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Review 综 述

乳杆菌胞外多糖合成基因簇及构效关系

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摘　要：肠道菌群稳态对维护人体的健康具有至关重要的作用。作为肠道天然微生物群“守卫兵”，益生菌参与改善体内微生态平衡。乳杆菌(*Lactobacillus*)是一种肠道益生菌的代表，其合成的胞外多糖(exopolysaccharide, EPS)既可通过增益肠道内其他益生菌的生长来优化肠道微生态，还具有抗肿瘤、抗氧化、降胆固醇、降血压和增强机体免疫力等益生功能。本文对近年来乳杆菌胞外多糖的遗传、生物学活性、构效关系等方面的研究进展，进行综述和展望。

关键词：乳杆菌；胞外多糖；基因簇；多糖结构；构效关系

Lactobacillus exopolysaccharide: gene clusters for synthesis and structure-activity relationship

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Abstract: Intestinal microflora homeostasis plays a crucial role in maintaining human health. As a guard of the natural intestinal microflora, probiotics improve the microecological balance

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in the host. *Lactobacillus* is a representative of intestinal probiotics. The exopolysaccharide (EPS) synthesized by *Lactobacillus* can optimize the intestinal microecology by promoting the growth of other probiotics in the intestine. Moreover, EPS is praised for anti-tumor, antioxidant, cholesterol-lowering, blood pressure-lowering, and immunity-enhancing activities. This article reviews the research progress in the genetics, biological activities, structure-activity relationship of *Lactobacillus* EPS in recent years.

Keywords: *Lactobacillus*; exopolysaccharide; gene cluster; polysaccharide structure; structure-activity relationship

人类的肠道中含有高度复杂的微生态系统——肠道菌群[1-2]，该微生态系统的稳态对维护人体健康具有极为关键的作用[3-4]。肠道菌群的调控已逐渐成为维护人体健康和预防治疗疾病的新方法[5]。益生菌是肠道菌群的重要组成部分，参与改善人体肠道微生态。作为具有代表性的一类肠道益生菌，肠道乳酸菌分布在多个属，最著名的属是乳酸杆菌、明串珠菌、趾球菌属、乳球菌属和链球菌属[6]。乳杆菌属(*Lactobacillus*)是乳酸菌中种数最多的属，有100 多种，并且乳杆菌属的许多菌株表现出益生菌特性，在全球食品工业益生菌产品中应用广泛[7]，并在发酵食品的生产中发挥重要作用，包括日用乳制品如酸奶、发酵蔬菜等生产过程。乳杆菌对人类的胃肠道系统具有健康益处，它们不仅能产生乳酸、芳香族化合物、过氧化氢、细菌素等，还能产生胞外多糖(exopolysaccharide, EPS)。EPS 是一种在微生物生长过程中合成并分泌到细胞外的生物聚合物，其分支程度不同，从线性分子到高支化分子不等[8]。目前乳杆菌胞外多糖的开发存在效率低、纯化困难、安全稳定性差等问题，胞外多糖的结构与功能的对应关系不甚明确；对乳杆菌胞外多糖的结构、合成过程及生理活性进行概述，可以为解决该问题提供借鉴依据。本文总结展望近年来乳杆菌合成胞外多糖的遗传、生物学活性和构效关系等方面的研究进展。

1 乳杆菌胞外多糖生物合成相关酶和基因簇

1.1 乳杆菌胞外多糖的合成与释放

依据单糖组成不同，乳杆菌胞外多糖可分为均多糖(homopolysaccharides, HoPS)和杂多糖(heteropolysaccharides, HePS)。乳杆菌在胞外合成 HoPS[8]，包括聚合和释放两个过程。在聚合过程中，果糖酶[9]和葡萄糖蔗糖酶[10]等相关酶将特定底物(如蔗糖)中的单糖转移到生长的多糖链上；聚合后的 HoPS 链被释放到细胞外环境中[11-12]。与 HoPS 相比，HePS 结构更多样，生物合成过程比较复杂。微生物通常通过 4 种途径(胞外合成途径、ABC 转运体依赖途径、合酶依赖途径和 Wzx/Wzy 依赖途径[13])来合成胞外多糖。乳球菌属、乳酸杆菌属和链球菌合成 HePS 的过程多属 Wzx/Wzy 依赖途径[14]。Wzx、Wzy 为 HePS 合成及释放过程中发挥关键作用的两种酶，Wzx 翻转酶将多糖重复单元易位至周质空间或外膜；Wzy 外膜聚合蛋白用于重复单元聚合，调节多糖链长。整个 Wzx/Wzy 依赖性途径可分为 2 个步骤，细胞质中的中心碳代谢产生活性糖前体(糖核苷酸)，多糖的组装与聚合。Wzx/Wzy 依赖性途径合成多糖是一个复杂的细胞内过程，该途径最初发现于革兰氏阴性菌脂多糖(lipopolysaccharide, LPS)的 O-抗原多糖的合成过程[15]，后在革兰氏阴性菌和革兰氏阳性菌的胞外多糖及荚膜多糖

的合成过程中也发现了 Wzx/Wzy 依赖性途径[13]。该合成途径(图 1)大致分为 5 步：(1) 单糖/双糖的转运及磷酸化；(2) 单糖活化——糖核苷酸形成；(3) 重复单元合成；(4) 重复单元由细胞膜内表面转运至外表面；(5) 重复单元聚合，长链释放。

1.2 乳杆菌胞外多糖生物合成基因簇

乳杆菌胞外多糖由 EPS 基因簇调控合成,该基因簇通常位于染色质或质粒上[16]。本实验室曾在综述中指出[17]乳酸乳球菌(*Lactococcus lactis*)和干酪乳酸菌(*Lactobacillus casei*)等 EPS 基因簇通常位于质粒上，而嗜热链球菌(*Stretococcus thermophilus*) Sfi6 的 EPS 基因簇则位于染色体上。HePS 中 Wzx/Wzy 依赖途径前两步涉及的酶通常处在 EPS 基因簇以外。值得注意的是,同一物种的不同菌株可能具有不同的 EPS 基因簇，因而具有不同的 EPS 特征[18]。一般来说，EPS 基因簇组织在一个 11−22 kb 的操纵子中[19]，由 13−23 个特定的功能区组成。但也在部分菌株中发现了多个EPS合成基因簇[20]。已有报道的乳杆菌胞外多糖合成基因簇中,必要基因包括转录调控因子、糖基转移酶(glycosyltransferase, GT)、多糖聚合酶(*wzy*)和翻转酶(*wzx*)等。其他基因如 LytR 转录调节基因(*epsA*)、磷酸酪氨酸磷酸酶基因(*epsD*)等常作为 EPS 合成基因簇的一部分，参与 EPS 的前体合成和化学装饰。EPS 的组成差异多源于糖基转移酶家族的数量和组成不同，见图 1，列举了目前已知的部分乳杆菌胞外多糖合成基因簇[20-28]。

2 乳杆菌胞外多糖的结构和组成

乳杆菌胞外多糖的结构可分为一级结构和高级结构。多糖的一级结构是指糖基的组成、排列顺序、相邻糖基的连接方式、糖链有无分支和分支的位置与长短等。糖基上可连接一些官能团如磷酸基团、硫酸基团、甲基化基团，这使得多糖的一级结构更为复杂。多糖的高级结构是在一级结构的基础上,各侧链通过非共价键相互作用结合而形成复杂结构[29]，可以理解为分子尺寸。分子生物学及物理化学新技术手段的涌现，为乳杆菌胞外多糖的结构解析提供了新的研究方法[30]。例如一级结构的解析除了常规的化学分析方法，还可利用凝胶柱层析、排阻色谱、气相色谱-质谱联用(gas chromatograph-mass spectrometer, GC-MS)、傅里叶变换红外光谱(Fourier transform infrared spectroscopy, FT-IR)以及核磁共振(nuclear magnetic resonance, NMR)等[31]。高级结构分析的常用方法包括 X 射线衍射法(X-ray diffraction, XRD)、原子力显微镜法(atomic force microscopy, AFM)与基于高分子稀溶液理论的联用技术、圆二色谱法(circular dichromatography, CD)等[32]。

乳杆菌 HoPS 通常只有糖苷键类型和分子聚合物大小的差异,这归因于细胞外合成依赖途径中合成基因编码的酶的唯一性。根据糖基类型、连接组成和碳键位置，HoPS 可分为几种类型，包括 D-α-葡聚糖、D-β-葡聚糖、果聚糖和半乳聚糖等[33-34]。乳杆菌 HoPS 分子量约在 4.0×10^4−6.0×10^6 之间[35]。HoPS 主要的聚糖重复单元结构见表 1 及图 2[36-49]，包括由葡萄糖、半乳糖及果糖组成的糖链结构及键型。

乳杆菌 HePS 基因簇的差异性决定了 HePS 生物合成步骤的复杂性[47]。HePS 生物合成位置以及 Wzx/Wzy 依赖途径中底物与酶的多样性，是造成 HePS 结构多样性的主要原因[38]。一方面,糖链长度与HePS的分子量和分支程度有关。另一方面,重复单元是由多种糖基转移酶调控合成的[48], 因此 HePS 聚合物具有从分子组成到框架键型的巨大结构多样性。中性 EPS 由两种或两种以上不同的中性单糖组成，而酸性 EPS 的组成比中性 EPS 更复杂，还含有一至多个醛酸

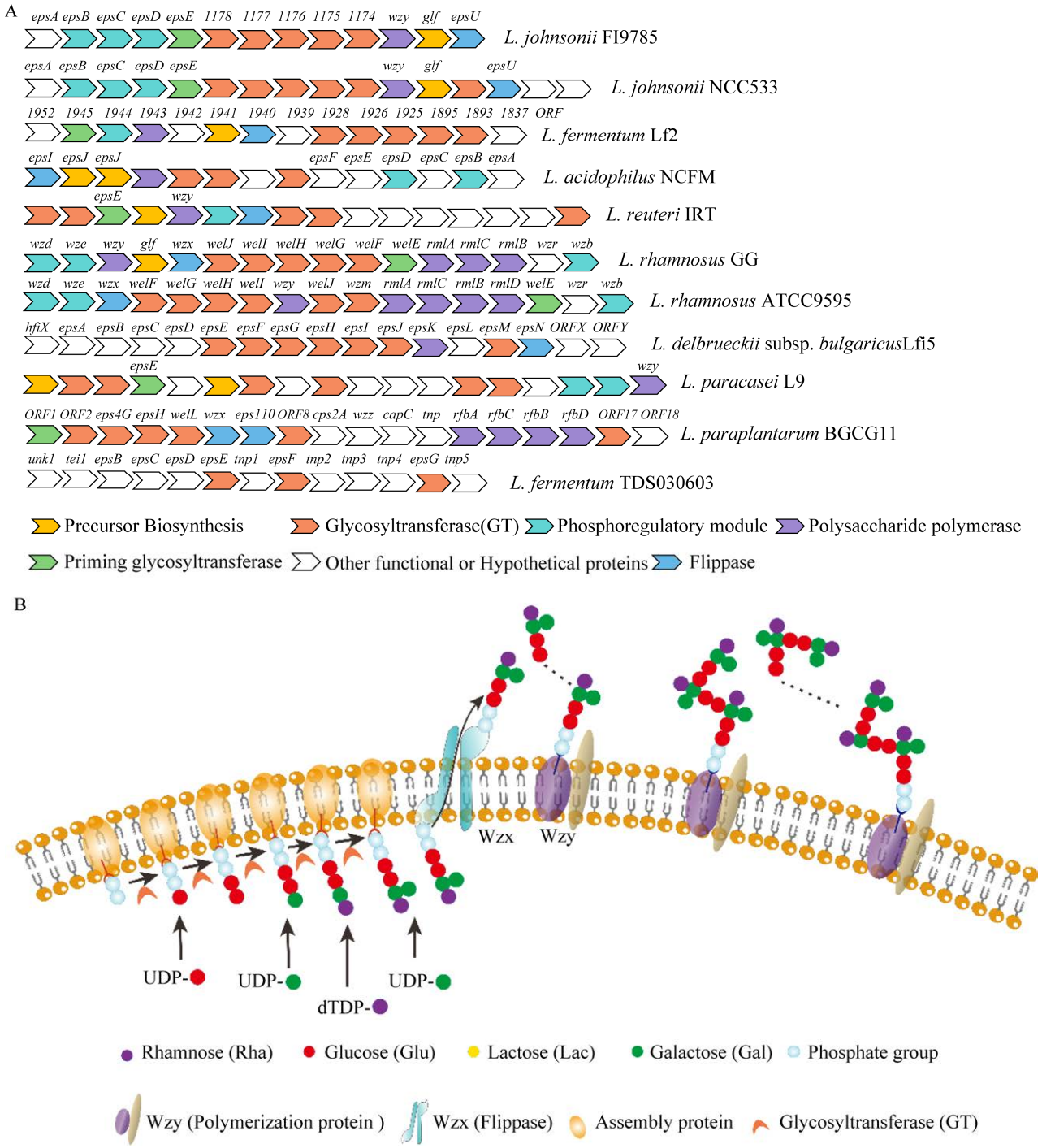


图 1　乳杆菌胞外多糖合成基因簇[20-28]　A：不同功能的基因由不同颜色的箭头进行注释. B：Wzx/Wzy 依赖途径合成胞外多糖过程. 不同的糖底物由不同颜色的圆形表示，Wzx 及 Wzy 由孔道及椭圆表示，糖基转移酶由月牙形表示

Figure 1　*Lactobacillus* extracellular polysaccharide synthesis gene cluster[20-28]. A: Genes with different functions are annotated with arrows of different colors. B: Synthesis of extracellular polysaccharides through the Wzx/Wzy pathway. Different sugar substrates are represented by circles of different colors, Wzx and Wzy are represented by pore and elliptical, while glycosyltransferase is represented by a crescent.

表 1　乳杆菌常见的均多糖聚糖重复单元结构

Table 1　Common repeat unit structures of homopolysaccharide glycans in *Lactobacillus*

EPS	Strain	Molecular weight	Linkage	References
α-D-glucan	*Lactobacillus johnsonii* FI9785	Unknown	α-D-Glcp(1→2)/(1→6)	[36]
	Lactobacillus. *plantarum* CIDCA 8327	Unknown	α-D-Glcp(1→3)/(1→4)	[37]
	Lactobacillus brevis E25	Unknown	α-D-Glcp (1→6)	[38]
	Lactobacillus sakei MN1	1.74×10^8 Da	α-D-Glcp (1→6)	[39]
Glucan	*Lactobacillus diolivorans* G-77	Unknown	α-D-Glcp(1→2)/β-D-Glcp(1→6)	[40]
β-D-glucan	*Lactobacillus suebicus* CUPV221	10^4–10^7 Da	β-D-Glcp(1→3)	[41]
	Lactobacillus brevis TMW 1.2112	Unknown	β-D-Glcp(1→3)/(1→2)	[42]
	Lactobacillus diolivorans G-77, *Lactobacillus ethanolidurans* CUPV141	Unknown	β-D-Glcp(1→2)/(1→3)	[40]
Fructan	*Lactobacillus reuteri* strain 121	1.5×10^5 & 2×10^6 Da	β-D-Fruf(2→6)	[43]
	Lactobacillus. *reuteri* strain 121	$>10^7$ Da	β-D-Fruf(2→1)	[44]
	Lactobacillus reuteri LTH 5448, *Lactobacillus sanfranciscensis* LTH 2590	Unknown	β-D-Fruf(1→6)	[45]
Mannan	*Lactobacillus crispatus* L1	Unknown	α-D-Manp-(1→2)/(1→6)/(1→3)	[36]
Galactan	*Lactobacillus mucosae* VG1	Unknown	α-D-Gal(1→6)/β-D-Gal(1→3)/(1→6)	[46]

单糖。此外，磺化和磷酸化等酸性取代基也会增加 HePS 的酸度，而乙酰基等中性取代基则不会影响到酸碱度的变化。HePS 最常见的单糖组成为葡萄糖(Glc)和半乳糖(Gal)，多为 β 型糖苷键。图 3 列举了由最常见的 2 种单糖——葡萄糖和半乳糖组成的聚糖重复单元结构[49]。另外，3 种及 3 种以上单糖组成的聚糖重复单元部分结构见图 4[49]，主要有岩藻糖(fucose, Fuc)、甘露糖(mannose, Man)、N-乙酰半乳糖胺(N-acetylgalactosamine, GalNAc)、葡糖胺(glucosamine, GlcN)等作为聚合物的组分。

3　乳杆菌胞外多糖构效关系

共生肠道微生物群对宿主的代谢和防御系统都有影响[50-51]。益生元如低聚半乳糖(galacto-oligosaccharides, GOS)[52-53]被广泛用于调节肠道菌群功能和丰度。乳杆菌胞外多糖的特异性免疫和非特异性免疫反应通路已经有多个实验验证[54]。几种宿主传感器[55]及其相关的信号通路[56]也已得到阐明。对乳杆菌益生菌活性的效应机制可以聚焦于肠-X 轴，例如有研究者称来自植物乳杆菌 HY7714 的胞外多糖通过皮肤-肠道轴通讯防止皮肤老化[57]。用 EPS 治疗癌症细胞系，是通过抑制相应基因的表达，导致核转录因子-κB (nuclear factor-kappa B, NF-κB)下调或失活[58]。简述了部分肠道微生态中乳杆菌免疫调节及修复肠道屏障的信号通路，乳杆菌多糖可以通过酶促和非酶促抗氧化系统的联合作用防止活性氧(reactive oxygen species, ROS)的代谢产物对肠上皮的损伤；还可以抑制促炎因子的表达，如 IL-1β、IL-6 和 TNF-α，这些因子是在激活树突状细胞和巨噬细胞的过程中产生的，以响应共生微生物群和 TLR 信号转导。乳杆菌胞外多糖具有抗癌和抗氧化活性[59]，具有抑菌、降低胆固醇、抑制 α-淀粉酶和癌细胞系的特性[60]。乳杆菌胞外多糖的抗癌活性与其抗增殖和促凋亡特性有关[61]，例如刺激免疫细胞(主要是 T 和 B 淋巴细胞、巨噬细胞和 NK 细胞)释放白细胞介素(图 5)[62]。EPS 在各种癌症细胞系中具有剂量和时间依赖性的抗增殖作用。这些性质与其单

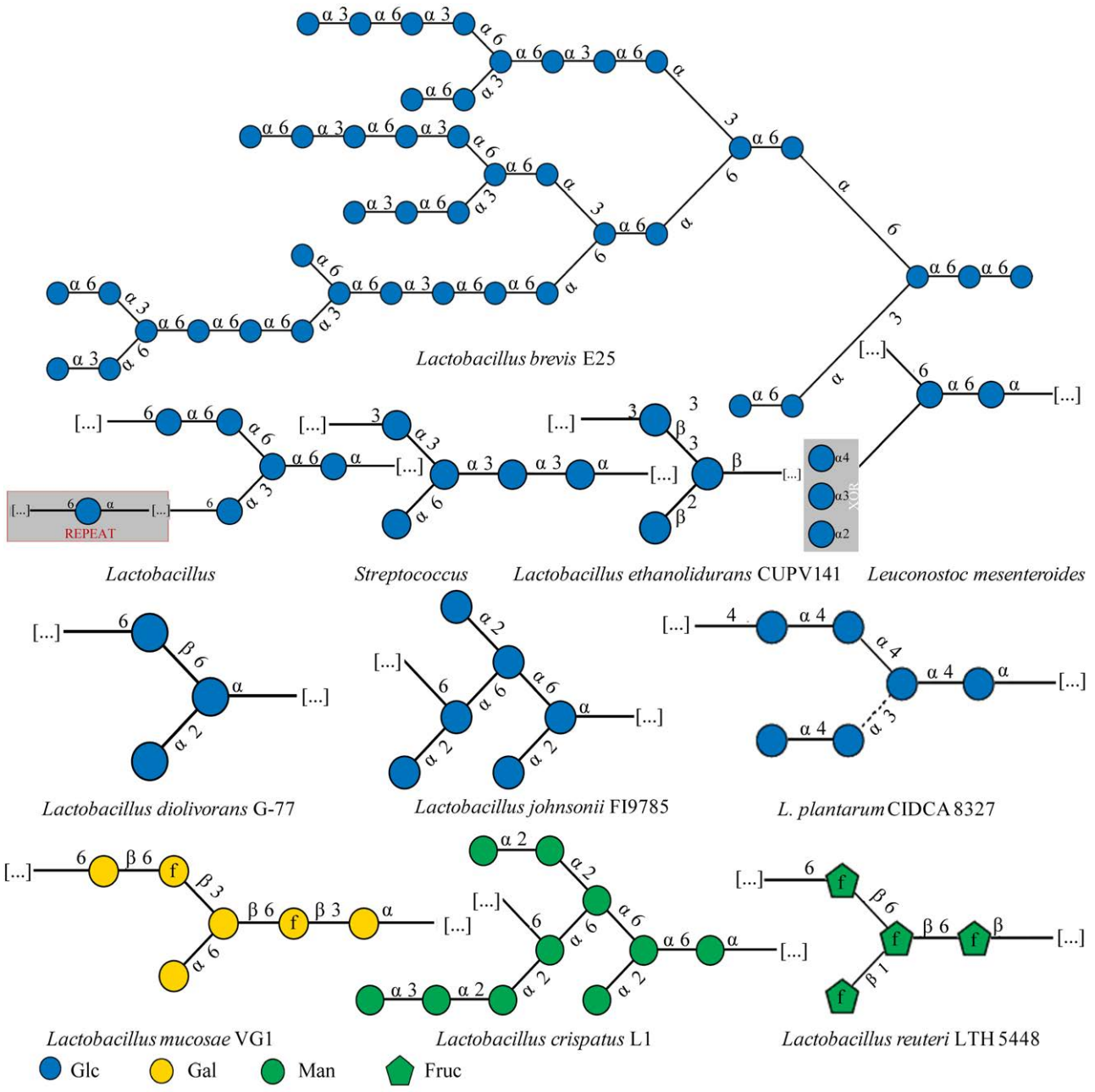


图 2　乳杆菌中常见的均多糖聚糖重复单元结构[49]

Figure 2　Common homoglycan repeat unit structures in *Lactobacillus*[49]. Blue circle represents glucose, yellow circle represents galactose, green circle represents mannose, and green pentagon represents fructose. The shaded portion is a repeat of the partial structure in the glycan unit structure.

糖组成、分子量、官能团、聚合主链和侧链结构以及分支点数量有关[63-66]，下面将综合论述。

3.1　乳杆菌胞外多糖主链糖苷键构效关系

在种类繁多的乳杆菌中，胞外多糖糖苷键对其活性影响较大。如乳杆菌胞外多糖中的 1,4-半乳糖或 1,4-葡萄糖在生物活性中发挥着关键作用。部分研究成果表明，主链结构中含有 1,4-半乳糖或 1,4-葡萄糖的胞外多糖具有更强的抗

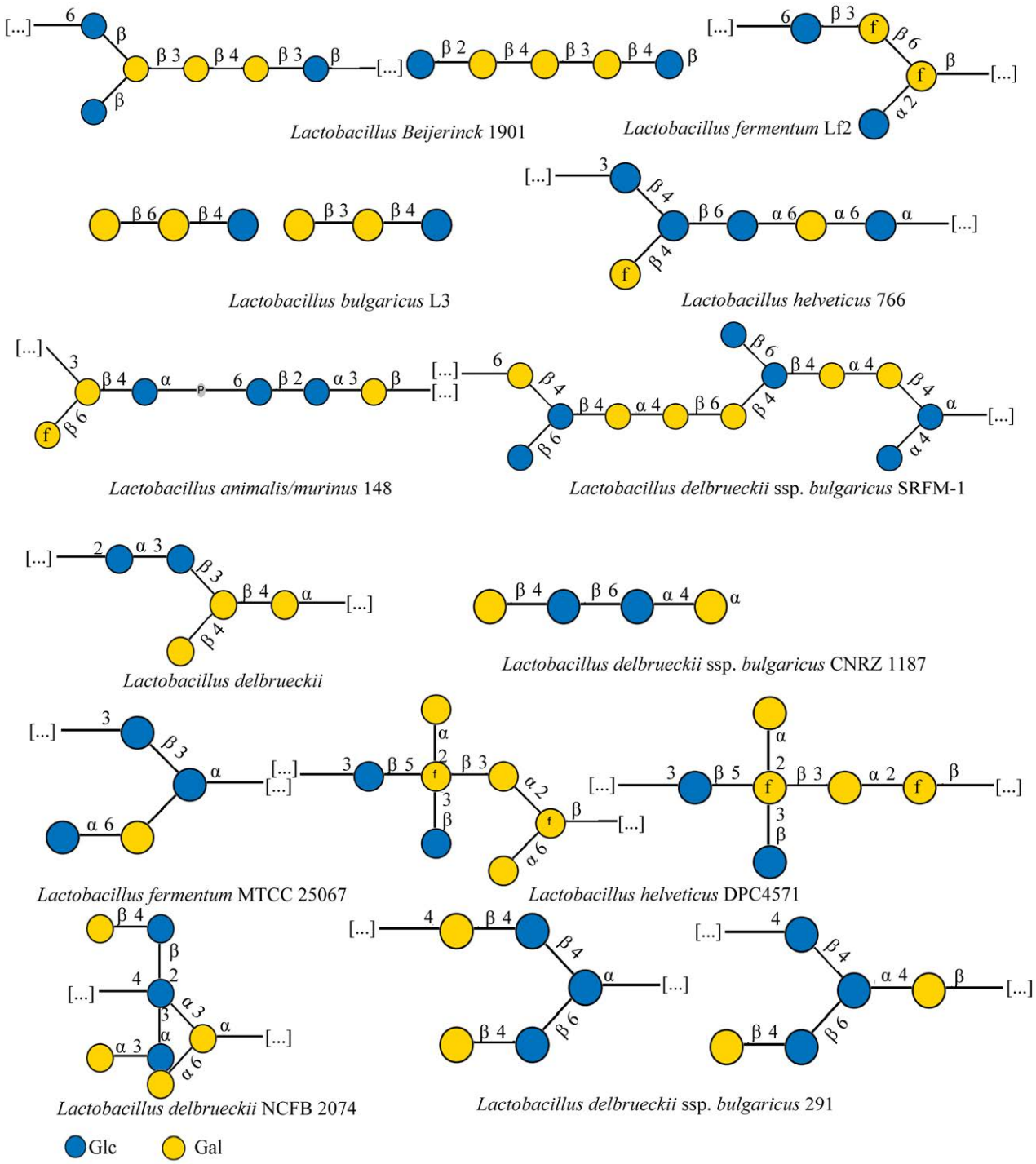
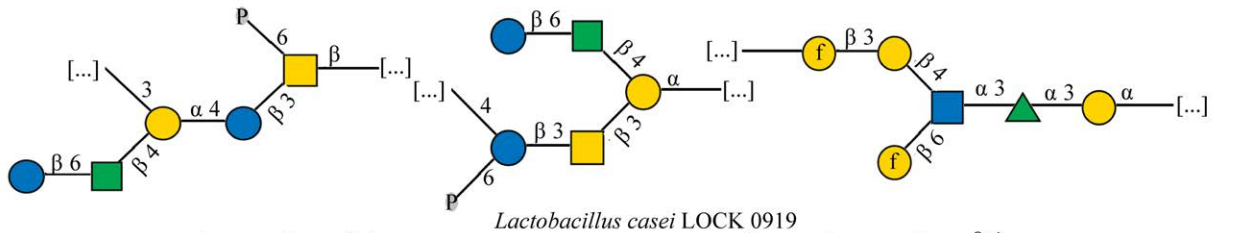


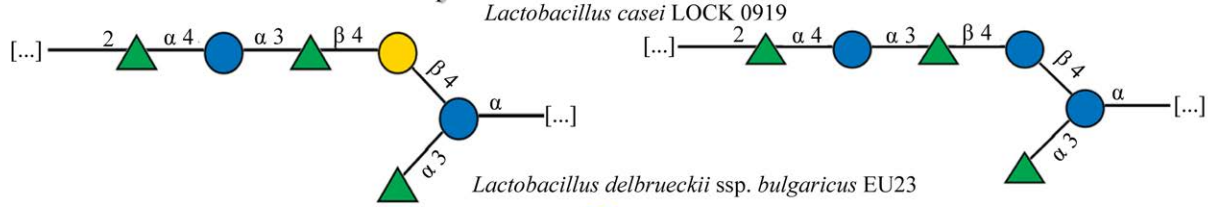
图 3　乳杆菌中杂多糖聚糖重复单元结构[49]

Figure 3　Structure of heteropolysaccharide repeat units in *Lactobacillus*[49] (HePS mainly composed of glucose and galactose as basic monosaccharides).

肿瘤活性[34]。Di 等对干酪乳杆菌 *L. casei* SB27 两种多糖进行结构鉴定，发现 2 种多糖主链均具 1,4-半乳糖或 1,4-葡萄糖结构，且对结肠癌抑制率可以达到 77.19%和 70.87%[67]；Li 等对瑞士乳杆菌 MB2-1 结构的研究表明，瑞士乳杆菌 MB2-1 的 EPS 组分一的主链含有 1,4-半乳糖和



Lactobacillus casei LOCK 0919



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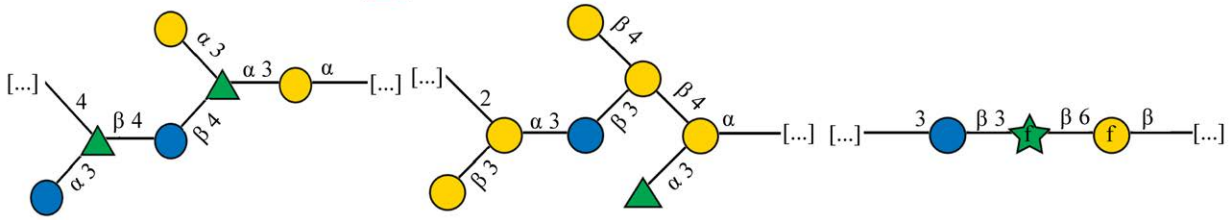
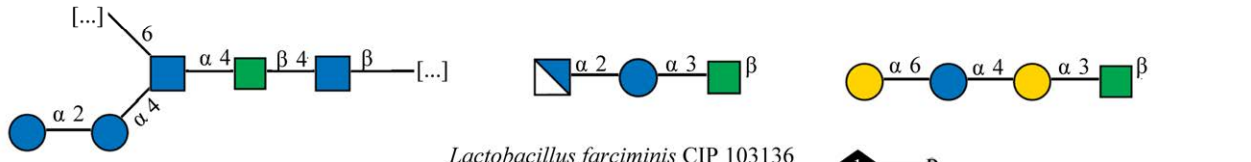
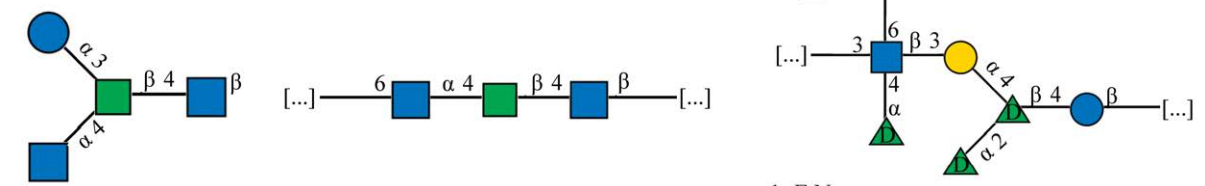


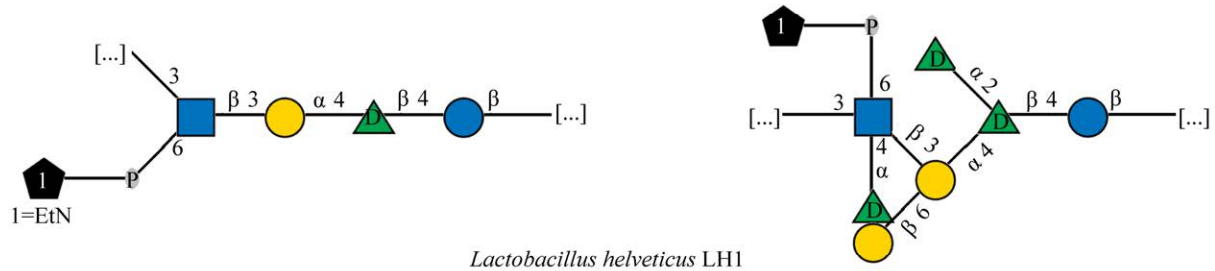
Lactobacillus delbrueckii ssp. *bulgaricus* 17 *Lactobacillus delbrueckii* ssp. *bulgaricus* LY03 *Lactobacillus fermentum* Lf2



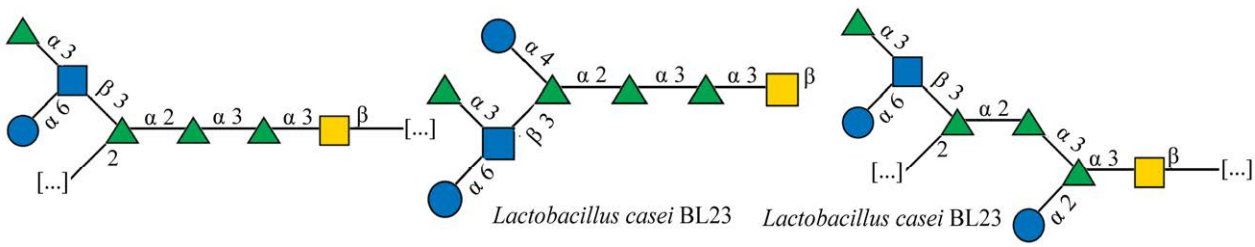
Lactobacillus farciminis CIP 103136



Lactobacillus farciminis CIP 103136 1=EtN *Lactobacillus helveticus* LH1



Lactobacillus helveticus LH1 1=EtN



Lactobacillus casei BL23 *Lactobacillus casei* BL23

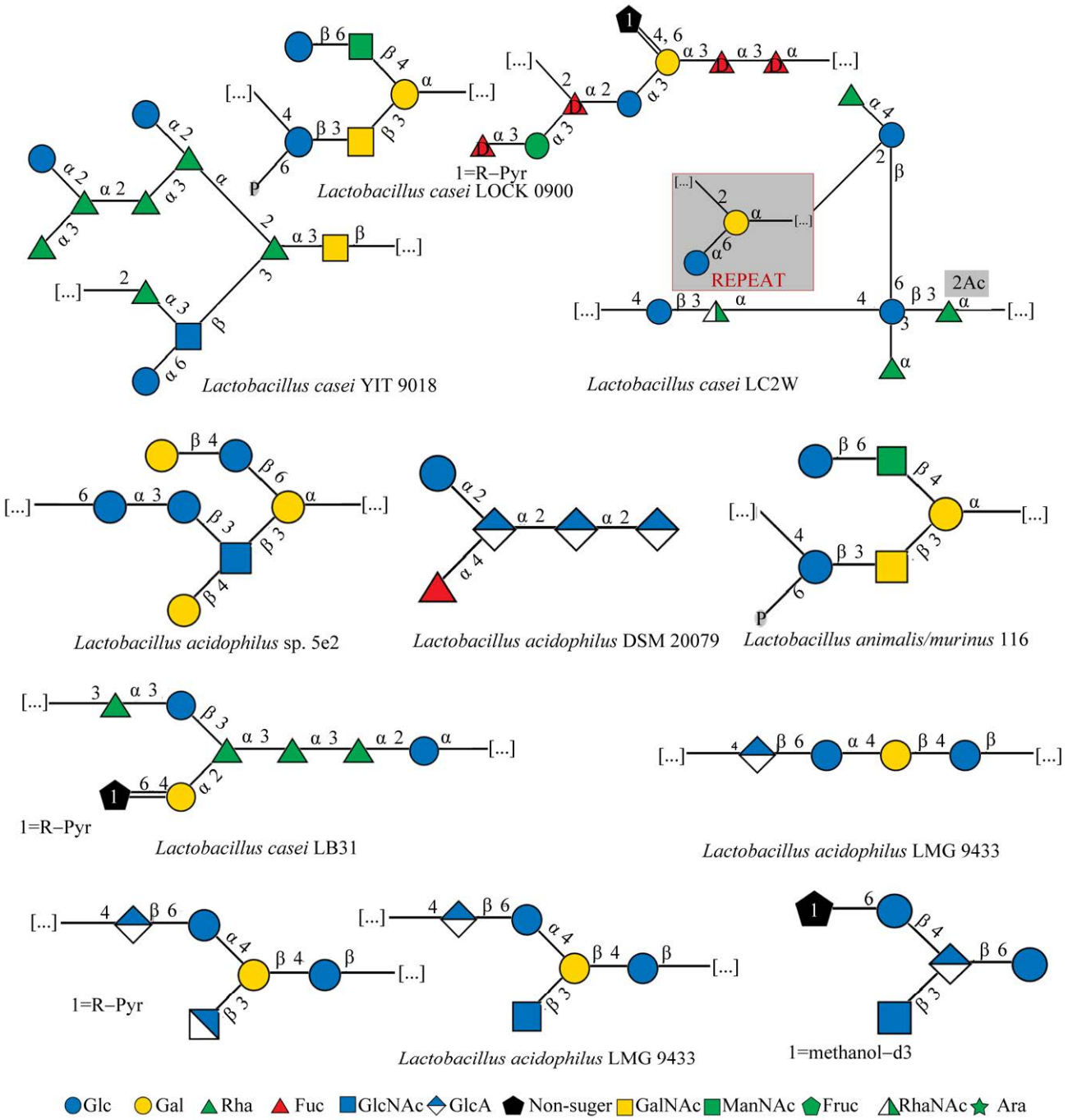


图 4　乳杆菌中杂多糖聚糖重复单元结构(以 3 种单糖及以上为基本单糖组成的 HePS)[49]

Figure 4　Structure of heteropolysaccharide glycan repeating unit in *Lactobacillus*[49]. HePS, which is composed of three or more monosaccharides as basic monosaccharides, uses green, red and white green triangles to represent rhamnose, fucose and N-acetylrhamnosamine, blue square to represent N-acetylglucosamine, blue white diamond to represent glucosamine, yellow square to represent N-acetyl galactose amine, green square to represent N-acetylmannosamine, and green star to represent arabinose. The shaded portion is a repeat of the partial structure in the glycan unit structure. Other illustrations are the same as figure 2.

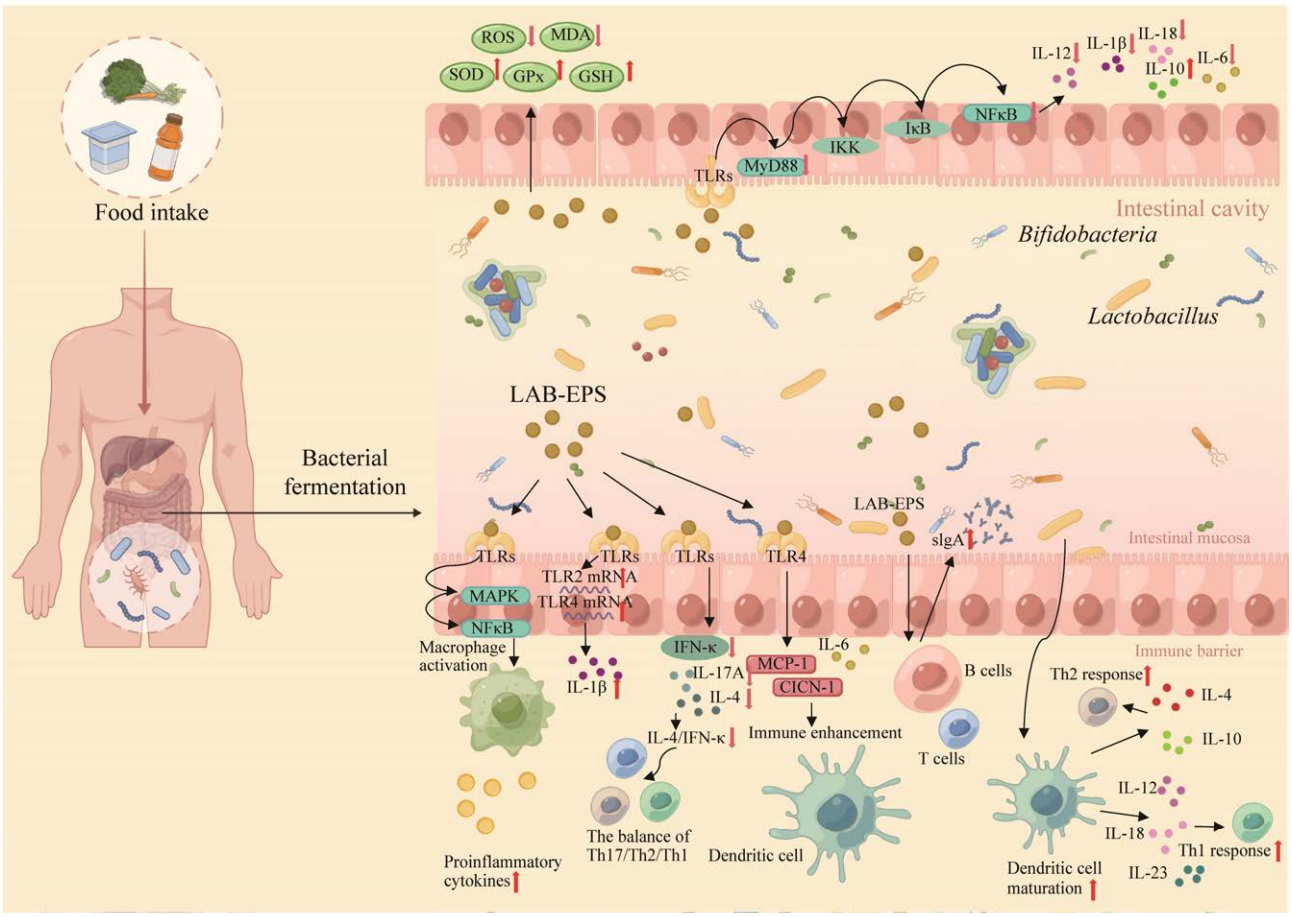


图 5　肠道稳态中乳杆菌胞外多糖的部分调节作用[58,62]

Figure 5　Partial regulatory effects of *Lactobacillus* extracellular polysaccharides in intestinal homeostasis[58,62]. The extracellular polysaccharides of *Lactobacillus* activate a series of signaling reactions by stimulating TLR receptors, regulating the secretion of various inflammatory signaling factors, thereby affecting the immune response of various immune cells (dendritic cells, B cells, T cells), and regulating intestinal homeostasis. Using different colored balls to represent various inflammatory factors, and brown balls to represent *Lactobacillus* extracellular polysaccharides.

1,4-葡萄糖，并且该 EPS 对结肠癌细胞 Caco-2 的增殖有显著的抑制效果[68]。

乳杆菌胞外多糖的活性与糖链上糖苷键构象有关。β-葡聚糖是具有生物活性的多糖，最经典的结构是以 β-1,3 糖苷键为主链，且含有 β-1,6 糖苷键连接的支链。β-糖苷键比 α-糖苷键构成的葡聚糖的抗肿瘤活性高[63]。*L. lactis* subsp. *cremoris* B40 菌株产生的 EPS 中，由 β-1,4 糖苷键连接比 β-1,3 或 α-1,4 糖苷键连接具有更强的刚性，并且 α 型键比 β 型键构成的链更具有柔性，进而影响多糖的黏度[69]。Haroun 等[70]从 *L. plantarum* NRRL B-4496 中分离得到一种以 β-1,4 和 β-1,6 糖苷键连接而成的葡聚糖，发现它在体外可以抑制 6 种癌细胞。刘宇等[71]从保加利亚乳杆菌 OLL1073R-1 分离得到 EPS-B16，发现糖苷键较少的酸性多糖能够有效的促进小鼠淋巴细胞增殖，具有抗肿瘤活性。刘滔[72]分离出的植物乳杆菌 *L. plantarum* HY

的 EPS 的糖环全为吡喃糖环，主要为 α 构型，且存在 α-D-甘露糖和 α-D-葡萄糖残基，HY-EPS 能够还原 Fe^{3+}和 Mo^{6+}，具有体外抗氧化能力，对 α-淀粉酶的活性具有抑制作用，并且具有降血糖潜力。

对乳杆菌胞外多糖的高级结构与抗肿瘤活性之间的构效关系研究可以植物多糖作为参考[63,73-75]，三股螺旋构型是多糖最具活性的空间构象，与 α 型相比，β 螺旋形立体结构由于能形成三股绳状螺旋立体构型，因此抗肿瘤活性更高。目前普遍认为多糖链上以 β-1,6 主链或 β-1,3 主链葡聚糖时具有较好的抗肿瘤活性[76]。

3.2 乳杆菌胞外多糖分子量构效关系

乳杆菌胞外多糖的活性与多糖相对分子质量有关。Asker 等[77]表明需土乳杆菌的 EPS 分子量越小，抗氧化能力越强，推测是因为低分子量的多糖更易结合自由基。在 *Lactococcus lactis* subsp. cremoris B40 菌株产生的 EPS 中，黏度与多糖分子量增加相关[69]。相对分子质量较小的多糖，无法形成有活性的复杂的高级聚合结构，而相对分子质量高的多糖分子体积大，不利于多糖跨膜运输进入生物体内发挥作用[78]。研究推测，小分子量和负电荷的 EPS 是强免疫调节剂，而分子量较大的中性聚合物是弱免疫调节剂，具有免疫抑制活性[79]。对 *L. confuses* TISTR 1498[80]合成的 EPS (5.06×10^8 g/mol))进行降解，发现 EPS (2.9×10^4 g/mol)更能显著促进巨噬细胞分泌 NO，并可提高多种细胞因子(iNOS、IL-10、IL-6 和 IL-1β)的 mRNA 表达水平。这是由于低分子量 EPS 与细胞受体之间具有更好的结合能力，从而促进细胞因子的表达和分泌。*L. casei* LC2W[81]合成的 2 种中性 EPS 组分中，分子质量较低的组分(2.14×10^4 Da)能够更好地促进小鼠 T 淋巴细胞增殖。

3.3 乳杆菌胞外多糖单糖摩尔比构效关系

对 3 株植物乳杆菌 *L. plantarum* SKTl 09、*L. plantarum* Ywl 1 和 *L. plantarum* Yw 32[36]所合成 EPS 进行抗癌实验发现，*L. plantarum* Ywl 1 EPS (单糖组成摩尔比 Glc:Gal=2.71:1)对人结肠癌细胞 HT-29 细胞的抑制率高于另外 2 种菌株的 EPS (单糖组成摩尔比 Fru:Glc=3:1 和 Man:Fru:Gal:Gal:Glc=8.2:1.0:4.1:4.2)。酸性单糖的含量对多糖的体外抗氧化能力具有重要影响。瑞士乳杆菌 MB2-1[82]3 种胞外多糖体外抗氧化能力的顺序依次为 LHEPS-3>LHEPS-2>LHEPS-1，均是由甘露糖、葡萄糖和半乳糖组成，其各自单糖组成的摩尔比为 1:2.75:1.33、9.34:1.43:1 和 2.96:1:1.17；中性糖含量分别为 97.85%、94.54%和 67.62%；糖醛酸含量分别为 0.53%、1.96%和 2.53%；硫酸基团含量分别为 0.27%、0.42%和 0.87%。瑞士乳杆菌 SNA12[83]中分离的胞外多糖(SNA12-EPS)，富含半乳糖和葡萄糖，摩尔比为 1.1:0.12，通过人体粪便发酵的体外模拟消化实验，证明其可提高肠道微生物群产生短链脂肪酸的能力。*L. rhamnosus* 发酵产生的 NCVP-F 新型 α-吡喃多糖有较高的甘露糖和葡萄糖醛酸摩尔比，通过提高抗氧化酶活性(SOD、GSH 和 GSH-Px)，抑制细胞因子水平(IL-6、IL-1β、TNF-α 和 IL-18)，可以更有效地减轻镉损伤小鼠模型的肝肾损伤。在过去 10 年中，口服益生菌，例如嗜酸乳杆菌(*L. acidophilus*)、双歧杆菌(*B. bifidum*)、阿克曼菌(*Akkermansia* spp.)、丙酸杆菌(*Propionibacterium* spp.)和益生元补充剂(例如甘露糖、半乳糖、果糖、木糖、异麦芽糖和乳果糖的低聚物)已被用于促进肠道益生菌在结肠中的黏附和定植，缓和肠道微生物的生态失调[84-85]。

3.4 乳杆菌胞外多糖主链分支构效关系

LAB-EPS 的活性与主链上分支点有关。活性最强的多糖分支度一般在 0.20−0.33[86]。有研

究者提出相对分子质量较高的多糖形成三螺旋结构是需要 β-D-吡喃葡萄糖基分支侧链来提高稳定性的[87]。已有研究证明，乳杆菌可以通过刺激抗癌作用来调节和缓解癌症，例如，EPS 结构中甘露糖和葡萄糖残基以及重复单元分支点的存在会增加它们的抗癌活性[68]；再如增强癌细胞的凋亡和保护它们免受氧化应激的影响[88]。

3.5 乳杆菌胞外多糖糖链基团及糖链修饰构效关系

乳杆菌胞外多糖的抗肿瘤活性随着多糖支链中的羟基数目的增多而增强，这与糖链上存在的羟基与氧自由基之间存在相互作用密切相关，同时随着糖链上羟基数目的增多，其抗氧化活性增强[89-90]。

研究表明，经过羧甲基、硫酸基、乙酰基、苯甲基及磷酸基等化学修饰后的胞外多糖的生物学活性可以得到较大幅度的提高[91]。Tsiapali 等[92]对葡聚糖及其衍生物的抗氧化活性进行了测定，发现磷酸化和硫酸化后的葡聚糖抗氧化能力更强，并且硫酸化程度越高，抗氧化活性越强。Li 等[66]对 *Streptococcus thermophilus* ASCC 1275 胞外多糖进行硫酸化修饰，通过超氧化物和羟基自由基清除试验及 Fe^{3+}还原试验对硫酸化 EPS 的抗氧化活性进行测定，结果表明，硫酸化修饰 EPS 的抗氧化活性显著提高(P<0.05)。乳酸菌可以与病毒直接相互作用、产生抑制物质或刺激免疫等来发挥抗病毒活性[93]。硫酸化多糖即使在粗形式下也具有抗病毒作用[94]。从发酵蔬菜中分离出的 *L. plantarum* IMB19[95]，其荚膜多糖富含鼠李糖，为线性九糖重复单元，并由 α-葡萄糖和丙氨酸进行修饰；在体外脾细胞培养系统中，该荚膜多糖可增强免疫刺激性细胞因子(IFNγ、TNF-α、IL-6 和 IL-12)或免疫调节性(IL-10)细胞因子。Zhao 等[96]将多糖硒化修饰后，与未修饰的多糖分别作为抗氧化剂，探究对 DPPH、羟基自由基和超氧自由基的清除能力，发现硒化修饰的多糖可以显著增强抗氧化的活性，这也是多糖化学改性的实例[97]。刘鹭等[98]研究了硒化修饰后 EPS 对小鼠腹腔巨噬细胞游离 Ca^{2+}的影响，结果表明 Se-EPS 能够显著提高巨噬细胞中游离 Ca^{2+}的浓度，增加巨噬细胞被激活的机会，分泌更多的细胞因子，进而发挥其免疫细胞的功能。乳杆菌胞外多糖具有机体免疫调节作用。对 *L. plantarum* KF 5[99]发酵合成的 EPS 单糖组成进行分析，依据 T 淋巴细胞增殖实验结果，发现有氨基基团且单糖种类多的胞外多糖免疫调节活性更高。另有研究发现，乳杆菌合成的酸性 EPS 中，磷酸取代基能有效提高巨噬细胞的活性，使抗体的柄端能更有效的与吞噬细胞表面受体进行结合，进而被吞噬；而这些是中性 EPS 所不能实现的[100]。

4 总结与展望

乳杆菌胞外多糖能够通过调节人体免疫系统从而增强机体的抗逆作用，是一种天然的提高机体免疫力的活性物质。可广泛应用在益生保健食品及药品领域中。本实验室曾对肠道共生菌的组成、利用进入肠道内多糖的机制以及产生的代谢产物可能对人体健康存在的潜在影响等方面进行过综述。帮助深入了解肠道菌群的组成结构以及多糖代谢机制，以便未来可以通过某些特定多糖或益生元来精准调控肠道多糖[101]。

由于技术限制，乳杆菌胞外多糖的产量仍不尽如人意。如何提高产量及安全稳定性是急需解决的问题，乳杆菌胞外多糖结构及功能的差异意味着很难建立一个通用的生产流程及标准。

随着糖化学、糖组学、高分辨质谱技术等前沿科学的快速发展，多糖科学迎来了技术发展的新浪潮，也加速推动了多糖微生物学在医药、保健食品和生物材料等领域的应用。微生物胞外多

糖介导许多重要的生物过程,如乳杆菌调节人类肠道稳态的作用等。利用细菌表面多糖来研发疫苗和益生制剂已经屡见不鲜。由于微生物多糖结构高度的复杂性和可变性，对其进行系统研究，找出细菌表面多糖共有糖结构仍然具有挑战性。在本实验室相关研究中[102]，对 CSDB (碳水化合物结构数据库)中的所有微生物多糖结构进行筛选,并分析了所有亚结构的出现次数和物种分布数量。结果表明，不同微生物中存在共同的多糖亚结构。进一步分析表明，这些多糖亚结构可能与细菌的种类、致病性和进化有关。总的来说，这为发现隐藏的信息和聚糖的生物学功能提供了一种替代方法或线索。

多糖的发展还有很长的路要走。在目前的技术水平下,多糖的结构表征和分析仍然是一项极具挑战性的任务。虽然目前对乳杆菌胞外多糖的遗传研究，单糖组成及结构，生理活性及免疫特性的研究已经取得一定的进展。对于乳杆菌 EPS 的一级结构及构效关系的研究已较为明确,但对其提高机体抗肿瘤和免疫能力的高级结构特征及构效机制的研究还存在明显的不足,尚未形成构效从低级到高级的系统性框架。所以今后应侧重对乳杆菌胞外多糖结构特性与功能特性的系统性研究,从而使其能够在人体共生菌群稳态领域中得到进一步的发展和应用。

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