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Review 综述

生物法制备壳寡糖的研究进展

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摘　要：壳寡糖(chitooligosaccharide, COS)是几丁质(chitin, CI)或壳聚糖(chitosan, CS)的降解产物。由于其生物相容性好、可降解、无毒且具有强生物活性，在食品、化妆品、复合材料、污水处理、生物制药等领域展现出广阔的应用前景。当前，国内外学者主要采用物理、化学、生物酶等方法制备 COS。然而，物理和化学方法存在较大的局限性，难以高效且绿色地合成特定要求的目标产物。相比之下，生物酶法制备 COS 的反应过程温和、可控，且对环境更加友好，能够克服物理、化学法的缺点。借助膜分离法、凝胶过滤色谱法、CM-SephadexC-25 离子交换柱法和固定化金属亲和层析法等先进的分离纯化手段，可以有效提升 COS 的纯度。本文综述了近年来利用生物酶法技术制备 COS 的研究进展，旨在为实现高质量 COS 的工业化制备奠定一定的理论基础。同时，对 COS 的结构、性能及应用等方面进行综述，为 COS 的制备与分离研究提供基础。

关键词：几丁质；壳聚糖；壳寡糖；生物酶法；分离纯化；应用

Recent advances in bioproduction of chitooligosaccharides

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Abstract: chitooligosaccharide (COS) are degradation products of chitin or chitosan, demonstrating good biocompatibility, degradability, non-toxicity, and multiple bioactivities. COS have been widely used in food, cosmetics, composite materials, wastewater treatment, and

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biomedical industries. Currently, researchers mainly use physical, chemical, and biological enzyme methods to prepare COS. Physical and chemical methods have large limitations, and it is difficult to synthesize the target products with specific requirements in an efficient and green way. Bio-enzymatic preparation of COS shows a mild, controllable, and environmental friendly reaction process, overcoming the drawbacks of physical and chemical methods. The purity of COS can be improved by separation and purification techniques such as membrane separation, gel filtration chromatography, CM-SephadexC-25 ion-exchange column chromatography, and immobilized metal affinity chromatography. This review summarized the research progress in COS preparation using bio-enzymatic technology, aiming to lay theoretical foundation for high-quality industrial COS preparation. It also gave an overview of the structure, properties, and application of COS, contributing for the research on COS preparation and isolation.

Keywords: chitin; chitosan; chitooligosaccharide; bio-enzymatic method; separation and purification; application

壳寡糖(chitooligosaccharide, COS)，也称作寡聚氨基葡糖或甲壳低聚糖，是由 2−10 个氨基葡萄糖单元通过 β-1,4 糖苷键连接形成的低聚糖系列，它们通常是几丁质或壳聚糖经过降解过程的产物，COS 是目前已知的少数碱性寡糖之一，由于其生物相容性好、可降解、无毒、生物活性强，在食品、化妆品、复合材料、污水处理、生物制药等领域有着广阔的应用前景[1]。COS 的制备技术途径包括物理法、化学法和生物酶法。在此基础上，利用色谱、离子交换、电渗析等方法可以对高聚合度甲壳素/壳聚糖进行降解，从而获得 COS。COS 的物理制备方法主要有紫外线辐照、超声波破碎、微波辐照等。虽然物理方法操作简便，但产品产率低、降解能力有限，从而制约了其规模化生产，并且未能产生显著的社会效益[2]。COS 的化学制备方法主要有酸分解(如盐酸、亚硝酸、磷酸)和氧化(双氧水、臭氧、次氯酸) 2 种方式[3-5]。其中，氢离子能够与壳聚糖的自由氨基团结合，破坏其分子间氢键，从而得到不同聚合度的 COS[5]。然而，目前采用的酸法生产 COS 存在着产品安全性不高、副产物分离困难、残酸及所产有害废物难处理等问题，这些问题容易导致环境污染等问题[6]。在水相中，氧化剂生成的自由基能够断裂壳聚糖的糖苷键，进而生成不同聚合度的 COS[5]。当采用氧化剂对壳聚糖进行氧化处理时，如果氧化剂的浓度和温度太高，则会导致壳聚糖的过度水解，使产品的氨基流失更多，同时还会因褐变反应而加深颜色，从而降低产品的质量。相比之下，生物酶法制备 COS 反应过程温和、可控且对环境更加友好。本文概述了 COS 在食品工业中的应用现状，并对近年来 COS 的生物制备与提纯技术进行了综述，为高质量 COS 的制备及工业化生产奠定了一定的理论基础。

1 壳寡糖的应用

COS 由于其无毒、生物相容性好、可生物降解等特点，在食品、农业、医药等领域得到了广泛的应用。在食品方面，COS 是一种广谱的抑菌物质，它能有效地抑制病原菌和腐败细菌的繁殖，并能延长水果、蔬菜等食物的货架期，例如，在面包中添加 COS 可以抑制食源性病原菌及根霉菌的生长[7]。此外，COS 还具有成膜特性，可用于鲜切苹果涂膜处理，有效抑制微生物生长，保持苹果品质，调控苹果呼吸

强度，稳定可溶性固形物和可滴定酸含量，延缓果实软化[8]。在农业领域，施加 COS 于农田中可抗病毒、抑菌，并调节植物生长。研究表明，COS 能增加烟叶中的防卫酶活力，降低烟草花叶病毒病的发生率，同时减缓叶绿素的下降幅度[9]。此外，作为一种创新的抗菌剂，COS 在管理果蔬采后病害[10]、激发植物的先天免疫反应[11]以及提高植物的生理学活性等方面，展现出了显著的应用潜力。在化妆品领域，COS 具有良好的抗氧化性能，能有效保护肌肤免受氧化损伤。作为化妆品添加剂，COS 还能阻止精油和维生素等其他有效成分的氧化[12]。将 COS 涂抹于皮肤表面，有助于预防紫外线引起的皮肤老化[13]。在医药领域，COS 是一种重要的生物活性物质，经肠道上皮细胞摄取后，COS 能够进入机体的不同部位，发挥其生理功能[14]。例如，在治疗神经系统疾病方面，COS 可通过抑制胆碱酯酶的活性，对阿尔茨海默病的治疗具有潜在作用[15]，为之后的临床治疗提供了新的思路。同时，研究发现 COS 对呼吸系统疾病、肾脏疾病具有潜在的有益作用，对慢性肾衰竭大鼠的肾脏具有保护功能[16]。关于 COS 作为治疗糖尿病的药物或补充剂的研究已持续十年，蛋白质组学数据表明，口服 COS 对 ob/ob 小鼠具有抗糖尿病和抗肥胖作用[17]。目前，COS 已被用作糖尿病治疗的食品补充剂，随餐补充 COS 可有效降低餐后血糖水平，与预防糖尿病有关[18-20]，并展现出抗肿瘤作用[21-23]。此外，COS 已被证明是一种促进伤口愈合的生物材料。例如，通过激活 TGF-β/Smad 途径，COS 能加速伤口愈合过程[24-25]。

2 生物法制备壳寡糖

近年来，酶法水解几丁质/壳聚糖制备 COS 一直是研究的热点。几丁质(chitin, CI)也称甲壳素，是一类在自然界中含量仅次于纤维素的大分子多糖，其主要成分是 N-乙酰-D-葡萄糖胺(N-acetyl-D-glucosamine, GlcNAc)[26-27]，在真菌类、藻类、节肢动物等组织中均有发现[28]。壳聚糖(chitosan, CS)是 CI 中的 GlcNAc 键水解后得到的脱乙酰产物，是自然界中唯一存在的天然碱性多糖[29]，通常脱乙酰度大于 55% 才能被定义为 CS。由于 CS 不溶于水，这限制了其应用范围；然而，CS 可以溶于酸性介质，如稀乙酸和甲酸[30-31]。酶法制备 COS 的途径主要有 2 条：(1) CI 通过几丁质脱乙酰酶制备 CS，CS 再经壳聚糖酶制备 COS，另一条途径是；(2) CI 直接在几丁质酶的作用下制备几丁寡糖，几丁寡糖再经进一步脱乙酰化处理制备 COS (图 1)，生物酶法的绿色生产技术能够克服物理化学方法的缺点，是未来发展的主要方向。

3 几丁质酶解制备几丁寡糖/壳寡糖

几丁寡糖是通过几丁质降解而得到的产物，几丁寡糖(N-acetylchito-oligosaccharides, NAc-COS)是指由 N-乙酰葡萄糖胺连接而成的 2−10 个单元的寡糖($GluNAc_2$−$GluNAc_{10}$)，NAc-COS 不仅具有水溶性和易于分解吸收的特点，还展现出多种功能[32]，如促进乳酸菌增殖、增强免疫机能、改善肠道功能、消除体内霉素以及抑制肿瘤细胞生长等重要生理效应。因此，在食品和医药领域，NAc-COS 具有广泛的应用前景。几丁质酶(chitinase)是一类能够水解 CI 的糖苷水解酶的总称[33]。根据酶切位点的不同，几丁质酶被分为外切几丁质酶和内切几丁质酶。其中，内切几丁质酶主要水解产生低聚合度的寡糖，如几丁二糖[$(GlcNAc)_2$]到几丁六糖[$(GlcNAc)_6$]等[34-35]；而外切几丁质酶则主要生成几丁二糖作为终产物[36]。单糖和葡萄糖会抑制几丁质酶

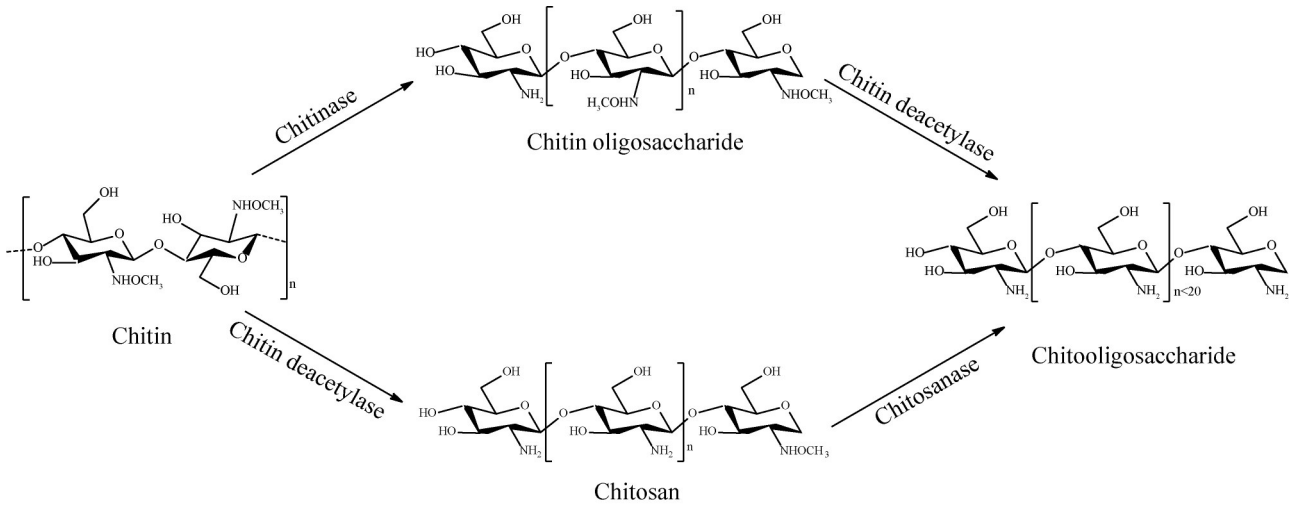

图1 生物法制备壳寡糖途径

Figure 1 Pathway of chitooligosaccharide biosynthesis from chitin. There are two routes to prepare chitooligosaccharides: (1) Chitin was degraded chitin oligosaccharide catalyzed by chitinase, then chitin oligosaccharide deacetylated to chitooligosaccharide catalyzed chitin deacetylase (the upper route); (2) Chitin deacetylated to produce chitosan catalyzed by deacetylase, then chitooligosaccharide was degraded to chitosanase catalyzed by chitosanase (the lower route).

的表达和分泌[37]。几丁质酶活性通过 3,5-二硝基水杨酸(3,5-dinitrosalicylicacid, DNS)法进行测定。

几丁质酶不仅参与外源 CI 的分解，还参与真菌细胞壁的降解和形态发生过程。其中，CI 的裂解对于菌丝生长、隔的形成和孢子萌发至关重要[38-39]。真菌几丁质酶在处理渔业的 CI 废物方面具有潜在应用价值。Le 等[40]从盐虾样品中分离得到一株能够降解 CI 的盐弧菌(*Salinivibrio* sp.) BAO-1801，并从中分离纯化出几丁质酶 BAO-1801，使用该酶对 CI 进行水解，得到了 $(GlcNAc)_2$，在反应 8 h 后，产率为 71.5%。此外，丝状真菌曲霉菌(*Aspergillus niveus*)能够利用蟹壳作为碳源，产生胞外抗真菌几丁质酶来降解蟹壳[41]。从土壤中分离出的黄曲霉等曲霉具有高效生产 GlcNAc 的能力，主要产物为 GlcNAc (约 80%)和少量的$(GlcNAc)_4$ (约 20%)，其性能与中温嗜酸性单孢青霉 CFR2 和尖孢镰孢菌 CFR8 相似；当使用 10 mg/mL 的胶体几丁质和结晶 α 几丁质作为底物，经过 48 h 的反应后，这 2 种真菌的浓缩粗几丁质酶分别产生了约 90 mmol/L 和 10 mmol/L 的 GlcNAc[42]。潘梦妍等[43]为了进一步提升几丁质酶 Chisb 的催化活性，采用了易错式 PCR 方法，建立了一个几丁质酶 Chisb 的随机突变体库，实现了几丁质酶 Chisb 的定向进化，获得了 2 个催化效率进一步提高的突变体 C43D 和 E336R，其催化效率相较于对照分别提高了 1.35 倍和 1.57 倍，以胶体几丁质为基质产生的 NAc-COS 的含量分别为 2.53 g/L 和 2.06 g/L，较对照(0.89 g/L)分别增加了 2.84 倍和 2.31 倍，同时，底物转化率分别为 84.3% 和 68.7%，较对照(29.7%)分别提高了 54.6% 和 39.0%。Gao 等[44]从白色链霉菌(*Streptomyces albolongus*) ATCC 27414 中克隆到一个新型几丁质酶基因，并在大肠杆菌 BL21 中实现了高效表达，得到几丁质酶 SaChiA4，它可以水解胶体甲壳素产生 GlcNAc 和$(GlcNAc)_2$，产

率分别为 0.87 mg/mL 和 2.17 mg/mL。多项研究表明，几丁质酶的联合使用对于 CI 的高效降解具有重要意义[45-47]。

CI 也可通过几丁质酶和几丁质脱乙酰酶降解生成 COS，Krolicka 等[48]报道了从嗜热丝霉菌中提取几丁质酶的生产和特性，该酶能够将 CI 废物生物转化为 COS，他们发现这种几丁质酶在 40 (>140 h 90% 活性)、50 (>168 h 90% 活性)和 55 ℃ (半衰期 48 h)的条件下展现出高热稳定性；同时该酶对$(GlcNAc)_{3-6}$的降解速率分别为 0.02、0.20、0.17 和 0.18 mmol/L min。使用来自米黑根霉和哈茨木霉的几丁质酶也观察到了类似的结果，这些酶能够将胶体 CI 水解成完全乙酰化的 COS[49-50]。目前，已经分离鉴定出的几丁质酶的特征见表 1。

4 几丁质脱乙酰化制备壳聚糖

几丁质脱乙酰酶(chitin deacetylase, CDA)能够催化 CI 中 N-乙酰胺基水解生成 CS。Araki 等[51]于 1974 年首次从鲁氏毛霉(*Mucor rouxii*)中成功提取并鉴定了 CDA，揭示了其作为金属酶的特性[52]，该酶对 CI 中的乙酰基具有高度的专一性去除能力。随后的多项研究表明，红串红球(*Rhodococcus erythropolis*)、杂色曲霉(*Aspergillus versicolor*)和蓝色犁头霉(*Absidia coerulea*)等都是能够产生 CDA 的菌株[53-54]。然而，由于 CDA 在脱乙酰过程中会释放乙酸盐，这限制了其在工业化生产中的应用[55-56]。CDA 的活性通常是通过使用无色的对硝基乙酰苯胺(无色)作为底物，并采用黄色的对硝基苯胺进行比色法测定来评估的[57-58]。目前，研究者已经从不同微生物中鉴定到多种 CDA，其特征见表 2。

日本和美国先后开始对微生物发酵法生产 CS 进行研究，90 年代初我国也进行了相关研究。与从虾、蟹壳中提取 CS 相比，从真菌细胞壁中提取 CS 具有诸多显著优势。例如，大部分真菌可通过发酵技术实现大规模培养，这一过程不受季节、地域限制，且分离工艺简单，仅需使用稀酸或稀碱进行处理。近年来，利用菌种发酵生产 CS 的研究日益受到关注，涉及的菌种包括毛霉、犁头霉、黑曲霉和根霉

表1 已经分离鉴定到的几丁质酶的特征

Table 1 Chitinase characteristics have been isolated and identified

Sources of enzymes	Carbon source	Matrix	Product	Productivity	References
Salinivibrio sp. BAO-1801	–	Colloidal chitin	$(GlcNAc)_2$	71.5%	[41]
Penicillium monoverticillium CFR2, *Aspergillus flavus* CFR10, *Fusarium oxysporum* CFR8	Wheat bran	Crystalline α-chitin	GlcNAc	10.11, 6.85, and 10.70 mmol/L	[42]
Penicillium monoverticillium CFR2, *Aspergillus flavus* CFR10, *Fusarium oxysporum* CFR8	Wheat bran	Colloidal chitin	GlcNAc	95.6, 96.6, and 96.1 mmol/L	[42]
Streptomyces albolongus ATCC27414	–	Colloidal chitin	GlcNAc $(GlcNAc)_2$	0.87 mg/mL and 2.17 mg/mL	[44]
Trichoderma harzianum	–	Colloidal chitin	COS	–	[49]
Rhizopus oryzae	Oat flour	Colloidal chitin	–	–	[50]

–表示无明确相关数据；Carbon source表示除基质以外的碳源。

– indicates that there is no clear data. Carbon source indicates the carbon source other than the matrix.

表2　部分来源CDA的相关理化性质

Table 2　Part of the source of CDA related physicochemical properties

Sources of enzymes	Enzyme site	Enzyme activity	Optimum temperature (℃)	Optimum pH	Inhibitor	Activator	References
Rhodococcus	Intracellular	254.43 U/mL	50	7.0	Mg^{2+}, Zn^{2+}, Fe^{2+}, Co^{2+} and high concentrations of Ca^{2+}, Mn^{2+}, K^{+}	Low-concentrations of Ca^{2+}, Mn^{2+}, K^{+}	[54]
Bacillus subtilis	Periplasmic space	0.212 U/mL	50	5.0	Zn^{2+}	Ca^{2+}	[57]
Mucor rouxii	Periplasmic space	0.434 μmol/(mL·10 min)	50	4.5	High concentrations of Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Cu^{2+}, EDTA, EGTA	–	[59]
Colletotrichum lindemuthianum	Extracellular	2.4 μmol/(mL·24 h)	50	8.5	Zn^{2+}, Mn^{2+}, Cu^{2+}	Co^{2+}	[60]
Absidia coerulea	Periplasmic space	2.048 μmol/(mL·100 min)	37	5.5	Fe^{3+}	–	[61]

–表示无明确相关数据。

– indicates that no specific data is available.

菌等[62]。毛霉属真菌能够利用其细胞内存在的CDA的催化作用，实现CI向CS的转化，从而直接从其菌丝体中高效提取CS，为后续研究奠定了坚实的基础[63]。魏光等[64]则从蓝色犁头霉细胞壁中成功提取了CS，其天然CS产量占菌丝干重的11.72%，经脱乙酰基处理后，CS的产量进一步提升至菌丝体干重的2.58%。曹健等[65]利用黑曲霉进行发酵生产CS，以蔗糖和玉米浆为培养液，不仅实现了9.72%的CS得率，还揭示了Mg^{2+}对黑曲霉具有生长刺激作用。然而，CS生产领域仍面临着产酶条件优化等挑战。秦汪艳等[66]经紫外诱变，获得一株高产CDA的丝状真菌*Penicilium janthinellum*菌株，并在优化产酶条件后，该菌株的酶活显著提升，增幅高达52%；Zhang等[67]通过优化培养基条件和产酶工艺，使得去乙酰化活性达到613.25 U/mL，制备出脱乙酰度大于90%的CS，实现了CS的绿色制备。为了进一步降低发酵成本，可以通过更换廉价的发酵碳源来实现。Yang等[68]创新性地采用稀酸蒸爆预处理后的玉米秸秆作为碳源，利用米根霉(*Rhizopus oryzae*) AS3.819进行发酵生产，CS产量为高达90 g/kg，为CS生产的工业化应用开辟了新路径。已经报道的通过几丁质生产壳聚糖的菌株见表3。

表3　几丁质制备壳聚糖

Table 3　Chitosan produced by chitin

Strains	Carbon source	Productivity	References
Absidia coerulea	Glucose	11.72%	[64]
Aspergillus niger	Sucrose, corn syrup	9.72%	[65]
Rhizopus oryzae AS3.819	Corn stalk	90 g/kg	[68]

5 壳聚糖酶解制备壳寡糖

COS 的另一种合成途径涉及 CI 的降解与去乙酰化过程，最终转化为 CS，随后在壳聚糖酶的催化作用下，CS 进一步降解为 COS。壳聚糖酶(chitosanase, Csn)在 1973 年由 Monaghan 在研究细菌和真菌的过程中首次提出，并于 1992 年被国际酶学大会正式系统命名[69]。Csn 的核心功能是催化 CS 的降解，从而产生 COS。该酶来源广泛，不仅普遍存在于细菌[70-72]和真菌[73-75]中，还存在于一些植物组织中[76-77]。根据作用机制，Csn 可分为内切型和外切型 2 种：内切型酶主要产生 COS 作为水解产物，而外切型酶则逐步降解 CS 至最终的氨基单糖[78]。Csn 展现出高度的水解专一性，对 CI 等类似多糖几乎无水解活性，CS 的去乙酰度(degree of deacetylation, DDA)越高，Csn 的相对活性也越高[79]。研究中常用 DNS 法与产生的还原产物发生显色反应，从而确定 Csn 的活性[80]。此外，Csn 的水解活性不仅受到 pH 值的调控[81]，还显著地受到金属离子种类及浓度、温度等条件的影响。多数 Csn 分子量为 20−75 kDa，且多为酸性酶，其最适 pH 值均低于 7.0，这主要是因为 CS 仅在酸性条件下才能溶解；研究表明，在 CS 工业应用中，通过构建微酸性环境可以有效提高 Csn 的催化水解效率[82]。值得注意的是，这些酶多来源于细菌，且多数为嗜温型酶，其最佳作用温度范围通常在 30−60 ℃之间[83]。此外，Csn 的催化活性还会受到不同金属阳离子的显著影响[79]，如 Fe^{2+}和 Mg^{2+}被证实对多种 Csn 具有激活作用，而 Cu^{2+}和 Ba^{2+}则显著抑制其酶活性，不同来源的壳聚糖酶及其酶学性质见表 4。

自然界中的多数产酶菌株是由诱导物和降解产物共同调控的野生型菌株，这类菌株的酶产量通常较低且酶活性不稳定。因此，通过提高酶活性及其稳定性，可以有效提升 COS 的产量。1997 年，Samain 等[90]在 2 L 生物反应器中成功利用大肠杆菌的重组技术，首次实现了

表4 不同来源的壳聚糖酶及其酶学性质

Table 4 Csn from different sources and their enzymatic properties

Source	Strain	Molecular weight (kDa)	Optimum pH	Optimum temperature (℃)	Enzyme activity	Inhibitor	Activator	References
Bacteria	*Butyrivibrio* sp. MC2013	35	8.0	45	146 U/mg	−	−	[71]
	Staphylococcus capitis	35	7.0	30	−	Ba^{2+}, Mg^{2+}, Ca^{2+}, Ni^{2+}	Zn^{2+}, Cu^{2+}, Mn^{2+}	[84]
	Pseudoalteromonas sp. SY39	28	5.9	40	370 U/mL	Ni^{2+}, Co^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}, Al^{3+}	Li^{+}, K^{+}, Mg^{2+}	[85]
Fungus	*Aspergillus cervinus*-ZJOUAC1		5.9	30	8.26 U/mL	−	−	[86]
	Penicillium oxalicum M2	42	5.5	60	60.45 U/mg	Ca^{2+}, Mn^{2+}, nonionic surfactants	−	[87]
	Aspergillus sp. QD-2		5.6	55	85.816 U/mL	−	−	[88]
Plant	*Ficus awkeotsang*	21	4.5	50	−	−	−	[89]

−表示无明确相关数据。

− indicates that no specific data is available.

5-N-乙酰基壳五丁糖及其去乙酰化衍生物4-N-乙酰基壳五丁糖酶的生物合成，标志着微生物生产法在COS合成领域取得了突破性进展。为了提高重组大肠杆菌生产COS的产量，Zhang等[91]测试了多种碳源和前体底物，并开发了一种两步发酵程序，成功实现了COS的高产。De Araújo等[92]则利用芽孢杆菌和埃希姆芽孢杆菌的酶提取物高效地将CS转化为低聚物，在CS与埃希氏芽孢杆菌酶作用12 h后，转化率高达99.2%，且产物中单体含量低、生物活性高，有效降低了生产成本。赵华等[93]通过响应面法优化了蜡样芽孢杆菌(*Bacillus cereus*)发酵上清液中Csn的酶解条件，最终使得COS的产物浓度达到35.73 μmol/mL。Chen等[94]利用细胞表面展示Csn (CHI-1)，在枯草芽孢杆菌(*Bacillus subtilis*)和醋杆菌属(*Acetobacter* sp.)的共同发酵体系下，建立了以虾糠为原料生产COS的高产方法，COS产率高达41%，这充分证明了该方法的经济高效性与大规模生产的可行性。

6 壳寡糖的分离纯化

COS主要作为益生元、药物和功能食品供人类食用，对产品纯度要求很高[95]。CS降解可得到不同分子量的COS混合物。开发一种从反应混合物中分离COS的技术确实是一项具有挑战性的任务，这需要将化学工程知识与膜分离过程等技术有效地结合起来。COS的潜在应用要求其富集组分具有确定的分子量。目前，COS的分离纯化方法包括膜分离法、凝胶过滤色谱法、CM-SephadexC-25离子交换柱法、固定化金属亲和层析法。

除了分子量的差异之外，不同聚合度的壳寡糖由于解离后所带电荷的不同，其极性也会相应改变。例如，杜昱光等[96]研究了一种酶法降解和膜分离相耦合的制备活性壳寡糖的方法，实现了水解反应和分离一体化，有效防止了活性壳寡糖的二次降解，使得聚合度为3−6的壳寡糖含量达到了80%以上。高丽霞等[97]采用聚丙烯酰胺P6、P6 Fine为填料，对脱乙酰程度95%的CS进行了二段式分离，并以0.1 mol/L NH_4HCO_3作为洗脱剂，并将洗脱流速设定为0.4 mL/min，通过这种方法制备的壳三糖至壳七糖的纯度超过了95%，由于解离后所带电荷的不同，其极性也会相应改变。相比之下，李克成[98]采用CM-SephadexC-25离子交换柱分离从虾壳中提取的脱乙酰度为82%的α-CS，用50 mmol/L的NaCl-NaAc缓冲液(pH 4.8)和不同浓度的NaCl (0−2 mol/L)-HAc缓冲液以3 mL/min的速度逐级洗脱，用苯酚-硫酸法在490 nm处收集和监测馏分，最终获得了3个高纯度壳三糖、壳五糖和壳六糖单体，其纯度分别为95.7%、85.6%和89.5%。此外，Le Dévédec等[99]将亚氨基二乙酸(imino diacetic acid, IDA)键合到琼脂糖凝胶上，并与铜螯合，制备出稳定的壳聚糖(COS)固定相，使其静态吸附能力大幅提高，经咪唑梯度洗脱后，获得纯度大于90%以上的壳二至四糖。COS的分离纯化方法见表5和图2。

表5 壳寡糖的分离纯化

Table 5 Isolation and purification of chitosan oligosaccharides

Method	Product	Purity (%)
Membrane separation	COS trimer-hexamer	>80
Polyacrylamide Bio Gel P6 and P6 Fine (gel filtration chromatography)	COS trimer-heptamer	>90
CM-SephadexC-25 ion exchange column	COS trimers, pentamer and hexamers	95.70, 85.65, 89.50
Immobilized metal affinity chromatography	COS dimer-tetramer	>90

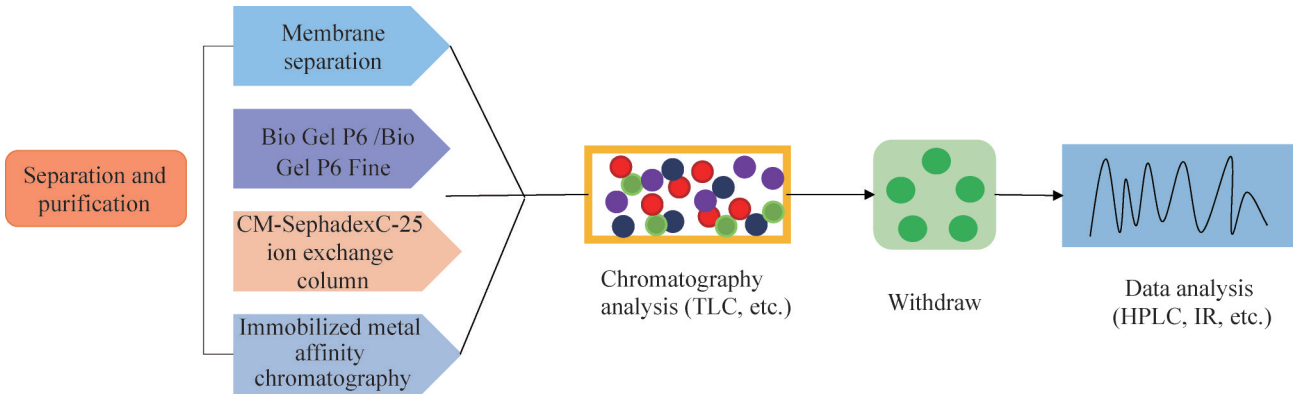

图2 壳寡糖的分离纯化方法

Figure 2 Isolation and purification methods of chitooligosaccharides. The separation and purification process of chitooligosaccharides includes separation and purification by membrane separation, gel filtration chromatography, CM-SephadexC-25 ion exchange column, and immobilized metal affinity chromatography, and then thin-layer chromatography analysis and extraction, and finally high performance liquid chromatography and infrared absorption spectrum data analysis.

7 总结与展望

生物酶法生产壳寡糖是一种可行且环保的方法，利用几丁质脱乙酰酶(CDA)等生物酶对壳聚糖进行催化降解以合成壳寡糖具有一定的优势。在不依赖化学合成和污染性溶剂的情况下，生物酶法能够高效地生产出壳寡糖，在医药、保健品、食品等领域展现出广阔的应用前景。通过对生物酶的特性、酶活性以及适宜的工艺条件进行深入研究和优化，可以进一步提高壳寡糖的产量和纯度，以满足不同领域的应用需求。本综述对生物法生产壳寡糖的 2 种通路进行了阐述，对通路中涉及的酶的特征及产酶微生物进行了总结。随着分子生物学技术的发展，对这些酶进行分子改造，在微生物中过表达以转化这些酶，有望显著提高它们的转化效率，从而实现工业化生产。丝状真菌由于具有强大的蛋白分泌和环境适应能力，许多丝状真菌在天然生长环境中就具备降解几丁质的能力。因此，对丝状真菌进行分子生物学改造，用于生产壳寡糖是未来发展的重要方向之一。目前，农杆菌介导的遗传转化体系经成功在米曲霉和蛹虫草等丝状真菌建立[100-102]，通过分子生物学方法对这些真菌进行遗传改造，有望使其成为壳寡糖工业化生产的优质底盘细胞。

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