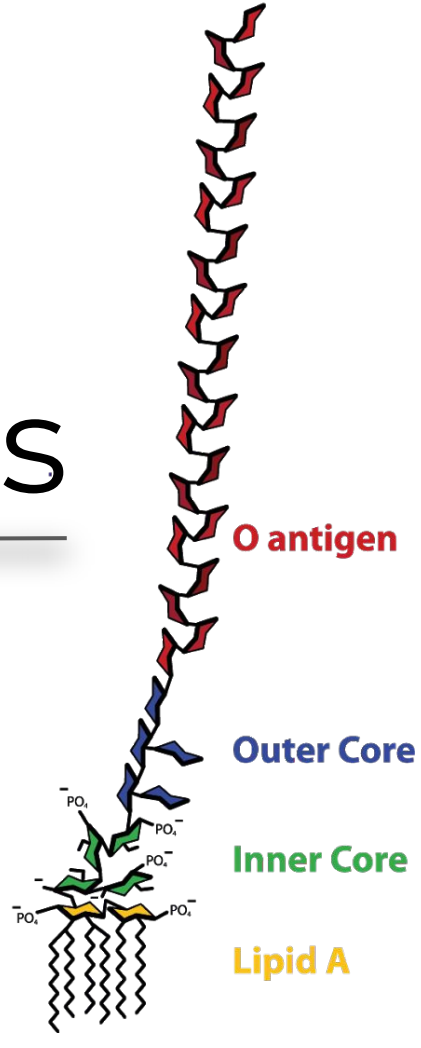
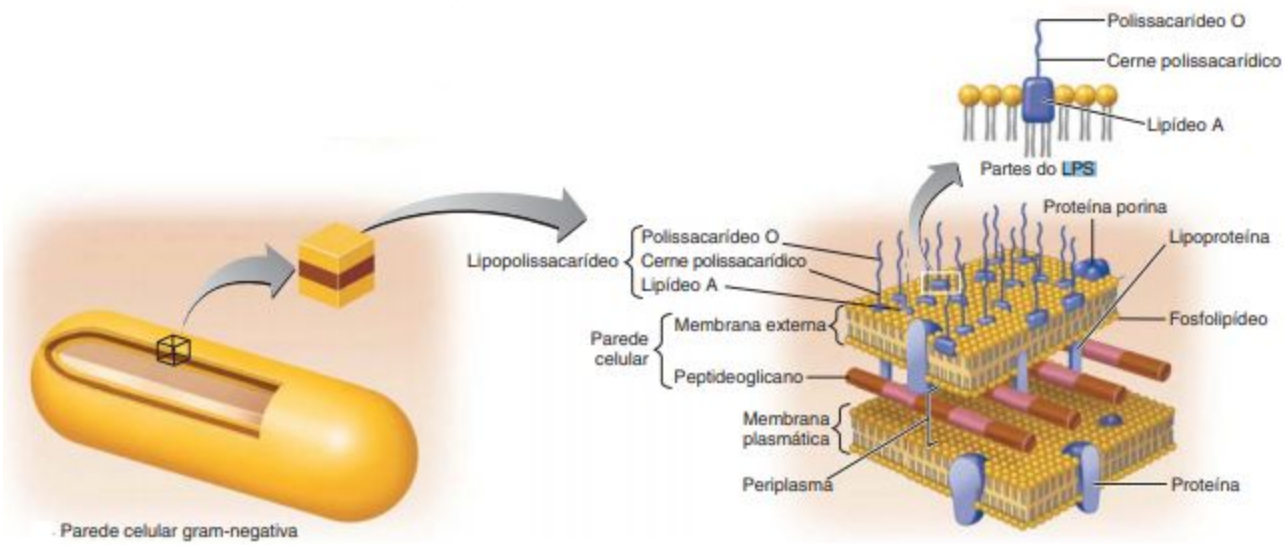
Envoltórios Bacterianos (BTC5901)

Lipopolissacarídeos

Grupo 01:
Nayara Del Santos
Filipe Barbalho
Mirla Henostroza
Lucas Guimarães

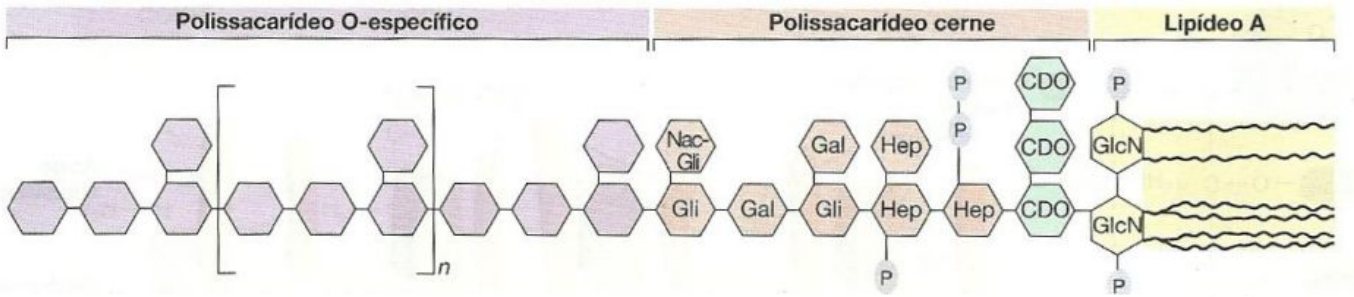


O QUE É O LPS?



TORTORA, G.J.; FUNKE, B.R.; CASE, CL. Microbiologia. 10. ed., Porto Alegre: Artmed, 2010.

ESTRUTURA DO LPS

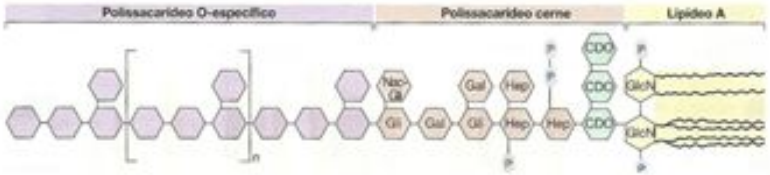


ANTÍGENO **ESTRUTURAL** **ENDOTOXINA**

Sua estrutura é composta por uma parte **hidrofóbica (antígeno A)**, e a parte hidrofílica ou **cadeia O.**

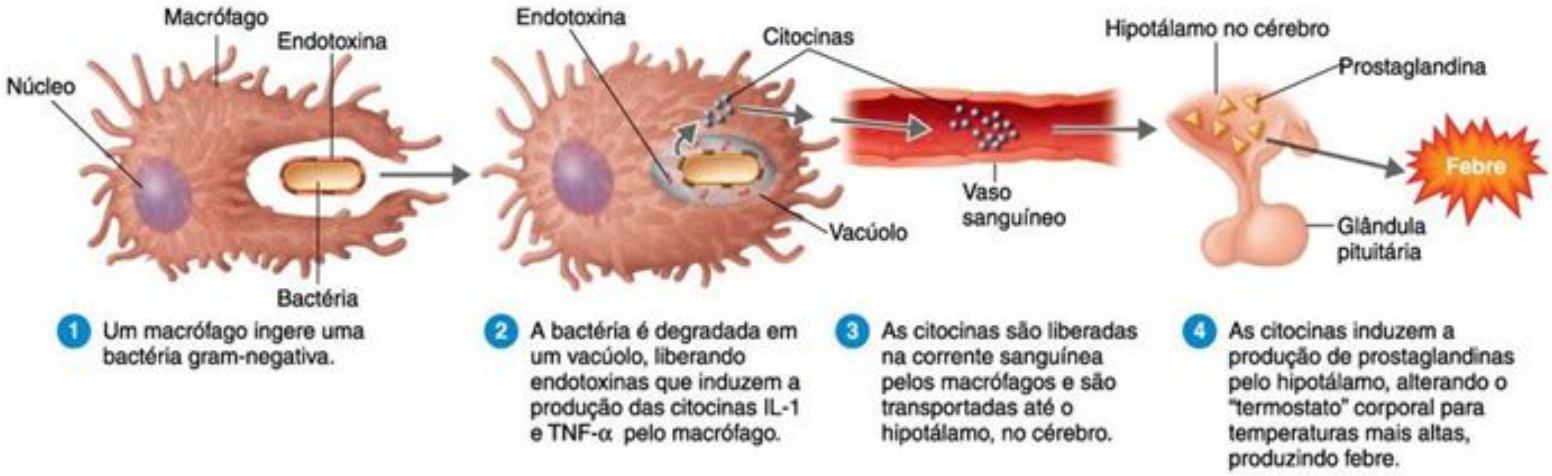
MADIGAN, Michael T; MARTINKO, John M; DUNLAP, Paul V; CLARK, David P. Microbiologia de Brock. 12ª edição. ed. [*S. l.*]: Artmed, 2010.

FUNÇÕES DO LPS



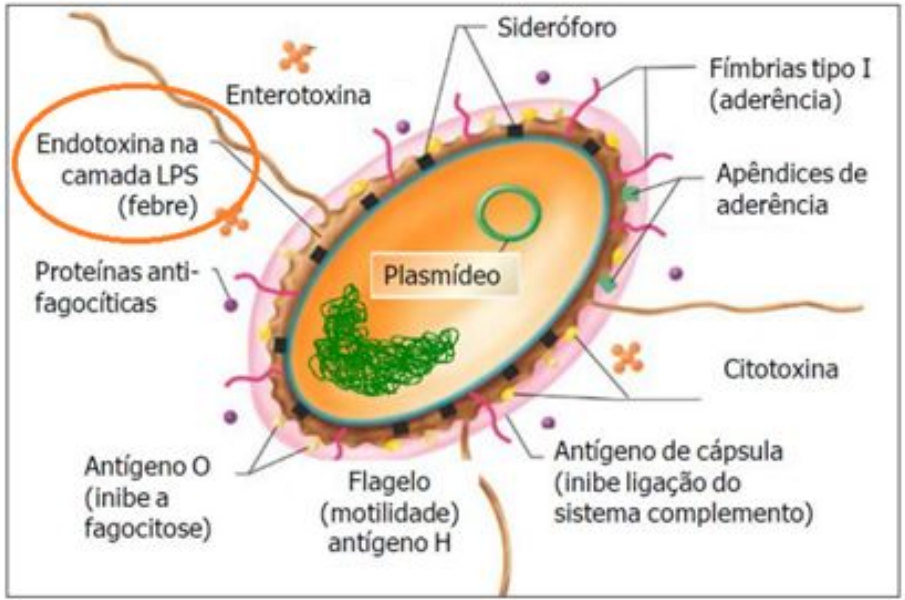
- ✓ Conferir carga à porção externa do envoltório Gram-negativa (Impede a entrada livre de compostos hidrofóbicos na célula, incluindo antibióticos);
- ✓ Assimetria da membrana externa (Permite a adoção de conformação de β-barril pelas proteínas da membrana externa);
- ✓ Interação bactéria-hospedeiro via sistema imune (Tanto em relações parasíticas, patógenos, quanto mutualísticas, microbiota intestinal);
- ✓ Proteção contra sais biliares.

MECANISMO DE RESPOSTA PIROGÊNICA



TORTORA, G.J.; FUNKE, B.R.; CASE, CL. Microbiologia. 10. ed., Porto Alegre: Artmed, 2010.

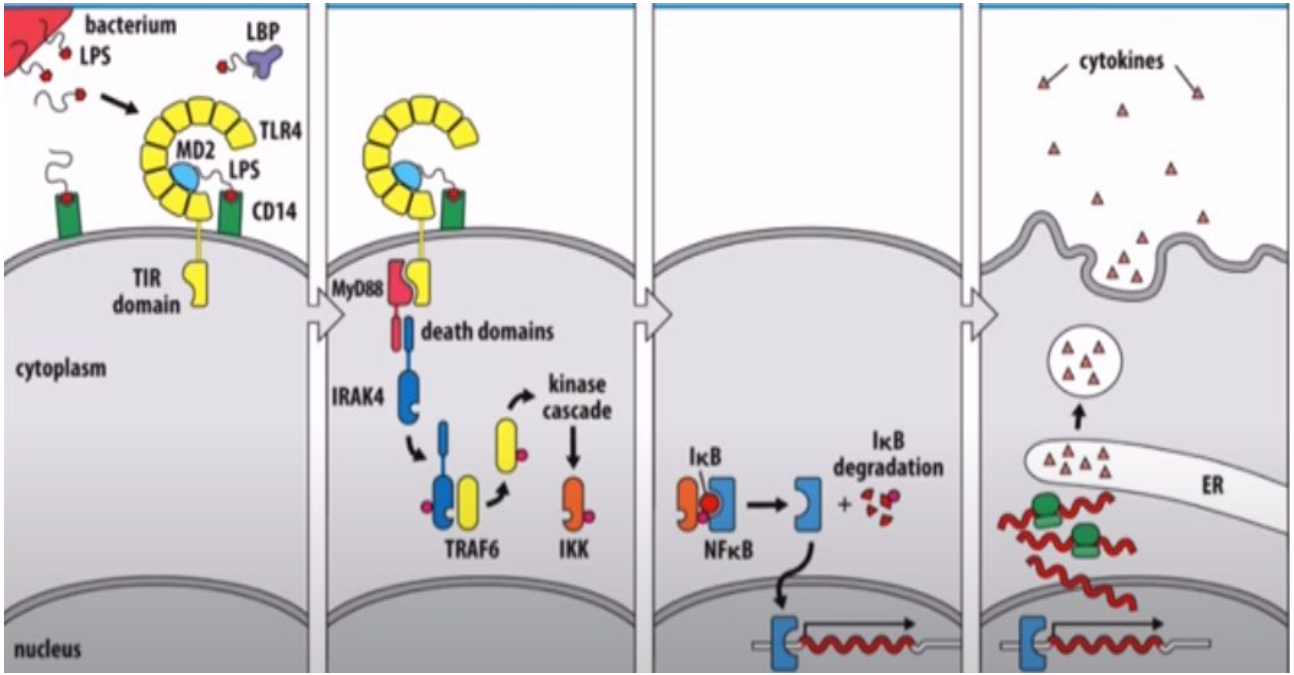
FATORES DE VIRULÊNCIA BACTERIANA



Estratégias de virulência em bactérias

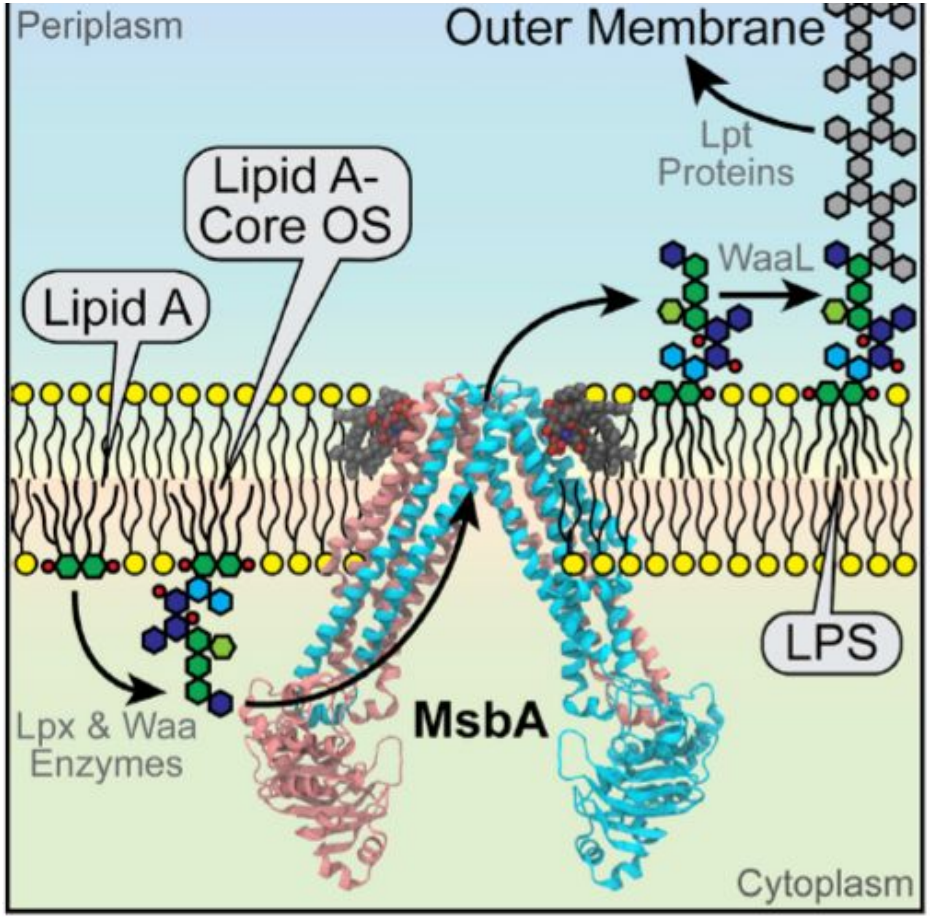
Mecanismos de patogenicidade: Aderência e invasão, evasão das defesas do hospedeiro, captação de nutrientes e **produção de citocinas**.

RECONHECIMENTO DO LPS PELO SISTEMA IMUNE



- Ativação das vias de sinalização (transcrição NFKB);
- Sinalização variada para outras células do organismo (neutrófilos, linfócitos B).

ESTRUTURA, SÍNTESE E TRANSPORTE DO LPS



(1) Padayatti, P. S.; Lee, S. C.; Stanfield, R. L.; Wen, P. C.; Tajkhorshid, E.; Wilson, I. A.; Zhang, Q. Structural Insights into the Lipid A Transport Pathway in MsbA. Structure 2019, 27 (7), 1114–1123.e3. https://doi.org/10.1016/j.str.2019.04.007.

Lipídeo A

- **Funções e Interações**
  - Endotoxina
  - Porção anfipática que permite a ancoragem na membrana externa- Cadeias acil + fosfato
  - Causador de choque séptico

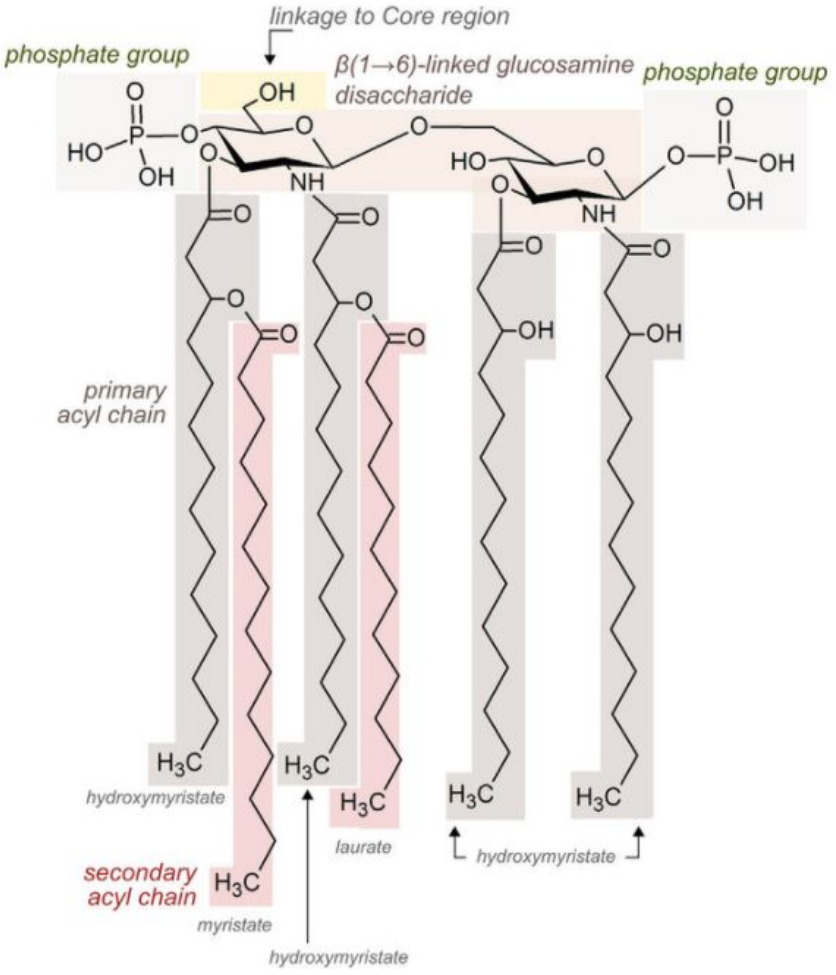
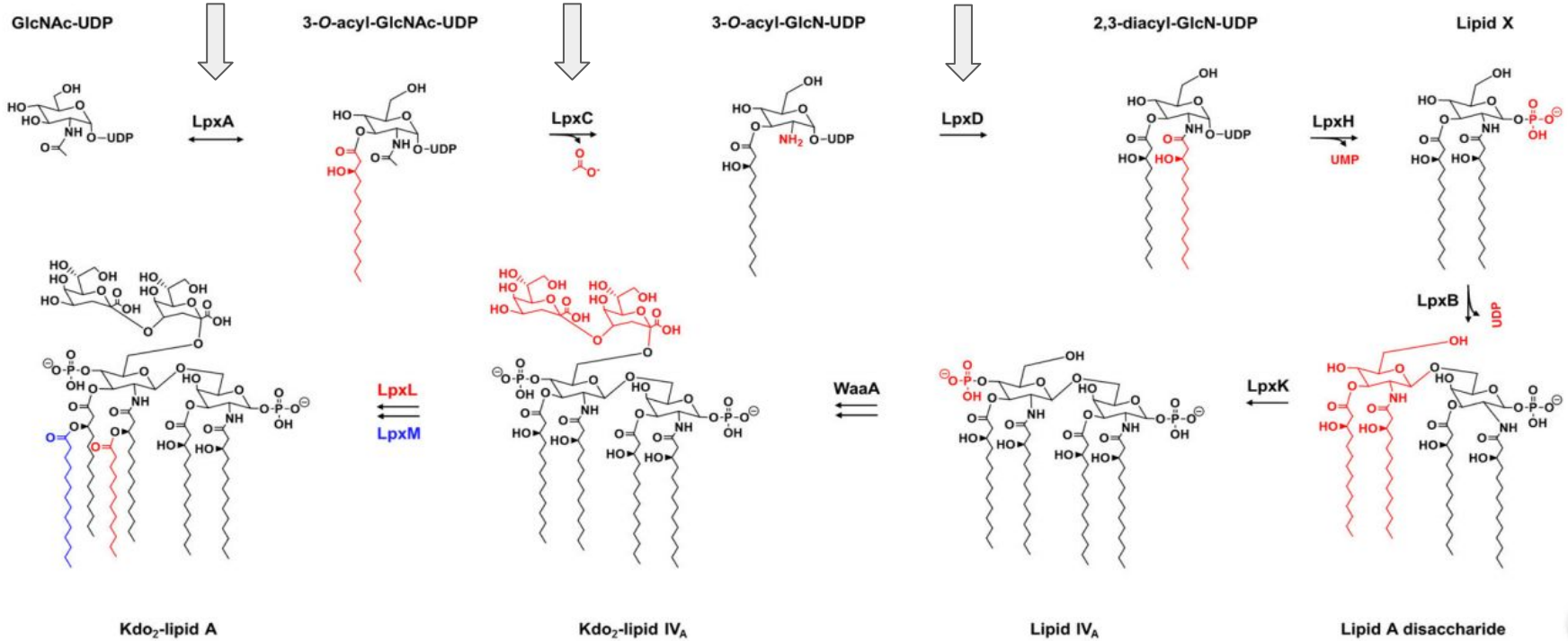


Imagem retirada de A. Steimle et al. / International Journal of Medical Microbiology 306 (2016) 290–301

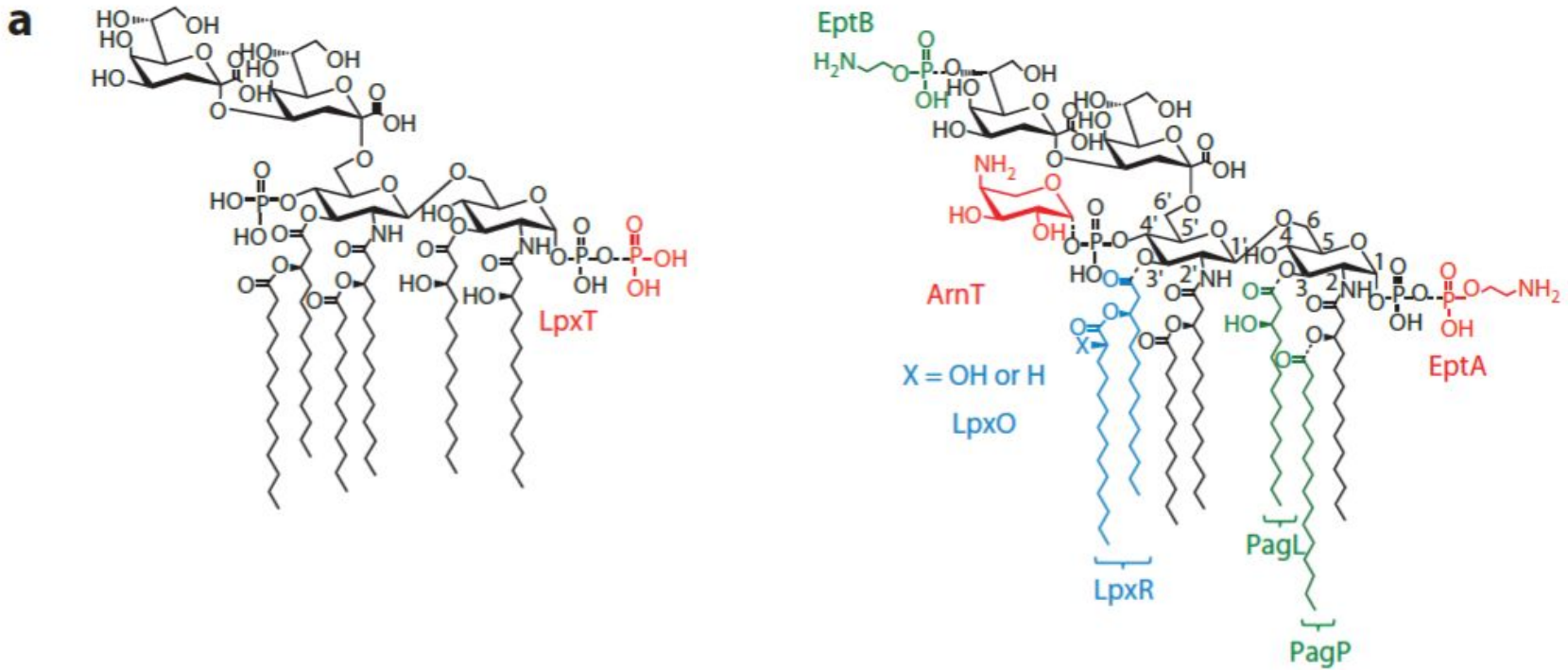
Síntese

Lipídeo A - Via de Raetz



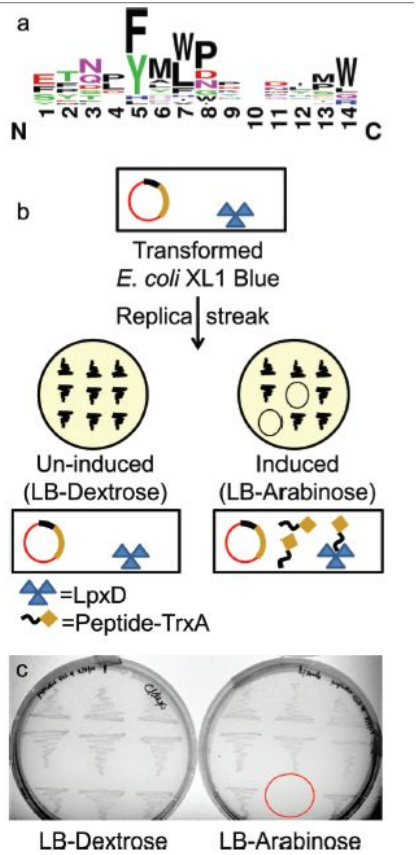
Bertani, B.; Ruiz, N. Function and Biogenesis of Lipopolysaccharides. EcoSal Plus 2018, 8 (1). https://doi.org/10.1128/ecosalplus.ESP-0001-2018.

Modificação do Lipídeo A

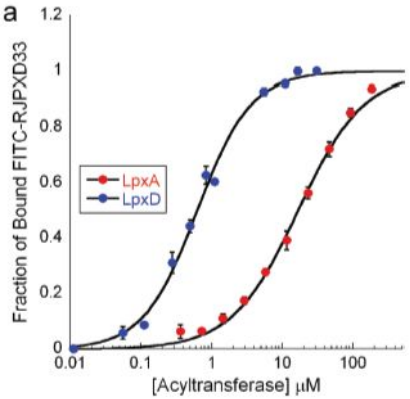


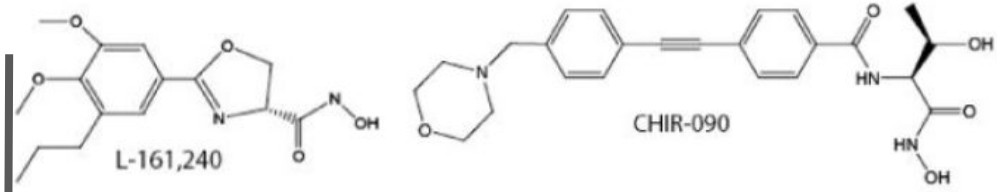
Whitfield, C.; Stephen Trent, M. Biosynthesis and Export of Bacterial Lipopolysaccharides. Annu. Rev. Biochem. 2014, 83 (February), 99–128. https://doi.org/10.1146/annurev-biochem-060713-035600.

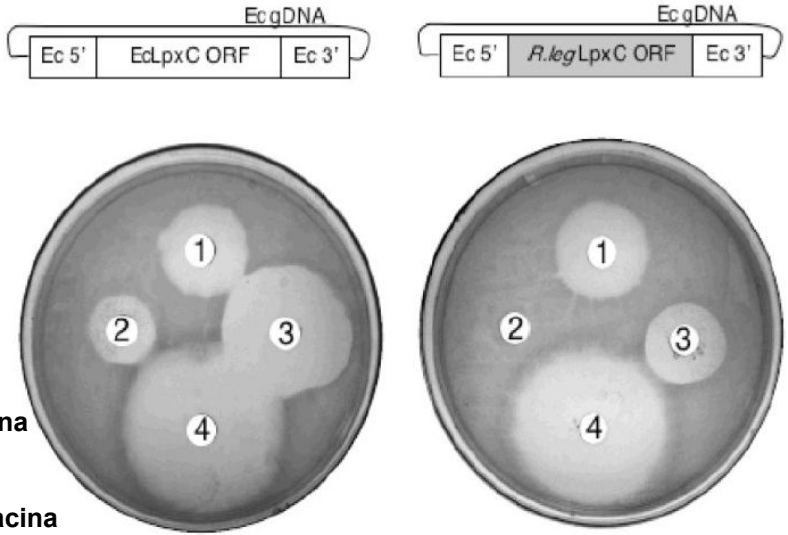
Inibidores da síntese de lipídeo A



RJPXD33
(TNLYMLPKWDIP)







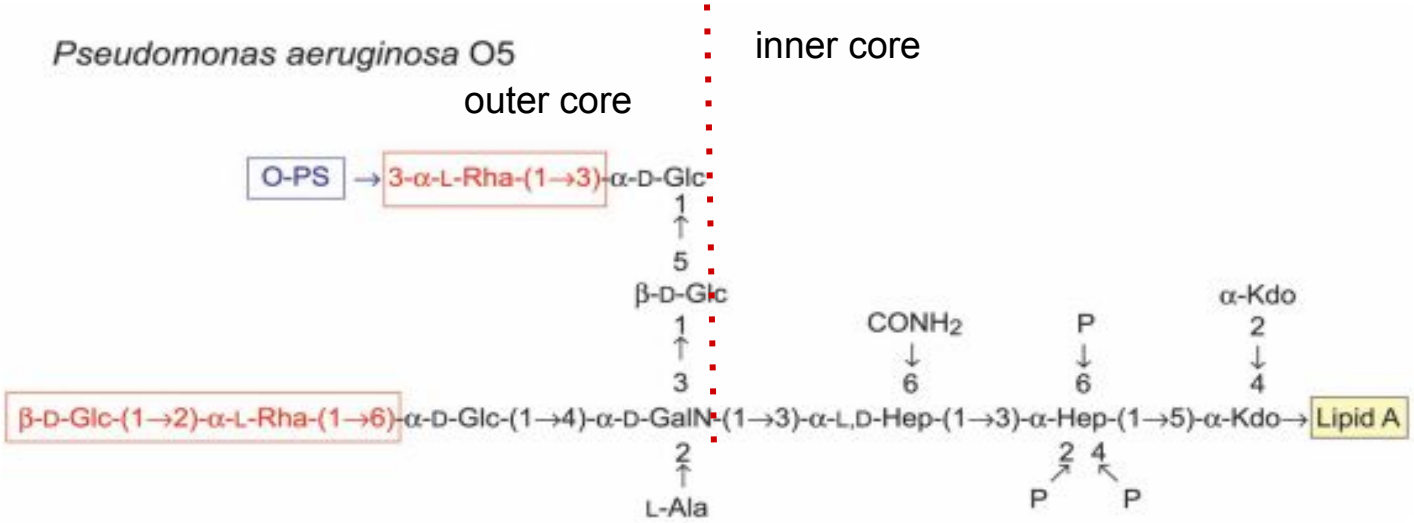
Legenda
1- tobramicina
2- L-161,240
3- CHIR-090
4- ciprofloxacina

Jenkins, R. J.; Dotson, G. D. Dual Targeting Antibacterial Peptide Inhibitor of Early Lipid a Biosynthesis. ACS Chem. Biol. 2012, 7 (7), 1170–1177. https://doi.org/10.1021/cb300094a.

Barb, AW et al. Inhibition of Lipid A Biosynthesis as the Primary Mechanism of CHIR-090 Antibiotic Activity in Escherichia coli. Biochemistry. 2007 March 27; 46(12): 3793–3802. doi:10.1021/bi6025165.

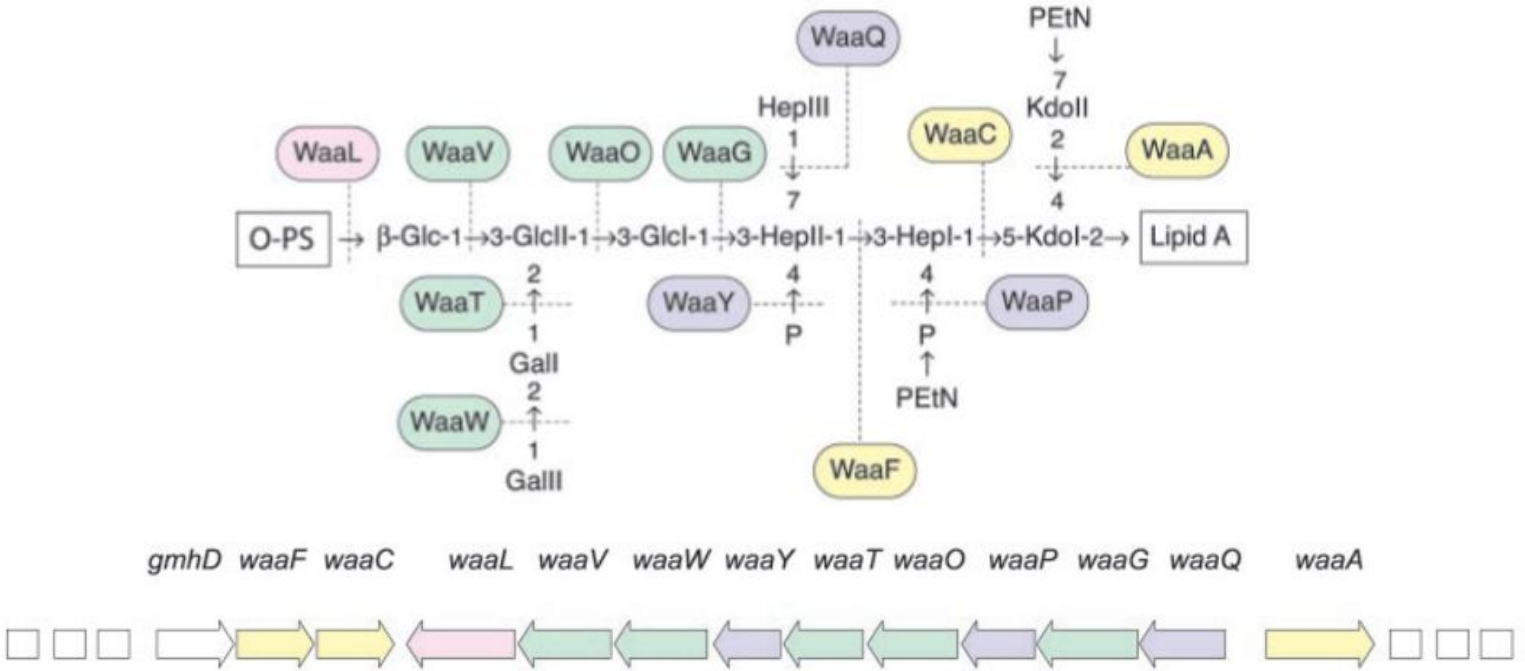
Polissacarídeo Cerne

- **Funções e Interações**
  - Interação com anticorpos que ativam o sistema complemento
  - Normalmente Espécie-específico
  - Mantenimento da homeostase celular



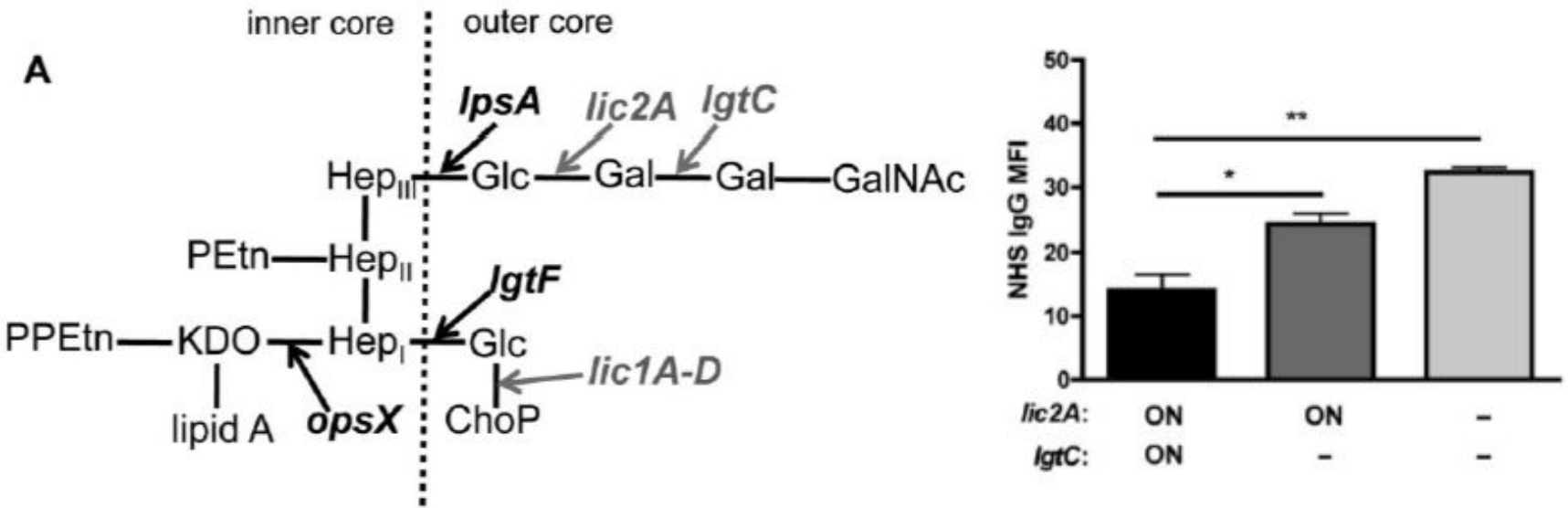
Whitfield, C.; Stephen Trent, M. Biosynthesis and Export of Bacterial Lipopolysaccharides. Annu. Rev. Biochem. 2014, 83 (February), 99–128. https://doi.org/10.1146/annurev-biochem-060713-035600.

Síntese



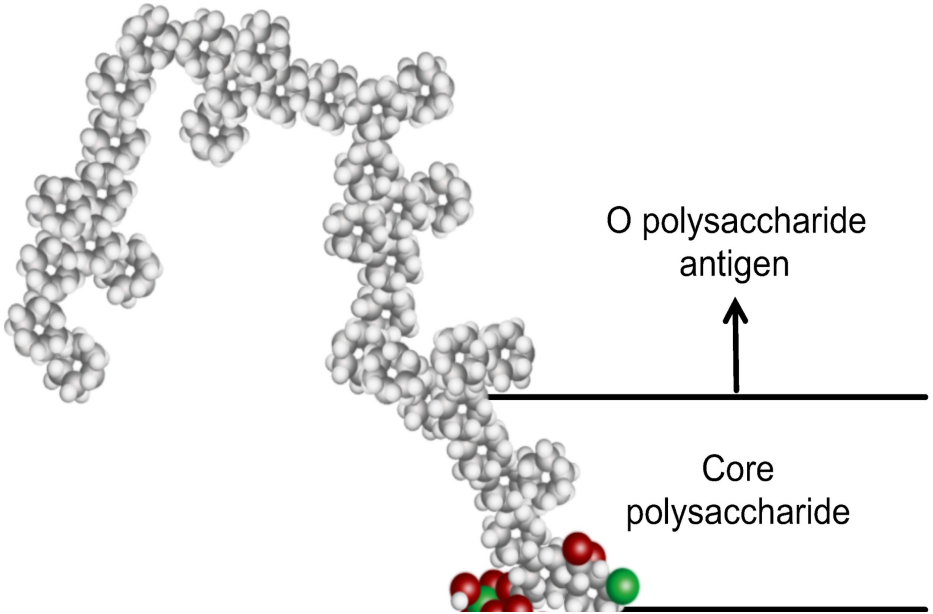
Whitfield, C.; Stephen Trent, M. Biosynthesis and Export of Bacterial Lipopolysaccharides. Annu. Rev. Biochem. 2014, 83 (February), 99–128. https://doi.org/10.1146/annurev-biochem-060713-035600.

Mudança de fase (Phase variation)



Clark, S. E.; Eichelberger, K. R.; Weiser, J. N. Evasion of Killing by Human Antibody and Complement through Multiple Variations in the Surface Oligosaccharide of Haemophilus Influenzae. Mol. Microbiol. 2013, 88 (3), 603–618. https://doi.org/10.1111/mmi.12214.

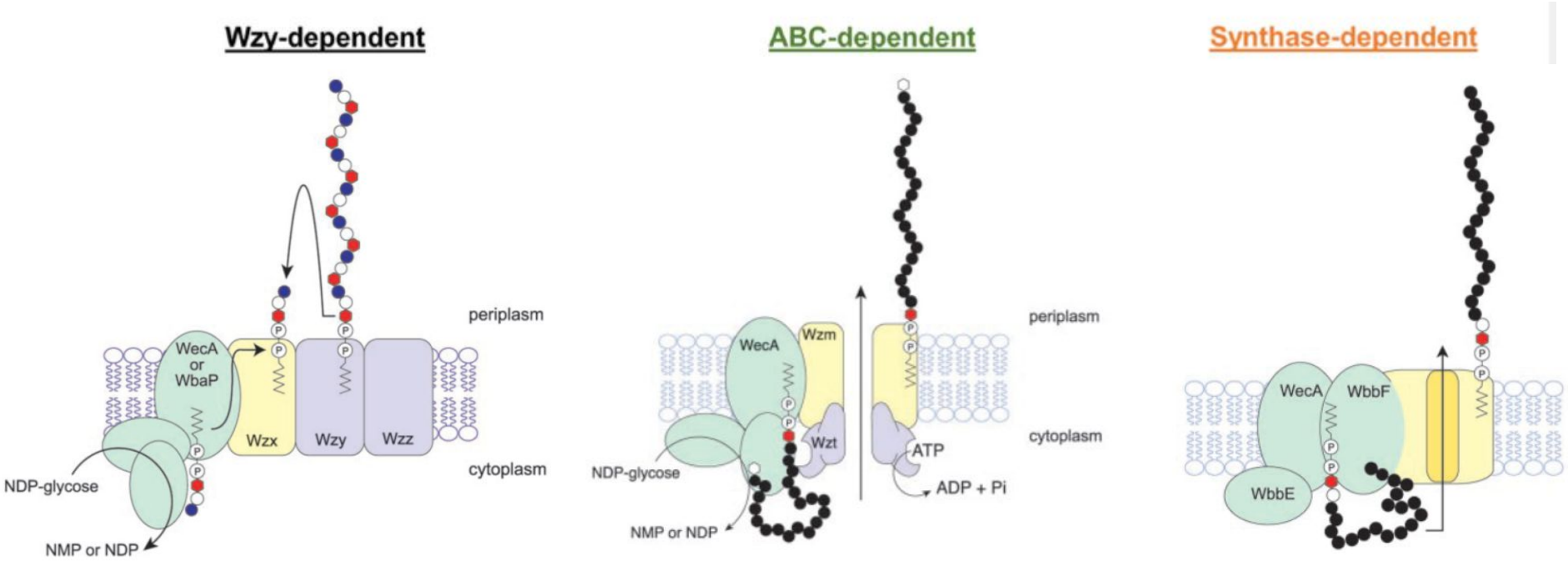
Polissacarídeo O-específico



- **Funções e Interações**
  - Resistência ao complemento- impede o acesso ao polissacarídeo cerne
  - Marcador antigênico sorovariante-específico (*E. coli, Salmonella*)
  - Reconhecimento por bacteriófagos
  - Resistência à fagocitose

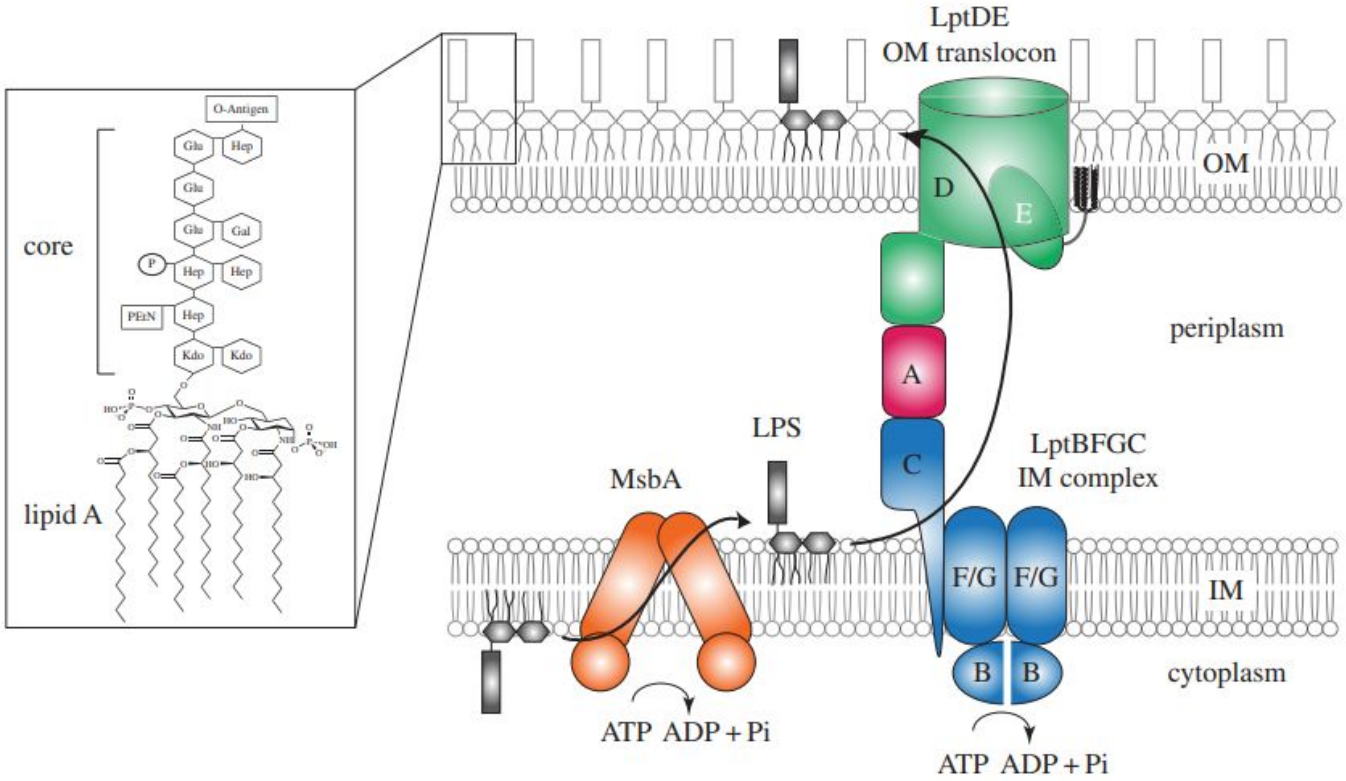
Stromberg LR, Hengartner NW, Swingle KL, Moxley RA, Graves SW, Montaño GA, et al. (2016) Membrane Insertion for the Detection of Lipopolysaccharides: Exploring the Dynamics of Amphiphile-in-Lipid Assays. PLoS ONE 11(5): e0156295. https://doi.org/10.1371/journal.pone.0156295

Vias de síntese do polissacarídeo O

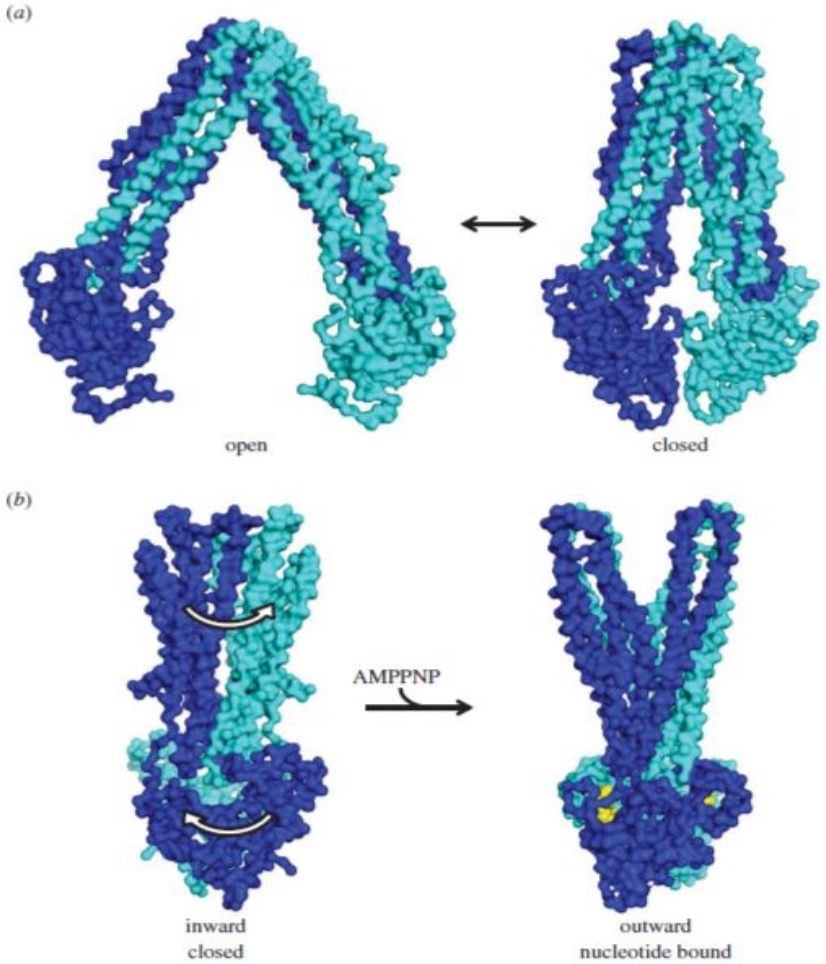


Whitfield, C.; Stephen Trent, M. Biosynthesis and Export of Bacterial Lipopolysaccharides. Annu. Rev. Biochem. 2014, 83 (February), 99–128. https://doi.org/10.1146/annurev-biochem-060713-035600.

Transporte até a Membrana Externa

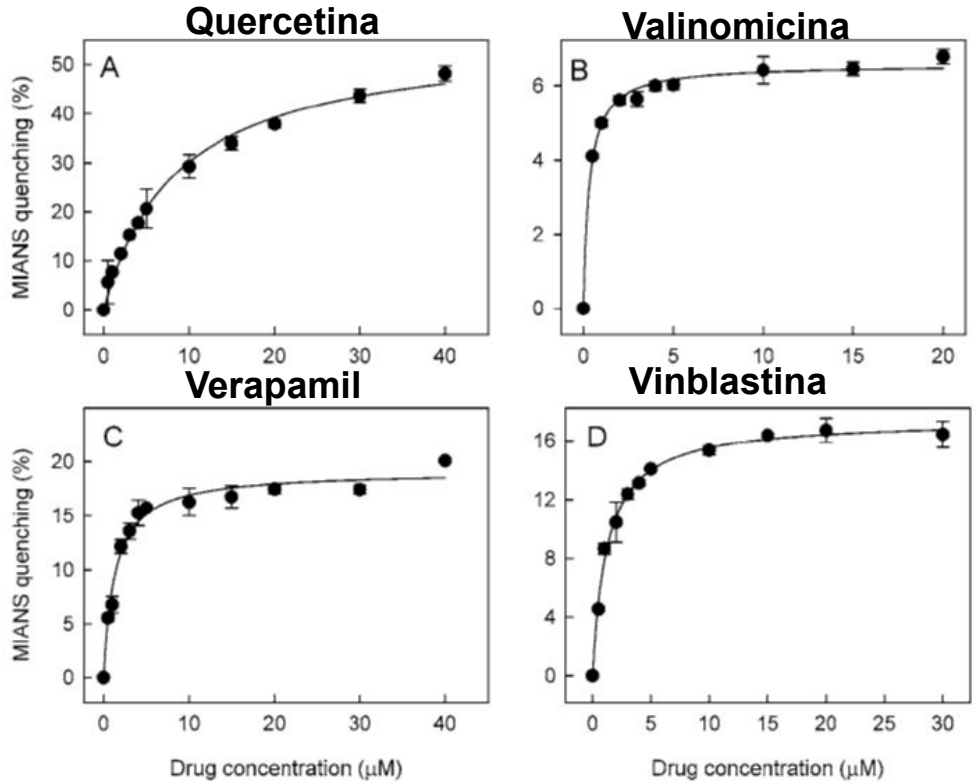


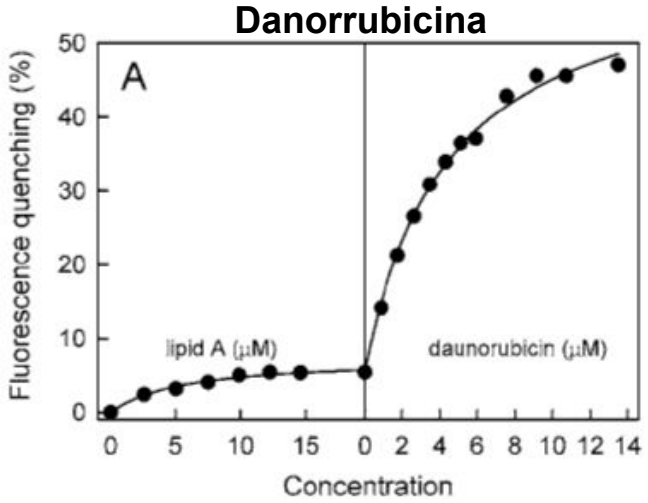
Simpson BW, May JM, Sherman DJ, Kahne D, Ruiz N. 2015 Lipopolysaccharide transport to the cell surface: biosynthesis and extraction from the inner membrane. Phil. Trans. R. Soc. B 370: 20150029. http://dx.doi.org/10.1098/rstb.2015.0029



Simpson BW, May JM, Sherman DJ, Kahne D, Ruiz N. 2015 Lipopolysaccharide transport to the cell surface: biosynthesis and extraction from the inner membrane. Phil. Trans. R. Soc. B 370: 20150029. http://dx.doi.org/10.1098/rstb.2015.0029

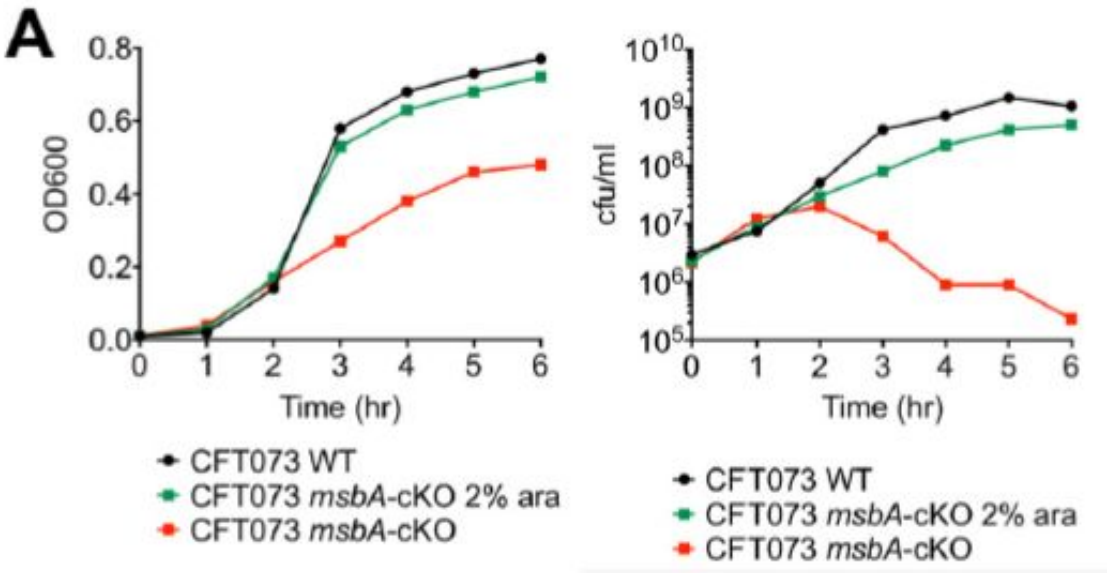
MsbA: transportador de drogas anfipáticas





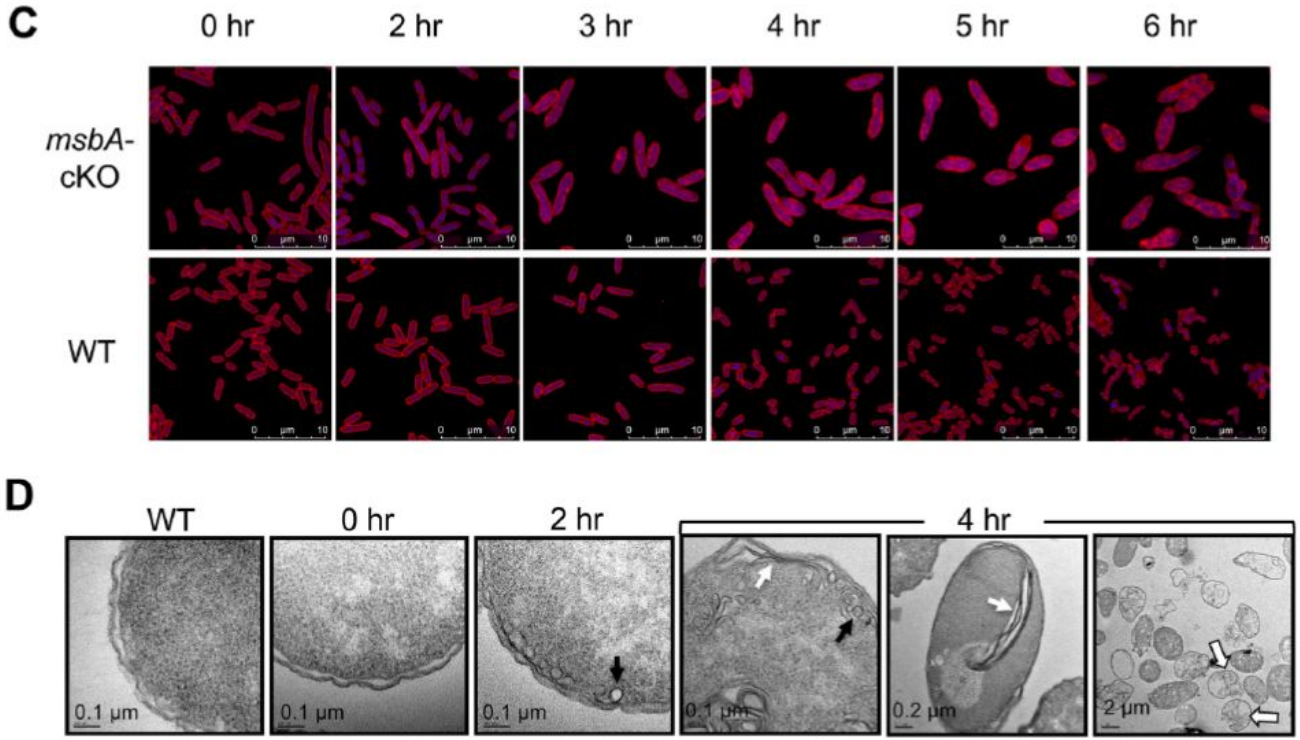
Siarheyeva, A.; Sharom, F. J. The ABC Transporter MsbA Interacts with Lipid A and Amphipathic Drugs at Different Sites. Biochem. J. 2009, 419 (2), 317–328. https://doi.org/10.1042/BJ20081364.

Nocaute de MsbA



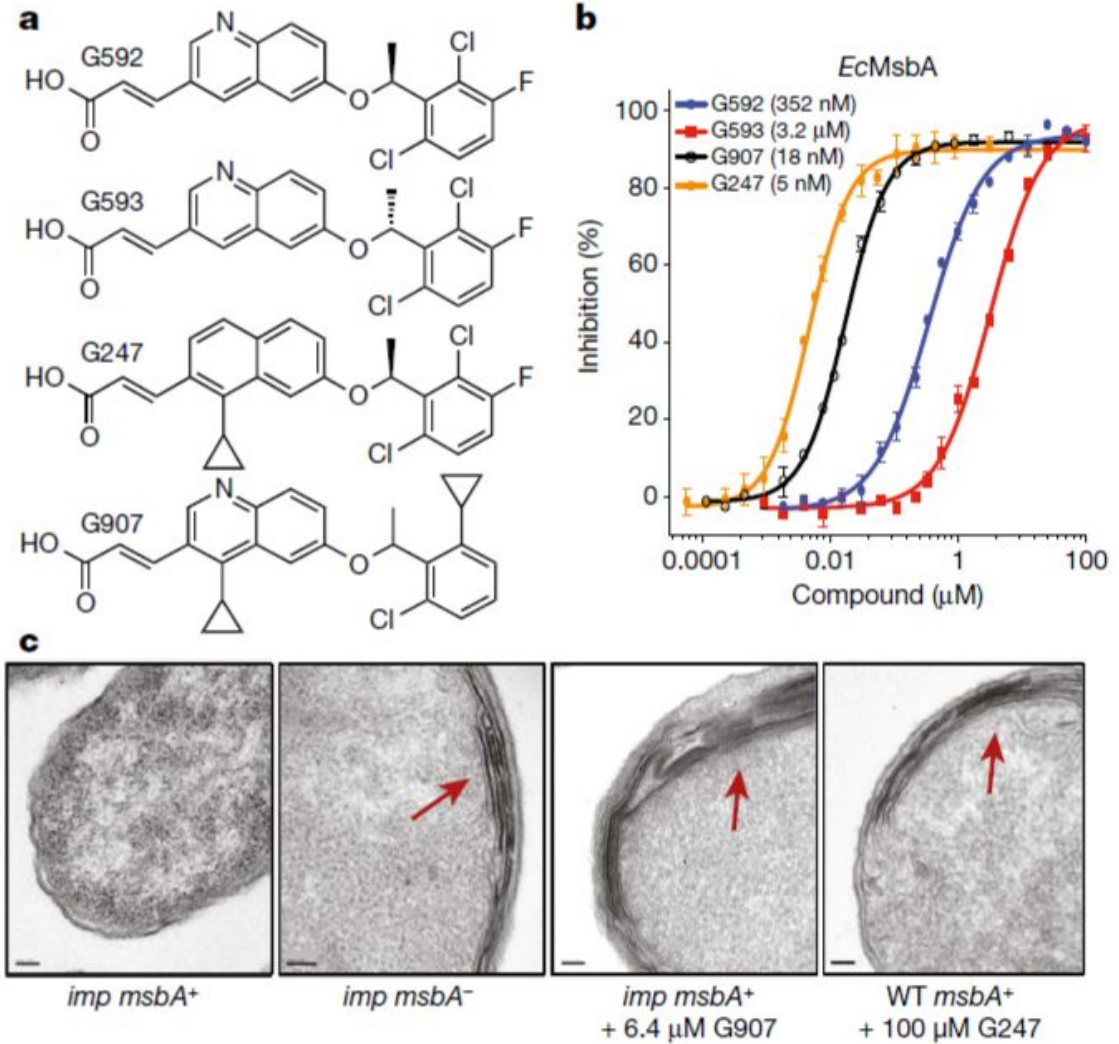
Alexander, M. K.; Miu, A.; Oh, A.; Reichelt, M.; Ho, H.; Chalouni, C.; Labadie, S.; Wang, L.; Liang, J.; Nickerson, N. N.; et al. Disrupting Gram-Negative Bacterial Outer Membrane Biosynthesis through Inhibition of the Lipopolysaccharide Transporter MsbA. Antimicrob. Agents Chemother. 2018, 62 (11), 1–15. https://doi.org/10.1128/AAC.01142-18.

Nocaute de MsbA



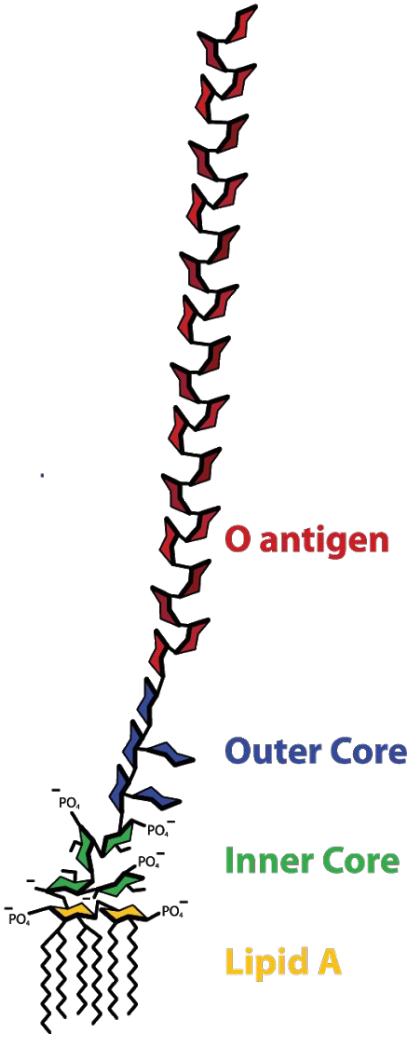
Alexander, M. K.; Miu, A.; Oh, A.; Reichelt, M.; Ho, H.; Chalouni, C.; Labadie, S.; Wang, L.; Liang, J.; Nickerson, N. N.; et al. Disrupting Gram-Negative Bacterial Outer Membrane Biosynthesis through Inhibition of the Lipopolysaccharide Transporter MsbA. Antimicrob. Agents Chemother. 2018, 62 (11), 1–15. https://doi.org/10.1128/AAC.01142-18.

Inibição de MsbA

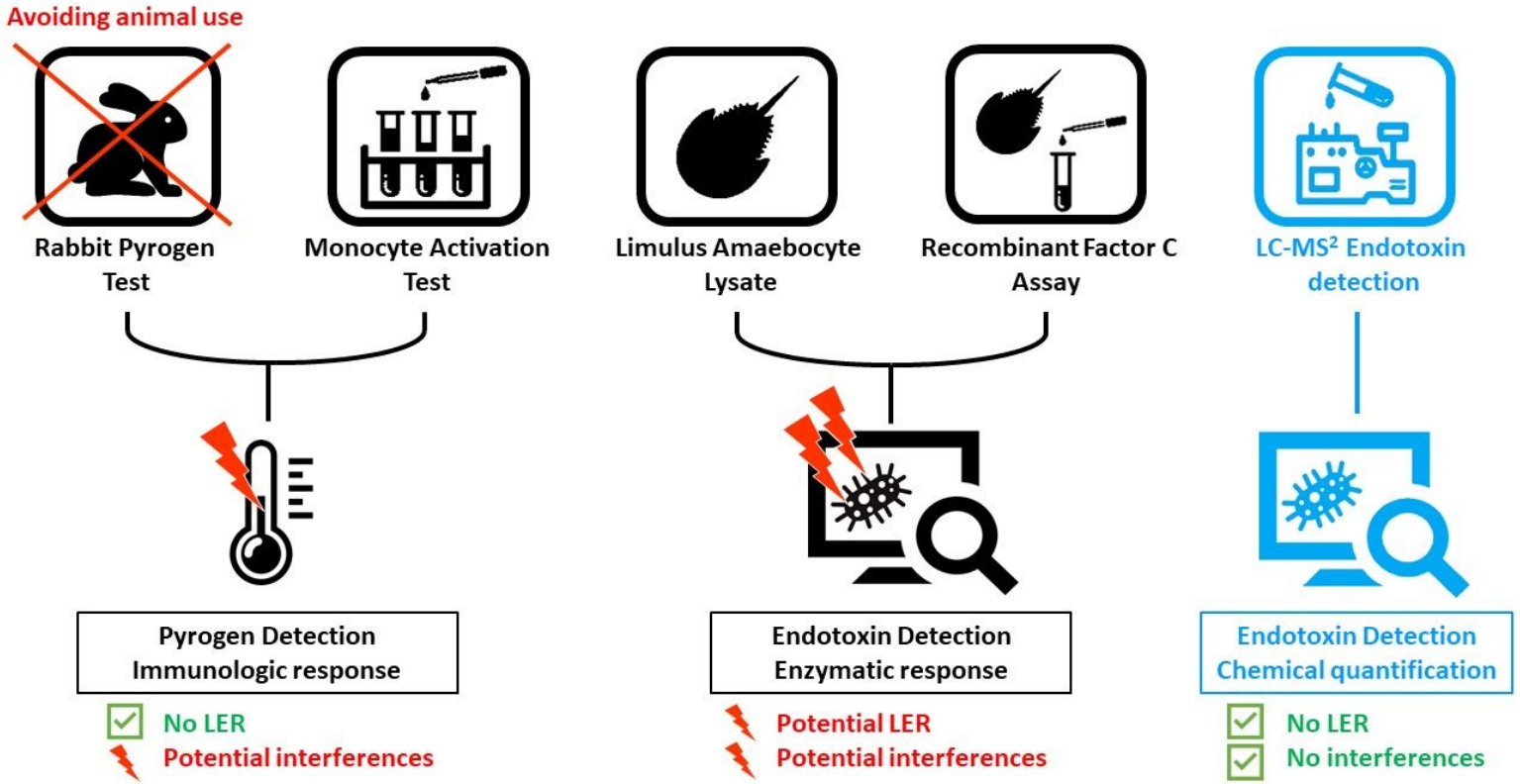


Ho, H.; Miu, A.; Alexander, M. K.; Garcia, N. K.; Oh, A.; Zilberleyb, I.; Reichelt, M.; Austin, C. D.; Tam, C.; Shriver, S.; et al. Structural Basis for Dual-Mode Inhibition of the ABC Transporter MsbA. Nature 2018, 557 (7704), 196–201. https://doi.org/10.1038/s41586-018-0083-5.

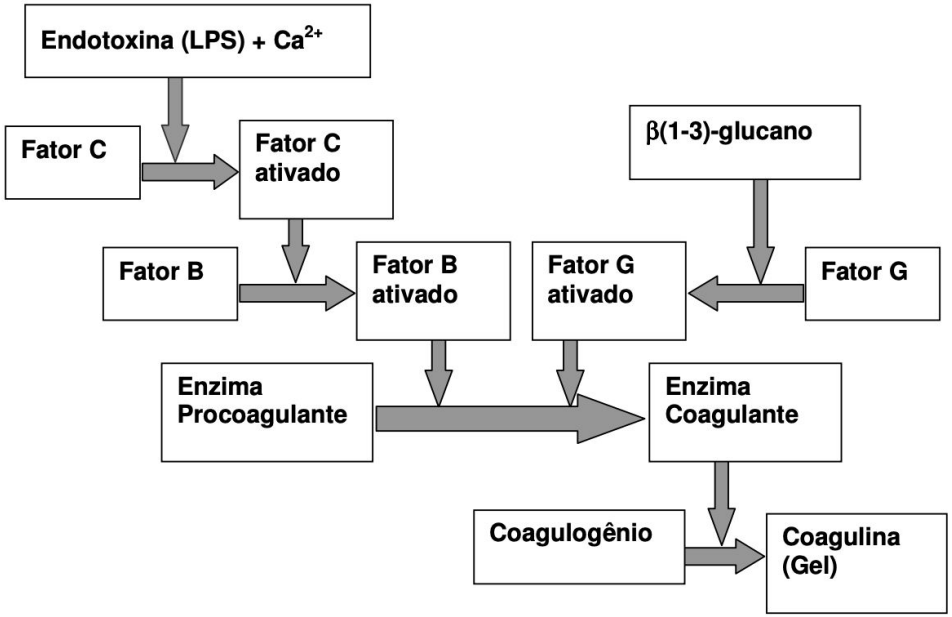
APLICAÇÕES DO LPS



Quantificação do LPS



Quantificação do LPS



(FUKUMORI, 2008)

Endotoxinas na Farmacopéia

Como método de detecção de endotoxinas bacterianas, o ensaio de LAL foi pela primeira vez introduzido na USP XX (1980). No Brasil, foi incluso na 4a. Edição da Farmacopéia em 1996.

Importante não só para IFA mas como componentes da cadeia produtiva, em especial para vias parenterais.

Limites de Endotoxina definidos a partir da quantidade a ser aplicada no paciente, UE (Unidade de Endotoxina)

Endotoxinas na Farmacopéia

VACINA HEPATITE B (RECOMBINANTE)

Vaccinum hepatitidis B ADN recombinatum

TESTES DE SEGURANÇA BIOLÓGICA

Esterilidade (5.5.3.2.1). Proceder conforme descrito na monografia de *Vacinas para uso humano*.

Pirogênios (5.5.2.1). Cumpre o teste.

Endotoxinas bacterianas (5.5.2.2). No máximo 10 UE/mL.

Vacinas Low LPS

RESEARCH PAPER

Human Vaccines & Immunotherapeutics 9:2, 339–348; February 2012; © 2012 Landes Bioscience

An improved whole cell pertussis vaccine with reduced content of endotoxin

Waldely Oliveira Dias,[1,*] Arno A.J. van der Ark,[2] Maria Aparecida Sakauchi,[3] Flávia Saldanha Kubrusly,[1] Ana Fabíola R.O. Prestes,[1] Monamaris Marques Borges,[4] Noemi Furuyama,[3] Denise S.P.Q. Horton,[4] Wagner Quintilio,[5] Marta Antoniazi,[6] Betsy Kuipers,[2] Bernard A.M. van der Zeijst[2] and Isaias Raw[1]

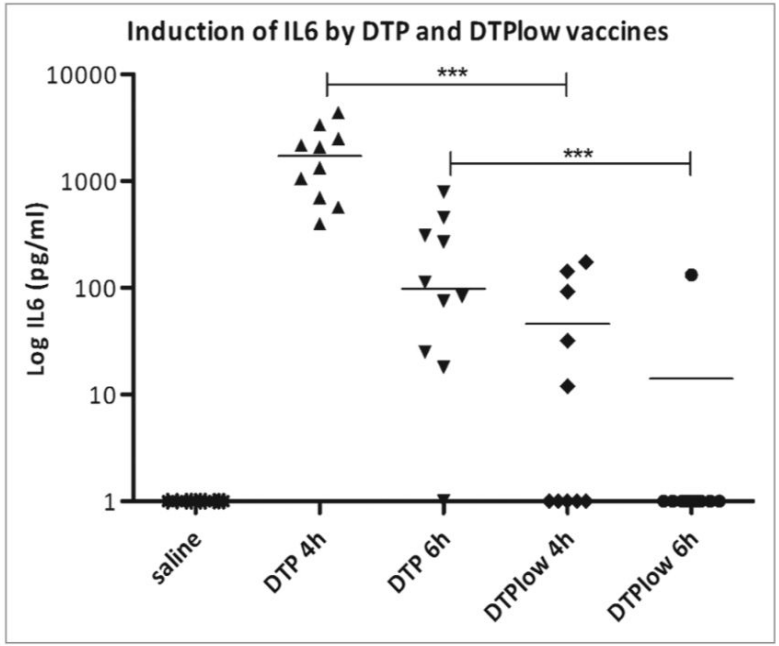
[1]Center of Biotechnology; Instituto Butantan; São Paulo, Brazil; [2]Research and Development; Netherlands Vaccine Institute (NVI)/ National Institute for Public Health and the Environment (RIVM); Bilthoven, Utrecht the Netherlands; [3]Division of Technological Development and Production; Instituto Butantan; São Paulo, Brazil; [4]Laboratory of Bacteriology; Instituto Butantan; São Paulo, Brazil; [5]Quality Control Service; Instituto Butantan; São Paulo, Brazil; [6]Laboratory of Genetic; Instituto Butantan; São Paulo, Brazil

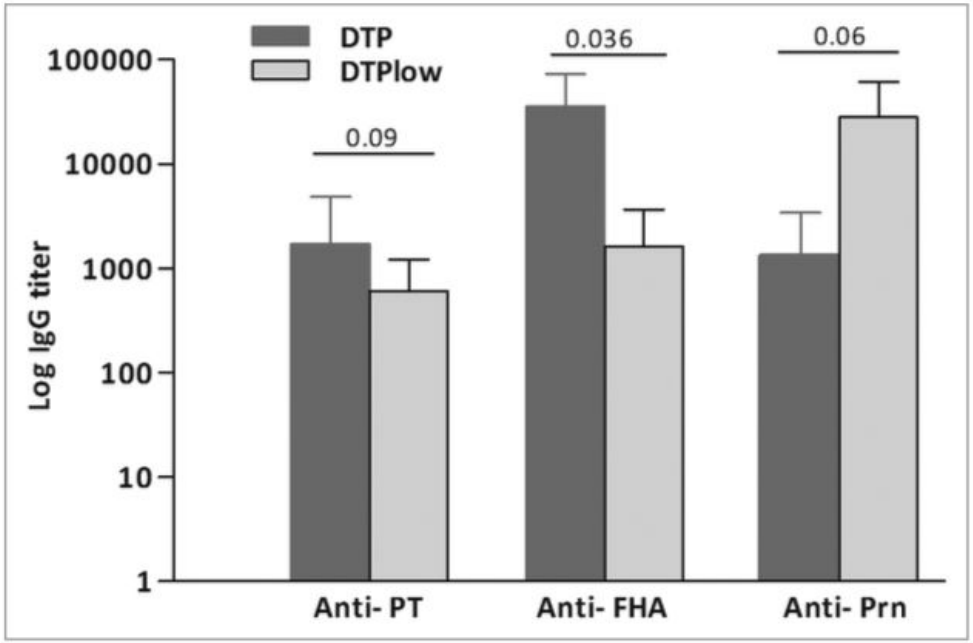
(DIAS et al., 2013)

Table 1. Quantitative analysis of LOS content and activity of 15 batches of Plow and bulk products in the GC and LAL assay, respectively

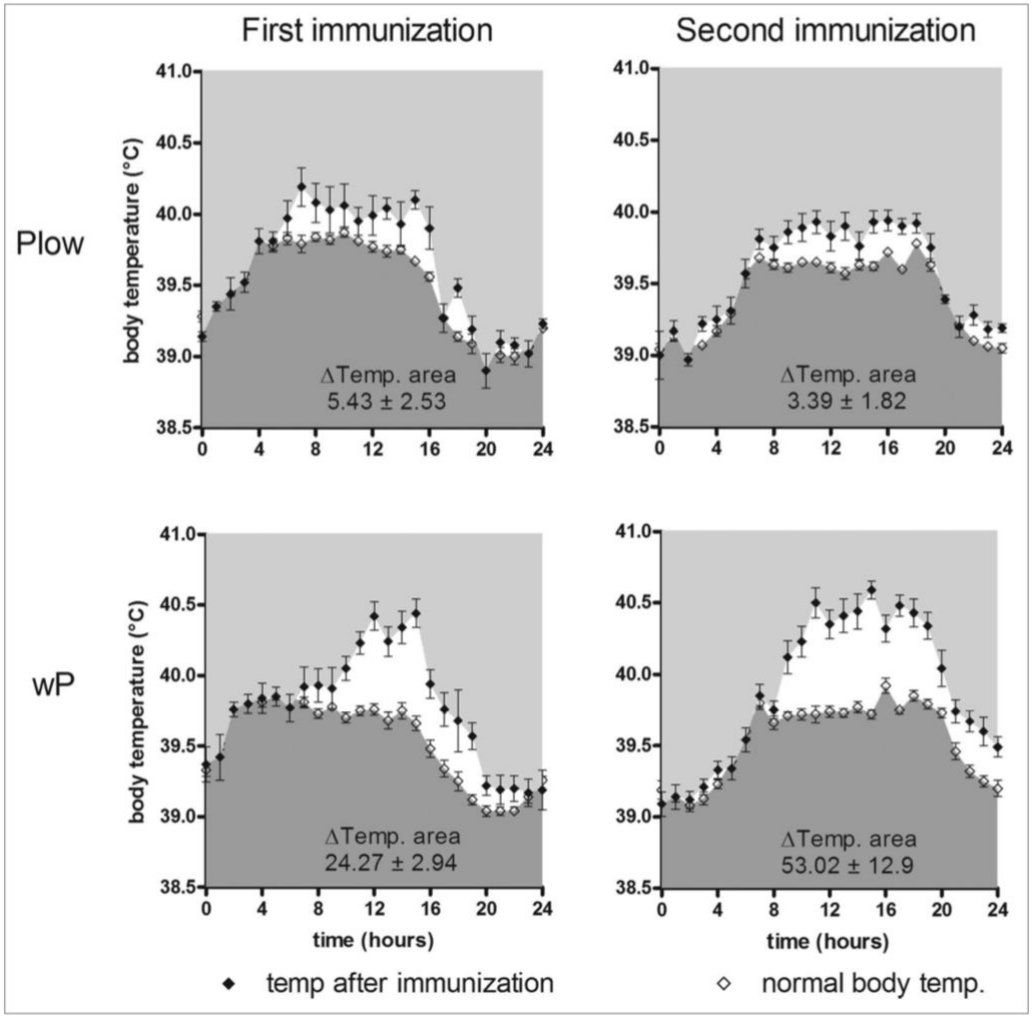
	Plow	wP	p (t-Test)
LOS content (μg LOS/mg protein)	5.27 ± 0.88	6.57 ± 0.87	**< 0.001***
Proportion of O-linked fatty acyl chains (C10:0–3OH/C14:0–3OH/C14:0)	0.9/2.2/1.0	0.9/2.2/1.0	
Endotoxin activity (IU endotoxin/mg protein)	**15,380 ± 13,851**	**533,317 ± 259,915**	**< 0.001****

*t-Test assuming equal variances; **t-Test assuming unequal variances.



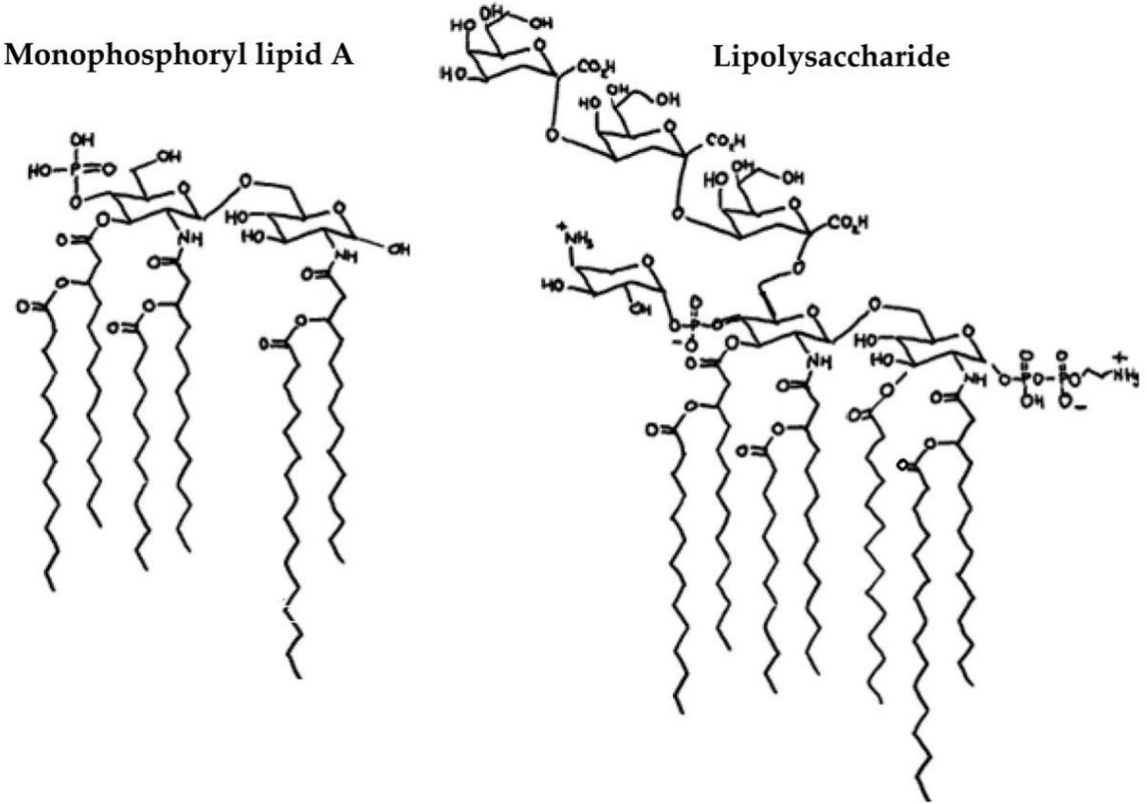


(DIAS et al., 2013)



(DIAS et al., 2013)

Monofosforil Lipídio A



(ARENAS, 2012)

Referências (Aplicações)

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CASELLA, C. R.; MITCHELL, T. C. Putting endotoxin to work for us: MPLA as clinical adjuvant. **Cell Mol Life Sci**, v. 65, n. 20, p. 3231–3240, 2008.

DIAS, W. O.; ARK, A. A. J. VAN DER; SAKAUCHI, M. A.; et al. An improved whole cell pertussis vaccine with reduced content of endotoxin. **Human Vaccines and Immunotherapeutics**, v. 9, n. 2, p. 339–348, 2013.

FUKUMORI, N. T. O. Determinação de endotoxina bacteriana (pirogênio) em radiofármacos pelo método de formação de gel. validação. , p. 77, 2008.

Vídeo

https://twitter.com/nature/status/906759694445940736