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Review 综 述

厌氧氨氧化菌内含物——糖原颗粒

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摘 要：厌氧氨氧化菌(anaerobic ammonium-oxidizing bacteria, AnAOB)是分类学上新近建立的细菌类群。由于生长缓慢，培养困难，迄今没有获得纯培物。与已知细菌类群相比，AnAOB 具有诸多特异性细胞结构和功能。AnAOB 是化能自养型细菌，但在其细胞内经常可见贮藏性的内含物——糖原颗粒。探讨这种糖原颗粒的性状与动态，可深化人们对 AnAOB 的认识。本文结合文献报道及前期研究基础，对厌氧氨氧化菌糖原颗粒的结构、代谢和功能特性进行了探讨，分析认为 AnAOB 糖原颗粒分布于核糖细胞质内，且处于多途径合成与多位点消耗的动态平衡中；此外，糖原颗粒具有提供能量、碳架和应激保护等能力，对逆境下 AnAOB 的生存具有重要意义。本综述可为厌氧氨氧化菌的深入研究和工程应用提供支撑。

关键词：厌氧氨氧化；厌氧氨氧化菌；糖原颗粒；糖原代谢

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Research progress in glycogen particles in anaerobic ammonium-oxidizing bacteria

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Abstract: Anaerobic ammonium-oxidizing bacteria (AnAOB) are a newly developed taxonomic group. Due to the slow growth and difficult cultivation, pure cultures of AnAOB have not yet been obtained. AnAOB exhibit unique cellular structures and functions compared with the well-known bacterial groups. Although being chemolithotrophic bacteria, AnAOB often contain cellular inclusions, e.g., glycogen particles. Studying the characteristics and dynamics of glycogen particles will deepen our understanding into AnAOB. According to relevant literature and our previous work, we systematically summarized the properties of the structure, metabolism, and function of the glycogen particles in AnAOB. The glycogen particles in AnAOB exist in the riboplasm and are in dynamic balance between synthesis via various pathways and degradation at multiple sites. Additionally, glycogen particles can serve as energy and carbon sources and provide stress protection, which are of great significance for the survival of AnAOB under adverse conditions. This review is expected to underpin the further investigation and application of AnAOB.

Keywords: anaerobic ammonium-oxidizing; anaerobic ammonium-oxidizing bacteria; glycogen particle; glycogen metabolism

厌氧氨氧化菌(anaerobic ammonium-oxidizing bacteria, AnAOB)广泛分布于海洋、淡水和陆地等环境中，对含氮物质的转化发挥着重要作用[1]。随着对 AnAOB 的研究和系统发育学的发展，新近出版的《伯杰氏系统细菌学手册》中，新设了厌氧氨氧化菌科，即 *Candidatus* Brocadiaceae。

AnAOB 是化能自养型细菌。它们能将铵和亚硝酸转化为氮气，合成 ATP 和 NADPH[2-3]；并能以二氧化碳或碳酸氢盐作为碳源，合成有机物质[4]。对于 AnAOB，有机物质不仅是其不易获得的稀缺物质，也是其构建新细胞的必要物质。值得关注的是，在 AnAOB 细胞中可观察到糖原颗粒[5]。

糖原是一种葡萄糖多聚物，在生物体内具有能量储备、渗透调节和 pH 维持等功能[6]。近期研究表明，糖原在 AnAOB 的生长和代谢中具有重要作用，特别是在基质缺乏的生境中，AnAOB 通过降解糖原来获取能量和碳源，从而维持生长和代谢[5]。

本文结合有关文献报道以及团队前期研究基础，对 AnAOB 中糖原颗粒的结构、代谢和功能特性作一综述和讨论，以期为 AnAOB 的理论研究和实际应用提供理论支撑。

1 厌氨氧化菌的种类与分布

AnAOB 是一类能在无氧条件下将铵(NH_4^+)和亚硝酸盐(NO_2^-)转化成氮气(N_2)的化能自养型细菌[7]。由于生长缓慢，培养困难，迄今没有获得 AnAOB 纯培物。基于 16S rRNA 基因的系统发育分析以及其他性状观测，AnAOB 被归入浮霉菌门(*Planctomycetes*)厌氧氨氧化菌科(*Candidatus* Brocadiaceae)。《伯杰氏系统细菌学手册》中收录的 AnAOB 共 5 属 8 种，包括 *Candidatus* Brocadia 属(*Candidatus* Brocadia anammoxidans 和 *Candidatus* Brocadia fulgida)、*Candidatus* Kuenenia 属(*Candidatus* Kuenenia stuttgartiensis)、*Candidatus* Anammoxoglobus 属(*Candidatus* Anammoxoglobus propionicus)、*Candidatus* Scalindua 属(*Candidatus* Scalindua brodae、*Candidatus* Scalindua sorokinii 和 *Candidatus* Scalindua wagneri)和 *Candidatus* Jettenia 属(*Candidatus* Jettenia asiatica)。随着研究的推进，又陆续发现了一些 AnAOB 新种，目前文献报道的 AnAOB 有 6 属 26 种(表 1)。

表 1 AnAOB 的分类、来源及特性

Table1 The clarification, sources and characteristics of anaerobic ammonium-oxidizing bacteria

Genus	Species	Sources	Accession No.	References
Brocadia	***Ca*. Brocadia anammoxidans**	Wastewater	AF375994	[8]
	***Ca*. Brocadia fulgida**	Wastewater	DQ459989	[9-10]
	Ca. Brocadia brasiliensis	Wastewater	GQ896513	[11]
	Ca. Brocadia caroliniensis	Nitrogen removal system	JF487828	[12]
	Ca. Brocadia sapporoensis	Membrane bioreactor	KY659581	[13]
	Ca. Brocadia sinica	Nitrogen removal reactor	AB565477	[14-15]
Kuenenia	***Ca*. Kuenenia stuttgartiensis**	Trickling filter reactor	AF375995	[16-17]
Anammoxoglobus	***Ca*. Anammoxoglobus propionicus**	Laboratory bioreactor	DQ317601	[18]
	Ca. Anammoxoglobus sulfate	Biological reactor	–	[19]
Scalindua	***Ca*. Scalindua brodae**	Wastewater	AY254883	[20]
	***Ca*. Scalindua wagneri**	Wastewater	AY254882	[20]
	***Ca*. Scalindua sorokinii**	Seawater	AY257181	[21]
	Ca. Scalindua marina	Marine sediments	EF602038	[22]
	Ca. Scalindua sinooilfield	Petroleum reservoirs	HM208769	[23]
	Ca. Scalindua arabica	Seawater	EU478593	[24]
	Ca. Scalindua profunda	Marine sediments	EU142947	[25]
	Ca. Scalindua zhenghei	Marine sediments	GQ331167	[26]
	Ca. Scalindua richardsii	Seawater	DQ368233	[27]
	Ca. Scalindua rubra	Brine in the Red Sea	MAYW01000056	[28]
	Ca. Scalindua flavia	Marine sediments	KP126678	[29]
	Ca. Scalindua pacifica	Marine sediments	JX537310	[30]
Anammoximicrobium	*Ca*. Anammoximicrobium moscowii	Moscow River silt	KC467065	[31]
Jettenia	***Ca*. Jettenia asiatica**	Anammox reactor	DQ301513	[32]
	Ca. Jettenia caeni	Membrane bioreactor	AB057453	[33]
	Ca. Jettenia ecosi	Anaerobic bioreactor	MH220407	[34]
	Ca. Jettenia moscovienalis	Bioreactor	KF720711	[35]

The bolded species in the table are AnAOB included in *Bergey's Manual of Systematic Bacteriology*, second edition, volume four.

本课题组前期研究表明，在自然生态系统中，AnAOB 广泛分布于陆地土壤(表层)、淡水淤泥(浅层)和油田(深层)等生境，也广泛存在于海洋深层缺氧海水和底部沉积物。在人工生态系统中，AnAOB 也在人工湿地、污水处理池、厌氧氨氧化反应器等生境内广泛发现[1]。由于生境异质性，陆地生境中的 AnAOB 的多样性较高[36]。陆地生境中优势 AnAOB 多为 *Candidatus* Brocadia 和 *Candidatus* Kuenenia 属[1]。海洋生境中的 AnAOB 多样性较低，多为 *Candidatus* Scalindua 属，这可能与海水的高盐环境有关。相比于自然生态系统，人工生态系统的基质种类相对稳定且浓度较高，因此 AnAOB 种类也相对单一，富集程度更高。值得一提的是，相比于寡营养生境中的 AnAOB，人工生态系统中的 AnAOB 更易积聚内含物——糖原颗粒。

2　厌氨氧化菌的糖原颗粒

糖原是一种葡萄糖多聚物，是原核生物细胞内一种重要储能物质[37-39]。糖原由葡萄糖残基通过 α-1,4-糖苷键连接而成，支链则通过 α-1,6-糖苷键引入。生物源糖原具有多态性，链长不一[40]，支链位置多样，因此具有相同质量的分子亦可能具有不同化学结构。

Goldsmith 等[41]提出糖原分层模型，将组成糖原分子的链分为内 B 链和外 A 链，内 B 链一般包含 2 个支链，而外 A 链则没有分支(图 1)。基于该模型，在一个典型的糖原颗粒中，每个 B 链含有 2 个分支点，最多拥有 12 个同心层[41-42]。换言之，理想(完整)的糖原由 12 层葡萄糖残基组成，葡萄糖残基总数约为 55 000 个，分子量约为 10^7 kDa，直径约为 42 nm。

糖原颗粒无法采用光学显微镜观察，采用化学染色也只能显示一般组织的糖原颗粒聚集体。糖原颗粒的结构可分为 3 个层次：短链寡聚物(长度约为 3 nm)、β 颗粒(直径约为 10–30 nm)和 α 颗粒(直径约 100–300 nm)[6]。短链寡聚物通过分支聚合，形成更大的 β 颗粒，β 颗粒相互黏附，形成玫瑰花状的 α 颗粒(图 2)，但其聚合机制尚不明确[43]。长期以来，研究认为原核

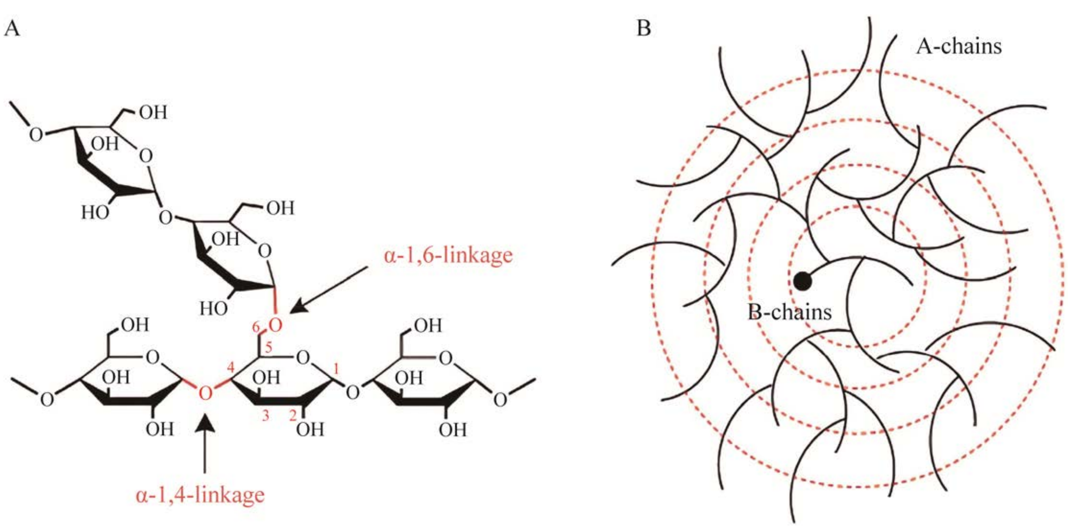

图 1　糖原结构模式示意图[42]

Figure 1　Glycogen structure[42]. A: Schematic diagram of α-1,4-glycosidic linkages and α-1,6-glycosidic linkages in glycogen. B: The tired model for glycogen, in which inner B chains carry an average of two branches, while the outer A chains are unbranched, the black circle in B denotes glycogenin.

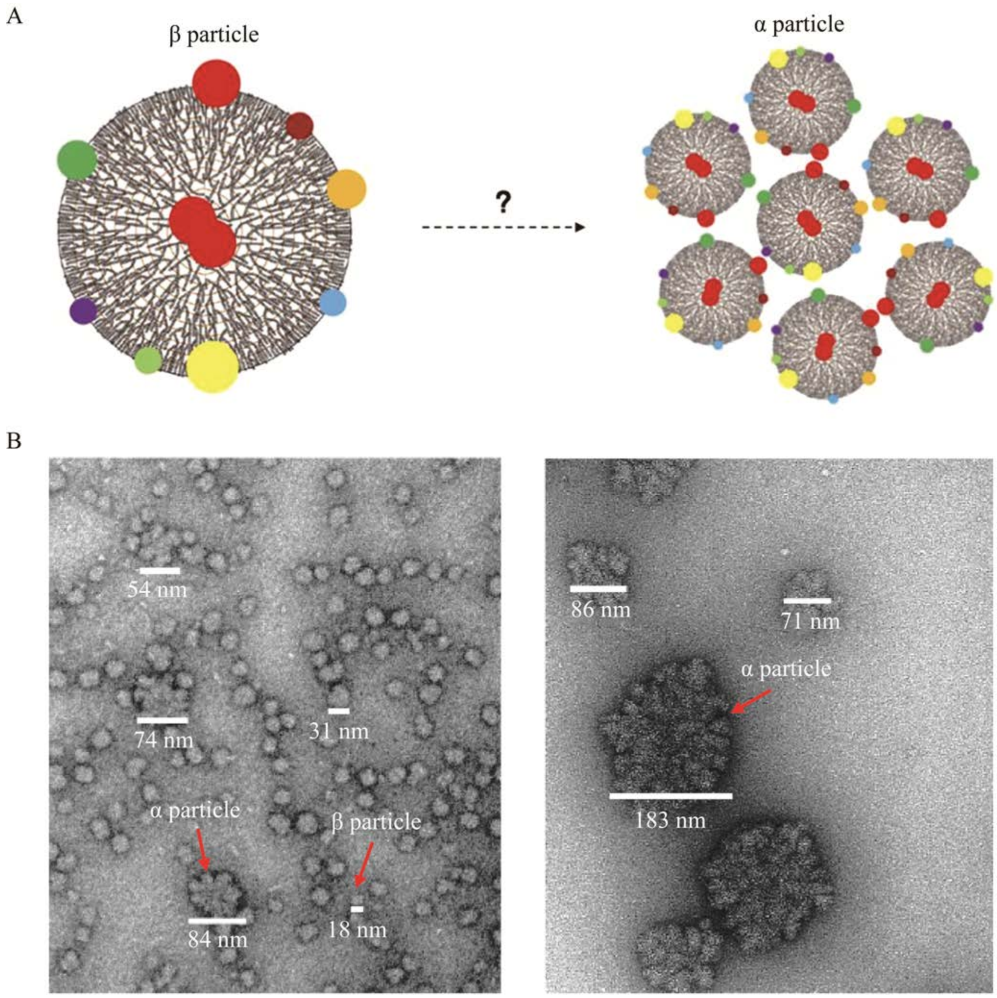

图 2 糖原的 α 与 β 颗粒示意图(A)[6]与大鼠肝糖原的 α 与 β 颗粒透射电子显微镜图(B)[43]

Figure 2 Schematic diagram of α and β glycogen particles (A)[6] and transmission electron microscope images of α and β glycogen particles in rat liver (B)[43].

生物积累糖原的形式只有小 β 颗粒。然后，近年来随着透射电镜和核磁共振分析的应用，在结核杆菌(*Mycobacterium tuberculosis*)和大肠杆菌(*Escherichia coli*)中发现了糖原 α 颗粒[44-45]。据报道，糖原 α 颗粒普遍存在于古菌、细菌和真核生物中，表明其具有进化保守性[46]。

糖原颗粒也已在多个 AnAOB 种属细胞中被观察到，如 *Candidatus* Kuenenia stuttgartiensis、*Candidatus* Brocadia fulgida、*Candidatus* Brocadia sinica、*Candidatus* Anammoxoglobus propionicus 和 *Candidatus* Scalindua spp.等(图 3)[47]。一般而言，AnAOB 细胞分隔成厌氧氨氧化体(anammoxosome)、核糖细胞质(riboplasm)和外室细胞质(paryphoplasm) 3 部分[48]。糖原染色结合透射电镜观察表明，糖原颗粒分布于胞浆内膜(intracytoplasmic membrane)和厌氧氨氧化体膜(anammoxosome membrane)之间的核糖细胞质(riboplasm)内。其数量从几颗到几百颗不等，与外界基质浓度呈正相关。糖原颗粒较少时，其在核糖细胞质中呈局域分布；数量较多时，

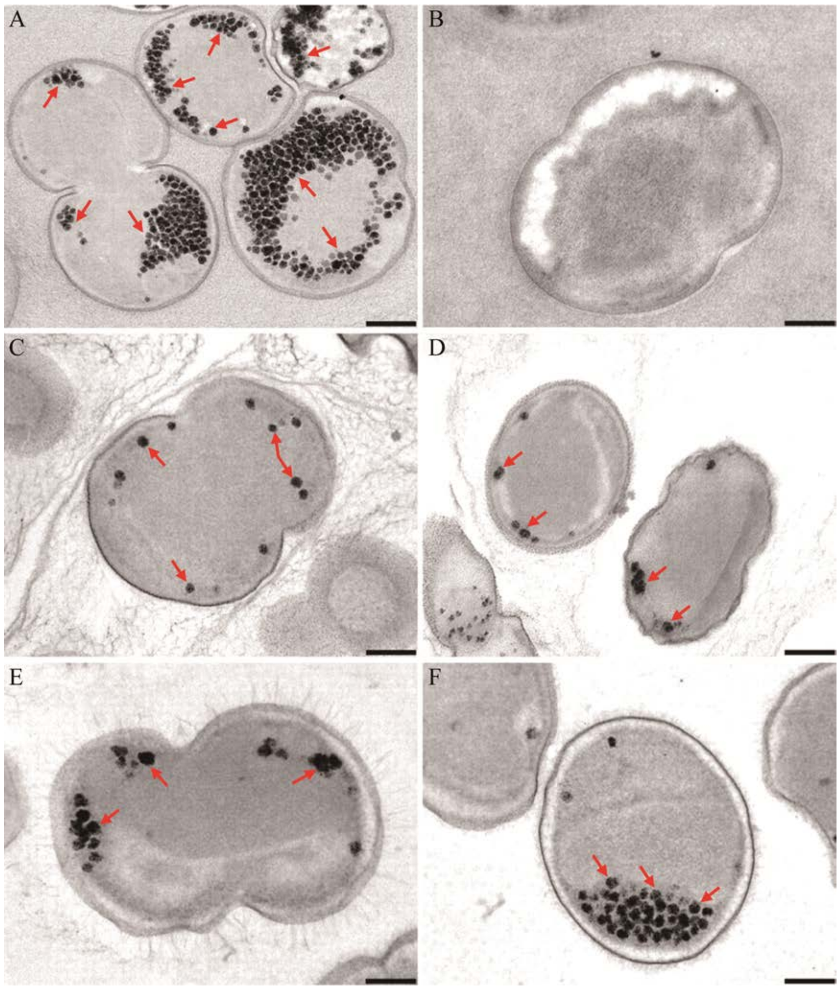

图 3　厌氧氨氧化菌胞内糖原颗粒染色电镜图[47]

Figure 3　Transmission electron microscopy images of glycogen staining in partial anaerobic ammonia oxidation bacteria[47]. A and B: Positive and negative control of glycogen staining in *Candidatus* Kuenenia stuttgartiensis. C–F: Glycogen staining images of *Candidatus* Anammoxoglobus propionicus, *Candidatus* Brocadia fulgida, *Candidatus* Scalindua spp. with and without pilus-like appendages, the scale bar represents 200 nm, and red arrows point to the glycogen particles.

呈均匀分布，甚至遍布核糖细胞质。经测量，AnAOB 中的糖原颗粒平均粒径约为 20 nm，据此推测为 β 颗粒。颗粒较多时，β 颗粒并未进一步聚集成更大的 α 颗粒。

3　厌氧氨氧化菌的糖原代谢

AnAOB 中的糖原颗粒并非永久性结构，而是处于生成与消耗的动态变化过程中。本课题组在 AnAOB 长期低温保藏中的研究发现，保藏初期，反应器中培育的营养态 AnAOB 中糖原颗粒含量丰富，遍布于核糖细胞质中，保藏第 60–150 天糖原颗粒逐渐消耗，保藏至 210 d，AnAOB 中糖原颗粒几乎耗尽(图 4)。当生境中能源和碳源基质充沛时，AnAOB 通过还原性乙酰辅酶 a 途径同化二氧化碳，并将过量合成的有机物质转化成糖原积累于核糖细胞质中；生

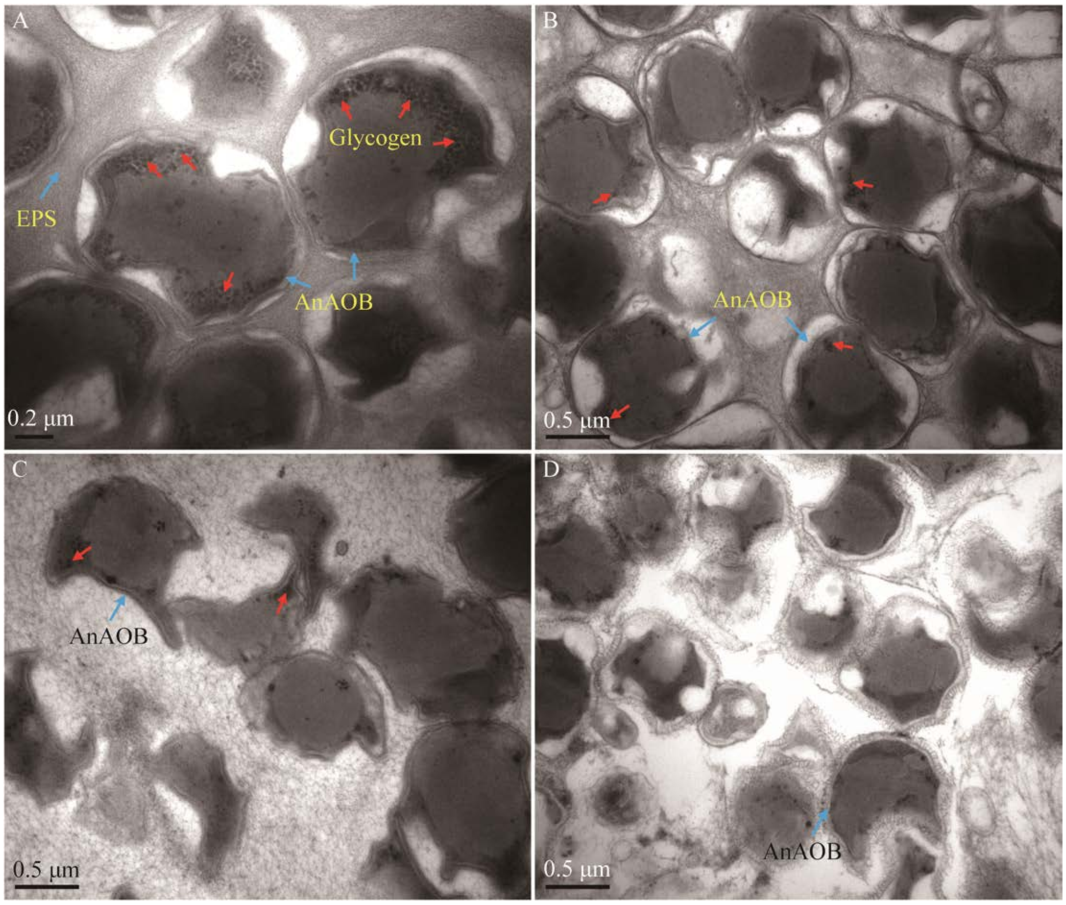

图 4 *Candidatus* Kuenenia stuttgartiensis 在 4 ℃长期低温保藏过程中的糖原消耗过程

Figure 4 The process of glycogen consumption during the long-term cryopreservation of *Candidatus* Kuenenia stuttgartiensis (A–D corresponds to day 0, 60, 150, and 210 during preservation and red arrows point to the glycogen particles).

境中能源基质不足时，糖原被降解，为细胞提供生存所需的能量。对真核生物糖原代谢的研究表明，糖原合成由糖原蛋白(glycogenin, GYG)引发。GYG 是一种自催化酶，其产生短链寡聚物和 β 颗粒包括以下步骤：(1) 将 UDP-葡萄糖中的葡萄糖添加到酪氨酸-194 上；(2) 添加约 7 个葡萄糖残基形成麦芽寡糖链作为引物；(3) 糖原合成酶通过 α-1,4-糖苷键连接线性寡糖链进行延伸；(4) 通过糖原分支酶连接 α-1,6-糖苷键进一步分支；(5) 重复 1–4 过程。在原核生物中，除了初始阶段不需要糖原蛋白外，其余步骤与上述真核生物的糖原合成过程类似[49]。糖原分解则可从多个位点开始，在糖原磷酸化酶和脱支酶的作用下分解成葡萄糖 1-磷酸。

关于 AnAOB 的糖原代谢，根据 *Candidatus* Brocadia sinica 基因组测定和分析，初步确定了中心碳代谢途径的基本基因，其中包括糖原合成(GlgC-GlgA 途径)和分解(GlgX-GlgP 途径)等相关基因[5]。电子显微镜观察证实，在 AnAOB 生长期、近零生长期以及饥饿期，其胞内均伴有糖原颗粒的积累和消耗(图 5)。

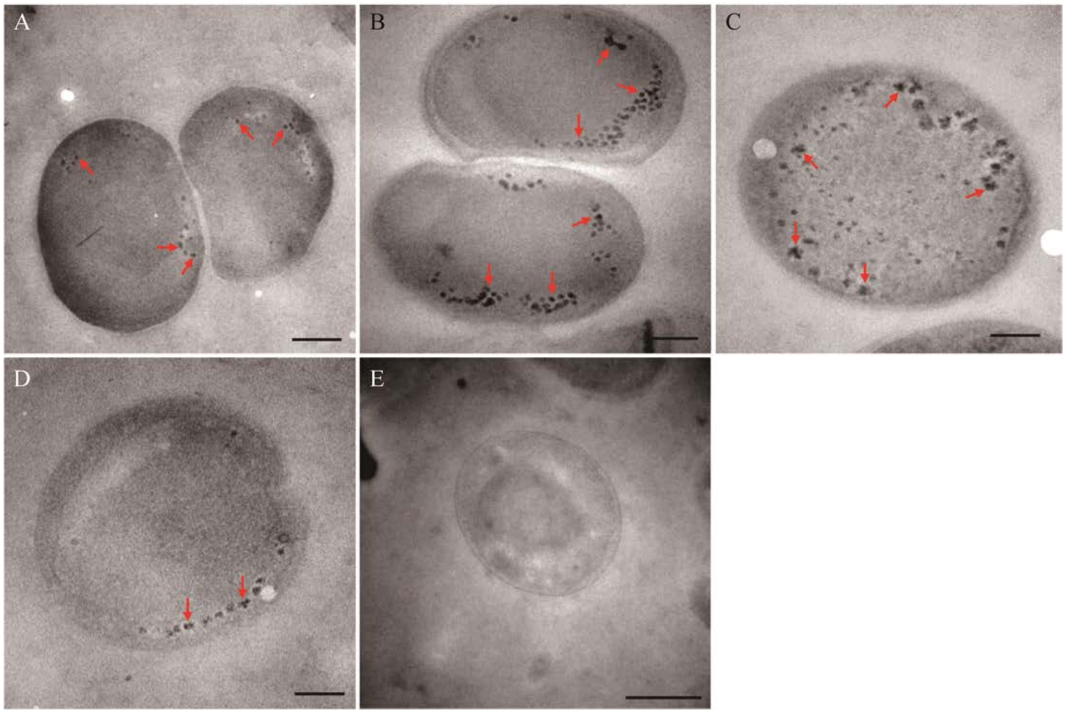

图 5 *Candidatus* Brocadia sinica 在培养过程中胞质室内糖原的积累与消耗[5]

Figure 5 Accumulation and consumption of glycogen in the cytoplasmic compartment of *Candidatus* Brocadia sinica during cultivation[5]. A: Growth phase. B and C: Near-zero growth phase. D: Starvation phase. E: Negative control incubated with water, scale bars in A–D are 200 nm and in E is 500 nm, and red arrows point to the glycogen particles.

基于基因组和蛋白质组的进一步分析，研究推断 *Candidatus* Brocadia sinica 具有 3 条糖原合成途径：GlgC-GlgA 途径、GlgE 途径和 Rv3032 途径[5]，如图 6A 所示。

在 GlgC-GlgA 途径中，首先，葡萄糖 1-磷酸经葡萄糖-1-磷酸腺苷转移酶(GlgC)催化，生成腺苷二磷酸葡萄糖(ADP-葡萄糖)；接着，糖原合成酶(GlgA)将 ADP-葡萄糖聚合成线性 α-1,4 葡聚糖；最后，α-1,4 葡聚糖支链酶(GlgB)介导 α-1,6-葡萄糖苷键的形成，将线性葡聚糖转化为糖原。在 GlgE 途径中，首先，海藻糖由 α-D-葡萄糖转移酶/α-淀粉酶(TreS)催化生成麦芽糖；接着，麦芽糖经麦芽激酶(Pep2)催化生成麦芽糖 1-磷酸，再经麦芽糖转移酶(GlgE)催化生成线性 α-1,4 葡聚糖；最后，线性葡聚糖经 α-1,4 葡聚糖支链酶(GlgB)催化生成糖原。在 Rv3032 途径中，尿苷二磷酸葡萄糖(UDP-葡萄糖)经 Rv3032 酶(具有 α-1,4 葡聚糖转移酶活性)催化生成线性 α-1,4 葡聚糖，再由分支酶 Rv3031 催化形成分支(α-1,6-葡萄糖苷键)，将线性葡聚糖转化为糖原。

关于糖原的分解，高度保守的糖原磷酸化酶(GlgP)可将糖原降解为葡萄糖 1-磷酸，这是重要的糖基重组中间体。由于 GlgP 只能切割 α-1,4-糖苷键将糖原降解成含有 4 个葡萄糖残基的寡糖，因此需要糖原分枝酶(GlgX)和 α-1,4-葡聚糖转移酶(MalQ)协助糖原的进一步分解，以便通过葡萄糖基转移酶(从 4 个葡萄糖残基的糖原支链上转移 3 个葡萄糖残基)和葡萄糖苷酶(断裂 α-1,6-糖苷键)催化降解，生成葡萄糖和 α-1,4-葡

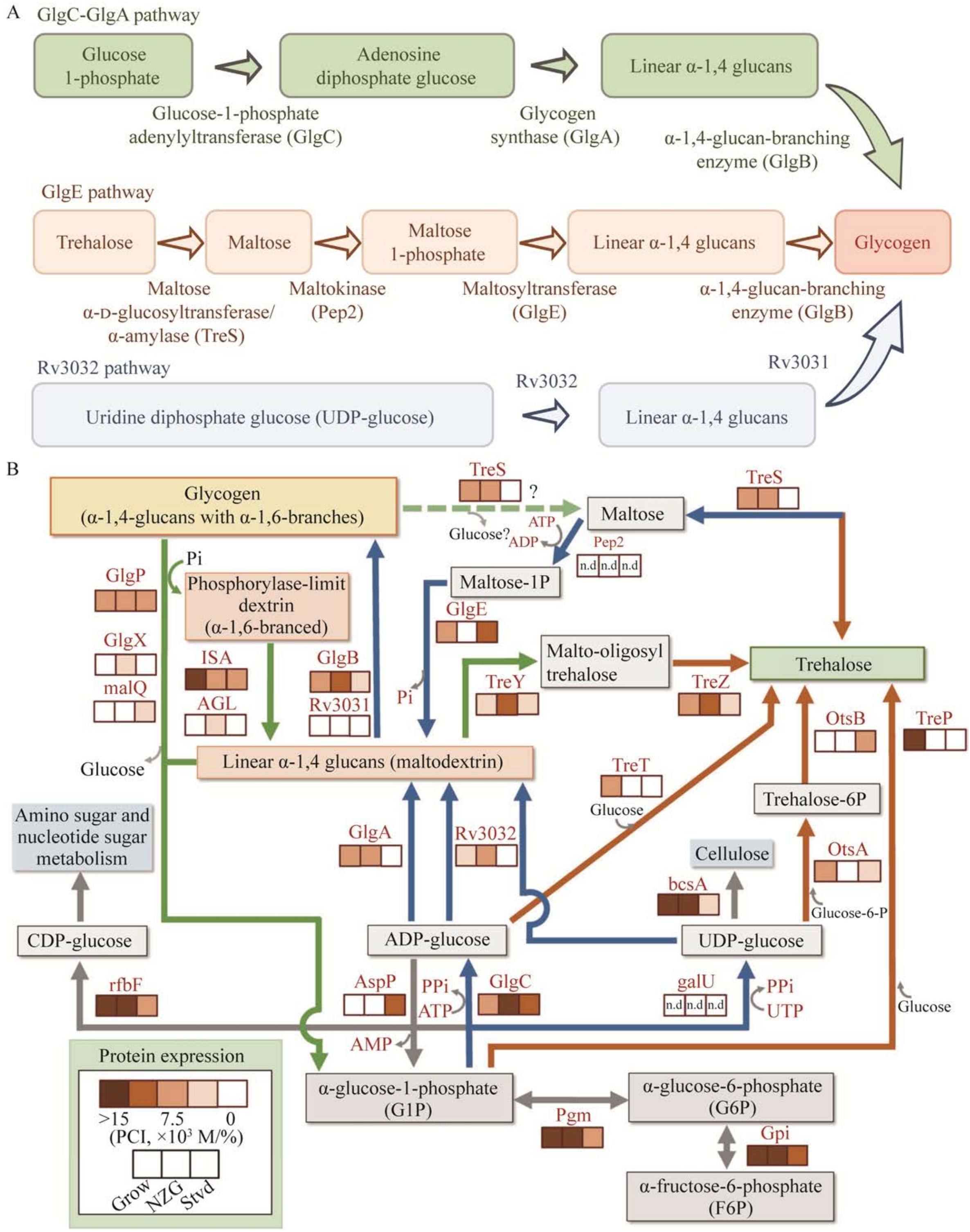

图 6　基于基因组和蛋白质组学分析的“*Candidatus* Brocadia sinica”中糖原代谢途径示意图(A)及相关酶的相对丰度(B)[5]

Figure 6　Schematic diagram of glycogen metabolism pathway (A) and relative abundance of glycogen metabolic enzymes in *Candidatus* Brocadia sinica (B) based on genomic and proteomic analysis[5].

聚糖线性链。此外，已检测到糖原分枝酶/异淀粉酶(isoamylase，ISA, EC: 3.2.1.68)，ISA 只有 α-1,6-葡糖苷酶活性，产物为麦芽糊精和麦芽糖。ISA 与古菌中的糖原分支酶 TreX 类似(同源性为 74%)，推测糖原也可以通过(ISA)-TreY-TreZ 或 ISA-TreS 的另一途径降解为海藻糖，而后者可用于重组葡聚糖。

4 厌氧氨氧化菌糖原颗粒功能分析

如上分析，糖原颗粒在 AnAOB 已被广泛观察到。对 AnAOB 的基因组和蛋白质组分析也表明，AnAOB 中存在糖原合成与分解的编码基因和相关酶。在异养型细菌中，糖原既是能源物质，也是碳源物质[50]。然而，AnAOB 是自养型细菌。一般认为，AnAOB 的能源物质是铵和亚硝酸，碳源物质是二氧化碳和水溶性碳酸盐[51-52]。对于其他细菌类群，生境中能源物质充裕时可在细胞内积累聚 β-羟基丁酸、聚磷酸盐、硫粒等储能物质[53-54]。对于 AnAOB，糖原颗粒的功能究竟是什么，笔者推测如下。

(1) 提供能量。在自然界，AnAOB 的基质(铵和亚硝酸)丰度不高，在长期进化中，AnAOB 形成了适应自然生境的代谢系统[55-57]。在培养或人工生境中，铵和亚硝酸丰度大幅提高，使 AnAOB 代谢系统偏离了原生的代谢平衡状态，多余的能量以糖原的形式储存于细胞中。

(2) 提供碳架。AnAOB 生长和繁殖需要细胞构建材料，其中碳素占细胞干重 50%以上。AnAOB 是自养型细菌。由二氧化碳和水溶性碳酸盐合成有机物质不仅需要消耗大量 ATP，也需要消耗大量还原力(NADH/NADPH)[2]，有机物质得来不易。作为细胞构建原料，糖原可高效节能地转化为各种细胞碳架，满足 AnAOB 生长和繁殖所需。

(3) 提供应激保护。糖原呈中等氧化还原状态，具有多功能性[6]。能量不足时，可作为能源物质补充能量。碳架不足时，可转化为碳架补充细胞构建材料。遇强氧化剂(如氧气)时，可产生抗氧化剂。遇强还原剂(如硫化氢)时，则可产生抗还原剂。在逆境中，糖原可助力 AnAOB 抵抗和渡过生境胁迫。

5 小结与展望

AnAOB 是分类学新建的细菌类群。由于生长缓慢，培养困难，迄今未获纯培养物。然而，AnAOB 具有诸多细胞结构和功能特异性，可丰富微生物学知识宝库和作为资源开发。AnAOB 中糖原代谢的存在，使其能够积聚来之不易的有机物质-糖原颗粒，抵抗逆境条件。关于 AnAOB 的糖原颗粒仍有许多方面值得深入研究，包括：(1) AnAOB 是自养菌，但其细胞内可积累有机物质——糖原颗粒。AnAOB 中糖原颗粒成因与去路值得深入研究；(2) AnAOB 可储存糖原颗粒，但其“仓贮”方式依然不明，AnAOB 中糖原颗粒的组成、结构和分布值得深入观察；(3) AnAOB 可形成和消耗糖原颗粒，但未知其是正常生理还是应激生理，AnAOB 中糖原颗粒形成和消耗的触发条件值得深入探索。

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