Grupo 3
CÁPSULA

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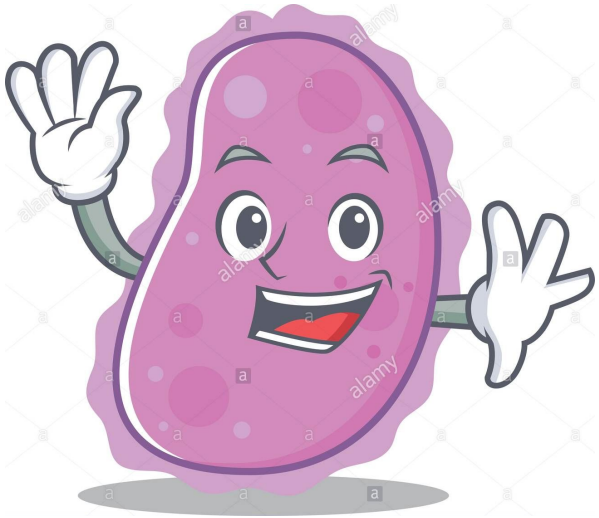
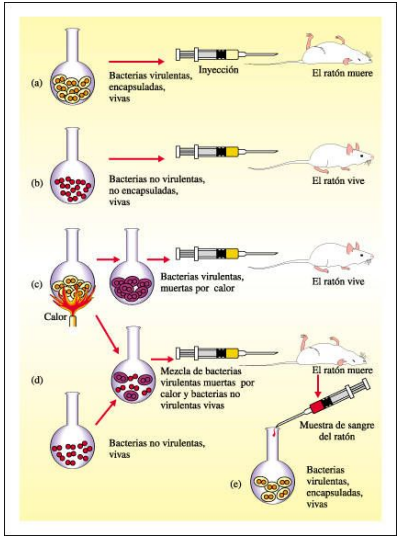
Disciplina: Estudo do Envoltório de Bactérias Gram-Positivas e Gram-Negativas (BTC5843)

INTRODUÇÃO



Quando foi primeiramente descrita



1881 — Pasteur e seus colegas observaram a cápsula de *S. pneumoniae*

1885 — Friedländer descreveu o primeiro método para corar a cápsula de *S. pneumoniae*

1891 — Kruse e Pansini observaram que a perda das cápsulas levava à atenuação da virulência de *S. pneumoniae*

1928 — Griffiths demonstrou seu "princípio de transformação" por meio do famoso "Experimento de Griffith".

Zhensong, Wen & Zhang, Jing-Ren. (2015). Bacterial Capsules. doi:10.1016/B978-0-12-397169-2.00003-2.

Em que bactérias geralmente é encontrada

TABLE 3.1 Pathogenic Bacteria with Capsules

Organism	Number of Serotypes	Chemical Nature	Reference
Acinetobacter baumannii	UD[1]	Polysaccharide	[16,17]
Campylobacter jejuni	47	Polysaccharide	[18,19]
Escherichia coli	80	Polysaccharide	[20]
Haemophilus influenzae	6	Polysaccharide	[21]
Klebsiella pneumoniae	82	Polysaccharide	[22]
Neisseria meningitidis	13	Polysaccharide	[23]
Pasteurella multocida	4	Polysaccharide	[24]
Pseudomonas aeruginosa	1	Polysaccharide	[25]
Salmonella enterica serovar Typhi	1	Polysaccharide	[26]
Streptococcus pneumoniae	94	Polysaccharide	[27,28]
Streptococcus agalactiae	9	Polysaccharide	[29]
Streptococcus pyogenes	1	Polysaccharide	[30]
Streptococcus suis	35	Polysaccharide	[31]
Staphylococcus aureus	11	Polysaccharide	[32]
Staphylococcus haemolyticus	1	Polysaccharide	[33]
Bacillus anthracis	1	Polyglutamate	[34]

Zhensong, Wen & Zhang, Jing-Ren. (2015). Bacterial Capsules. doi:10.1016/B978-0-12-397169-2.00003-2.

Zhensong, Wen & Zhang, Jing-Ren. (2015). Bacterial Capsules. doi:10.1016/B978-0-12-397169-2.00003-2.

COMPOSIÇÃO

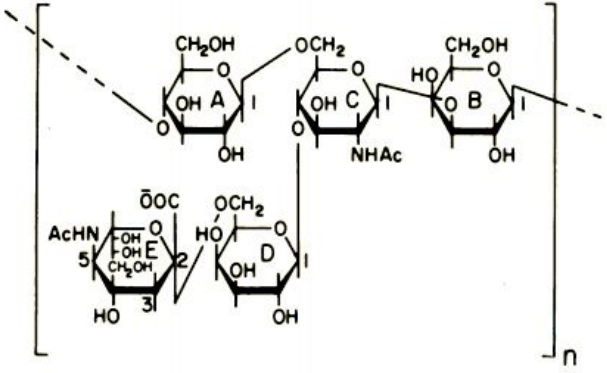
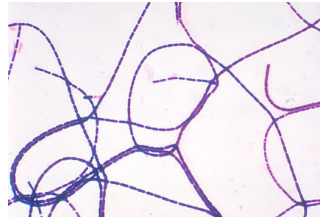
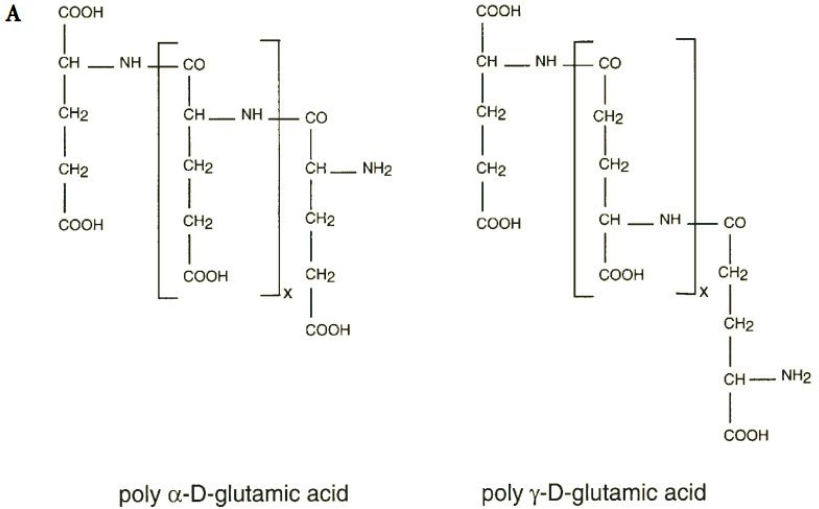


Figure 1. Chemical structure of a single repeating unit of the type III group B streptococcal capsular polysaccharide. Structure represents the following sugars:

→ D-glucose → *N*-acetyl glucosamine → D-galactose →
↓
D-galactose
↓
N-acetyl neuraminic acid

Outras bactérias com cápsula





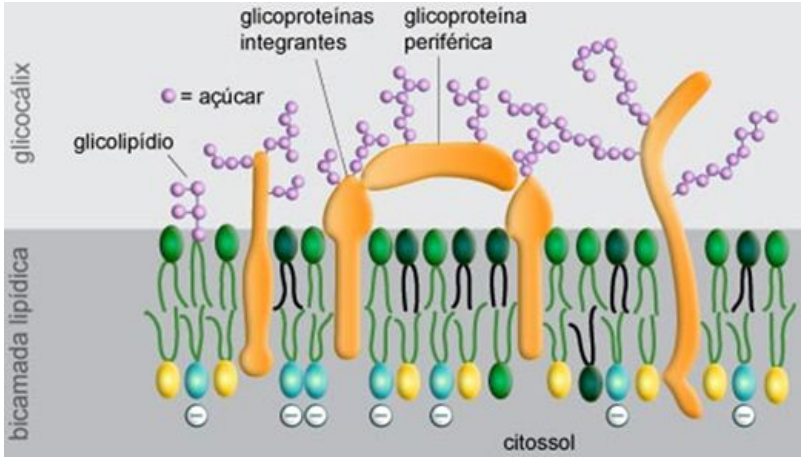
poly α-D-glutamic acid

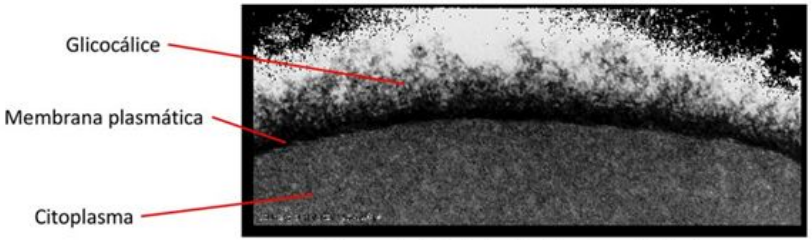
poly γ-D-glutamic acid

Bacillus anthracis

GLICOCÁLIX

- Envoltório externo da célula, composto de polissacarídeos e proteínas
- Espessura de 10 a 20 nm
- Papel no reconhecimento celular e na adesão
- Proteção, resistência mecânica

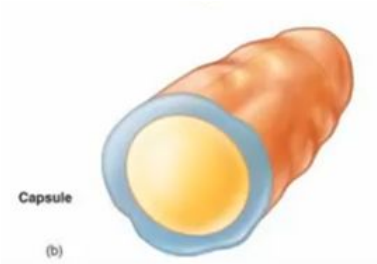




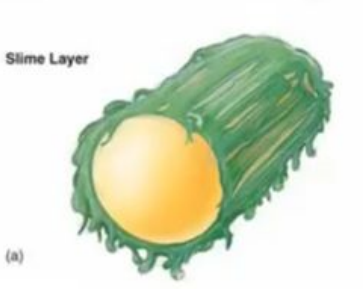
Microscopia eletrônica do glicocálice de um eritrócito humano

ESTRUTURA

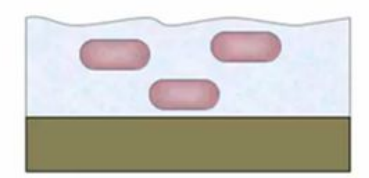
Cápsulas: rígida, organizada, compacta

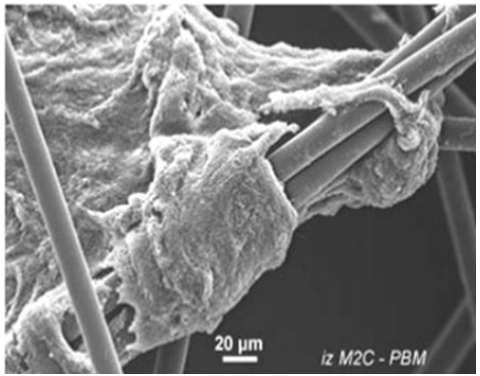


Camada limosa: frouxa, desorganizada, amorfa



Biofilme: comunidades bacterianas, aspecto mucóide

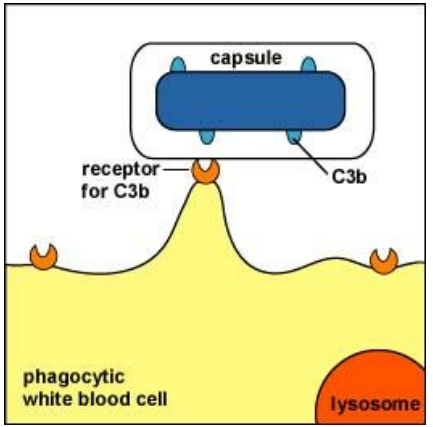




* Ambos: *Streptococcus salivarius*

FUNÇÕES

- Fator de virulência: adesão ao hospedeiro
- Proteção: fagocitose, dessecação
- Reserva de nutrientes e água



As defesas imunológicas do corpo podem eventualmente contornar a cápsula, produzindo anticorpos opsonizantes (IgG) contra a cápsula. O anticorpo então cola a cápsula ao fagócito.

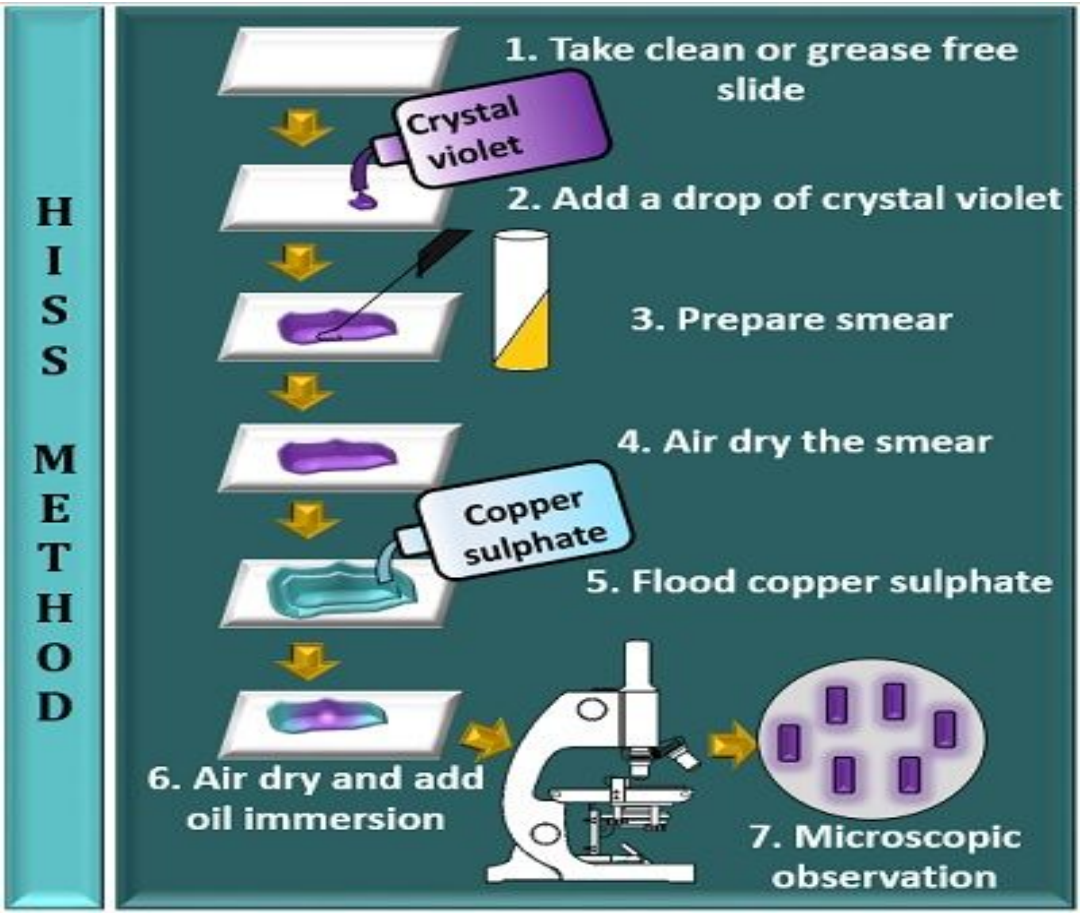
COMO IDENTIFICAR A CÁPSULA?

TÉCNICAS DE COLORAÇÃO

A coloração da cápsula pode ser feita por:

1. Método de coloração Hiss
2. Método de Maneval
3. Método de tinta nanquim
4. Método de Anthony
5. Técnica Imunoquímica: reação de Neufeld ou reação de Quellung

Método Hiss



Fonte: https://biologyreader.com/capsule-staining.html

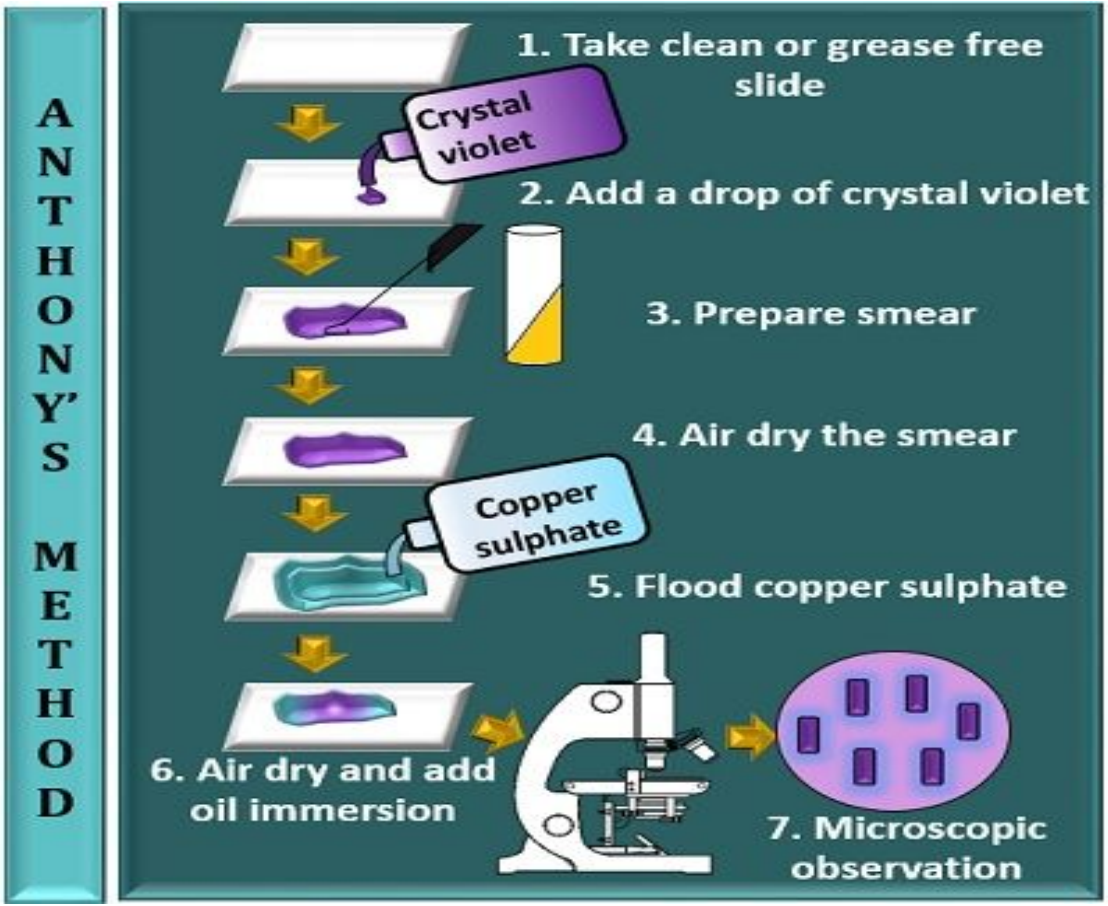
Método de Maneval

Fonte: https://biologyreader.com/capsule-staining.html

Método de Tinta Nanquim

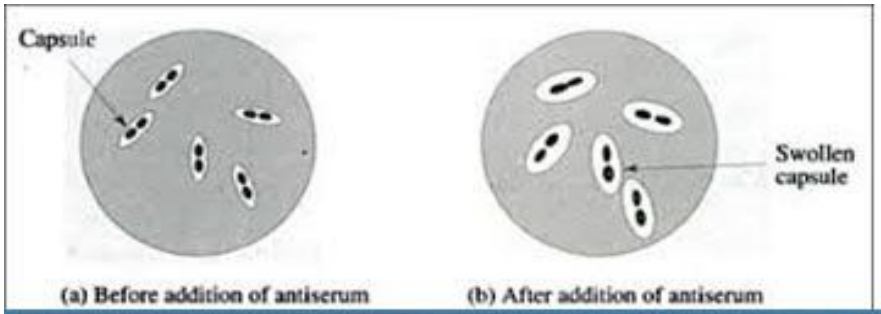
Fonte: https://biologyreader.com/capsule-staining.html

Método de Anthony

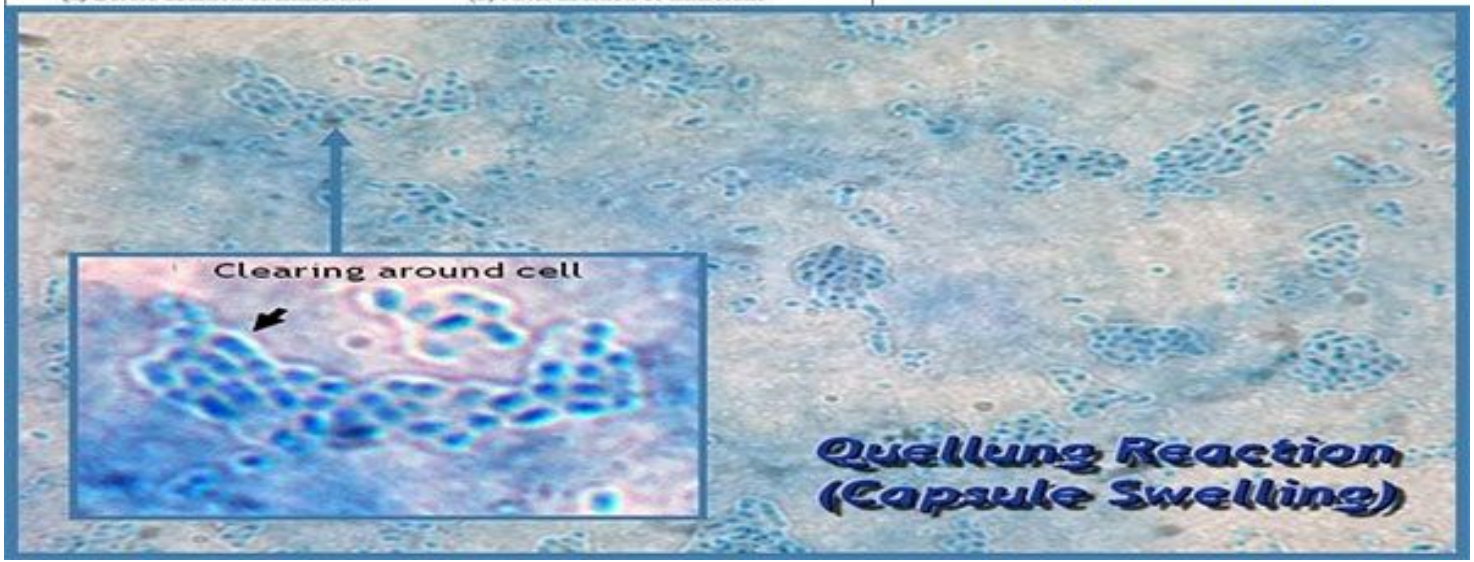


Fonte: https://biologyreader.com/capsule-staining.html

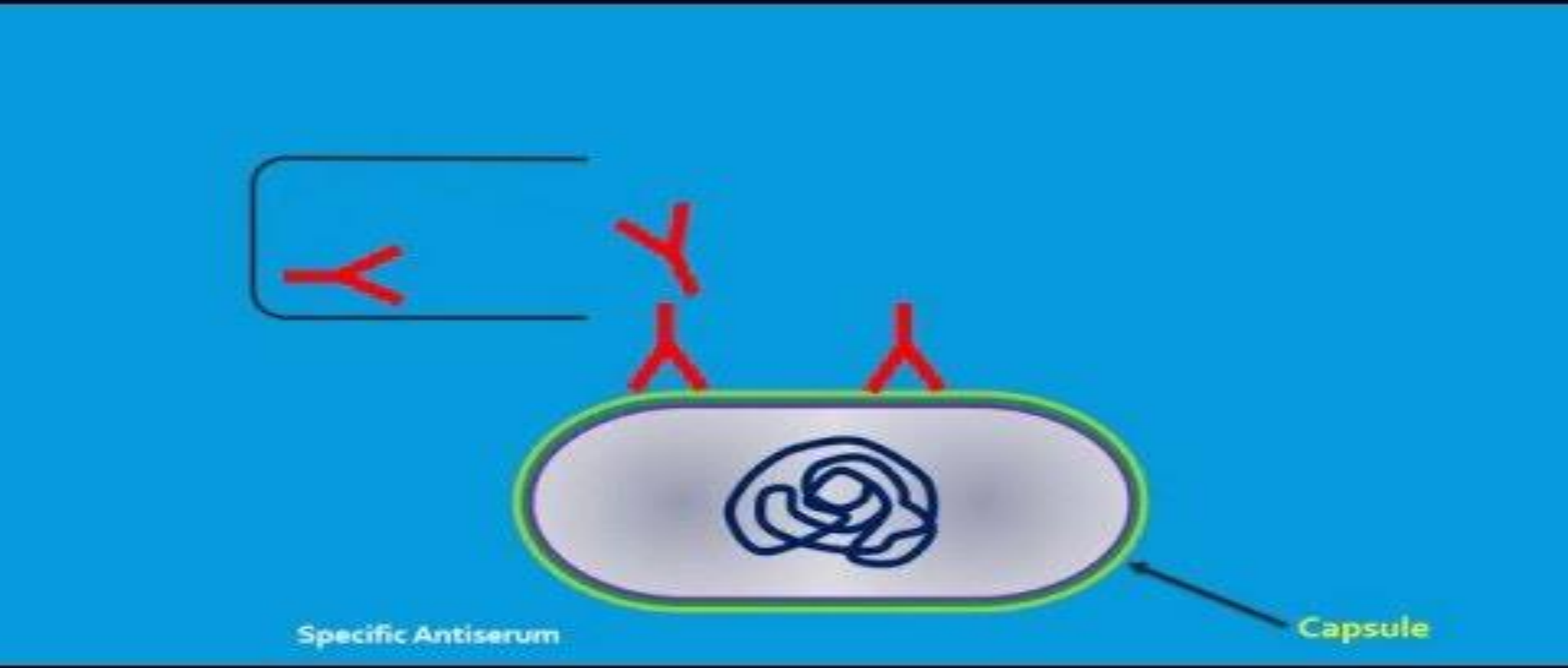
Técnica Imunoquímica: reação de Neufeld ou reação de Quellung



Quellung reaction form the basis of serotyping and depends on the swelling of capsule upon binding of homologous antibody.



Fonte: https://microbiologynotes.com/quellung-reaction-for-pneumococci/



Fonte: https://youtu.be/Y-mfCShGmeg

BIOSSÍNTESE DA CÁPSULA

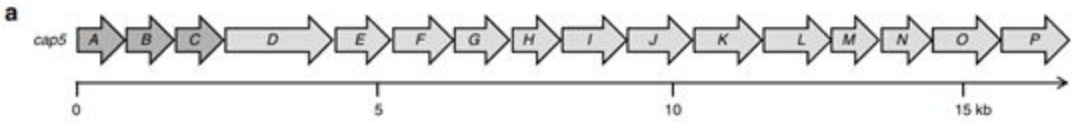
Gram-positiva (*Staphylococcus aureus*)

Nat Commun. 2019; 10: 1404.
Published online 2019 Mar 29. doi: 10.1038/s41467-019-09356-x

PMCID: PMC6441080
PMID: 30926919

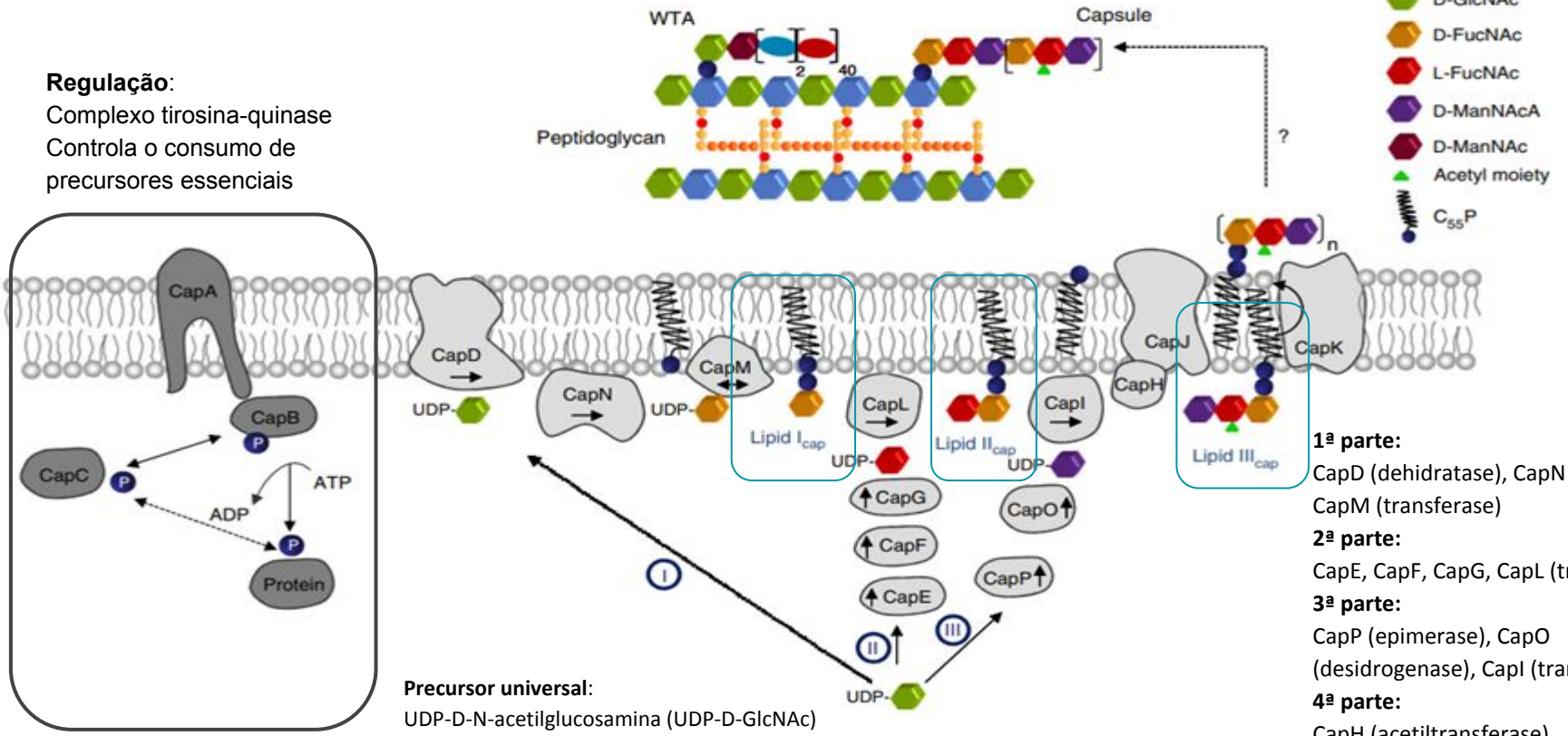
Coordination of capsule assembly and cell wall biosynthesis in *Staphylococcus aureus*

Marvin Rausch,[#1,2] Julia P. Deisinger,[#1,2] Hannah Ulm,[#1] Anna Müller,[1] Wenjin Li,[3] Patrick Hardt,[1] Xiaogang Wang,[4] Xue Li,[4] Marc Sylvester,[5] Marianne Engeser,[6] Waldemar Vollmer,[7] Christa E. Müller,[3] Hans Georg Sahl,[8] Jean Claire Lee,[4] and Tanja Schneider[✉1,2]



Ligação ao MurNAc do PG ainda é desconhecido, provavelmente proteína LCP

Regulação:
Complexo tirosina-quinase
Controla o consumo de precursores essenciais



Precursor universal:
UDP-D-N-acetilglucosamina (UDP-D-GlcNAc)

1ª parte:
CapD (dehidratase), CapN (redutase), CapM (transferase)
2ª parte:
CapE, CapF, CapG, CapL (transferase)
3ª parte:
CapP (epimerase), CapO (desidrogenase), CapI (transferase)
4ª parte:
CapH (acetiltransferase), CapK (flipase), CapJ (polimerase)

Rausch et al. (2019)

Gram-negativa (*Escherichia coli*)

ELSEVIER

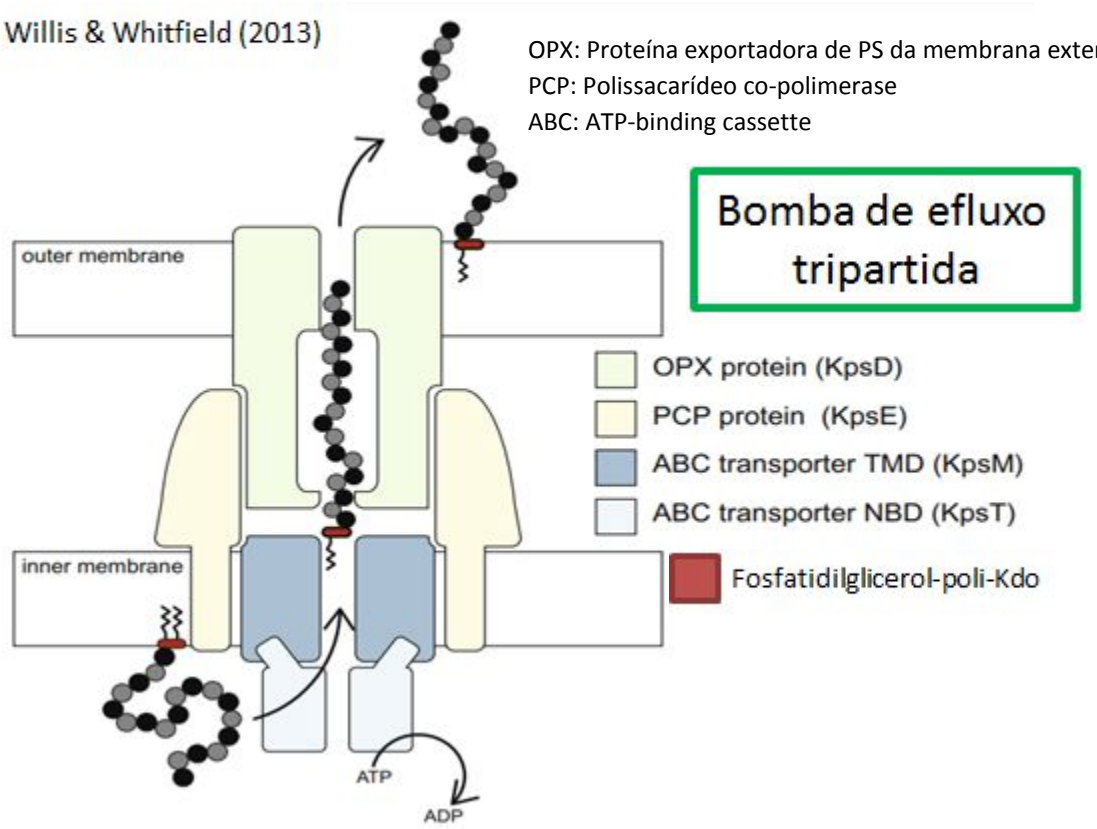
Carbohydrate Research

Volume 378, 30 August 2013, Pages 35-44

Structure, biosynthesis, and function of bacterial capsular polysaccharides synthesized by ABC transporter-dependent pathways

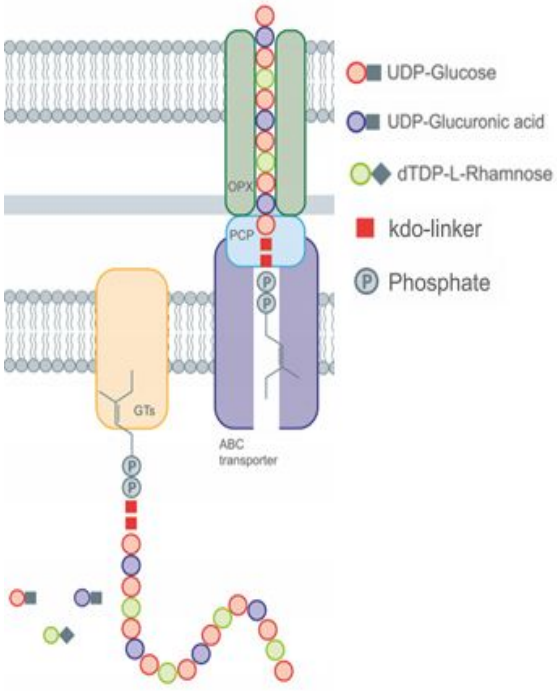
Lisa M. Willis, Chris Whitfield

Via dependente de Transportador ABC



1. KpsC e KpsS (transferases) sintetizam ligante poli-Kdo utilizando diacil ou monoacil fosfatidilglicerol.
2. Enzima iniciadora transfere o primeiro resíduo repetitivo de carboidrato ao ligante poli-Kdo.
3. Extensão dos polissacarídeos feita pelas glicosiltransferases, que adiciona unidades repetitivas na cadeia glicana.
4. Várias bactérias também sintetizam polissacarídicos capsulares de ácido polisiálico, que são feitos pela ação da enzima polisialiltransferase.

Transportadores ABC nesse contexto:
2 domínios transmembranas (TM)
2 domínios de ligação a nucleotídeo (NBD)
NBD utilizam hidrólise de ATP para translocar substrato pela membrana através de mudanças estruturais nas TM



Schmid J, Sieber V and Rehm B (2015). *Front. Microbiol*. 6:496.

Gram-negativa (*Escherichia coli*)

Review > Annu Rev Biochem. 2006;75:39-68. doi: 10.1146/annurev.biochem.75.103004.142545.

Biosynthesis and assembly of capsular polysaccharides in Escherichia coli

Chris Whitfield [1]

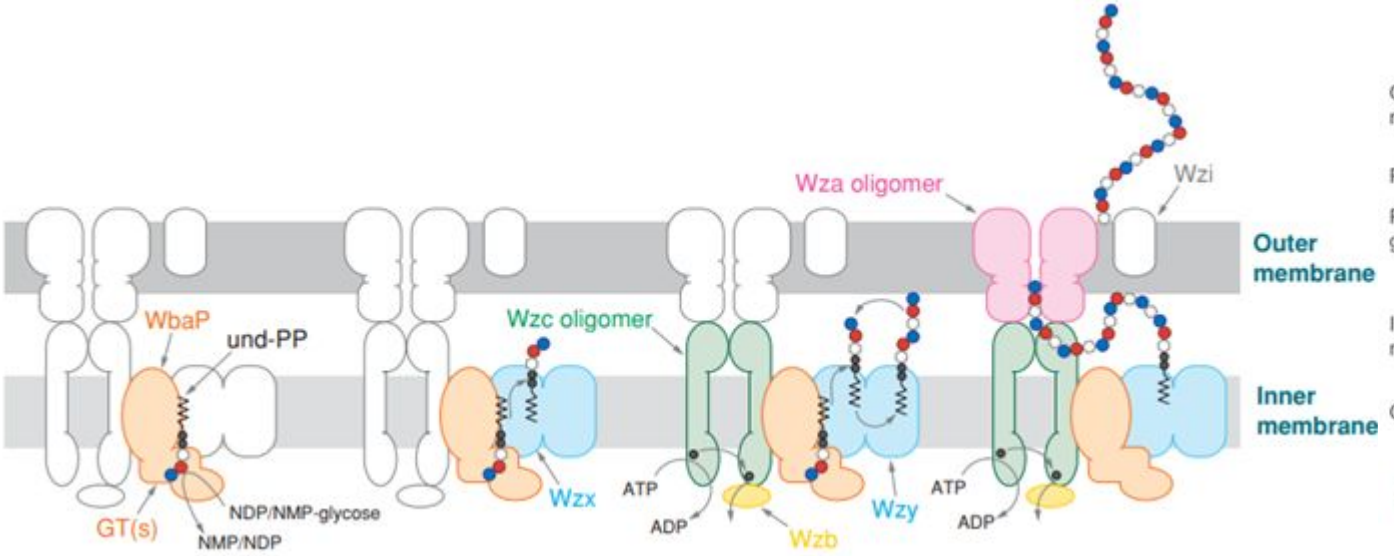
Affiliations + expand

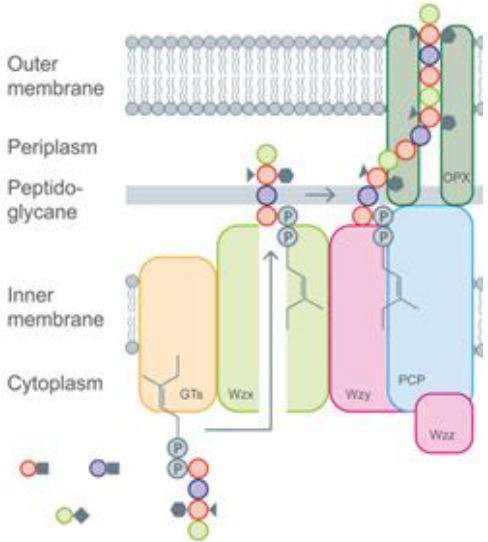
PMID: 16756484 DOI: 10.1146/annurev.biochem.75.103004.142545

Via dependente de Wzx/Wzy

Whitfield (2006)

Via dependente de Wzx/Wzy





1. Iniciação pela WbaP (transferase) e glicosiltransferases (GTs) no undecaprenil-fosfato
2. **Wzx** é uma flipase que permite o elongamento da cadeia glicana pela **Wzy**, uma polimerase
3. Elongamento requer ação da Wzb (fosfatase) pela transfosforilação do oligômero Wzc
4. Polímero é translocado para Wza, que age como um canal
5. Wzi modula a associação do polímero à superfície

Schmid J, Sieber V and Rehm B (2015).

Micobactéria (*M. tuberculosis*)

Biochem J. Author manuscript; available in PMC 2019 Aug 17.
Published in final edited form as:
Biochem J. 2019 Jul 18; 476(14): 1995–2016.
Published online 2019 Jul 18. doi: 10.1042/BCJ20190324

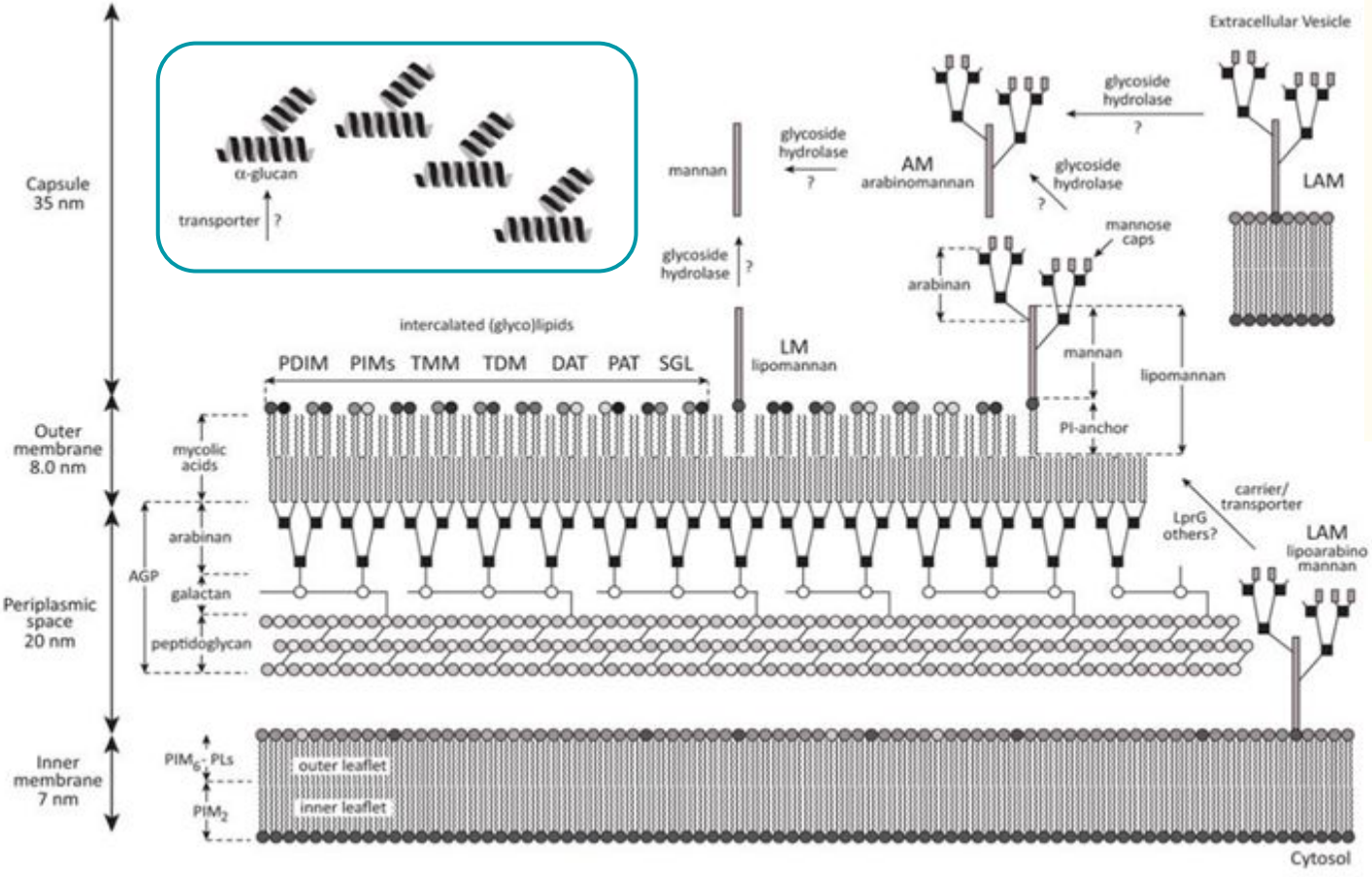
PMCID: PMC6698057
NIHMSID: NIHMS1046039
PMID: 31320388

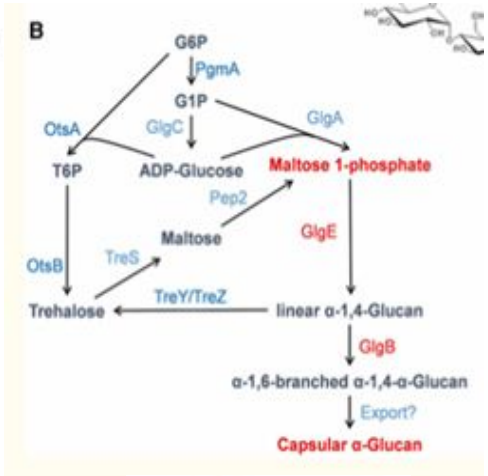
The *Mycobacterium tuberculosis* capsule: a cell structure with key implications in pathogenesis

Rainer Kalscheuer,[1] Ainhoa Palacios,[2] Itxaso Anso,[2] Javier Cifuente,[2] Juan Anguita,[2,3] William R. Jacobs, Jr,[4,5] Marcelo E. Guerin,[2,3] and Rafael Prados-Rosales[2,4,6]

- A cápsula é composta de polissacarídeos neutros principalmente o **α-glucano**, semelhante ao glicogênio, e menor quantidade de arabinomanana e manana.
- Cápsula fracamente aderida à célula, perdida por agitação ou adição de detergentes.

Kalscheuer et al. (2019)

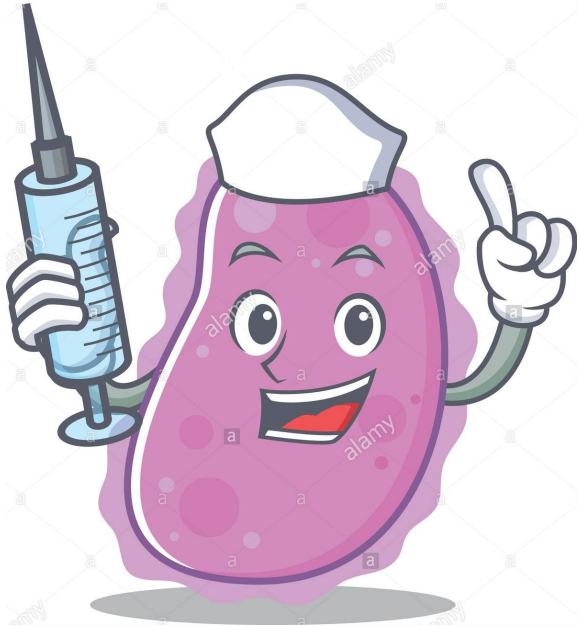




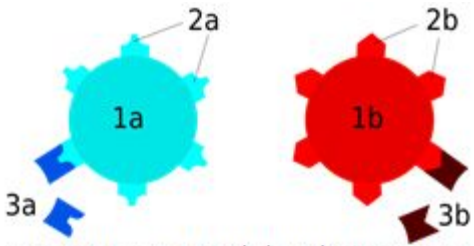
GlgE e GlgB

"a dedicated transporter for α-glucan secretion likely exists in Mtb, which has not yet been identified"

IMPORTÂNCIA CLÍNICA



SOROTIPAGEM



Two serotypes 1a and 1b with antigens 2a and 2b on surface, which are recognized by two distinct antibodies, 3a and 3b, respectively

Examples of CPS repeat units from encapsulated pathogenic bacteria

Strain	Serotype	CPS repeat unit
Escherichia coli	K1	–8)-NeuAc-α(2–
	K2	–4)-Gal-α(1,2)-Gro-3-PO_4–
	K4	–4)-[Fru-β(1,3)-]GlcA-β(1,3)-GalNAc-β(1–
	K5	–4)-GlcA-β(1,4)-GlcNAc-α(1–
	K12	–3)-Rha-α(1,2)-Rha-α(1,5)-Kdo-β(2–
	K54	–4)-[threonine-6-]GlcA-β(1,3)-Rha-α(1–
	K92	–8)-NeuAc-α(2,9)-NeuAc-α(2–
Neisseria meningitidis	Serogroup A	–6)-ManNAc-α-1-(PO_4–
	Serogroup B	–8)-NeuAc-α(2–
	Serogroup C	–9)-NeuAc-α(2–
	Serogroup W-135	–4)-NeuAc-α(2,6)-Gal-α(1-
	Serogroup Y	–4)-NeuAc-α(2,6)-Glc-α(1–
	Serogroup X	–4)-GlcNAc-α-1-(PO_4–
Haemophilus influenzae	Serogroup a	–4)-Glc-β(1,4)-ribitol-5-(PO_4–
	Serogroup b	–3)-Ribf-β(1,1)-ribitol-5-(PO_4–
	Serogroup e	–3)-GlcNAc-β(1,4)-ManANAc-β(1–
	Serogroup f	–3)-GalNAc-β(1,4)-GalNAc-α-1-(PO_4–

Willis, L. M., & Whitfield, C. (2013). *Carbohydrate Research, 378, 35–44.*

VACINAS

CPS: desenvolvimento de vacinas, por causa de sua localização na superfície e suas diferenças dos glicanos humanos

Pouco imunogênicos, necessitando conjugação a proteínas carreadoras

Alta variabilidade antigênica da cápsula de algumas bactérias requer vacinas conjugadas multivalentes onerosas

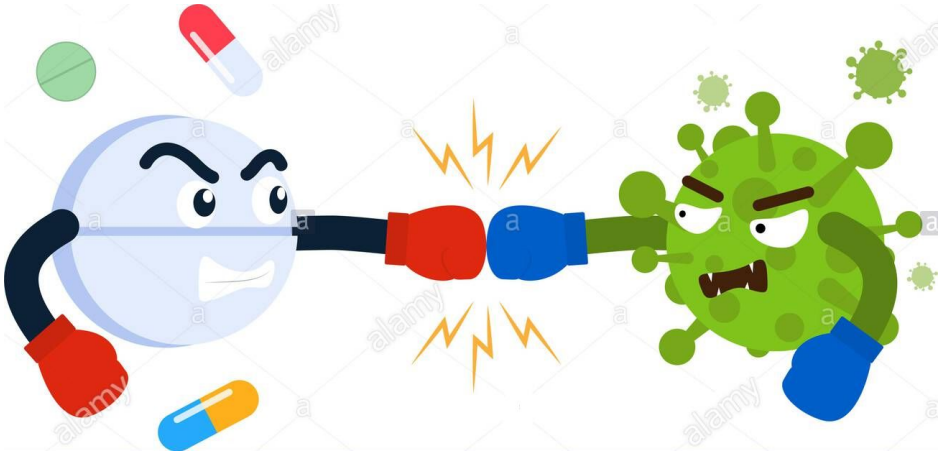
Substituição de sorotipos: aumento de sorotipos ausentes leva à redução na eficácia

Streptococcus pneumoniae:
98 sorotipos conhecidos

Paton JC, Trappetti C. 2019. Microbiol Spectrum 7(2):GPP3-0019- 2018. doi:10.1128/microbiolspec.GPP3-0019-2018.

Vacina conjugada mais abrangente:
13 sorotipos

RESISTÊNCIA A ANTIMICROBIANOS



› PLoS One. 2012;7(5):e36312. doi: 10.1371/journal.pone.0036312. Epub 2012 May 15.

Chemical inhibition of bacterial protein tyrosine phosphatase suppresses capsule production

Alistair J Standish [1], Angela A Salim, Hua Zhang, Robert J Capon, Renato Morona

Affiliations + expand

PMID: 22629313 PMCID: PMC3356977 DOI: 10.1371/journal.pone.0036312

Free PMC article

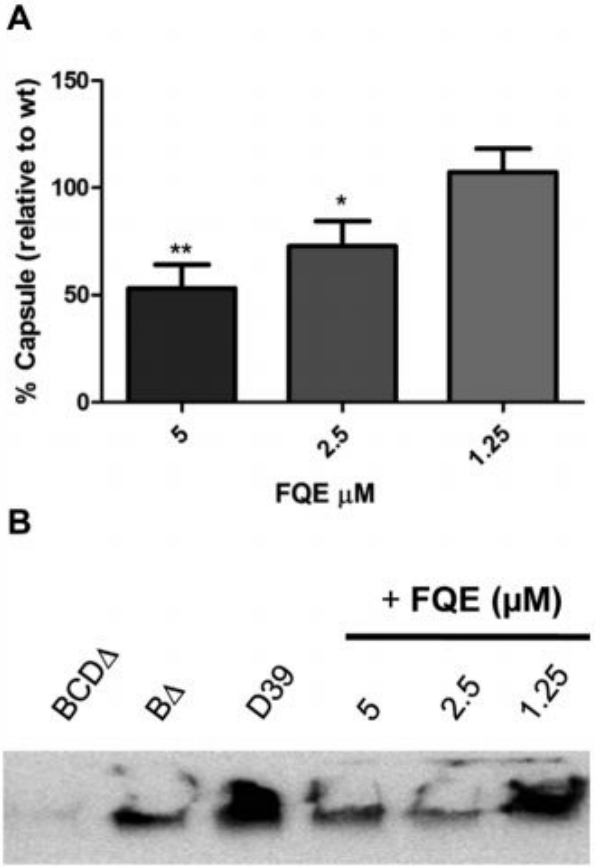


Figure 4. FQE decreases capsule synthesis in *S. pneumoniae* D39. Total CPS preparations were isolated from equal numbers of bacteria after incubation with FQE for 1 h. CPS levels were analysed by either (A) uronic acid assay or alternatively (B) by separating CPS on SDS-PAGE, transferring to Nylon and the probing with α-cps2 as described in the materials and methods. Data in (A) is from ≥3 independent experiments (5 μM vs 1.25 μM; * - $P<0.05$ by Student's *t*-test).

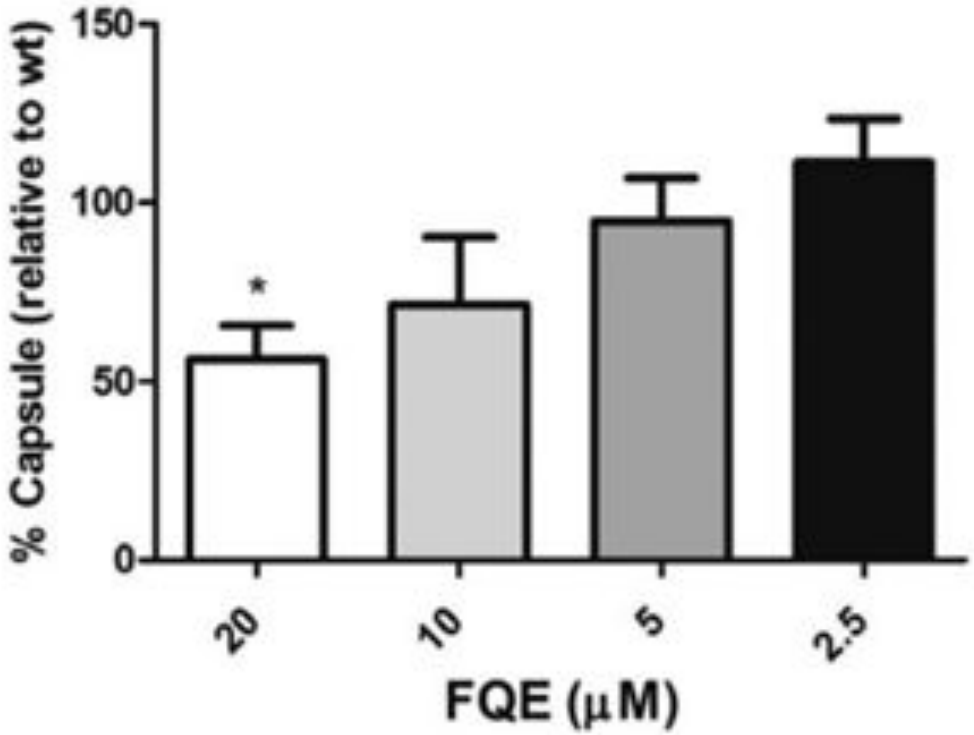


Figure 6. FQE also inhibits *E. coli* Wzb and CPS synthesis in *K. pneumoniae O1.* (A) His_6Wzb dephosphorylation of *p*NPP in presence of FQE in 1 M Tris pH 7.0 at 37°C. (B) Total CPS preparations from *K. pneumoniae* incubated with FQE were analysed by uronic acid assay. Data is from four independent experiments (20 μM vs 5 μM; * - $P<0.05$ by Student's *t*-test:).

frontiers in Microbiology

REVIEW
published: 31 January 2017
doi: 10.3389/fmicb.2017.00070

Review > Front Microbiol. 2017 Jan 31;8:70. doi: 10.3389/fmicb.2017.00070. eCollection 2017.

E. coli Group 1 Capsular Polysaccharide Exportation Nanomachinary as a Plausible Antivirulence Target in the Perspective of Emerging Antimicrobial Resistance

Shivangi Sachdeva [1], Raghuvamsi V Palur [1], Karpagam U Sudhakar [1], Thenmalarchelvi Rathinavelan [1]

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Shin'iseihaito (Xinyiqingfeitang) Suppresses the Biofilm Formation of *Streptococcus pneumoniae* In Vitro

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Affiliations + expand

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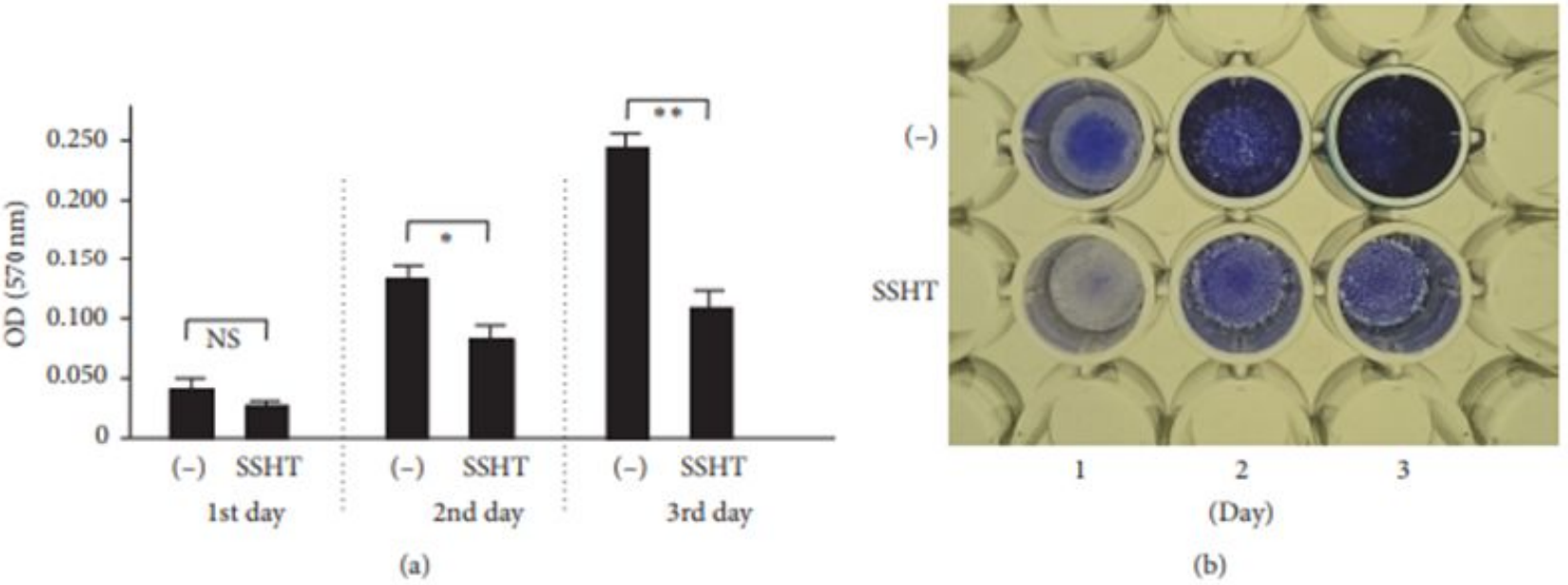


FIGURE 1: SSHT inhibits the biofilm formation of *S. pneumoniae* in time-dependent manners. (a) *S. pneumoniae* was treated with or without SSHT (500 μg/mL) for 1–3 days, and its antibiofilm activity was quantified by crystal violet adsorption by measuring absorbance at 570 nm. Data shown represent the mean ± SD ($n = 6$). $^{*}P < 0.05$; $^{**}P < 0.01$. NS: not significant. (b) Image of microplate. SSHT: Shin'iseihaito extract.

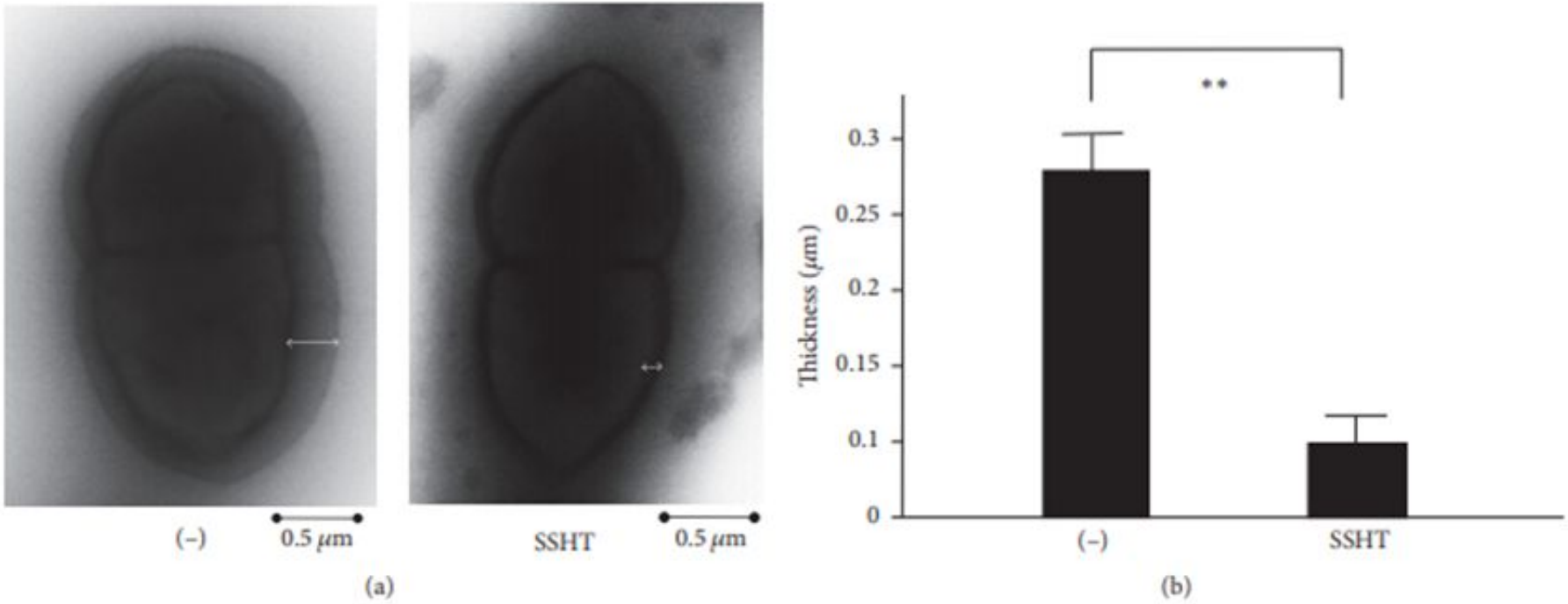
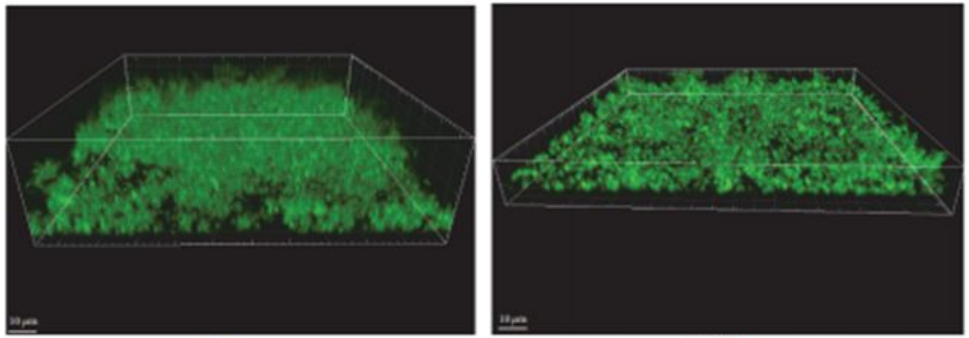
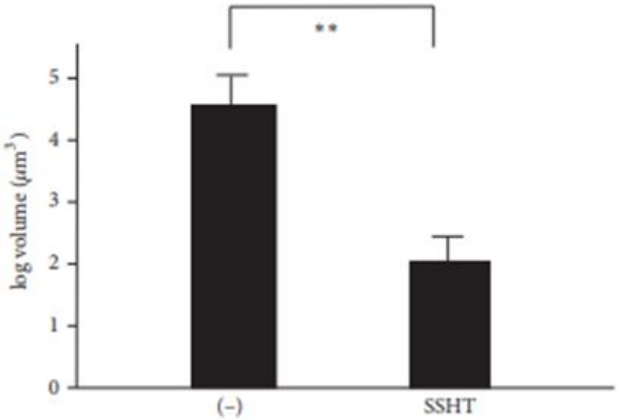


FIGURE 6: Morphological change of *S. pneumonia* treated with SSHT. (a) Representative photo of *S. pneumoniae* treated with or without SSHT (500 μg/mL) for 1 day using electron microscopy by negative staining. Double-headed white arrow represent the size of bacterial capsule. (b) The change of capsule thickness of *S. pneumonia* treated with SSHT. *S. pneumoniae* was treated with or without SSHT (500 μg/mL) for 1 day, and the thickness was measured at six arbitrary points in the bacteria treated with or without SSHT. Data shown represent the mean ± SD ($n = 6$). $^{**}p < 0.01$. SSHT: Shin'iseihaito extract.



(a)



(b)

FIGURE 4: Effect of SSHT on the thickness of *S. pneumonia* biofilm. (a) 3D analysis of biofilm formation of *S. pneumonia* treated with SSHT. Bacteria were cultured with or without SSHT (500 μg/mL) under static conditions at 37°C for 2 days in THY. Formed biofilms were stained with FITC. Three-dimensional images were reconstructed from confocal optical sections using Imaris software. Thickness was measured at nine arbitrary points in each field using Imaris software. (b) The quantification of the biofilm of *S. pneumonia* treated with SSHT. The bacteria were cultured with or without SSHT (500 μg/mL) under static conditions at 37°C for 2 days in THY, and the thickness was measured at nine arbitrary points in each field using Imaris software. Data shown represent the mean ± SD ($n = 6$). $^{**}P < 0.01$. SSHT: Shin'iseihaito extract.

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Integrated proteomic and metabolomic analysis reveals that rhodomyrtone reduces the capsule in *Streptococcus pneumoniae*

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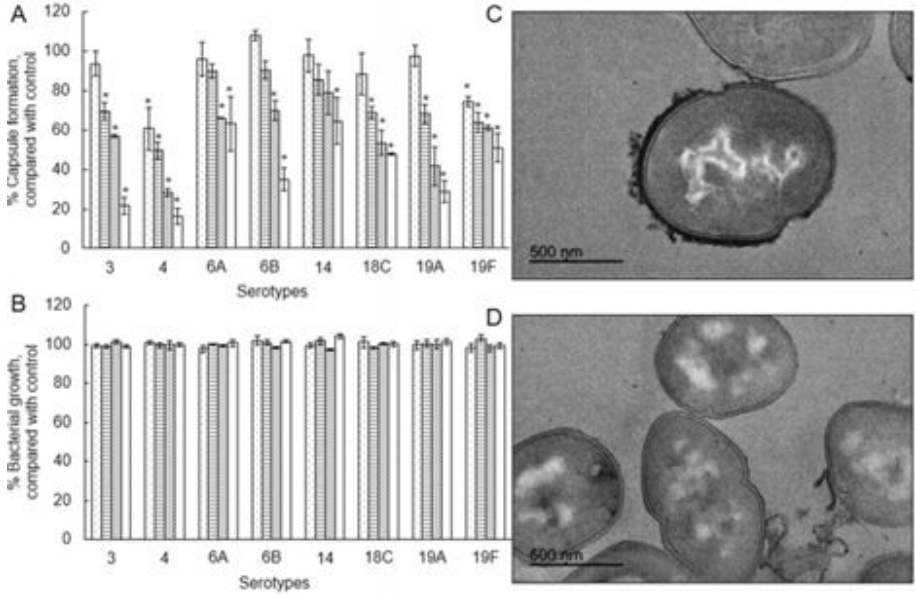

Antibacterial agents	Antibacterial activity (μg/ml)				
	S. pneumoniae clinical isolates				*S. pneumoniae* ATCC 700673
	MIC_{50}/MBC_{50}	MIC_{90}/MBC_{90}	MIC range	MBC range	MIC/MBC
Ethanol extract	64/128	128/512	16–256	16–512	32/128
Purified rhodomyrtone	0.50/1	2/4	0.125–4	0.125–4	0.50/1
Synthetic rhodomyrtone	0.50/2	2/4	0.125–4	0.25–4	1/4
Erythromycin	0.03/0.03	0.25/0.50	0.03–2	0.03–4	0.125/0.25

viruses

MDPI

Article

Capsule-Targeting Depolymerase, Derived from *Klebsiella* KP36 Phage, as a Tool for the Development of Anti-Virulent Strategy

Grażyna Majkowska-Skrobek [1,*], Agnieszka Łątka [1], Rita Berisio [2], Barbara Maciejewska [1], Flavia Squeglia [2], Maria Romano [2], Rob Lavigne [3], Carsten Struve [4,5] and Zuzanna Drulis-Kawa [1]

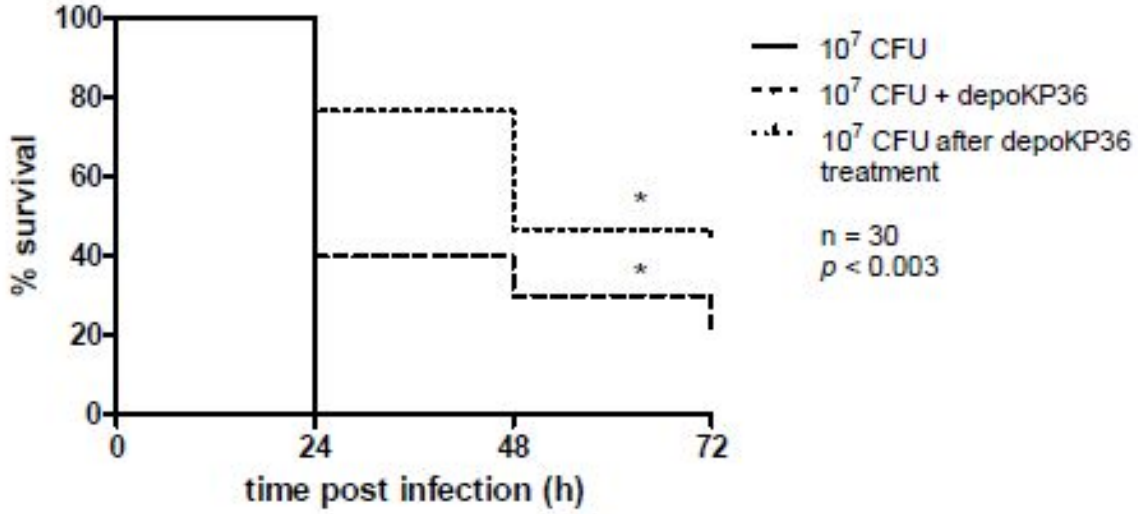

Figure 4. Inhibition of *K. pneumoniae*–induced mortality by depoKP36 using *G. mellonella* model. Larvae ($n = 30$) were injected with either bacteria (10^7 CFUs/per larvae), enzyme (final concentration, 280 μg/mL) administered within 5 min after untreated bacteria inoculation, or depoKP36-treated bacteria, and monitored for mortality. The experiments were controlled by observation of uninfected larvae, PBS-injected larvae and larvae receiving the enzyme only. Survival for each control group was 100%, so for simplicity, these groups were not included in the figure. Survival curves were plotted using the Kaplan-Meier method, and differences in survival were calculated by using the log-rank test (GraphPad); * $p < 0.003$ (considered to be statistically significant); Results are the means of at least three independent experiments.

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