低(通常<30%)仍是制约其工业化应用的关键瓶颈。

2.1.2. 枯草芽孢杆菌表达系统

枯草芽孢杆菌是革兰氏阳性菌，相较于大肠杆菌，具备更强的外源蛋白分泌能力，能够将合成蛋白质分泌到培养基中[14] [15]，避免胞内蛋白酶降解，同时简化了下游纯化工艺。此外，其无显著密码子偏好性，适用于复杂真核基因的表达。研究表明，枯草芽孢杆菌可合成 α-淀粉酶[16]、碱性蛋白酶等工业酶，且 90%以上为可溶形式。这一特性使其适用于鱼类饲料添加剂[17]和口服疫苗[18]等需要分泌表达功能蛋白的生产。该系统存在局限性，一是外源质粒介导的不稳定性，可通过同源重组将外源基因插入枯草芽孢杆菌基因组来避免；二是转化效率低，需通过电穿孔或原生质体法优化。目前，已经设计了诱导型启动子系统[19]，可用于在枯草芽孢杆菌中高效生产重组蛋白。

2.1.3. 链霉菌表达系统

链霉菌作为放线菌的重要类群，具有高 GC 含量基因组，近年来因其卓越的蛋白分泌能力[20]和工业发酵成熟度，逐渐成为原核表达系统的新兴选择。其特点包括：单层细胞壁结构有利于蛋白分泌[21]，可避免胞内蛋白酶降解；发酵产物中内毒素含量极低[22]，适用于药用蛋白生产；具备部分翻译后修饰能力(如磷酸化)，可提升重组蛋白活性。在鱼类研究中，链霉菌表达系统已被用于表达鲑鱼降钙素[23]等具有药用价值的肽类分子，产量可达 200 mg/L，且无需复杂复性处理。然而，该系统存在一些缺陷，主要包括：遗传操作复杂，转化效率低；外源基因整合位点有限，表达调控元件开发滞后[24]。为此，通过构建链霉菌过表达穿梭载体[25]，与自诱导启动子结合，使蛋白表达量显著提高，为规模化应用奠定了基础。

2.2. 真核表达系统

真核表达系统主要包括酵母表达系统、昆虫/杆状病毒表达系统、哺乳动物表达系统等。

2.2.1. 酵母表达系统

酵母是一种易于操作和培养的单细胞微生物，具有像真核生物一样处理蛋白质的能力，即组装、折叠和翻译后修饰[26]。酵母表达系统凭借其真核修饰能力和高密度发酵优势，在鱼类复杂蛋白表达中占据重要地位。其中，毕赤酵母(*Pichia pastoris*)和酿酒酵母(*Saccharomyces cerevisiae*)应用最广泛。毕赤酵母通过甲醇诱导型启动子 AOX1 实现高密度发酵，已成功表达多种鱼类蛋白。如鱼类重组激素的生产[27]，以及生产用于预防和治疗尼罗罗非鱼(*Oreochromis niloticus*)细菌感染的 Nk-lysin [28]。尽管毕赤酵母具有许多优势，但在使用该系统时存在一定的限制。如无法保证每个目的蛋白都能被稳定表达，某些蛋白质可能会遇到与 RNA 稳定性、糖基化过程、蛋白质折叠或分泌过程相关的各种障碍[29]。与原核表达系统相反，酿酒酵母具有进行翻译后修饰和分泌的能力，这大大降低了发酵后体外纯化和修饰的成本[30]。酿酒酵母也更耐低 pH 值、高糖和乙醇浓度以及高渗透压，这使其适用于工业发酵[31]。在鱼类口服疫苗开发中，酿酒酵母表达系统表现突出。例如，表达 ORF132 的重组酿酒酵母口服疫苗对 CyHV-2 起到保护性免疫[32]。口服表达 MSRV 蛋白的重组酿酒酵母疫苗可在大口黑鲈(*Micropterus salmoides*)中引发保护性免疫[33]。

2.2.2. 昆虫细胞－杆状病毒表达系统

昆虫细胞－杆状病毒表达系统可以在昆虫细胞中高效表达多种外源蛋白[34] [35]。典型的杆状病毒表达载体系统基于苜蓿银纹夜蛾核型多角体病毒(AcMNPV)或家蚕核型多角体病毒(BmNPV)，前者通常用于培养细胞的表达[36] [37]，后者用于昆虫(家蚕)的表达[38]。该系统的优势在于能够正确折叠和修饰复杂蛋白质，与细菌或酵母表达系统相比具有较高的表达和较低的毒性[39]，其最高表达量可达昆虫细胞蛋白总量的 50%。该系统凭借其强大的翻译后修饰能力和多亚基复合物组装特性，在鱼类疫苗研发中占据核心地位。Lyu 等人[40]通过杆状病毒表达系统合成病毒糖蛋白，用于开发鱼类口服疫苗。Li 等人[41]通过昆虫－杆状病毒表达系统构建了二价嵌合病毒样颗粒疫苗(ND-IBD cVLPs)，并评价其免疫原性和免疫保护效果。

2.2.3. 哺乳动物细胞表达系统

哺乳动物细胞一直是治疗性蛋白质商业生产的主要表达系统。目前，超过 80%的治疗性蛋白质是使用哺乳动物细胞生产的，这主要是由于哺乳动物细胞能够合成在分子结构和生化特性方面与天然存在蛋白质相似的蛋白质[42]。且该系统在重组蛋白上可以产生有效的翻译后修饰[43]。用于生产重组蛋白的哺乳动物细胞主要有中国仓鼠卵巢细胞(CHO)、小鼠骨髓瘤细胞(NS0 和 Sp2/0)、非洲绿猴肾细胞(COS)、仓鼠肾细胞(BHK-21)和人胚胎肾细胞(HEK-293)等[44]-[46]。在鱼类疫苗制备中，哺乳动物系统的优势尤为显著。Ling 等人[47]成功构建并测试了一种携带高免疫原性 Vapa 基因的重组腺病毒候选疫苗，可感染 HEK-293 细胞并表达 A 层蛋白，为预防虹鳟鱼(*Oncorhynchus mykiss*)杀鲑气单胞菌 A450 感染提供了一种候选疫苗。CHO 细胞系广泛用于生产正确折叠和翻译后加工的重组蛋白[48] [49]。Li [50]的研究中使用线性聚乙烯亚胺瞬时转染 CHO 细胞，成功生产出虹鳟鱼重组 IFN-γ 蛋白，且适用于无血清操作。此外，哺乳动物表达系统主要用于产生分泌蛋白而不是胞内蛋白，已经开发了 CHO 和 HEK293 细胞系的无血清培养基，简化了分泌型重组蛋白的纯化[51]-[53]。未来，结合无血清培养基优化和基因编辑技术，哺乳动物系统在鱼类药物领域的应用将更加广泛。

3. 总结

原核与真核表达系统在鱼类研究中各具优劣。原核系统(如大肠杆菌、枯草芽孢杆菌、链霉菌)凭借材料、技术等成本低的优势，适用于大规模生产简单蛋白；而真核系统(如酵母、昆虫－杆状病毒、哺乳动物细胞)则更适合复杂修饰蛋白生产。未来，跨系统协同(如原核分泌载体与真核修饰酶共表达)及合成生物学技术的研发及进步，将推动鱼类重组蛋白应用迈向更高维度。

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