微生物学报
Acta Microbiologica Sinica
2024, 64(9): 3168–3199
http://journals.im.ac.cn/actamicrocn
DOI: 10.13343/j.cnki.wsxb.20240120

Review 综 述

肺炎链球菌荚膜多糖结构与合成的研究进展

赵文旭[1,2]，杨帆[1,2]，黄蕾[1,2]，董文博[1,2]，杨静华[1,2*]

1 中国科学院微生物研究所，北京 100101
2 中国科学院大学，北京 101408

赵文旭，杨帆，黄蕾，董文博，杨静华. 肺炎链球菌荚膜多糖结构与合成的研究进展[J]. 微生物学报，2024，64(9): 3168-3199.
ZHAO Wenxu, YANG Fan, HUANG Lei, DONG Wenbo, YANG Jinghua. Advances in the chemical structures and biosynthesis of capsular polysaccharides of *Streptococcus pneumoniae*[J]. Acta Microbiologica Sinica, 2024, 64(9): 3168-3199.

摘　要：肺炎链球菌(*Streptococcus pneumoniae*)是一种能够引发人类肺炎和脑膜炎等严重疾病的病原体。其中，包裹着细菌的荚膜多糖(capsular polysaccharide, CPS)是关键的致病因子和重要抗原，已被制成多糖疫苗和多糖蛋白结合疫苗，在抗细菌感染中发挥了巨大作用。荚膜多糖由寡糖单位重复聚合而成，每个寡糖单位通常含有 2−8 个单糖残基，其结构复杂，具有不同的抗原表位。荚膜多糖也是细菌分型的依据，目前已发现肺炎链球菌有 107 种血清型，每种血清型都有特定的荚膜多糖结构、遗传基础和血清学特征。荚膜多糖结构的多样性和不断变化是肺炎链球菌难以被根除的主要原因。本文总结了目前已知的 95 个肺炎链球菌荚膜多糖的化学结构，探讨了荚膜多糖的遗传基础、合成机制和纯化方法，旨在提高对荚膜多糖结构的全面认知，为深入研究荚膜多糖的功能和进化机制以及多糖疫苗的制备提供参考。

关键词：肺炎链球菌；荚膜多糖；结构；合成机制；纯化方法

资助项目：国家自然科学基金(31972919)；国家重点研发计划合成生物学重点专项(2019YFA0905600)
This work was supported by the National Natural Science Foundation of China (31972919) and the National Key Research and Development Program of China for Synthetic Biology (2019YFA0905600).
*Corresponding author. E-mail: yangjh@im.ac.cn
Received: 2024-02-27; Accepted: 2024-04-09; Published online: 2024-04-15

Advances in the chemical structures and biosynthesis of capsular polysaccharides of *Streptococcus pneumoniae*

ZHAO Wenxu[1,2], YANG Fan[1,2], HUANG Lei[1,2], DONG Wenbo[1,2], YANG Jinghua[1,2*]

1 Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
2 University of Chinese Academy of Sciences, Beijing 101408, China

Abstract: *Streptococcus pneumoniae* causes serious diseases such as pneumoniae and meningitis in humans. Capsular polysaccharides (CPSs) surrounding bacteria are not only key virulence factors but also major antigens. Therefore, CPSs have been prepared into polysaccharide vaccines and polysaccharide conjugate vaccines, which have greatly reduced the infection of pneumococci. CPSs are formed by polymerization of oligosaccharide repeating units which generally have 2−8 monosaccharides. CPSs present complex structures with diverse antigenic epitopes, being the basis of bacterial serotyping. Currently, 107 serotypes of *S. pneumoniae* have been identified. Each serotype has a unique CPS structure, a stable genetic basis, and specific serological characteristics. The diversity and constant changes of CPS structures explain the difficulty in the eradication of pneumococci. This review summarizes the known chemical structures of 95 CPSs and discusses the genetic basis, biosynthesis mechanism, and purification methods of CPSs. This review aims to enrich the knowledge about CPS diversity and provide a reference for probing into the functions and evolution of CPSs as well as for preparing polysaccharide vaccines.

Keywords: *Streptococcus pneumoniae*; capsular polysaccharide; structure; biosynthesis mechanism; purification method

肺炎链球菌定殖在上呼吸道的黏膜表面，一般呈现无症状携带。然而，一旦细菌侵入到无菌部位，便可引起中耳炎、支气管炎和鼻窦炎等非侵袭性疾病；若细菌进一步侵入肺、血液和脑脊液等深层组织，则可引起肺炎、败血症和脑膜炎等侵袭性疾病(invasive pneumococcal disease, IPD)[1]。2019 年的全球疾病负担(global burden of diseases, GBD)统计分析表明，肺炎链球菌是全球范围内第三位高致死率细菌[2]。同时，肺炎链球菌也是社区获得性肺炎(community-acquired pneumoniae, CAP)的主要病原菌，非致死性的肺部感染增加了社会经济成本和医疗负担。

1881 年 Pasteur 和 Sternberg 首次从病人的唾液中分离到肺炎链球菌[3]，并证明将其注射到兔子体内会导致致命的疾病。随后发现肺炎链球菌的临床菌株都被一层荚膜包裹。1917 年，Dochez 等[4]报道在肺炎链球菌培养液的过滤物中以及病人的血清和尿液中都分离出可溶性物质，具有菌株特异性，可以用于血清型(serotype)分型。由于这种可溶性物质具有免疫原性，最初以为是蛋白质，直到 1925 年才证明是血清型特异的荚膜多糖，这是历史上第一个被识别的多糖抗原、非蛋白质抗原，也由此让人们认识到多糖和细菌的致病性是相关的[5]。

20 世纪初发现肺炎链球菌有多个血清型，用不同菌株免疫兔子会产生不同的抗血清，拥有独一无二的荚膜多糖结构和血清学特征的菌株被定义为一个血清型；与同一个抗血清有免疫交叉反应的血清型被归属为一个血清群(serogroup)[6-7]。传统的血清学分型方法是用Neufeld在1902年发明的荚膜肿胀反应(quellung reaction)通过抗血清鉴定血清型[8]。近些年，多项研究用单克隆抗体发现血清群6和11中存在多个新的血清型[9-13]。另外，随着测序技术的快速发展，分子分型(genotyping)的方法如PCR和全基因组测序被用于鉴定肺炎链球菌。目前发现肺炎链球菌有107个血清型，分为46个血清群。然而，并不是所有血清型的致病力都一样，只有少数血清型能够引起严重的侵袭性疾病[14]。流行病学监测表明，不同国家或地域流行的血清型不完全一样。比如，在中国的流行株为血清型6A、6B、9V、14、19F、19A和23F[15]，在美国的流行株为血清型4、6B、9V、14、18C、19F和23F[16]。

研究发现引起疾病的临床菌株都有荚膜，而失去荚膜导致细菌不能引起侵袭性疾病，但是仍然可以引起非侵袭性疾病[17]。另外，荚膜的厚度和结构组成影响细菌的流行和毒力[18-20]。然而，不同血清型表现出明显不同的毒力[21-22]。例如，血清型3、11A、6A、6B和19F对人的致死率高，血清型1和7F对人的感染力强，但是并不引起死亡[21]。当同一个菌株的荚膜被置换成不同血清型的荚膜后，细菌对小鼠的致死力不同。例如，血清型4、6A的致死力最强，低剂量感染小鼠即可导致其全部死亡；其次是血清型1、2、3、5和8是强毒菌株；而血清型6B、7F、9V、14、18C、19A、19F和23F都属于低毒菌株，只有在高剂量感染小鼠时才能致其死亡[22-23]。基于这些特征，荚膜多糖被认为是肺炎链球菌最关键的致病因子[24]。

荚膜多糖主要通过抑制补体和抗体等调理素(opsonin)沉积在细菌表面，从而抑制调理素介导的中性粒细胞和巨噬细胞吞噬细菌(opsonophagocytosis)[25-26]。荚膜多糖也抑制中性粒细胞胞外诱捕网(neutrophil extracellular trap, NET)捕获杀死细菌[27-28]；荚膜多糖还通过覆盖细菌表面的Toll样受体(Toll-like receptors, TLR)，使其不被免疫细胞的髓样分化因子88(myeloid differentiation primary response 88, Myd88)识别，进而降低了宿主的杀菌能力[29]。大多数荚膜多糖带有负电荷，能够通过静电排斥作用阻止黏液和黏膜纤毛清除细菌[1]。肺炎链球菌不同血清型的荚膜厚度不同，有的厚度可达约400 nm，占细菌体积的一半以上[30]。荚膜的厚度在细菌感染的不同时期会发生改变。在细菌黏附宿主上皮细胞时，荚膜的合成减少，荚膜变薄，暴露出细胞壁和细菌表面蛋白等结构帮助细菌黏附细胞，这种相变(phase variant)多发生在鼻咽道；一旦细菌侵入宿主细胞，在血液中细菌又恢复荚膜多糖的合成，覆盖细菌表面结构，以抵抗免疫系统识别和调理吞噬细菌[31-32]。

荚膜多糖的结构十分复杂，多种多样。每个多糖结构中都含有多个抗原表位(epitope)，能够刺激宿主产生多克隆抗体，从而获得血清型特异的免疫保护作用[33]。因此，侵袭力强的血清型的荚膜多糖被制成糖疫苗，通过群体免疫达到预防细菌感染和传播的目的。目前有2种类型的肺炎球菌疫苗被使用：肺炎链球菌多糖疫苗和肺炎球菌结合疫苗。1983年商业化的23价肺炎链球菌多糖疫苗(23-valent pneumococcal polysaccharide vaccine, PPSV23)是由23个血清型的荚膜多糖组成，每个血清型含有25 μg纯化的荚膜多糖。PPSV23对成年人有保护作用，但是对2岁以下的儿童和65岁以上有慢性病的老年人的保护作

用差[34-35]。这是因为多糖是不依赖于 T 细胞的抗原(T independent antigen, TI-Ag)，多糖与 B 细胞受体作用进而刺激细胞产生抗体，这些抗体的亲和力低，主要是 IgM，没有 T 细胞辅助，B 细胞不能分化产生记忆性 B 细胞，不能激发长久的免疫保护[36]。随着抗体水平的下降，需要定期重复接种多糖疫苗。由于婴幼儿的 B 细胞发育还不成熟，因此 PPSV23 对 2 岁以下婴幼儿的保护效果差[36]，后来美国又研发了七价肺炎链球菌疫苗(7-valent pneumococcal conjugate vaccine, PCV7)，是将 7 个强毒血清型的多糖结合到无毒的白喉毒素 CRM_{197} 上得到的疫苗[37]。糖蛋白结合疫苗为 T 细胞依赖性抗原(T dependent antigen, TD-Ag)，可以被消化成多肽片段，装载至主要组织相容性复合体 II (major histocompatibility complex-II, MHC-II)分子，递呈至细胞表面，被 T 细胞识别并激活 B 细胞，活化的 B 细胞克隆增殖产生浆细胞和记忆细胞，产生高亲和力的抗体如 IgG1，在二次接种时具有免疫记忆功能，可产生持久的免疫效果[36]。PCV7 对所有群体都表现出很好的免疫保护作用，使肺炎链球菌引起的婴幼儿侵袭性疾病的病例减少了 90%[38]。然而，随着糖疫苗在全世界的广泛应用，出现了血清型取代(serotype replacement)。流行病学调查发现，疫苗血清型得到很好的控制，但是其他非疫苗血清型引起的感染日益增多，并逐渐成为新的流行株[39]。为此，又在 PCV7 的基础上加入 6 个新的流行株(血清型 1、3、5、6A、7F 和 19A)，研发了 13 价糖蛋白结合疫苗(13-valent pneumococcal conjugate vaccine, PCV13)，成为目前应用广泛的肺炎链球菌疫苗，同时也是最贵的疫苗之一。新流行株产生的原因可能是疫苗诱导的抗体抑制了疫苗菌株的生长，使非疫苗菌株获得更多生态位点(ecologic niche)并逐渐成为新的流行株[40]。可以预见，随着疫苗的普及，新的流行株还会陆续出现，因此，对肺炎链球菌荚膜多糖结构的鉴定和全面了解显得更为重要。

基于荚膜多糖在肺炎链球菌的致病性和糖疫苗中的重要作用，本文对荚膜多糖的化学结构、合成机制、分离和纯化方法进行了综述。

1 肺炎链球菌荚膜多糖的结构

鉴定多糖的结构要比鉴定蛋白质的结构困难复杂得多，一般需要确定以下几个参数：(1) 单糖的组成和数量；(2) 单糖的排列顺序以及糖环大小，即单糖是吡喃糖还是呋喃糖；(3) 糖苷键类型以及异头碳的构型；(4) 非糖成分如甘油、核糖醇、胆碱等；(5) 化学修饰如乙酰化修饰、磷酸化修饰等[41]。对多糖的鉴定在技术上极具挑战性，早期只能用传统的化学方法鉴定一些简单的多糖结构，例如 1941 年第一个被鉴定的血清型 3 的荚膜多糖仅由 2 个单糖组成。然而，由于技术的限制，早期的许多结构都是不完整或不准确的。随着分析技术如气相色谱(gas chromatography, GC)、液相色谱(liquid chromatography, LC)、质谱(mass spectrometry, MS)和核磁共振(nuclear magnetic resonance, NMR)的发展，彻底改变了多糖的结构研究[10,41-43]。单糖的组成和单糖之间的连接键可用 GC 分析确定；MS 可以确定寡糖的相对分子质量，分析重复单位的大小；NMR 的一维色谱和二维色谱可以解析多糖的完整结构。我们也分离纯化了荚膜多糖，并通过 NMR 和 GC-MS 技术鉴定了 5 个肺炎链球菌血清型(10F、10B、10C、35F 和 35C)的荚膜多糖完整的化学结构[44-46]。在目前发现的 107 个血清型中，有 95 个血清型的荚膜多糖结构已知，都总结在表 1 中。

表 1　肺炎链球菌血清型和荚膜多糖的化学结构

Table 1　The serotypes of *Streptococcus pneumoniae* and the biochemical structures of capsular polysaccharides

Type	Structure	References
1	→3)-α-AATGal*p*-(1→4)-α-D-Gal*p*A$2_{0.3}$,$3_{0.3}$Ac$_2$-(1→3)-α-D-Gal*p*A-(1→	[47]
2	→4)-β-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-L-Rha*p*-(1→ 2 ↑ 1 α-D-Glc*p*A-(1→6)-α-D-Glc*p*	[48]
3	→3)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→	[49]
4	→3)-β-D-Man*p*NAc-(1→3)-α-L-Fuc*p*NAc-(1→3)-α-D-Gal*p*NAc(1→4)-α-D-Gal*p*2,3(S)Pyr-(1→	[50]
5	→4)-β-D-Glc*p*-(1→4)-α-L-Fuc*p*NAc-(1→3)-β-D-Sug*p*-(1→ 3 ↑ 1 α-L-Pne*p*NAc-(1→2)-β-D-Glc*p*A	[51]
6A	→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-D-Rib-ol-(5→*P*→	[52]
6B	→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-Rib-ol-(5→*P*→	[53]
6C	→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→3)-D-Rib-ol-(5→*P*→	[54]
6D	→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-Rib-ol-(5→*P*→	[54]
6E	→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-Rib-ol-(5→*P*→	[55]
6F	6F has both 6A and 6C repeating units	[56]
6G	6G has both 6B and 6D repeating units	[54]
6H	6H has both 6A and 6B repeating units	[12]
6I	6I has both 6C and 6D repeating units	[12]
7F	→6)-α-D-Gal*p*-(1→3)-β-L-Rha*p*2Ac-(1→4)-β-D-Glc*p*-(1→3)-β-D-Gal*p*NAc-(1→ 2 4 ↑ ↑ 1 1 β-D-Gal*p* α-D-Glc*p*NAc-(1→2)-α-L-Rha*p*	[57]
7A	→6)-α-D-Gal*p*-(1→3)-β-L-Rha*p*2Ac-(1→4)-β-D-Glc*p*-(1→3)-β-D-Gal*p*NAc-(1→ 4 ↑ 1 α-D-Glc*p*NAc-(1→2)-α-L-Rha*p*	[58]
7B	→6)-α-D-Glc*p*NAc-(1→2)-α-L-Rha*p*-(1→2)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→*P*→ 3 ↑ 1 β-D-Rib*f*-(1→4)-α-L-Rha*p*	[59]
7C	→6)-α-D-Glc*p*NAc-(1→2)-α-L-Rha*p*-(1→2)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→4)-α-D-Gal*p*NAc-(1→*P*→ 3 ↑ 1 β-D-Rib*f*-(1→4)-α-L-Rha*p*	[60]

(待续)

(续表 1)

Type	Structure	References
7D	1×→6)-α-D-Glc*p*NAc-(1→2)-α-L-Rha*p*-(1→2)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→*P*→ 3 ↑ 1 β-D-Rib*f*-(1→4)-α-L-Rha*p* 5×→6)-α-D-Glc*p*NAc-(1→2)-α-L-Rha*p*-(1→2)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→4)-α-D-Gal*p*NAc-(1→*P*→ 3 ↑ 1 β-D-Rib*f*-(1→4)-α-L-Rha*p*	[60]
8	→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Gal*p*-(1→	[61]
9A	→4)-α-D-Glc*p*A2$_{0.27}$,3$_{0.61}$Ac$_2$-(1→3)-α-D-Gal*p*-(1→3)-β-D-Man*p*NAc4Ac$_{0.3}$-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→	[62]
9L	→4)-α-D-Glc*p*A-(1→3)-α-D-Gal*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*NAc-(1→	[63]
9N	→4)-α-D-Glc*p*A-(1→3)-α-D-Glc*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*NAc-(1→	[64]
9V	→4)-α-D-Glc*p*A2$_{0.25}$,3$_{055}$Ac$_2$-(1→3)-α-D-Gal*p*-(1→3)-β-D-Man*p*NAc4$_{0.09}$,6$_{1.04}$Ac$_2$-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→	[62]
10F	β-D-Gal*f* 1 ↓ 6 →5)-β-D-Gal*f*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→4)-D-Rib-ol-(5→*P*→	[44]
10A	β-D-Gal*p* 1 ↓ 6 →5)-β-D-Gal*f*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→2)-D-Rib-ol-(5→*P*→	[65]
10B	→5)-β-D-Gal*f*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→4)-D-Rib-ol-(5→*P*→ 3 ↑ 1 β-D-Gal*f*	[45]
10C	β-D-Gal*f* 1 ↓ 6 →5)-β-D-Gal*f*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→2)-D-Rib-ol-(5→*P*→	[45]
10D	β-D-Gal*p* 1 ↓ 6 →6)-α-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→4)-β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→1)-D-Rib-ol-(5→P→ 3 ↑ 1 β-D-Gal*f*	[13]

(待续)

(续表 1)

Type	Structure	References
11F	→6)-α-D-Glc*p*NAc3Ac-(1→4)-α-D-Gal*p*-(1→3)-β-D-Gal*p*4$_{0.8}$,6$_{0.6}$Ac$_2$-(1→4)-β-D-Glc*p*-(1→ 4 ↑ Rib-ol-(1→*P*	[10]
11A	→6)-α-D-Glc*p*2$_{0.6}$,3$_{0.5}$Ac$_2$-(1→4)-α-D-Gal*p*-(1→3)-β-D-Gal*p*4,6$_{0.5}$Ac$_2$-(1→4)-β-D-Glc*p*-(1→ 4 ↑ Gro-(1→*P*	[10]
11B	→6)-α-D-Glc*p*NAc3Ac$_{0.8}$-(1→4)-α-D-Gal*p*2Ac$_{0.4}$-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 4 ↑ Rib-ol-(1→P	[10]
11C	→6)-α-D-Glc*p*NAc3Ac$_{0.9}$-(1→4)-α-D-Gal*p*2Ac$_{0.3}$-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 4 ↑ Gro-(1→*P*	[10]
11D	Gro-(1→*P* ↓ 4 →6)-α-D-Glc*p*NAc3Ac$_{0.8}$-(1→4)-α-D-Gal*p*-(1→3)-β-D-Gal*p*4,6$_{0.5}$Ac$_2$-(1→4)-β-D-Glc*p*-(1→ Or/And Gro-(1→*P* ↓ 4 →6)-α-D-Glc*p*2$_{0.6}$,3$_{0.5}$Ac$_2$-(1→4)-α-D-Gal*p*-(1→3)-β-D-Gal*p*4,6$_{0.5}$Ac$_2$-(1→4)-β-D-Glc*p*-(1→	[66]
11E	→6)-α-D-Glc*p*2,3$_{0.3}$Ac$_2$-(1→4)-α-D-Gal*p*-(1→3)-β-D-Gal*p*4Ac$_{0.3}$-(1→4)-β-D-Glc*p*-(1→ 4 ↑ Gro-(1→*P*	[67]
12F	75%: →4)-α-L-Fuc*p*NAc-(1→3)-β-D-Gal*p*NAc-(1→4)-β-D-Man*p*NAcA-(1→ 3 3 ↑ ↑ 1 1 α-D-Gal*p* α-D-Glc*p*-(1→2)-α-D-Glc*p* 25%: →4)-α-L-Fuc*p*NAc-(1→3)-β-Sug*p*-(1→4)-β-D-Man*p*NAcA-(1→ 3 3 ↑ ↑ 1 1 α-D-Gal*p* α-D-Glc*p*-(1→2)-α-D-Glc*p*	[68]
12A	→4)-α-L-Fuc*p*NAc-(1→3)-β-D-Glc*p*NAc-(1→4)-β-D-Man*p*NAcA-(1→ 3 3 ↑ ↑ 1 1 α-D-Gal*p*NAc α-D-Glc*p*-(1→2)-α-D-Glc*p*	[69]
12B	No information	[41]

(待续)

(续表 1)

Type	Structure	References
13	→4)-β-D-Gal*p*-(1→4)-β-D-Glc*p*2,3Ac_2-(1→3)-β-D-Gal*f*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Rib-ol-(5→P→	[70]
14	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 4 ↑ 1 β-D-Gal*p*	[71]
15F	→3)-α-D-Gal*p*6Ac-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*6Ac-(1→4)-β-D-Glc*p*-(1→ 3 ↑ $Cho_{0.2}$-*P*	[72]
15A	→3)-α-D-Gal*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 3 ↑ $Gro_{0.7}$-(2→*P*	[72]
15B	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 4 ↑ 1 α-D-Gal*p*$2_{0.06}$,$3_{0.12}$,$4_{0.12}$,$6_{0.55}Ac_4$-(1→2)-β-D-Gal*p* 3 ↑ $Gro_{0.7}$-(2→*P*	[73]
15C	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 4 ↑ 1 α-D-Gal*p*-(1→2)-β-D-Gal*p* 3 ↑ $Gro_{0.7}$-(2→*P*	[73]
15D	→3)-α-D-Gal*p*6Ac -(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ 3 ↑ $Cho_{0.2}$-*P*	[72]
16F	→3)-α-L-Rha*p*-(1-3)-α-D-Glc*p*- (1-3)-β-L-Rha*p*2Ac-(1-4)-β-D-Glc*p*-(1→ 4 6 ↑ ↑ Gro-(1→*P* Gro-(1→*P*	[74]
16A	→3)-β-D-Gal*f*$2_{0.7}$Ac-(1-3)-α-L-Rha*p*-(1-2)-α-L-Rha*p*-(1-3)-α-D-Gal*p*-(1-3)-β-D-Gal*p*-(1-4)-β-D-Glc*p*-(1→ 6 ↑ Gro-(1→*P*	[74]
17F	→3)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→3)-α-D-Gal*p*-(1→3)-β-L-Rha*p*2Ac-(1→4)-α-L-Rha*p*-(1→2)-D-Ara-ol-(1→*P*→ 4 ↑ 1 α-D-Gal*p*	[75]

(待续)

(续表 1)

Type	Structure	References
17A	→3)-β-Glc*p*-(1→3)-α-Gal*p*-(1→3)-β-L-Rha*p*2Ac-(1→4)-α-L-Rha*p*-(1→4)-β-Glc*p*A-(1→3)-β-D-Gal*f*-(1→ 4 2 ↑ ↑ 1 1 β-D-Gal*p* α-D-Glc*p*	[76]
18F	Gro-(1→*P* ↓ 3 →4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-α-D-Glc*p*-(1→3)-β-L-Rha*p*2Ac-(1→ 2 ↑ 1 α-D-Glc*p*6Ac	[77]
18A	Gro-(1→*P* ↓ 3 →4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-α-D-Glc*p*NAc-(1→3)-β-L-Rha*p*-(1→ 2 ↑ 1 α-D-Glc*p*	[78]
18B	Gro-(1→*P* ↓ 3 →4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-α-D-Glc*p*-(1→3)-β-L-Rha*p*-(1→ 2 ↑ 1 α-D-Glc*p*	[79]
18C	Gro-(1→*P* ↓ 3 →4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-α-D-Glc*p*-(1→3)-β-L-Rha*p*-(1→ 2 ↑ 1 α-D-Glc*p*6Ac$_{0.3}$	[80]
19F	→4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*-(1→2)-α-L-Rha*p*-(1→P→	[81]
19A	→4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→P→	[82]
19B	→4)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→4)-β-D-Man*p*NAc-(1→4)-α-L-Rha*p*-(1→P→ 3 ↑ 1 β-D-Rib*f*-(1→4)-α-L-Rha*p*	[83]

(待续)

(续表 1)

Type	Structure	References
19C	β-D-Glc*p* 1 ↓ 6 →4)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→4)-β-D-Man*p*NAc-(1→4)-α-L-Rha*p*-(1→P→ 3 ↑ 1 β-D-Rib*f*-(1→4)-α-L-Rha*p*	[83]
20A	β-D-Gal*f*2Ac$_{0.9}$ 1 ↓ 4 →3)-α-D-Glc*p*NAc-(1-*P*-6)-α-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→3)-β-D-Gal*f*5$_{0.9}$6$_{0.9}$Ac$_2$-(1→3)-β-D-Glc*p*-(1→	[84]
20B	β-D-Gal*f*2Ac$_{0.9}$ 1 ↓ 4 →3)-α-D-Glc*p*NAc-(1-*P*-6)-α-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→3)-β-D-Gal*f*5$_{0.9}$6$_{0.9}$Ac$_2$-(1→3)-β-D-Glc*p*-(1→ 6 ↑ 1 α-D-Glc*p*	[84]
21	Constituents: Glc, Gal, and GlcN	[85]
22F	→4)-β-D-Glc*p*A-(1→4)-β-L-Rha*p*2Ac$_{0.8}$-(1→4)-α-D-Glc*p*-(1→3)-α-D-Gal*f*-(1→2)-α-L-Rha*p*-(1→ 3 ↑ 1 α-D-Glc*p*	[86]
22A	No information	
23F	Gro-(2→*P* ↓ 3 →4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-β-L-Rha*p*-(1→ 2 ↑ 1 α-L-Rha*p*	[87]
23A	→4)-β-D-Glc*p*-(1→3)-β-L-Rha*p*-(1→ 4 ↑ 1 α-L-Rha*p*-(1→2)-β-D-Gal*p* 3 ↑ Gro-(2→*P*	[88]

(待续)

(续表 1)

Type	Structure	References
23B	→4)-β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-β-L-Rha*p*-(1→ 3 ↑ Gro-(2→*P*	[88]
24F	→4)-β-D-Glc*p*NAc-(1→4)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→ 3 3 ↑ ↑ 1 1 β-D-Rib*f*-(1→4)-α-D-Rha*p* Ara-ol-*P*	[89]
24A	Cho→*P* 1 ↑ 6 →4)-β-D-Glc*p*NAc-(1→4)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→ 3 3 ↑ ↑ 1 1 α-D-Rha*p* Ara-ol-*P*	[89]
24B	→4)-β-D-Glc*p*NAc-(1→4)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→ 3 3 ↑ ↑ 1 1 β-D-Rib*f*-(1→4)-α-D-Rha*p* Rib-ol-*P*	[89]
24C	→4)-β-D-Glc*p*NAc-(1→4)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→ 3 3 ↑ ↑ 1 1 β-D-Rib*f*-(1→4)-α-D-Rha*p* Ara-ol-*P* and →4)-β-D-Glc*p*NAc-(1→4)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→ 3 3 ↑ ↑ 1 1 β-D-Rib*f*-(1→4)-α-D-Rha*p* Rib-ol-*P*	[89]
25F	Constituents: Glc, Rha, GlcN, Rib, and Rib-ol-*P*	[41]
25A	No information	[41]
26	→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→4)-D-Rib-ol-(5→*P*→	[53]
27	→3)-β-D-Glc*p*NAc4,6(*S*)Pyr-(1→3)-α-D-Gal*p*-(1→4)-β-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→ 2 ↑ Cho→*P*	[90]
28F	Cho→*P* ↓ 6 →4)-α-L-Rhap-(1→3)-α-D-Glcp-(1→3)-β-L-Rhap2Ac-(1→4)-β-D-Glc*p*-(1→ 4 ↑ Gro-(2→*P*)	[91]

(待续)

(续表 1)

Type	Structure	References
28A	Cho→*P* ↓ 6 →4)-α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-L-Rha*p*2Ac-(1→4)-β-D-Glc*p*-(1→ 4 ↑ Gro-(2→*P*)	[91]
29	→4)-β-D-Gal*p*NAc-(1→6)-β-D-Gal*f*-(1→3)-β-D-Gal*p*-(1→6)-β-D-Gal*f*-(1→1)-D-Rib-ol-(5→*P*→	[92]
31	→3)-β-D-Gal*f*5,6Ac$_2$-(1→3)-β-D-Galp-(1→3)-β-L-Rha*p*2Ac-(1→2)-α-L-Rha*p*-(1→4)-β-D-Glc*p*A-(1→	[93]
32F	→4)-β-D-Glc*p*-(1→3)-α-D-Glc*p*-(1→4)-β-L-Rha*p*2Ac-(1→ 2 3 ↑ ↑ α-L-Rha*p*-(1→*P* Cho→*P*	[94]
32A	→4)-β-D-Glc*p*-(1→3)-α-D-Glc*p*4Ac -(1→4)-β-L-Rha*p*2Ac-(1→ 2 3 ↑ ↑ α-L-Rha*p*-(1→*P* Cho→*P*	[94]
33F	→3)-β-D-Gal*p*-(1→3)-α-D-Gal*p*-(1→3)-β-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→5)-β-D-Gal*f*2Ac$_{0.5}$-(1→ 2 ↑ 1 α-D-Gal*p*	[95]
33A	→3)-β-D-Gal*p*-(1→3)-α-D-Gal*p*-(1→3)-β-D-Gal*f*5,6Ac$_2$-(1→3)-β-D-Glc*p*-(1→5)-β-D-Gal*f*2Ac-(1→ 2 ↑ 1 α-D-Gal*p*	[96]
33B	→6)-β-Gal*f*2Ac-(1→3)-β-Gal*p*NAc-(1→3)-α-Gal*p*-(1→4)-Rib-ol-(5→*P*→2)-α-Glc*p*-(1→3)-β-Glc*p*-(1→ 2 ↑ 1 α-D-Gal*p*	[43]
33C	→6)-β-Gal*f*2Ac-(1→3)-β-Gal*p*NAc-(1→3)-α-Gal*p*-(1→3)-Rib-ol-(5→*P*→3)-α-Gal*p*-(1→3)-β-Gal*p*-(1→ 2 ↑ 1 α-D-Gal*p*	[43]
33D	→6)-β-Gal*f*2Ac-(1→3)-β-Gal*p*NAc-(1→3)-α-Gal*p*-(1→4)-Rib-ol-(5→*P*→2)-α-Gal*p*-(1→3)-β-Glc*p*-(1→ 2 ↑ 1 α-D-Gal*p*	[43]

(待续)

(续表 1)

Type	Structure	References
33E	→3)-β-D-Gal*p*-(1→3)-α-D-Gal*p*-(1→3)-β-D-Gal*f*-(1→3)-β-DD-Glc*p*-(1→5)-β-D-Gal*f*2Ac$_{0.5}$-(1→	[97]
33G	→6)-β-D-Gal*f*2Ac-(1→3)-β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→4)-Rib-ol-(5→*P*→5)-β-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→	[98]
34	→3)-β-D-Gal*f*-(1→3)-α-D-Glc*p*-(1→2)-β-D-Gal*f*6Ac$_{0.5}$-(1→3)-α-D-Gal*p*-(1→2)-Rib-ol-(5→*P*→	[41]
35F	→6)-β-D-Gal*f*2Ac-(1→3)-α-D-Gal*p*-(1→2)-Rib-ol-(5→*P*→3)-β-D-Gal*f*-(1→3)-β-D-Gal*p*-(1→	[46]
35A	→3)-β-D-Gal*p*-(1→3)-β-D-Gal*f*5,6Ac$_2$-(1→3)-β-D-Glc*p*-(1→6)-β-D-Gal*f*2Ac-(1→1)-Man-ol-(6→*P*→	[96]
35B	→4)-β-D-Gal*p*NAc-(1→6)-β-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→6)-β-D-Gal*f*2Ac$_{0.7}$-(1→1)-Rib-ol-(5→*P*→	[99]
35C	→3)-β-D-Gal*p*-(1→3)-β-D-Gal*f*5$_{0.7}$,6$_{0.3}$Ac$_2$-(1→3)-β-D-Glc*p*-(1→6)-β-D-Gal*f*2Ac-(1→1)-Man-ol-(6→*P*→ 2 ↑ 1 α-D-Glc*p*	[46,100]
35D	→4)-β-D-Gal*p*NAc-(1→6)-β-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→6)-β-D-Gal*f*-(1→1)-Rib-ol-(5→*P*→	[101]
36	No information	[41]
37	→3)-β-D-Glc*p*-(1→ 2 ↑ 1 β-D-Glc*p*	[102]
38	No information	[41]
39	β-D-Gal*p* 1 ↓ 6 →6)-β-D-Gal*f*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→1)- D-Rib-ol-(5→*P*→ 3 ↑ 1 β-D-Gal*f*3$_{0.35}$,6$_{0.65}$Ac$_2$	[103]
40	No information	[41]
41F	→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→3)-α-D-Rha*p*-(1→ 2 2 ↑ ↑ 1 1 α-D-Glc*p* β-D-Rha*p*2$_{0.4}$,3$_{0.35}$,4$_{0.15}$Ac$_3$	[104]
41A	→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*f*-(1→3)-β-D-Glc*p*-(1→3)-α-D-Rha*p*-(1→ 2 2 ↑ ↑ 1 1 α-D-Glc*p* β-L-Rha*p*	[104]
42	→3)-β-D-Gal*p*-(1→3)-β-D-Gal*f*5$_{0.7}$,6$_{0.3}$Ac$_2$-(1→3)-β-D-Glc*p*-(1→6)-β-D-Gal*f*-(1→1)-D-Man-ol-(6→P→ 2 ↑ 1 α-D-Glc*p*	[100,103]

(续表 1)

(续表 1)

Type	Structure	Reference
43	No information	[41]
44	No information	[41]
45	Gro-(1→*P*→6)-β-D-Glc*p*NAc 1 ↓ 4 →3)-α-D-Gal*p*-(1→3)-α-L-Fuc*p*NAc-(1→3)-β-D-Gal*p*NAc-(1→2)-α-L-Rha*p*-(1→ 6 ↑ 1 α-D-Gal*p*	[105]
46	Constituents: Gal, GalNAc, GlcNAc, and FucNAc	[41]
47F	→6)-β-D-Gal*f*3,5Ac$_2$-(1→3)-β-D-Gal*p*-(1→6)-β-D-Gal*f*2Ac-(1→3)-α-D-Gal*p*-(1→2)-D-Rib-ol-(5→*P*→	[103]
47A	β-D-Glc*p* 1 ↓ 6 →6)-β-D-Gal*f*3,5Ac$_2$-(1→3)-β-D-Gal*p*-(1→4)-α-D-Glc*p*NAc-(1→4)-α-D-Gal*p*-(1→2)-D-Rib-ol-(5→*P*→ 3 ↑ 1 β-D-Glc*p*	[106]
48	No information	[41]
CWPS 1	Cho-*P* ↓ 6 →6)-β-D-Glc*p*-(1→3)-α-AATGal*p*-(1→4)-α-D-Gal*p*NAc-(1→3)-β-D-Gal*p*NAc-(1→1)-D-Rib-ol-(5→*P*→	[41]
CWPS 2	Cho-*P* Cho-*P* ↓ ↓ 6 6 →6)-β-D-Glc*p*-(1→3)-α-AATGal*p*-(1→4)-α-D-Gal*p*NAc-(1→3)-β-D-Gal*p*NAc-(1→1)-D-Rib-ol-(5→*P*→	[41]
CWPS 3	Cho-*P* Cho-*P* ↓ ↓ 6 6 →6)-β-D-Gal*p*-(1→3)-α-AATGal*p*-(1→4)-α-D-Gal*p*NAc-(1→3)-β-D-Gal*p*NAc-(1→1)- D-Rib-ol-(5→*P*→	[41]

AATGal: 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose; Ac: Acetate; Ara-ol: Arabinitol; Cho: Choline; Fuc: Fucose; FucNAc: N-acetylfucosamine; Gal: Galactose; GalA: Galacturonic acid; GalN: Galactosamine; GalNAc: N-acetylgalactosamine; Glc: Glucose; GlcA: Glucuronic acid; GlcN: Glucosamine; GlcNAc: N-acetylglucosamine; Gro: Glycerol; ManNAc: N-acetylmannosamine; ManNAcA: N-acetylmannosaminuronic acid; Man-ol: Mannitol; P: Phosphate; PneNAc: N-acetylpneumosamine (2-acetamido-2,6-dideoxytalose); Pyr: Pyruvate; Rha: Rhamnose; Rib: Ribose; Rib-ol: Ribitol; Sug: 2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose; f: Furanose; p: Pyranose; ND: Not defined; CWPS: Cell wall polysaccharide, C-polysaccharide or teichoic acid.

这些荚膜多糖是由 2−8 个单糖组成的寡糖重复单位(repeat unit)聚合而成。其中常见的单糖有葡萄糖(α/β-D-glucose)、半乳糖(α/β-D-galactose)、鼠李糖(α/β-L-rhamnose)、N-乙酰葡萄糖胺(N-acetyl-α/β-D-glucosamine)、N-乙酰半乳糖胺(N-acetyl-α/β-D-galactosamine)、N-乙酰甘露糖胺

(N-acetyl-α/β-D-mannosamine)、N-乙酰岩藻糖胺(N-acetyl-α-L-fucosamine)和葡萄糖醛酸(α/β-D-glucuronic acid)。此外，少数血清型含有岩藻糖(α-L-fucose)、核糖(β-D-ribose)、半乳糖醛酸(α-D-galacturonic acid)。有的血清型还含有特殊的单糖，如血清型 1 的 2-乙酰氨基-4-氨基-2,4,6-三脱氧-半乳糖(2-acetamido-4-amino-2,4,6-trideoxy-α-D-galactose, AAT-Gal)、血清型 5 的 2-乙酰氨基-2,6-双脱氧己糖(2-acetamido-2,6-dideoxy-D-xylo-hexos-4-ulose, Sug)和 2-乙酰氨基-2,6-双脱氧塔罗糖(2-acetamido-2,6-dideoxytalose, PneNAc)。除了糖，荚膜多糖的结构中也出现 3 种醛醇，分别是核糖醇(D-ribitol)、阿拉伯糖醇(D-arabinitol)和甘露醇(D-mannitol)，有的多糖还含有胆碱。这些结构中，38 个多糖被 O-乙酰化修饰，50 个多糖被磷酸化修饰，2 个多糖被丙烯酰乙酰化修饰；有的修饰位于主链，有的修饰位于支链。单糖上乙酰化修饰的水平和位置多种多样，例如，血清型 9V 的多糖中有 2 个单糖被 O-乙酰化修饰，分别是葡萄糖醛酸(glucuronic acid, GlcpA)的第 2 个碳有 25%的乙酰化修饰，第 3 个碳有 55%的乙酰化修饰；N-乙酰甘露糖胺(N-acetylmannosamine, ManpNAc)的第 4 个碳有 9%的乙酰化修饰，第 6 个碳有 104%的乙酰化修饰，这些都增加了多糖结构的复杂性和多样性。

另外，同一个血清群中的不同血清型的多糖结构很相似。例如，血清群 10 含有 4 个血清型(10F、10A、10B 和 10C)，我们鉴定了 10F、10B、10C 的荚膜多糖结构，发现 10F 和 10C 多糖的唯一区别是主链上的一个糖苷键不同；10F 和 10B 多糖的唯一区别是支链半乳糖与主链连接的糖苷键不同[44-45]。通过糖工程技术，我们鉴定了血清群 10 中所有的糖基转移酶的功能，例如糖基转移酶 WcrD 负责合成荚膜多糖上的 β1-3Gal*f* 支链，糖基转移酶 WcrG 负责合成多糖上 β1-6Gal*p* 支链[44-45]。然而，我们发现 β1-6Gal*p* 支链能够被单克隆抗体识别，表明这个支链糖是多糖的抗原决定簇；还发现多糖上的 Gal*f*β1-6GalNAcβ1-3Gal 结构是放线菌识别结合的位点，但是当 Gal*f*β1-6 处于支链位置，细菌不再与放线菌结合[44-45]。这些研究揭示，多糖上的支链结构具有重要的生物学功能。另外，我们也研究了血清型 35F、35C 和 42 的荚膜多糖结构，发现血清型 35C 和 42 的多糖结构几乎完全一样，唯一的区别是血清型 35C 多糖的 6-β-Gal*f* 上有 O-乙酰化修饰，而血清型 42 多糖中不存在这个修饰，导致它们的血清学特征几乎一样，很难用 Quellung 反应区分[46]。我们进一步鉴定了 O-乙酰基转移酶 WciG，发现 *wciG* 基因在血清型 42 中突变导致多糖失去了 O-乙酰化修饰，同时也失去了和抗血清因子 35a 的反应，表明荚膜多糖的 O-乙酰化修饰也是细菌重要的抗原决定簇[100]。

值得注意的是，近年发现的血清群 6 中新的血清型 6F、6G、6H、6I 和血清群 7 中新的血清型 7D 的荚膜多糖是由 2 种寡糖重复单位组成[60]。如表 1 中所示，血清型 7D 即产生血清型 7B 的荚膜多糖又产生血清型 7C 的荚膜多糖，它们的比例是 1:5。这些发现表明，肺炎链球菌为了适应新的环境，可能是抗生素和疫苗的广泛应用带来的选择压力，其荚膜结构越来越趋向于更加复杂化。

2 肺炎链球菌荚膜多糖的合成

合成荚膜多糖的基因在基因组上成簇排列，称为 *cps* locus。除了血清型 37 外，其他所有血清型的荚膜多糖合成基因簇都位于高度保守的 *dexB* 和 *aliA* 基因之间。2006 年，Sanger 研究所破译了 90 个血清型的基因簇序列，发现它们的

长度分布在 13−30 kb 之间，由 10−20 多个基因组成，并作为一个转录单位在 δ 启动子的作用下进行转录表达[107]。这个 δ 启动子高度保守，转录的起始位点位于第一个基因的起始密码子上游 20−30 个碱基处[108]。预测了 72%的糖基转移酶(glycosyltransferase, GT)的功能包括供体糖、受体糖以及它们之间的糖苷键，并对 88 个血清型的基因簇做了进化分析，揭示了不同血清型在遗传上的关系[109]。同时发现肺炎链球菌荚膜多糖和口腔链球菌表面受体多糖的合成基因簇序列相似，表明肺炎链球菌可能是从口腔链球菌进化而来[110]。这些研究全面阐明了肺炎链球菌荚膜多糖多样性的遗传基础。

2.1 荚膜多糖合成基因簇

为了显示荚膜多糖合成基因簇的特征，比较了 PCV7 的 7 个疫苗血清型的 *cps* locus，如图 1 所示。

每个 *cps* locus 都含有调控基因、糖基转移酶基因、单糖合成酶基因、转位酶基因和聚合酶基因。此外，有的血清型还含有乙酰基转移酶基因、甘油磷酸转移酶基因。绝大多数基因簇两端都有转座酶基因(*tnp*)，推测合成荚膜多糖的基因簇是从其他地方通过横向转移进化而来[109]。位于基因簇 5′端的 4 个调控基因 *wzg*、*wzh*、*wzd*、*wze*，最早称为 *cpsA*、*cpsB*、*cpsC*、*cpsD*，是高度保守的基因，在不同血清型中序列相似性高，负责调控荚膜多糖链的合成和转移。然后是跨膜的磷酸糖基转移酶(phosphoglycosyl transferase, PGT)基因，负责将第一个单糖以磷酸糖的形式连接到脂载体(lipid-carrier)上起始多糖的合成。

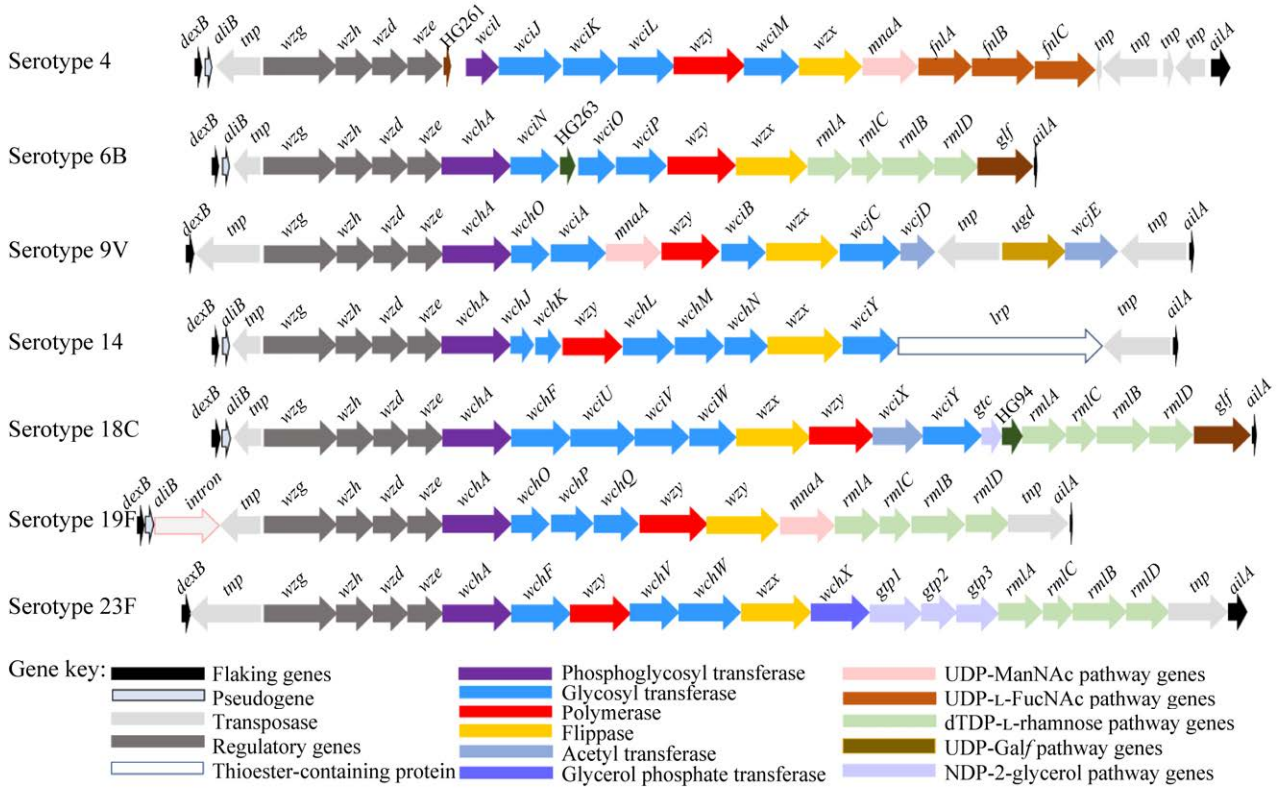

图 1 PCV7 疫苗中的 7 个血清型的荚膜多糖合成基因簇

Figure 1 Capsule biosynthesis gene clusters of PCV7 serotypes. The genes are presented on the forward and reverse strands by boxes which are colored according to the different functions.

绝大多数血清型的 PGT 基因是 *wchA*，合成磷酸葡萄糖基转移酶；在血清型 33C 和 47A 中是 *wcjG*，在血清型 29、35F、39、47F 中是 *wcjH*，都负责合成磷酸半乳糖基转移酶；在血清型 4、5、12F、12A、45 中是 *wciI*，合成磷酸-N-乙酰葡萄糖胺或 N-乙酰半乳糖胺转移酶。唯一的例外是血清型 1 的基因簇中无 PGT 基因，因为它的起始单糖(AAT-Gal)是细胞壁中磷壁酸的一个组分，可能由磷壁酸合成途径中的 *Spr1655* 将其转移到脂载体上[111]。一般寡糖中的每个单糖均需要一个相应的糖基转移酶负责转移。糖基转移酶基因的特异性非常高，拥有大量的糖基转移酶基因是肺炎链球菌荚膜多糖多种多样的遗传基础。根据糖基转移酶序列的相似性、反应机制和预测的结构，CAZy 数据库(carbohydrate-active enzyme database)将它们分为不同的组，绝大多数肺炎链球菌的糖基转移酶分布在 GT2 和 GT4 组中[112]。另外，每个基因簇中都有转位酶基因(*wzx*)和聚合酶基因(*wzy*)，但是它们在不同血清型中的相似性并不高，是血清型特异的基因。图 1 中的血清型 9V 和 18C 的基因簇中还有不同的 O-乙酰基转移酶基因，对多糖中不同的单糖进行 O-乙酰化修饰，增加多糖结构的多样性，O-乙酰化修饰在文献[113]中已经详细描述。

多糖的合成需要核苷二磷酸单糖作为供体糖。在组成荚膜多糖的 18 种单糖中，7 种最常见的单糖由细菌的管家代谢途径(house-keeping pathway)合成，9 种单糖以及糖醇磷酸和甘油磷酸都需要 *cps* locus 中特有的基因合成，如图 2 所示。

2.2 荚膜多糖的合成机制

2.2.1 Wzx/Wzy-依赖的合成途径(Wzx/Wzy-dependent pathway)

绝大多数荚膜多糖都依赖于 Wzx/Wzy 途径合成[114]，本文以血清型 14 为代表阐述该合成途径(图 3)。

血清型 14 的荚膜多糖含有 4 个单糖，包括主链上的 3 个单糖和 1 个支链半乳糖(图 3B)。合成 CPS14 的基因簇含有 13 个基因(图 3A)，其中 2 个糖基转移酶基因(*wchN*、*wciY*)内部突变失去功能，另外还有 4 个糖基转移酶基因是完整的，具有功能。多糖在嵌入细胞膜内侧的脂载体(lipid-carrier) 即磷酸十二丙烯酯(undecaprenyl-phosphate, Und-P)上进行合成(图 3C)。第 1 步：需要合成核苷二磷酸单糖作为供体糖(图 2)。第 2 步：在磷酸葡萄糖基转移酶 WchA 的作用下将 UDP-葡萄糖(UDP-glucose, UDP-Glc)的葡萄糖-1-磷酸(glucose-1-phosphate, Glc-1-P)连接到脂载体 Und-P 上合成 Und-PP-葡萄糖；随后在糖基转移酶 WchK 的作用下将 UDP-半乳糖(UDP-galactose, UDP-Gal)的半乳糖连接到葡萄糖上合成 Und-PP-双糖；然后在糖基转移酶 WchL 的作用下将 UDP-N-乙酰葡萄糖胺(UDP-N-acetylglucosamine, UDP-GlcNAc)的 N-乙酰葡萄糖胺连接到半乳糖上合成 Und-PP-三糖；最后在糖基转移酶 WchM 的作用下将 UDP-半乳糖的半乳糖作为支链连接到 N-乙酰葡萄糖胺上合成了 Und-PP-四糖，作为一个寡糖单元。第 3 步：跨膜的转位酶 Wzx 将寡糖单元从细胞膜内侧转移到细胞膜外侧。第 4 步：在周质区中，聚合酶 Wzy 将多个寡糖单元通过糖苷键连接在一起合成多糖链。第 5 步：多糖链的合成及长度受络氨酸激酶磷酸化系统(tyrosine kinase phosphoregulatory system)调控。在 4 个调控蛋白中，Wzg (CpsA)的功能未知；Wzh (CpsB)是一个依赖于镁离子的络氨酸磷酸化酶；Wze (CpsD)是一个酪氨酸激酶；Wzd (CpsC)是一个跨膜蛋白，其 N 端和 C 端都在细胞膜内侧起始 Wze (CpsD)的络氨酸自身磷酸化。相反，Wzh (CpsB)使 Wze (CpsD)去磷酸化，并阻止磷酸基团在该蛋白之间的转移。因此，通过 Wze (CpsD)蛋白

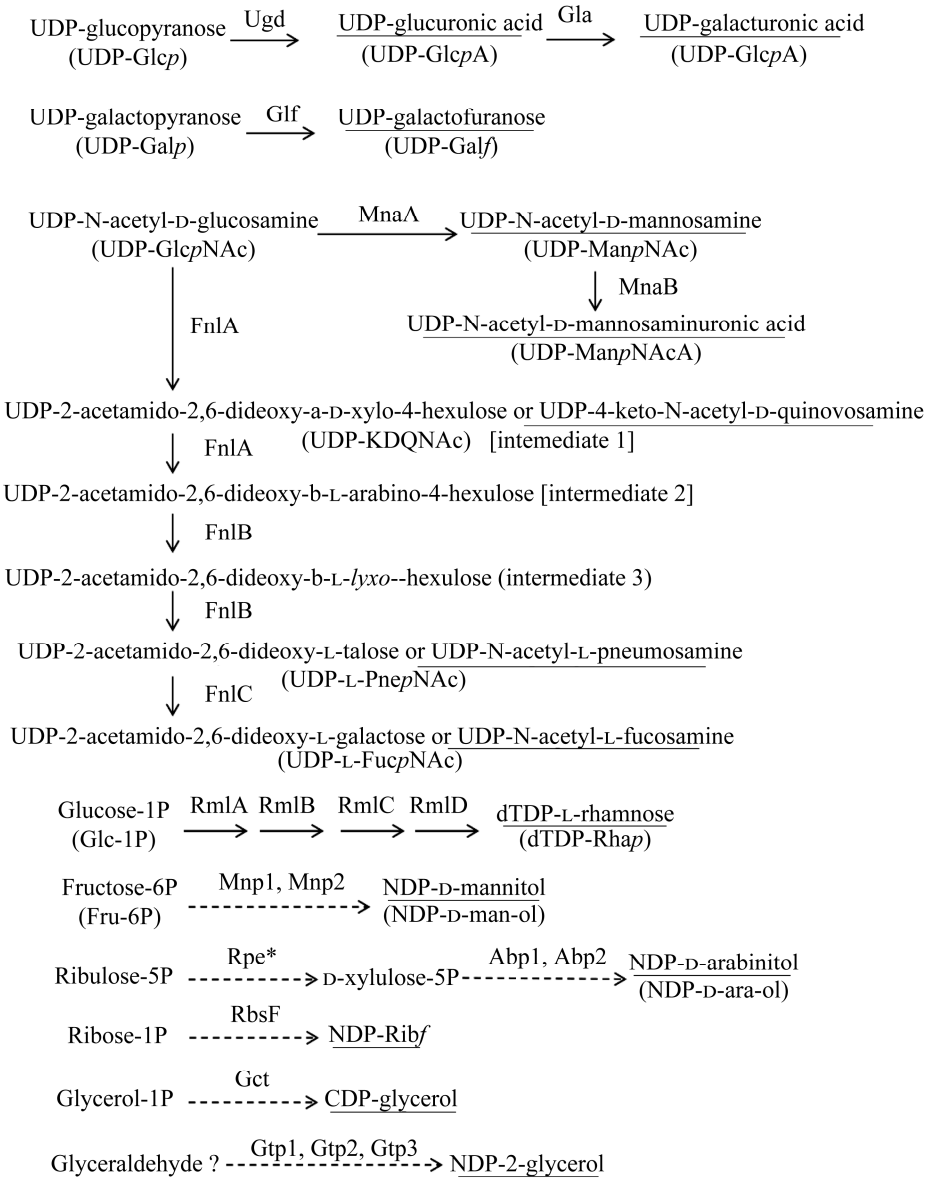

图 2　肺炎链球菌荚膜多糖中特殊组分的合成途径[109]

Figure 2　Biosynthetic pathways of CPS-specific components of *Streptococcus pneumoniae*[109]. Putative pathways are denoted by a dotted line, and the constituents of the repeat units are underlined. All gene products are encoded within the *cps* loci, except for ribulose phosphate 3-epimerase (Rpe), which is chromosomally encoded and marked by an asterisk. Ugd: UDP-glucose 6-dehydrogenase; Gla: UDP-galacturonate 4-epimerase; Gl*f*: UDP-galactopyranose mutase; MnaA: UDP-N-acetylglucosamine-2-epimerase; MnaB: UDP-N-acetylmannosamine dehydrogenase; FnlA: Steps 1 and 2 of UDP-FucNAc synthesis; FnlB: Steps 3 and 4 of UDP-FucNAc synthesis; FnlC: Step 5 of UDP-FucNAc synthesis; RmlA: Glucose-1-phosphate thymidylyltransferase; RmlB: dTDP-D-glucose 4,6-dehydratase; RmlC: dTDP-4-keto-6-deoxy-D-glucose3,5-epimerase; RmlD: dTDP-4-keto-L-rhamnose reductase; Mnp1: Putative nucleotidyl-transferase; Mnp2: Putative reductase; RbsF: Putative epimerase/dehydratase; Gct: CDP-glycerol biosynthetic protein; Gtp1-3: NDP-2-glycerol pathway; Abp1: Putative nucleotidyltransferase; Abp2: Putative reductase.

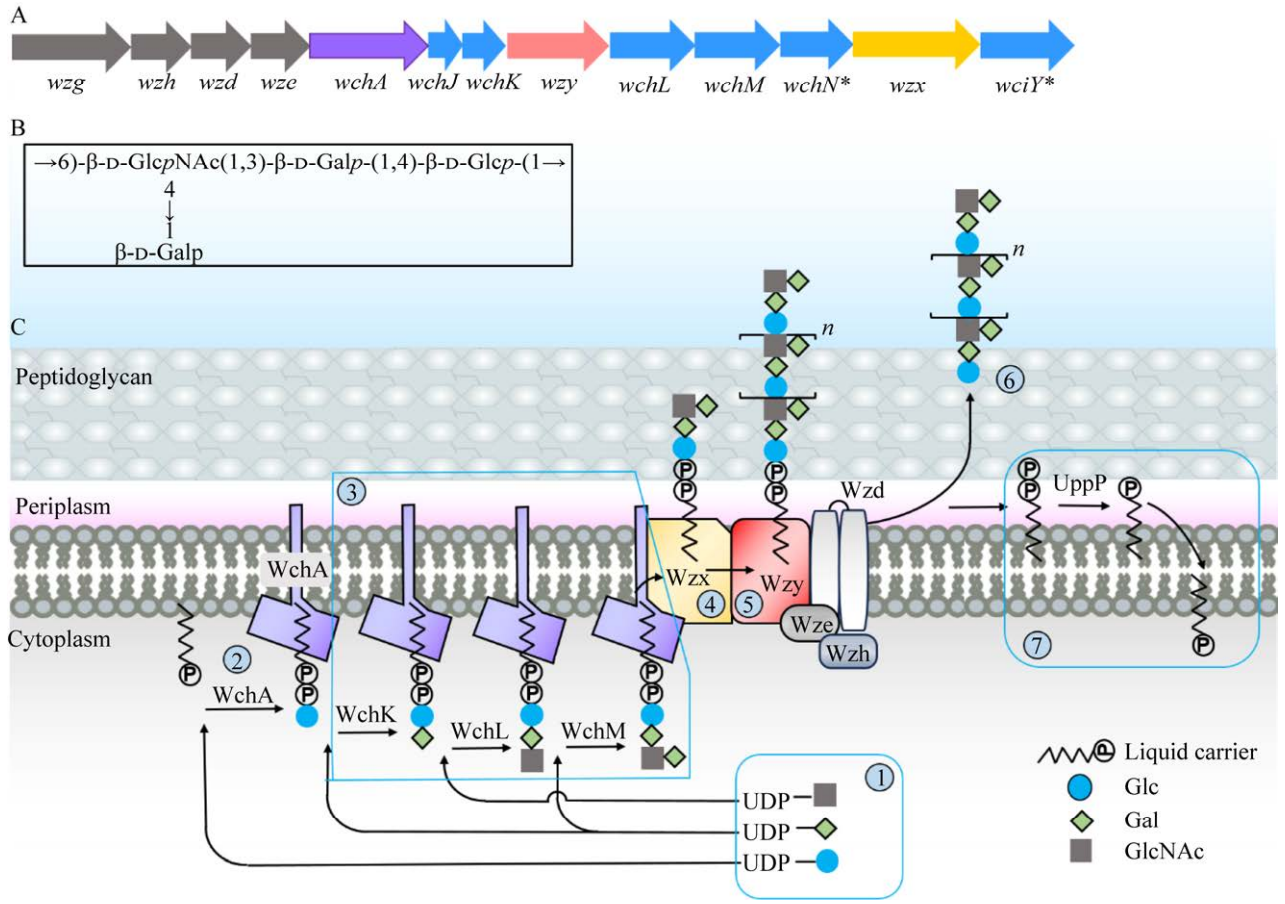

图 3　Wzx/Wzy-依赖的合成途径模式图　A：合成 CPS14 的基因簇. B：CPS14 的结构. C：CPS14 的合成过程

Figure 3　Representation of the Wzx/Wzy-dependent pathway for biosynthesis of capsular polysaccharide of serotype 14. A hypothetical model based on experimental evidence and theoretical speculation. A: *cps*14 locus. *: Pseudogene. B: CPS14 structure. C: Biosynthesis of CPS14.

的磷酸化和去磷酸化以及聚合酶共同调控糖链的合成和长度[115-116]。第 6 步：在调控蛋白和聚合酶的作用下将多糖链连接到细胞壁肽聚糖的 β-N-乙酰葡萄糖胺上[117-118]。目前将多糖链转移到细胞壁上的机制还不完全清楚。第 7 步：释放出的 Und-PP 被一个磷酸化酶切割成 Und-P，返回到细胞膜内侧被循环利用。

2.2.2　合酶-依赖的合成途径(synthase-dependent pathway)

血清型 3 和 37 的荚膜多糖是由合酶-依赖的途径合成。它们的多糖结构简单，都是由 2 个单糖组成，在血清型 3 中是线性排列，在血清型 37 中是支链排列(表 1)。合成机制并不复杂，现以血清型 3 为例阐述该合成途径，如图 4 所示。

血清型 3 荚膜多糖合成基因簇(图 4A)中的 *wzd*、*galU*、*pgm* 基因都突变失去功能，只有 2 个基因 *ugd* (又名 *cps3D*)和 *wchE* (又名 *cps3S*)是必需基因[119-125]。Cps3D 是一个葡萄糖脱氢酶，将 UDP-葡萄糖转化为 UDP-葡萄糖醛酸(UDP-glucuronic acid, UDP-GlcA)；Cps3S 是一

个跨膜的合酶(synthase)。首先，在细胞膜内侧，Cps3S 将 UDP-葡萄糖的葡萄糖连接到嵌入细胞膜的磷脂酰甘油(phosphatidyl glycerol)上；然后再将 UDP-葡萄糖醛酸的葡萄糖醛酸连接到葡萄糖上合成双糖(图 4B)；最后在细胞膜内侧将双糖单位连接在一起形成多糖链。在合适的条件下，多糖链被 Cps3S 转运到细胞膜外侧并继续延伸，多糖链的长度由 UDP-Glc 和 UDP-GlcA 的比例决定。当 GlcA 不足时，多糖停止合成并被释放到细胞外，并不是连接到细胞壁的肽聚糖上。至于血清型 37，其位于 *dexB* 和 *aliA* 基因之间的基因簇不能转录，失去功能，而负责其荚膜多糖合成的唯一合酶基因 *tts* 位于染色体的其他位置[126]。

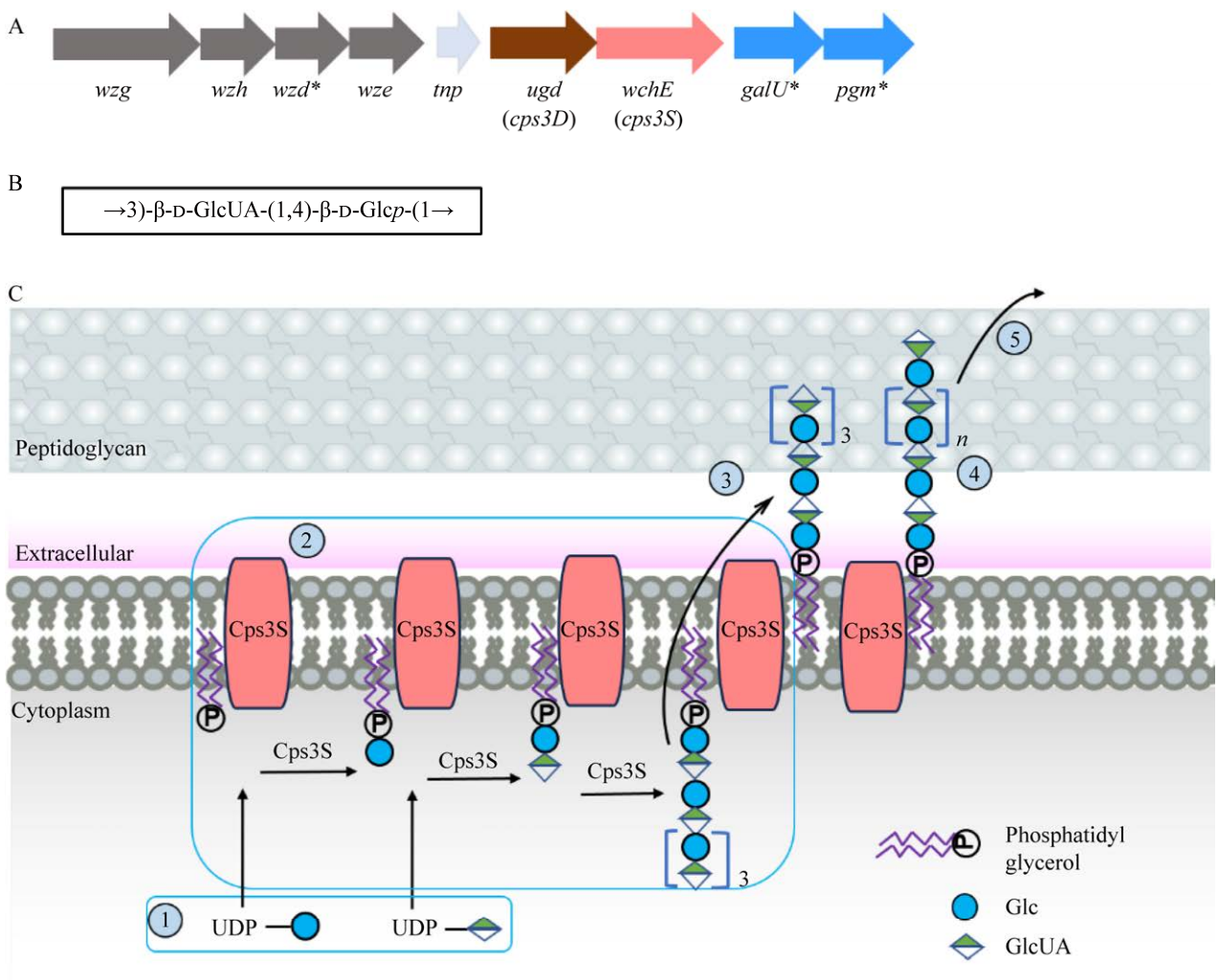

图 4　合酶依赖的合成途径模式图(修改自文献[109])　A：合成 CPS3 的基因簇. B：CPS3 的结构. C：CPS3 的合成过程

Figure 4　Representation of the synthase-dependent pathway for biosynthesis of capsular polysaccharide of serotype 3 (modified from reference [109]). A hypothetical model based on experimental evidence and theoretical speculation. A: *cps*3 locus. *: Pseudogene. B: CPS3 structure. C: Biosynthesis of CPS3.

3 荚膜多糖的纯化

高纯度的荚膜多糖对于准确鉴定多糖结构和制备合格的糖疫苗都十分重要。然而，纯化荚膜多糖是比较困难的，除了要去除大量的非糖物质，还要去除其他多糖尤其是细胞壁多糖(CWPS)的污染。CWPS 是由寡糖重复单位聚合而成线性多糖，含有磷酸胆碱，又称为 C-多糖或磷壁酸，有 3 种形式(表 1)，在血清型 4、7F、14 中含量很高。与荚膜多糖一样，CWPS 也带有负电荷，也是通过共价键连接到肽聚糖上，因此很难将 CWPS 完全除去，需要多个纯化步骤才能减少其污染，但是多糖的产量也会相应减少。其他的污染物还有蛋白质和核酸，根据世界卫生组织的数据，蛋白质和核酸污染物的理想量分别低于 3%和 2%[127]。总之，多糖的纯度和产量以及工艺简化都是纯化多糖中要考虑的因素。肺炎链球菌荚膜多糖的纯化方法主要包括醇分级沉淀、超滤、透析、离子交换柱层析、凝胶过滤层析和亲和色谱等方法，并在此基础上根据不同血清型的特征和多糖结构进行调整。

3.1 传统的荚膜多糖纯化方法

从细菌培养液的上清中纯化荚膜多糖要比从细胞中纯化荚膜多糖简单。1980 年，Cano 等[128]最早提出了荚膜多糖的纯化步骤，包括 5 步的乙醇分级沉淀，十六烷基三甲基溴化铵(cetyl trimethylammonium bromide, CTAB)沉淀去除上清中的蛋白质和核酸，用活性炭纯化多糖，通过透析去除小分子物质。这些过程去除了大部分污染物，同时保留了产品的免疫原性。然而，该方法步骤繁多，既复杂又耗时，而且为记录产量和最终的纯度结果。1979 年，默克公司的专利[129]提出了一种多糖纯化工艺，包括乙醇、异丙醇和西曲溴铵沉淀，蛋白酶和核酸酶处理和透析，最后从 14 L 培养物中提取了 0.35 g (1 型)至 4.6 g (2 型)纯化的荚膜多糖。1981 年，梅里埃研究所(Institut Mérieux)为另一种肺炎链球菌荚膜多糖纯化方法申请了专利[130]，他们用半合成培养基培养肺炎链球菌，用 0.1%脱氧胆酸(deoxycholic acid, DOC)裂解细胞，上清液用乙醇沉淀、苯酚抽提、活性炭过滤、超滤，经过 11 步纯化，最后血清型 1、2、4 的荚膜多糖回收率分别为 0.4、0.3、0.1 g/L。该专利强调了培养基对于多糖纯度的重要性。1998 年，Arnold[131]为 23 个血清型荚膜多糖的纯化方法申请了专利，该专利表明，除血清型 7F、14 和 33F 产生的中性多糖外，其他酸性多糖均与 1%−4%的 CTAB 沉淀后用活性炭过滤，最后用羟基磷灰石色谱法进行纯化，大大提高了多糖的纯度。

这些传统方法通常会使用某些有毒或腐蚀性试剂，如苯酚用于杀死细菌和去除蛋白质；用 DOC 裂解细菌时也将细胞内大量核酸和蛋白质释放到培养基中，会增加纯化步骤以去除这些污染物，最终导致产量降低和成本增加。因此，后来发展出了一些新的荚膜多糖纯化方法，旨在优化纯化流程并提高产量和纯度。

3.2 改进的荚膜多糖纯化方法

Suárez 等[132]建立了用大豆凝集素亲和色谱纯化血清型 14 的荚膜多糖，同时省去了用溶剂和酶处理样品；该方法比传统的纯化方法更快，实现了高于 99%的纯度。然而该方法的分离量仅限于几毫克荚膜多糖，而且凝集素昂贵，如果扩大生产成本会很高。Gonçalves 等[133-134]用超滤膜过滤上清液并结合乙醇沉淀纯化血清型 23F 和 6B 的荚膜多糖。根据多糖的分子量，使用截留量为 30 kDa 或 100 kDa 的超滤膜浓缩样品，用不同浓度的乙醇分级沉淀，实现了 89%的多糖回收率，蛋白质和核酸的污染小于 2%[133-134]。Jung 等[135]简化了血清型 19A 的纯化步骤，包括调节细菌裂解液的 pH 至 4.5 以沉淀可溶性蛋白

质或其他可溶性组分，然后用 50%−80%乙醇分级沉淀多糖，最终获得了 75%的回收率和 97%的纯度。然而，此方法仅适用于耐酸多糖。Macha等[136]利用 DOC 裂解细菌，然后使用 30 kDa 超滤膜过滤，乙醇沉淀并进行磷酸铝吸附，多糖回收率达到 65%−80%，杂质小于 1.5%。这种方法的优点是用磷酸铝代替了苯酚以及核酸酶和蛋白酶的使用。此外，Zanardo 等[137]评估了血清型 14 的新纯化工艺，包括用化学培养基(chemical medium, CDM)培养细菌，离心除去细菌并过滤上清液，然后用 50 kDa 超滤膜超滤浓缩上清液；用 30 kDa 超滤膜在十二烷基硫酸钠存在下进行渗滤去除 7%的蛋白质和 68%的核酸；再用 5%三氯乙酸进一步去除蛋白质，用 20%和 60%乙醇分级沉淀多糖；最后用琼脂糖树脂(Q-Sepharose FF resin)进行阴离子交换柱层析纯化多糖。该方法多糖的回收率为 65%，核酸含量小于2%和蛋白质含量小于3%。此外，Gaikwad等[138]在纯化血清型 2 荚膜多糖时，使用三氟乙酸处理多糖，使杂质被沉淀，同时 CPS 部分被解聚但不会失去抗原性，纯化出来的多糖可以直接用于疫苗生产。

为了减少 CWPS 的污染，Lee 等[139]通过改进超滤和 CTAB 沉淀步骤，包括在细菌裂解后用乙酸沉淀去除蛋白质，在超滤后用 20 倍体积的纯水清洗样品，并将CTAB浓度从原来的0.7%提高到 2.0%，显著降低了血清型 5 荚膜多糖中的 CWPS 污染，核酸污染也得到了改善，这种方法也成功地应用于其他 14 种血清型。然而，将纯化的多糖偶联蛋白免疫兔子后，抗体检测表明，用改进方法纯化的多糖具有更高的免疫原性[139]。此外，将多糖溶解在乙酸钠中再用亚硝酸盐处理可以去除 CWPS[140]。用 3%过氧化氢和核酸酶处理可以去除粗多糖中的蛋白质和核酸污染，该方法纯化的荚膜多糖适用于糖偶联疫苗生产[134]。为了解析多糖的结构，我们也纯化了多个血清型的荚膜多糖。通过 THB 培养基培养细菌，离心除去细胞并用 0.22 μmol/L 滤膜过滤上清液，然后用 100 kDa 超滤膜过滤浓缩上清液。用核酸酶和蛋白酶降解核酸和蛋白质，用变溶菌素(mutanolysin)降解荚膜多糖和 CWPS 以及细胞壁肽聚糖之间的糖苷键，用三氯乙酸去除所有蛋白质得到粗多糖。然后用 DEAE 阴离子交换柱层析和氯化钠梯度洗脱多糖，进一步用S300 分子筛纯化多糖，最后透析除去小分子物质，获得高纯度的荚膜多糖。其中蛋白质和核酸的含量都小于 1%；CWPS 的含量小于 5%，该纯度可以用 NMR 方法准确鉴定多糖结构[44-46,100,141]。

4 总结与展望

本文汇总了目前已知的肺炎链球菌 95 个血清型的荚膜多糖结构，探讨了荚膜多糖的生物合成和分离纯化方法。在细菌荚膜多糖的遗传、结构和生物学功能的研究中，多糖结构的鉴定扮演一个关键角色。尽管可以利用生物信息学分析推测基因编码的蛋白质的功能，但是，如果没有多糖结构，大量的高度特异性的糖基转移酶的确切功能很难被确定。同时有的 O-乙酰基转移酶基因的核苷酸序列是完整的，但是可能并不表达，只有知道多糖结构才能确定这些基因是否发挥功能。因此，解析多糖结构是鉴定血清型特异的基因功能的主要手段。糖工程技术是通过遗传改造多糖合成相关基因，并解析基因改变后菌株的多糖结构，最后比较多糖结构来鉴定基因的功能。通过该技术我们已经鉴定了链球菌中多个糖基转移酶和乙酰基转移酶的功能[100,142]，也正在研究荚膜多糖的支链结构和 O-乙酰化修饰与细菌毒力的关系。另一方面，荚膜多糖是抗原，多糖结构决定了细菌的血清学特征。多糖主链结构、支链结构、乙酰化修饰和磷酸化修饰等都可

能作为抗原决定簇刺激宿主产生不同的抗体。也只有知道多糖结构，才能揭示多糖的抗原表位，发现遗传基础-抗原表位-血清学特征之间的对应关系，以及多糖结构组成与细菌的致病性的关系。因此，深入解析多糖结构对于理解其功能至关重要，也为抗体和疫苗的研究奠定基础。

荚膜多糖的结构与肺炎链球菌的毒力密切相关。研究发现，肺炎链球菌荚膜多糖的结构影响荚膜的厚度，而荚膜越厚越有利于肺炎链球菌在鼻咽道中繁殖和传播[18,143]，荚膜多糖上的负电荷可阻止巨噬细胞的吞噬和黏液的清除[144]。此外，荚膜多糖的组成也影响细菌的感染，如血清型 19A/19F 和 6A/6B 的荚膜多糖中都含有葡萄糖-α1-2-鼠李糖(glucose-α1-2-rhamnose, Glcα1-2Rha)的结构，它们在鼻咽道中形成生物被膜的能力强，延长了细菌在鼻咽道中的存活时间[19]。我们的研究也表明荚膜多糖支链结构和O-乙酰化修饰影响多糖的抗原性和免疫原性[44,45,46,100]。2022 年 An 等发现，在脓毒症小鼠模型中，肝脏的巨噬细胞 Kupffer 细胞能够有效清除血清型 7F 和 14，因为它们的荚膜多糖结构可以被 Kupffer 细胞上的去唾液酸糖蛋白受体(sialic acid glycoprotein receptors, ASGR)识别[22]。2023 年 Chun 等[145]报道，荚膜多糖的结构影响肺炎链球菌在人呼吸道上皮细胞的增殖；他们发现富含鼠李糖的多糖和模拟宿主糖链的多糖能够影响细菌对呼吸道细胞的黏附；发现血清型 2 和 31 的荚膜多糖含有多个鼠李糖，血清型 14 的荚膜多糖结构几乎和人神经酰胺糖链乳糖-N-新四糖(lacto-N-neotetraose, nLC4)结构一样，这样的血清型更容易黏附呼吸道上皮细胞并引起炎症反应。这些研究都揭示多糖上部分糖结构(glycomotif)在细菌致病性上扮演重要角色。

测序技术的迅速发展使人们很容易获得野生菌株和临床菌株的荚膜多糖合成基因簇以及全基因组序列。然而对它们产生的多糖结构的鉴定要困难得多，滞后得多。目前的多糖鉴定技术如 NMR 和色谱技术都是 20 世纪 70 年代发展起来的技术，需要高纯度的多糖才能准确解析液态多糖的结构。然而多糖的纯化步骤繁多复杂，简化多糖的纯化工艺同时保证多糖纯度仍然是多糖结构研究中的一个瓶颈。另一方面需要分析技术的突破性进步。比如不用分离纯化多糖就能直接鉴定细菌表面多糖的结构。由于糖本身的柔性特点，很难用 X-射线衍射分析多糖特征。近年发展的固态 NMR (solid-state NMR, ssNMR)技术能够分析生理条件下如完整细胞、生物被膜中的多糖组成和构象变化，极大地推动了多糖结构和功能的研究[146-147]。目前利用固态 NMR 技术在细菌全细胞中直接分析了细胞壁肽聚糖的结构[148]；分析了完整脂多糖的结构[149]；在活的霉菌和真菌以及完整的植物组织中直接鉴定了细胞壁中的多糖组成[147,150]。固态 NMR 技术仍然在发展和完善中，相信在不远的将来，能够实现从肺炎链球菌中直接解析表面荚膜多糖结构的目标。

荚膜多糖的纯化对于制备肺炎链球菌疫苗至关重要。纯化方法的进步旨在提高多糖纯度和产量并简化纯化过程，以降低疫苗价格，使多糖疫苗和糖蛋白结合疫苗能够在中低收入国家大规模推广应用。需要注意的是，随着疫苗的应用，出现血清型替换，降低了糖疫苗的效率，也导致糖疫苗的组成需要不断更新，以加入新流行株的荚膜多糖。另外，随着荚膜多糖结构的解析，人们开始关注荚膜多糖的化学合成和生物合成。据报道，目前已经通过糖化学方法合成了血清型 1、2、3、4、5、6、7F、8、9V、12F、14、17F、19F、22F、23F 的低聚寡糖，并应用于疫苗的生产，但仍存在难以控制化学结构和低聚糖的长度以及去除杂质等问题[151]。2022 年，北京大学叶

新山团队自主研发了新型双模式液相糖自动合成仪，并利用该自动合成仪合成了复杂结构的寡糖和高分子量的阿拉伯聚糖[152]。随着糖化学合成技术的不断进步，荚膜多糖也成为越来越有吸引力的合成靶标。化学合成的荚膜多糖不仅可用于开发多糖疫苗[153-154]，也能用于多糖的生物学功能研究。

未来研究可以从以下 4 个方面展开。(1) 解析荚膜多糖的结构。利用 ssNMR 技术结合其他的结构分析方法，在不破坏细胞的条件下鉴定多糖结构和空间构象；研究新的技术方法快速纯化多糖。(2) 鉴定糖基转移酶的功能和特征。可通过糖工程技术鉴定糖基转移酶的功能[44-45]，也可表达糖基转移酶基因，与脂连接的糖受体和核苷糖供体进行糖基化反应来鉴定糖基转移酶的功能[155]；虽然越来越多的肺炎链球菌全基因组被测序，为预测基因的功能创造了条件，但是还缺乏利用糖基转移酶的信息来预测荚膜多糖结构的方法。(3) 荚膜多糖结构和功能的关系。研究荚膜多糖的结构组成和修饰对细菌毒力的影响，揭示细菌不断进化和产生新的血清型的机制。(4) 荚膜多糖的合成和调控机制。细菌在定殖和侵染宿主的过程中随着环境的改变调节其荚膜多糖的合成，调控蛋白以及外界因素如何共同作用调控多糖的合成还不完全清楚。鉴于荚膜多糖对于肺炎链球菌毒力的重要性，荚膜多糖合成和调控机制的研究可能是新型抗菌策略的突破口。

参考文献

[1] WEISER JN, FERREIRA DM, PATON JC. *Streptococcus pneumoniae*: transmission, colonization and invasion[J]. Nature Reviews Microbiology, 2018, 16(6): 355-367.

[2] IKUTA KS, SWETSCHINSKI LR, AGUILAR GR, SHARARA F, MESTROVIC T, AUTHIA P GRAY, WEAVER ND. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the global burden of disease study 2019[J]. The Lancet, 2022, 400(10369): 2221-2248.

[3] RUDNIC E. Actual considerations in pneumococcal meningitis[J]. Revista Medico-Chirurgicala a Societatii de Medici si Naturalisti Din Iasi, 2000, 104(3): 37-42.

[4] DOCHEZ AR, AVERY OT. The elaboration of specific soluble substance by pneumococcus during growth[J]. The Journal of Experimental Medicine, 1917, 26(4): 477-493.

[5] AVERY OT, MORGAN HJ. Immunological reactions of the isolated carbohydrate and protein of pneumococcus[J]. The Journal of Experimental Medicine, 1925, 42(3): 347-353.

[6] KAUFFMANN F, MØRCH E, SCHMITH K. On the serology of the pneumococcus-group[J]. Journal of Immunology, 1940, 39(5): 397-426.

[7] LUND E. On the nomenclature of the pneumococcal types[J]. International Journal of Systematic Bacteriology, 1970, 20(3): 321-323.

[8] BECKLER E, MACLEOD P. The neufeld method of pneumococcus type determination as carried out in a public health laboratory: a study of 760 typings[J]. Journal of Clinical Investigation, 1934, 13(6): 901-907.

[9] BRATCHER PE, KIM KH, KANG JH, HONG JY, NAHM MH. Identification of natural pneumococcal isolates expressing serotype 6D by genetic, biochemical and serological characterization[J]. Microbiology (Reading), 2010, 156(Pt 2): 555-560.

[10] CALIX JJ, NAHM MH, ZARTLER ER. Elucidation of structural and antigenic properties of pneumococcal serotype 11A, 11B, 11C, and 11F polysaccharide capsules[J]. Journal of Bacteriology, 2011, 193(19): 5271-5278.

[11] CAMILLI R, VESCIO MF, GIUFRÈ M, DAPRAI L, GARLASCHI ML, CERQUETTI M, PANTOSTI A. Carriage of haemophilus influenzae is associated with pneumococcal vaccination in Italian children[J]. Vaccine, 2015, 33(36): 4559-4564.

[12] PARK IH, GENO KA, YU JG, OLIVER MB, KIM KH, NAHM MH. Genetic, biochemical, and serological characterization of a new pneumococcal serotype, 6H, and generation of a pneumococcal strain producing three different capsular repeat units[J]. Clinical and Vaccine Immunology, 2015, 22(3): 313-318.

[13] GANAIE F, SAAD JS, MCGEE L, van TONDER AJ, BENTLEY SD, LO SW, GLADSTONE RA, TURNER P, KEENAN JD, BREIMAN RF, NAHM MH. A new pneumococcal capsule type, 10D, is the 100th serotype

and has a large *cps* fragment from an oral *Streptococcus*[J]. mBio, 2020, 11(3): e00937-20.

[14] ROBBINS JB, AUSTRIAN R, LEE CJ, RASTOGI SC, SCHIFFMAN G, HENRICHSEN J, MAKELA PH, BROOME CV, FACKLAM RR, TIESJEMA RH, PARKE JC. Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups[J]. The Journal of Infectious Diseases, 1983, 148(6): 1136-1159.

[15] CHEN KL, ZHANG XY, SHAN W, ZHAO GM, ZHANG T. Serotype distribution of *Streptococcus pneumoniae* and potential impact of pneumococcal conjugate vaccines in China: a systematic review and meta-analysis[J]. Human Vaccines & Immunotherapeutics, 2018, 14(6): 1453-1463.

[16] HAUSDORFF WP, BRYANT J, PARADISO PR, SIBER GR. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I[J]. Clinical Infectious Diseases: an Official Publication of the Infectious Diseases Society of America, 2000, 30(1): 100-121.

[17] KELLER LE, ROBINSON DA, MCDANIEL LS. Nonencapsulated *Streptococcus pneumoniae*: emergence and pathogenesis[J]. mBio, 2016, 7(2): e01792.

[18] WEINBERGER DM, TRZCIŃSKI K, LU YJ, BOGAERT D, BRANDES A, GALAGAN J, ANDERSON PW, MALLEY R, LIPSITCH M. Pneumococcal capsular polysaccharide structure predicts serotype prevalence[J]. PLoS Pathogens, 2009, 5(6): e1000476.

[19] DOMENECH M, ARAÚJO-BAZÁN L, GARCíA E, MOSCOSO M. *In vitro* biofilm formation by *Streptococcus pneumoniae* as a predictor of post-vaccination emerging serotypes colonizing the human nasopharynx[J]. Environmental Microbiology, 2014, 16(4): 1193-1201.

[20] ZAFAR MA, HAMAGUCHI S, ZANGARI T, CAMMER M, WEISER JN. Capsule type and amount affect shedding and transmission of *Streptococcus pneumoniae*[J]. mBio, 2017, 8(4): e00989-17.

[21] SJÖSTRÖM K, SPINDLER C, ORTQVIST A, KALIN M, SANDGREN A, KÜHLMANN-BERENZON S, HENRIQUES-NORMARK BH. Clonal and capsular types decide whether pneumococci will act as a primary or opportunistic pathogen[J]. Clinical Infectious Diseases, 2006, 42(4): 451-459.

[22] AN HR, QIAN CY, HUANG YJ, LI J, TIAN XB, FENG JY, HU J, FANG YJ, JIAO FF, ZENG YN, HUANG XT, MENG XB, LIU X, LIN X, ZENG ZT, GUILLIAMS M, BESCHIN A, CHEN YW, WU YZ, WANG J, et al. Functional vulnerability of liver macrophages to capsules defines virulence of blood-borne bacteria[J]. The Journal of Experimental Medicine, 2022, 219(4): e20212032.

[23] BRILES DE, CRAIN MJ, GRAY BM, FORMAN C, YOTHER J. Strong association between capsular type and virulence for mice among human isolates of *Streptococcus pneumoniae*[J]. Infection and Immunity, 1992, 60(1): 111-116.

[24] AUSTRIAN R. Some observations on the pneumococcus and on the current status of pneumococcal disease and its prevention[J]. Reviews of Infectious Diseases, 1981, 3(supplement_1): S1-S17.

[25] YOTHER J. Capsules of *Streptococcus pneumoniae* and other bacteria: paradigms for polysaccharide biosynthesis and regulation[J]. Annual Review of Microbiology, 2011, 65: 563-581.

[26] PATON JC, TRAPPETTI C. *Streptococcus pneumoniae* capsular polysaccharide[J]. Microbiology Spectrum, 2019, 7(2): 30977464.

[27] BEITER K, WARTHA F, ALBIGER B, NORMARK S, ZYCHLINSKY A, HENRIQUES-NORMARK B. An endonuclease allows *Streptococcus pneumoniae* to escape from neutrophil extracellular traps[J]. Current Biology, 2006, 16(4): 401-407.

[28] WARTHA F, BEITER K, ALBIGER B, FERNEBRO J, ZYCHLINSKY A, NORMARK S, HENRIQUES-NORMARK B. Capsule and D-alanylated lipoteichoic acids protect *Streptococcus pneumoniae* against neutrophil extracellular traps[J]. Cellular Microbiology, 2007, 9(5): 1162-1171.

[29] de VOS AF, DESSING MC, LAMMERS AJJ, de PORTO APNA, FLORQUIN S, de BOER OJ, de BEER R, TERPSTRA S, BOOTSMA HJ, HERMANS PW, van't VEER C, van der POLL T. The polysaccharide capsule of *Streptococcus pneumonia* partially impedes MyD88-mediated immunity during pneumonia in mice[J]. PLoS One, 2015, 10(2): e0118181.

[30] SKOV SØRENSEN UB, BLOM J, BIRCH-ANDERSEN A, HENRICHSEN J. Ultrastructural localization of capsules, cell wall polysaccharide, cell wall proteins, and F antigen in pneumococci[J]. Infection and Immunity, 1988, 56(8): 1890-1896.

[31] WEISER JN, AUSTRIAN R, SREENIVASAN PK, MASURE HR. Phase variation in pneumococcal opacity: relationship between colonial morphology and nasopharyngeal colonization[J]. Infection and Immunity, 1994, 62(6): 2582-2589.

[32] KIM JO, ROMERO-STEINER S, SØRENSEN UB, BLOM J, CARVALHO M, BARNARD S, CARLONE G, WEISER JN. Relationship between cell surface carbohydrates and intrastrain variation on opsonophagocytosis of *Streptococcus pneumoniae*[J]. Infection and Immunity, 1999, 67(5): 2327-2333.

[33] COLE R. Treatment of pneumonia by means of specific serums[J]. Journal of the American Medical Association, 1913, 61(9): 663-666.

[34] DOMINGUES CMAS, VERANI JR, MONTENEGRO RENOINER EI, de CUNTO BRANDILEONE MC, FLANNERY B, de OLIVEIRA LH, SANTOS JB, de MORAES JC, Brazilian Pneumococcal Conjugate Vaccine Effectiveness Study Group. Effectiveness of ten-valent pneumococcal conjugate vaccine against invasive pneumococcal disease in Brazil: a matched case-control study[J]. The Lancet Respiratory Medicine, 2014, 2(6): 464-471.

[35] JAIN S, WILLIAMS DJ, ARNOLD SR, AMPOFO K, BRAMLEY AM, REED C, STOCKMANN C, ANDERSON EJ, GRIJALVA CG, SELF WH, ZHU YW, PATEL A, HYMAS W, CHAPPELL JD, KAUFMAN RA, KAN JH, DANSIE D, LENNY N, HILLYARD DR, HAYNES LM, et al. Community-acquired pneumonia requiring hospitalization among U.S. children[J]. The New England Journal of Medicine, 2015, 372(9): 835-845.

[36] JONES C. Vaccines based on the cell surface carbohydrates of pathogenic bacteria[J]. Anais Da Academia Brasileira De Ciencias, 2005, 77(2): 293-324.

[37] DURANDO P, FAUST SN, FLETCHER M, KRIZOVA P, TORRES A, WELTE T. Experience with pneumococcal polysaccharide conjugate vaccine (conjugated to CRM197 carrier protein) in children and adults[J]. Clinical Microbiology and Infection, 2013, 19(Suppl 1): 1-9.

[38] ESPOSITO S, PRINCIPI N. Pneumococcal vaccines and the prevention of community-acquired pneumonia[J]. Pulmonary Pharmacology & Therapeutics, 2015, 32: 124-129.

[39] BOSCH A, van HOUTEN MA, BRUIN JP, WIJMENGA-MONSUUR AJ, TRZCIŃSKI K, BOGAERT D, ROTS NY, SANDERS EAM. Nasopharyngeal carriage of *Streptococcus pneumoniae* and other bacteria in the 7th year after implementation of the pneumococcal conjugate vaccine in the Netherlands[J]. Vaccine, 2016, 34(4): 531-539.

[40] WEINBERGER DM, MALLEY R, LIPSITCH M. Serotype replacement in disease after pneumococcal vaccination[J]. The Lancet, 2011, 378(9807): 1962-1973.

[41] KAMERLING JP. *Streptococcus pneumoniae*: Molecular Biology and Mechanisms of Disease[M]. Larchmont, NewYork: Mary Ann Liebert, Inc. Publishers, 2000: 88-114.

[42] PARK IH, PRITCHARD DG, CARTEE R, BRANDAO A, BRANDILEONE MCC, NAHM MH. Discovery of a new capsular serotype (6C) within serogroup 6 of *Streptococcus pneumoniae*[J]. Journal of Clinical Microbiology, 2007, 45(4): 1225-1233.

[43] LIN FL, VINOGRADOV E, DENG C, ZELLER S, PHELAN L, GREEN BA, JANSEN KU, PAVLIAK V. Structure elucidation of capsular polysaccharides from *Streptococcus pneumoniae* serotype 33C, 33D, and revised structure of serotype 33B[J]. Carbohydrate Research, 2014, 383: 97-104.

[44] YANG JH, SHELAT NY, BUSH CA, CISAR JO. Structure and molecular characterization of *Streptococcus pneumoniae* capsular polysaccharide 10F by carbohydrate engineering in *Streptococcus oralis*[J]. The Journal of Biological Chemistry, 2010, 285(31): 24217-24227.

[45] YANG JH, NAHM MH, BUSH CA, CISAR JO. Comparative structural and molecular characterization of *Streptococcus pneumoniae* capsular polysaccharide serogroup 10[J]. The Journal of Biological Chemistry, 2011, 286(41): 35813-35822.

[46] BUSH CA, CISAR JO, YANG JH. Structures of capsular polysaccharide serotypes 35f and 35c of *Streptococcus pneumoniae* determined by nuclear magnetic resonance and their relation to other cross-reactive serotypes[J]. Journal of Bacteriology, 2015, 197(17): 2762-2769.

[47] STROOP CJM, XU QW, RETZLAFF M, ABEYGUNAWARDANA C, BUSH CA. Structural analysis and chemical depolymerization of the capsular polysaccharide of *Streptococcus pneumoniae* type 1[J]. Carbohydrate Research, 2002, 337(4): 335-344.

[48] JANSSON PE, LINDBERG B, ANDERSON M, LINDQUIST U, HENRICHSEN J. Structural studies of

the capsular polysaccharide from *Streptococcus pneumoniae* type 2, a reinvestigation[J]. Carbohydrate Research, 1988, 182(1): 111-117.

[49] AVERY OT, GOEBEL WF. Chemoimmunological studies on the soluble specific substance of pneumococcus: i. The isolation and properties of the acetyl polysaccharide of pneumococcus type i[J]. The Journal of Experimental Medicine, 1933, 58(6): 731-755.

[50] JONES C, CURRIE F, FORSTER MJ. N.m.r. and conformational analysis of the capsular polysaccharide from *Streptococcus pneumoniae* type 4[J]. Carbohydrate Research, 1991, 221: 95-121.

[51] JANSSON PE, LINDBERG B, LINDQUIST U. Structural studies of the capsular polysaccharide from *Streptococcus pneumoniae* type 5[J]. Carbohydrate Research, 1985, 140(1): 101-110.

[52] REBERS PA, HEIDELBERGER M. The specific polysaccharide of type VI *Pneumococcus*. II.1 the repeating Unit2[J]. Journal of the American Chemical Society, 1961, 83(14): 3056-3059.

[53] KENNE L, LINDBERG B, MADDEN JK. Structural studies of the capsular antigen from *Streptococcus pneumoniae* type 26[J]. Carbohydrate Research, 1979, 73: 175-182.

[54] OLIVER MB, van der LINDEN MPG, KÜNTZEL SA, SAAD JS, NAHM MH. Discovery of *Streptococcus pneumoniae* serotype 6 variants with glycosyltransferases synthesizing two differing repeating units[J]. The Journal of Biological Chemistry, 2013, 288(36): 25976-25985.

[55] BURTON RL, GENO KA, SAAD JS, NAHM MH. *Pneumococcus* with the "6E" *cps* locus produces serotype 6b capsular polysaccharide[J]. Journal of Clinical Microbiology, 2016, 54(4): 967-971.

[56] WERREN JP, TROXLER LJ, OYEWOLE ORA, RAMETTE A, BRUGGER SD, BRUGGMANN R, van der LINDEN M, NAHM MH, GJUROSKI I, CASANOVA C, FURRER J, HILTY M. Carbon source-dependent changes of the structure of *Streptococcus pneumoniae* capsular polysaccharide with serotype 6F[J]. International Journal of Molecular Sciences, 2021, 22(9): 4580.

[57] MOREAU M, RICHARDS JC, PERRY MB, KNISKERN PJ. Application of high-resolution n.m.r. spectroscopy to the elucidation of the structure of the specific capsular polysaccharide of *Streptococcus pneumoniae* type 7F[J]. Carbohydrate research, 1988, 182(1): 79-99.

[58] BACKMAN-MARKLUND I, JANSSON PE, LINDBERG B, HENRICHSEN J. Structural studies of the capsular polysaccharide from *Streptococcus pneumoniae* type 7A[J]. Carbohydrate Research, 1990, 198(1): 67-77.

[59] JANSSON PE, LINDBERG J, WIMALASIRI KM, HENRICHSEN J. The structure of the capsular polysaccharide from *Streptococcus pneumoniae* type 7B[J]. Carbohydrate Research, 1991, 217: 171-180.

[60] KJELDSEN C, SLOTT S, ELVERDAL PL, SHEPPARD CL, KAPATAI G, FRY NK, SKOVSTED IC, DUUS JØ. Discovery and description of a new serogroup 7 *Streptococcus pneumoniae* serotype, 7D, and structural analysis of 7C and 7D[J]. Carbohydrate Research, 2018, 463: 24-31.

[61] SAKSOUK N, PELOSI L, COLIN-MOREL P, BOUMEDIENNE M, ABDIAN PL, GEREMIA RA. The capsular polysaccharide biosynthesis of *Streptococcus pneumoniae* serotype 8: functional identification of the glycosyltransferase WciS (Cap8H)[J]. The Biochemical Journal, 2005, 389(Pt 1): 63-72.

[62] CALIX JJ, SAAD JS, BRADY AM, NAHM MH. Structural characterization of *Streptococcus pneumoniae* serotype 9A capsule polysaccharide reveals role of glycosyl 6-O-acetyltransferase wcjE in serotype 9V capsule biosynthesis and immunogenicity[J]. The Journal of Biological Chemistry, 2012, 287(17): 13996-14003.

[63] RICHARDS JC, PERRY MB, KNISKERN PJ. Structural analysis of the specific polysaccharide of *Streptococcus pneumoniae* type 9L (American type 49)[J]. Canadian Journal of Biochemistry and Cell Biology, 1984, 62(12): 1309-1320.

[64] RUTHERFORD TJ, JONES C, DAVIES DB, ELLIOTT AC. NMR assignment and conformational analysis of the antigenic capsular polysaccharide from *Streptococcus pneumoniae* type 9N in aqueous solution[J]. Carbohydrate Research, 1994, 265(1): 79-96.

[65] JONES C. Full assignment of the NMR spectrum of the capsular polysaccharide from *Streptococcus pneumoniae* serotype 10A[J]. Carbohydrate Research, 1995, 269(1): 175-181.

[66] OLIVER MB, JONES C, LARSON TR, CALIX JJ, ZARTLER ER, YOTHER J, NAHM MH. *Streptococcus pneumoniae* serotype 11D has a bispecific glycosyltransferase and expresses two different capsular polysaccharide repeating units[J].

The Journal of Biological Chemistry, 2013, 288(30): 21945-21954.

[67] CALIX JJ, NAHM MH. A new pneumococcal serotype, 11E, has a variably inactivated *wcjE* gene[J]. Journal of Infectious Diseases, 2010, 202(1): 29-38.

[68] SACKETT K, BROWN P, DUTTA K, SCULLY IL, GANGOLLI S, LOOI K, NEMANI S, YU AYH, KLEVEN M, XIE J, MORAN J, PRIDE MW, ANDERSON AS, LOTVIN J. Identification of a novel keto sugar component in *Streptococcus pneumoniae* serotype 12F capsular polysaccharide and impact on vaccine immunogenicity[J]. The Journal of Immunology, 2023, 210(6): 764-773.

[69] LEONTEIN K, LINDBERG B, LÖNNGREN J, CARLO DJ. Structural studies of the capsular polysaccharide from *Streptococcus pneumoniae* type 12A[J]. Carbohydrate Research, 1983, 114(2): 257-266.

[70] WATSON MJ, TYLER JM, BUCHANAN JG, BADDILEY J. The type-specific substance from *Pneumococcus* type 13[J]. The Biochemical Journal, 1972, 130(1): 45-54.

[71] LINDBERG B, LöNNGREN J, POWELL DA. Structural studies on the specific type-14 pneumococcal polysaccharide[J]. Carbohydrate Research, 1977, 58(1): 177-186.

[72] LI CX, ANDERSEN KB, ELVERDAL PL, SKOVSTED IC, DUUS JØ, KJELDSEN C. Full NMR assignment, revised structure and biosynthetic analysis for the capsular polysaccharide from *Streptococcus pneumoniae* serotype 15F[J]. Carbohydrate Research, 2021, 508: 108418.

[73] JANSSON PE, LINDBERG B, LINDQUIST U, LJUNGBERG J. Structural studies of the capsular polysaccharide from *Streptococcus pneumoniae* types 15B and 15C[J]. Carbohydrate Research, 1987, 162(1): 111-116.

[74] LI C, DUDA KA, ELVERDAL PL, SKOVSTED IC, KJELDSEN C, DUUS J. Structural, biosynthetic, and serological cross-reactive elucidation of capsular polysaccharides from *Streptococcus pneumoniae* serogroup 16[J]. Journal of Bacteriology, 2019, 201(20): e00453-19.

[75] JONES C, AGUILERA B, van BOOM JH, BUCHANAN JG. Confirmation of the D configuration of the 2-substituted arabinitol 1-phosphate residue in the capsular polysaccharide from *Streptococcus pneumoniae* type 17F[J]. Carbohydrate Research, 2002, 337(21/22/23): 2353-2358.

[76] JANSSON PE, LINDBERG B, LINDQUIST U. Structural studies of the capsular polysaccharide from *Streptococcus pneumoniae* type 17A[J]. Carbohydrate Research, 1981, 95(1): 73-80.

[77] JANSSON PE, KUMAR NS, LINDBERG B, WIDMALM G, HENRICHSEN J. Structural studies of the capsular polysaccharide from *Streptococcus pneumoniae* type 18F[J]. Carbohydrate Research, 1988, 173(2): 217-225.

[78] JANSSON PE, SAVITRI N, KUMAR, BENGT, LINDBERG, GöRAN. Structural studies of the capsular polysaccharide from *Streptococcus pneumoniae* type 18a[J]. Carbohydrate Research, 1988, 172(2): 227-233.

[79] KARLSSON C, JANSSON PE, WIDMALM G, SKOV SøRENSEN UB. Structural elucidation of the capsular polysaccharide from *Streptococcus pneumoniae* type 18B[J]. Carbohydrate Research, 1997, 304(2): 165-172.

[80] LUGOWSKI C, JENNINGS HJ. Structural determination of the capsular polysaccharide of *Streptococcus pneumoniae* type 18C (56)[J]. Carbohydrate Research, 1984, 131(1): 119-129.

[81] JENNINGS HJ, ROSELL KG, CARLO DJ. Structural determination of the capsular polysaccharide of *Streptococcus pneumoniae* type-19 (19F)[J]. Canadian Journal of Chemistry, 1980, 58(11): 1069-1074.

[82] LEE CJ, FRASER BA, BOYKINS RA, LI JP. Effect of culture conditions on the structure of *Streptococcus pneumoniae* type 19A(57) capsular polysaccharide[J]. Infection and Immunity, 1987, 55(8): 1819-1823.

[83] BEYNON LM, RICHARDS JC, PERRY MB, KNISKERN PJ. Antigenic and structural relationships within group 19 *Streptococcus pneumoniae*: chemical characterization of the specific capsular polysaccharides of types 19B and 19C[J]. Canadian Journal of Chemistry, 1992, 70(1): 218-232.

[84] CALIX JJ, PORAMBO RJ, BRADY AM, LARSON TR, YOTHER J, ABEYGUNWARDANA C, NAHM MH. Biochemical, genetic, and serological characterization of two capsule subtypes among *Streptococcus pneumoniae* serotype 20 strains: discovery of a new pneumococcal serotype[J]. The Journal of Biological Chemistry, 2012, 287(33): 27885-27894.

[85] SHABAROVA ZA, BUCHANAN JG, BADDILEY J. The composition of pneumococcus type-specific substances containing phosphorus[J]. Biochimica et Biophysica Acta, 1962, 57: 146-148.

[86] RICHARDS JC, PERRY MB, KNISKERN PJ. Structural analysis of the specific capsular polysaccharide of *Streptococcus pneumoniae* type 22F[J]. Canadian Journal of Chemistry, 1989, 67(6): 1038-1050.

[87] RICHARDS JC, PERRY MB. Structure of the specific capsular polysaccharide of *Streptococcus pneumoniae* type 23F (American type 23)[J]. Biochemistry and Cell Biology, 1988, 66(7): 758-771.

[88] RAVENSCROFT N, OMAR A, HLOZEK J, EDMONDS-SMITH C, FOLLADOR R, SERVENTI F, LIPOWSKY G, KUTTEL MM, CESCUTTI P, FARIDMOAYER A. Genetic and structural elucidation of capsular polysaccharides from *Streptococcus pneumoniae* serotype 23A and 23B, and comparison to serotype 23F[J]. Carbohydrate Research, 2017, 450: 19-29.

[89] GANAIE F, MARUHN K, LI C, PORAMBO RJ, ELVERDAL PL, ABEYGUNWARDANA C, van der LINDEN M, DUUS J, SHEPPARD CL, NAHM MH. Structural, genetic, and serological elucidation of *Streptococcus pneumoniae* serogroup 24 serotypes: discovery of a new serotype, 24c, with a variable capsule structure[J]. Journal of Clinical Microbiology, 2021, 59(7): e0054021.

[90] BENNETT LG, BISHOP CT. Structure of the type XXVII *Streptococcus pneumoniae* (pneumococcal) capsular polysaccharide[J]. Canadian Journal of Chemistry, 1977, 55(1): 8-16.

[91] LI CX, DUDA KA, ELVERDAL PL, SKOVSTED IC, KJELDSEN C, TEZE D, DUUS JØ. Structural, biosynthetic and serological cross-reactive elucidation of capsular polysaccharides from *Streptococcus pneumoniae* serogroup 28[J]. Carbohydrate Polymers, 2021, 254(1): 117323.

[92] KENNE L, LINDBERG B. The structure of the *Streptococcus pneumoniae* type 29 polysaccharide: a re-examination[J]. Carbohydrate Research, 1988, 184: 288-291.

[93] SUN TT, MAI SY, MAO HZ, LI HT, DUAN YY, MENG S, BAO JL, DING N, ZONG CL. Conjugate of structurally reassigned pneumococcal serotype 31 polysaccharide with CRM197 elicited potent immune response[J]. Carbohydrate Polymers, 2022, 289: 119414.

[94] KARLSSON C, JANSSON PE, SØRENSEN UB. The chemical structures of the capsular polysaccharides from *Streptococcus pneumoniae* types 32F and 32A[J]. European Journal of Biochemistry, 1998, 255(1): 296-302.

[95] LEMERCINIER X, JONES C. Full assignment of the 1H and 13C spectra and revision of the O-acetylation site of the capsular polysaccharide of *Streptococcus pneumoniae* type 33F, a component of the current pneumococcal polysaccharide vaccine[J]. Carbohydrate Research, 2006, 341(1): 68-74.

[96] LIN FL, VINOGRADOV E, DENG C, ZELLER S, GREEN BA, JANSEN KU, PAVLIAK V. Identification of the common antigenic determinant shared by *Streptococcus pneumoniae* serotypes 33A, 35A, and 20 capsular polysaccharides[J]. Carbohydrate Research, 2013, 380: 101-107.

[97] GANAIE FA, SAAD JS, LO SW, MCGEE L, van TONDER AJ, HAWKINS PA, CALIX JJ, BENTLEY SD, NAHM MH. Novel pneumococcal capsule type 33E results from the inactivation of glycosyltransferase WciE in vaccine type 33F[J]. Journal of Biological Chemistry, 2023, 299(9): 105085.

[98] MANNA S, WERREN JP, ORTIKA BD, BELLICH B, PELL CL, NIKOLAOU E, GJUROSKI I, LO S, HINDS J, TUNDEV O, DUNNE EM, GESSNER BD, BENTLEY SD, RUSSELL FM, MULHOLLAND EK, MUNGUN T, von MOLLENDORF C, LICCIARDI PV, CESCUTTI P, RAVENSCROFT N, et al. *Streptococcus pneumoniae* serotype 33G: genetic, serological, and structural analysis of a new capsule type[J]. Microbiol Spectr, 2024, 12(1): e0357923.

[99] BEYNON LM, RICHARDS JC, PERRY MB, KNISKERN PJ. Characterization of the capsular antigen of *Streptococcus pneumoniae* serotype 35B[J]. Canadian Journal of Chemistry, 1995, 73(1): 41-48.

[100] GENO KA, BUSH CA, WANG MN, JIN C, NAHM MH, YANG JH. WciG O-acetyltransferase functionality differentiates pneumococcal serotypes 35C and 42[J]. Journal of Clinical Microbiology, 2017, 55(9): 2775-2784.

[101] GENO KA, SAAD JS, NAHM MH. Discovery of novel pneumococcal serotype 35D, a natural WciG-deficient variant of serotype 35B[J]. Journal of Clinical Microbiology, 2017, 55(5): 1416-1425.

[102] LARSSON EA, SJÖBERG M, WIDMALM G. Synthesis of oligosaccharides related to the repeating unit of the capsular polysaccharide from *Streptococcus pneumoniae* type 37[J]. Carbohydrate Research, 2005, 340(1): 7-13.

[103] PETERSEN BO, MEIER S, PAULSEN BS, REDONDO AR, SKOVSTED IC. Determination of native capsular polysaccharide structures of

Streptococcus pneumoniae serotypes 39, 42, and 47F and comparison to genetically or serologically related strains[J]. Carbohydrate Research, 2014, 395: 38-46.

[104] PETERSEN BO, SKOVSTED IC, PAULSEN BS, REDONDO AR, MEIER S. Structural determination of *Streptococcus pneumoniae* repeat units in serotype 41A and 41F capsular polysaccharides to probe gene functions in the corresponding capsular biosynthetic loci[J]. Carbohydrate Research, 2014, 400: 26-32.

[105] MOREAU M, RICHARDS JC, PERRY MB, KNISKERN PJ. Structural analysis of the specific capsular polysaccharide of *Streptococcus pneumoniae* type 45 (American type 72)[J]. Biochemistry, 1988, 27(18): 6820-6829.

[106] PETERSEN BO, HINDSGAUL O, PAULSEN BS, REDONDO AR, SKOVSTED IC. Structural elucidation of the capsular polysaccharide from *Streptococcus pneumoniae* serotype 47A by NMR spectroscopy[J]. Carbohydrate Research, 2014, 386(complete): 62-67.

[107] BENTLEY SD, AANENSEN DM, MAVROIDI A, SAUNDERS D, RABBINOWITSCH E, COLLINS M, DONOHOE K, HARRIS D, MURPHY L, QUAIL MA, SAMUEL G, SKOVSTED IC, KALTOFT MS, BARRELL B, REEVES PR, PARKHILL J, SPRATT BG. Genetic analysis of the capsular biosynthetic locus from all 90 pneumococcal serotypes[J]. PLoS Genetics, 2006, 2(3): e31.

[108] MUÑOZ R, MOLLERACH M, LÓPEZ R, GARCÍA E. Molecular organization of the genes required for the synthesis of type 1 capsular polysaccharide of *Streptococcus pneumoniae*: formation of binary encapsulated pneumococci and identification of cryptic dTDP-rhamnose biosynthesis genes[J]. Molecular Microbiology, 1997, 25(1): 79-92

[109] AANENSEN DM, MAVROIDI A, BENTLEY SD, REEVES PR, SPRATT BG. Predicted functions and linkage specificities of the products of the *Streptococcus pneumoniae* capsular biosynthetic loci[J]. Journal of Bacteriology, 2007, 189(21): 7856-7876.

[110] MAVROIDI A, AANENSEN DM, GODOY D, SKOVSTED IC, KALTOFT MS, REEVES PR, BENTLEY SD, SPRATT BG. Genetic relatedness of the *Streptococcus pneumoniae* capsular biosynthetic loci[J]. Journal of Bacteriology, 2007, 189(21): 7841-7855.

[111] DENAPAITE D, BRÜCKNER R, HAKENBECK R, VOLLMER W. Biosynthesis of teichoic acids in *Streptococcus pneumoniae* and closely related species: lessons from genomes[J]. Microbial Drug Resistance, 2012, 18(3): 344-358.

[112] SU T, NAKAMOTO R, CHUN YY, CHUA WZ, CHEN JH, ZIK JJ, SHAM LT. Decoding capsule synthesis in *Streptococcus pneumoniae*[J]. FEMS Microbiology Reviews, 2021, 45(4): fuaa067.

[113] 王晟旭, 曹禹琪, 财音青格乐, 乔建军, 杨静华. 肺炎链球菌荚膜多糖 O-乙酰化修饰的研究进展[J]. 微生物学报, 2021, 61(61): 2316-2337.
WANG CX, CAO YQ, CAIYIN ZL, QIAO JJ, YANG JH. Research progress in O-acetylation of capsular polysaccharide of *Streptococcus pneumoniae*[J]. Acta Microbiologica Sinica, 2021, 61(61): 2316-2337 (in Chinese).

[114] GENO KA, GILBERT GL, SONG JY, SKOVSTED IC, KLUGMAN KP, JONES C, KONRADSEN HB, NAHM MH. Pneumococcal capsules and their types: past, present, and future[J]. Clinical Microbiology Reviews, 2015, 28(3): 871-899.

[115] WHITFIELD C, WEAR SS, SANDE C. Assembly of bacterial capsular polysaccharides and exopolysaccharides[J]. Annual Review of Microbiology, 2020, 74: 521-543.

[116] NAKAMOTO R, KWAN JMC, CHIN JFL, ONG HT, FLORES-KIM J, MIDONET C, VANNIEUWENHZE MS, GUAN XL, SHAM LT. The bacterial tyrosine kinase system CpsBCD governs the length of capsule polymers[J]. Proceedings of the National Academy of Sciences of the United States of America, 2021, 118(45): e2103377118.

[117] EBERHARDT A, HOYLAND CN, VOLLMER D, BISLE S, CLEVERLEY RM, JOHNSBORG O, HÅVARSTEIN LS, LEWIS RJ, VOLLMER W. Attachment of capsular polysaccharide to the cell wall in *Streptococcus pneumoniae*[J]. Microbial Drug Resistance, 2012, 18(3): 240-255.

[118] LARSON TR, YOTHER J. *Streptococcus pneumoniae* capsular polysaccharide is linked to peptidoglycan *via* a direct glycosidic bond to β-D-N-acetylglucosamine[J]. Proceedings of the National Academy of Sciences of the United States of America, 2017, 114(22): 5695-5700.

[119] DILLARD JP, YOTHER J. Genetic and molecular characterization of capsular polysaccharide biosynthesis in *Streptococcus pneumoniae* type 3[J]. Molecular Microbiology, 1994, 12(6): 959-972.

[120] KELLY T, DILLARD JP, YOTHER J. Effect of genetic switching of capsular type on virulence of *Streptococcus pneumoniae*[J]. Infection and Immunity, 1994, 62(5): 1813-1819.

[121] ARRECUBIETA C, GARCÍA E, LÓPEZ R. Sequence and transcriptional analysis of a DNA region involved in the production of capsular polysaccharide in *Streptococcus pneumoniae* type 3[J]. Gene, 1995, 167(1/2): 1-7.

[122] DILLARD JP, VANDERSEA MW, YOTHER J. Characterization of the cassette containing genes for type 3 capsular polysaccharide biosynthesis in *Streptococcus pneumoniae*[J]. The Journal of Experimental Medicine, 1995, 181(3): 973-983.

[123] CAIMANO MJ, HARDY GG, YOTHER J. Capsule genetics in *Streptococcus pneumoniae* and a possible role for transposition in the generation of the type 3 locus[J]. Microbial Drug Resistance, 1998, 4(1): 11-23.

[124] HARDY GG, CAIMANO MJ, YOTHER J. Capsule biosynthesis and basic metabolism in *Streptococcus pneumoniae* are linked through the cellular phosphoglucomutase[J]. Journal of Bacteriology, 2000, 182(7): 1854-1863.

[125] HARDY GG, MAGEE AD, VENTURA CL, CAIMANO MJ, YOTHER J. Essential role for cellular phosphoglucomutase in virulence of type 3 *Streptococcus pneumoniae*[J]. Infection and Immunity, 2001, 69(4): 2309-2317.

[126] MORONA JK, PATON JC, MILLER DC, MORONA R. Tyrosine phosphorylation of CpsD negatively regulates capsular polysaccharide biosynthesis in *Streptococcus pneumoniae*[J]. Molecular Microbiology, 2000, 35(6): 1431-1442.

[127] World Health Organization. Recommendations for the production and control of pneumococcal conjugate vaccines[J]. World Health Organization Technical Report Series, 2005, 927(2): 64-98.

[128] CANO FKJ, QUERRY M. Purification of pneumococcal capsular polysaccharides: patent US 4242501A[P]. 1980-12-30.

[129] CARLO DJNKH, STOUDT TH, WALTON RB, ZELTNER JY. Pneumococcal vaccine and a process for its preparation: patent EP0002404A1[P]. 1979-06-13.

[130] MERIEUX SIAF, DONIKIAN R. Procede de purification de polyosydes de *Streptococcus pneumoniae* et vaccin a base de polyosides ainsi purifies: WIPO (PCT) WO1982001995A1[P]. 1982-06-24.

[131] ARNOLD FSM. Alcohol-free pneumococcal polysaccharide purification process: patent US 5714354A[P]. 1998-02-03.

[132] SUÁREZ N, FRAGUAS LF, TEXEIRA E, MASSALDI H, BATISTA-VIERA F, FERREIRA F. Production of capsular polysaccharide of *Streptococcus pneumoniae* type 14 and its purification by affinity chromatography[J]. Applied and Environmental Microbiology, 2001, 67(2): 969-971.

[133] GONçALVES VM, TAKAGI M, LIMA RB, MASSALDI H, GIORDANO RC, TANIZAKI MM. Purification of capsular polysaccharide from *Streptococcus pneumoniae* serotype 23F by a procedure suitable for scale-up[J]. Biotechnology and Applied Biochemistry, 2003, 37(Pt 3): 283-287.

[134] GONçALVES VM, TAKAGI M, CARMO TS, BARBOSA RM, ALBANI SM, PINTO JV, GIORDANO RDC, TANIZAKI MM. Simple and efficient method of bacterial polysaccharides purification for vaccines production using hydrolytic enzymes and tangential flow ultrafiltration[J]. Communicating Current Research and Educational Topics and Trends in Applied Microbiology, 2007: 450-457.

[135] JUNG SJ, SEO ES, YUN SI, MINH BN, JIN SD, RYU HJ, KIM D. Purification of capsular polysaccharide produced by *Streptococcus pneumoniae* serotype 19A[J]. Journal of Microbiology and Biotechnology, 2011, 21(7): 734-738.

[136] MACHA C, LAVANYA A, NANNA R. Purification of *Streptococcus pneumoniae* capsular polysaccharides using aluminium phosphate and ethanol[J]. International Journal of Pharmacy & Pharmaceutical Sciences, 2014, 6: 385-387.

[137] ZANARDO RT, FERRI ALS, FIGUEIREDO DB, KRASCHOWETZ S, CABRERA-CRESPO J, GONÇALVES VM. Development of a new process for purification of capsular polysaccharide from *Streptococcus pneumoniae* serotype 14[J]. Brazilian Journal of Chemical Engineering, 2016, 33(3): 435-443.

[138] GAIKWAD WK, KODAM KM, DHERE RM, JANA SK, GAUTAM M, MALLYA AD, SONI D, BHAGADE S, GULAHNE A. Simultaneous purification and depolymerization of *Streptococcus pneumoniae* serotype 2 capsular polysaccharides by trifluoroacetic acid[J]. Carbohydrate Polymers, 2021, 261: 117859.

[139] LEE C, CHUN HJ, PARK M, KIM RK, WHANG YH, CHOI SK, BAIK YO, PARK SS, LEE I. Quality improvement of capsular polysaccharide in *Streptococcus pneumoniae* by purification process optimization[J]. Frontiers in Bioengineering and Biotechnology, 2020, 8: 39.

[140] ZOU W, LI JJ, VINOGRADOV E, COX A. Removal of cell wall polysaccharide in pneumococcal capsular polysaccharides by selective degradation *via* deamination[J]. Carbohydrate Polymers, 2019, 218: 199-207.

[141] BUSH CA, YANG JH, YU BW, CISAR JO. Chemical structures of *Streptococcus pneumoniae* capsular polysaccharide type 39 (CPS39), CPS47F, and CPS34 characterized by nuclear magnetic resonance spectroscopy and their relation to CPS10A[J]. Journal of Bacteriology, 2014, 196(18): 3271-3278.

[142] YANG JH, RITCHEY M, YOSHIDA Y, BUSH CA, CISAR JO. Comparative structural and molecular characterization of ribitol-5-phosphate-containing *Streptococcus oralis* coaggregation receptor polysaccharides[J]. Journal of Bacteriology, 2009, 191(6): 1891-1900.

[143] HATHAWAY LJ, BRUGGER SD, MORAND B, BANGERT M, ROTZETTER JU, HAUSER C, GRABER WA, GORE S, KADIOGLU A, MÜHLEMANN K. Capsule type of *Streptococcus pneumoniae* determines growth phenotype[J]. PLoS Pathogens, 2012, 8(3): e1002574.

[144] LI Y, WEINBERGER DM, THOMPSON CM, TRZCIŃSKI K, LIPSITCH M. Surface charge of *Streptococcus pneumoniae* predicts serotype distribution[J]. Infection and Immunity, 2013, 81(12): 4519-4524.

[145] CHUN YY, TAN KS, YU LS, PANG M, WONG MHM, NAKAMOTO R, CHUA WZ, HUEE-PING WONG A, LEW ZZR, ONG HH, CHOW VT, TRAN T, YUN WANG DY, SHAM LT. Influence of glycan structure on the colonization of *Streptococcus pneumoniae* on human respiratory epithelial cells[J]. Proceedings of the National Academy of Sciences of the United States of America, 2023, 120(13): e2213584120.

[146] CAÑADA FJ, CANALES Á, VALVERDE P, de TORO BF, MARTÍNEZ-ORTS M, PHILLIPS PO, PEREDA A. Conformational and structural characterization of carbohydrates and their interactions studied by NMR[J]. Current Medicinal Chemistry, 2022, 29(7): 1147-1172.

[147] FERNANDO LD, ZHAO WC, GAUTAM I, ANKUR A, WANG T. Polysaccharide assemblies in fungal and plant cell walls explored by solid-state NMR[J]. Structure, 2023, 31(11): 1375-1385.

[148] ROMANIUK JAH, CEGELSKI L. Bacterial cell wall composition and the influence of antibiotics by cell-wall and whole-cell NMR[J]. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences, 2015, 370(1679): 20150024.

[149] LAGURI C, SILIPO A, MARTORANA AM, SCHANDA P, MARCHETTI R, POLISSI A, MOLINARO A, SIMORRE JP. Solid state NMR studies of intact lipopolysaccharide endotoxin[J]. ACS Chemical Biology, 2018, 13(8): 2106-2113.

[150] LIU XY, BRČIĆ J, CASSELL GH, CEGELSKI L. CPMAS NMR platform for direct compositional analysis of mycobacterial cell-wall complexes and whole cells[J]. Journal of Magnetic Resonance Open, 2023, 16/17: 100127.

[151] GENING ML, KURBATOVA EA, NIFANTIEV NE. Synthetic analogs of *Streptococcus pneumoniae* capsular polysaccharides and immunogenic activities of glycoconjugates[J]. Russian Journal of Bioorganic Chemistry, 2021, 47(1): 1-25.

[152] YAO WL, XIONG DC, YANG Y, GENG CM, CONG ZS, LI FF, LI BH, QIN XJ, WANG LN, XUE WY, YU NF, ZHANG HY, WU X, LIU M, YE XS. Automated solution-phase multiplicative synthesis of complex glycans up to a 1 080 mer[J]. Nature Synthesis, 2022, 1(11): 854-863.

[153] HECHT ML, STALLFORTH P, SILVA DV, ADIBEKIAN A, SEEBERGER PH. Recent advances in carbohydrate-based vaccines[J]. Current Opinion in Chemical Biology, 2009, 13(3): 354-359.

[154] HEVEY R, LING CC. Recent advances in developing synthetic carbohydrate-based vaccines for cancer immunotherapies[J]. Medicinal Chemistry, 2012, 4(4): 545-584.

[155] MUSUMECI MA, HUG I, SCOTT NE, IELMINI MV, FOSTER LJ, WANG PG, FELDMAN MF. *In vitro* activity of *Neisseria* meningitidis PglL O-oligosaccharyltransferase with diverse synthetic lipid donors and a UDP-activated sugar[J]. The Journal of Biological Chemistry, 2013, 288(15): 10578-10587.