CARRERA DE ESPECIALIZACIÓN EN ESTERILIZACIÓN

ASIGNATURA: MICROBIOLOGÍA APLICADA

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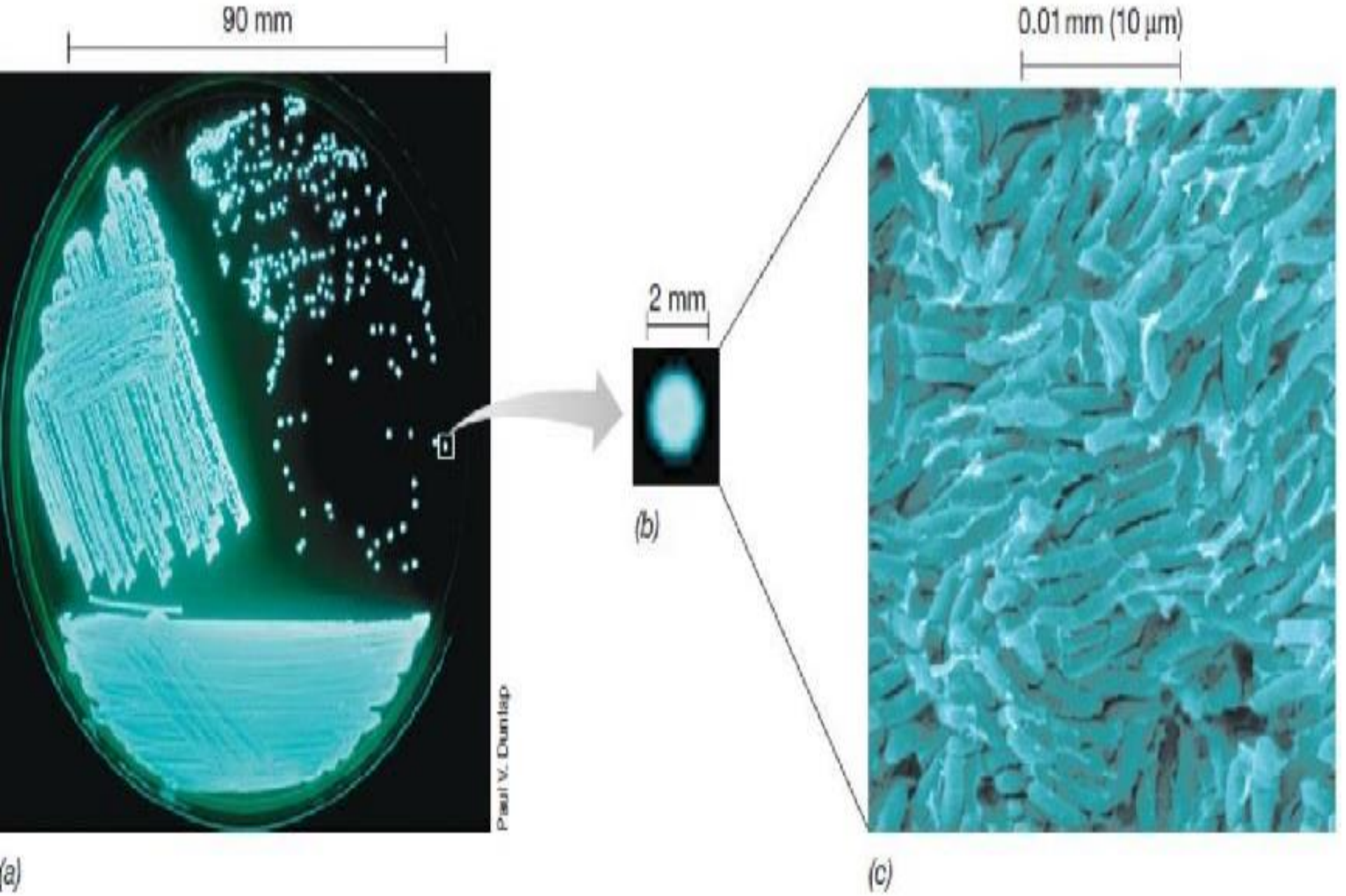
Clases 3 y 4

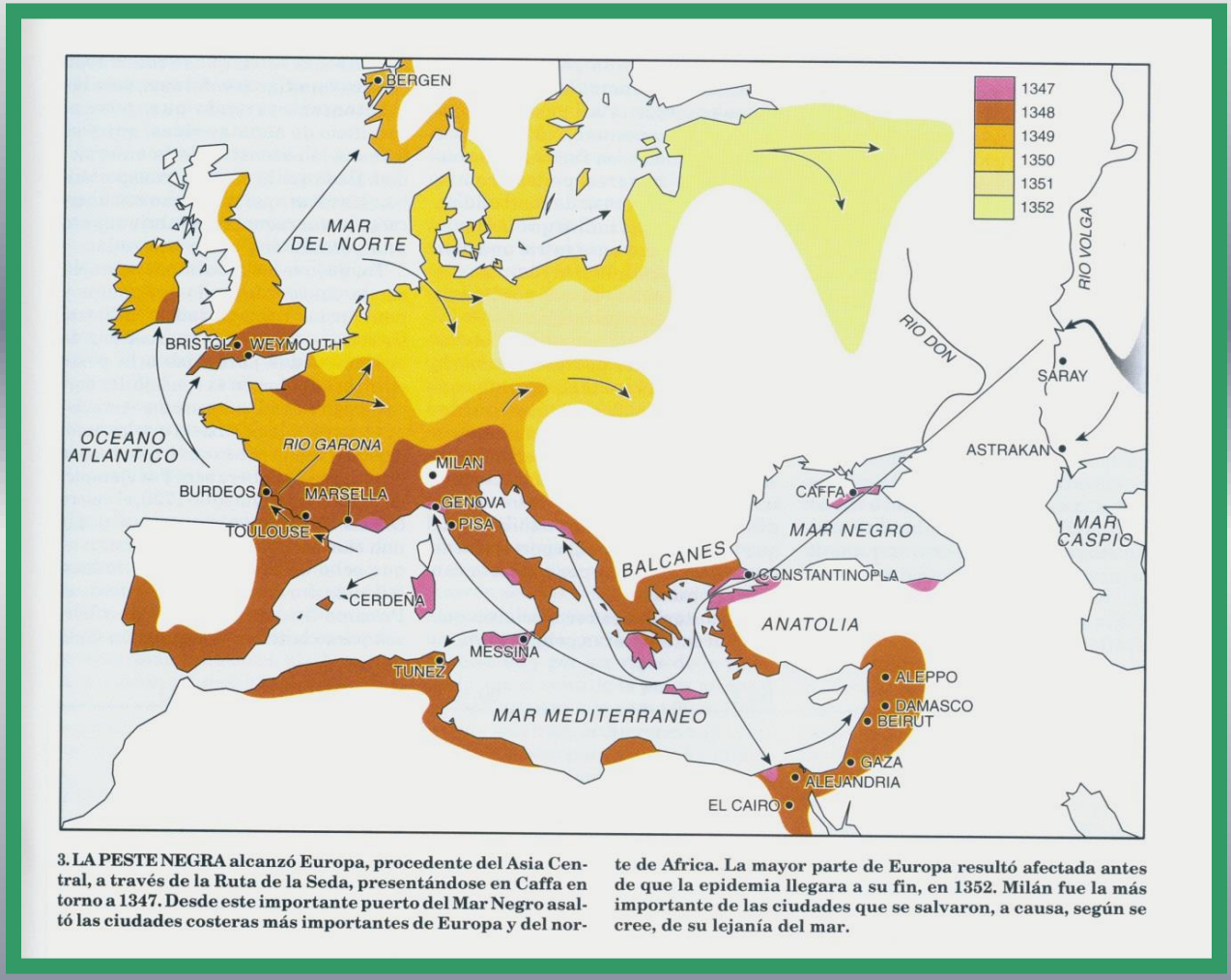
18 de Mayo 2018

Rosario


90 mm
0.01 mm (10 µm)
2 mm
(b)
Pasi V. Dunlap
(a)
(c)

2. VESTIMENTA USADA POR LOS MEDICOS durante el brote de peste que afectó a Marsella en 1720. Recubría a su portador de pies a cabeza; a ésta se le daba apariencia de pájaro, creyéndose que protegía del contagio. En el pico se depositaban hierbas aromáticas a modo de filtro del aire contagiado; la vara contenía incienso para despejar los miasmas. Incluso los orificios de los ojos ofrecían protección, recubiertos de cristal.

FINES DEL SIGLO XIV, POR LA RUTA DE LA SEDA ENTRADA DE LA PESTE NEGRA



3. LA PESTE NEGRA alcanzó Europa, procedente del Asia Central, a través de la Ruta de la Seda, presentándose en Caffa en torno a 1347. Desde este importante puerto del Mar Negro asaltó las ciudades costeras más importantes de Europa y del norte de Africa. La mayor parte de Europa resultó afectada antes de que la epidemia llegara a su fin, en 1352. Milán fue la más importante de las ciudades que se salvaron, a causa, según se cree, de su lejanía del mar.

IMPORTANCIA DEL BIOFILM

ESTRUCTURA BACTERIANA

PARED CELULAR DE BACTERIAS GRAM-POSITIVAS

PARED CELULAR DE BACTERIAS GRAM-NEGATIVAS

PARED CELULAR DE MICOBACTERIAS

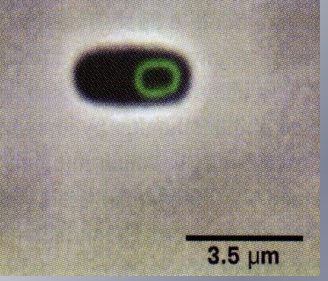
PARED CELULAR O ENVOLTURA DE ESPORAS

OTROS TIPOS DE PAREDES Y ENVOLTURAS

APÉNDICES BACTERIANOS

MOTILIDAD BACTERIANA

BIOFILMS



FINES DEL SIGLO XVII
DESCUBRIMIENTO DE LAS BACTERIAS Y PROBABLEMENTE DE LOS BIOFILMS

PADRE DE LA MICROBIOLOGÍA

Observations, communicated to the Publisher by Mr. Antony van Leewenhoeck, *in a Dutch Letter of the 5th of* Octob. 1676. *here Englished: Concerning little Animals by him observed in Rain- Well- Sea- and Snow-water; as also in water wherein Pepper had lain infused.*

IN the year 1675. I discover'd living creatures in Rain water, which had stood but few days in a new earthen pot, glased blew within. This invited me to view this water with great attention, especially those little animals appearing to me ten thousand times less than those represented by Monsr. *Swammerdam*, and by him called *Water-fleas* or *Water-lice*, which may be perceived in the water with the naked eye.

The *first* sort by me discover'd in the said water, I divers times observed to consist of 5, 6, 7, or 8 clear globuls, without being able to discern any film that held them together, or contained them. When these *animalcula* or living Atoms did move, they put forth two little horns, continually moving themselves: The place between these two horns was flat, though the rest of the body was roundish, sharpning a little towards the end, where they had a tayl, near four times the length of the whole body, of the thickness (by my Microscope) of a Spiders-web; at the end of which appear'd a globul, of the bignes of one of those which made up the body; which tayl I could not perceive, even in very clear water, to be mov'd by them. These little creatures, if they chanced to light upon the least filament or string, or other such particle, of which there are many in water, especially after it hath stood some days, they stook intangled therein, extending their body in a long round, and striving to disintangle their tayl; whereby it came to pass, that their whole body lept back towards the globul of the tayl, which then rolled together Serpent-like, and after the manner of Copper- or Iron-wire that having been wound about a stick, and unwound again, retains those windings and turnings. This motion of extension and contraction continued a while; and I have seen several hundreds of these poor little creatures, within the space of a grain of gross sand, lye fast cluster'd together in a few filaments.

I also discover'd a *second* sort, the figure of which was oval; and I imagined their head to stand on the sharp end. These were a little bigger than the former. The inferior part of their body is flat, furnished with divers incredibly thin feet, which moved very

THE BIOFILM LIFESTYLE

J.W. COSTERTON
ZBIGNIEW LEWANDOWSKI

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Adv Dent Res 11(2):192-195, April, 1997

in certain medical, industrial, or environmental systems. Our objective was to take the same approach as the organisms themselves, which show no obvious regard for anthrocentric points of view and simply make themselves as safe and as comfortable as possible by adhering to available surfaces and forming biofilms in virtually all aquatic systems. Similarly, we have sought to understand the basic advantages of the "biofilm lifestyle" for bacteria growing in any and all aquatic ecosystems.

THE STRUCTURE OF BIOFILMS

It is a distinct pleasure to address the dental research community on the subject of microbial biofilms because it was this same community, more years ago than I care to admit, that inspired many of our initial thoughts in this area. Gibbons and van Houte, of the Forsyth Dental Center, had already addressed that most obvious and troublesome biofilm—dental plaque—in direct observations and elegant experiments, before we began our own oddessey, and our early 1978 article in Scientific American (Costerton *et al.*, 1978) made liberal use of their ideas. Their successors

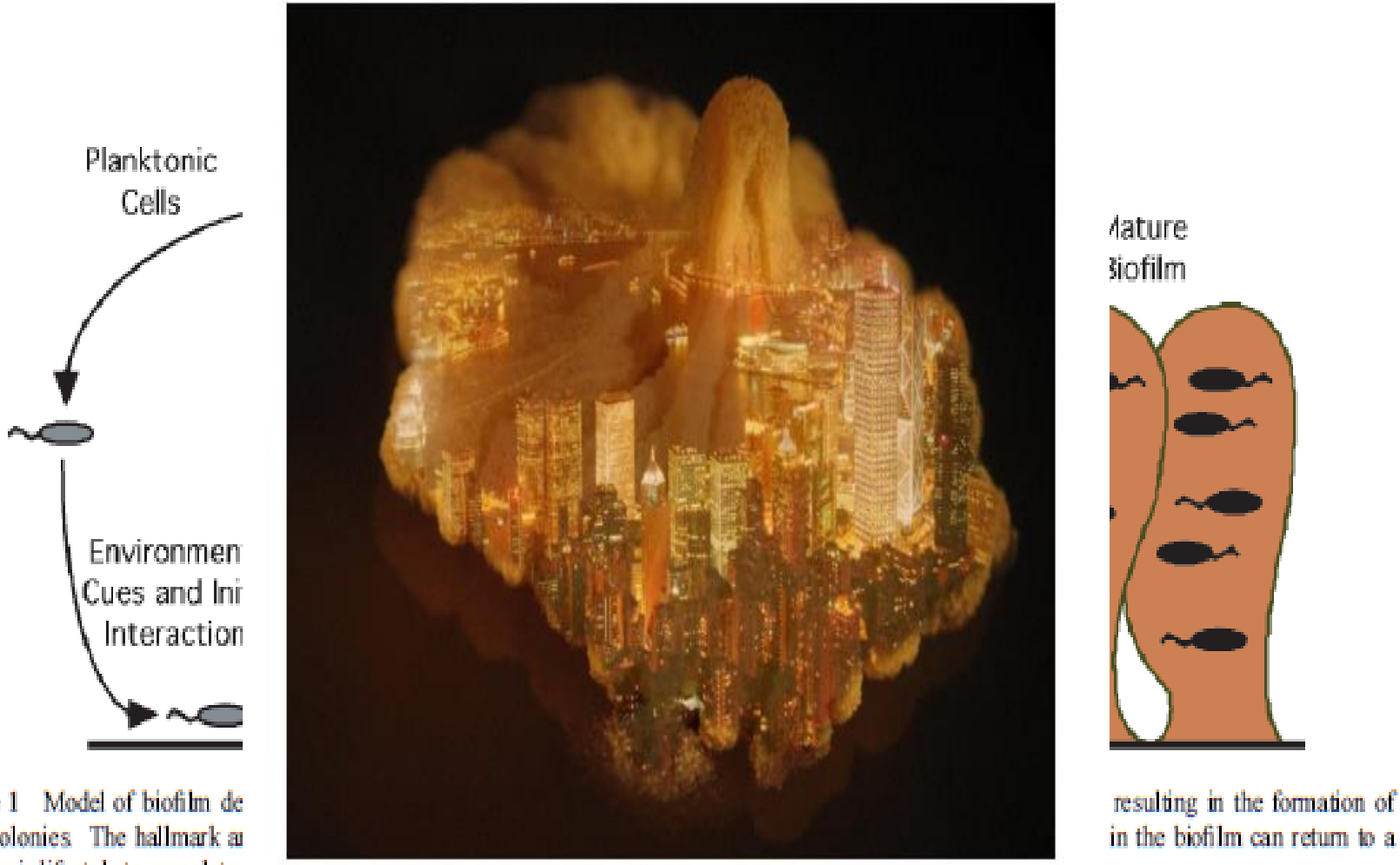
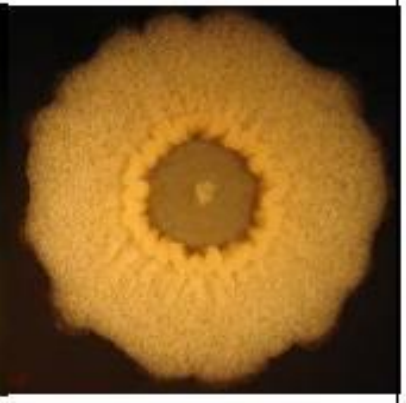
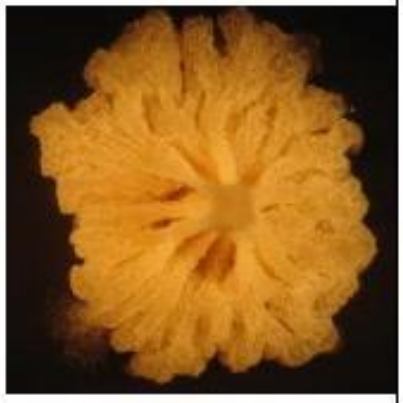


Figure 1 Model of biofilm de
microcolonies. The hallmark a
planktonic lifestyle to complete the cycle of biofilm development.

resulting in the formation of
in the biofilm can return to a

BIOFILMS DESARROLLADOS

	JH642	HS	PR2
Tubo			
Microplaca			
Colonia			

BIOFILMS DESARROLLADOS

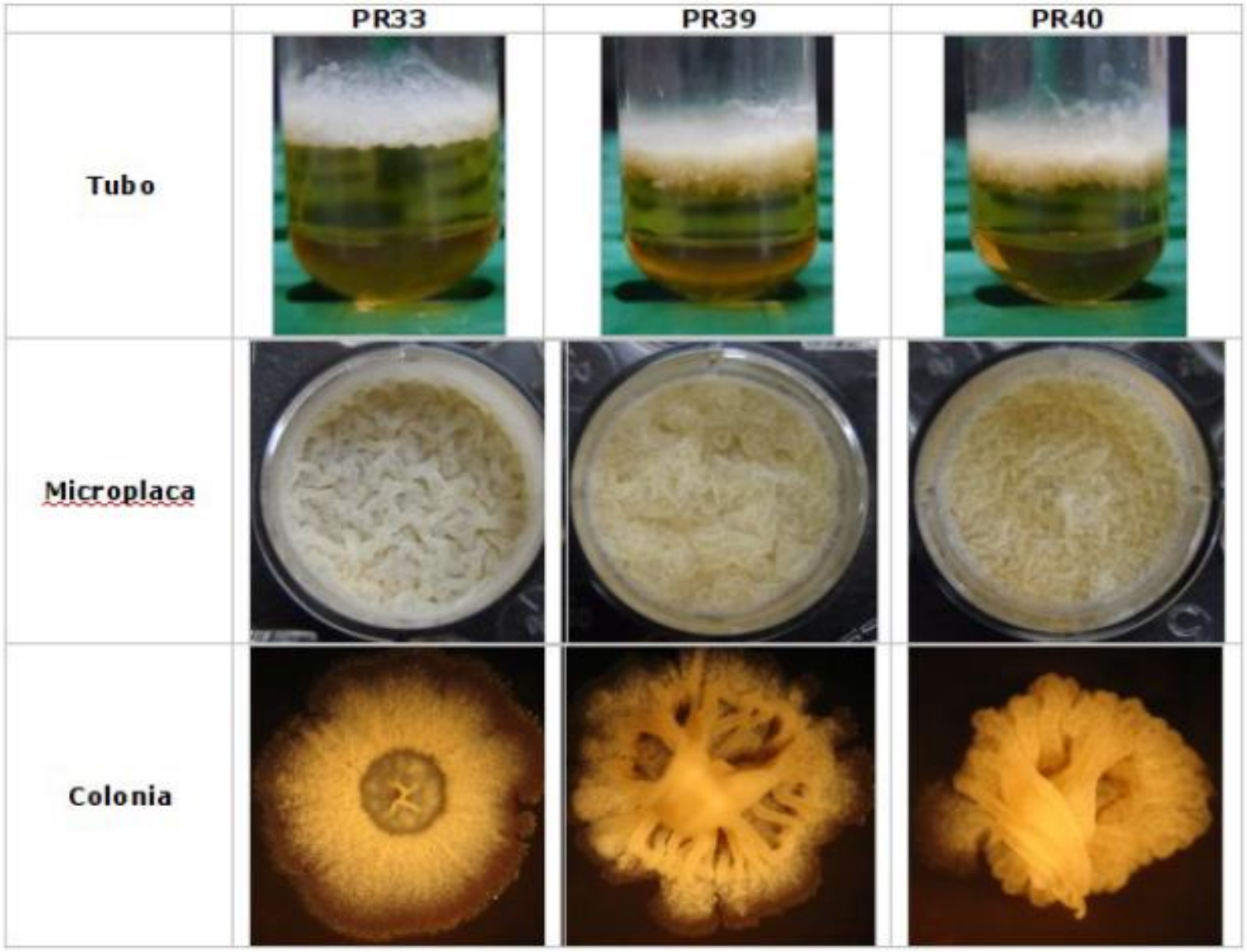
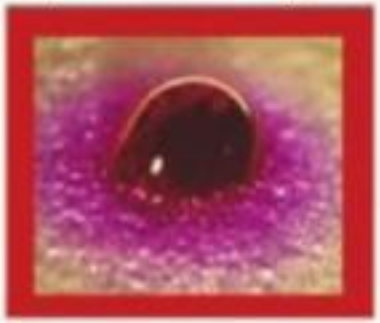
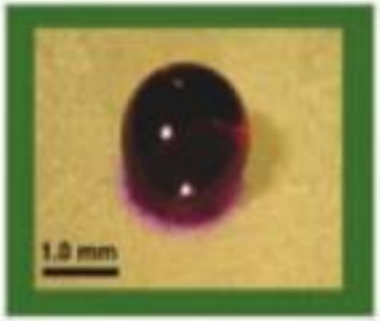


Figura 27: Capacidad de formar biofim.

Hidrofobicidad
repelencia al agua



NCIB3610 (◆)
(wt)



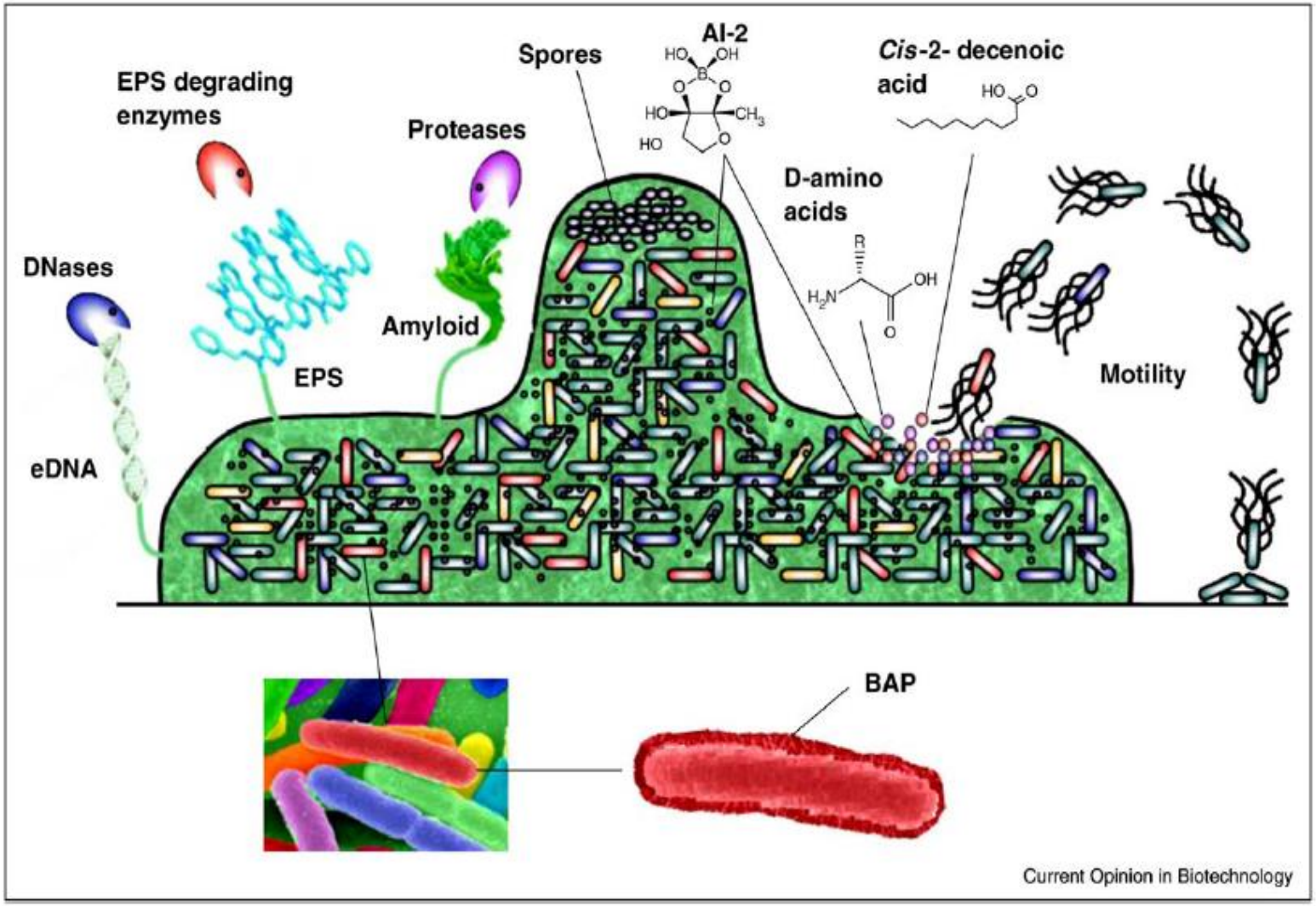
RG4365 (●)
(wt)

bslA (■)

Hidrofabicidad celular

Reparto

	NCIB36110	RG4365	*bslA*
Reparto$_{hexano}$	1.2 ± 0.02	2.2 ± 0.02	< 0.02
Reparto$_{octano}$	1.45 ± 0.02	3.87 ± 0.02	< 0.02



Schematic presentation showing mechanisms and components involved in biofilm formation and dispersal. Biofilms can contain various extracellular biopolymers like extracellular DNA (eDNA), extracellular polysaccharides, amyloid fibers, and biofilm-associated proteins (BAP). These matrix components might be good targets for (combinations of) putative enzymes such as DNases, proteases, and extracellular polysaccharide degrading enzymes to prevent formation of biofilms or to stimulate dispersal of already formed biofilms. Communication between cells during biofilm formation and dispersal of biofilms is dependent on quorum sensing systems and molecules like autoinducer 2 (AI-2), D-amino acids, and *cis*-2-decenoic acid. Furthermore, motility is an important factor in the establishment of new biofilms and the dispersal of cells from mature biofilms. Also, aerial structures of the biofilm serve as specific sites for the generation of spores (see text for details and corresponding references).

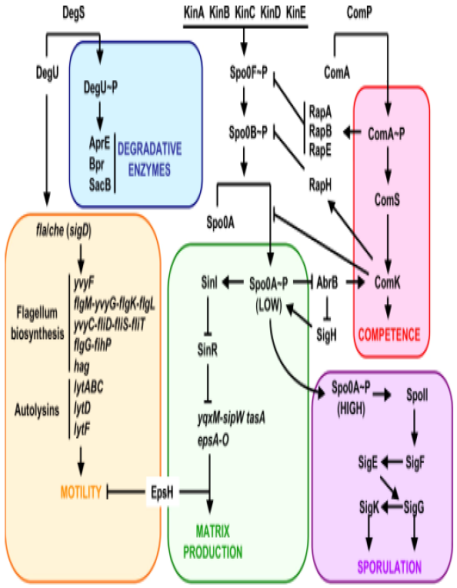
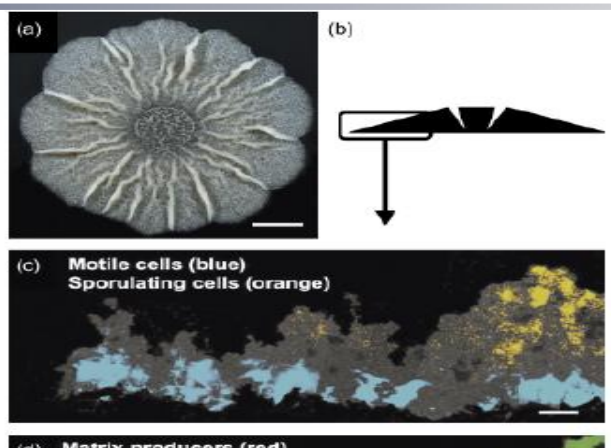


Fig. 3. Network of the different genetics pathways related to cell differentiation in *Bacillus subtilis*. Genes related specifically for each differentiation process are located within the specific frame.

FEMS Microbiol Rev **33** (2009) 152–163



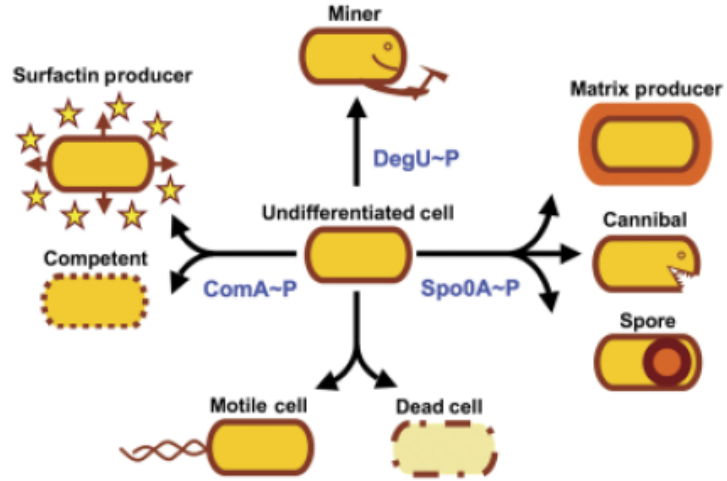


Fig. 1. Schematic representation of the distinct cell types that differentiate in the communities of *Bacillus subtilis*. Each cell type has been caricaturized, considering its most representative attribute. All the cell types were classified into subgroups, according to the master regulator that triggers their differentiation. Arrows indicate the process of differentiation. The master regulator involved is presented in blue. Notice that motile cells do not require the activation of any master regulator for their differentiation. The subpopulation of cells subjected to the action of the cannibalism toxins, labeled as dead cells, is also represented, and yet they cannot be considered strictly as a differentiated cell type.

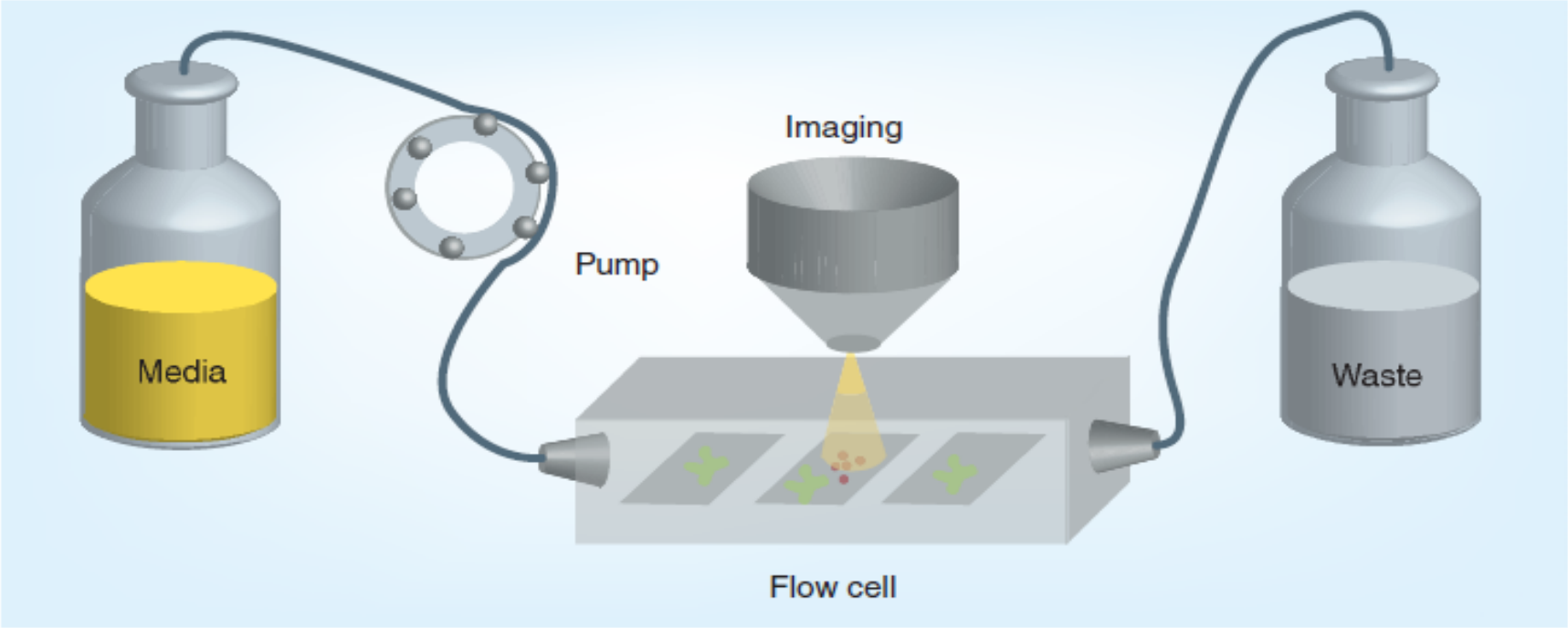
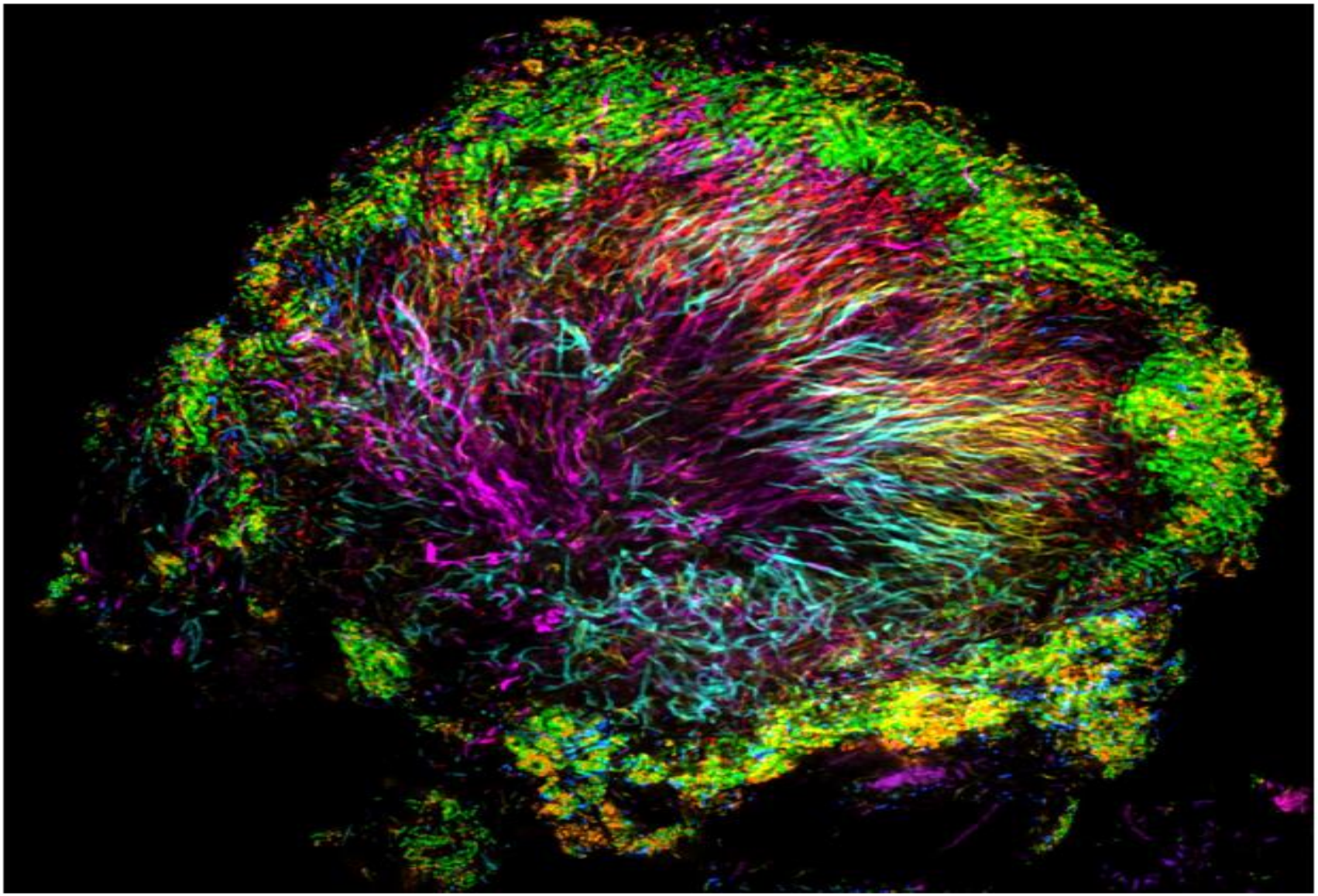
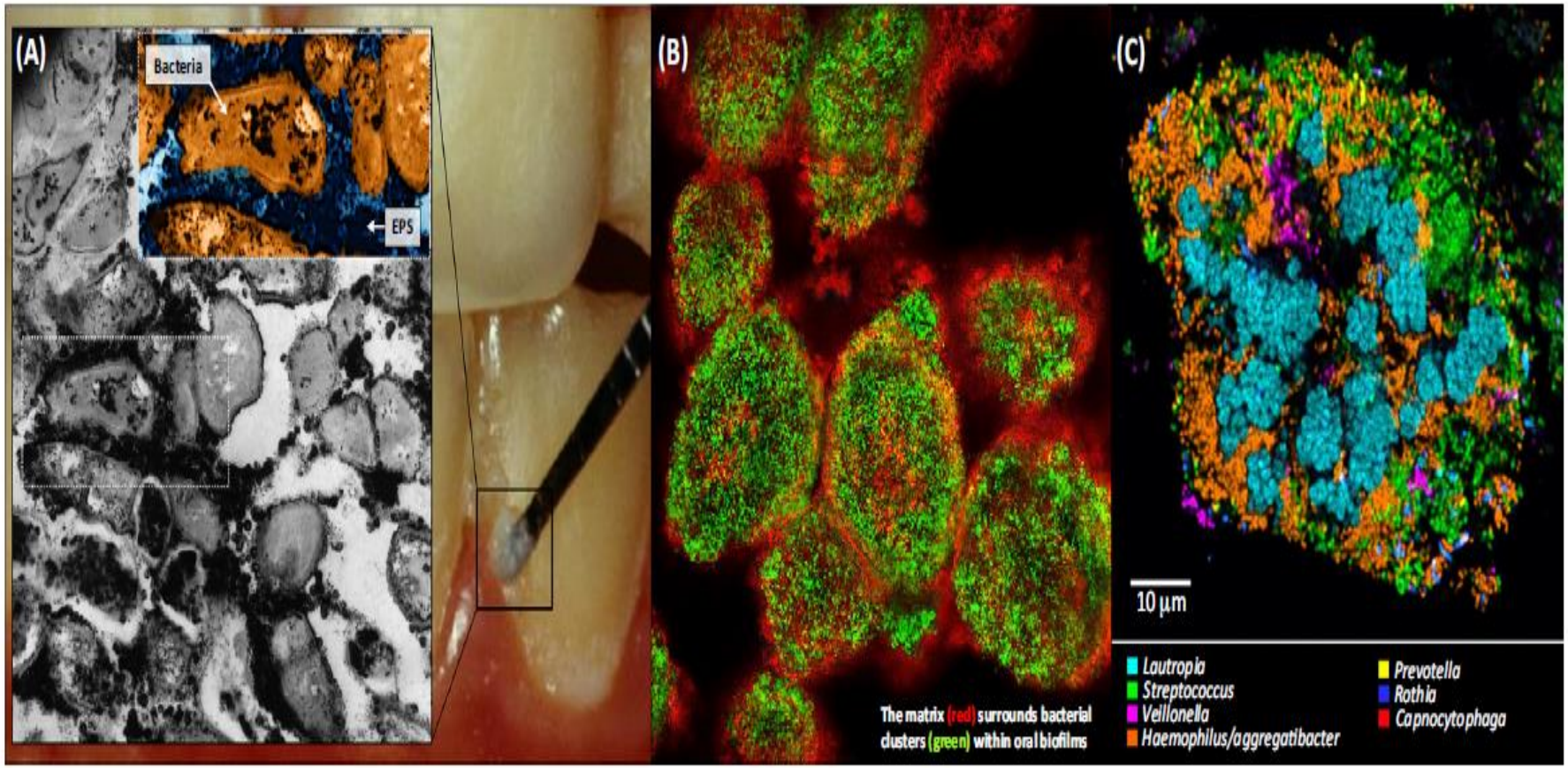


Figure 3. Basic schematic of a dynamic biofilm system. The goal of dynamic systems is to simulate the natural environment by replenishing nutrients and providing shear conditions. Typically, fresh media from a receptacle is pumped through a flow-cell and into a waste receptacle. The speed at which the media flows through the system will dictate the amount of shear force to which the biofilm cells are exposed. An alternative to the common peristaltic pump setup is to allow media to flow through the system by gravity, as in the drip flow reactor model. This generates much lower shear forces, which flow down an angled substratum. Other variations on this basic experimental setup include using different flow-cell apparatuses, such as a Robbins device, or the use of different substrates or surfaces within the flow-cell. Once a biofilm has developed within the flow-cell it can be imaged, either in real-time or after removal from the flow-cell or other apparatus. The main advantages of dynamic systems include the ability to visualize biofilm development in real-time under flow conditions, and for longer periods of time due to the replenishment of nutrients.



Trends in Microbiology

Figure 1. Image of the Oral Biofilm as seen by the CLASI-FISH Technique. Different colours indicate different bacteria present in a human dental plaque sample, with the outstanding presence of long filaments of *Corynebacterium* (labeled in magenta). Image courtesy Gary G. Borisy, The Forsyth Institute, and Jessica L. Mark Welch, Marine Biological Laboratory.



Trends in Microbiology

Figure 1. Dental Plaque Architecture: The EPS Matrix, Spatial Organization, and Polymicrobial Composition. (A) Plaque biofilm from a caries-active subject (photo courtesy of Dr Jaime A. Cury): microscopic image (inset) of plaque-biofilm showing a selected area containing bacterial cells (highlighted in orange) enmeshed in EPS (in dark blue); the image was pseudo-colored using Adobe Photoshop software for visualization purposes (adapted from [19]). (B) Bacterial clusters (green) surrounded by EPS matrix (red) detected in mature mixed-species oral biofilms formed in sucrose (adapted from [5]). (C) Spatial organization of human dental plaque showing multiple clusters of varying sizes containing different microbial species (adapted from [31]). Abbreviation: EPS, extracellular polymeric substances.

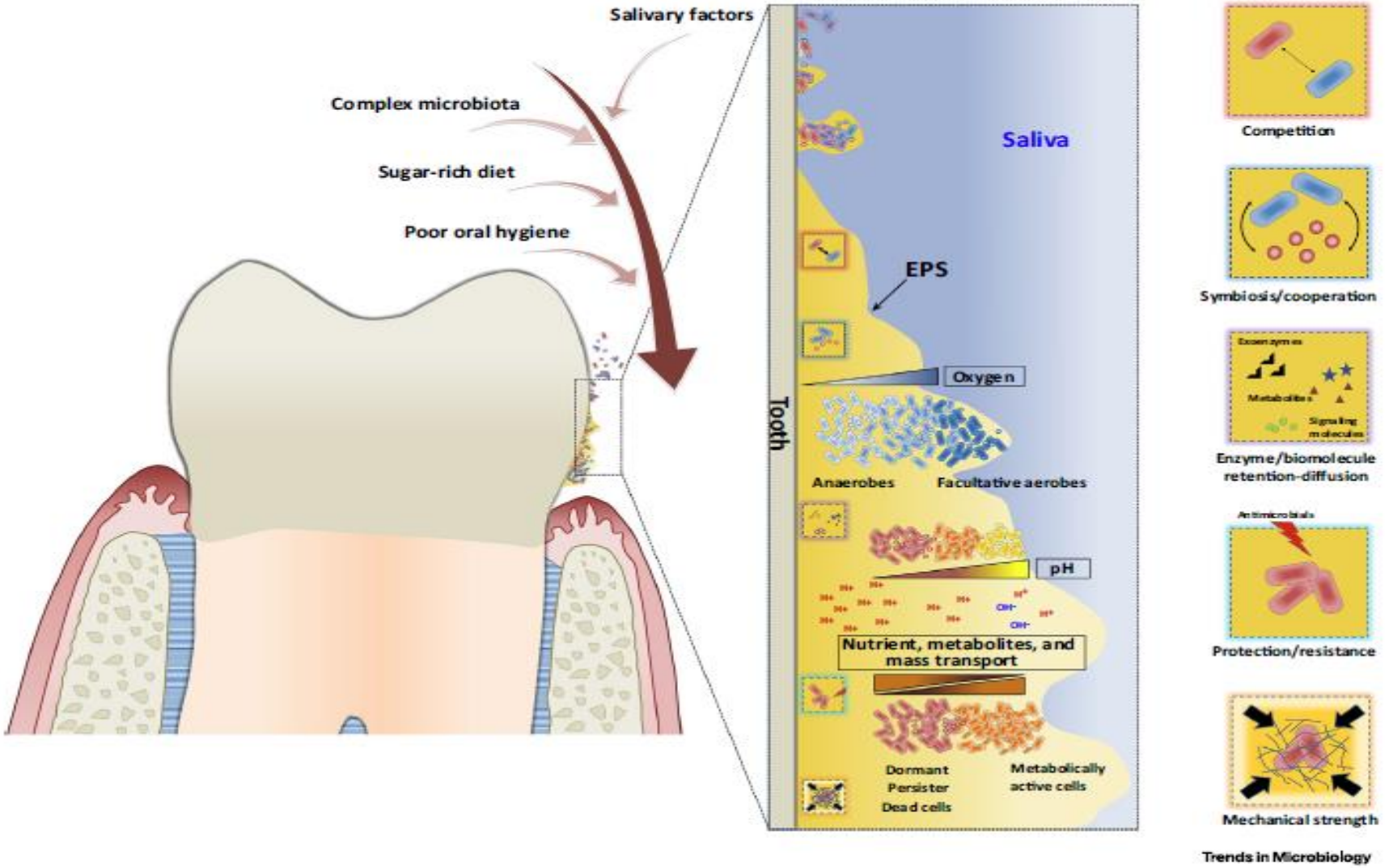


Figure 2. The Biofilm Properties: Assembling a Complex Microenvironment. In the oral cavity, a diet rich in sugar, particularly sucrose, provide a substrate for the production of extracellular polysaccharides, which form the core of the extracellular matrix in cariogenic biofilms. The matrix drastically changes the physical and biological properties of the biofilm. The exopolysaccharides enhance microbial adhesion–cohesion and accumulation on the tooth surface, while forming a polymeric matrix that embeds the cells. The matrix provides a multifunctional scaffold for structured organization and stability of the biofilm's microbial community, where microorganisms coexist and compete with each other. The diffusion-modifying properties of the matrix, combined with the metabolic activities of embedded organisms, help to create a variety of chemical microenvironments, including localized gradients of oxygen and pH. Furthermore, the matrix can trap or sequester a diverse range of substances, including nutrients, metabolites, and quorum-sensing molecules. Similarly, enzymes can be retained and stabilized, transforming the matrix into a *de facto* external digestive system [1]. These properties provide the distinctive characteristics of the biofilm lifestyle, including mechanical stability, spatial and chemical heterogeneity, and drug tolerance [1].

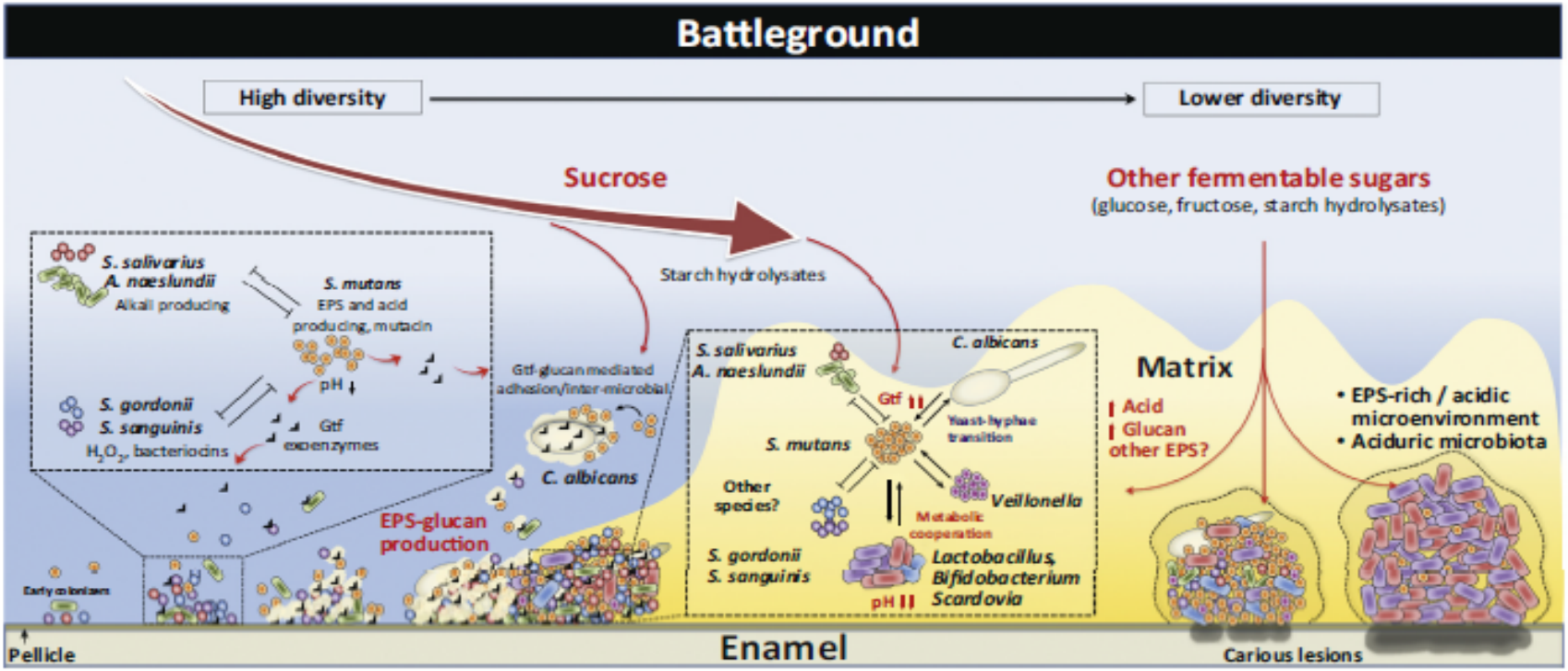


Figure 3. The Biofilm Battleground: Antagonistic, Synergistic, and Mutualistic Interactions to Build a Pathogenic Habitat. The social interactions of the oral microbial community start with early colonizers that can rapidly adhere to the tooth surface, and then coadhere with other microorganisms. During this process the various species interact physically and metabolically to shape the initial biofilm community. The interactions can be both antagonistic and cooperative, which can dynamically change depending on the host. Certain interactions are beneficial as *Streptococcus gordonii*/*Streptococcus salivarius* and *Actinomyces naeslundii* interfere with caries pathogens such as *Streptococcus mutans* by secreting bacteriocins and hydrogen peroxide as chemical weapons or counter the deleterious effects of acidification by producing alkali. However, the balance between commensals and pathogens can be disrupted by frequent sugar consumption and poor oral care. When sucrose is available, EPS-producing exoenzymes such as Gtfs, present in the pellicle and also bound to different microbes (including *Candida albicans*), produce copious amounts of glucans. Furthermore, the Gtfs can also use starch hydrolysates (from α-amylase activity on pellicle and bacterial surfaces) to produce hybrid glucan polymers. The surface-formed glucans provide novel binding sites for adhesion and coadhesion, which mediate new interspecies interactions and microbial clustering on the tooth surface, while assembling a polymeric matrix that provides protection and mechanical stability, making the biofilm recalcitrant to antimicrobials and difficult to remove. Competitive and synergistic interactions continue to develop between the microbes embedded in the biofilm structure. If the biofilm remains on teeth, and the consumption of carbohydrate-rich diets persists, the amount of EPS and the extent of acidification of the matrix increases. The diffusion-modifying properties of the matrix, combined with microbial metabolic activities, help to create a highly acidic and increasingly anaerobic (hypoxic) niche. Such conditions elicit biochemical, ecological, and structural changes that favor the survival and dominance of highly acid-stress-tolerant organisms that can synergize with each other. The microbial diversity can further reduce in favor of an aciduric microbiota, helping to maintain an acidified microenvironment. The low-pH condition at the tooth–biofilm interface promotes demineralization of the enamel, leading to the onset and progression of dental caries. This model may explain the rapid accumulation of cariogenic plaque in the presence of sucrose (and other fermentable sugars) in the diet, even if the initial population of pathogens, such as *S. mutans*, is numerically low. Abbreviations: EPS, extracellular polymeric substances; *S. sanguinis*, *Streptococcus sanguinis*.

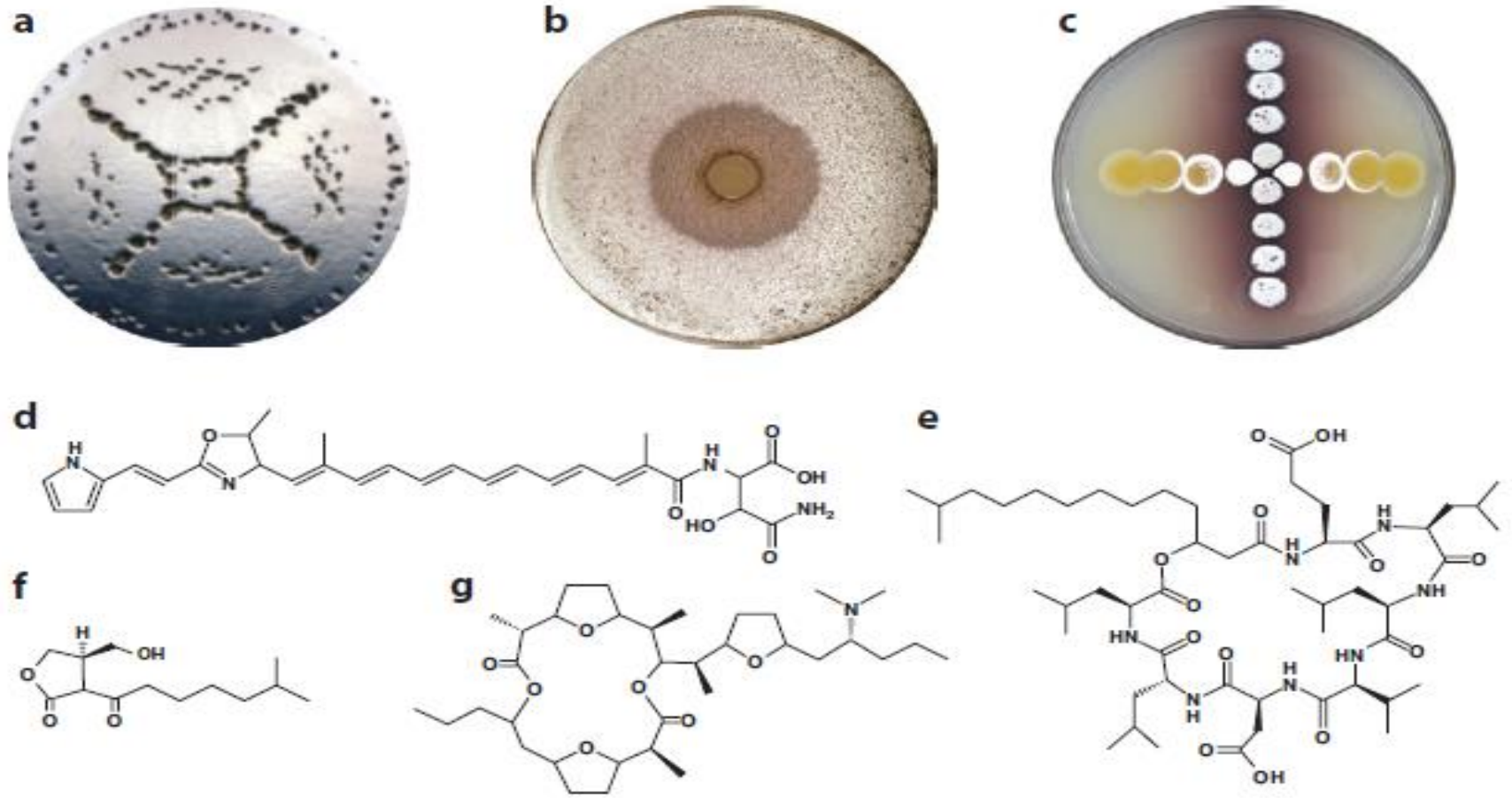


Figure 2

Free-living soil bacteria such as the myxococci, bacilli, and streptomycetes undergo dramatic developmental changes in response to environmental stimuli and are the advanced organic chemists of the microbial world. Secondary metabolism and bacterial development collide when these soil bacteria encounter each other in dual-species interactions. (*a*) Swarms of *Myxococcus xanthus* converge from four directions on a colony of prey bacteria, leaving a cross pattern of fruiting bodies where their paths meet, and the prey is exhausted. (Image courtesy of J. Kirby). (*b*) Sporulation by a lawn of *Streptomyces coelicolor*, visible as white aerial hyphae, is disrupted by *Bacillus subtilis* (colony in center of plate) through the action of surfactin. (*c*) Two streptomycetes, *S. sviceus* (*vertical*) and *S. griseoflavus* (*horizontal*), are plated as spots in a crosswise pattern on agar medium. A diffusible substance from *S. sviceus* induces aerial development by *S. griseoflavus* (white on colony surface) (image by J. Stewart). (*d*) The structure of a yellow polyene pigment from *M. xanthus* representing the DKxanthene family required for sporulation. (*e*) The structure of surfactin from *B. subtilis*, the agent active in blocking aerial growth by *S. coelicolor* (see panel *b*) and signaling development via K^+ efflux (see text). (*f*) The A-factor from *S. griseus*, γ-butyrolactone. (*g*) Chemical structure of pamamycin-607, a macrodiolide antibiotic from *Streptomyces alboniger*, which acts to induce development by multiple species of *Streptomyces*.

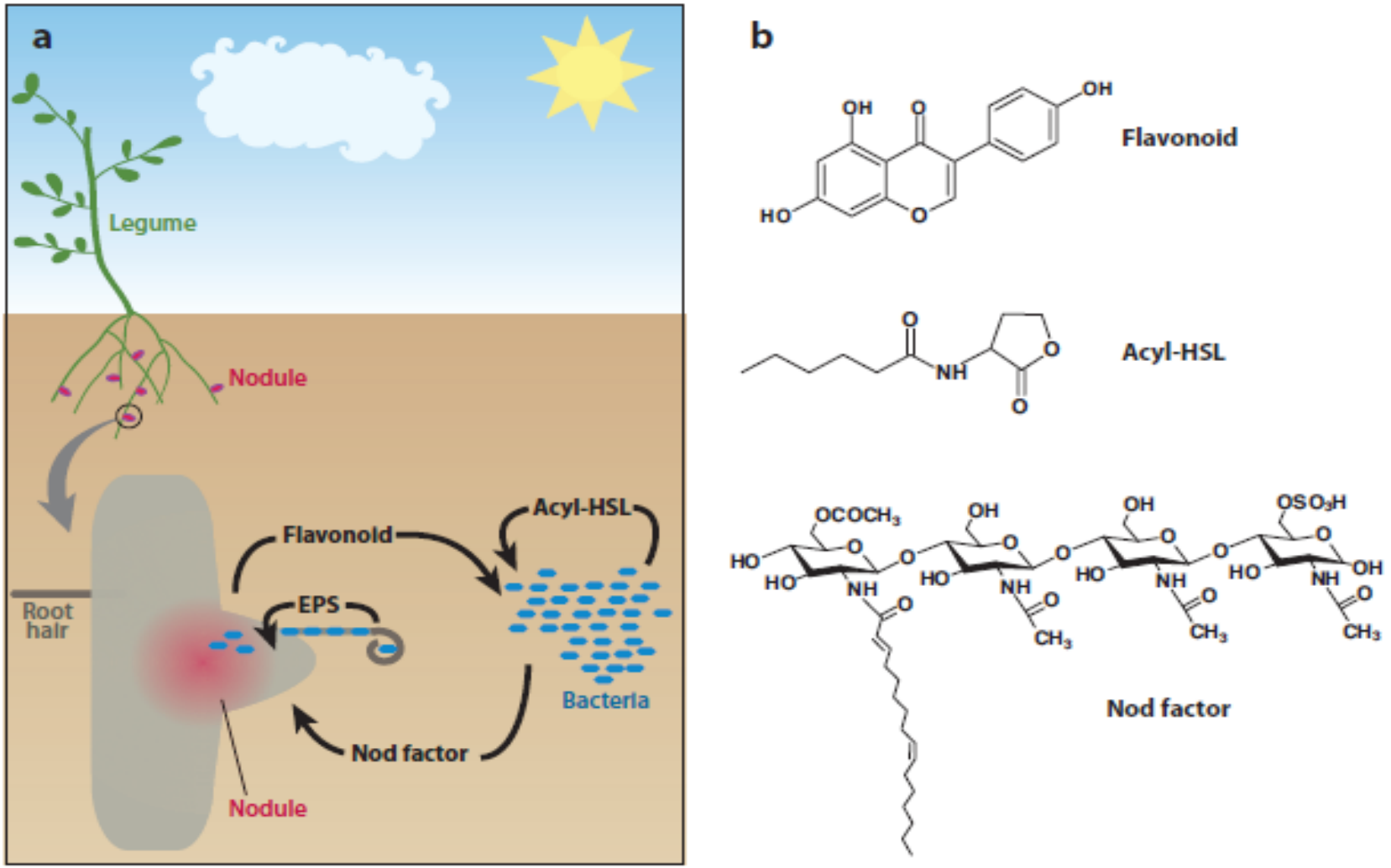
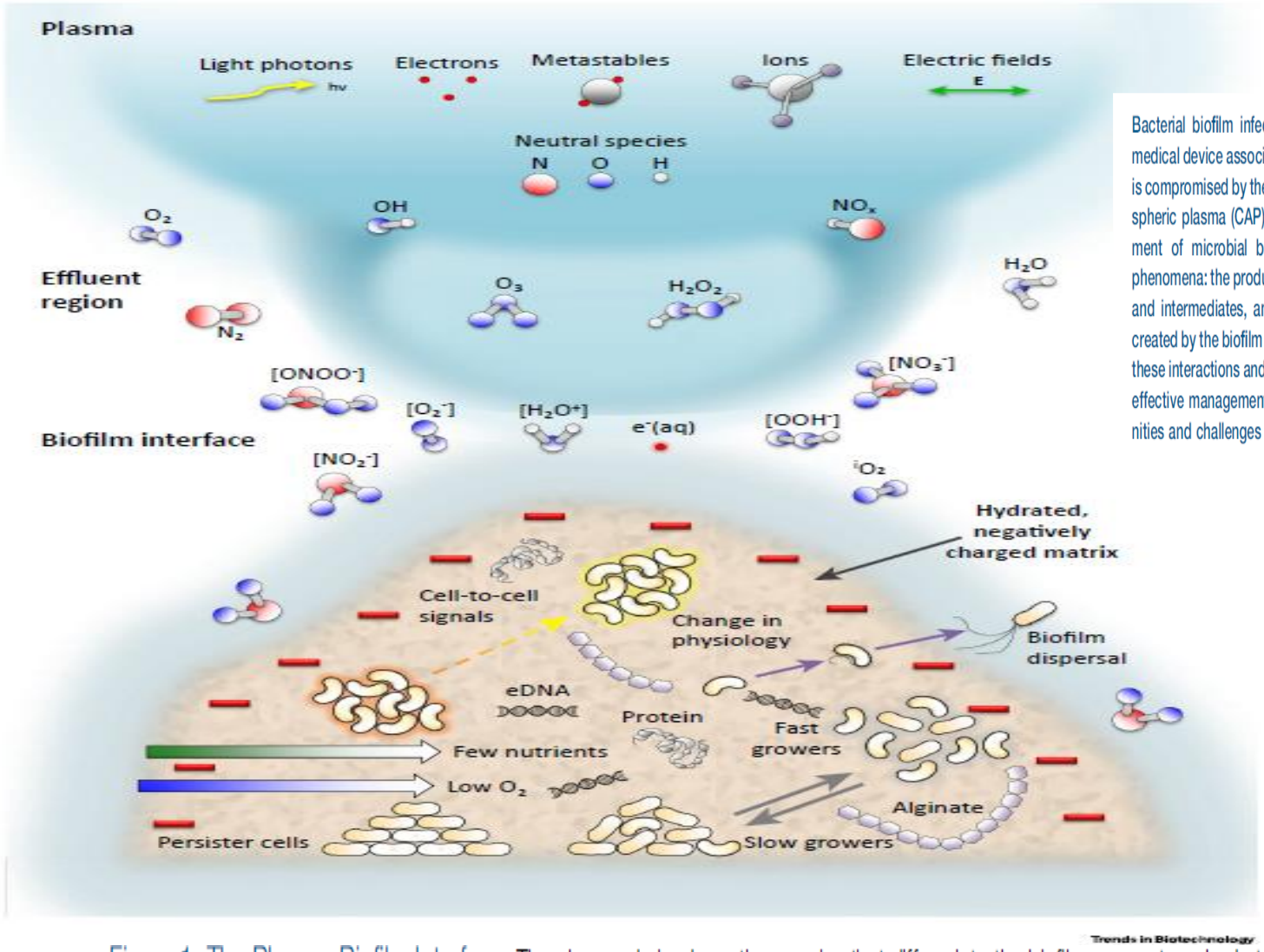


Figure 1

A two-way chemical exchange occurs between bacteria in the family *Rhizobiaceae* and leguminous plants during the establishment of N_2-fixing symbioses. (*a*) Nodules on the plant roots (*magenta*) arise as a result of a progression of developmental changes in bacteria and plant during a controlled infection. Flavonoid compounds excreted by the roots signal the bacteria to assemble near the root surface. At a sufficiently high cell density, bacterial acylated homoserine lactone (acyl-HSL) quorum signals activate the production of Nod factors. The Nod factors instruct the plant root hairs to prepare for invasion. Bacterial exopolysaccharides [succinoglycan (EPSI) and EPSII] facilitate entry into root hairs and passage into the root cortex. Modified with permission from Reference 42. (*b*) Chemical structures of a flavonoid (genistein), a quorum signal (acyl-HSL), and a Nod factor (lipochitooligosaccharide).



Bacterial biofilm infections account for a major proportion of chronic and medical device associated infections in humans, yet our ability to control them is compromised by their inherent tolerance to antimicrobial agents. Cold atmospheric plasma (CAP) represents a promising therapeutic option. CAP treatment of microbial biofilms represents the convergence of two complex phenomena: the production of a chemically diverse mixture of reactive species and intermediates, and their interaction with a heterogeneous 3D interface created by the biofilm extracellular polymeric matrix. Therefore, understanding these interactions and physiological responses to CAP exposure are central to effective management of infectious biofilms. We review the unique opportunities and challenges for translating CAP to the management of biofilms.

Trends in Biotechnology

Figure 1. The Plasma–Biofilm Interface. The plasma-derived reactive species that diffuse into the biofilm encounter a hydrated, cationic extracellular polymeric matrix which may sequester RONS and attenuate plasma cidal efficacy and maintains a 3D architecture supporting heterogeneous microenvironments that in turn support multispecies microcolonies. Growth rate may be reduced due to nutrient and O_2 limitations within the biofilm, leading to elevated tolerance and persister formation. Quorum sensing, leading to alterations in microbial physiology may also affect microbial tolerance to plasma-derived RONS. Finally, RONS-mediated dispersal of microbes from the biofilm may reverse plasma tolerance. Adapted from [7,87]. Abbreviations: eDNA, extracellular DNA; RONS, reactive oxygen and nitrogen species.

Table 1. Microbiologic factors that can facilitate surface environment-mediated transmission of selected pathogens

Pathogen able to survive for prolonged periods of time on environmental surfaces (all)
Ability to remain virulent after environmental exposure (all)
Contamination of the hospital environment frequent (all)
Ability to colonize patients (*Acinetobacter*, *C difficile*, MRSA, VRE)
Ability to transiently colonize the hands of health care workers (all)
Transmission via the contaminated hands of healthcare workers (all)
Small inoculating dose (*C difficile*, norovirus)
Relative resistance to disinfectants used on environmental surfaces (*C difficile*, norovirus)

C difficile, *Clostridium difficile*; *MRSA*, methicillin-resistant *Staphylococcus aureus*; *VRE*, vancomycin-resistent *Enterococcus* spp.

Table 2. Microbiologic and epidemiologic features of norovirus that promote epidemics

Large human reservoir of infection
Widespread host susceptibility
Strain-specific immunity is short lived (weeks to months)
Multiple routes of transmission (fecal-oral, foodborne, waterborne, aerosol)
High infectivity
Very low inoculating dose (<10 virions)
Stable in the environment
Prolonged shedding
No vaccine available
No specific chemotherapy

Table 3. Evidence supporting role of environmental contamination in transmission of emerging health care-associated pathogens

Characteristic	Norovirus	*Clostridium difficile*	*Acinetobacter* spp
Able to survive for prolonged periods in the environment	Yes	Yes	Yes
Environmental contamination frequently found in rooms of infected patients	Yes	Yes	Yes
Contaminated environmental reservoir demonstrated to be source of an outbreak	—	Yes	Yes
Contamination of health care worker hands demonstrated	—	Yes	Yes
Human challenge studies demonstrate that contaminated health care worker hands can transfer pathogen	Yes	—	Yes
Level of environmental contamination associated with frequency of health care worker hand contamination	—	Yes	—
Prevalence of environmental contamination associated with incidence of patient acquisition/infection	—	Yes	—
Admission to a room previously occupied by an infected patient associated with risk of colonization/infection	—	Yes	—
Enhanced cleaning demonstrated to reduce hospital incidence of infection	—	Yes	Yes

ESTRUCTURA BACTERIANA

PARED CELULAR DE BACTERIAS GRAM-POSITIVAS

PARED CELULAR DE BACTERIAS GRAM-NEGATIVAS

PARED CELULAR DE MICOBACTERIAS

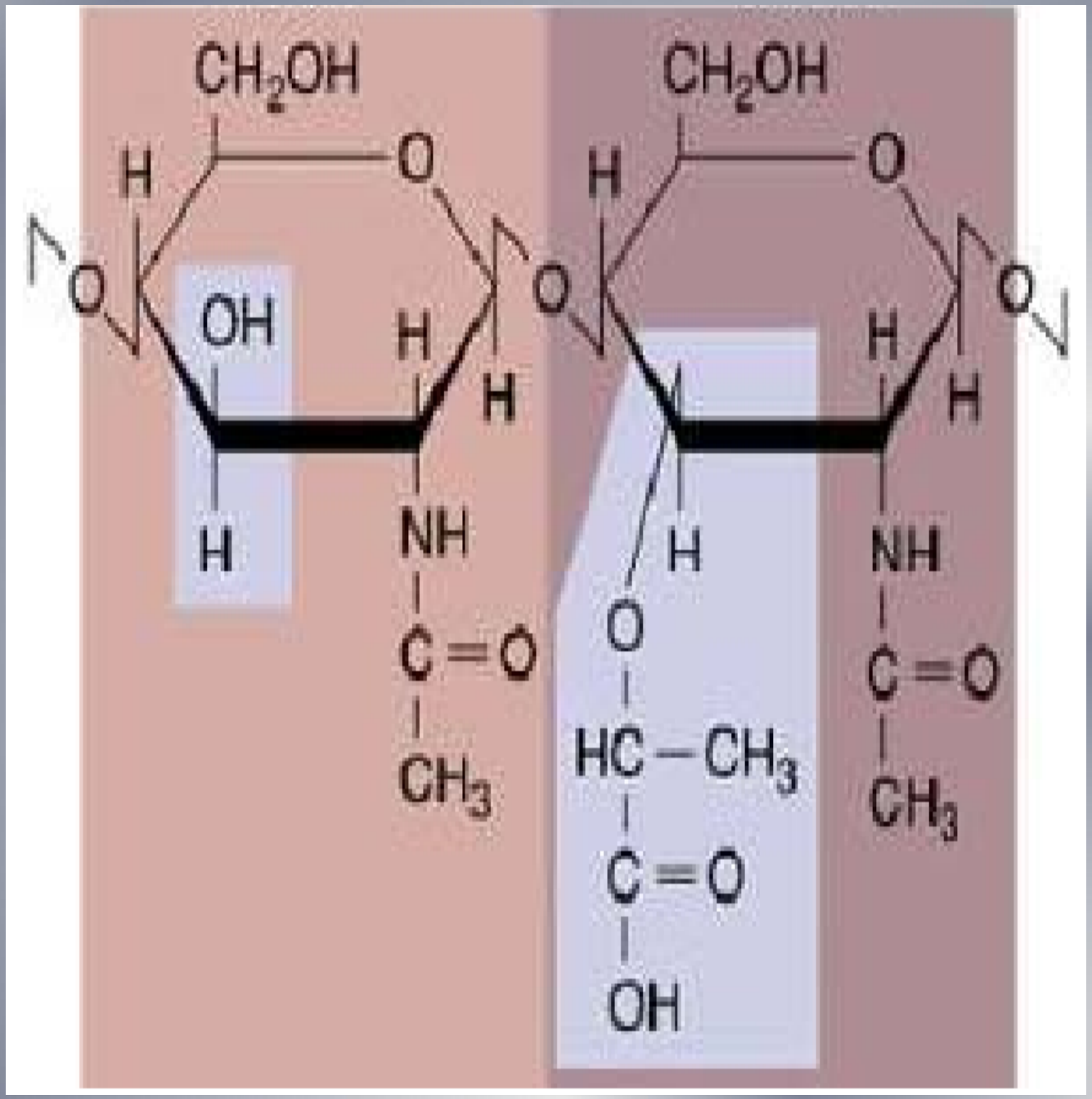
PARED CELULAR O ENVOLTURA DE ESPORAS

OTROS TIPOS DE PAREDES Y ENVOLTURAS

APÉNDICES BACTERIANOS

MOTILIDAD BACTERIANA

BIOFILMS


CH2OH
O
H
OH
H
H
H
NH
C=O
CH3
O
CH2OH
O
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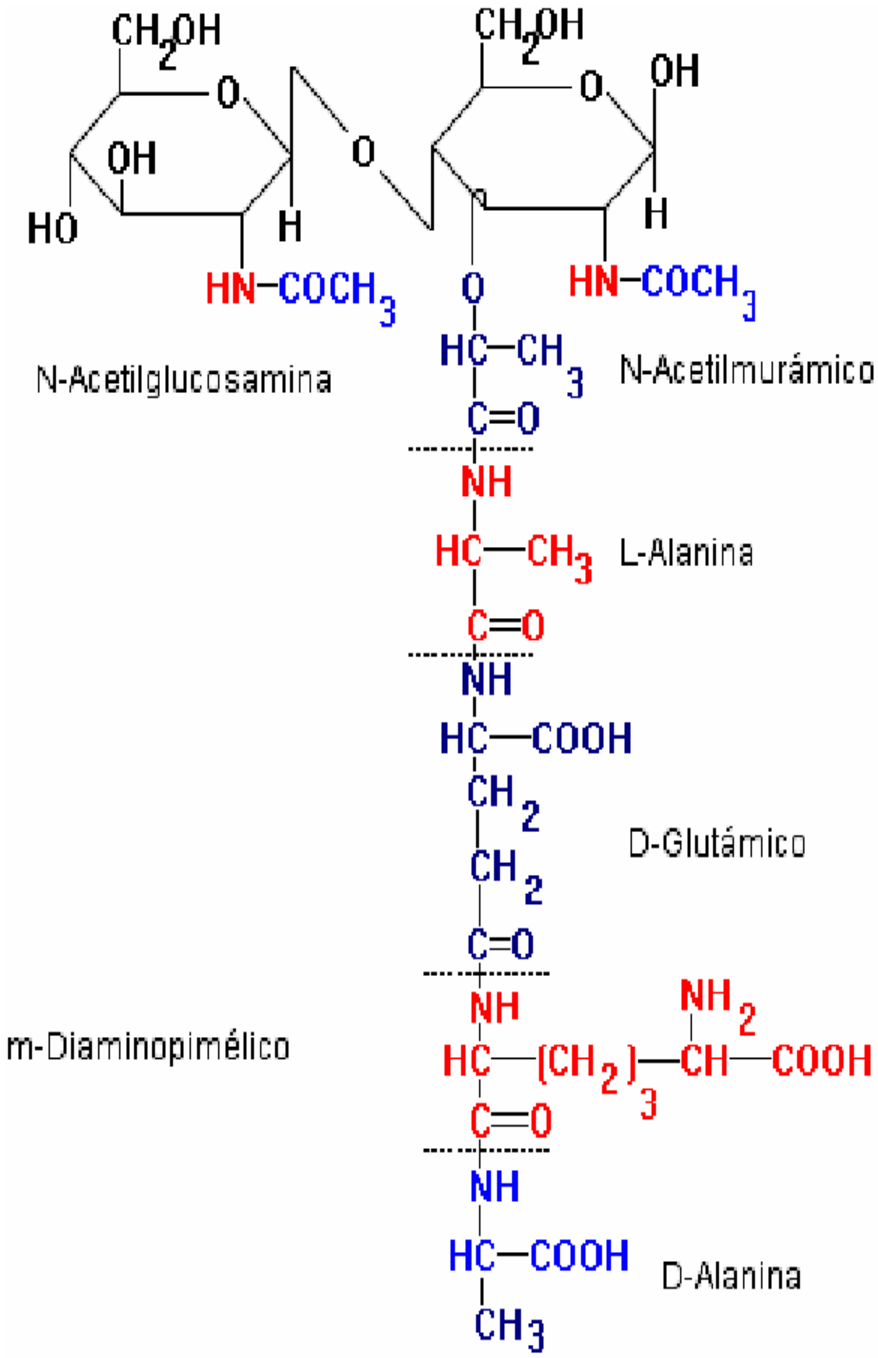
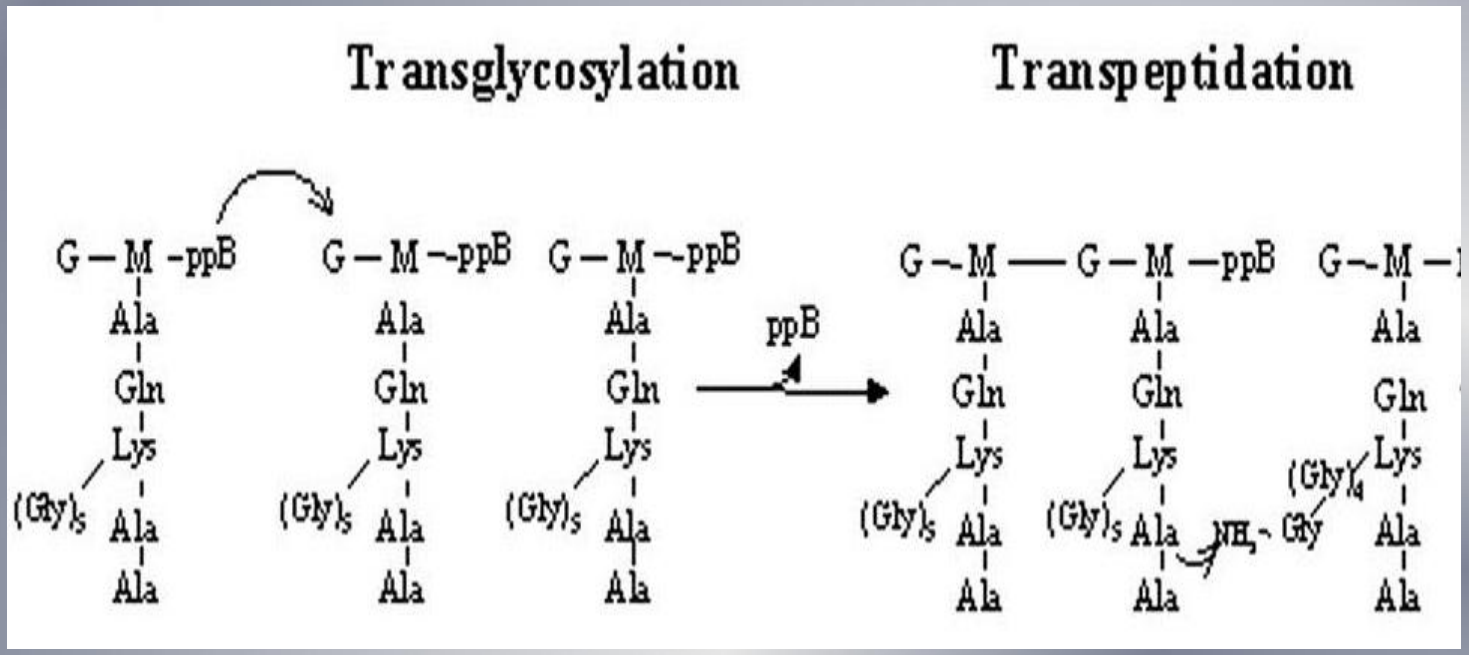
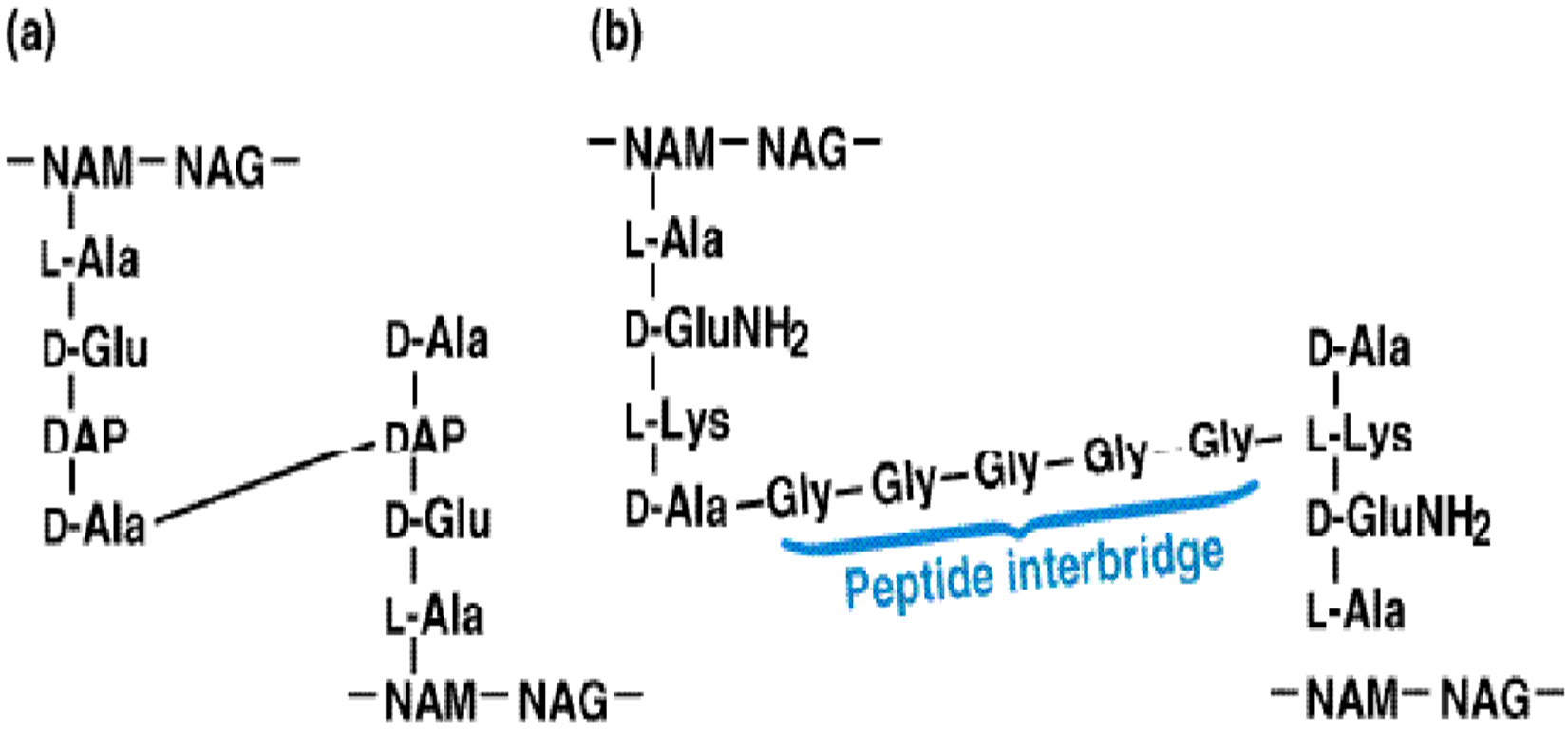
HC—CH3
C=O
OH
NH
C=O
CH3


N-Acetilglucosamina
N-Acetilmurámico
L-Alanina
D-Glutámico
m-Diaminopimélico
D-Alanina

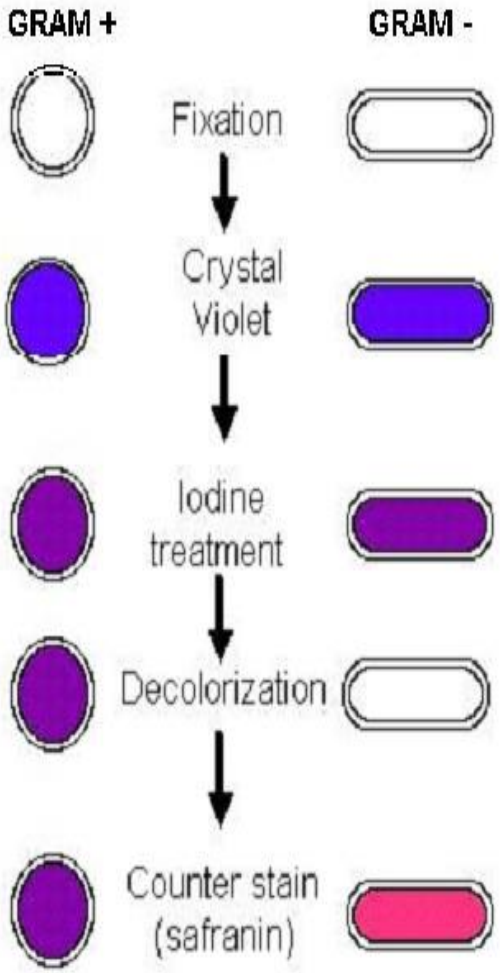

Transglycosylation
Transpeptidation
G – M –ppB
Ala
Gln
Lys
(Gly)5
Ala
Ala
ppB
NH2
Gly
(Gly)4



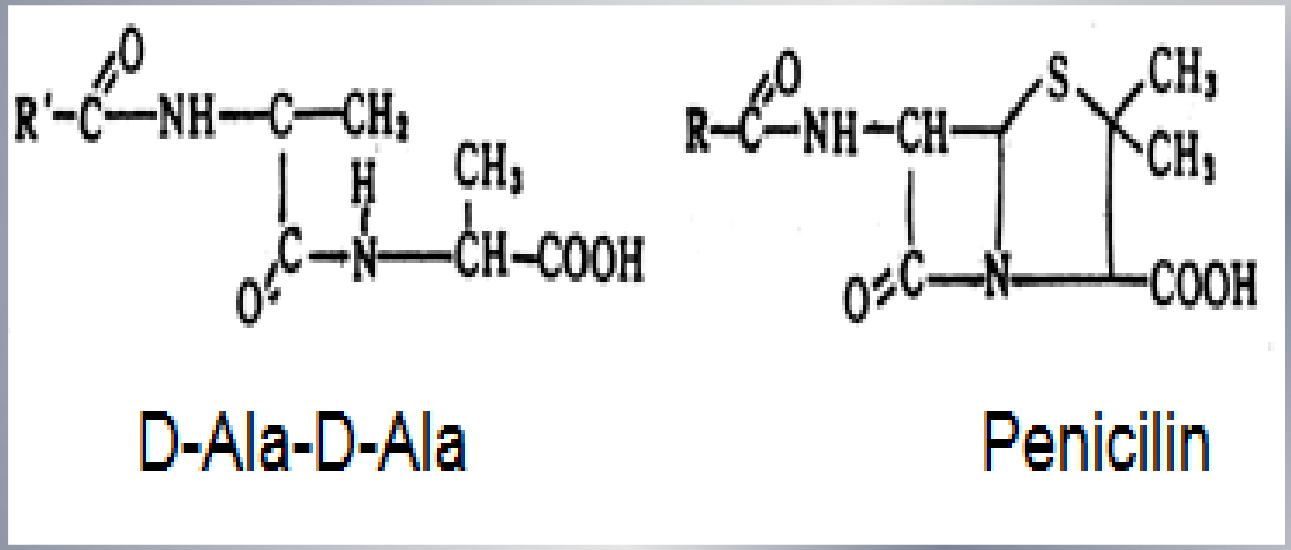
Directo (muchas Gram-negativas)

Puente pentaglicina (algunas Gram positivas

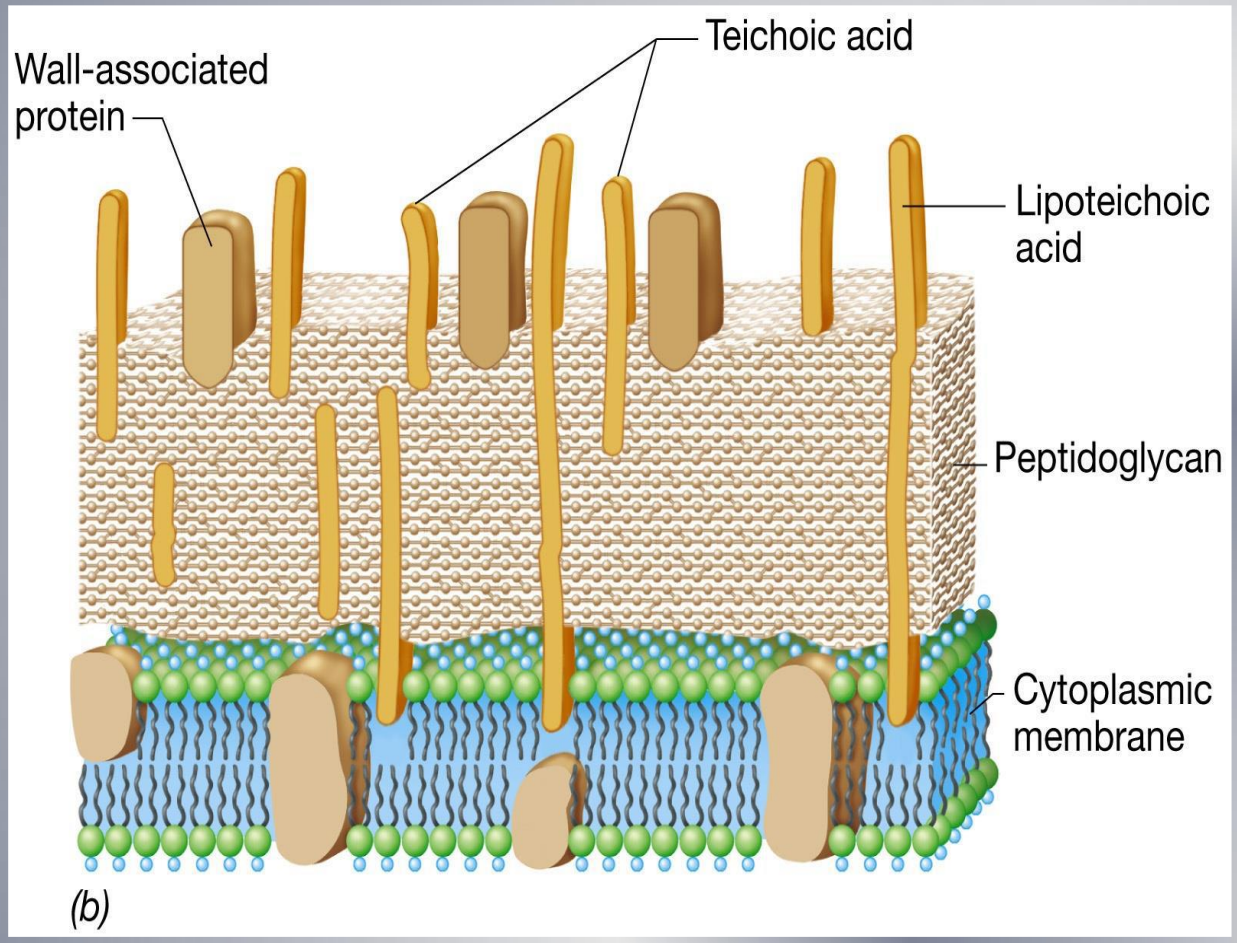
Christian Gram, 1884



ANTIBIÓTICOS BETA-LACTÁMICOS



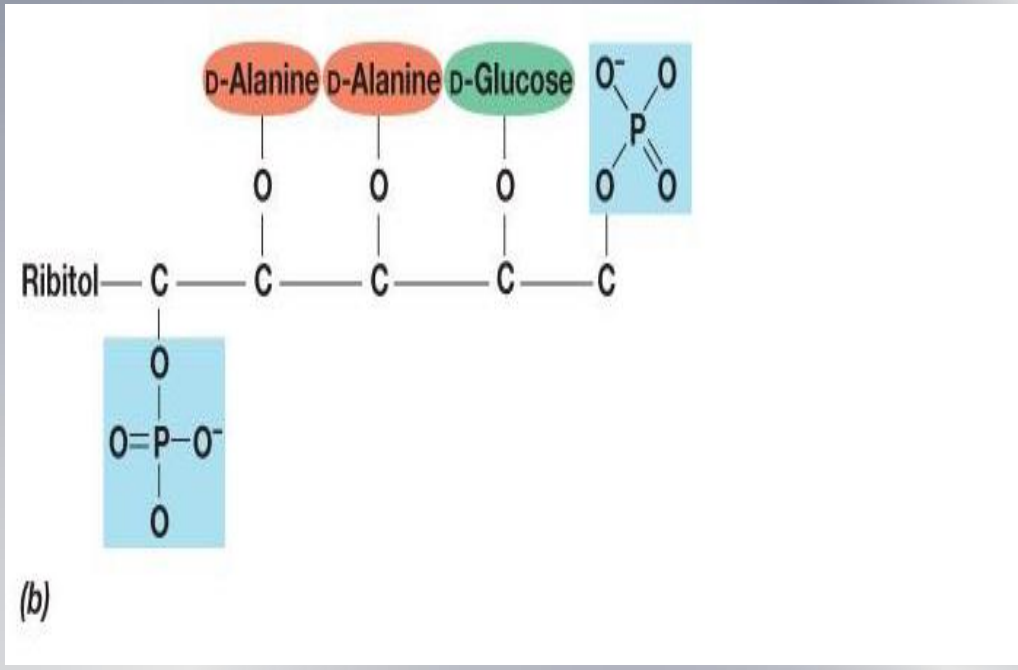
ESTRUCTURA BACTERIANA
COMPONENTES EXCLUSIVOS DE LA PARED CELULAR DE BACTERIAS GRAM-POSITIVAS

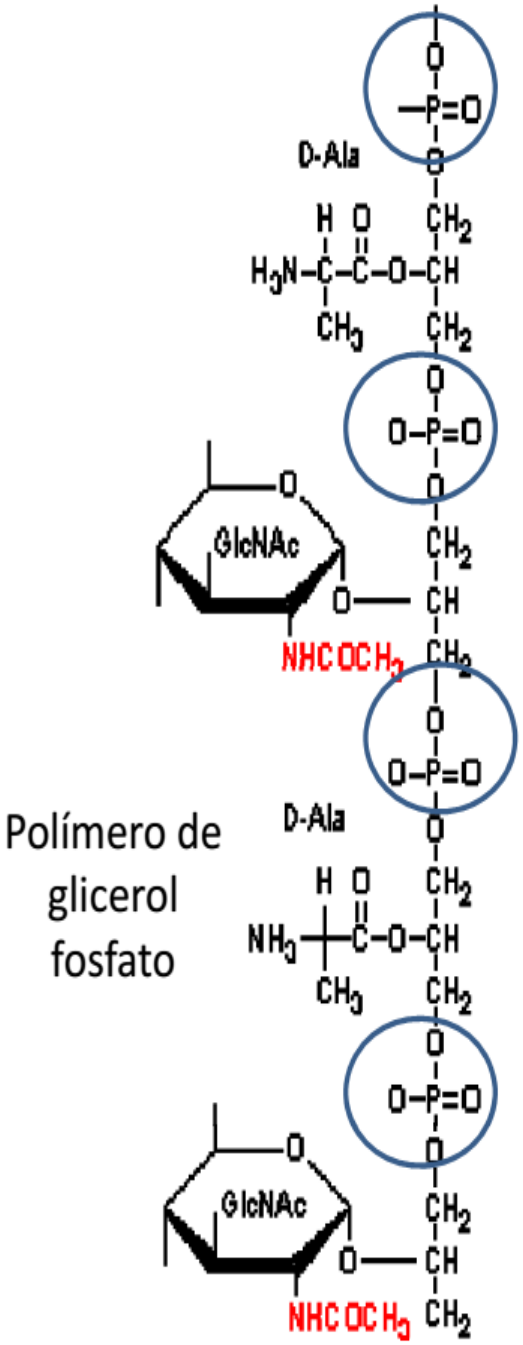


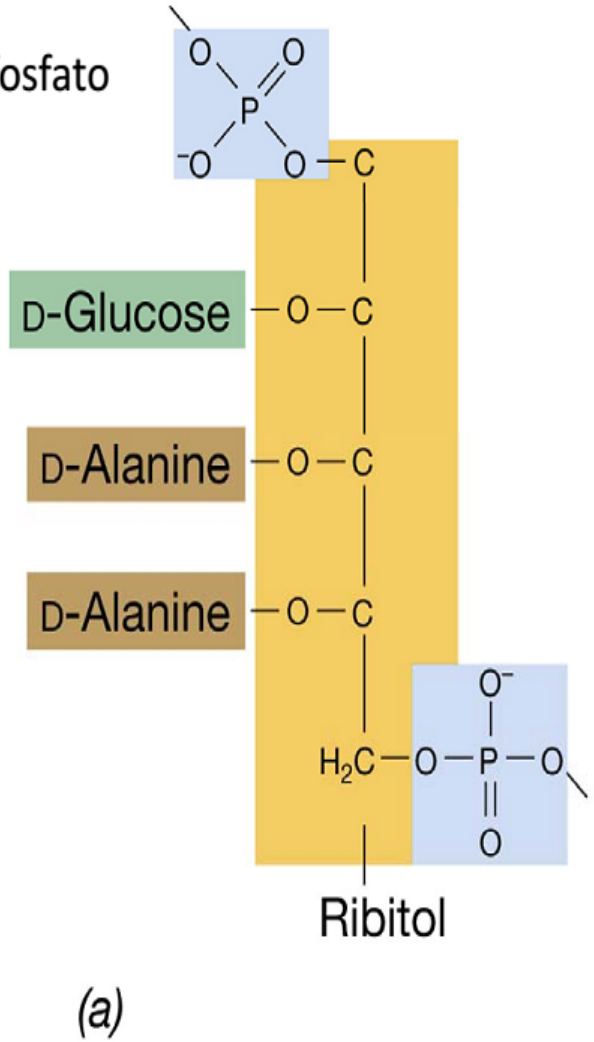
ÁCIDOS TEICOICOS

ÁCIDOS LIPOTEICOICOS

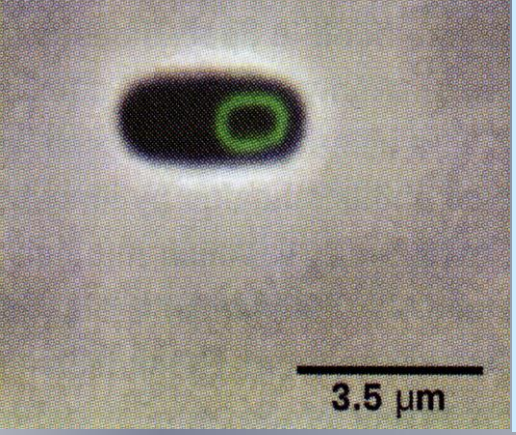
ÁCIDOS TEICURÓNICOS



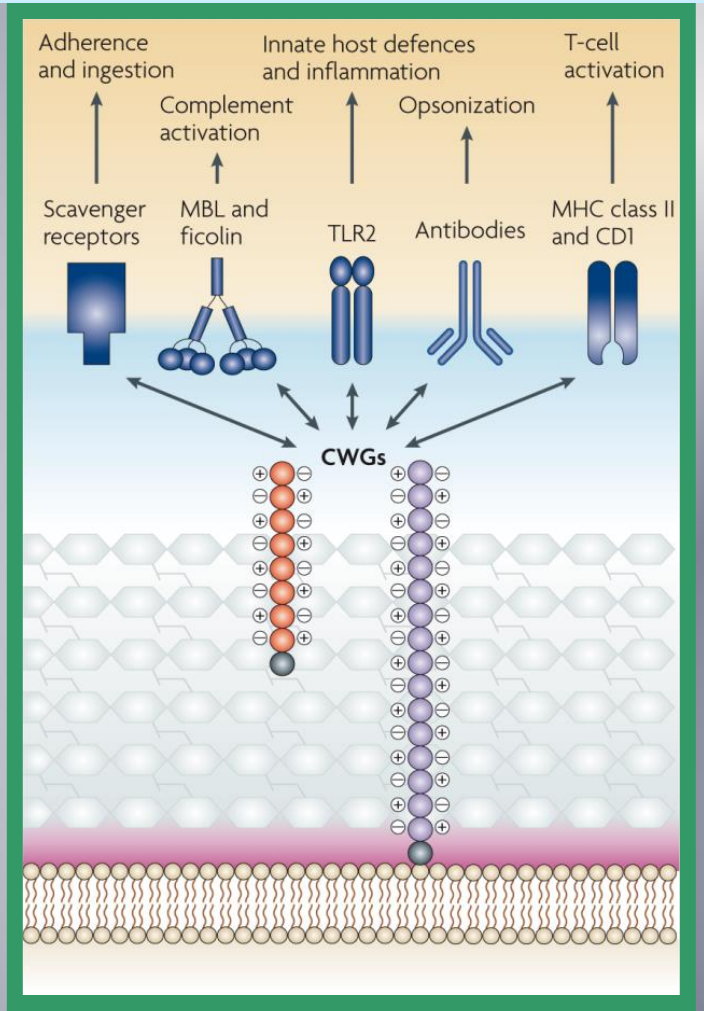




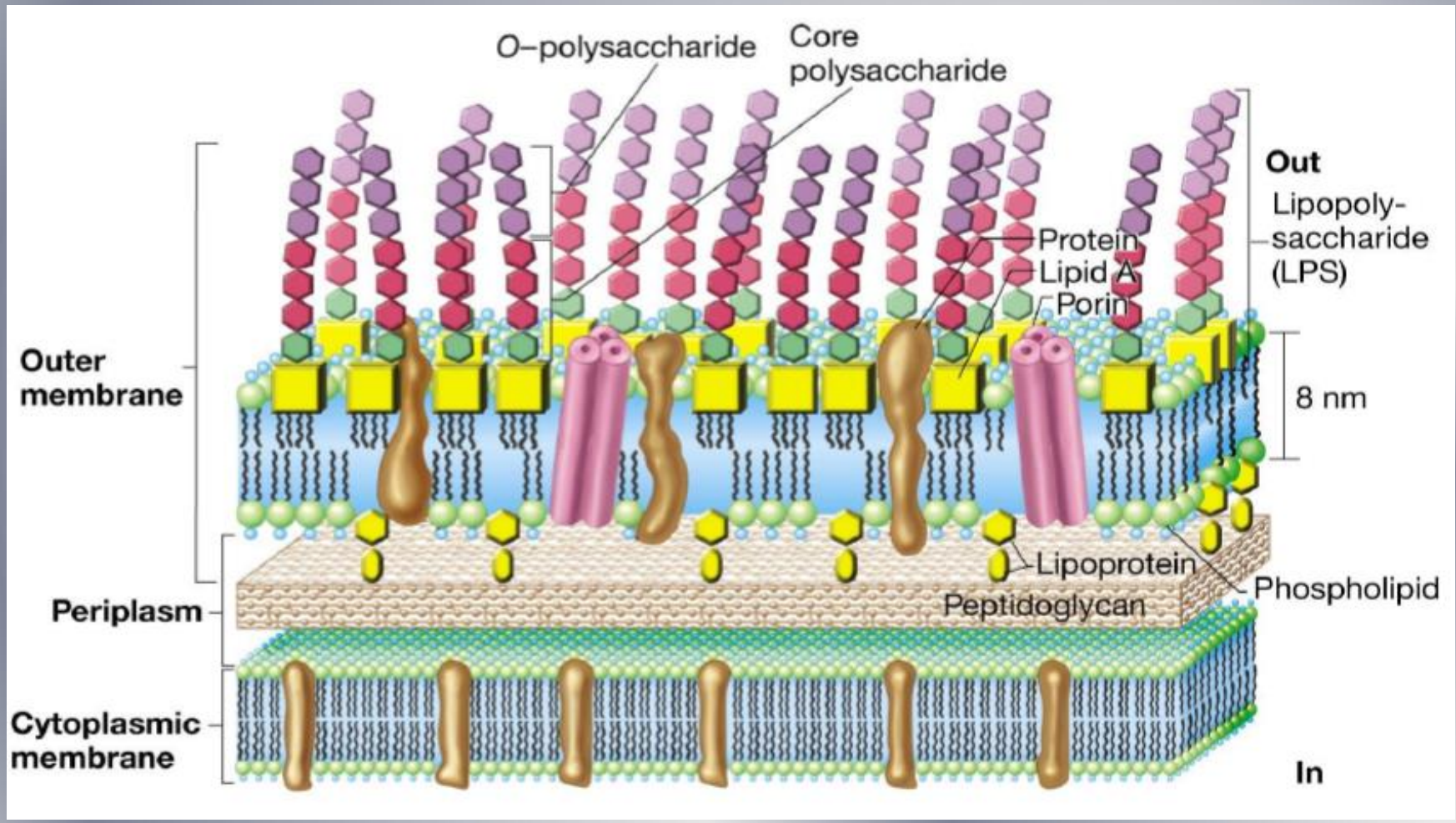
Se unen a través de azucares variables al PG

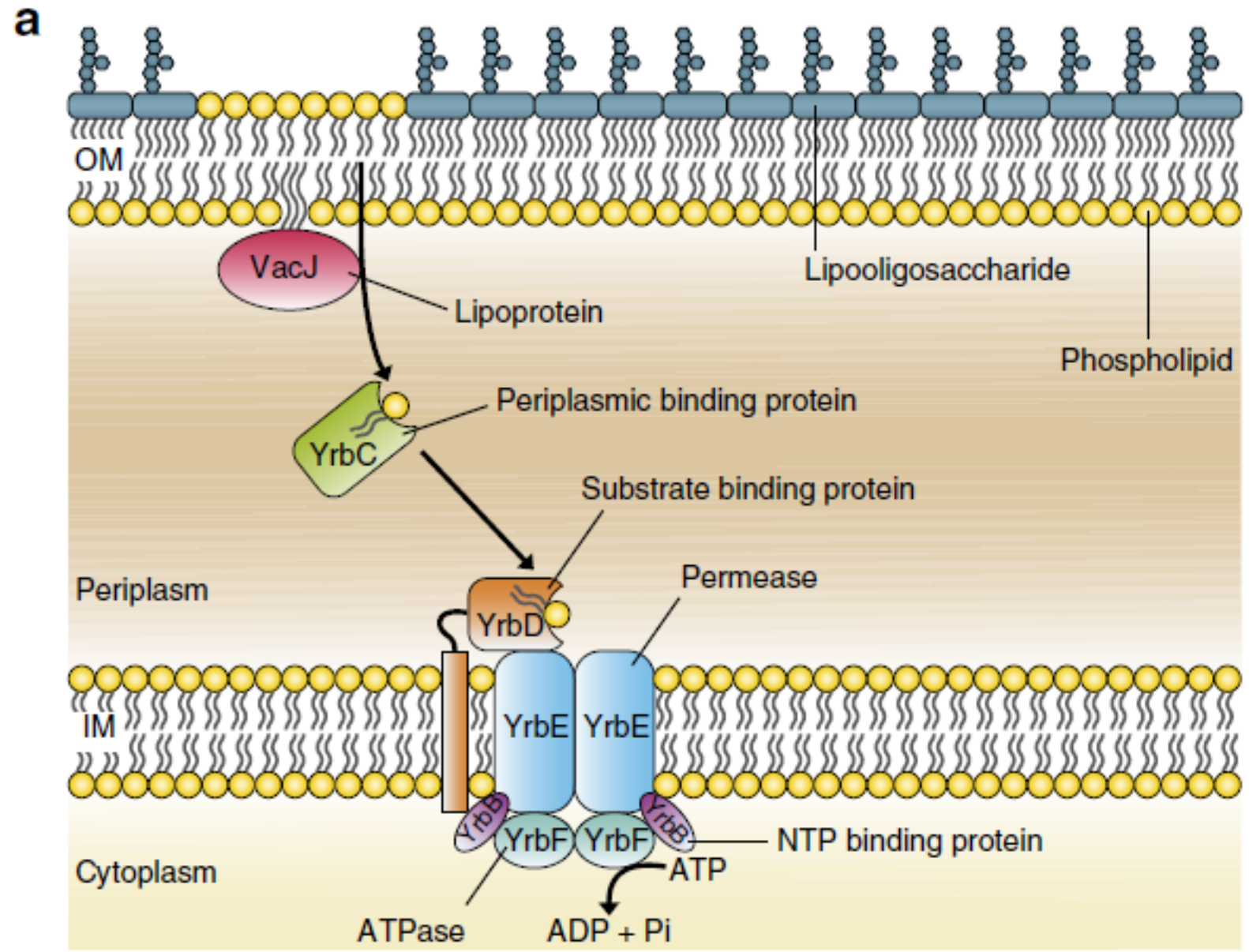


INTERACCIÓN ENTRE LA FLORA INTESTINAL Y EL SISTEMA INMUNOLÓGICO



ESTRUCTURA BACTERIANA
COMPONENTES EXCLUSIVOS DE LA PARED CELULAR DE BACTERIAS GRAM-NEGATIVAS





A conserved ABC transport system putatively prevents PL accumulation in the outer leaflet of the OM.

FORMACIÓN DE VESÍCULAS DE MEMBRANA EXTERNA EN BACTERIAS GRAM-NEGATIVAS

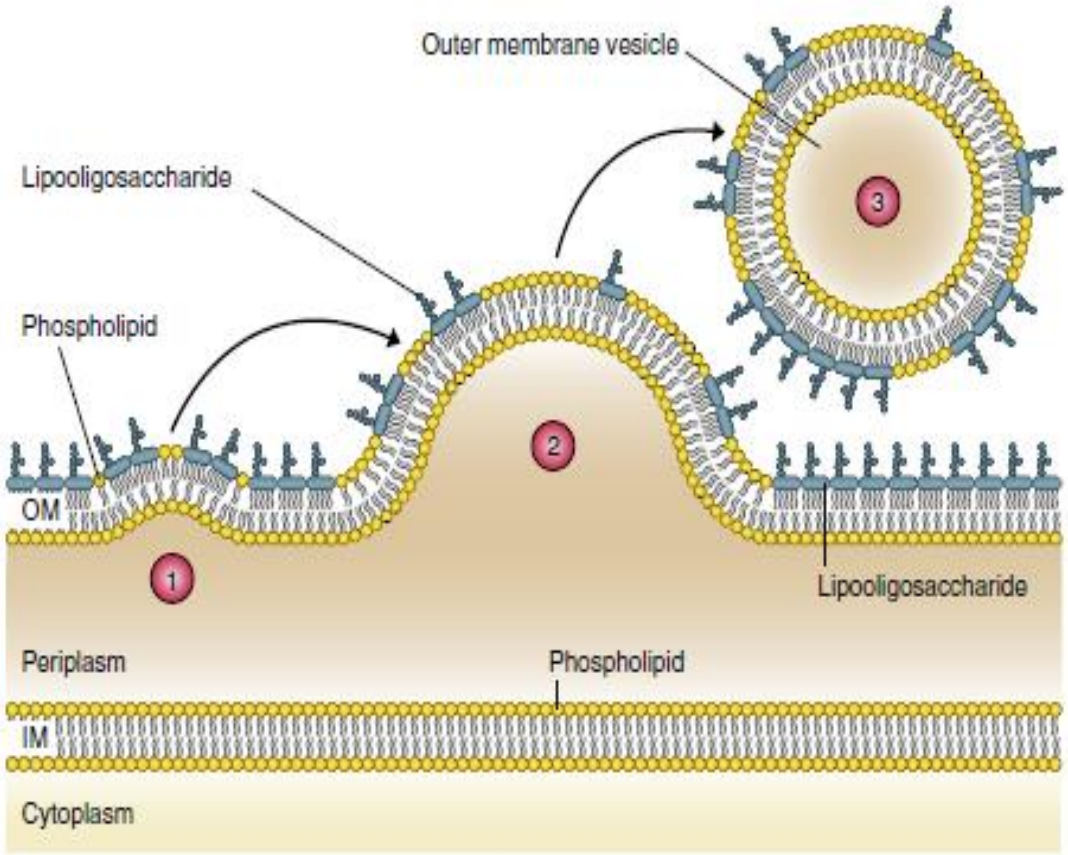
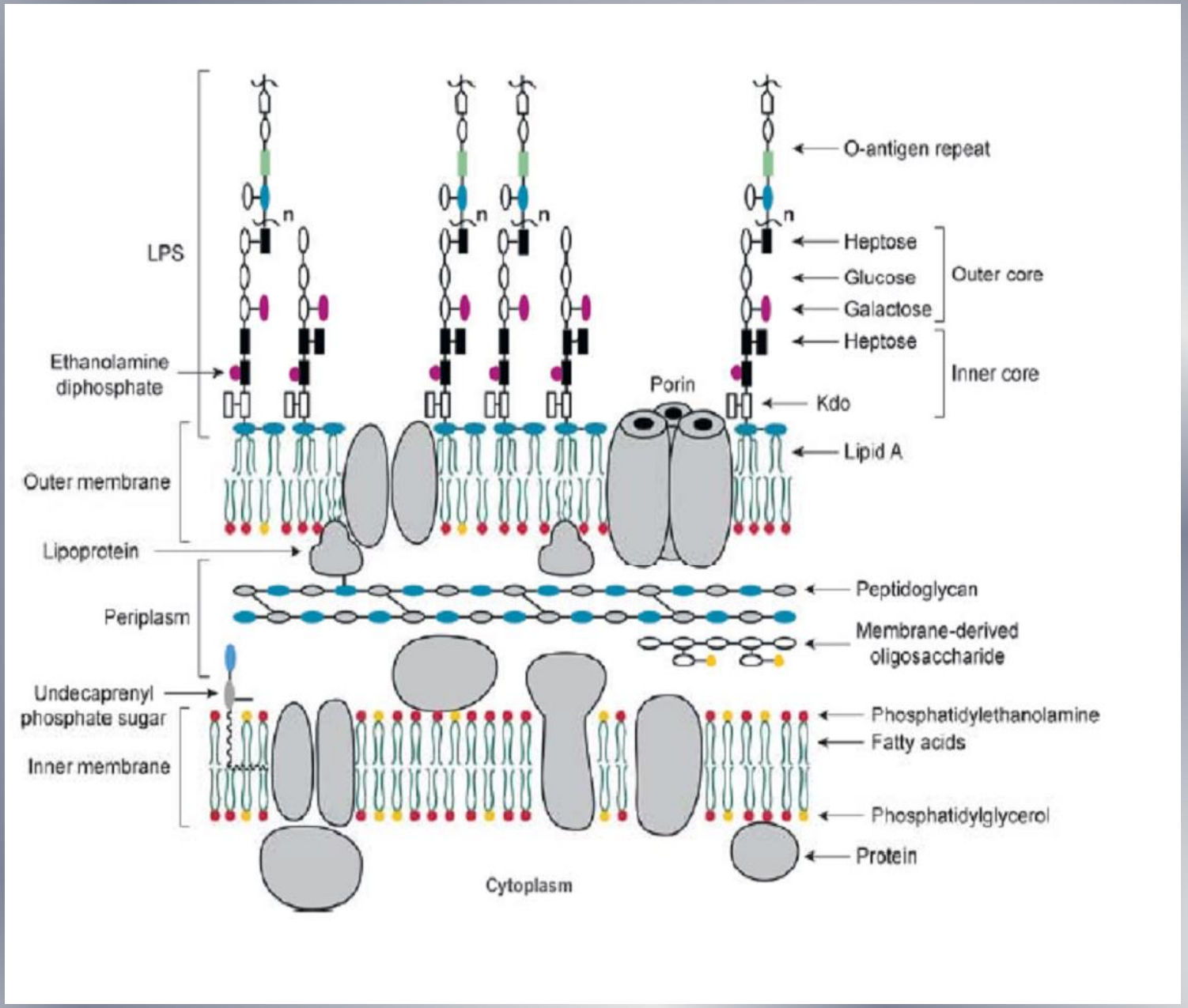
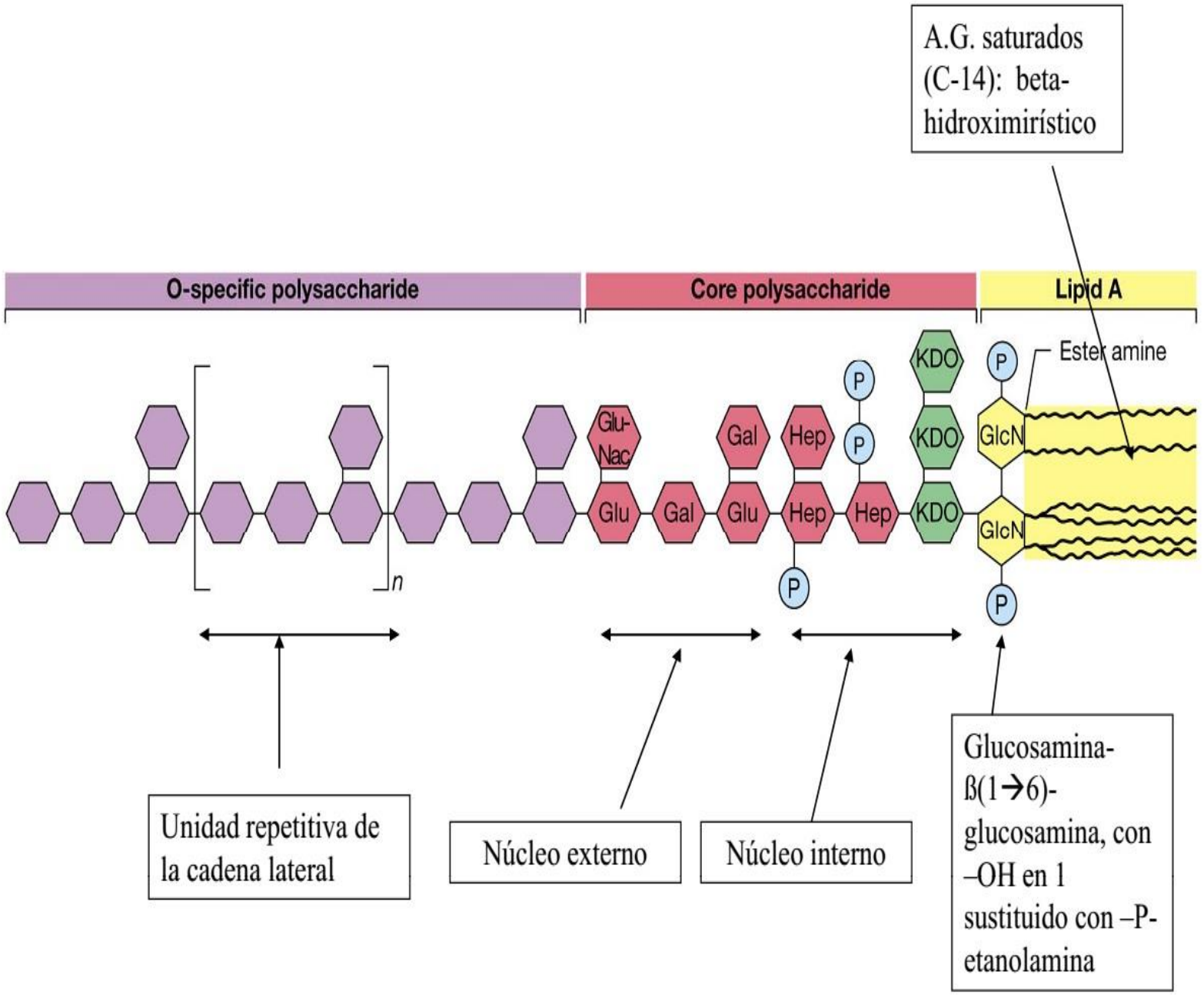


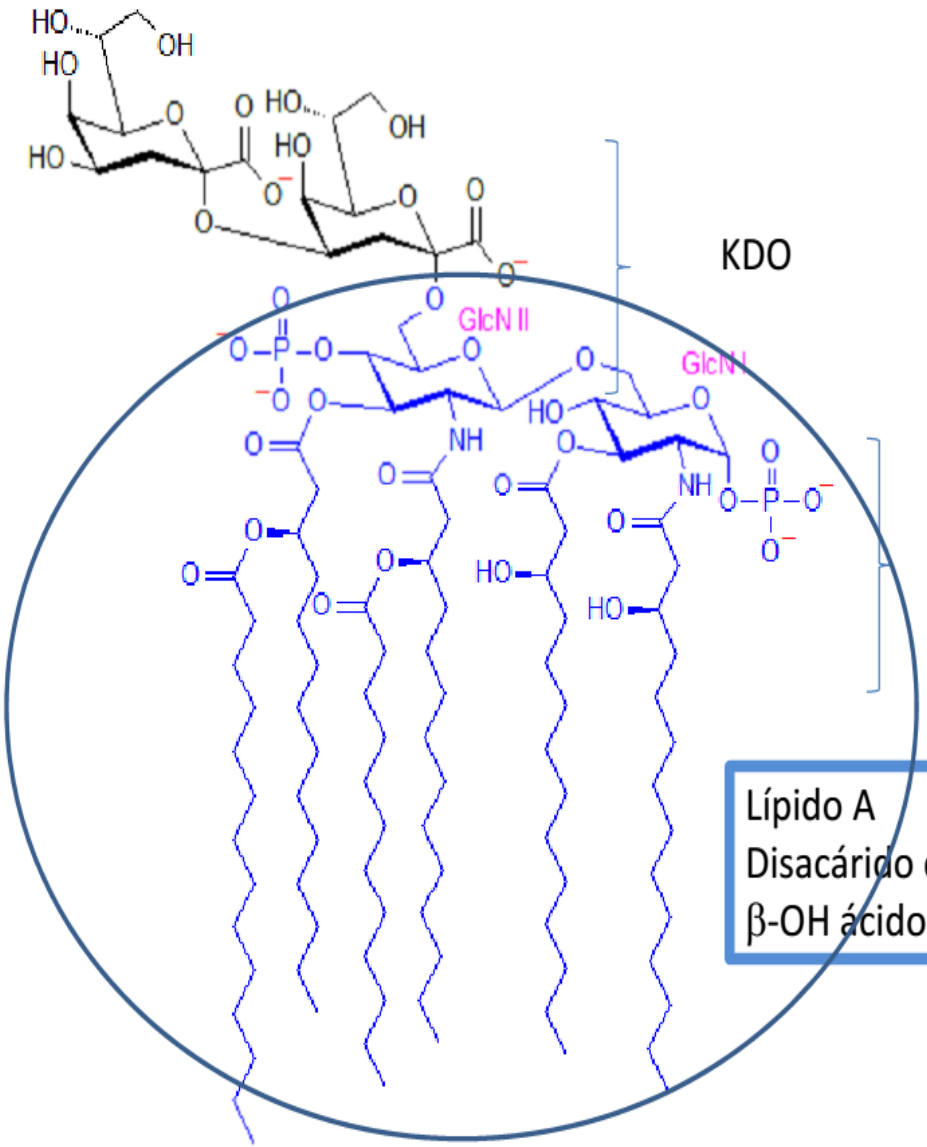
Figure 8 | A new model of OMV formation in Gram-negative bacteria. Step 1: Decreased expression or deletion of *vacJ* and/or *yrb* genes results in PL accumulation in the outer leaflet of the OM. This asymmetric expansion of the outer leaflet initiates an outward bulging of the OM. Step 2: Further enrichment of positive and negative curvature-inducing PLs in both leaflets supports the budding of the OM, which finally pinches off to form an OMV. Step 3: The released OMV is enriched in PLs incorporated into the outer leaflet of the vesicle membrane.

LIPOPOLISACÁRIDO



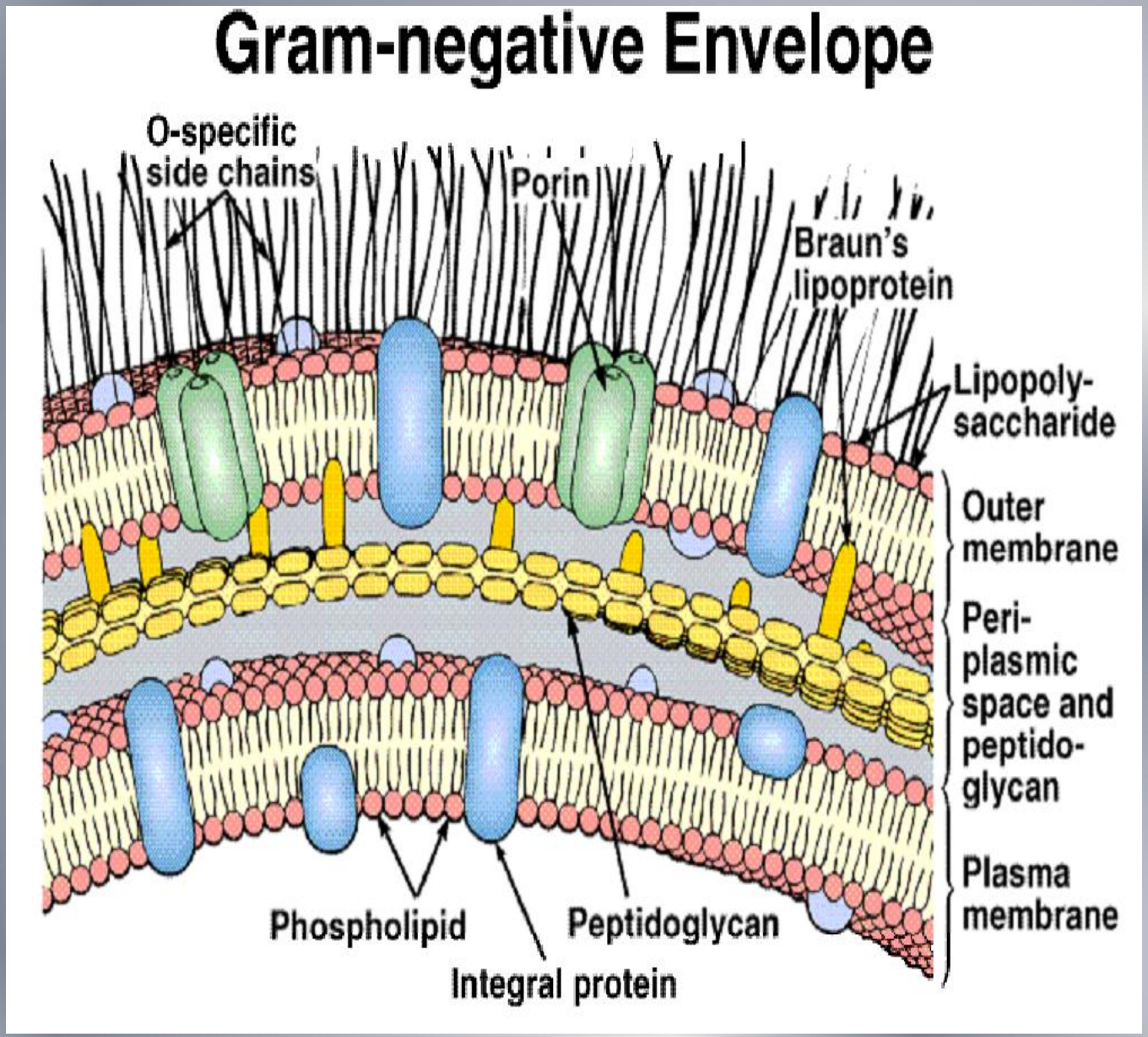

A.G. saturados (C-14): beta-hidroximirístico
O-specific polysaccharide
Core polysaccharide
Lipid A
Ester amine
KDO
P
Glu-Nac
Gal
Hep
Glu
GlcN
n
Unidad repetitiva de la cadena lateral
Núcleo externo
Núcleo interno
Glucosamina-ß(1→6)-glucosamina, con –OH en 1 sustituido con –P-etanolamina

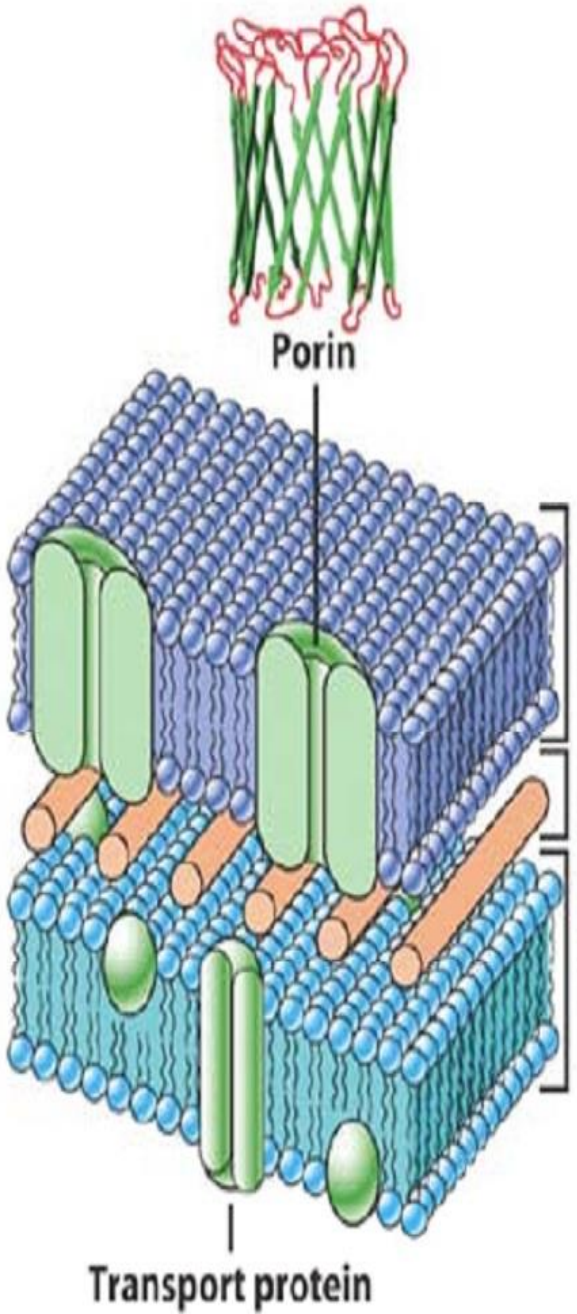
ENDOTOXINA BACTERIANA



The basic lipopolysaccharide of *E. coli*, incorporating lipid A (blue portion of the structure).

PORINAS


Gram-negative Envelope
O-specific side chains
Porin
Braun's lipoprotein
Lipopoly-saccharide
Outer membrane
Peri-plasmic space and peptido-glycan
Plasma membrane
Phospholipid
Peptidoglycan
Integral protein



Porinas mayoritarias

OmpF: poro grande

OmpC: poro chico

PhoE

OmpC y OmpF están presentes siempre

OmpC/OmpF aumenta cuando aumenta

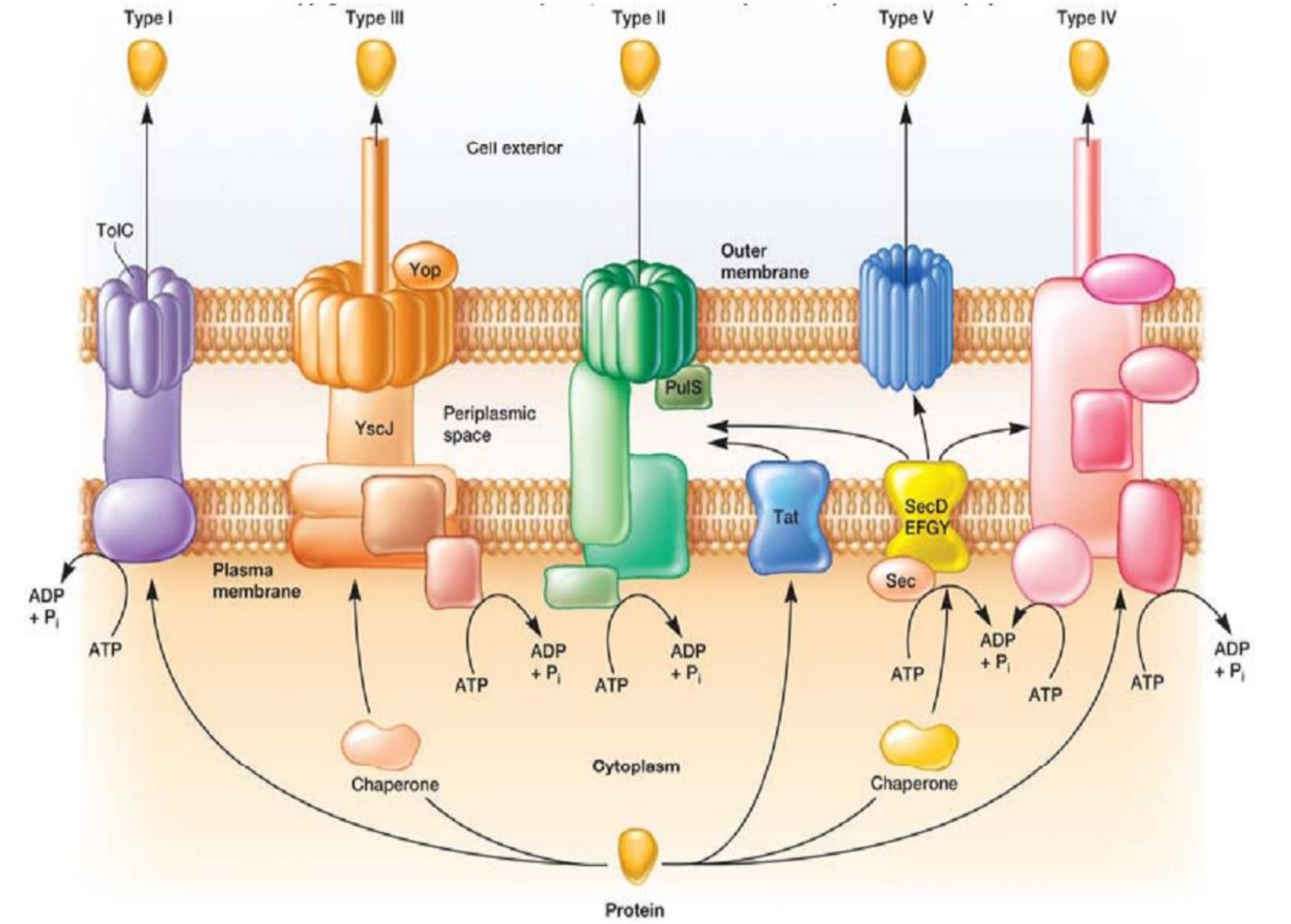
La osmolaridad

La temperatura

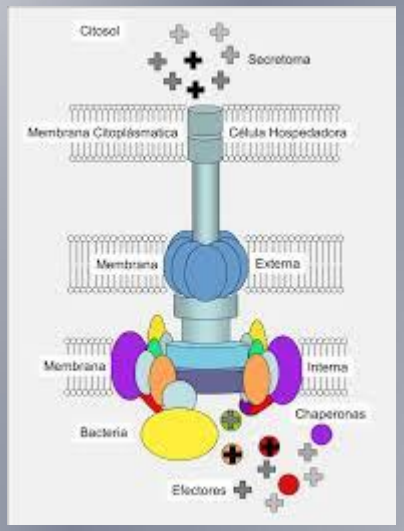
PhoE solo se induce en condiciones de

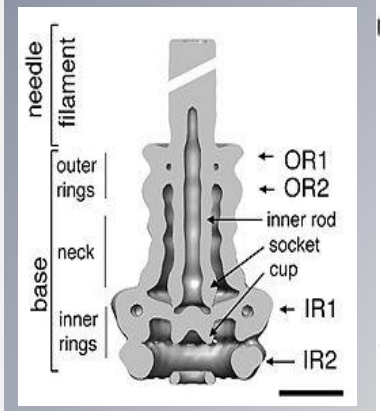
Limitación de fosfatos y otros aniones

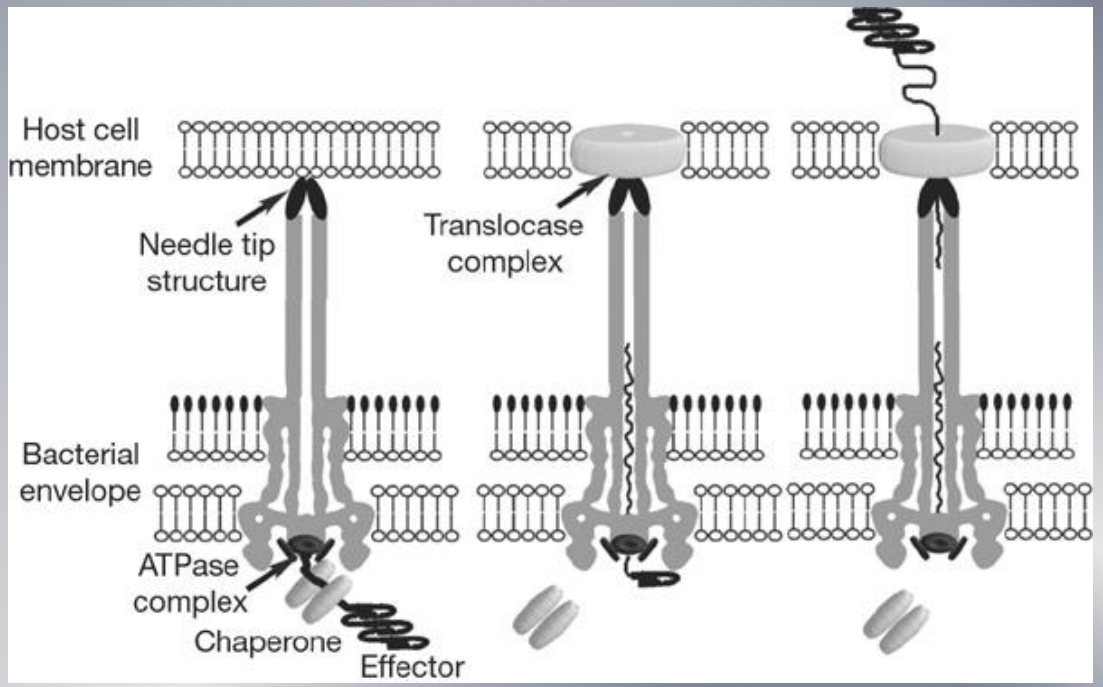
SISTEMAS DE SECRECIÓN ALGUNOS EXCLUSIVOS DE GRAM-NEGATIVOS Y OTROS COMUNES A GRAM-NEGATIVOS Y GRAM-POSITIVOS

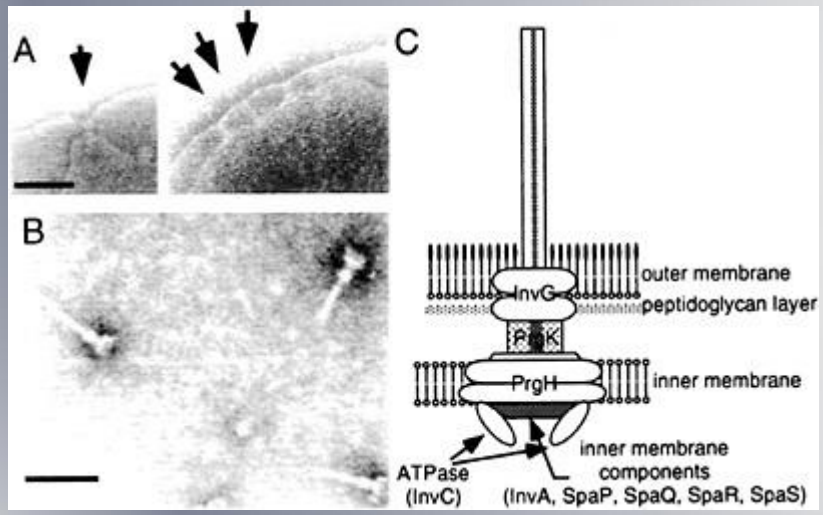


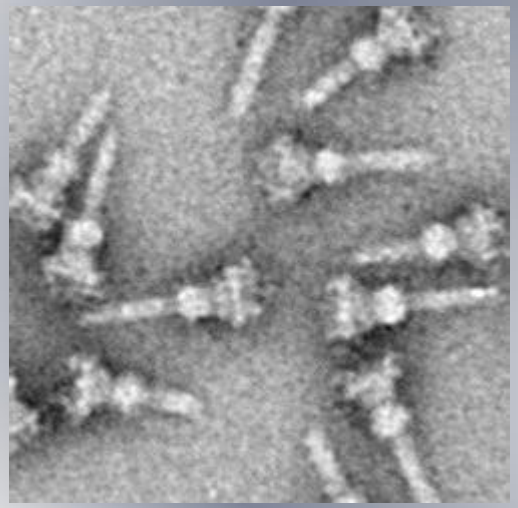
SISTEMAS DE SECRECIÓN TIPO III











ESTRUCTURA BACTERIANA

PARED CELULAR DE BACTERIAS GRAM-POSITIVAS

PARED CELULAR DE BACTERIAS GRAM-NEGATIVAS

PARED CELULAR DE MICOBACTERIAS

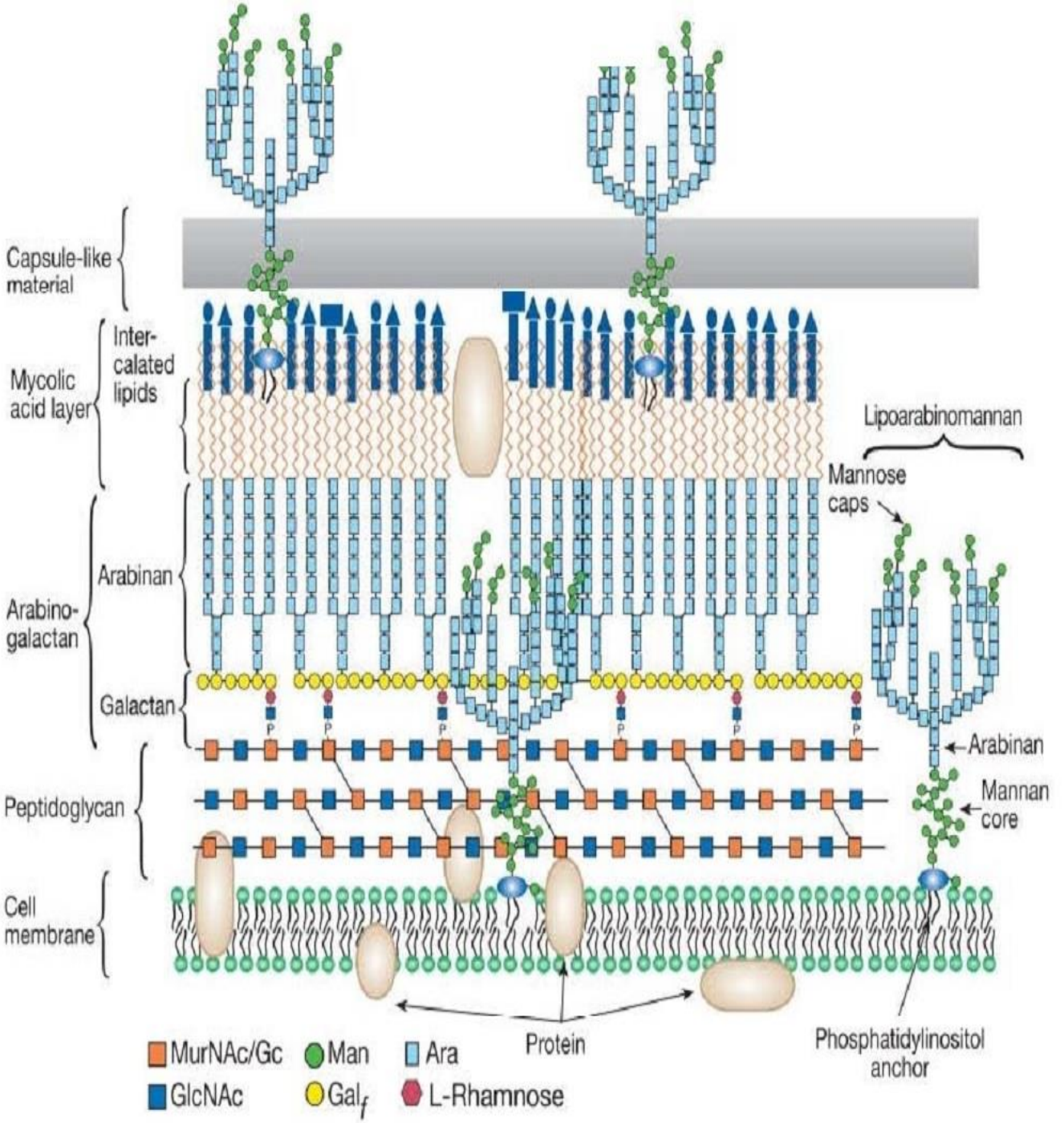
PARED CELULAR O ENVOLTURA DE ESPORAS

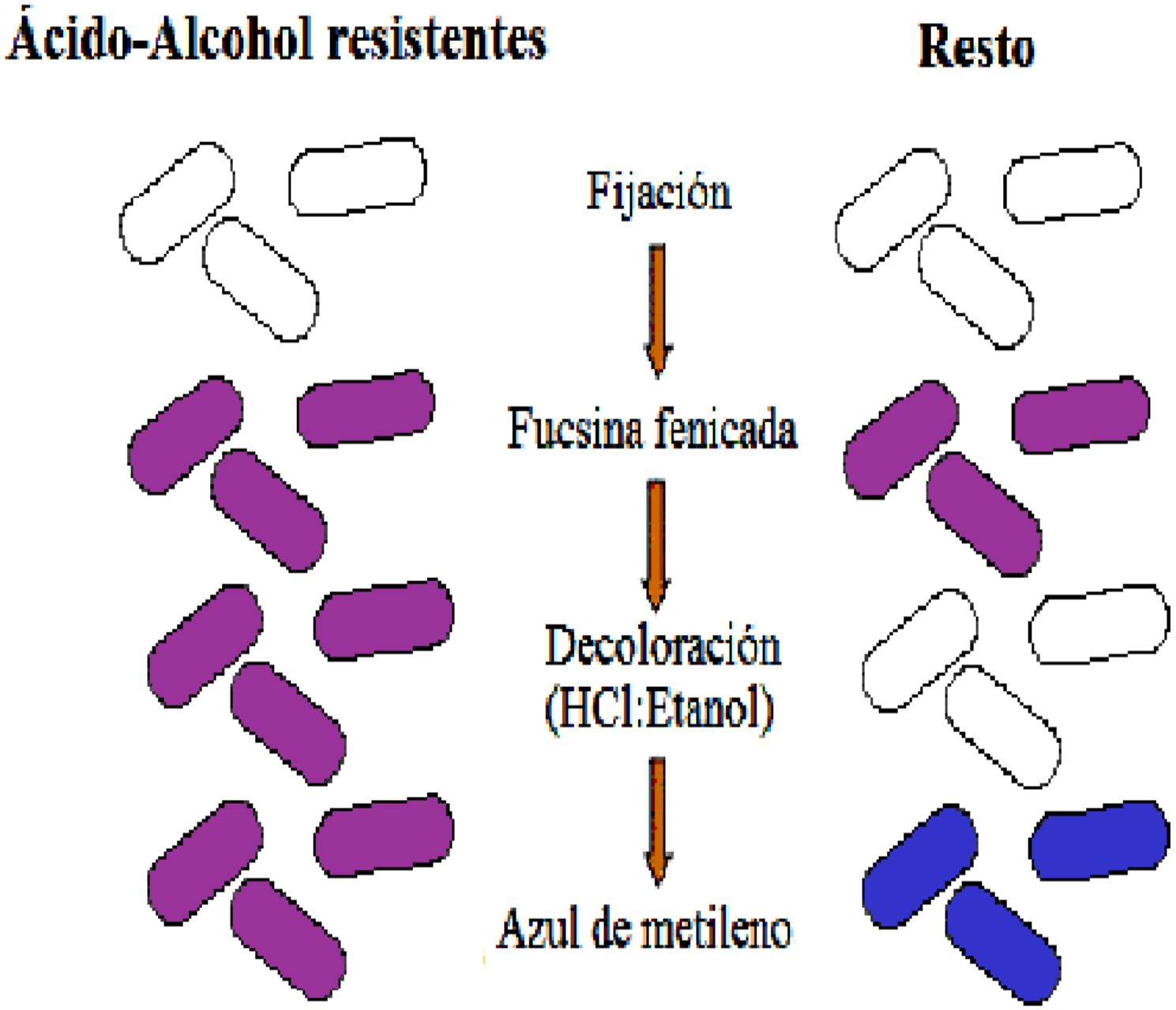
OTROS TIPOS DE PAREDES Y ENVOLTURAS

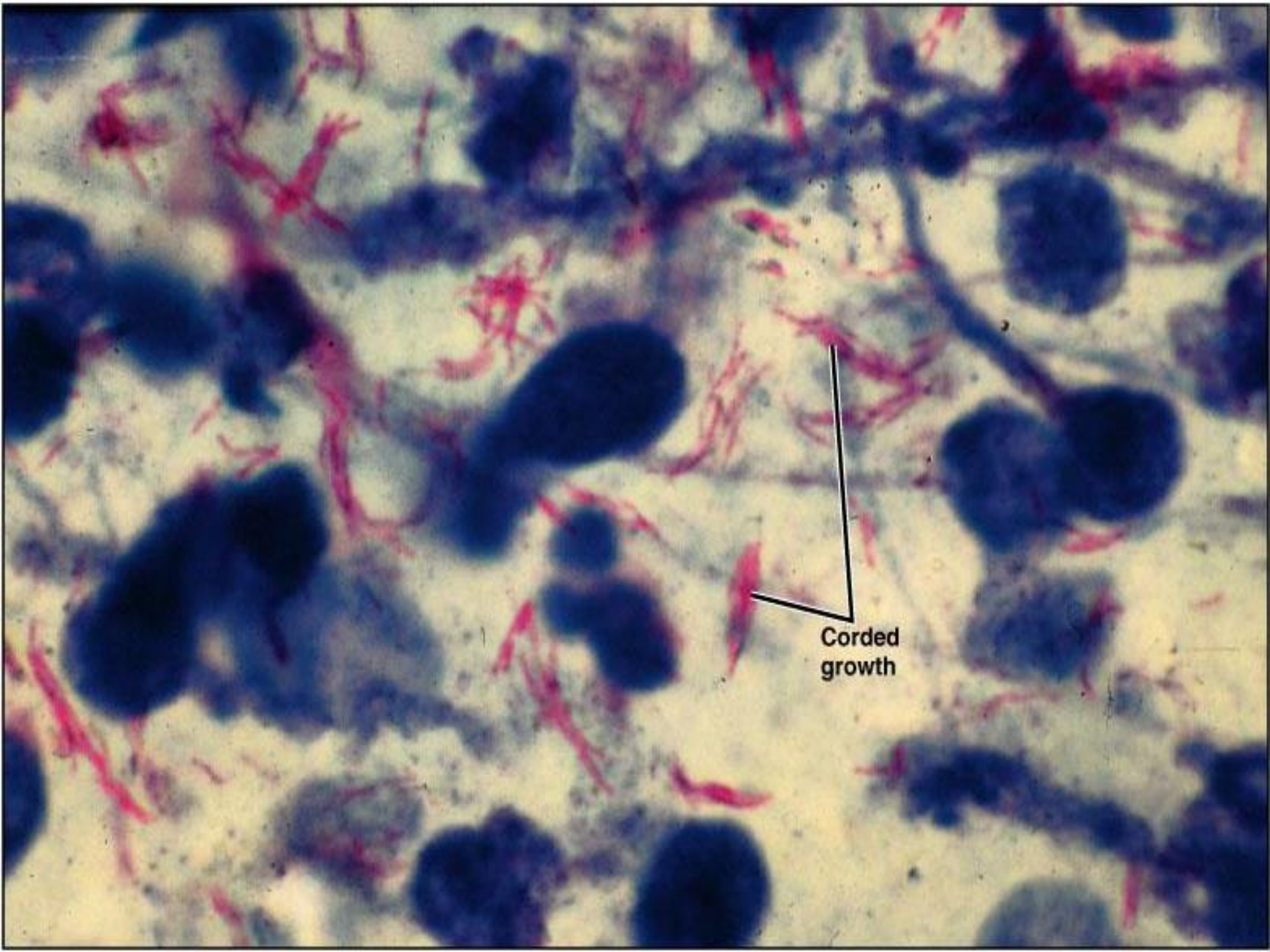
APÉNDICES BACTERIANOS

MOTILIDAD BACTERIANA

BIOFILMS


Capsule-like material
Mycolic acid layer
Inter-calated lipids
Arabino-galactan
Arabinan
Galactan
Peptidoglycan
Cell membrane
Lipoarabinomannan
Mannose caps
Arabinan
Mannan core
Protein
Phosphatidylinositol anchor
MurNAc/Gc
GlcNAc
Man
Gal$_f$
Ara
L-Rhamnose

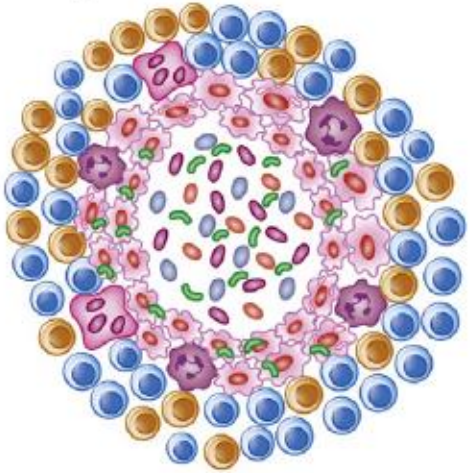

Ácido-Alcohol resistentes
Resto
Fijación
Fucsina fenicada
Decoloración
(HCl:Etanol)
Azul de metileno


Corded
growth

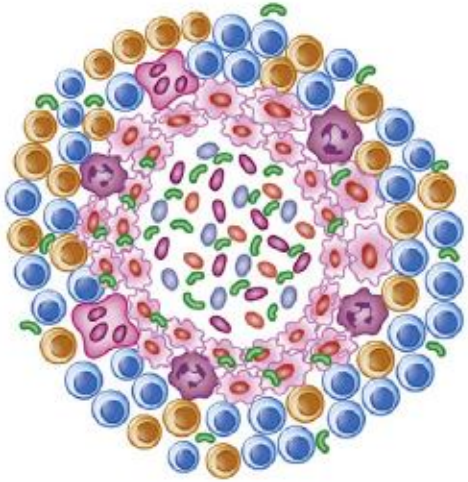
Opening Pandora's Box: Mechanisms of *Mycobacterium tuberculosis* Resuscitation

Latency:
Clinical term
No visible symptoms
Not infectious

Reactivation:
Clinical term
Visible symptoms
Infectious



Dormancy:
Bacterial phenotype
Low levels of replication
Minimal metabolism
Thick cell wall



Resuscitation:
Bacterial phenotype
Active replication
Active metabolism

Trends in Microbiology

Figure 1. Definition of Clinical and Bacterial Terms. Latency and reactivation refer to clinical states of disease during TB. Latency is a period during which there are no symptoms of the disease and the patient is not infectious. Reactivation is the return of symptoms, and the patient is infectious. During latency, the bacilli are hypothesized to be dormant. Dormancy is defined as low levels of replication, minimal metabolism, and a thick cell wall. In favorable conditions, the bacilli are thought to resuscitate and begin replication and metabolism again, and this is believed to lead to reactivation of disease.

Opening Pandora's Box: Mechanisms of *Mycobacterium tuberculosis* Resuscitation

Bacterial Changes from Dormancy to Resuscitation

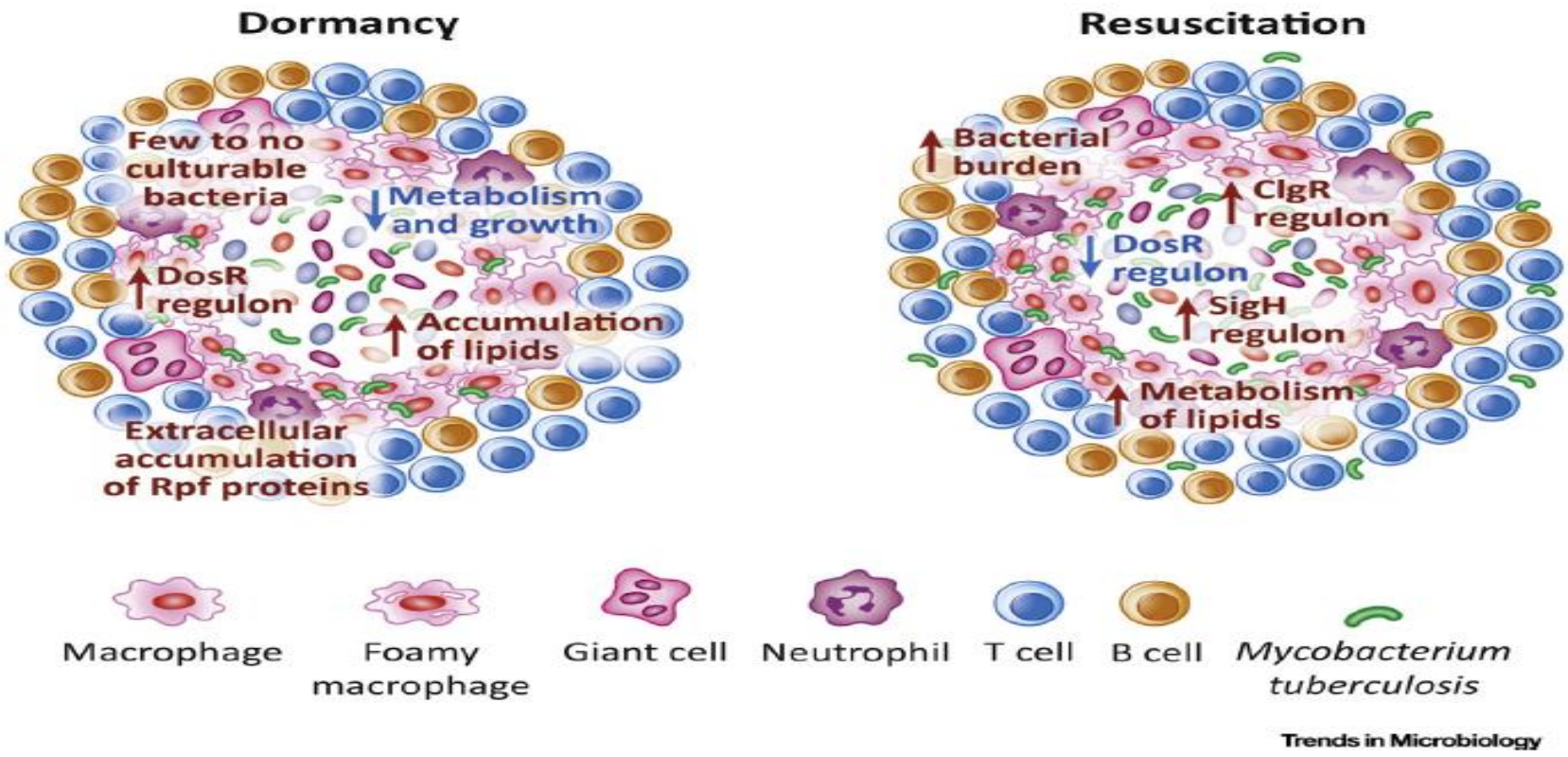


Figure 3. Dormancy is characterized by low metabolism and growth, and increased lipid accumulation and DosR regulon expression. Accumulation of Rpf proteins may be part of the groundwork for future resuscitation. Resuscitation is characterized by decreased DosR and increased ClgR regulon expression and increased metabolism using lipids as an energy source —pathways that are different from aerobic respiratory metabolism. The bacilli will increase in number and escape the granuloma.

ESTRUCTURA BACTERIANA

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PARED CELULAR DE BACTERIAS GRAM-NEGATIVAS

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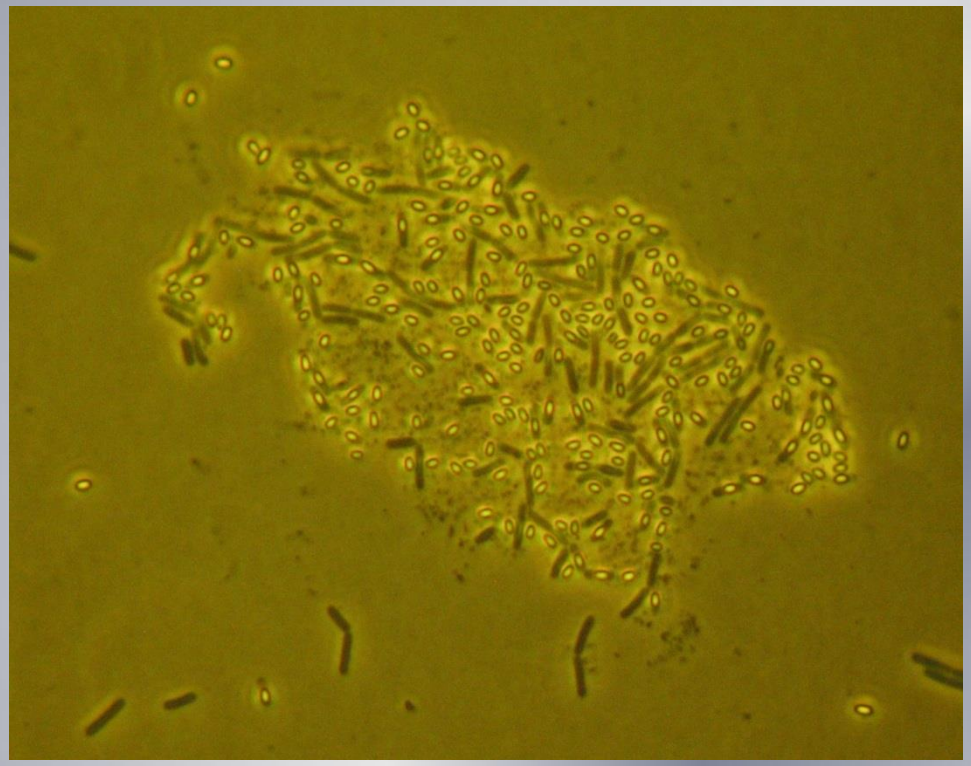
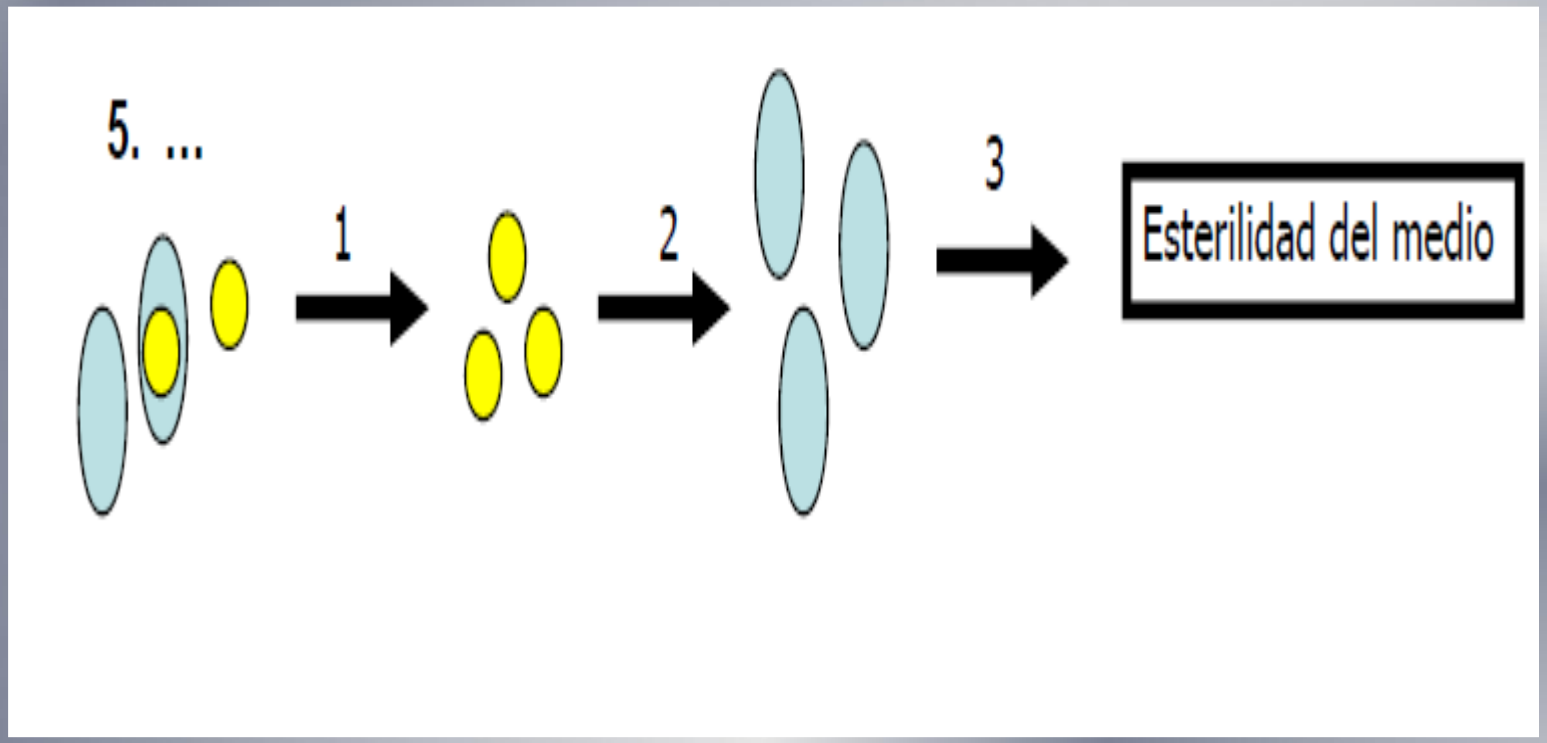
PARED CELULAR O CUBIERTA DE LAS ESPORAS

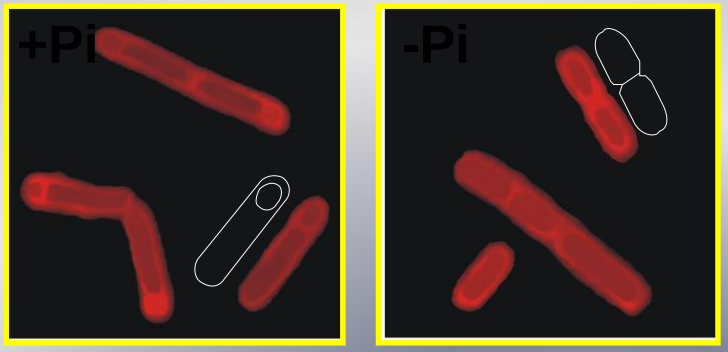


PASTEUR Y TYNDAL

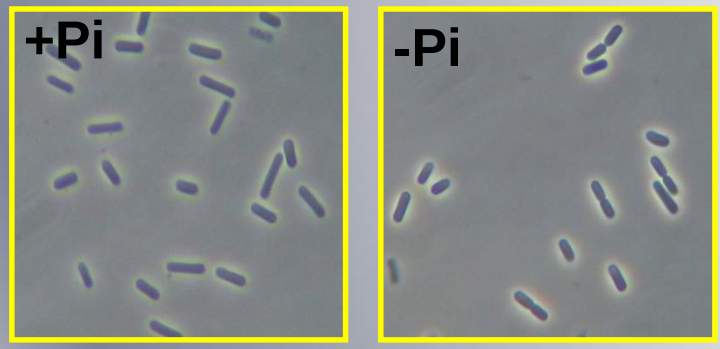


SM101 (cepa tipo salvaje)

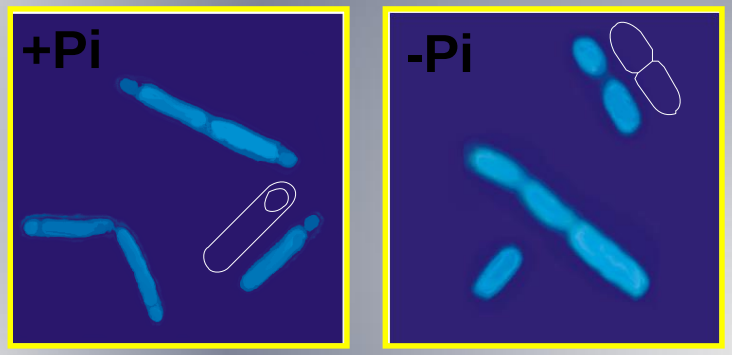
SM101 teñida con FM4-64

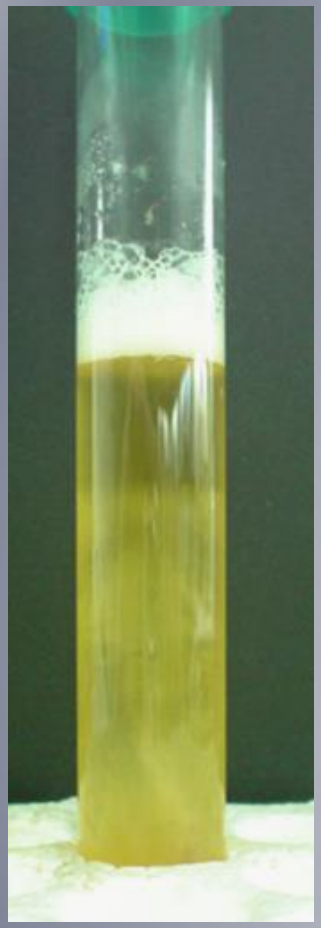


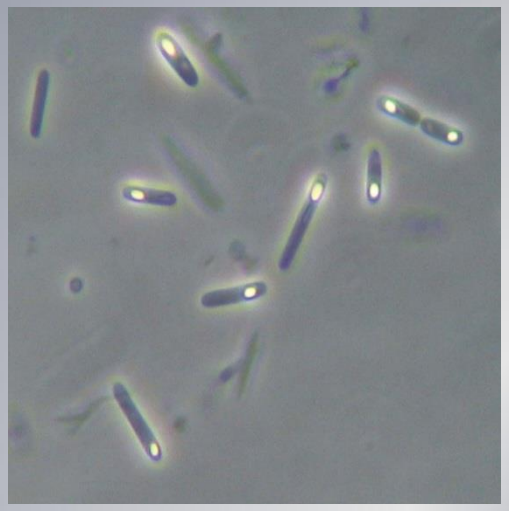
IH101 (mutante *spo0A*)

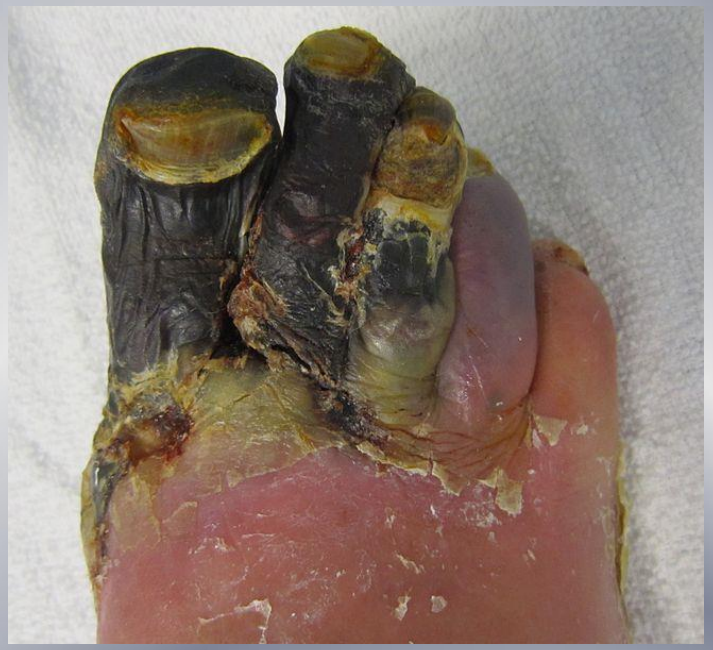


SM101 teñida con DAPI

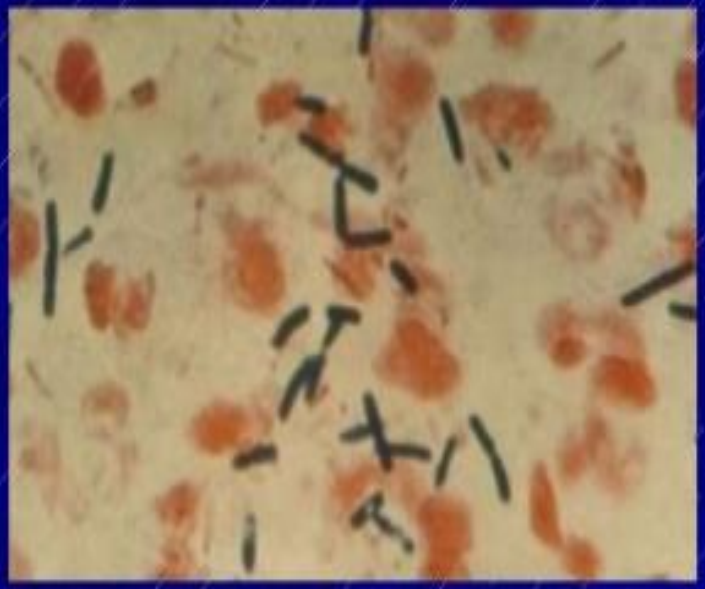






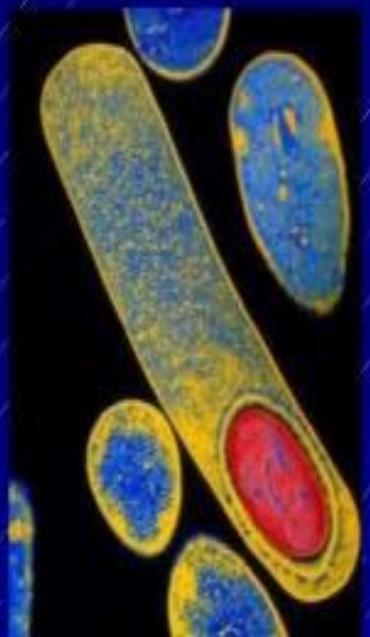


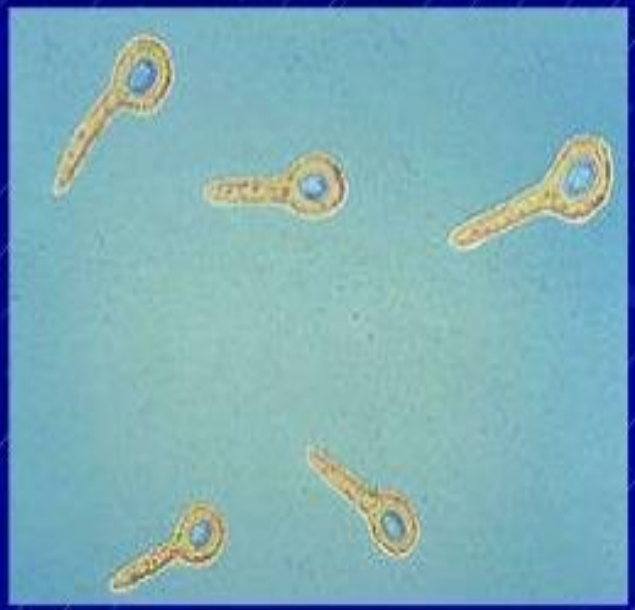
C. botulinum



C. perfringes

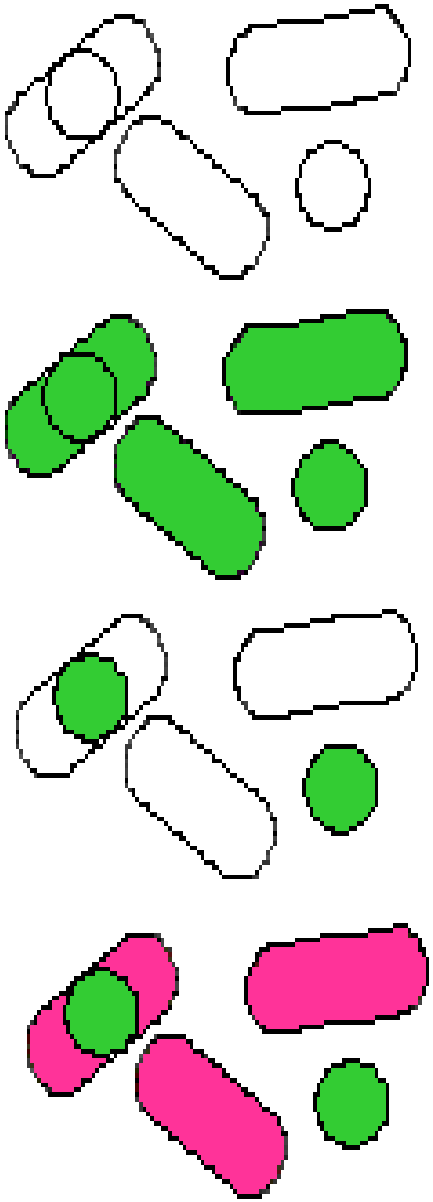
C. difficile





C. Tetani

Bacilos+Esporas

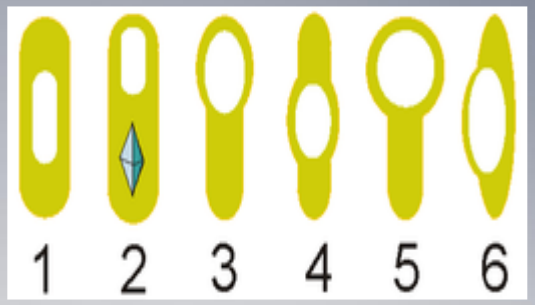


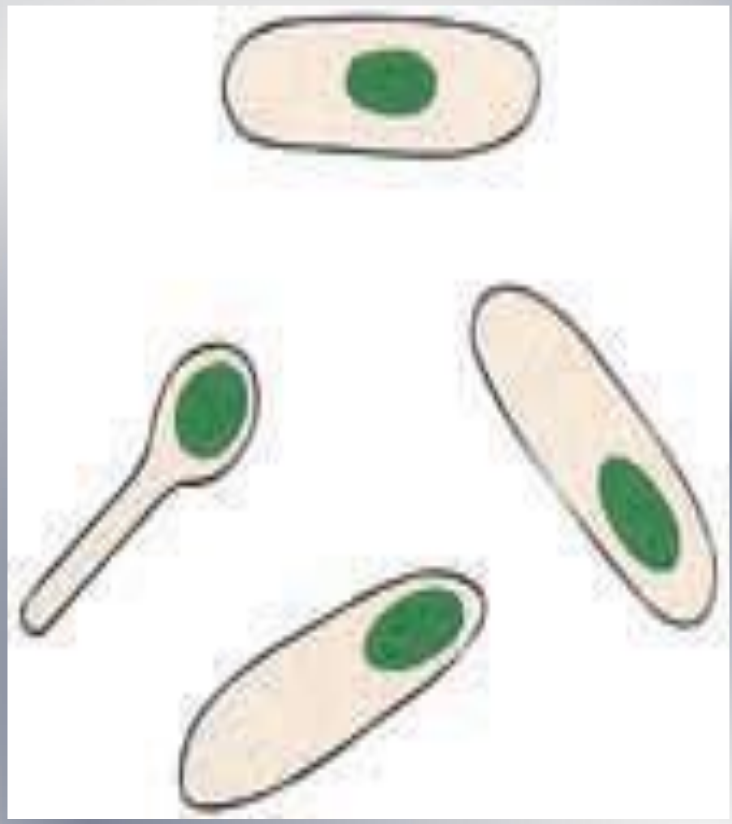
Fijación

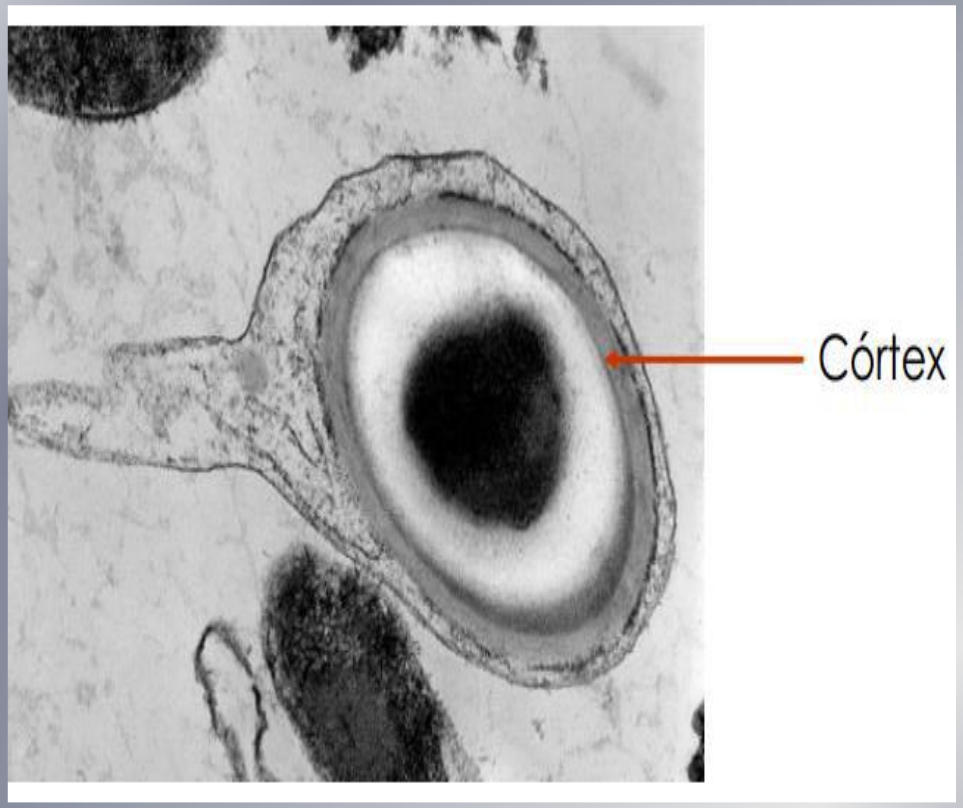
Verde Malaquita

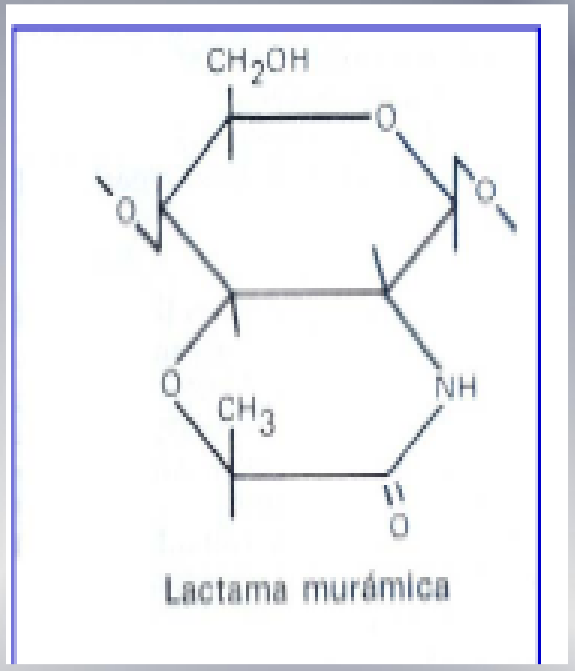
Agua abundante

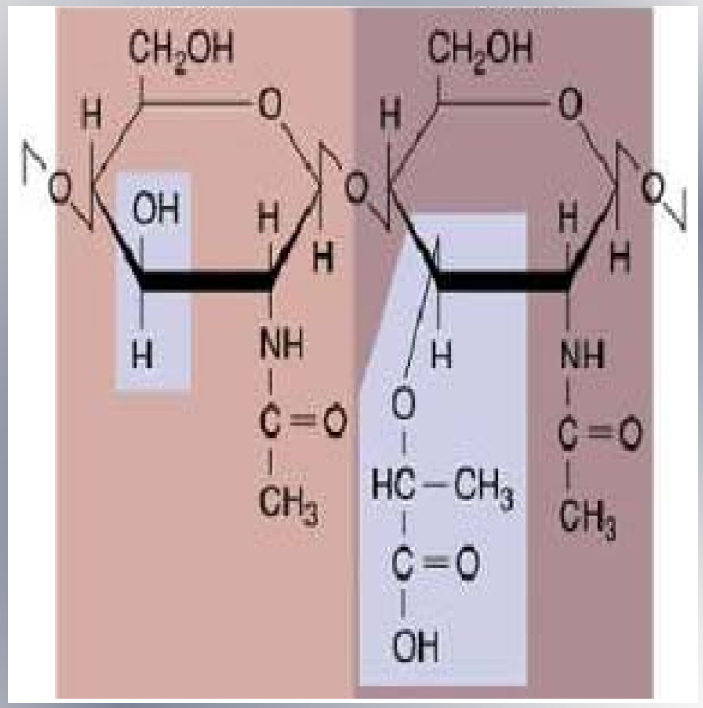
Safranina






Córtex


CH2OH
O
O
O
O
NH
CH3
O
Lactama murámica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CH3
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C=O
OH

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Review article Open Access

Bacterial Spores and its Relatives as Agents of Mass Destruction

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Abstract

The term bioterrorism has acquired full force for the planetary consciousness from the very beginning of the new century. Indeed, from the events that occurred during October and November of 2001 with the intentional contamination with spores of pathogenic *Bacillus anthracis*, of letters distributed by the US public postal service and the terrorist attacks in the last months of 2015 in Egypt, France, Mali, Afghanistan, Turkey, USA and other countries have warned again about the reality of the bioterrorist threat and its immeasurable cultural and undesirable economic and political consequences. In this review, we summarize the main structural characteristics that make the spores of Bacilli and Clostridia as the ideal agents for use in bioterrorism. In addition, we discuss the properties of non-sporulating *Coxiella burnetii*, the causative agent of Q fever, because of its peculiar resistance, high infectivity and environmental persistence that resembles true spores.

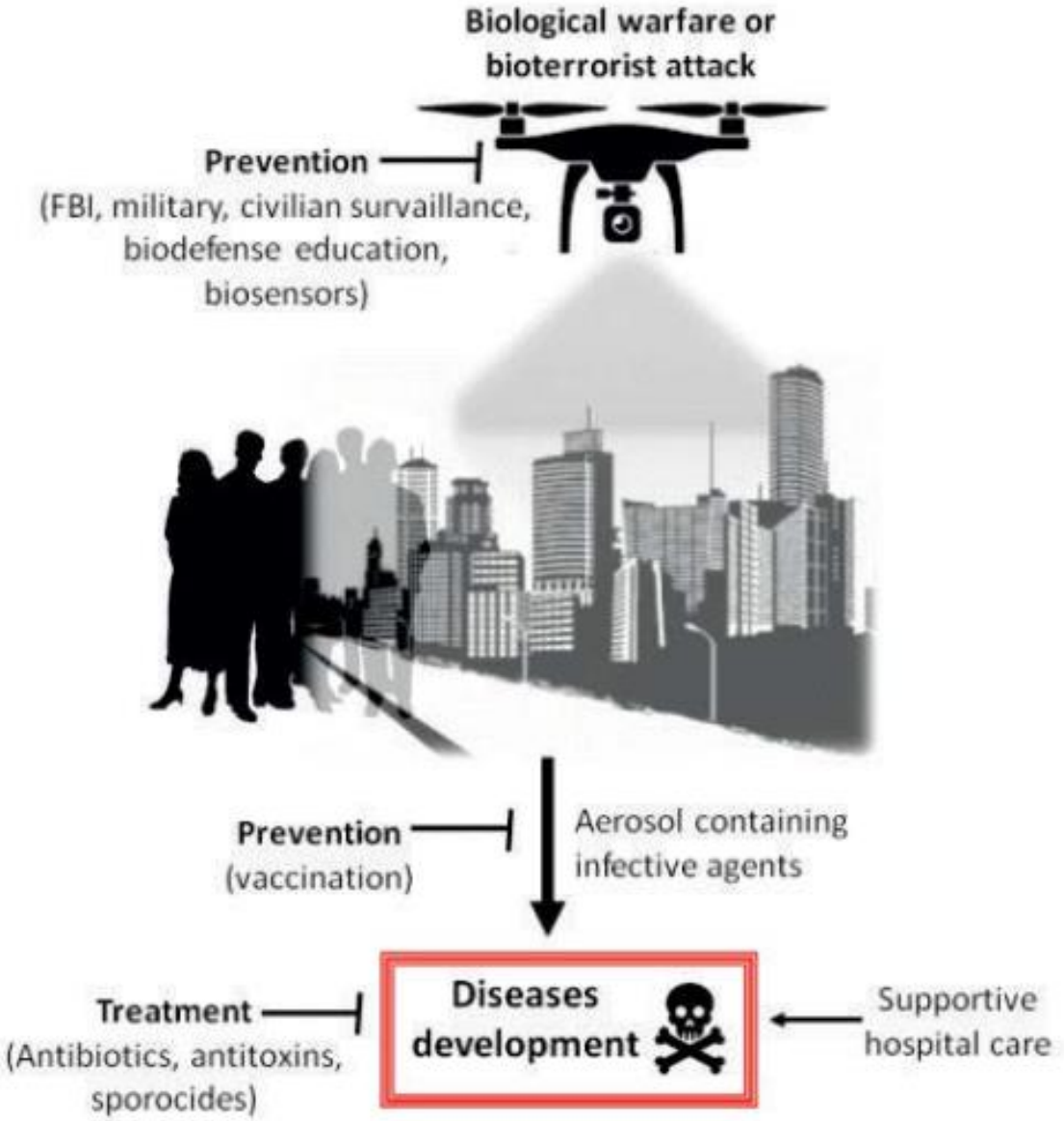


Figure 3: A hypothetical scenario showing measures that should be taken to prevent a bioterrorist attack using aerosolized spores or SCV of *BacillusClostridium* or *Coxiella burnetii*, respectively, or in the worst case mitigate its effects. See text for details.

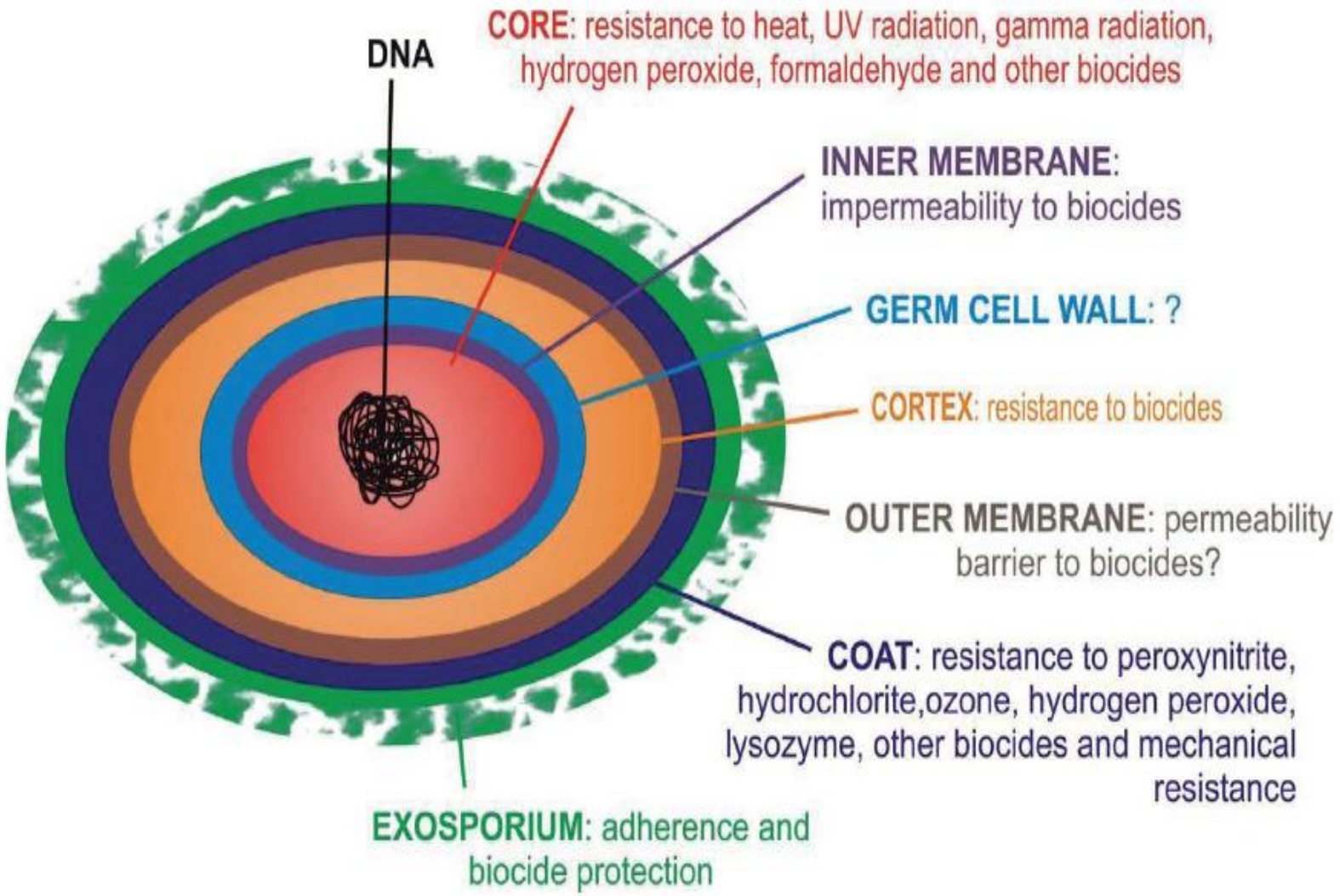


Figure 1: Cartoon of a typical bacterial spore. The different structural components of the spores of *Bacilli* and *Clostridia* and their roles in resistance to biocides and chemical/physical treatments are indicated here and explained in the text.

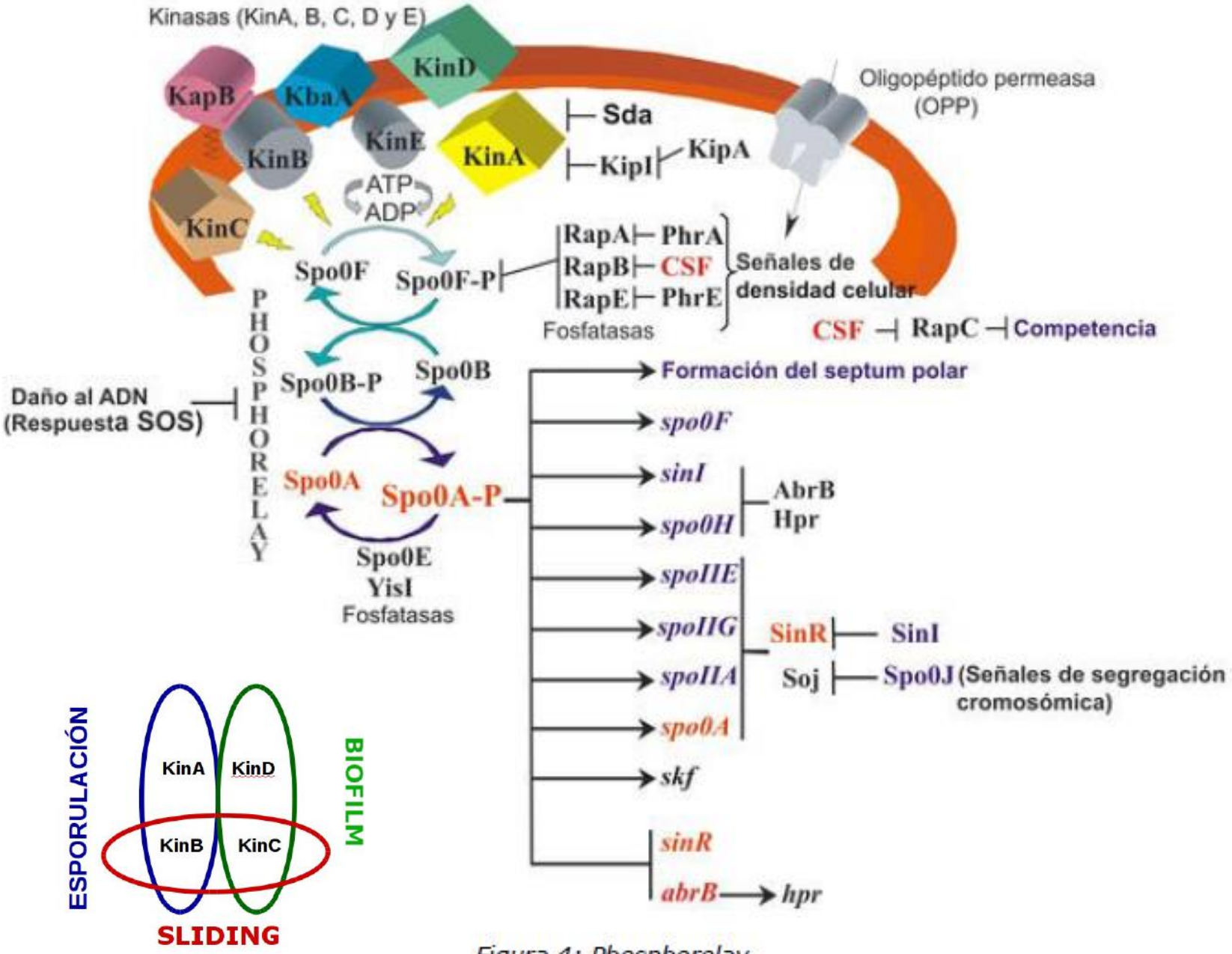
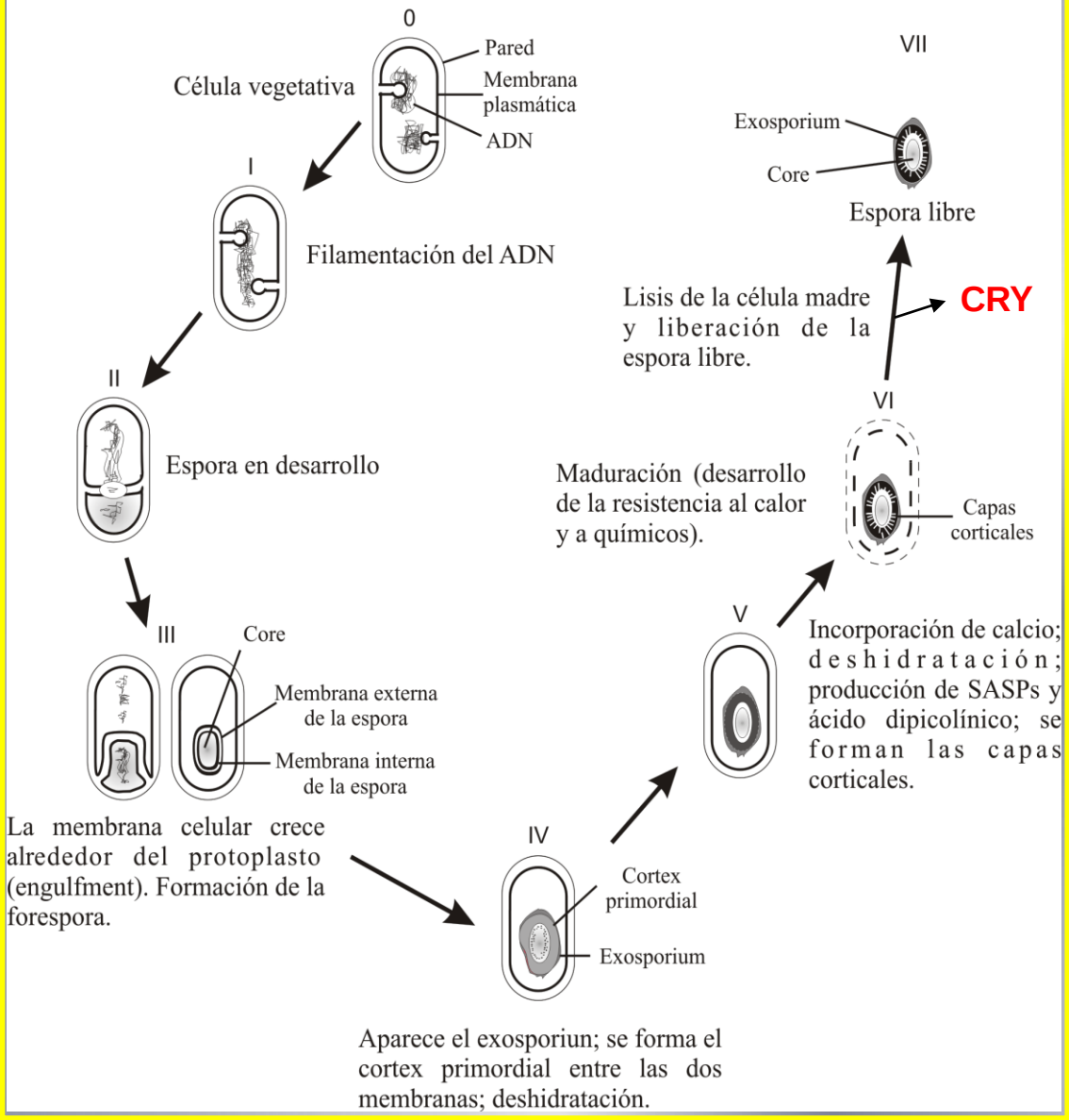


Figura 4: Phosphorelay.

ALGUNAS APLICACIONES: *BACILLUS THURINGIENSIS*

Estadíos de desarrollo de la espora



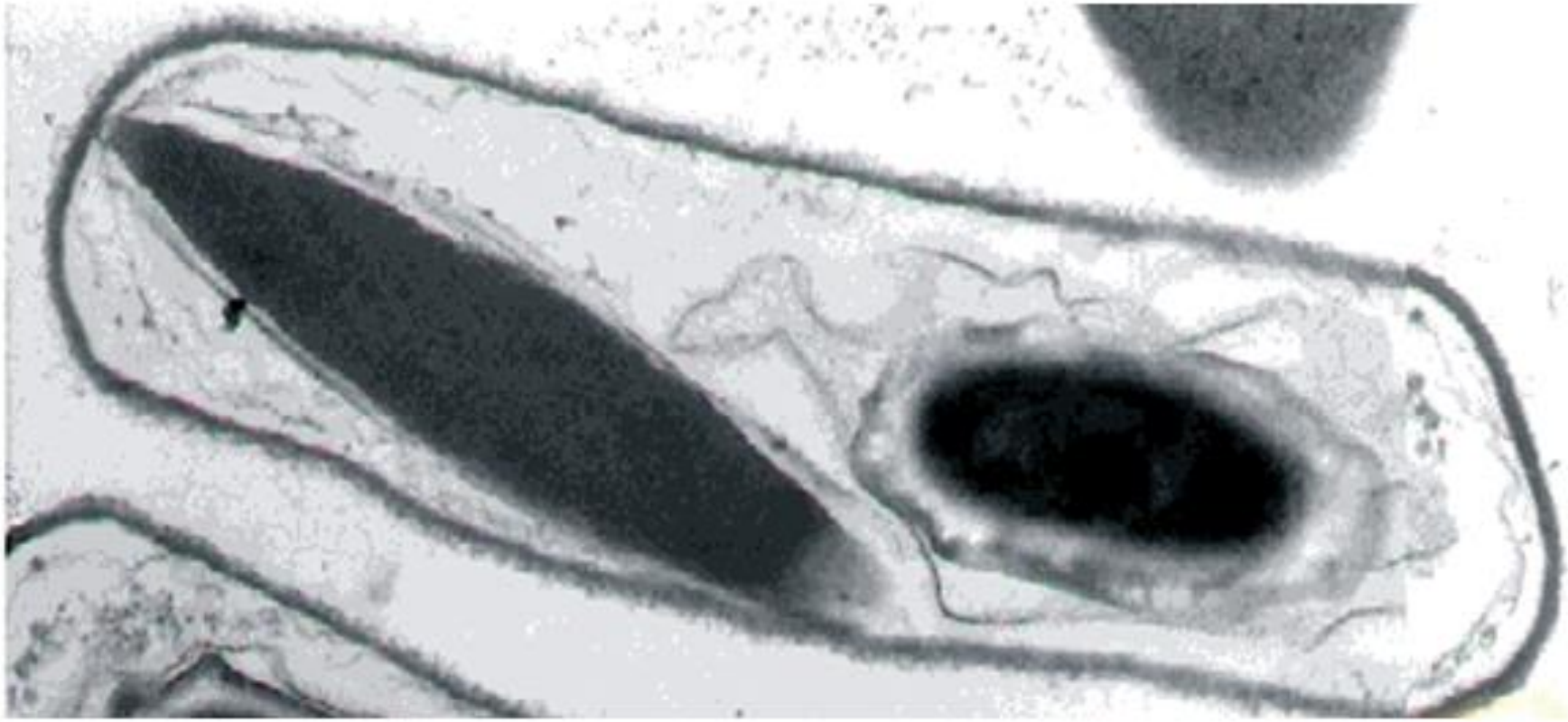
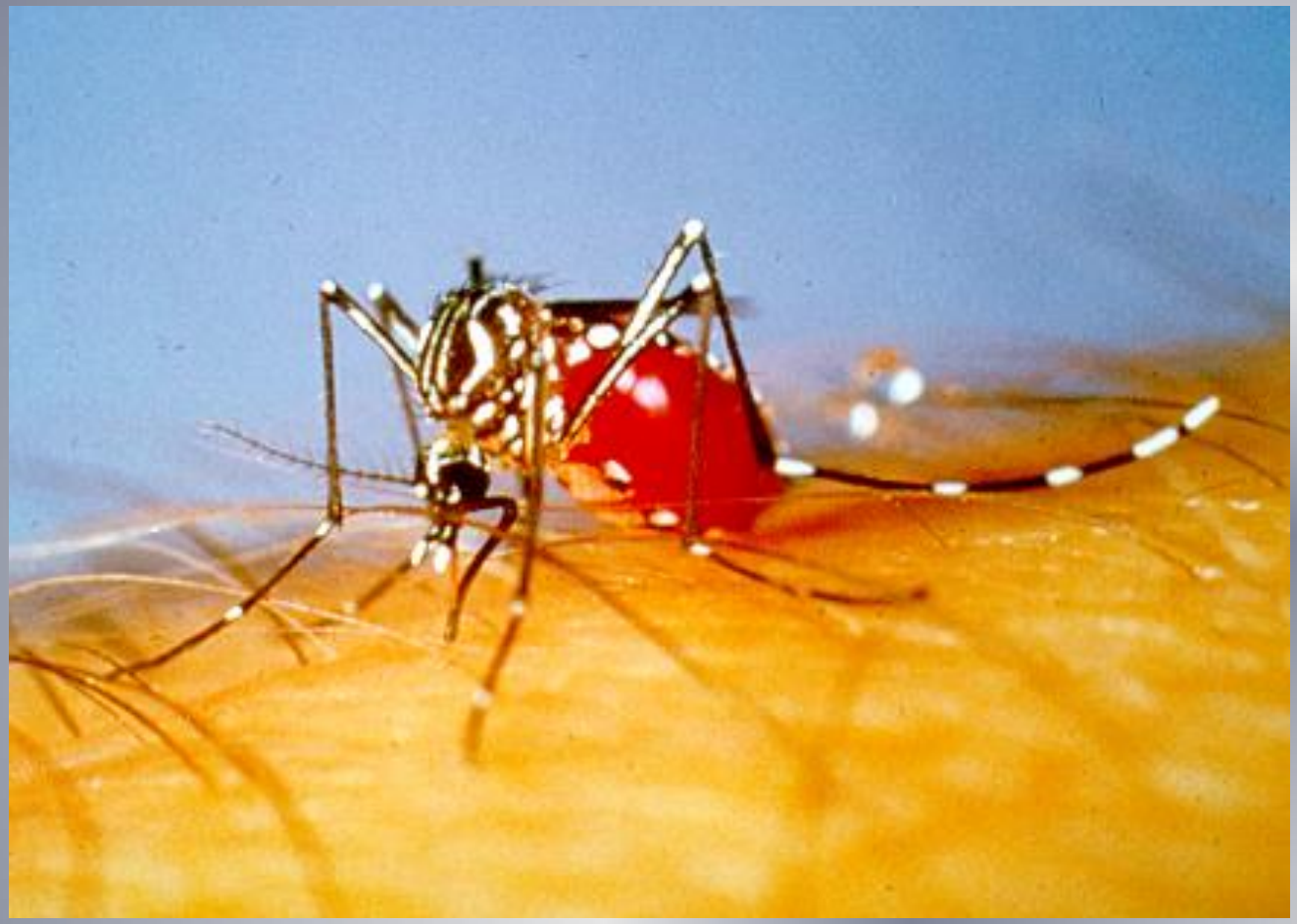


Figura 1.
Microfotografía de *Bacillus thuringiensis* en microscopio electrónico de transmisión. Se muestra el cristal proteínico romboide compuesto de toxinas Cry y una espora en proceso.

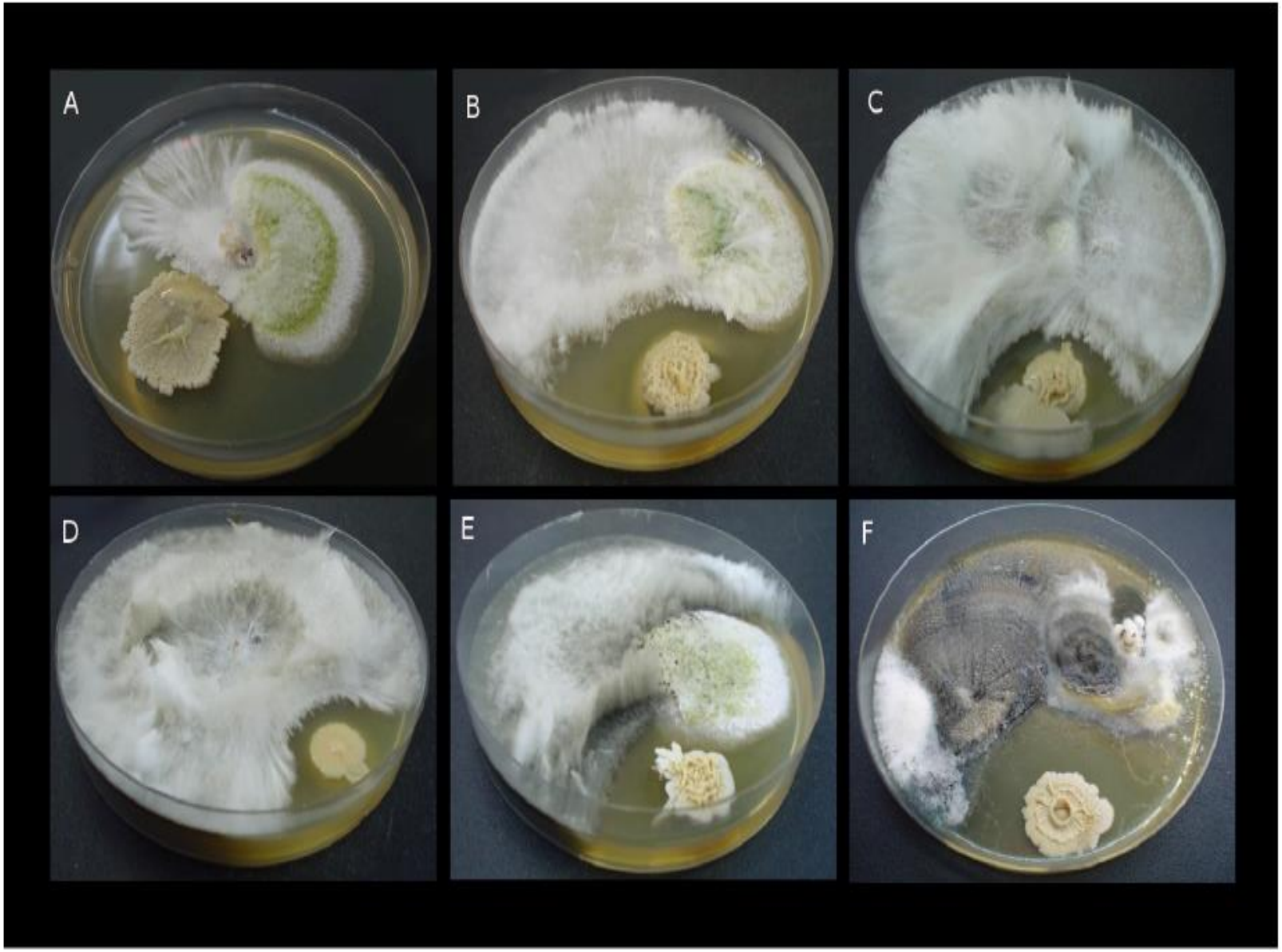
Biolarvicida VECTOGREEN

Mosquito *Aedes aegypti*



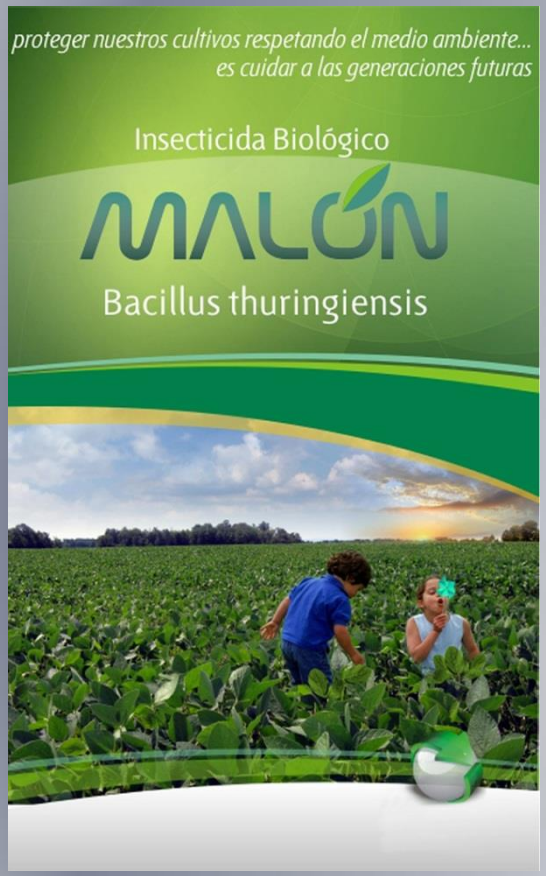
BIOLARVICIDA VECTOGREEN

BIOFUNGUICIDA LÍMITE



MALÓN LÍMITE BIOCROP

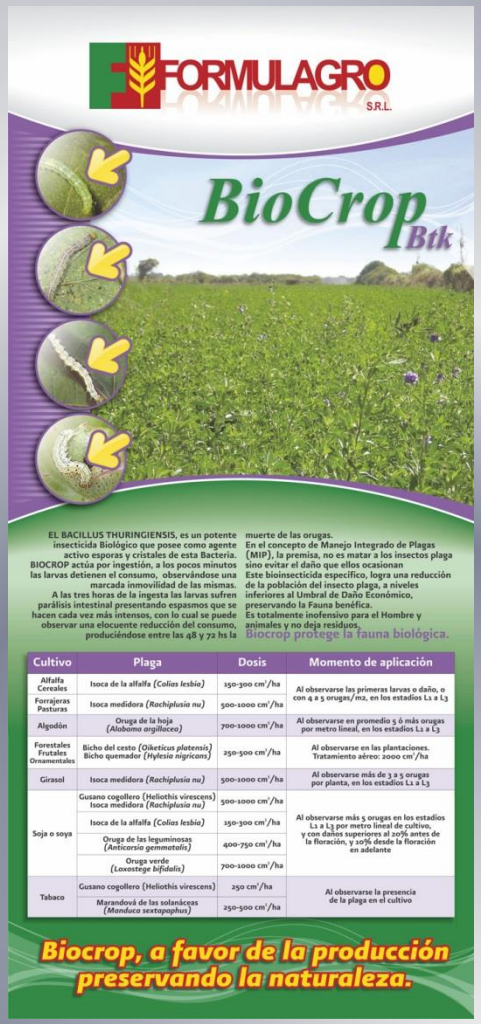
Bioinsecticida Promotor de crecimiento Biofunguicida



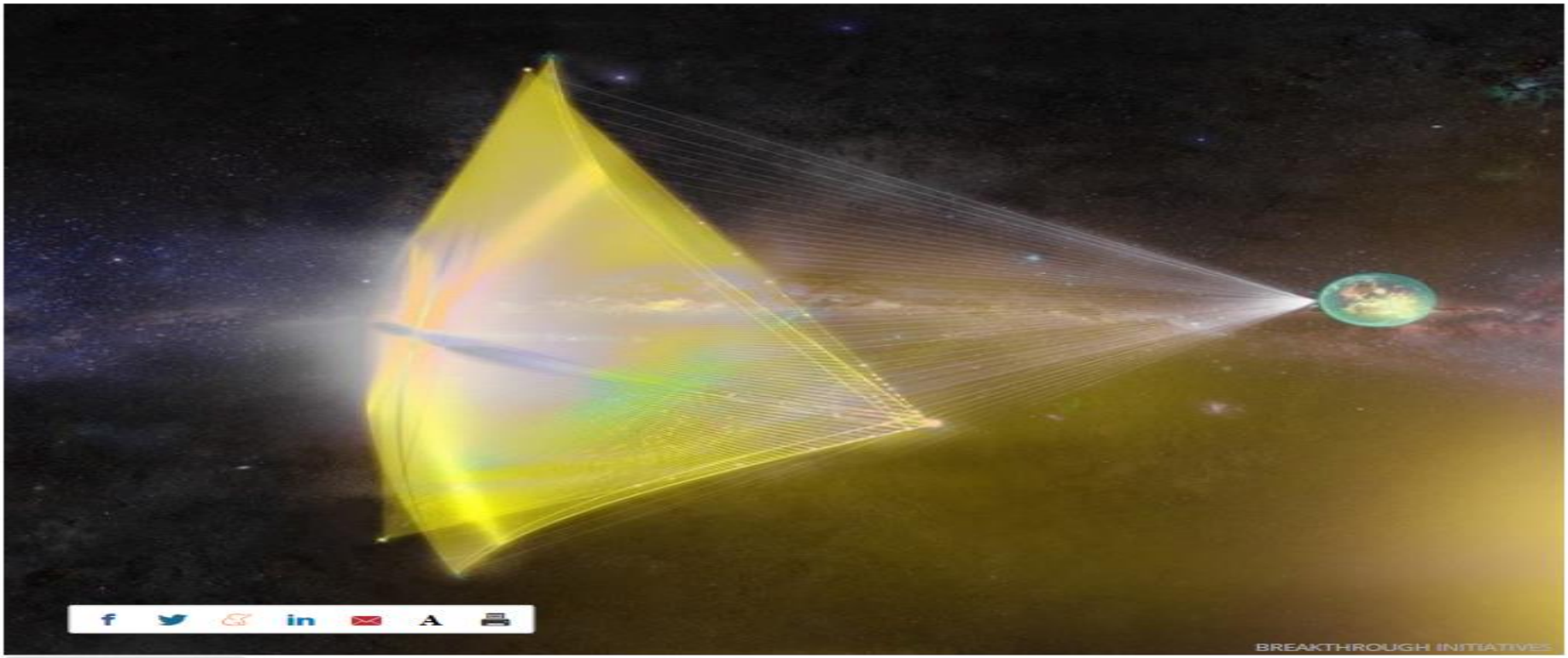
limite

limite
Bacillus subtilis
Evolución Tecnológica con máximo perfil ecológico

PGPR



Se estudia enviar bacterias a las estrellas para probar la panspermia



Así lo ha asegurado el físico de la Universidad de Hawai Jeff Kuhn, asesor de **Breaktrough Starshot**, iniciativa que trabaja en desarrollar tecnología para acelerar pequeñas naves espaciales a vela hasta un 20 por ciento de la velocidad de la luz, usando potentes láseres. Su destino sería el planeta Proxima b, **tras un viaje de 20 años.**

"Creo que sería divertido colocar en una de estas naves una pequeña colonia de Bacillus, enviarla durante 20 años, encenderla, darle algunos nutrientes y ver si todavía está viva, solo para decidir experimentalmente si la panspermia funciona sobre distancias interestelares", dijo Kuhn durante una conferencia celebrada en Stanford por Breaktrough Discuss, **informa Space.com**.

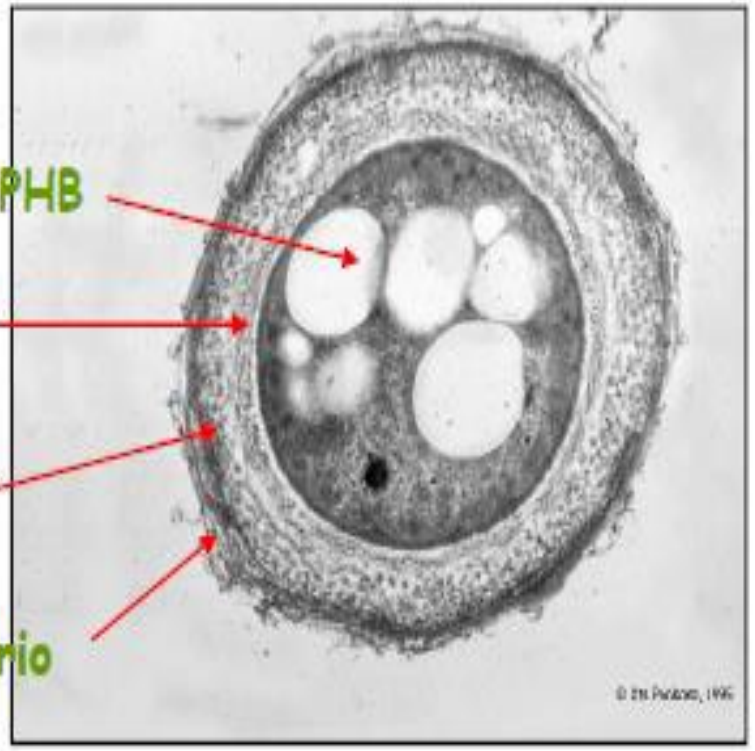
ESTRUCTURA BACTERIANA

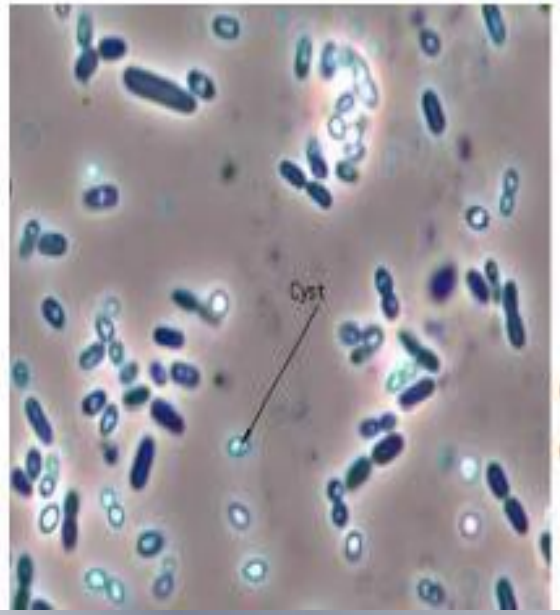
OTROS TIPOS DE ESTRUCTURAS BACTERIANAS

QUISTES O CISTOS

endosporas).

Ej. Quistes de *Azotobacter*



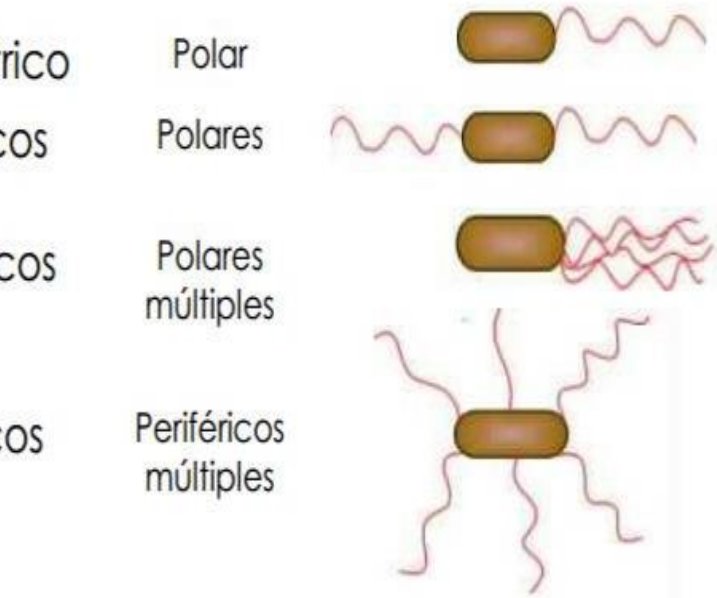


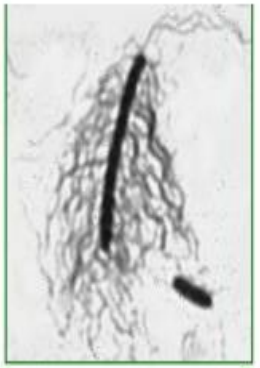
Quistes y células de *Azotobacter vinelandii* a 1000 X. Los quistes son los mas brillantes y las células las mas oscuras.

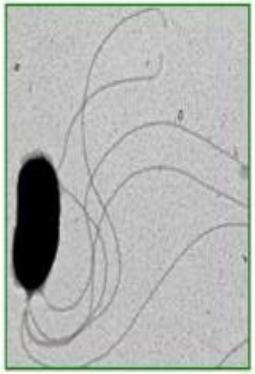
APÉNDICES BACTERIANOS

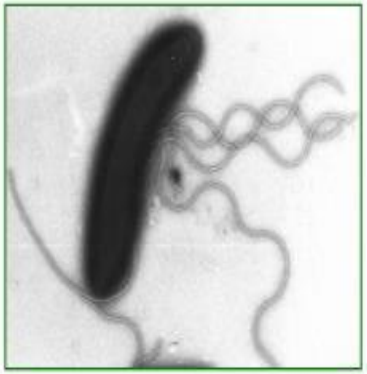
Flagelos localización y número

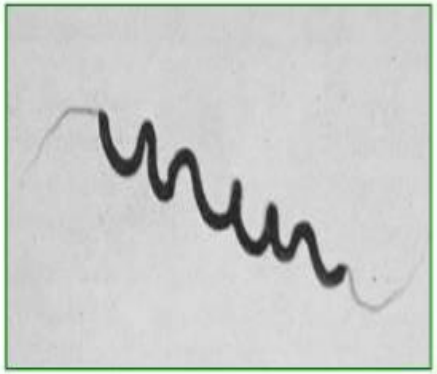
- Monótrico — Polar
- Anfítricos — Polares
- Lofótricos — Polares múltiples
- Perítricos — Periféricos múltiples



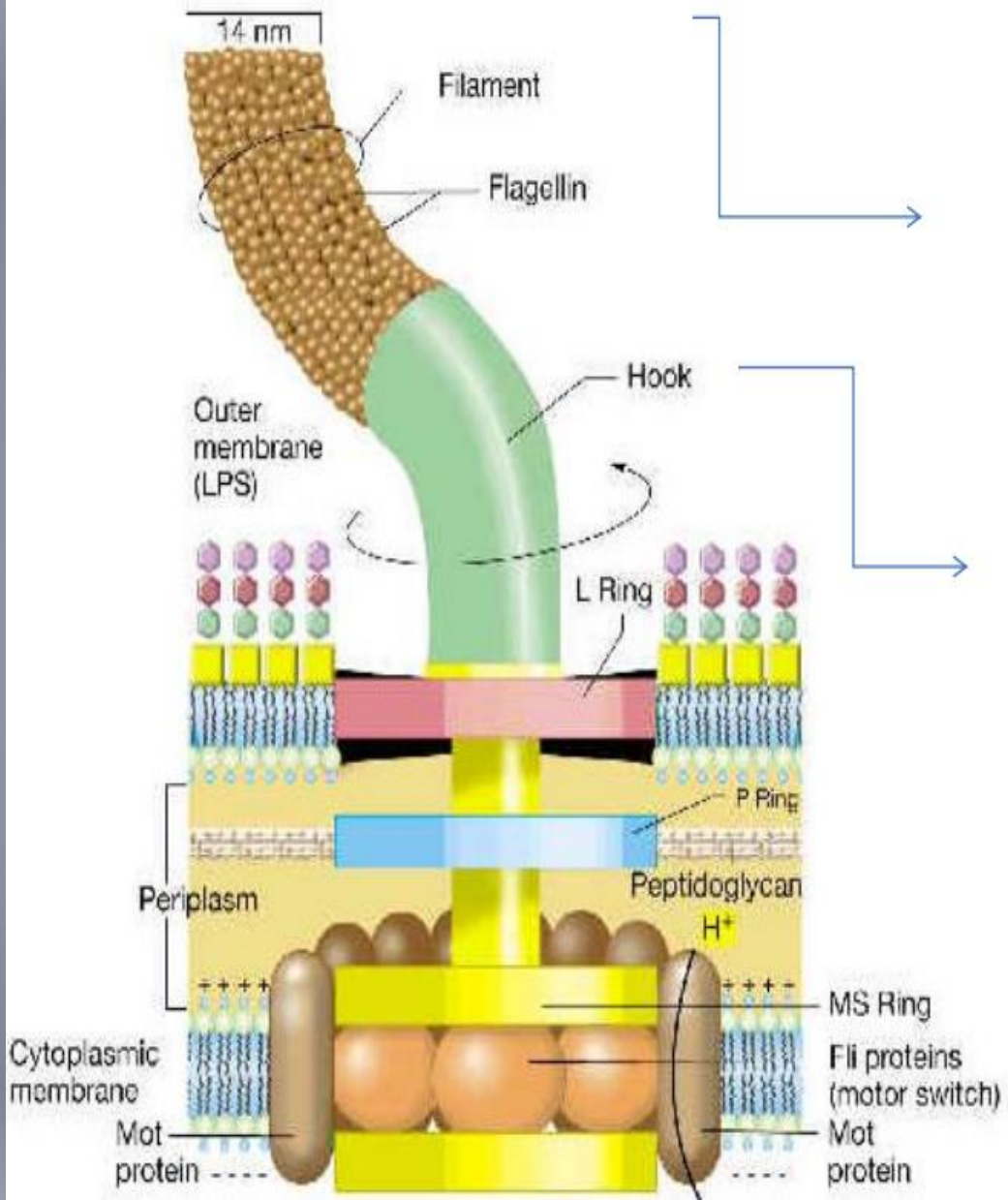








Estructura

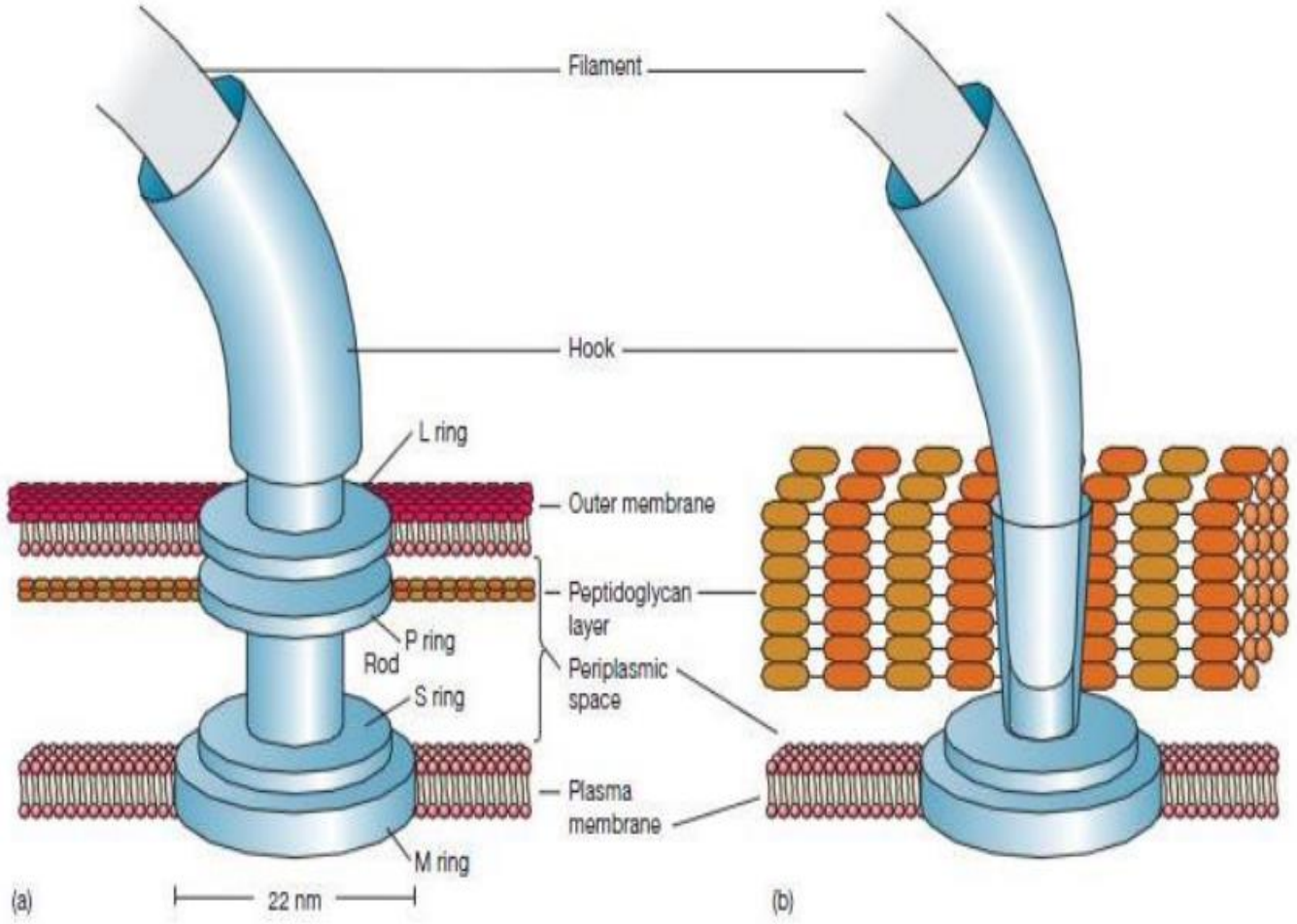


Filamento

Gancho o codo

Cuerpo basal

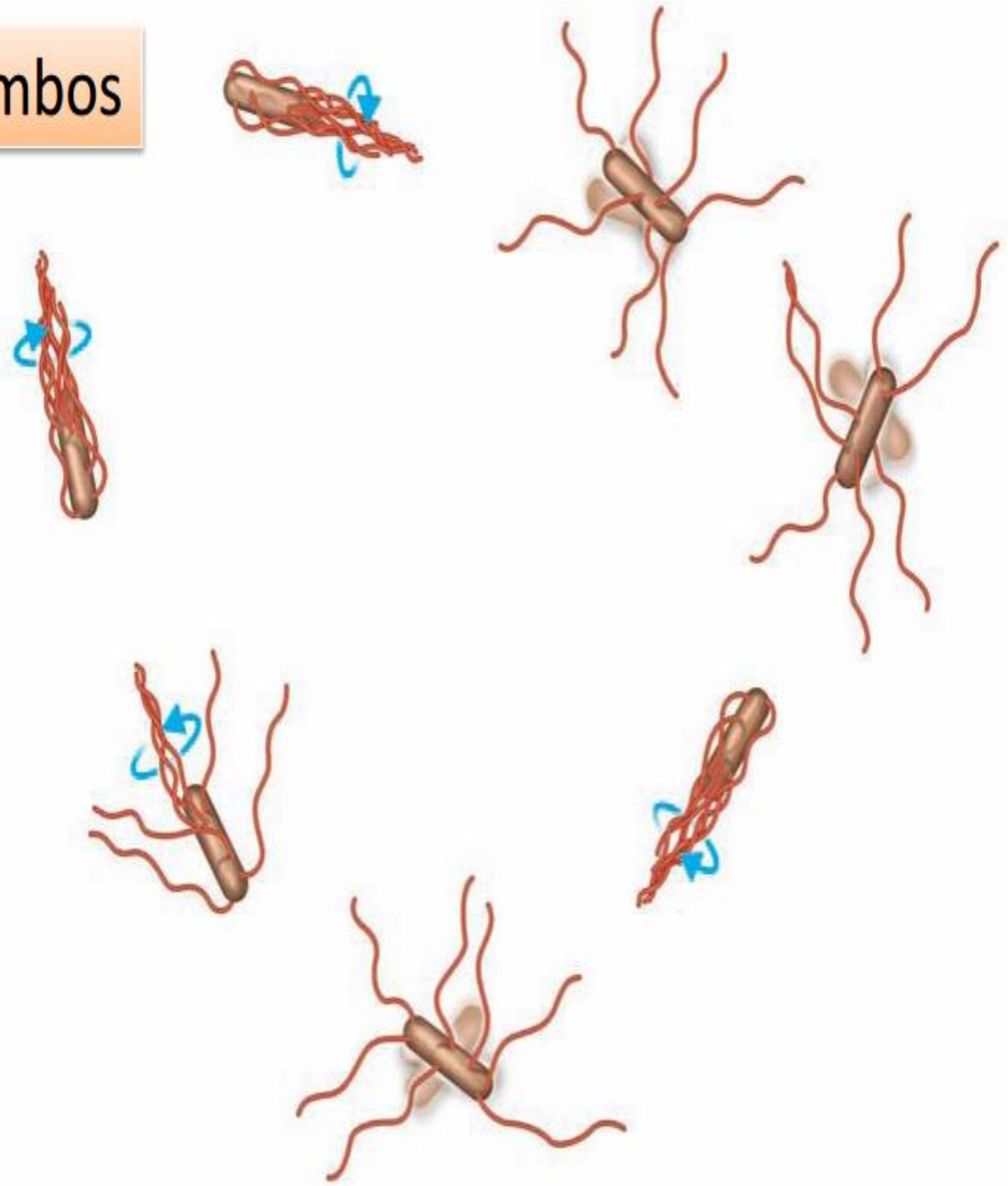
Diferencias entre G(+) y G (-)



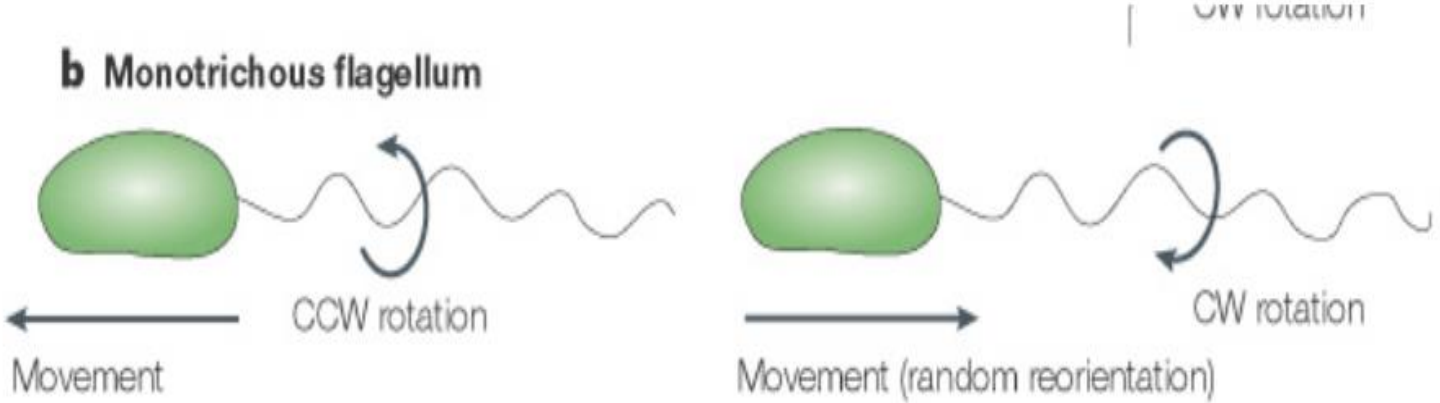
El flagelo se encuentra anclado en la membrana citoplasmática y la pared celular.

el cuerpo basal esta formado esencialmente por dos pares de **discos o anillos**, el par externo (anillo L y anillo P) se encuentra a la altura de la pared y membrana externa atravesados por un cilindro. Cada letra hace referencia al sitio de localización del anillo

Nado y tumbos



Descripción del movimiento de bacterias monótricas

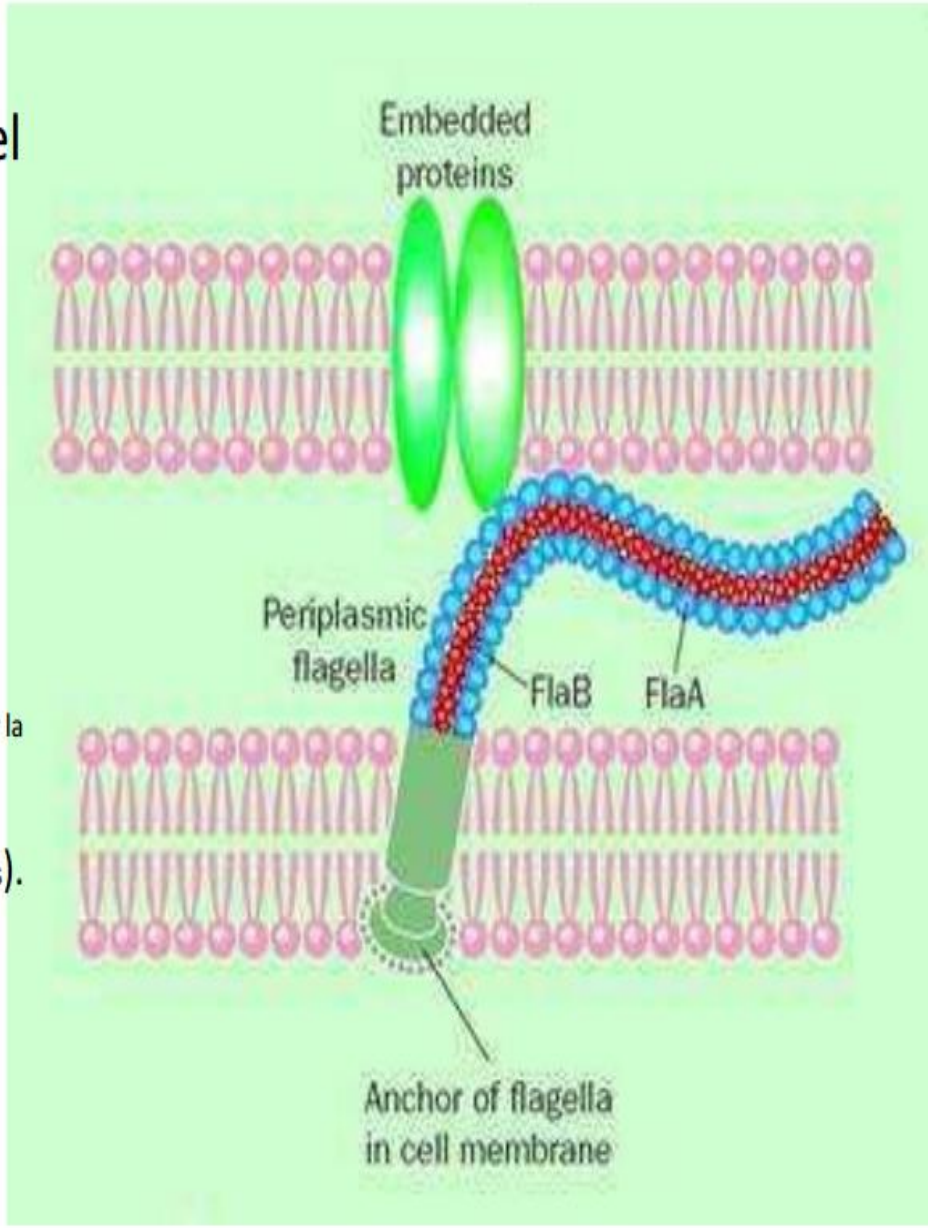


Flagelos periplásmicos

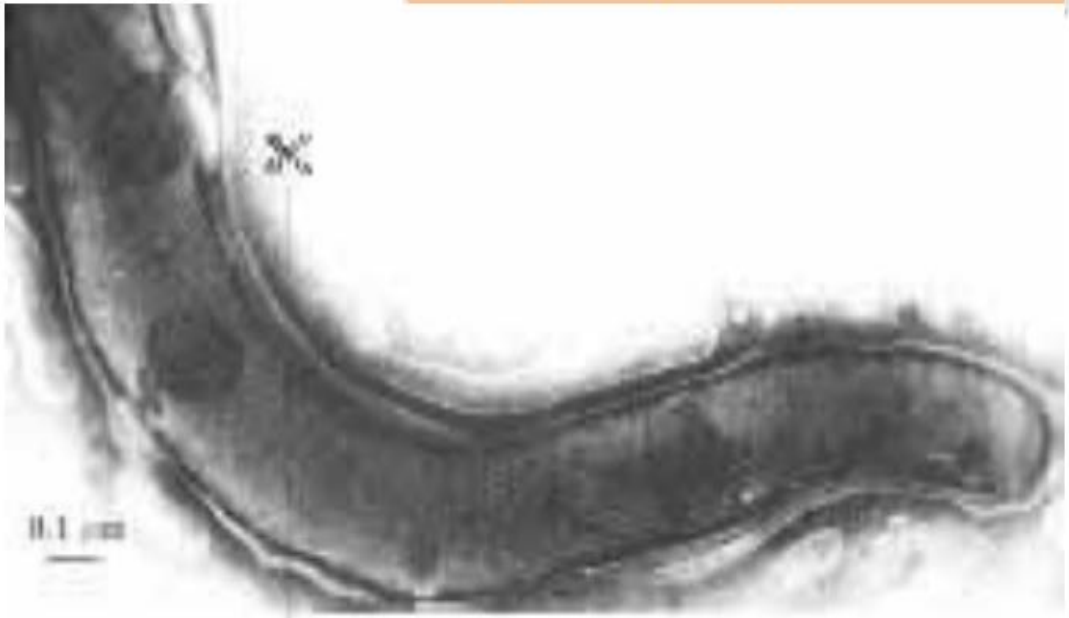
Presentes exclusivamente el grupo de las Espiroquetas. Estas bacterias Gram-negativas de forma helicoidal.

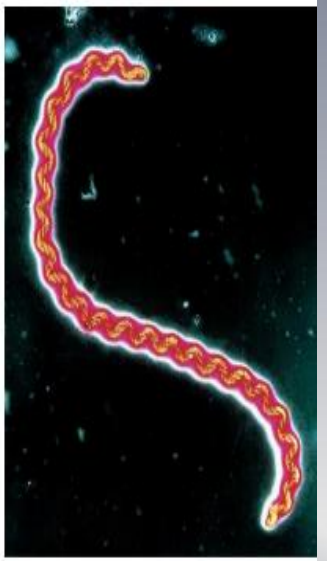
- no sobresale de la superficie celular
- Flagelos. Entre el cilindro protoplasmático y la membrana externa, insertados subpolarmente y enrollados alrededor del cilindro. Estos flagelos se denominan flagelos periplásmicos o filamentos axiales).

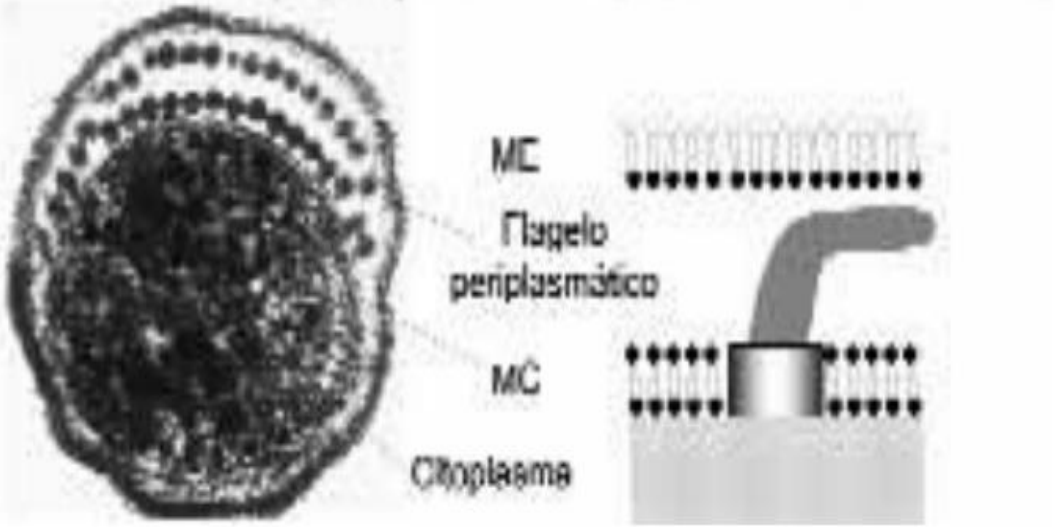
Tiene dos flagelos polares que se unen en el medio



Flagelos periplásmicos

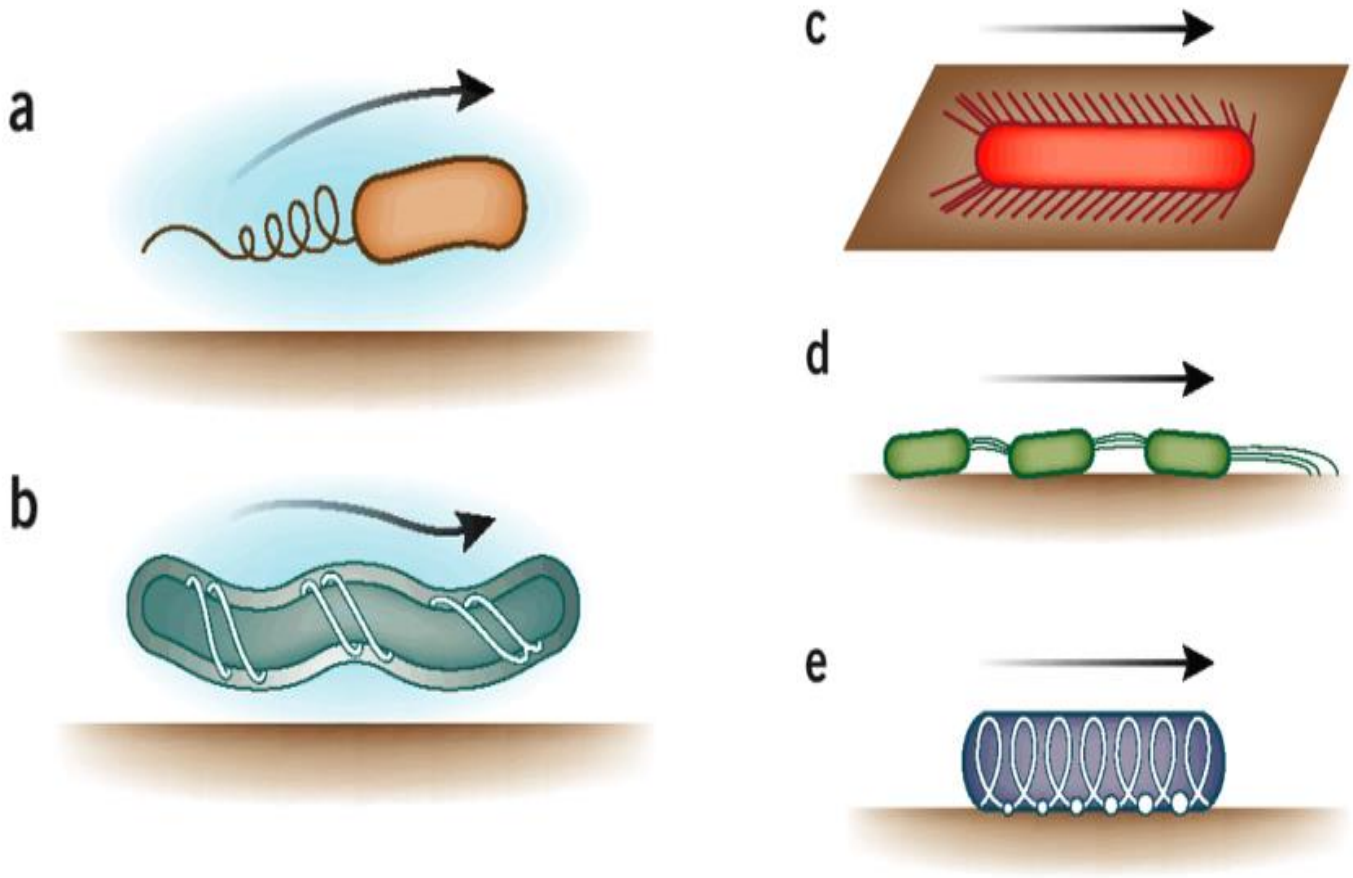




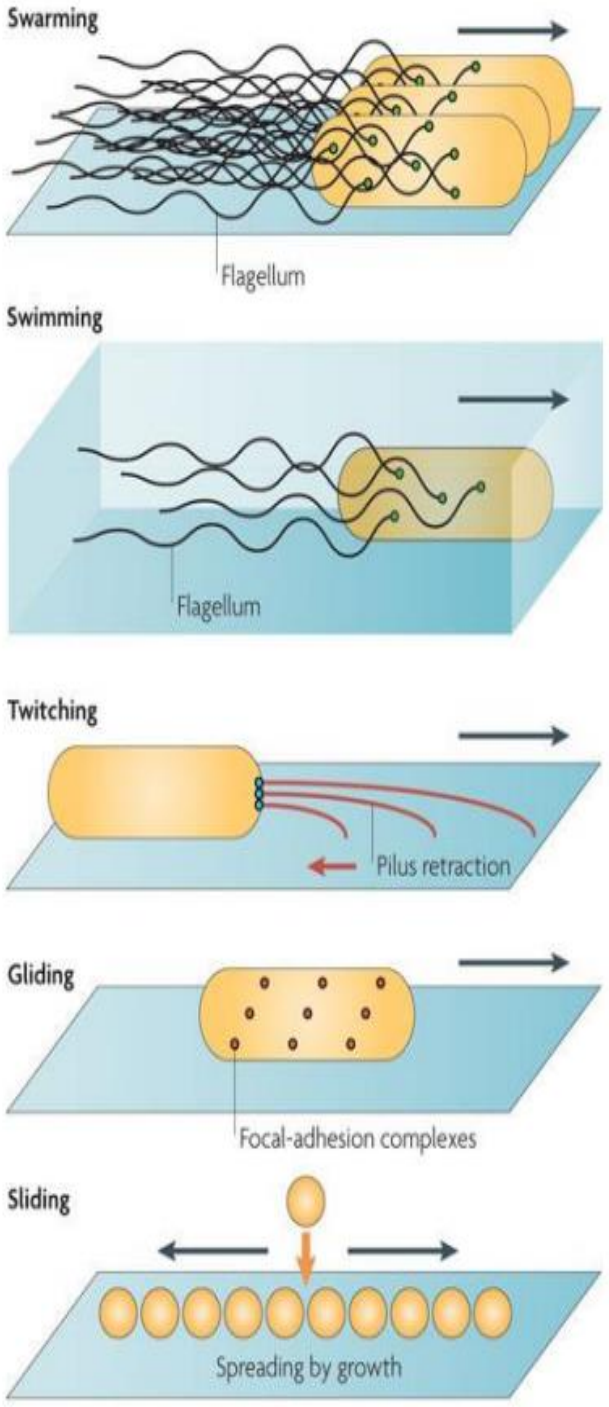


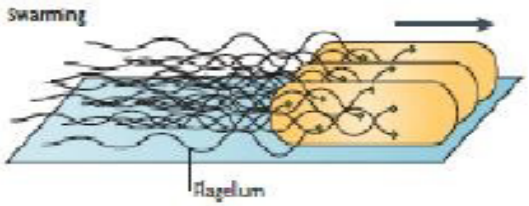
Cortes transversales de espiroquetas. ME: membrana externa; MC: membrana celular

Diferentes tipos de movilidad

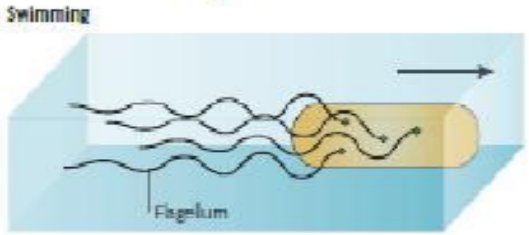


Sistemas de movimiento celular			
Tipo	Movimiento atribuido a..	Las células se mueven juntas	Las células se mueven aisladas
swarming	flagelo	+	
swimming	flagelo		+
gliding	Polisacárido? Actividad celular	+	
Twitching o gliding	Pili tipo IV, actividad celular		+
darting	Fuerzas de tensión en los agregados de células que las eyecta de la colonia	+	
sliding	Fuerza centrifuga que expande la colonia	+	

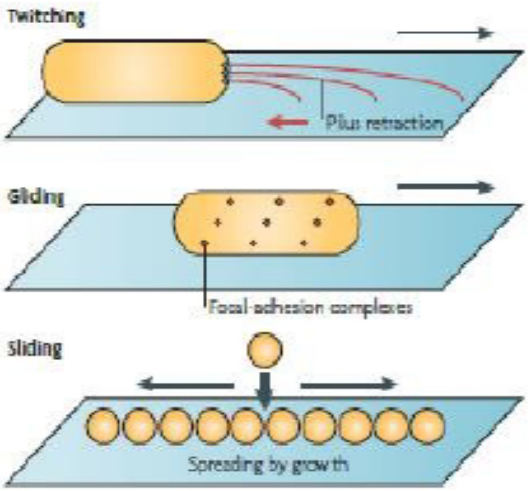




Swarming: Es un movimiento multicelular y coordinando de las bacterias a través de las superficies y es accionado por flagelos helicoidales.



Swimming: Es el movimiento de bacterias individuales en medio líquido, generalmente impulsado por rotación de flagelos.



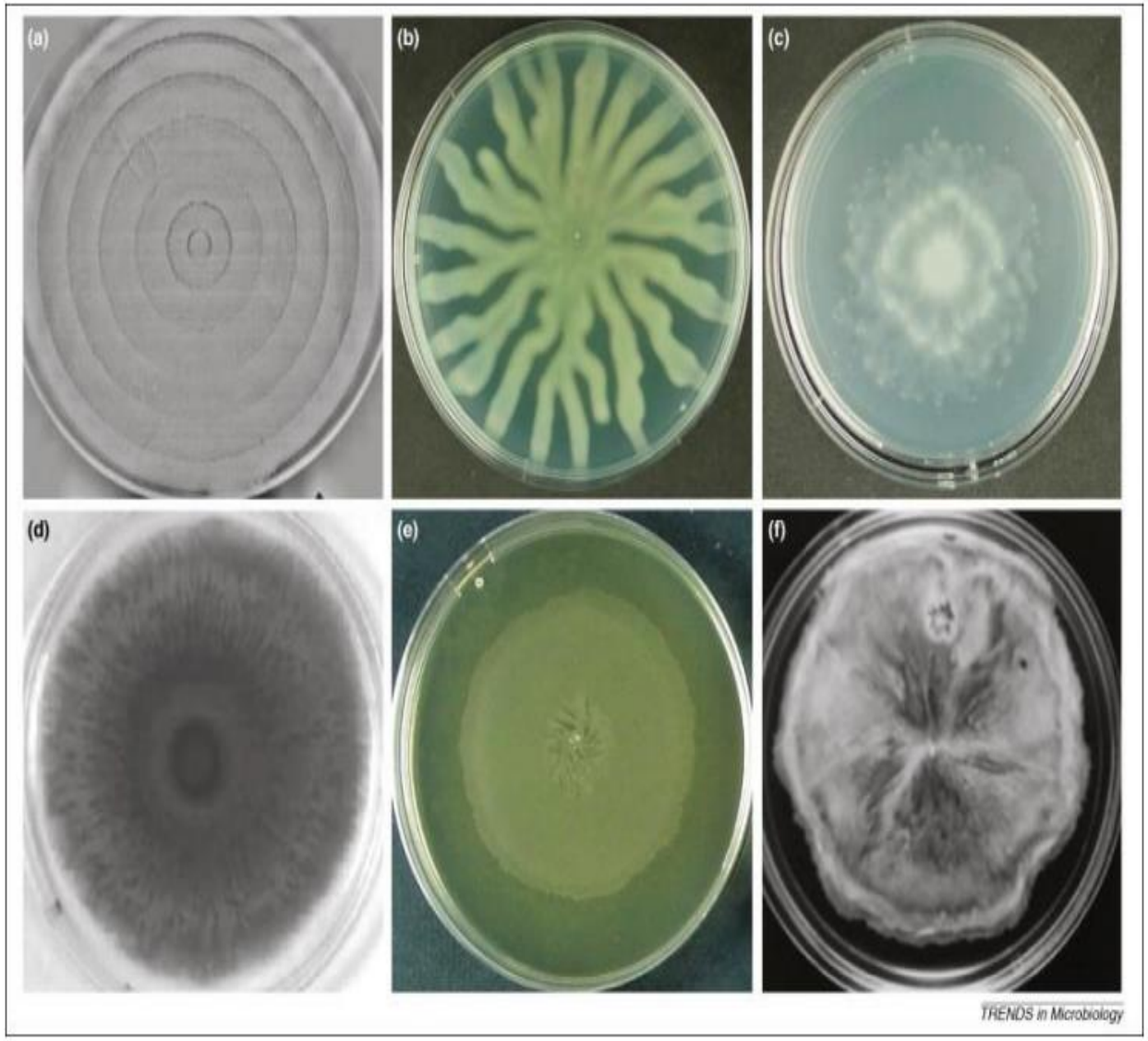
Twiching: Es un movimiento individual o social sobre superficies de las bacterias, accionado por extensiones de pili, que "ataca" las superficies y consecutivamente se contrae, impulsando la célula cerca del sitio que atacó.

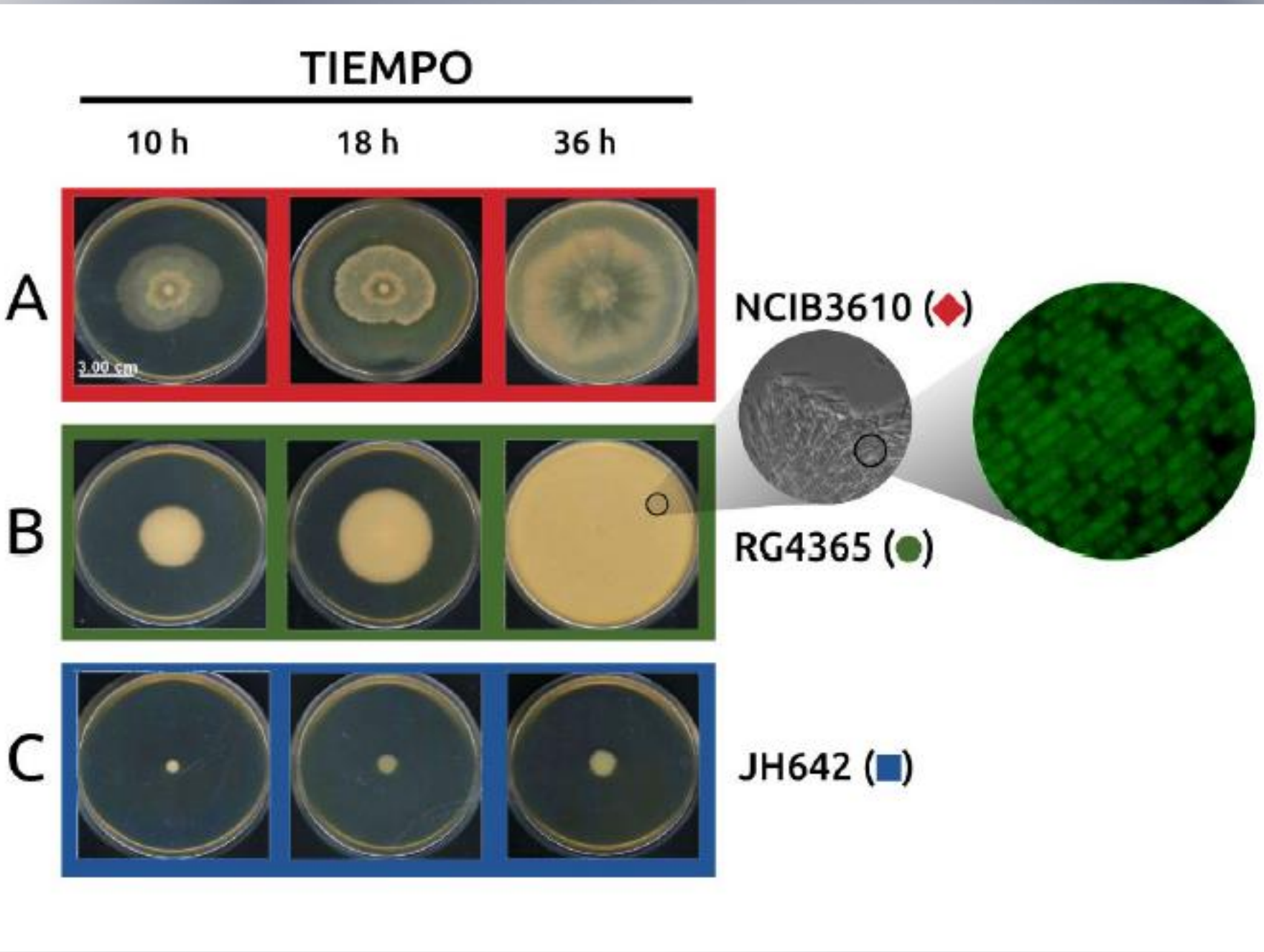
Gliding: es un movimiento individual o social "activo" sobre la superficie que no requiere ni flagelos ni pili que involucra complejos de adhesión focal y complejos proteicos de membrana que se deslizan sobre la superficie.

Sliding: es una translocación "pasiva" social o colectiva, sobre la superficie que sería impulsada por el crecimiento cooperativo de las células y facilitada por la producción de surfactante.

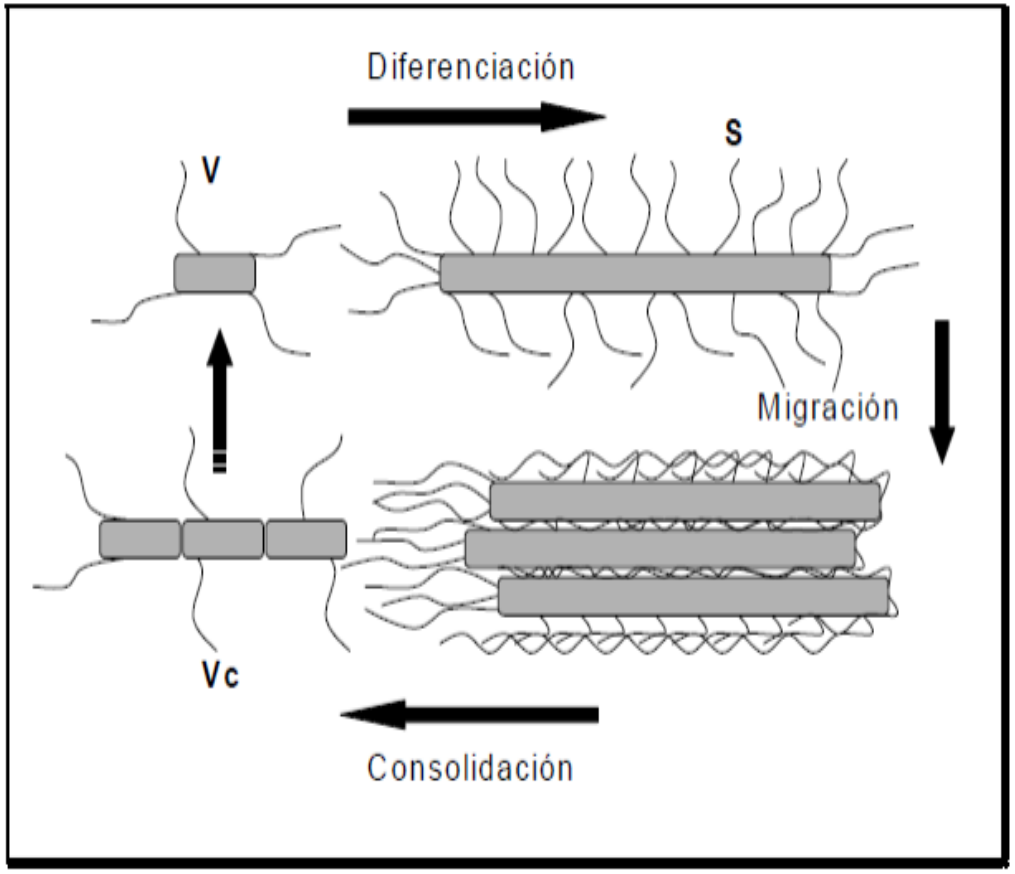
Swarming. Movimiento social

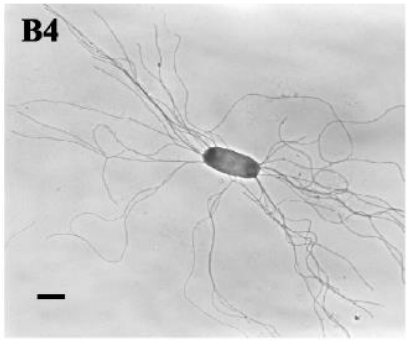
Swarming colonies
a) *P. mirabilis*
b) *P. aeruginosa*
c) *R. etli*
d) *S. marcescens*
e) *S. typhymurium*
f) *E. coli*




TIEMPO
10 h
18 h
36 h
A
B
C
3.00 cm
NCIB3610 (◆)
RG4365 (●)
JH642 (■)

Proteus mirabilis: ciclo de swarming





Fraser-Hughes, 1999. Curr Opin Microbiol 2(6)

Ultrastructure of *Proteus mirabilis* Swarmer Cell Rafts and Role of Swarming in Catheter-Associated Urinary Tract Infection

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Received 15 January 2004/Returned for modification 28 February 2004/Accepted 17 March 2004

Proteus mirabilis **is a common cause of catheter-associated urinary tract infection (C-UTI). It blocks indwelling urethral catheters through the formation of extensive crystalline biofilms. The obstruction of urine flow can induce episodes of pyelonephritis, septicemia, and shock. *P. mirabilis* exhibits a type of motility referred to as swarming, in which multicellular rafts of elongated, hyperflagellated swarmer cells form and move rapidly in concert over solid surfaces. It has been suggested that swarming is important in the pathogenesis of C-UTI. In this study we generated a set of stable transposon mutants deficient in swarming and used them to assess the role of swarming in the migration of *P. mirabilis* over urinary catheters. Swarming was found to be essential for migration over all-silicone catheters. Swarming-deficient mutants were attenuated in migration over hydrogel-coated latex catheters, but those capable of swimming motility were able to move over and infect these surfaces. A novel vapor fixation technique for the preparation of specimens and scanning electron microscopy were used to resolve the ultrastructure of *P. mirabilis* multicellular rafts. The flagellar filaments of *P. mirabilis* were found to be highly organized during raft migration and were interwoven in phase to form helical connections between adjacent swarmer cells. Mutants lacking these novel organized structures failed to swarm successfully. We suggest that these structures are important for migration and formation of multicellular rafts. In addition, the highly organized structure of multicellular rafts enables *P. mirabilis* to initiate C-UTI by migration over catheter surfaces from the urethral meatus into the bladder.**

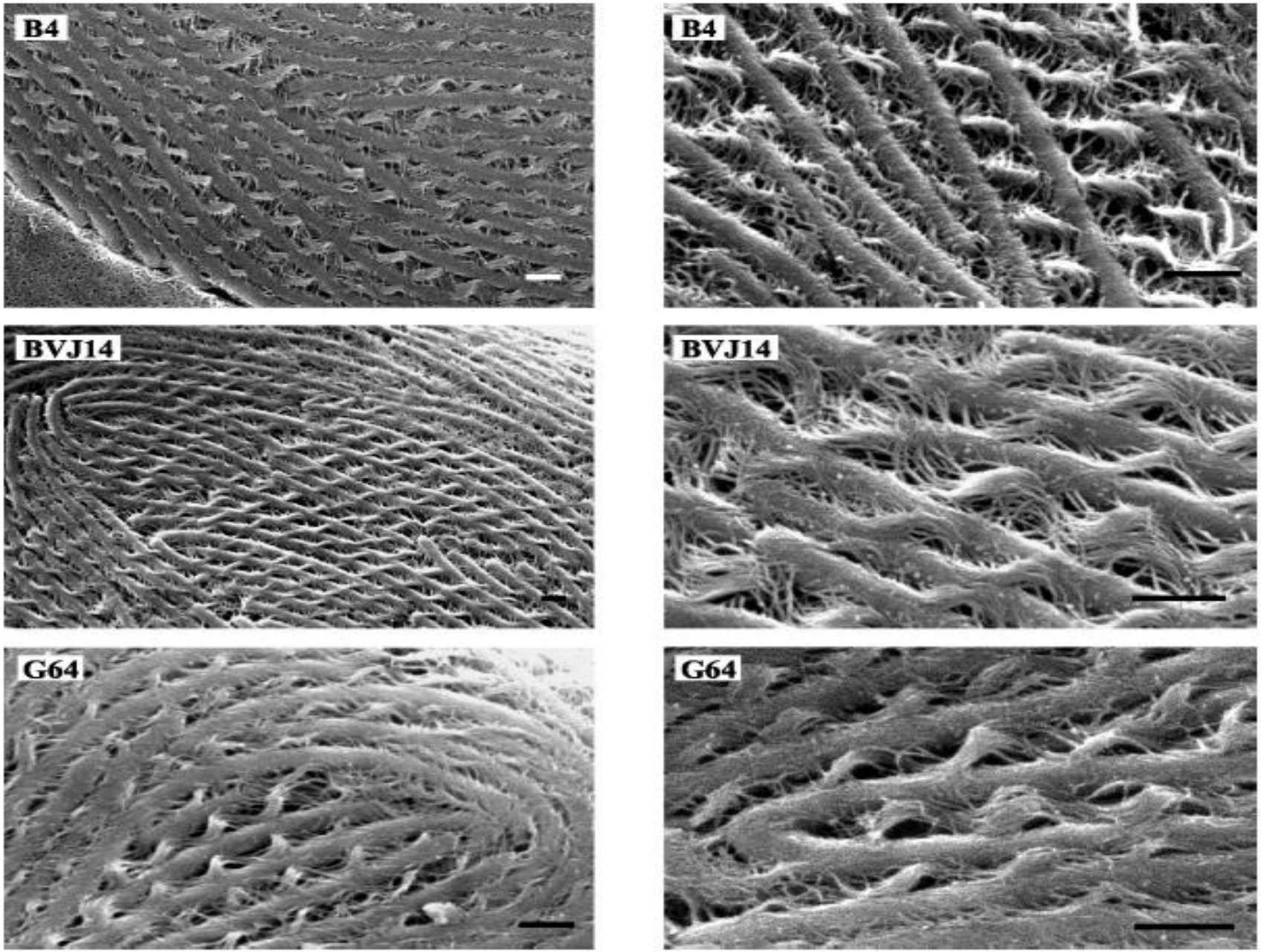


FIG. 3. SEM of in situ vapor-fixed swarm fronts of wild-type *P. mirabilis* and transposon mutants exhibiting helical connections. Strains: B4, wild type, BVJ14, transposon-containing control mutant, G64, poor-swarming mutant. Bars = ~1 μm.

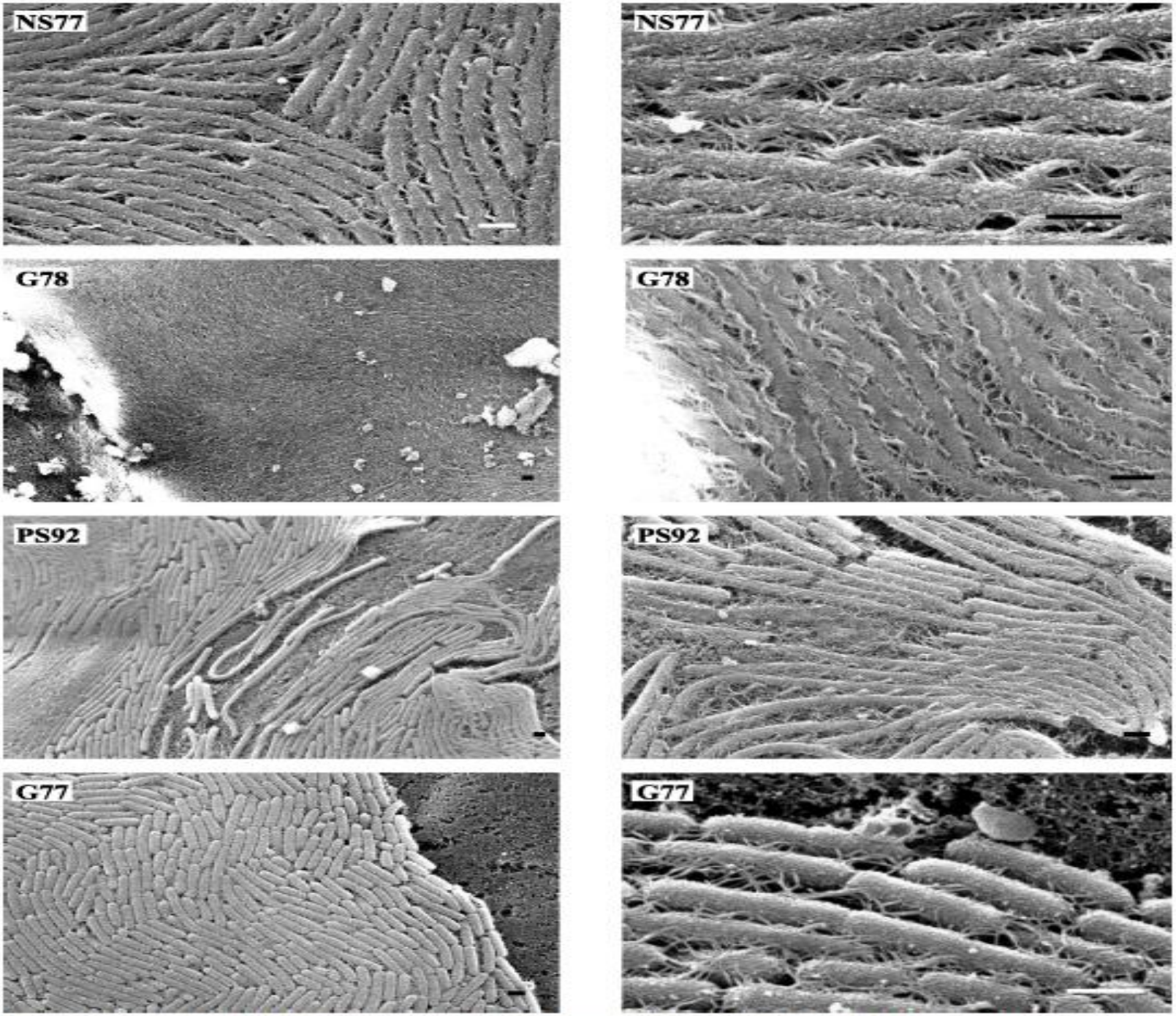


FIG. 4. SEM observations of in situ vapor-fixed swarm fronts of poor-swarming mutants exhibiting abnormal helical connections. Bars = ~1

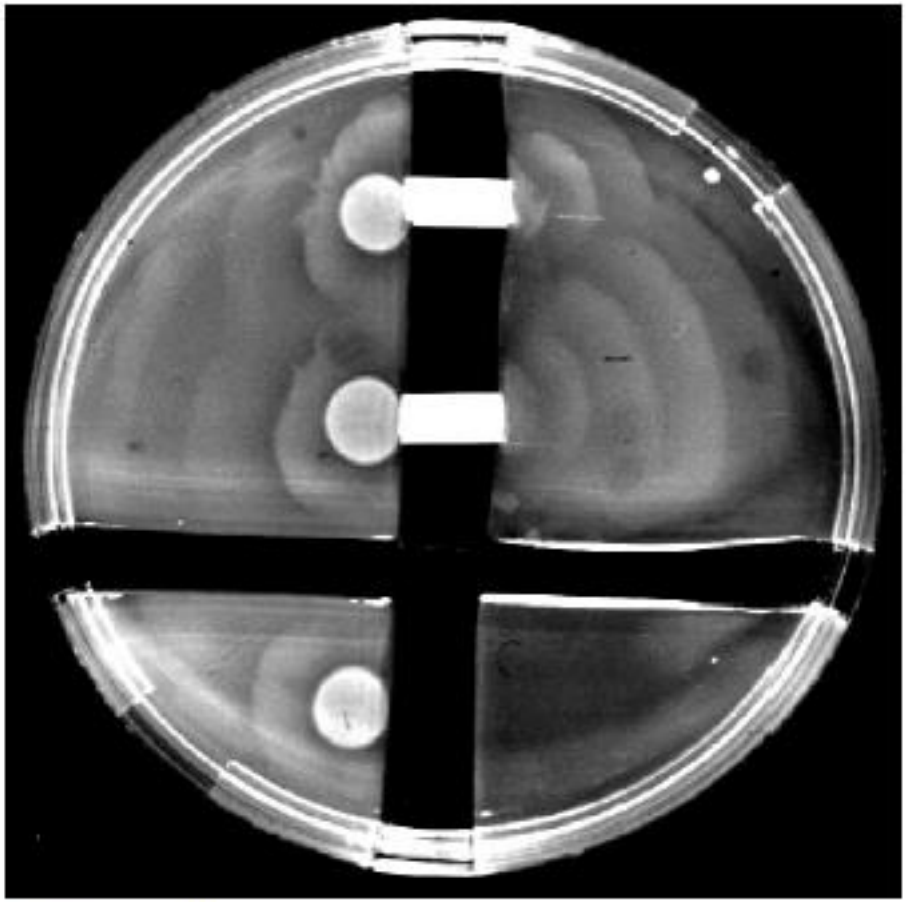


FIG. 1. *P. mirabilis* migrating over 1-cm sections of hydrogel-coated latex catheter.

From Catheter to Kidney Stone: The Uropathogenic Lifestyle of *Proteus mirabilis*

Allison N. Norsworthy[1] and Melanie M. Pearson[1,2,3,*]

Proteus mirabilis **is a model organism for urease-producing uropathogens. These diverse bacteria cause infection stones in the urinary tract and form crystalline biofilms on indwelling urinary catheters, frequently leading to polymicrobial infection. Recent work has elucidated how *P. mirabilis* causes all of these disease states. Particularly exciting is the discovery that this bacterium forms large clusters in the bladder lumen that are sites for stone formation. These clusters, and other steps of infection, require two virulence factors in particular: urease and MR/P fimbriae. Highlighting the importance of MR/P fimbriae is the cotranscribed regulator, MrpJ, which globally controls virulence. Overall, *P. mirabilis* exhibits an extraordinary lifestyle, and further probing will answer exciting basic microbiological and clinically relevant questions.**

Introduction to *Proteus mirabilis*

P. mirabilis is a gastrointestinal (GI) organism notorious for causing catheter-associated urinary tract infections (**CAUTIs**, see Glossary) and urinary stones. It is a model organism for urease$^+$ bacteria, a phylogenetically diverse group of bacteria encoding the virulence factor urease, that often form resilient crystalline biofilms on catheters and potentially deadly stones in the urinary system. Recent studies have elucidated key steps in *P. mirabilis*'*s* virulence lifestyle and the intracellular regulatory mechanisms that control these stages of infection. The combination of this new information with previous foundational studies has shed light on how this organism, and perhaps other urease$^+$ bacteria, successfully initiates and establishes infection of the urinary system.

Trends

Proteus mirabilis is a model organism for urease$^+$ organisms that are associated with CAUTIs and infectious stones.

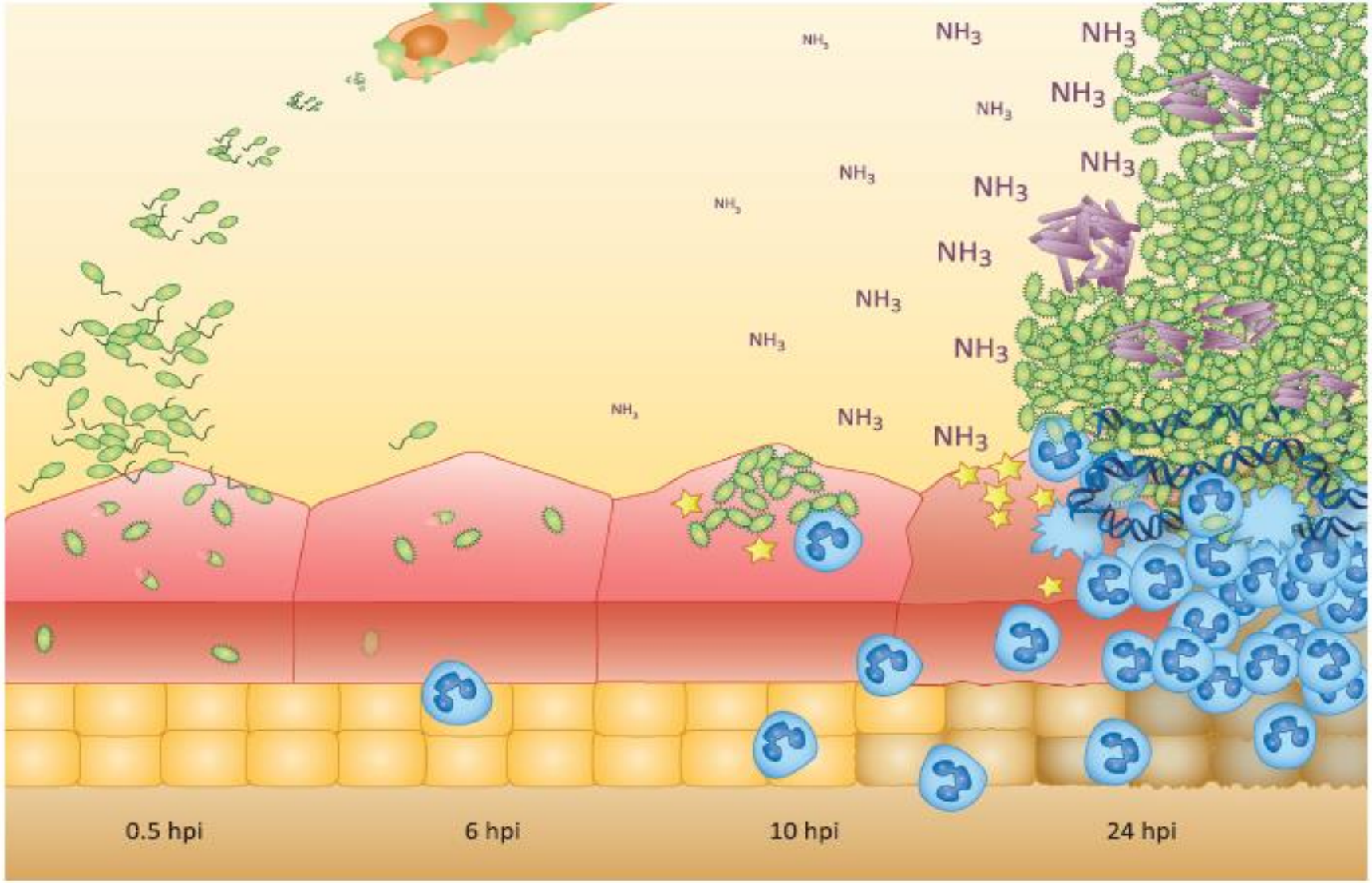
P. mirabilis forms extensive crystalline biofilms on catheters that are infamously difficult to treat.

Once in the bladder, *P. mirabilis* can form luminal clusters that are the site of bladder stone formation.

At least two virulence factors, urease and MR/P fimbriae, are required for both cluster formation and crystalline biofilm formation on catheters.

The MR/P regulator, MrpJ, controls a variety of other known and putative virulence factors.

Polymicrobial infections can lead to synergistic enhancement of UTI and may have clinically relevant consequences.



Trends in Microbiology

Figure 1. Lifestyle of *Proteus mirabilis*. *P. mirabilis* cells (green) form crystalline biofilms on the surface of catheters. Once inside the bladder [0.5–6 hours post-infection (hpi)], this organism can invade into urothelial cells of the bladder. As early as 10–24 hpi, *P. mirabilis* forms intraluminal clusters that can extend the length of the bladder and are associated with urothelial cell destruction [perhaps through the production of toxins (yellow stars) or an increase in urine pH] and mineral deposition (purple rods). Host innate immune cells such as neutrophils (blue) are recruited to the site of infection and can form NETs (neutrophil extracellular traps). Figure adapted, with permission, from Schaffer *et al.* [1].

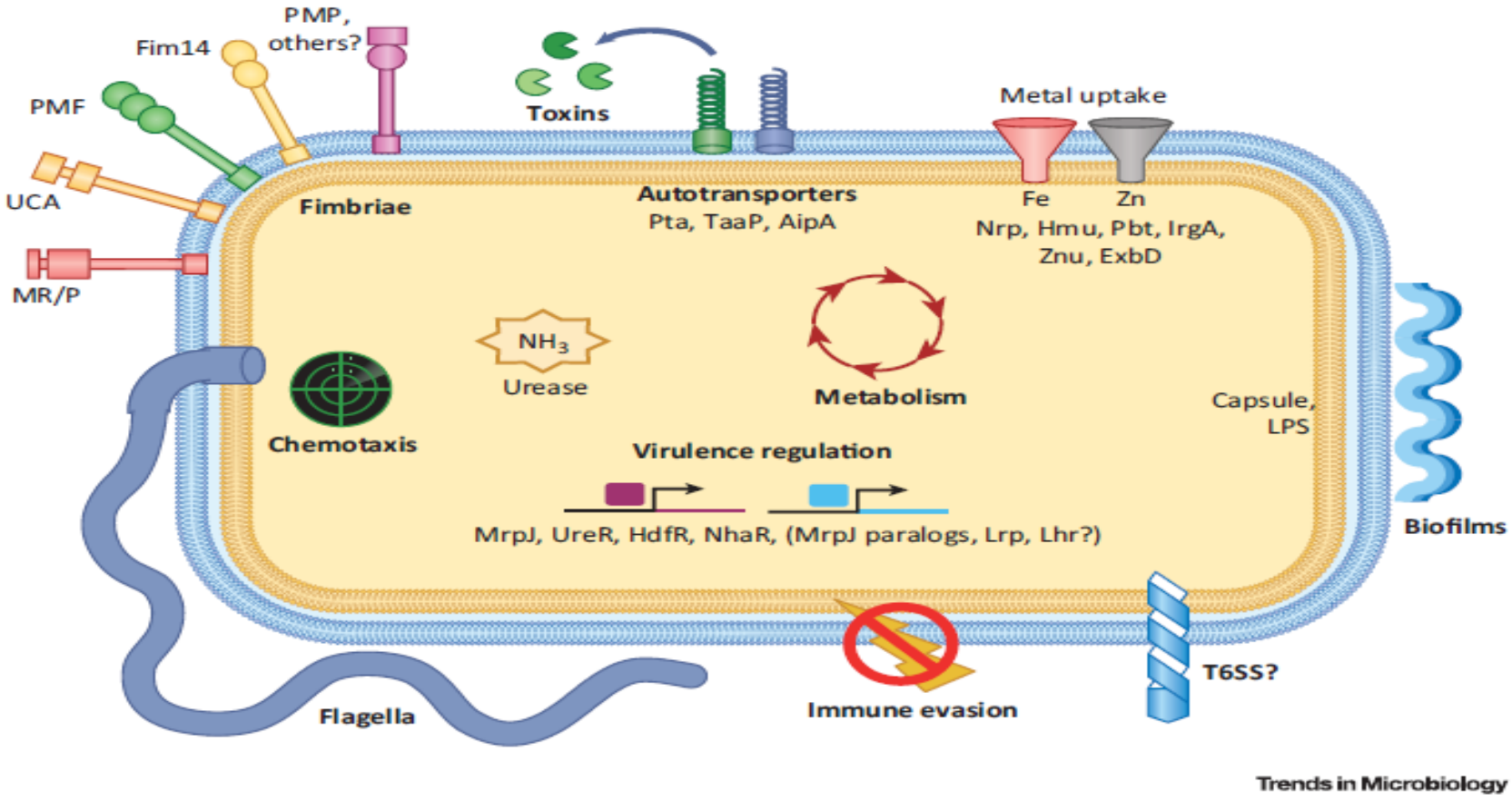


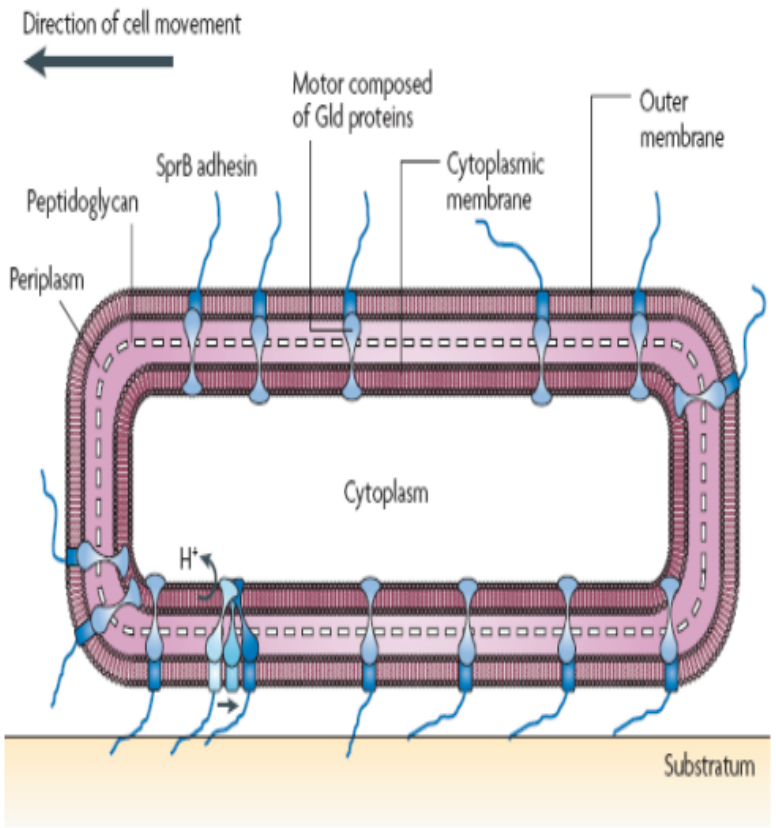
Figure 2. Concepts of *Proteus mirabilis* Pathogenesis during Urinary Tract Infection (UTI). Bolded classes of virulence factors have genes that are regulated by MrpJ. For more extensive information on these virulence factors, see references [21,22,99,100]. *Adherence:* binding catheters, host tissues, and neighboring bacteria may all contribute to disease. Adherence is mediated by chaperone-usher fimbriae and autotransporter adhesins. *Urease:* involved in stones, crystalline biofilms, and possibly nutrition or host sensing. *Motility: P. mirabilis* swarms across catheters and may ascend to the kidneys using swimming motility. Both forms of motion are mediated by flagella. Chemotaxis proteins allow the bacteria to follow chemical gradients. *Metabolism:* likely permits establishment of a nutritional niche, competition with other species, and response to host cues. *Metal scavenging:* iron and zinc uptake are essential for growth, but are sequestered by the host; therefore, specialized proteins are required for bacteria to scavenge these metals. *Toxins:* proteins such as HpmA and Pta may aid in nutrient accessibility, immune evasion, or provision of surfaces to colonize. *Biofilm formation:* Crystalline biofilms readily form on catheters, and bacterial clusters in the bladder may be a biofilm-mediated process. *Immune evasion:* this can include antibody and antimicrobial peptide degradation, polymyxin resistance, lipopolysaccharide (LPS) variation, and physical obstruction of phagocytosis. *Virulence regulation:* required to coordinate all steps of infection. MrpJ-controlled systems in this figure are bolded. *Type 6 secretion system (T6SS):* involved in self-recognition; unknown role during UTI.

Gliding o deslizamiento sin pili

Maquinaria Involucra 10 proteínas: formación de complejos de adhesión focal. SprB el componente móvil y Gld parte del motor

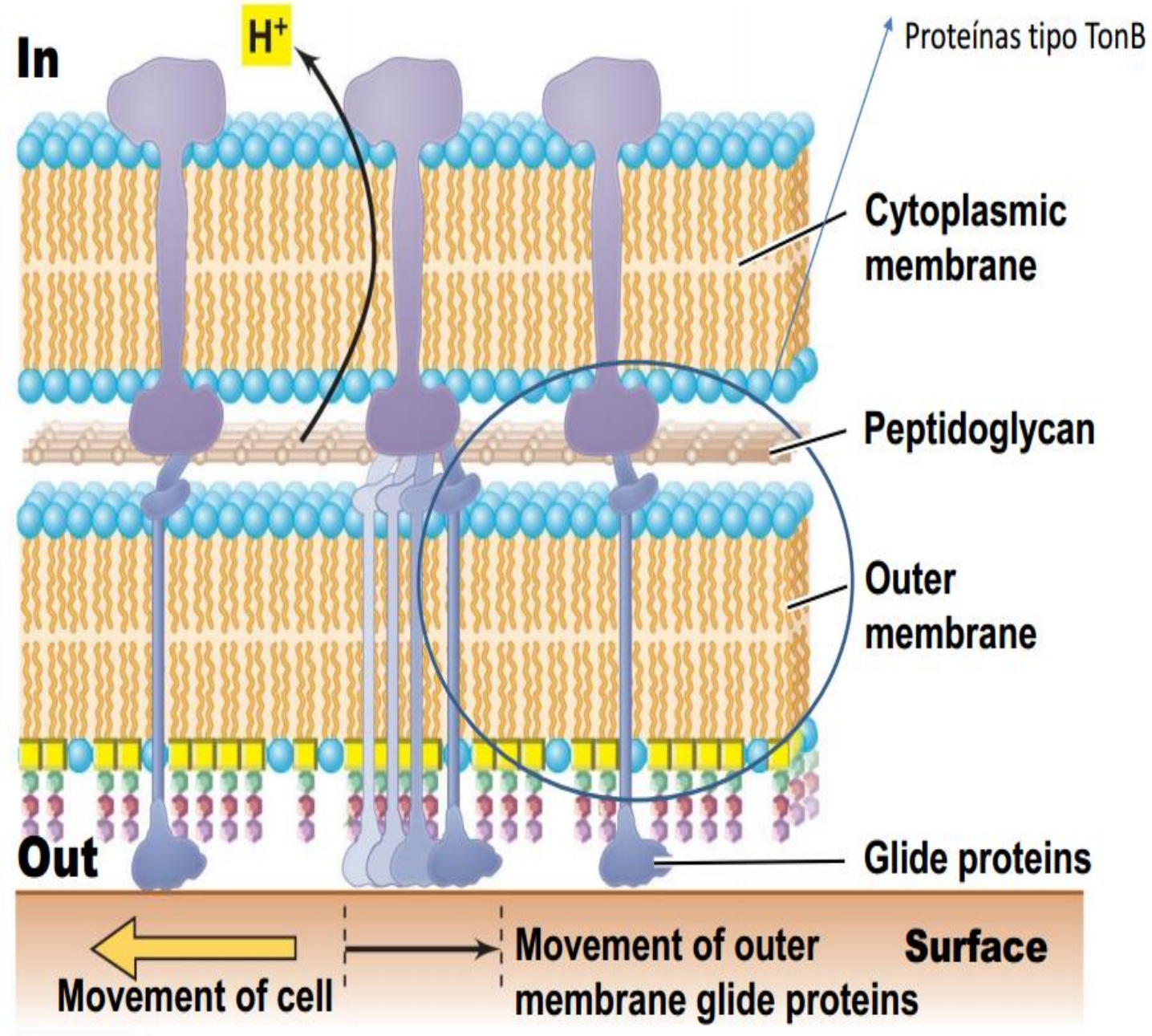
Movimiento de las proteínas citoplasmáticas por fuerza protón motriz..impulsa….

Movimiento lateral de las adhesinas de superficie

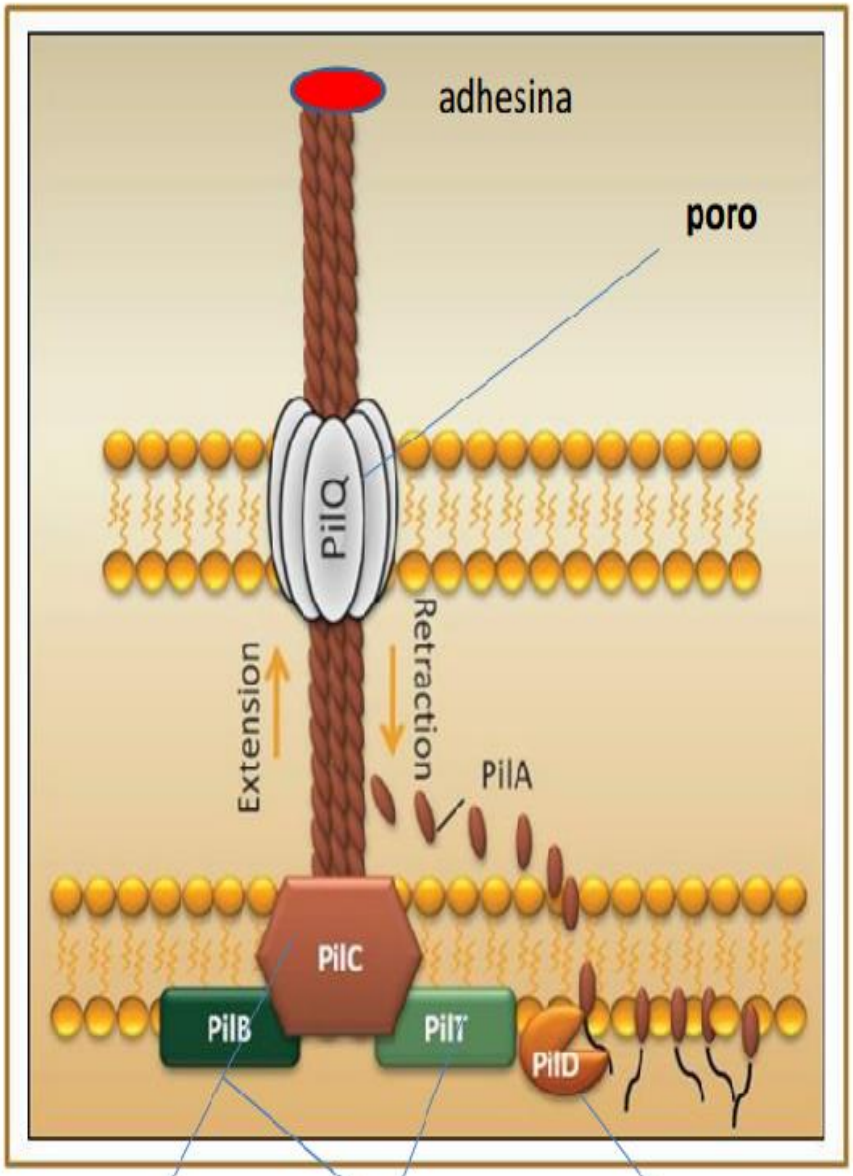


En *Flavobacterium* hay un movimiento de las proteínas (adhesinas) de la superficie de la célula

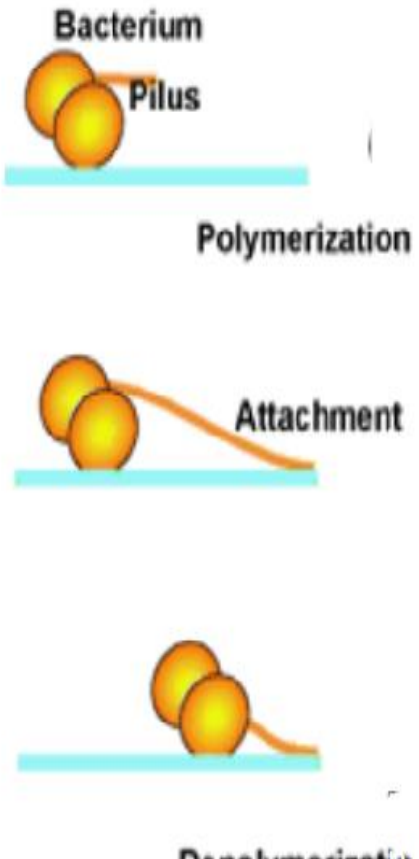
http://www.youtube.com/watch?v=0BuVarYDurA


In
H+
Proteínas tipo TonB
Cytoplasmic membrane
Peptidoglycan
Outer membrane
Out
Glide proteins
Movement of cell
Movement of outer membrane glide proteins
Surface

Organela de motilidad: Pili Tipo 4



Modo de propulsión



Apéndices y estructuras superficiales

- Flagelos (swimming y swarming)
- Estructura y composición química y ensamblaje
- Movimiento flagelar
- Las taxias , quimiotaxis y su base molecular
- Bacterias flageladas dispersivas (swarming)
- Flagelos periplásmicos de espiroquetas
- **Capsulas y limos**
- **Fimbrias (adhesión)**
- **Pilis (sexualidad y movillidad)**
- **Prostecas**
- **Tallos o pedúnculos**

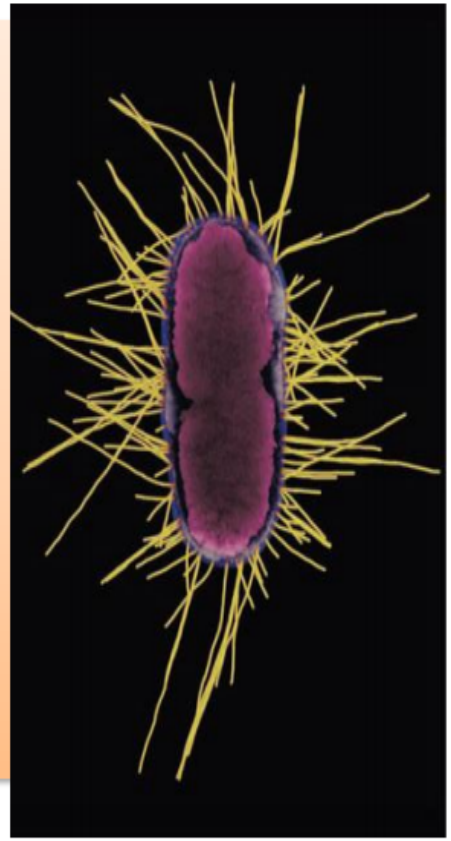
ADHESINAS: FIMBRIADAS O NO-FIMBRIADAS

Fimbrias y pilis

Existen dos tipos principales de *pili* (*pilus*, en singular):

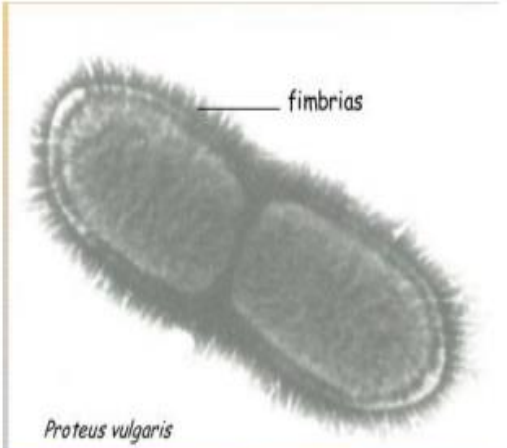
Fibrillas adhesivas=fimbrias adhesivas

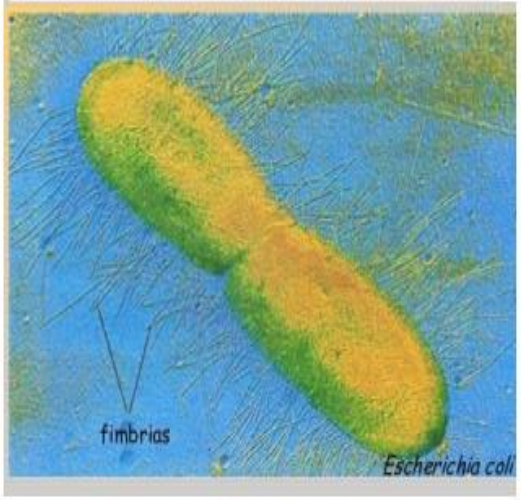
pelos sexuales y de motilidad



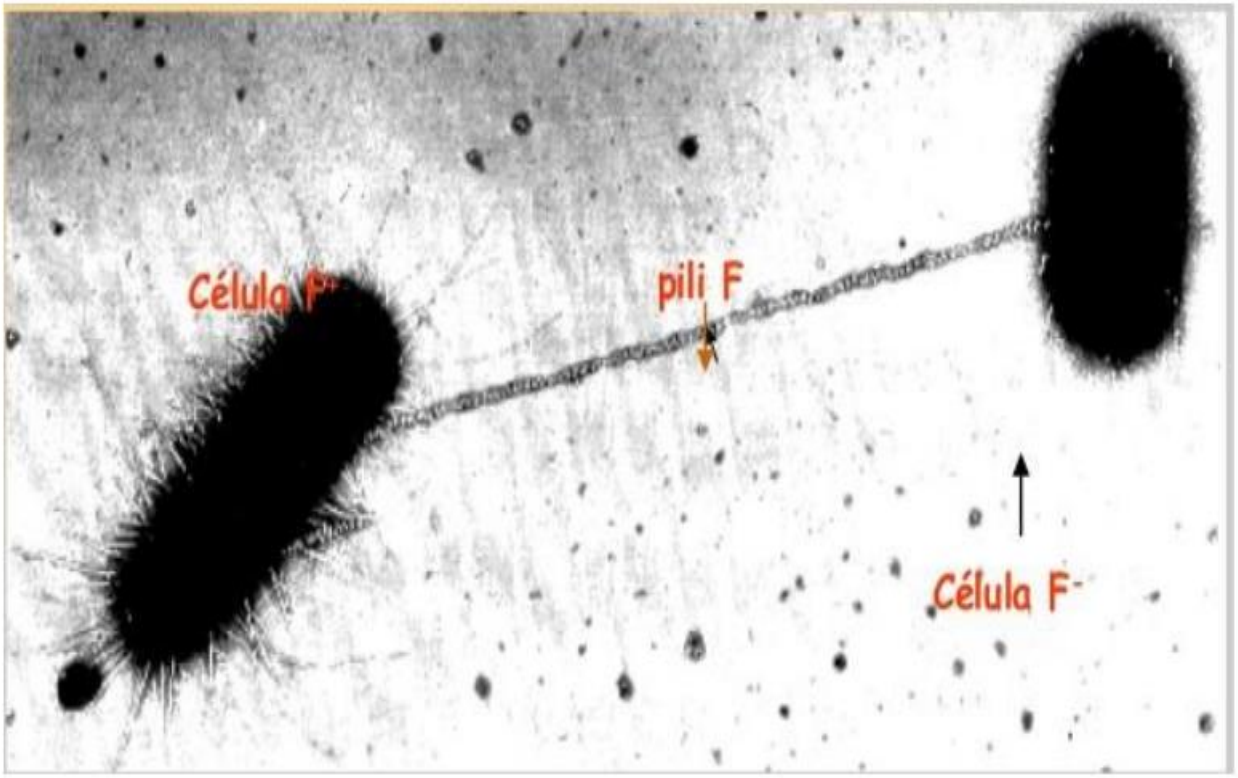
Características

•**Son apéndices filamentosos rectos y rígidos, más cortos y más finos** (3-10 nm de diámetro) que los flagelos,
•Presentes en muchas bacterias (sobre todo Gram-negativas).
•Número variable: desde 1 a varios cientos o miles por célula.
•Disposición: **alrededor de todo el perímetro celular**, y a veces, de inserción polar.
• La mayoría están compuestos por **un solo tipo de proteína (la pilina)**, de unos 17-25 kDa, proteina globular cuyas subunidades se disponen en una matriz helicoidal que deja un pequeño hueco central.
•Están implantados **a nivel de membrana citoplásmica** (no en todos)
•Ensamblaje: **inserción de subunidades de pilina en la base del pelo en crecimiento**, a partir de pre-pilina, que se procesa por escisión del correspondiente péptido-líder a su paso por la membrana citoplásmica.



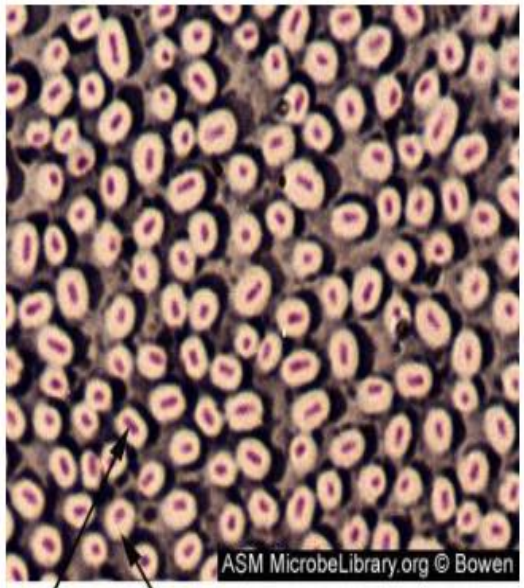


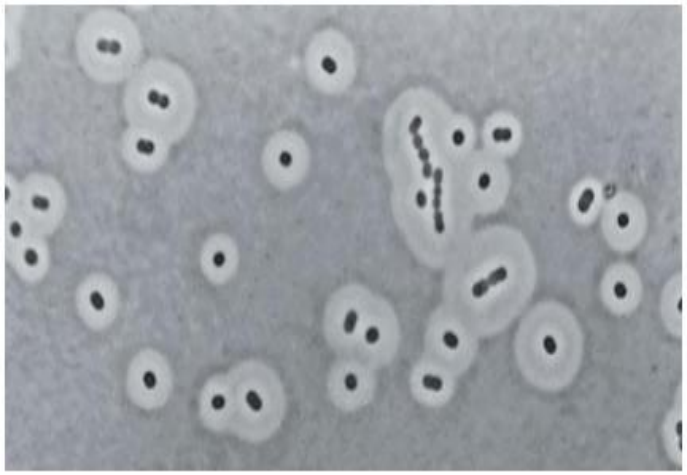
Pilis o pelos sexuales

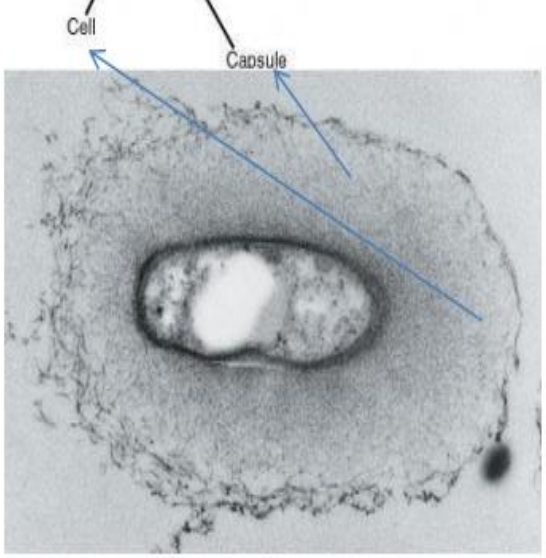


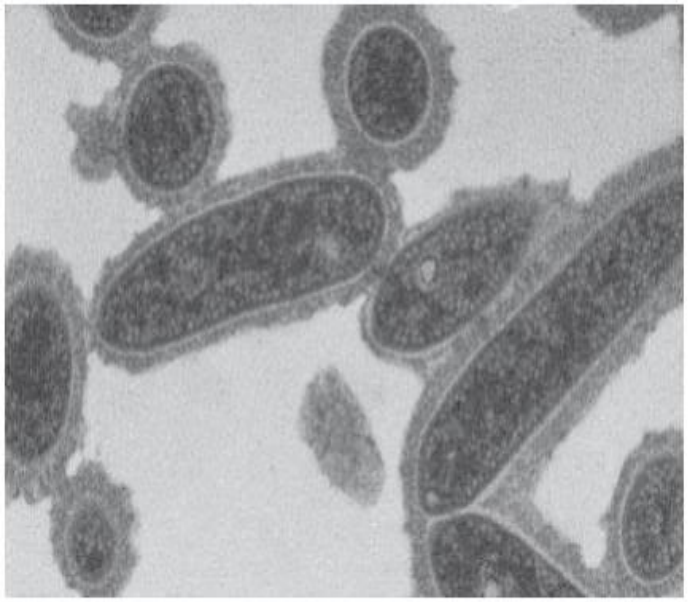
- Son mas largos y gruesos que las fimbrias y están presentes en menor numero (1-10)
- Permiten ponerse en contacto con otra célula para transmitir el DNA
- Están codificados por plásmidos conjugativos (plásmido F)
- Permiten la conjugación.El mecanismo es ponerse en contacto con el receptor en la célula
- aceptora,retracción (depolimerización) y transferencia del DNA
- Son receptores específicos de fagos

Cápsulas y capas mucilaginosa observación

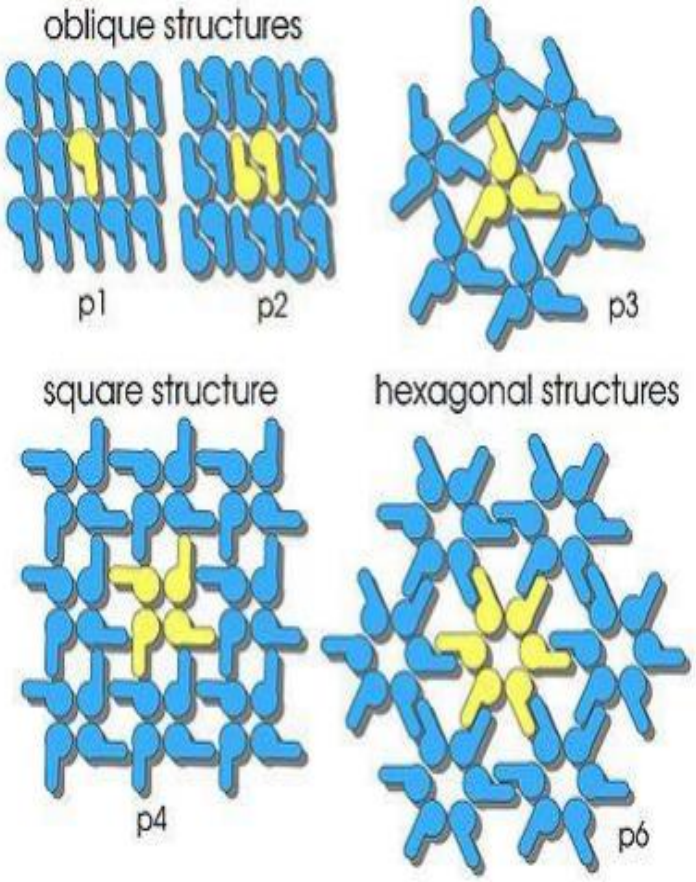








CAPA “S” (Capa superficial cristalina)



Capa que, en muchas eubacterias (sobre todo Gram-positivas), envuelve a la pared celular, formada por el ensamblaje regular de subunidades idénticas de proteínas o glucoproteínas.

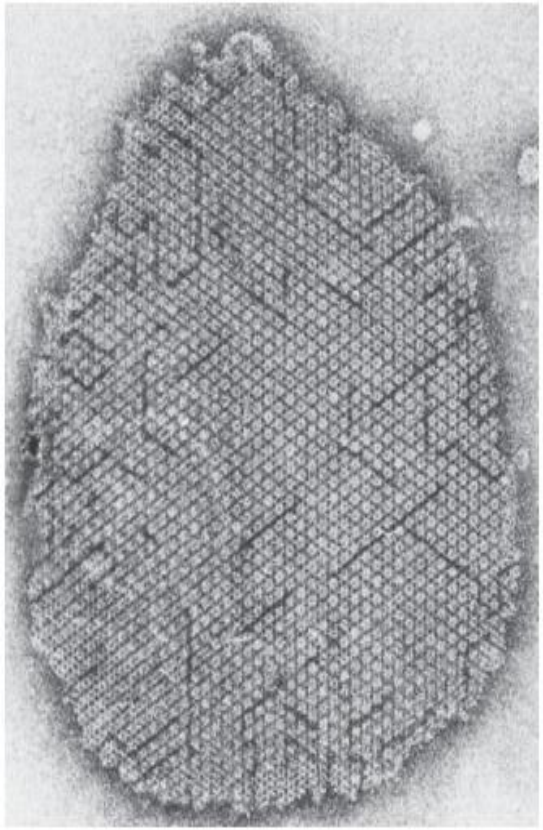
Estructura cristalina que adopta simetría variada

En Gram negativas la capa S se une a la ME

En Gram (+) positivas lo hace al PG.

En muchas Arqueas es la única capa que rodea al protoplasto, por lo que en ellas cumple funciones de auténtica pared celular.

Funciones



En eubacterias provistas de pared celular:

Tamiz Molecular

Protección impide la entrada de agentes antibacterianos y frente a fluctuaciones iónicas y de pH, estrés osmótico, etc.

Factor de virulencia protege a la bacteria frente al ataque del complemento y de los fagocitos, en patógenos

Es la capa mas externa en contacto con el medio

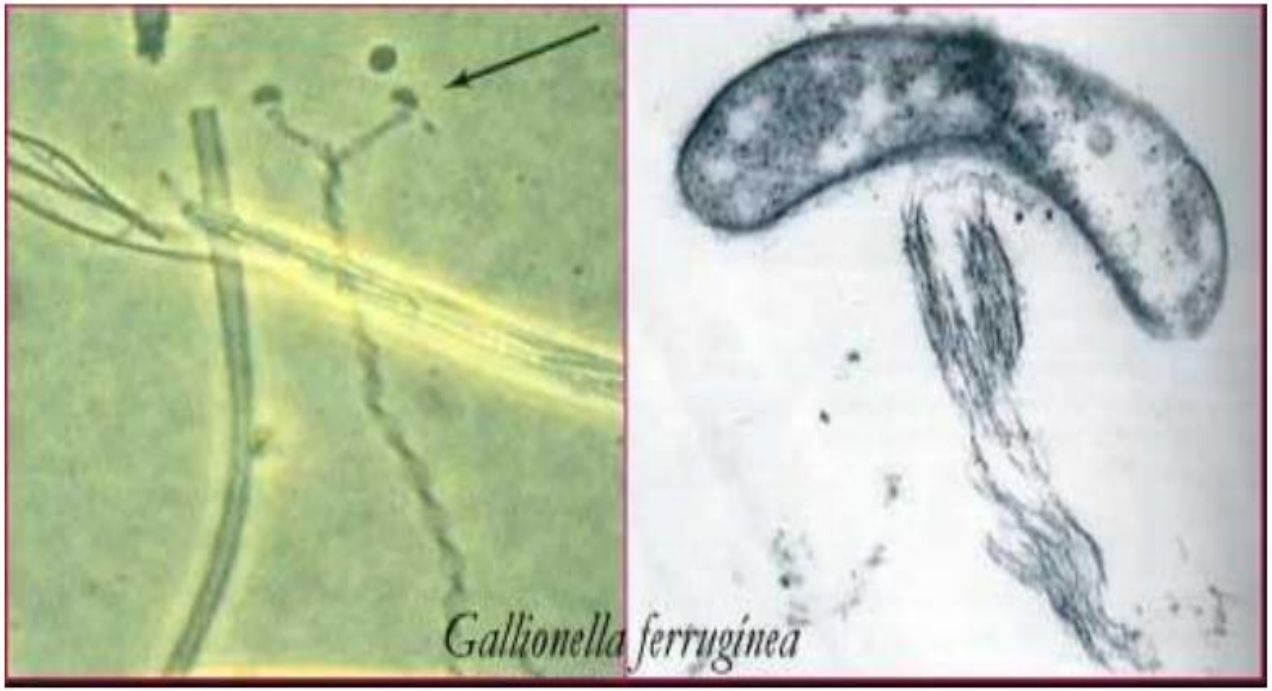
Adhesion de enzimas

Tiene aplicación biotecnológica, nanobiotech.

En Arqueas :

Forma y rigidez a muchas especies, ejerciendo papeles equivalentes al de una pared celular.

Bacterias con tallos o pedúnculos

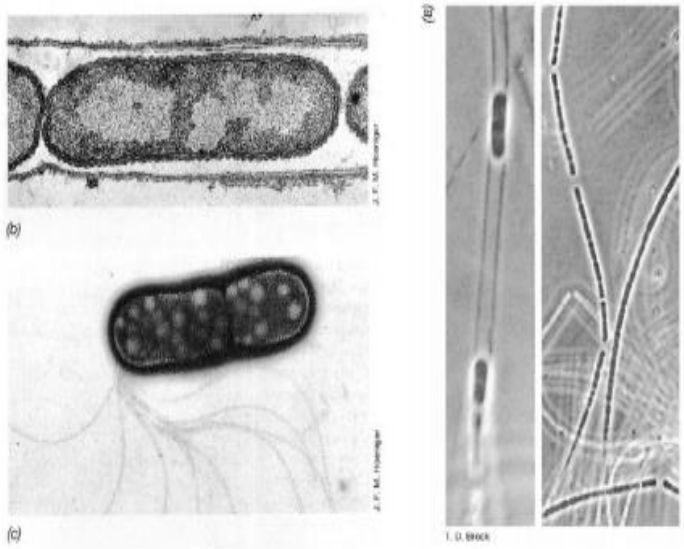


Estructuras filamentosas no vivas terminadas en botones de anclaje (discos adhesivos) producidas por secreción continua de materiales polisacarídicos en una zona concreta de la superficie bacteriana.

Función: Permite la unión de ciertas bacterias de hábitats acuáticos a sustratos sólidos, vivos o no.

Vaina y botones de anclaje

Sphaerotilus y *Leptothrix*



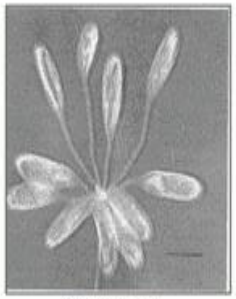
Vaina

Estructura tubular que engloba a células bacilares y conjuntos de células.
Heteropolímero, a base de proteína, lípido y polisacárido que puede recubrirse de oxido de Fe o Mn
En contacto con la pared celular subyacente, pero no hay enlaces entre ambas
Función : mantener las células agrupadas

Las Arqueas *Methanothrix* y *Methanospirillum* poseen vainas proteicas con subunidades dispuestas en anillos, y son las que confieren la forma.

Botones de anclaje

Material mucilaginoso agregado
En puntos concretos de los extremos de prostecas y pedúnculos
Adhesión a sustrato



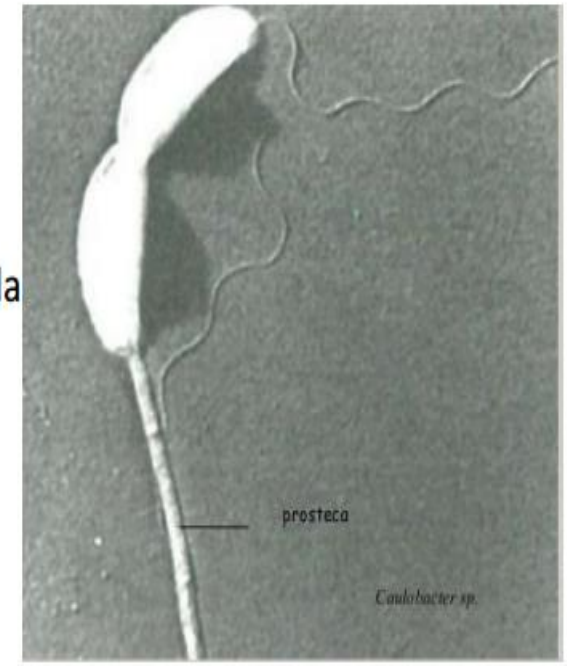
Caulobacter

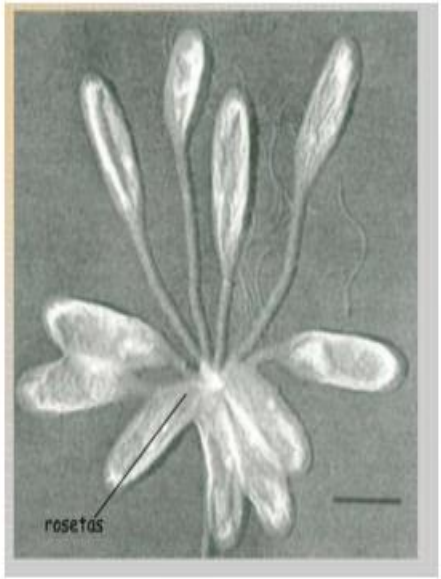
Prostecas

•Son prolongaciones de MP semirígidas
presentes en un grupo especifico de bacterias prostecada
(gemantes y/o con apendices)

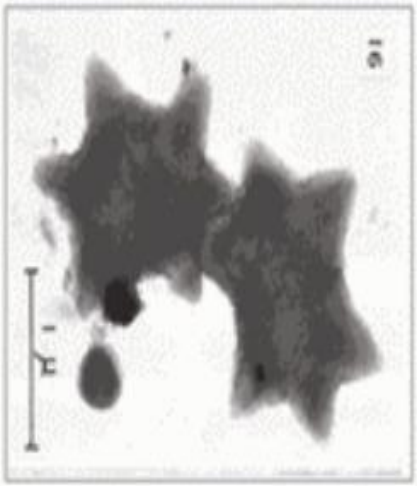
•Tiene un diámetro menor que el cuerpo celular
y están rodados de membrana y pared celular

•Tienen forma de yema, hifa o pedúnculo

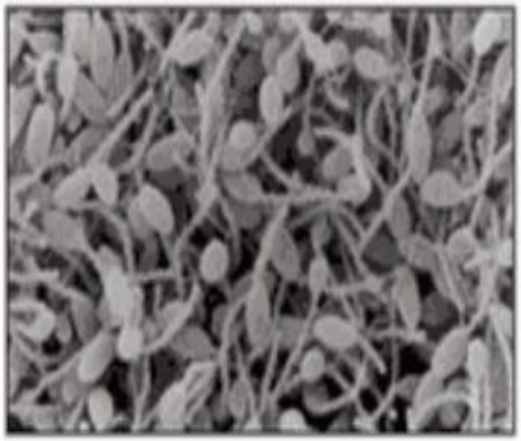




Funciones de las prostecas



Prosthecomicrobium



Hyphomicrobium

Caulobacter

Función:

- Sirven como estructura de unión a los sustratos inertes
- Aumento de la relación superficie /volumen lo que favorece
  - la flotabilidad para algunas bacterias planctónicas
  - y mayor superficie para la captación de nutrientes en ambiente oligotróficos , en *Prosthecomicrobium*
- Tiene alguna función en el proceso de reproducción en bacterias del género *Hyphomicrobium*
- Permiten la agrupación en rosetas en la bacteria del género *Caulobacter*

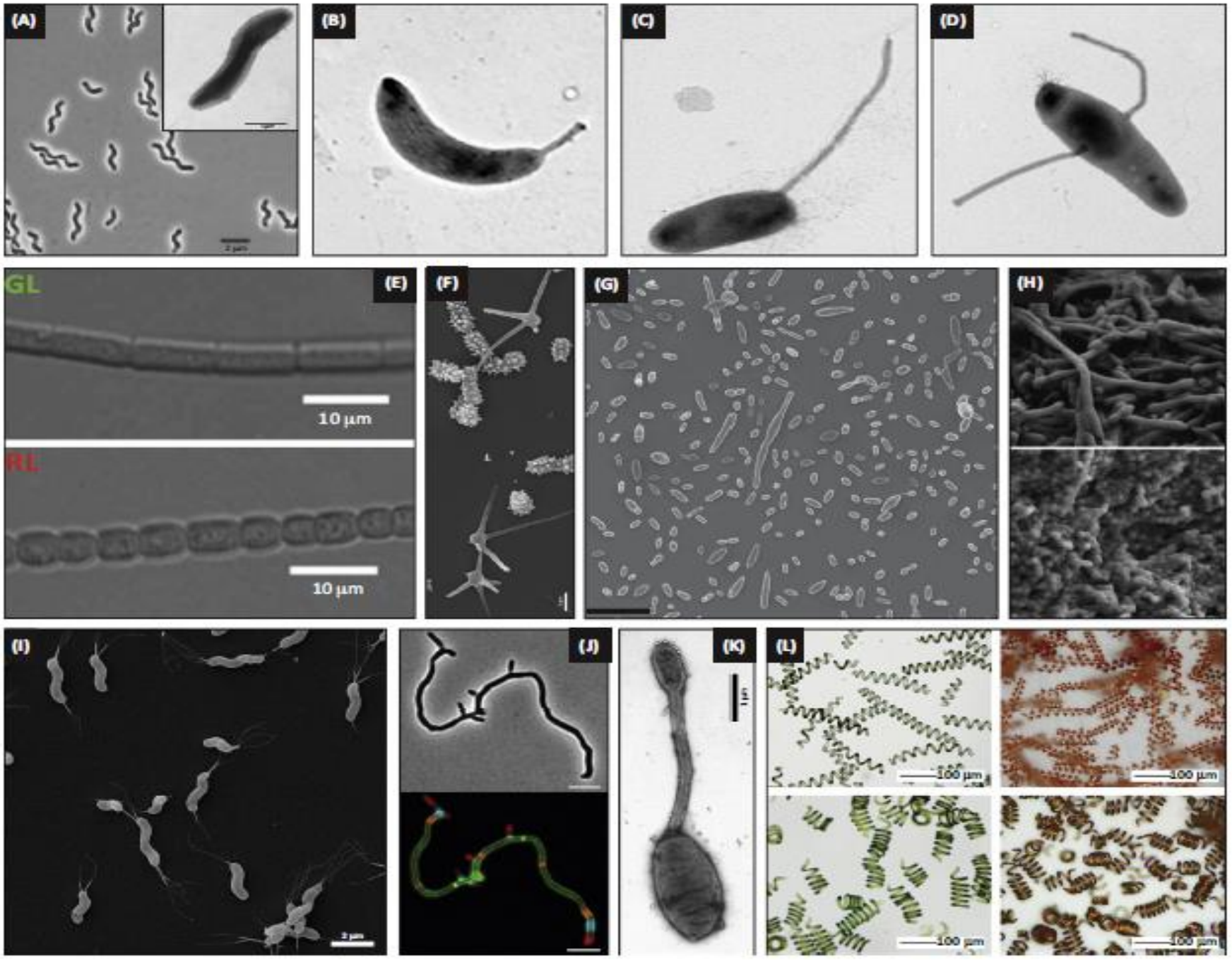

(A)
(B)
(C)
(D)
GL
(E)
10 µm
RL
10 µm
(F)
(G)
(H)
(I)
(J)
(K)
(L)
100 µm
100 µm
100 µm
100 µm

Table 1. Genes That Impact Bacterial Morphology

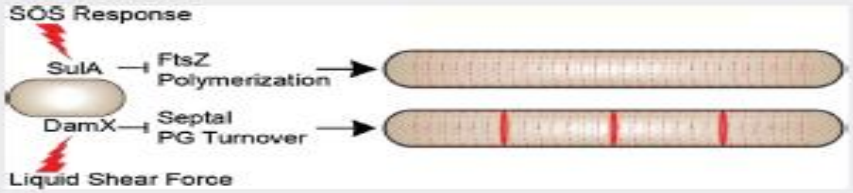
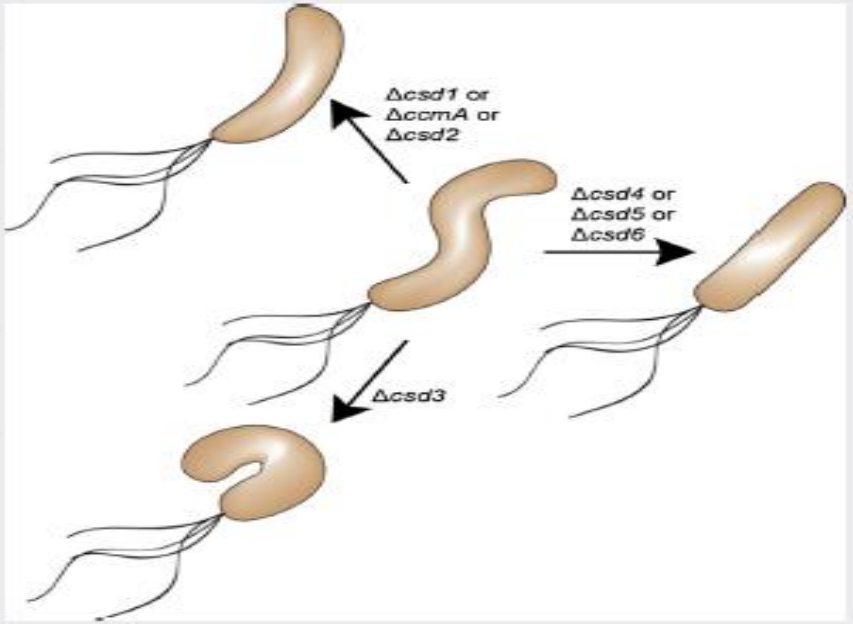
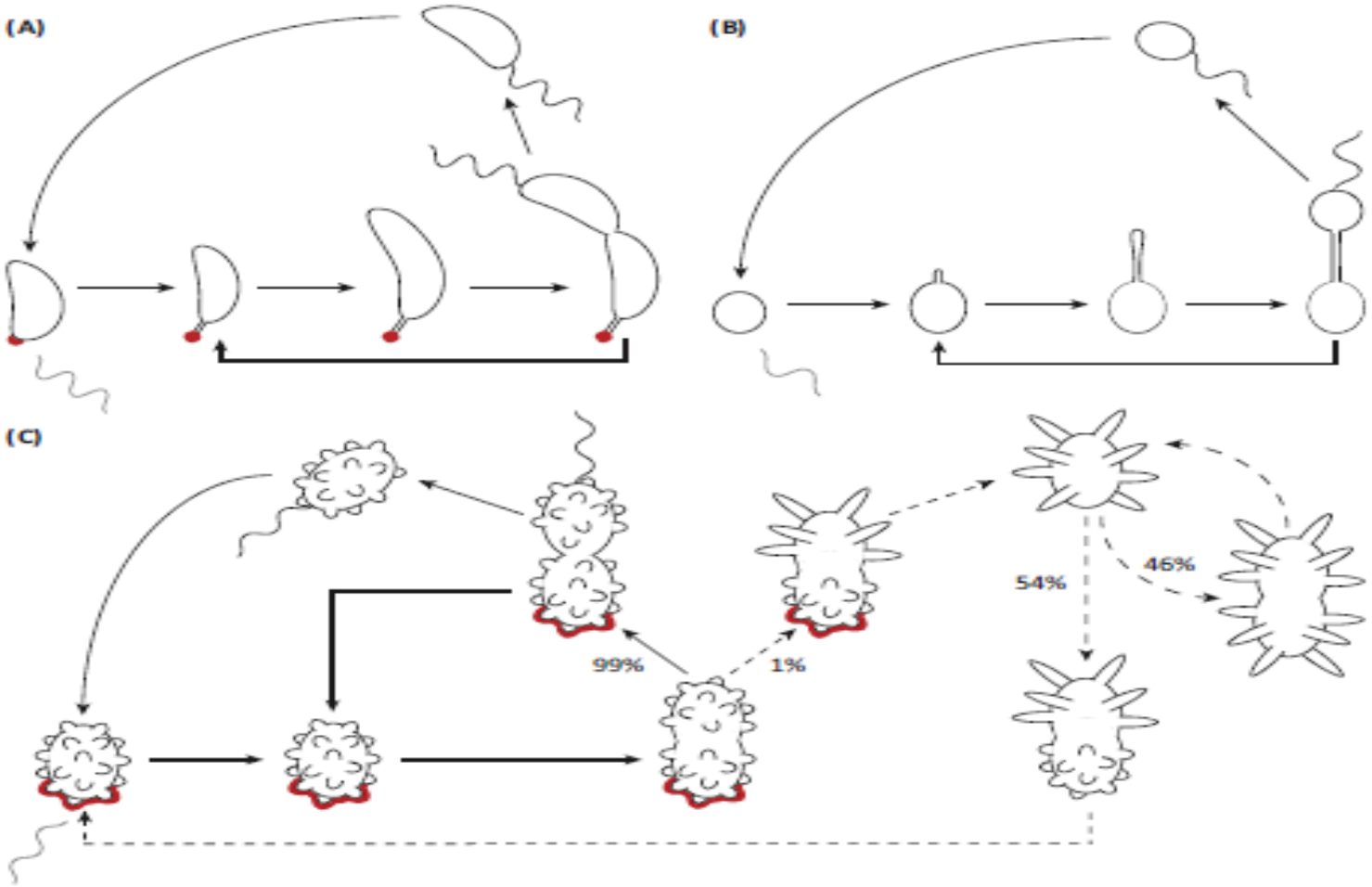
Organism	Gene	Protein description	Localization, functional effect, or mutational effect
Nostoc punctiforme	*amiC2*	Cell wall amidase	
Uropathogenic *Escherichia coli* (UPEC)	*sulA*	Binds FtsZ in presence of GTP; prevents Z-ring formation	
	damX	SPOR domain containing protein; localizes to the septal ring and binds PG	
Asticcacaulis excentricus and *Asticcacaulis biprosthecum*	*spmX*	Phage muramidase domain containing protein; required for stalk synthesis	
Helicobacter pylori	*csd1*	LytM (peptidase family M23) DD-endopeptidase	
	ccmA	Bactofilin; cytoskeletal, polymer-forming, proteins	
	csd2	LytM (peptidase family M23) DD-endopeptidase	
	csd3	DD-endopeptidase and DD-carboxypeptidase activity	
	csd4	DL-carboxypeptidase (note that because Csd4 removes a terminal, uncross-linked amino acid, it is referred to as a carboxypeptidase and not an endopeptidase)	
	csd5	No known enzymatic domain but may modulate Csd4 activity (directly interacts with Csd4 and the dipeptide product of the Csd4-catalyzed PG reaction)	
	csd6	LD-carboxypeptidase	
Streptomyces	*divIVA*	Coiled coil-rich protein; assembles polarisome	
	scy	Coiled coil-rich protein; component of polarisome	

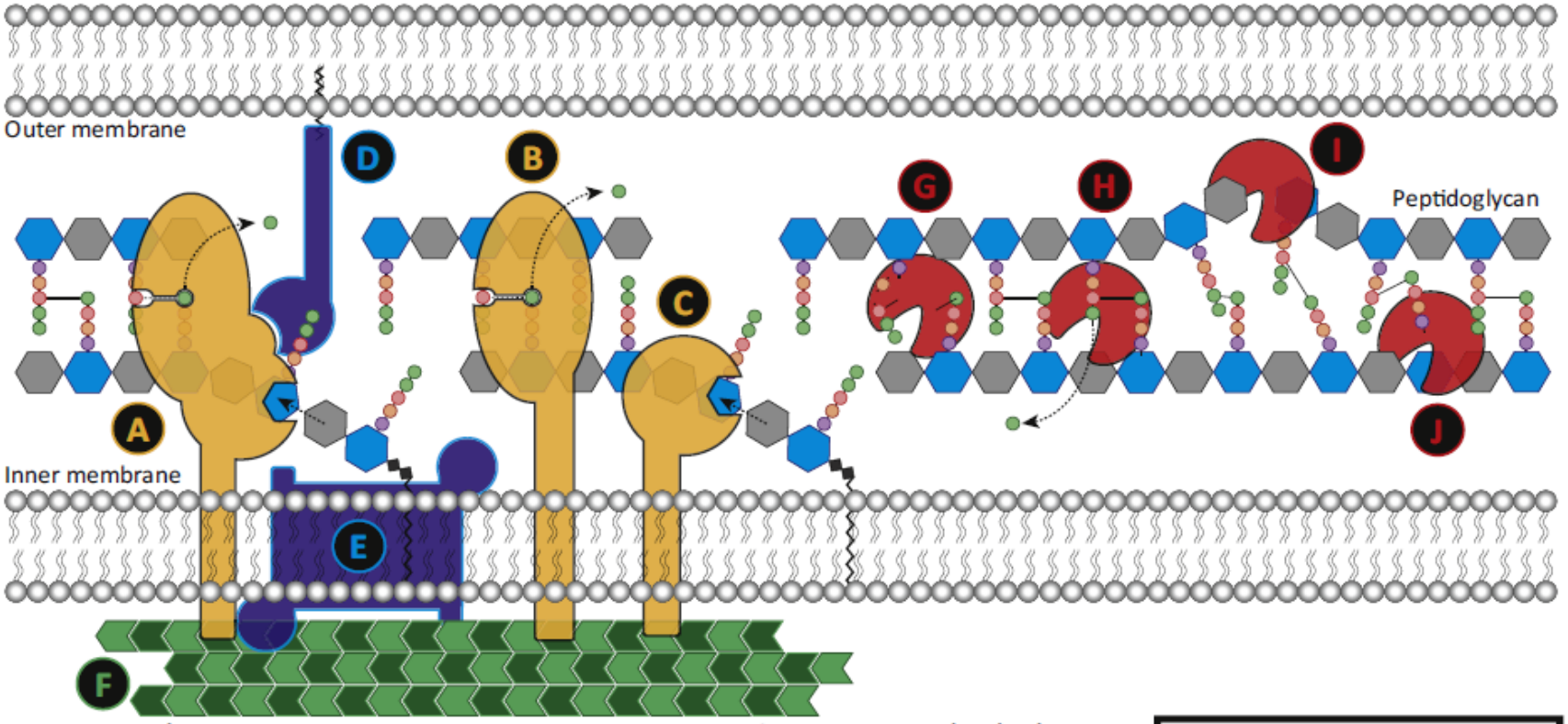
Table 1. (continued)

Organism	Gene	Protein description	Localization, functional effect, or mutational effect
	filP	Coiled coil-rich protein; component of polarisome	
Hyphomonas neptunium	WT	n/a	
	dacB	DD-carboxypeptidase	
	dacL	DD-carboxypeptidase	DacL localizes to the new pole at the start of prostheca synthesis
	lmdC	LytM (peptidase family M23) DD-endopeptidase	Could not be deleted. Essential?
	lmdE	LytM (peptidase family M23) DD-endopeptidase	
	amiC	Cell wall amidase	
	pbp1X	Bifunctional DD-transpeptidase	
	pbp2	Monofunctional DD-transpeptidase	Diffuse or patchy localization pattern until the onset of prostheca formation, when it condenses at the prosthecate pole; once a visible bud has formed, the polar complex disappears followed by patchy foci appearing in the mother cell, the prostheca, and the bud. This suggests that PBP2 may contribute to all aspects of *H. neptunium* growth. Could not be deleted. Essential?
	pbp3	Monofunctional DD-transpeptidase	PBP3 predominantly localizes to the division site of late budding cells where it remains briefly associated with the new pole but then disperses evenly within the cell once a visible prostheca has formed. Consistent with its role in other bacteria, PBP3 thus appears to be an integral part of the divisome involved in septal cell wall remodeling. Could not be deleted. Essential?
	mreB	Actin homolog; serves as the scaffolding protein to organize the elongasome	 MreB coalesces at the future prosthecate pole during early stages of the cell cycle, where it remains until the prostheca has formed and budding initiates. In early budding cells, patchy fluorescence is apparent in the bud and the prostheca, but MreB becomes largely confined to the mother and bud cell compartments as division progresses.
	rodZ	Cytoskeletal protein that mediates MreB circumferential movement and couples MreB to cell wall synthesis enzymes	Exhibits localization patterns similar to those of MreB.



Trends in Microbiology

Figure 3. Multimorphic Life Cycles of Prosthecate Alphaproteobacteria. (A) Dimorphic life cycle of *Caulobacter crescentus*. The prosthecate mother cell produces an adhesive holdfast (shown in red) at the tip of the prostheca. Cell division results in a motile, nonreplicating swarmer cell that differentiates into a prosthecate cell. (B) Dimorphic life cycle of *Hyphomonas neptunium*. The prosthecate mother cell produces a bud at the distal end of the prostheca. Upon septation, a motile, nonreplicating swarmer cell is released that differentiates into a prosthecate cell. (C) Multimorphic life cycle of *Prosthecomicrobium hirschii*. Most of the time, short-prosthecate *P. hirschii* cells follow a *C. crescentus*-like life cycle (solid arrows): a unipolar polysaccharide (UPP, an adhesin similar to *C. crescentus* holdfast; shown in red)-producing mother cell gives rise to a motile, nonreplicating swarmer cell that differentiates into a short-prosthecate cell. A rare event, short-prosthecate mother cells can give rise to nonmotile, long-prosthecate cells that do not produce UPP (dashed arrow). Long-prosthecate mother cells can produce either long-prosthecate cells or short-prosthecate cells at roughly equal frequency (dashed arrows), though it should be noted that the observed frequency of morphotype conversion was observed on MMB (minimal medium broth) agarose pads and might differ in other conditions.



PG synthases

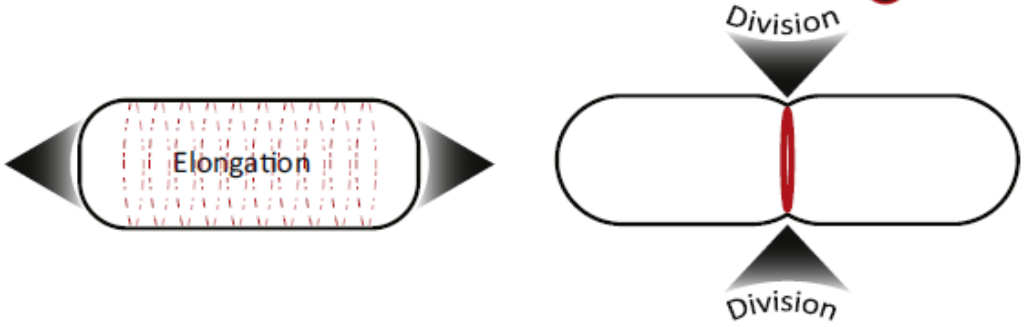
- A Bifunctional (transpeptidase & glycosyltransferase)
- B Monofunctional transpeptidase
- C Monofunctional glycosyltransferase

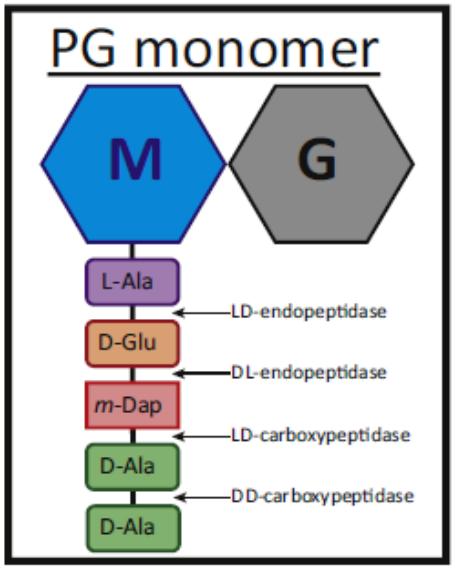
Accessory proteins

- D OM PG synthase activator
- E IM associated proteins
- F Cytoplasmic scaffold

PG hydrolases

- G Endopeptidase
- H Carboxypeptidase
- I Glucosidase
- J Amidase





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REVIEW

The role of environmental cleaning in the control of hospital-acquired infection

S.J. Dancer*

Summary Increasing numbers of hospital-acquired infections have generated much attention over the last decade. The public has linked the so-called 'superbugs' with their experience of dirty hospitals but the precise role of environmental cleaning in the control of these organisms remains unknown. Until cleaning becomes an evidence-based science, with established methods for assessment, the importance of a clean environment is likely to remain speculative. This review will examine the links between the hospital environment and various pathogens, including meticillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, norovirus, *Clostridium difficile* and acinetobacter. These organisms may be able to survive in healthcare environments but there is evidence to support their vulnerability to the cleaning process. Removal with, or without, disinfectants, appears to be associated with reduced infection rates for patients. Unfortunately, cleaning is often delivered as part of an overall infection control package in response to an outbreak and the importance of cleaning as a single intervention remains controversial. Recent work has shown that hand-touch sites are habitually contaminated by hospital pathogens, which are then delivered to patients on hands. It is possible that prioritising the cleaning of these sites might offer a useful adjunct to the current preoccupation with hand hygiene, since hand-touch sites comprise the less well-studied side of the hand-touch site equation. In addition, using proposed standards for hospital hygiene could provide further evidence that cleaning is a cost-effective intervention for controlling hospital-acquired infection.

Table 1. Microbiologic factors that can facilitate surface environment-mediated transmission of selected pathogens

Pathogen able to survive for prolonged periods of time on environmental surfaces (all)
Ability to remain virulent after environmental exposure (all)
Contamination of the hospital environment frequent (all)
Ability to colonize patients (*Acinetobacter*, *C difficile*, MRSA, VRE)
Ability to transiently colonize the hands of health care workers (all)
Transmission via the contaminated hands of healthcare workers (all)
Small inoculating dose (*C difficile*, norovirus)
Relative resistance to disinfectants used on environmental surfaces (*C difficile*, norovirus)

C difficile, *Clostridium difficile*; *MRSA*, methicillin-resistant *Staphylococcus aureus*; *VRE*, vancomycin-resistent *Enterococcus* spp.

Table 2. Microbiologic and epidemiologic features of norovirus that promote epidemics

Large human reservoir of infection
Widespread host susceptibility
Strain-specific immunity is short lived (weeks to months)
Multiple routes of transmission (fecal-oral, foodborne, waterborne, aerosol)
High infectivity
Very low inoculating dose (<10 virions)
Stable in the environment
Prolonged shedding
No vaccine available
No specific chemotherapy

Table 3. Evidence supporting role of environmental contamination in transmission of emerging health care-associated pathogens

Characteristic	Norovirus	*Clostridium difficile*	*Acinetobacter* spp
Able to survive for prolonged periods in the environment	Yes	Yes	Yes
Environmental contamination frequently found in rooms of infected patients	Yes	Yes	Yes
Contaminated environmental reservoir demonstrated to be source of an outbreak	—	Yes	Yes
Contamination of health care worker hands demonstrated	—	Yes	Yes
Human challenge studies demonstrate that contaminated health care worker hands can transfer pathogen	Yes	—	Yes
Level of environmental contamination associated with frequency of health care worker hand contamination	—	Yes	—
Prevalence of environmental contamination associated with incidence of patient acquisition/infection	—	Yes	—
Admission to a room previously occupied by an infected patient associated with risk of colonization/infection	—	Yes	—
Enhanced cleaning demonstrated to reduce hospital incidence of infection	—	Yes	Yes

What is 'clean'?

If we state that a hospital is clean, we assume that it looks clean and that it is safe for patients. Unfortunately, the microbes responsible for HAI are invisible to the naked eye. This means that visual assessment is insufficient for defining cleanliness, nor will it accurately predict the infection risk for patients.[2] Cleanliness should not actually be defined without indicating how it is assessed. A recent study compared visual assessment against both biochemical (ATP bioluminescence) and microbiological screening of the hospital environment.[3] The results showed that whereas most surfaces looked clean, less than a quarter were free from organic soil (ATP) and less than half were microbiologically clean.[3] Given the risk of acquiring hospital pathogens from a hospital ward, visual assessment is outdated, inadequate and scientifically obsolete. The only benefit from a visual inspection is to appease aesthetic obligations.

There has been suggestion that hospitals would benefit from cleaning standards emulating those implemented in the food industry.[2,48] Food prep-

Where to clean?

There is increasing evidence regarding the importance of hand-touch sites in the transmission of pathogens to healthy persons, as well as to patients.[51,52] It is also becoming apparent that the sites closer to the patient are more likely to furnish an infection risk than those situated further away.[7,8] The role of these near-patient hand-touch sites in MRSA transmission and, indeed, other hospital pathogens, has not been given the priority that it deserves. Ward cleaners work to a set specification that encompasses general surfaces and bathrooms, with emphasis on the cleaning of floors and toilets.[53] These are not necessarily near-patient hand-touch sites. Examples of the latter include bed rails, bedside lockers, infusion pumps, door handles and various switches,

How to clean?

Most of the studies describing the benefits from cleaning in this review used disinfectants to clean the hospital environment. Virtually all were reported as part of the response to an outbreak. Only a few UK-based studies used detergent and water, and even fewer reported cleaning benefits in the absence of an outbreak.[18,30] It appears that when reviewing the evidence for the role of cleaning in the control of HAI, there are several issues which still require clarification. First, is there any difference between the quantity, quality and methods for routine cleaning compared with what is needed in the event of an outbreak; and second, is it sufficient to proclaim the benefits from cleaning with disinfectants without establishing what can be achieved using soap and water alone? These questions require an evidence-based approach before we can set the best specification for cleaning in our hospitals. In addition, no one has yet modelled different cleaning methods against the infection risk for patients, their degree of vulnerability and the clinical area in which they are exposed.

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How long do nosocomial pathogens persist on inanimate surfaces? A systematic review

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Abstract

Background: Inanimate surfaces have often been described as the source for outbreaks of nosocomial infections. The aim of this review is to summarize data on the persistence of different nosocomial pathogens on inanimate surfaces.

Methods: The literature was systematically reviewed in MedLine without language restrictions. In addition, cited articles in a report were assessed and standard textbooks on the topic were reviewed. All reports with experimental evidence on the duration of persistence of a nosocomial pathogen on any type of surface were included.

Results: Most gram-positive bacteria, such as Enterococcus spp. (including VRE), *Staphylococcus aureus* (including MRSA), or *Streptococcus pyogenes*, survive for months on dry surfaces. Many gram-negative species, such as Acinetobacter spp., *Escherichia coli*, Klebsiella spp., *Pseudomonas aeruginosa*, *Serratia marcescens*, or Shigella spp., can also survive for months. A few others, such as *Bordetella pertussis*, *Haemophilus influenzae*, *Proteus vulgaris*, or *Vibrio cholerae*, however, persist only for days. Mycobacteria, including *Mycobacterium tuberculosis*, and spore-forming bacteria, including *Clostridium difficile*, can also survive for months on surfaces. *Candida albicans* as the most important nosocomial fungal pathogen can survive up to 4 months on surfaces. Persistence of other yeasts, such as *Torulopsis glabrata*, was described to be similar (5 months) or shorter (*Candida parapsilosis*, 14 days). Most viruses from the respiratory tract, such as *corona*, *coxsackie*, *influenza*, *SARS* or *rhino* virus, can persist on surfaces for a few days. Viruses from the gastrointestinal tract, such as *astro*virus, *HAV*, *polio*- or *rota* virus, persist for approximately 2 months. Blood-borne viruses, such as HBV or HIV, can persist for more than one week. Herpes viruses, such as CMV or HSV type 1 and 2, have been shown to persist from only a few hours up to 7 days.

Conclusion: The most common nosocomial pathogens may well survive or persist on surfaces for months and can thereby be a continuous source of transmission if no regular preventive surface

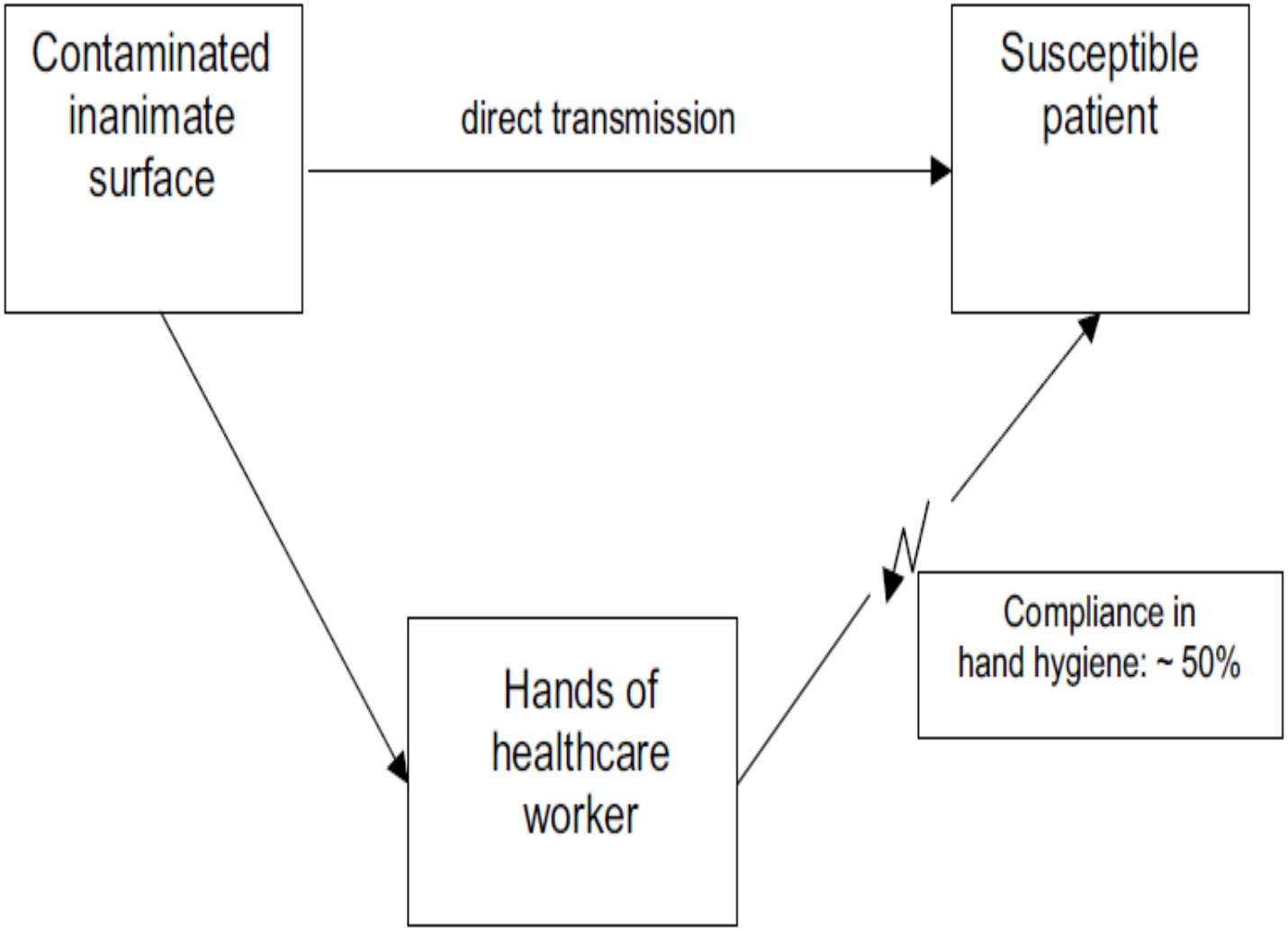


Figure 1
Common modes of transmission from inanimate surfaces to susceptible patients.

Table 1: Persistence of clinically relevant bacteria on dry inanimate surfaces.

Type of bacterium	Duration of persistence (range)	Reference(s)
Acinetobacter spp.	3 days to 5 months	[18, 25, 28, 29, 87, 88]
Bordetella pertussis	3 – 5 days	[89, 90]
Campylobacter jejuni	up to 6 days	[91]
Clostridium difficile (spores)	5 months	[92–94]
Chlamydia pneumoniae, C. trachomatis	≤ 30 hours	[14, 95]
Chlamydia psittaci	15 days	[90]
Corynebacterium diphtheriae	7 days – 6 months	[90, 96]
Corynebacterium pseudotuberculosis	1–8 days	[21]
Escherichia coli	1.5 hours – 16 months	[12, 16, 17, 22, 28, 52, 90, 97–99]
Enterococcus spp. including VRE and VSE	5 days – 4 months	[9, 26, 28, 100, 101]
Haemophilus influenzae	12 days	[90]
Helicobacter pylori	≤ 90 minutes	[23]
Klebsiella spp.	2 hours to > 30 months	[12, 16, 28, 52, 90]
Listeria spp.	1 day – months	[15, 90, 102]
Mycobacterium bovis	> 2 months	[13, 90]
Mycobacterium tuberculosis	1 day – 4 months	[30, 90]
Neisseria gonorrhoeae	1 – 3 days	[24, 27, 90]
Proteus vulgaris	1 – 2 days	[90]
Pseudomonas aeruginosa	6 hours – 16 months; on dry floor: 5 weeks	[12, 16, 28, 52, 99, 103, 104]
Salmonella typhi	6 hours – 4 weeks	[90]
Salmonella typhimurium	10 days – 4.2 years	[15, 90, 105]
Salmonella spp.	1 day	[52]
Serratia marcescens	3 days – 2 months; on dry floor: 5 weeks	[12, 90]
Shigella spp.	2 days – 5 months	[90, 106, 107]
Staphylococcus aureus, including MRSA	7 days – 7 months	[9, 10, 16, 52, 99, 108]
Streptococcus pneumoniae	1 – 20 days	[90]
Streptococcus pyogenes	3 days – 6.5 months	[90]
Vibrio cholerae	1 – 7 days	[90, 109]

Table 3: Persistence of clinically relevant viruses on dry inanimate surfaces.

Type of virus	Duration of persistence (range)	Source
Adenovirus	7 days – 3 months	[32, 34, 38–41, 111]
Astrovirus	7 – 90 days	[38]
Coronavirus	3 hours	[112, 113]
SARS associated virus	72 – 96 hours	[114]
Coxsackie virus	> 2 weeks	[34, 111]
Cytomegalovirus	8 hours	[115]
Echovirus	7 days	[39]
HAV	2 hours – 60 days	[35, 38, 41]
HBV	> 1 week	[116]
HIV	> 7 days	[117–119]
Herpes simplex virus, type 1 and 2	4.5 hours – 8 weeks	[34, 111, 118, 120]
Influenza virus	1 – 2 days	[39, 43, 121, 122]
Norovirus and feline calici virus (FCV)	8 hours – 7 days	[42, 45]
Papillomavirus 16	> 7 days	[123]
Papovavirus	8 days	[118]
Parvovirus	> 1 year	[118]
Poliovirus type 1	4 hours – < 8 days	[35, 118]
Poliovirus type 2	1 day – 8 weeks	[34, 38, 111]
Pseudorabies virus	≥ 7 days	[124]
Respiratory syncytial virus	up to 6 hours	[44]
Rhinovirus	2 hours – 7 days	[33, 125]
Rotavirus	6 – 60 days	[36 – 38, 41]
Vacciniavirus	3 weeks – > 20 weeks	[34, 126]

CUARTA CLASE

Antibióticos

- **Qué son, cuál es su origen, cómo se clasifican?**
- **Modo de acción selectivo**
- **Quienes los producen**
- **Origen y desarrollo de resistencia a antibióticos**
- **Necesidad de nuevos antibióticos**

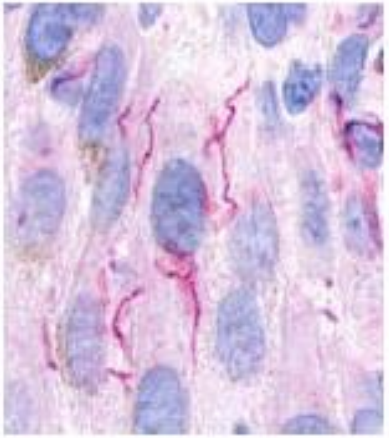
Antibióticos

Qué son?

Sustancia químicas capaz de actuar sobre los microorganismos, inhibiendo su crecimiento o destruyéndolos

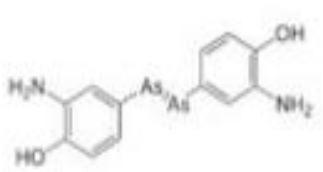
Desde cuando se conocen?

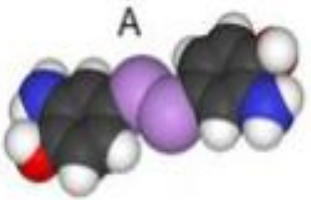
Antecedentes Históricos

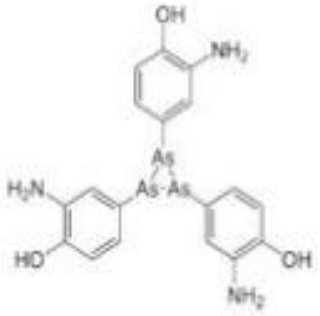


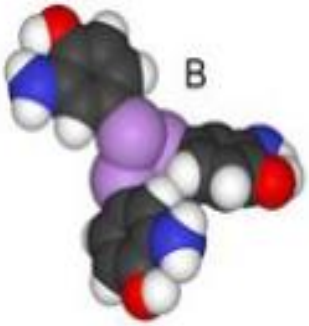
Treponema pallidum

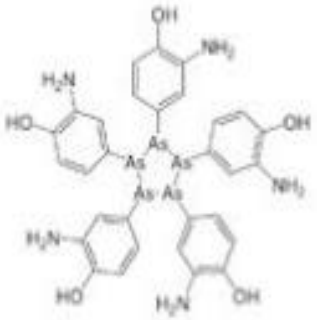
Estructura química del
Salvarsan o arsfenamina
"La bala mágica"

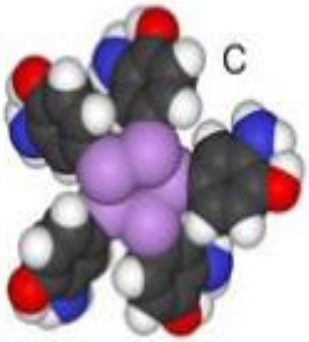












Paul Ehrlich

Concepto basico de la quimiter ápia: **Toxicidad selectiva**

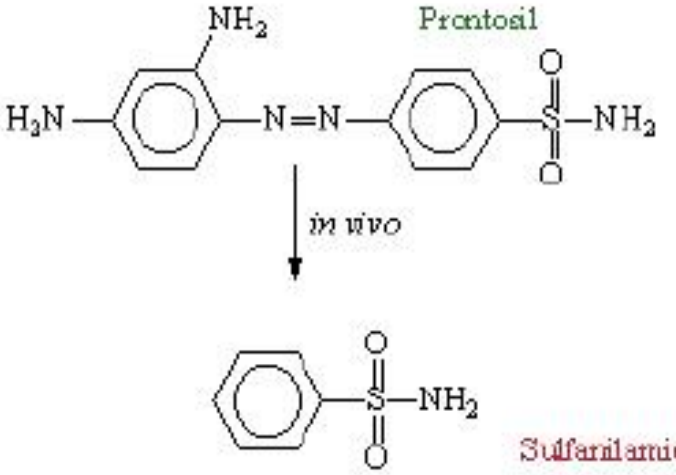
Creó el primer compuesto químico sintético (Salvarsan en 1901, 606 derivados) que podía curar una infección, la sífilis

Premio Nobel Medicina: 1908

Antecedentes Históricos

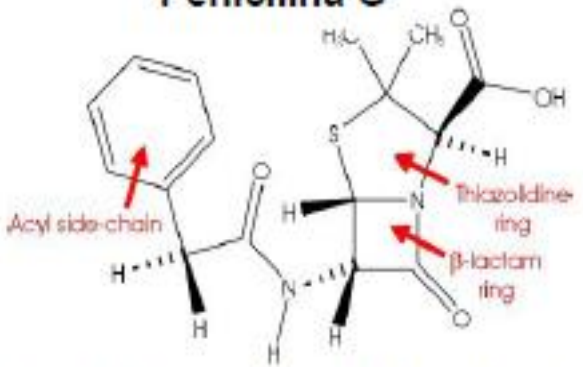
Sulfamidas

Descubiertas por Gerhard Domagk (1932) mientras buscaba colorantes para teñir *S. aureus*. Un colorante rojo (Prontosil Rubrum) protegía a ratones y conejos contra dosis letales de estáfilos y estreptococos hemolíticos.



Antecedentes Históricos

Penicilina G



A la sustancia se le dio el nombre de Penicilina, porque el hongo contaminante fue identificado como *Penicillium notatum*. Efectiva contra las peores enfermedades infecciosas del momento, como la tuberculosis, sífilis, cólera o neumonía.

Alexander Fleming

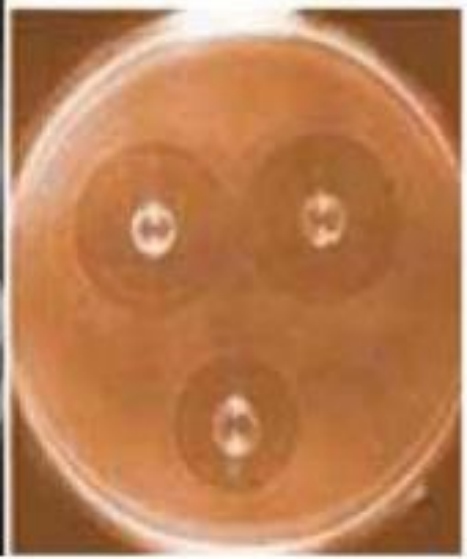
Descubrió el primer antibiótico, cuando por accidente se contaminó un cultivo de *Staphylococcus aureus* con un hongo y observó un halo transparente de inhibición de crecimiento de este microorganismo alrededor del hongo (1928).
Premio Nobel de Medicina 1945

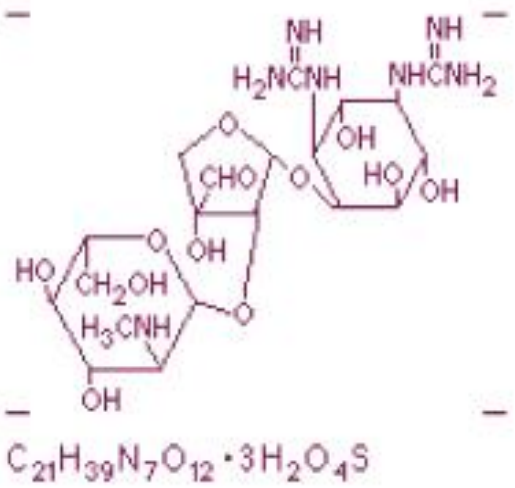
Antecedentes Históricos

Streptomicina

Fue aislada en 1944 por el Albert Schatz en el laboratorio de Selman Waksman a partir de *Streptomyces griseus* debido a las evidencias de que cepas de *Mycobacterias* se inactivaban al mezclarlos con muestras del suelo. Introduce el término "**antibiótico**".

Selman Waksman recibe el premio Nobel de medicina 1952



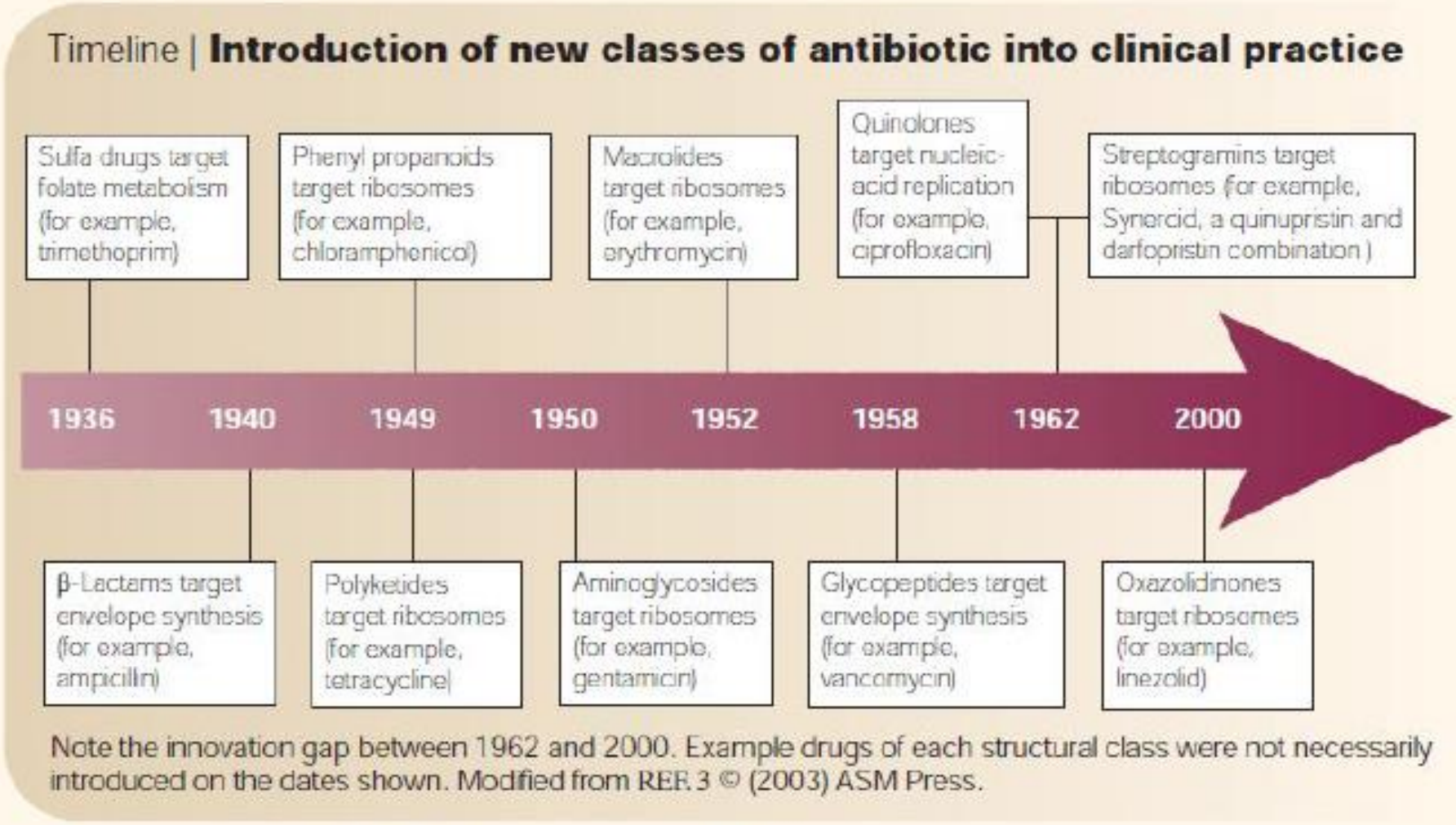


$C_{21}H_{39}N_7O_{12} \cdot 3H_2O_4S$

Aminoglicósidos

Son azúcares complejos unidos por enlaces glicosídicos. Atraviesan la membrana citoplasmática por un mecanismo oxígeno-dependiente. Los grupos $-NH_2$ y -OH interactúan con proteínas del ribosoma.

Antecedentes Históricos

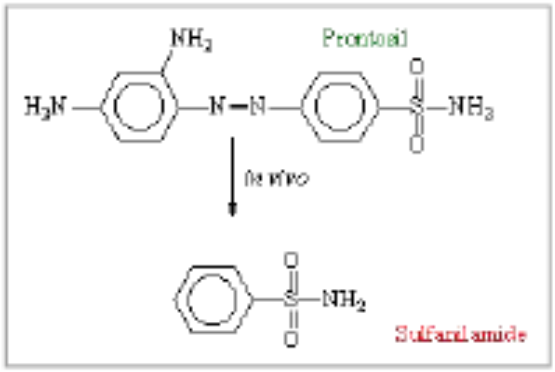


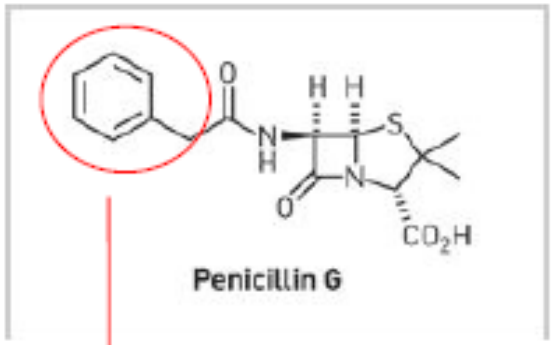
daptomycin (2000) descubierto 1980
pleuromutilins (2007) se usó en vetrinaria por mas de 30 años
fidaxomicin (2011) primer reporte en 1970

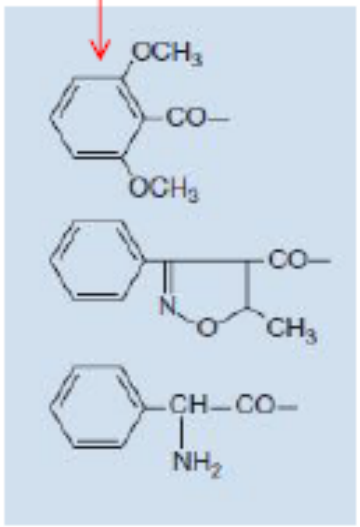
Clasificación de los antibióticos

1- Según su origen
2- Según su estructura química
3- Según su actividad sobre microorganismos
4- Según su espectro de acción
5- Según su mecanismo de acción

Clasificación según su origen







Quimioterapéutico o Sintético

Sustancia producida de manera sintética que posee la propiedad de inhibir el crecimiento o destruir microorganismos

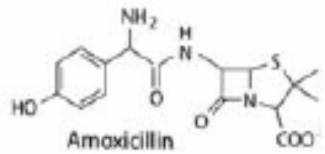
Productos Naturales

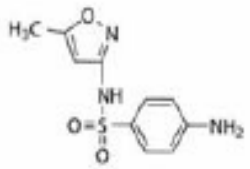
Sustancia producida por el metabolismo de organismos vivos, principalmente hongos y bacterias, que posee la propiedad de inhibir el crecimiento o destruir microorganismos

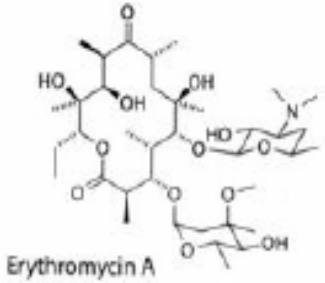
Semisintéticos

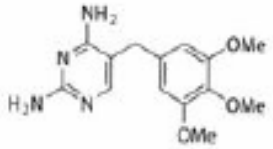
Productos naturales con modificaciones químicas en su estructura, que poseen mejoras en sus propiedades fisicoquímicas y/o farmacológicas

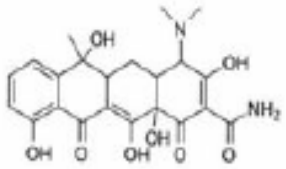
Clasificación según su estructura

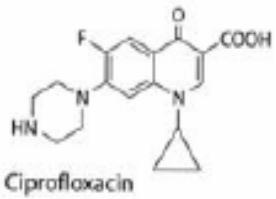

Amoxicillin

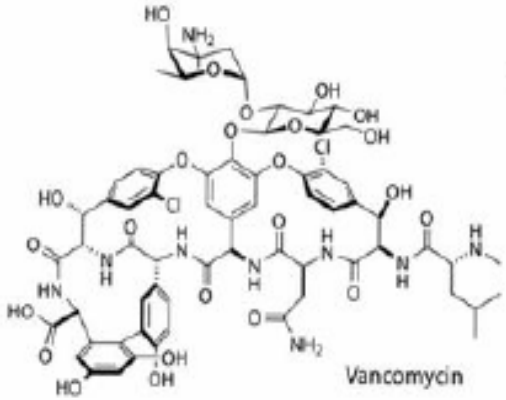

Sulfamethoxazole

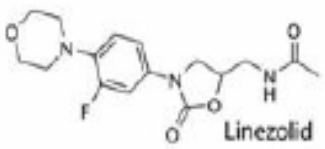

Erythromycin A

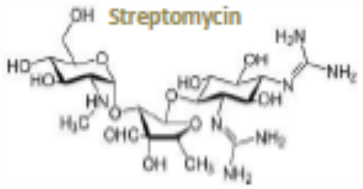

Trimethoprim


Tetracycline


Ciprofloxacin


Vancomycin


Linezolid


Streptomycin

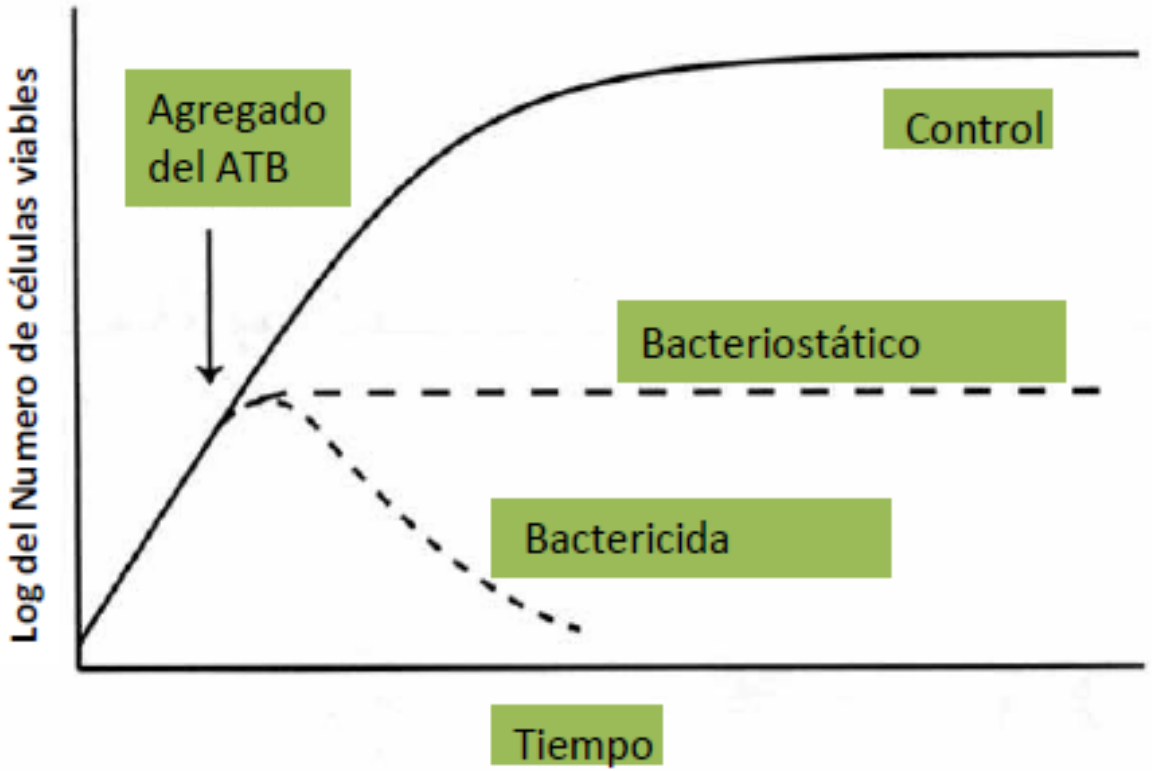
- β-lactámicos
  - penicilinas
  - cefalosporinas
- Tetraciclinas
- Aminoglicósidos
- Quinolonas
- Polipéptidos (síntesis ribosomal o no ribosomal)
- Macrólidos
- Cloramfenicol

Esta diversidad estructural les permite interactuar con diferentes sitios blancos en las bacteria

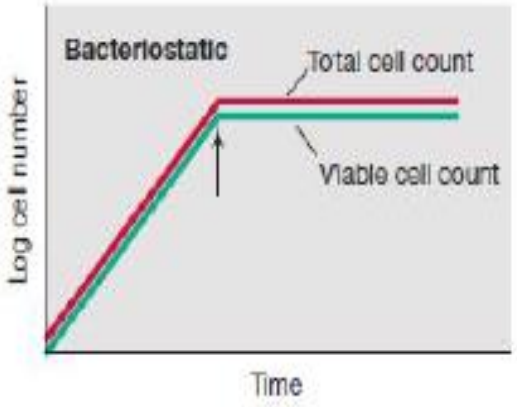
Efecto de los antimicrobianos sobre el crecimiento bacteriano

Bactericidas: producen la muerte de los agentes infecciosos

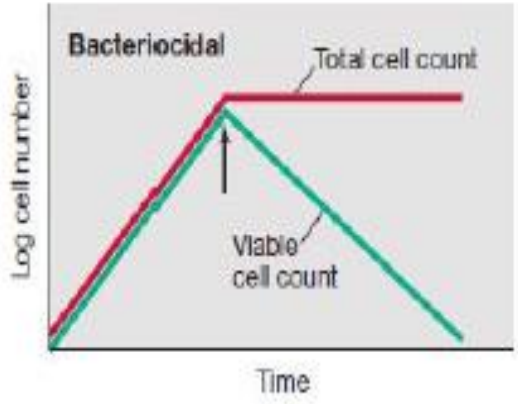
Bacteriostáticos: inhiben el crecimiento bacteriano aunque el microorganismo permanece viable



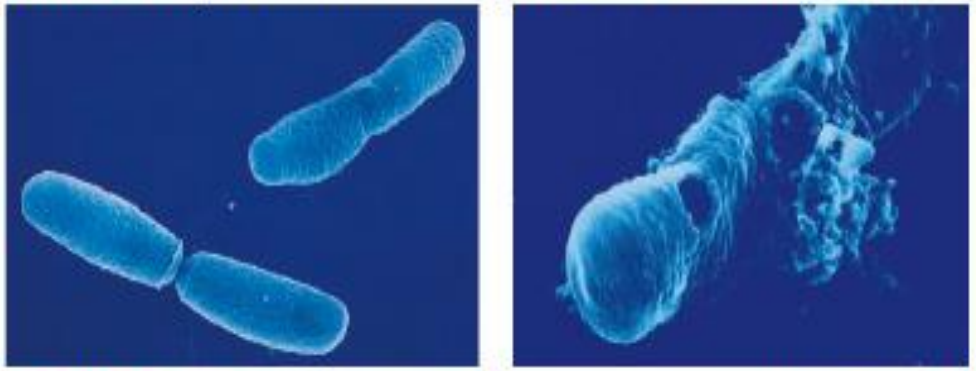
Crecimiento bacteriano: N° de células totales vs Viables

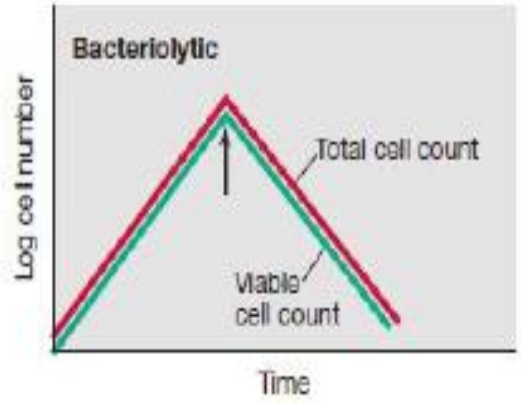


Bacteriostáticos: inhiben el crecimiento bacteriano



Bactericidas: producen la muerte sin necesidad de lísis

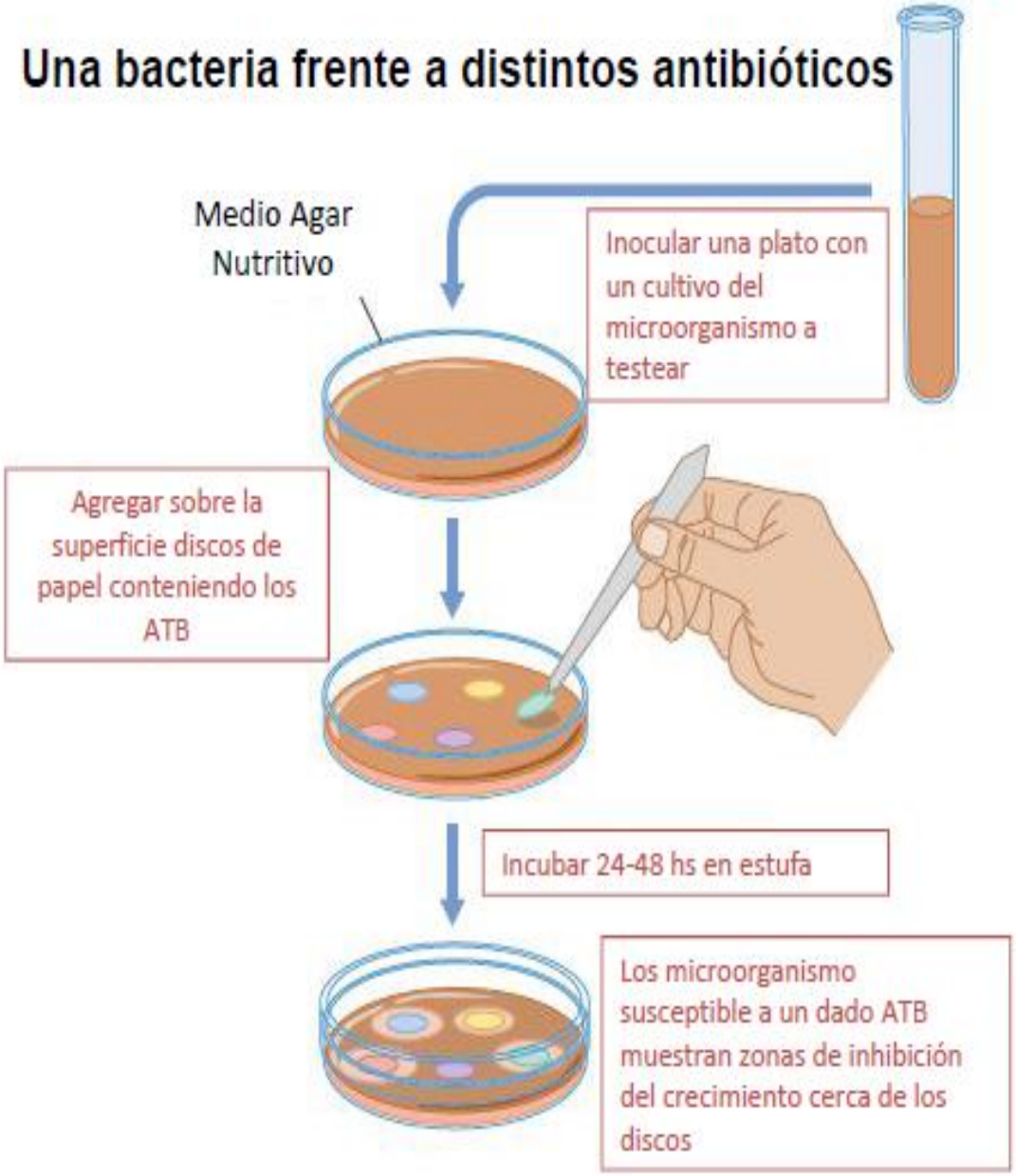




Bacteriolíticos: producen la muerte mediante la lísis

Determinación de la sensibilidad: Antibiograma

Una bacteria frente a distintos antibióticos



Clasificación de según su espectro de acción

Un antibiótico frente a distintas bacterias

- **Espectro reducido**:

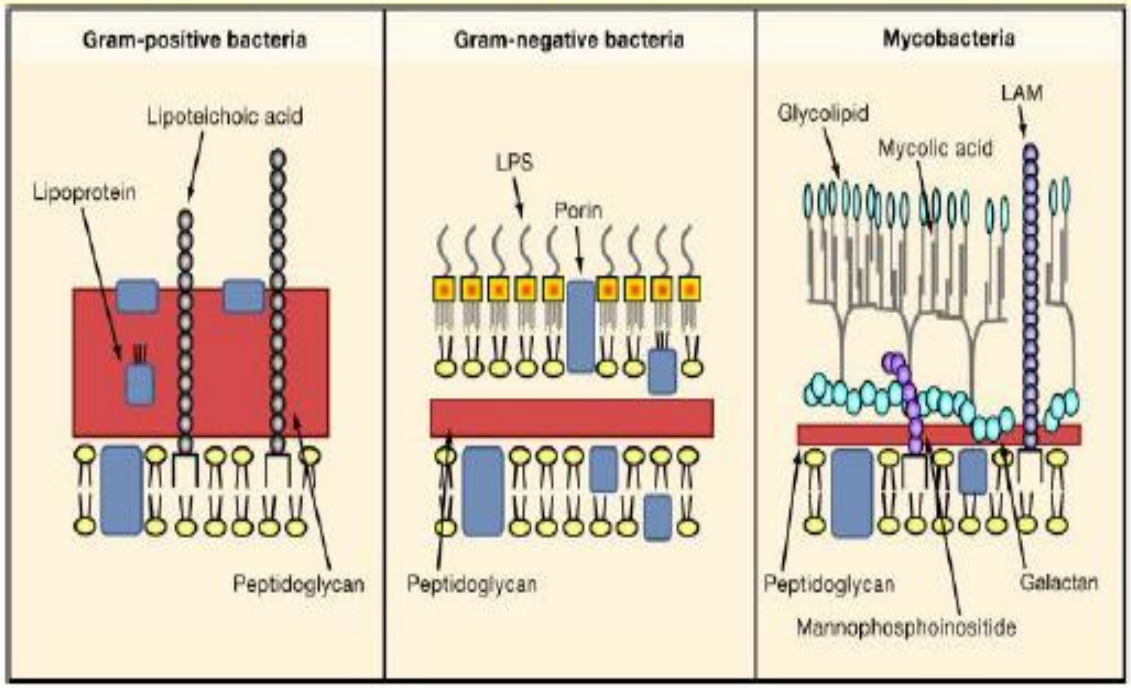
  Son activos selectivamente frente a un grupo determinado de bacterias

  Ej: **Macrólidos:** cocos Gram (+)
  Gentamicina: bacilos Gram (-)

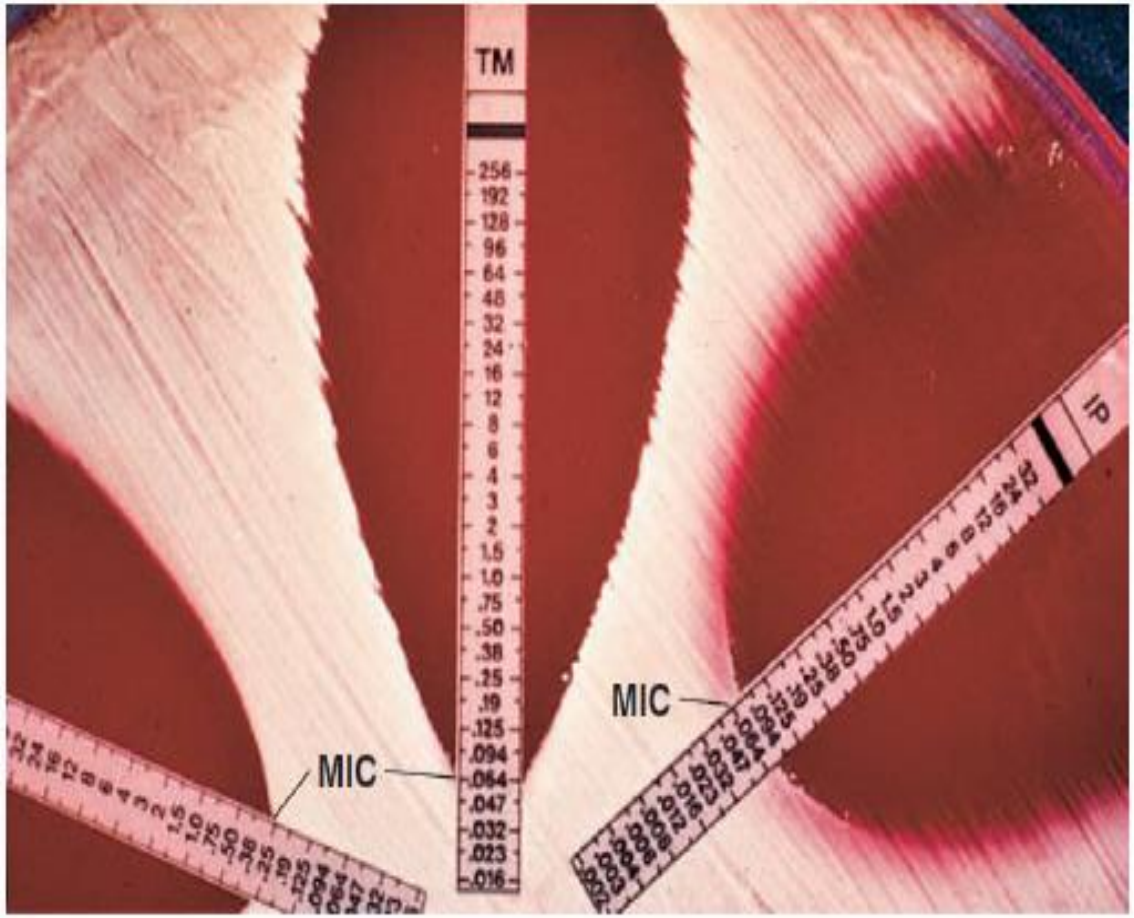
- **Espectro amplio**:

  Presentan actividad frente a la mayoría de los grupos bacterianos de importancia clínica

  Ej: **Penicilina:** cocos Gram (+), cocos Gram (-), bacilos Gram (+)
  Ampicilina: cocos Gram (+) y Gram (-), algunos bacilos Gram (-)

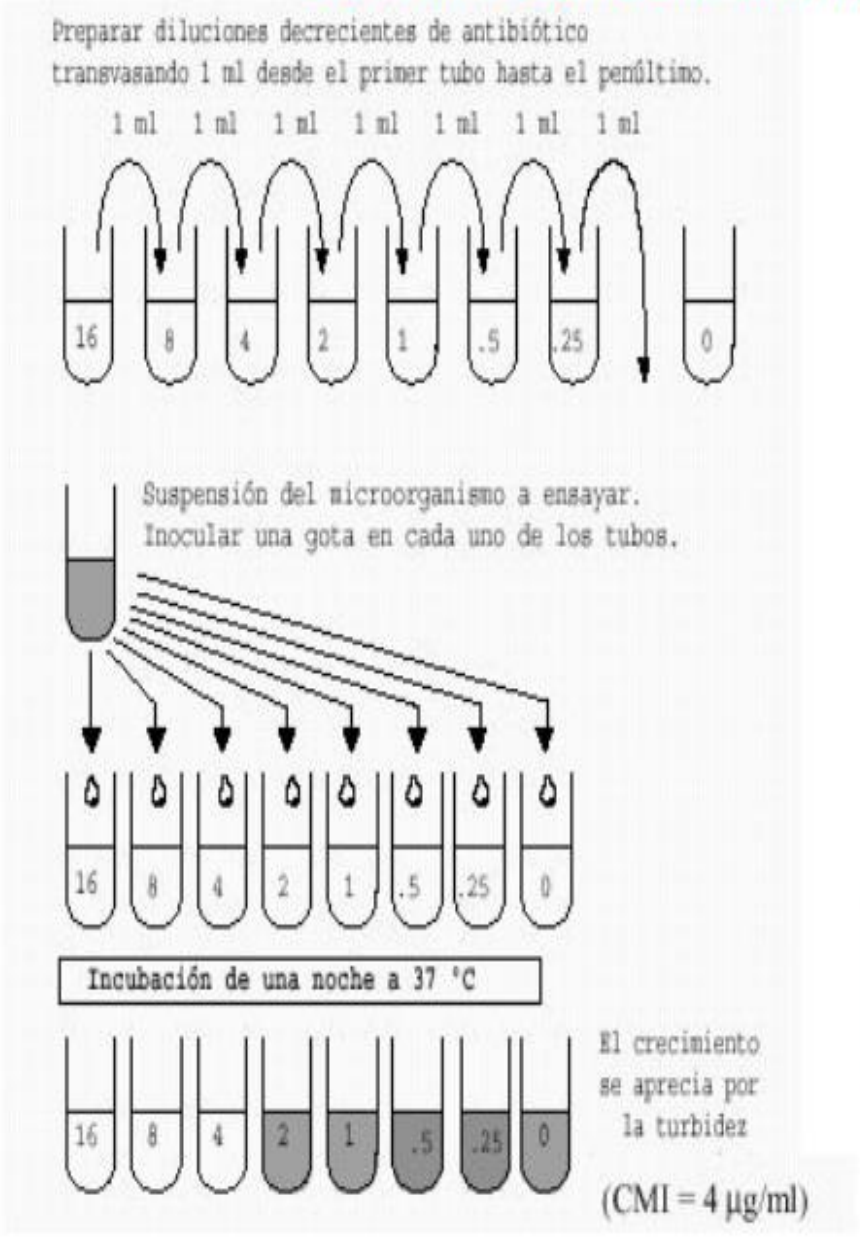


Determinación de CIM: Antibiograma

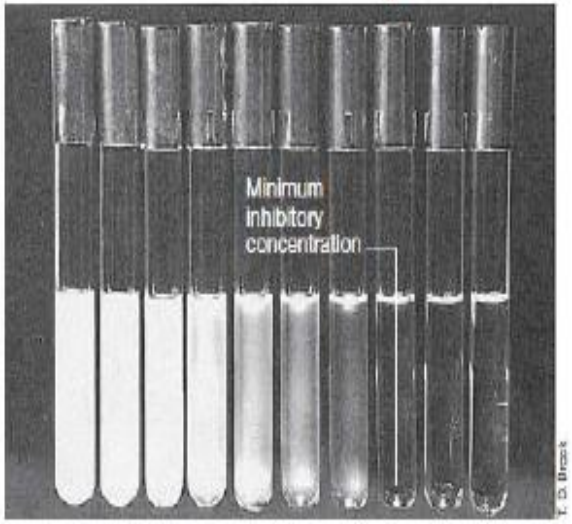


Antibiograma cuantitativo: la tira de antibiotico posee una gradiente de la droga que permite a simple vista determinar la CIM de la bacteria en estudio.

Determinación de la CIM: Método Clásico

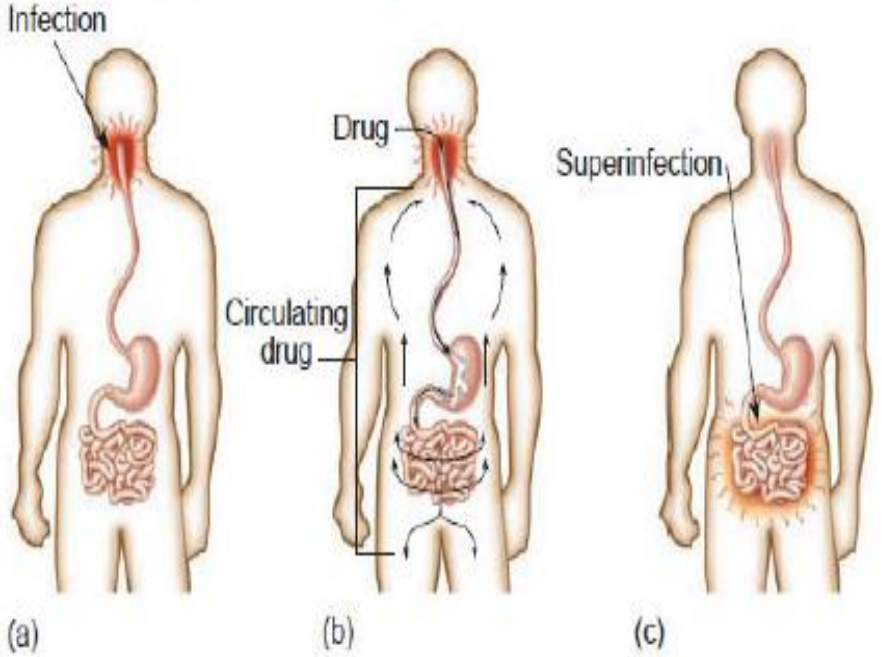


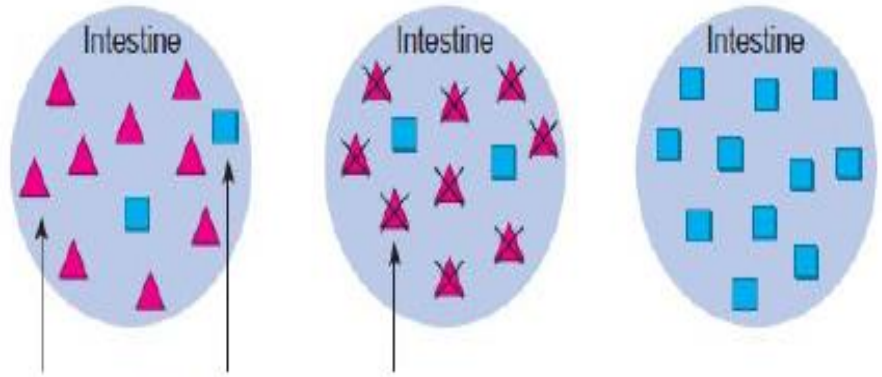
Concentración Inhibitoria Mínima = CIM



Indice Terapéutico
IT = Dosis Inhibicion Humana/CIM

Acción de antibióticos sobre flora normal





Bacteria Intestinal Normal

Patógeno

Inhibición

Porque es importante conocer los blancos y los mecanismos de acción de los ATB?

1- Sentar las bases de la toxicidad selectiva

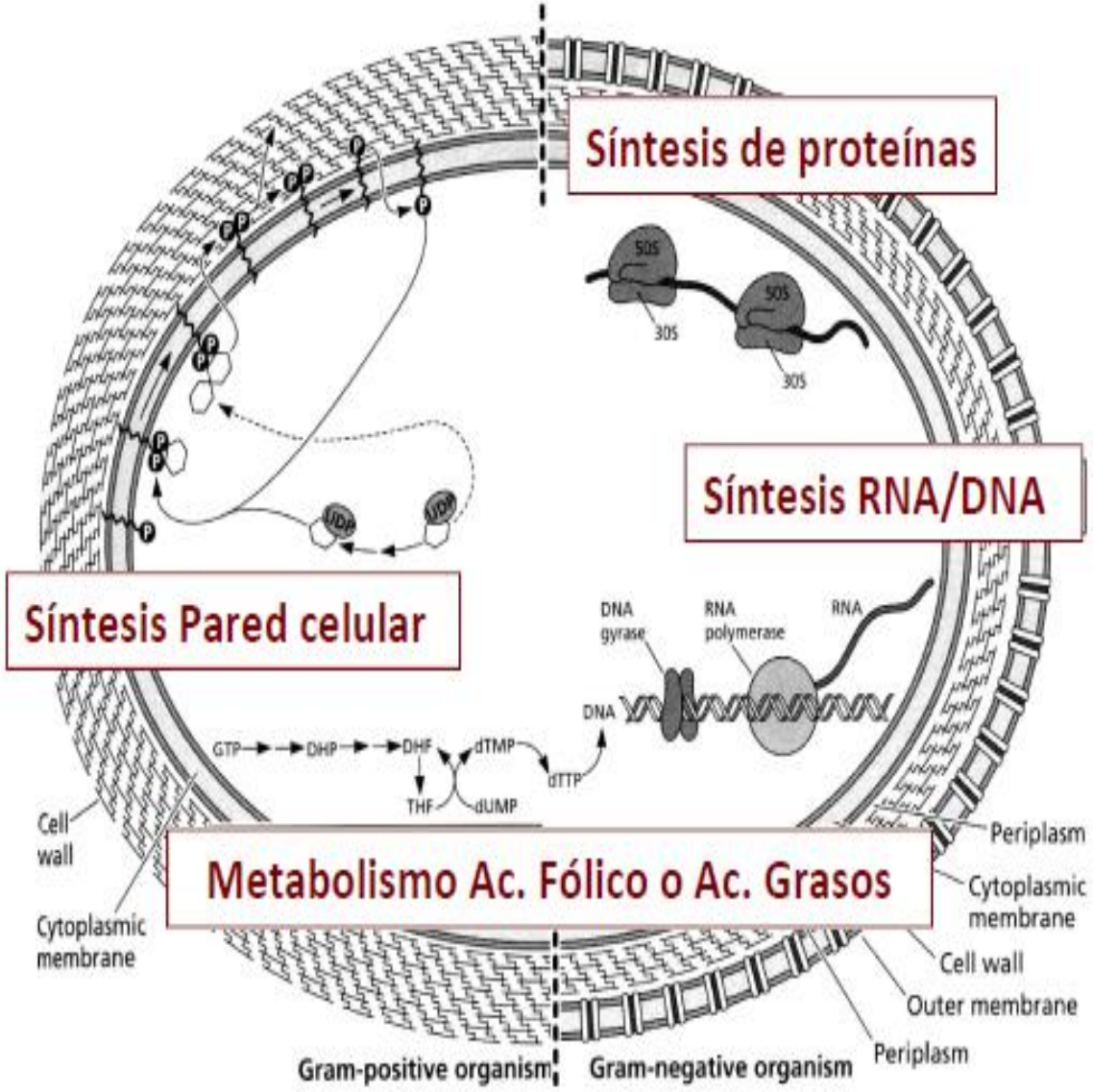
2- Categorizar los nuevos ATB

3- Encontrar un nuevo sitio blanco

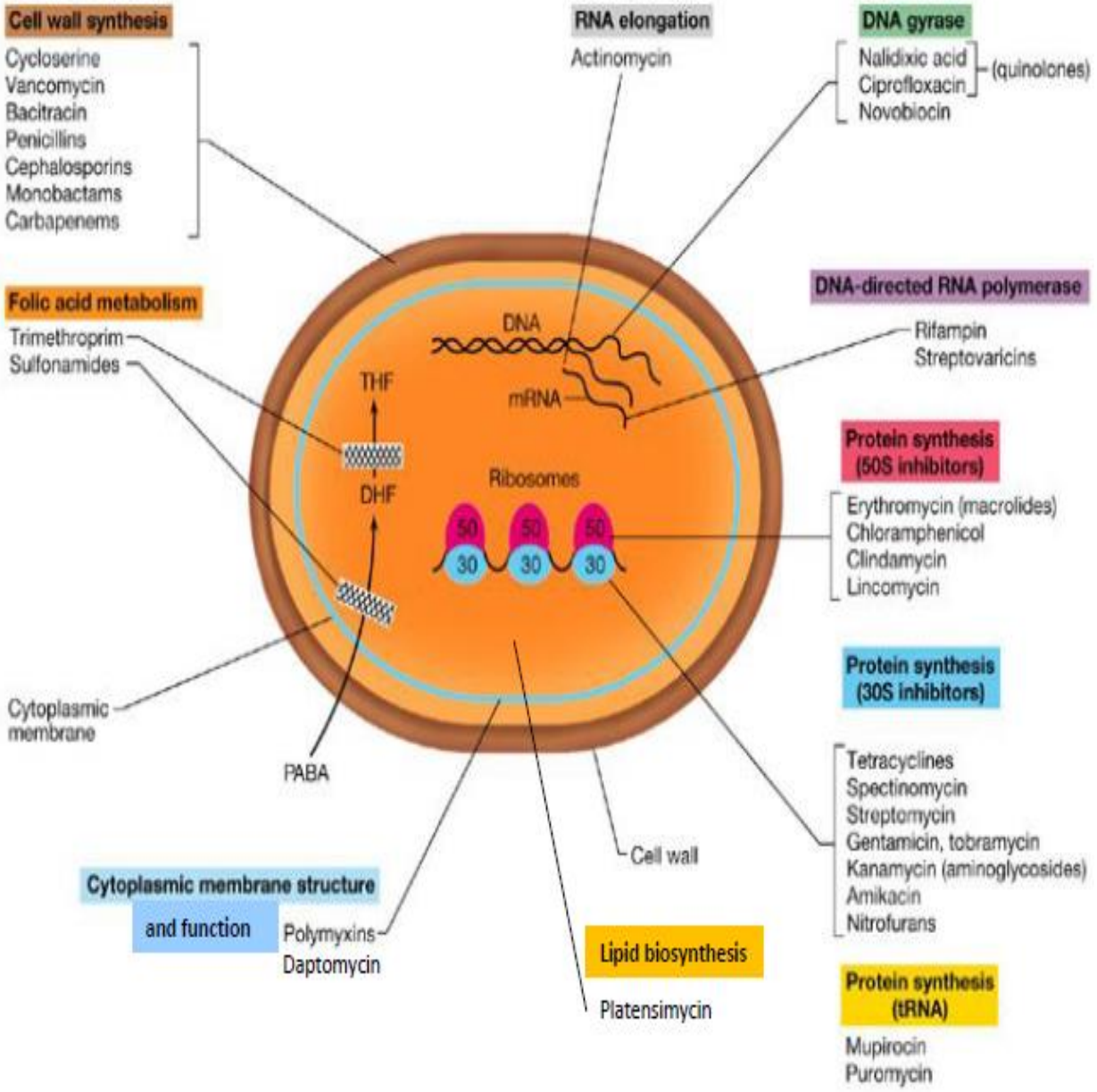
Cuál es la base de la toxicidad selectiva de los antibióticos?

- Procesos celulares presentes solo en microorganismos → Síntesis de pared o folato
- Procesos similares pero con diferencia estructurales suficientes → Ribosomas

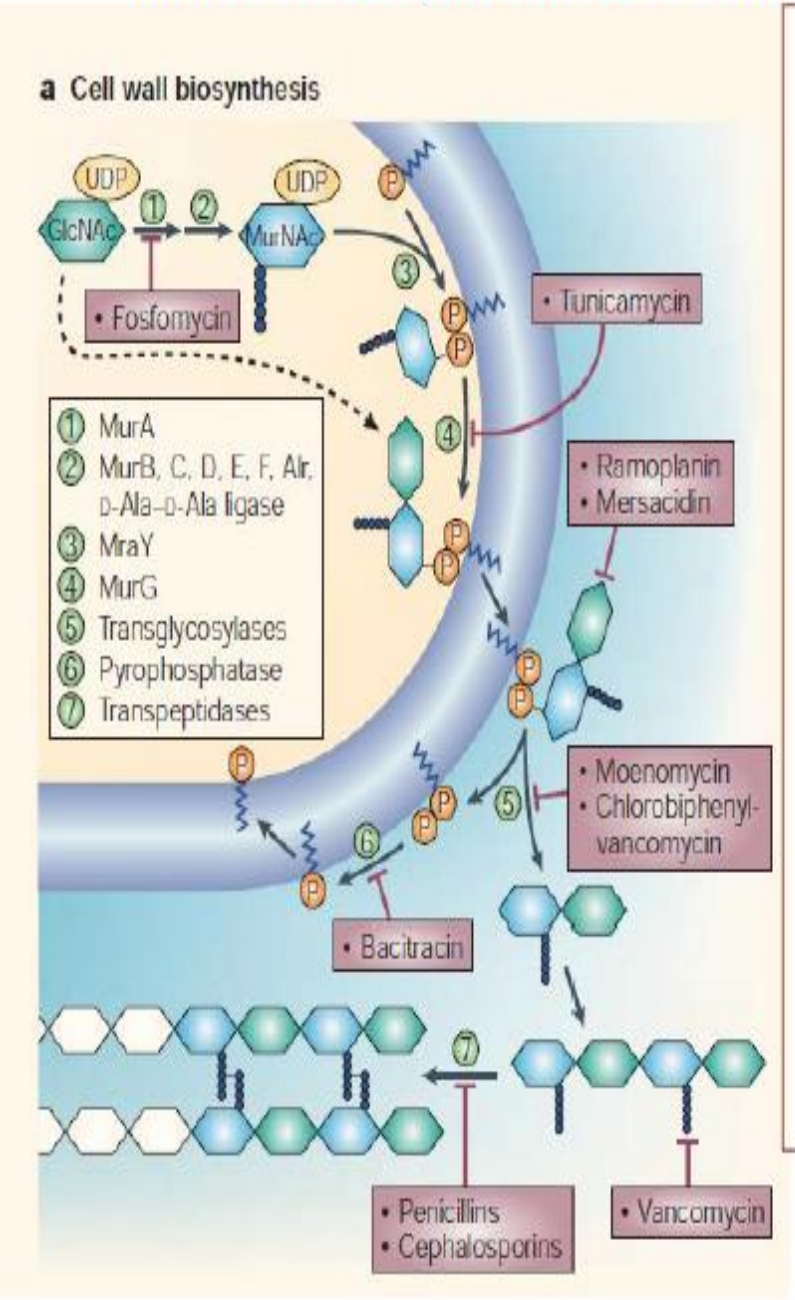
Clasificación de los antibióticos según su mecanismo de acción



Sitios blanco de acción de los principales antibacterianos



Antibióticos que inhiben la síntesis de pared celular



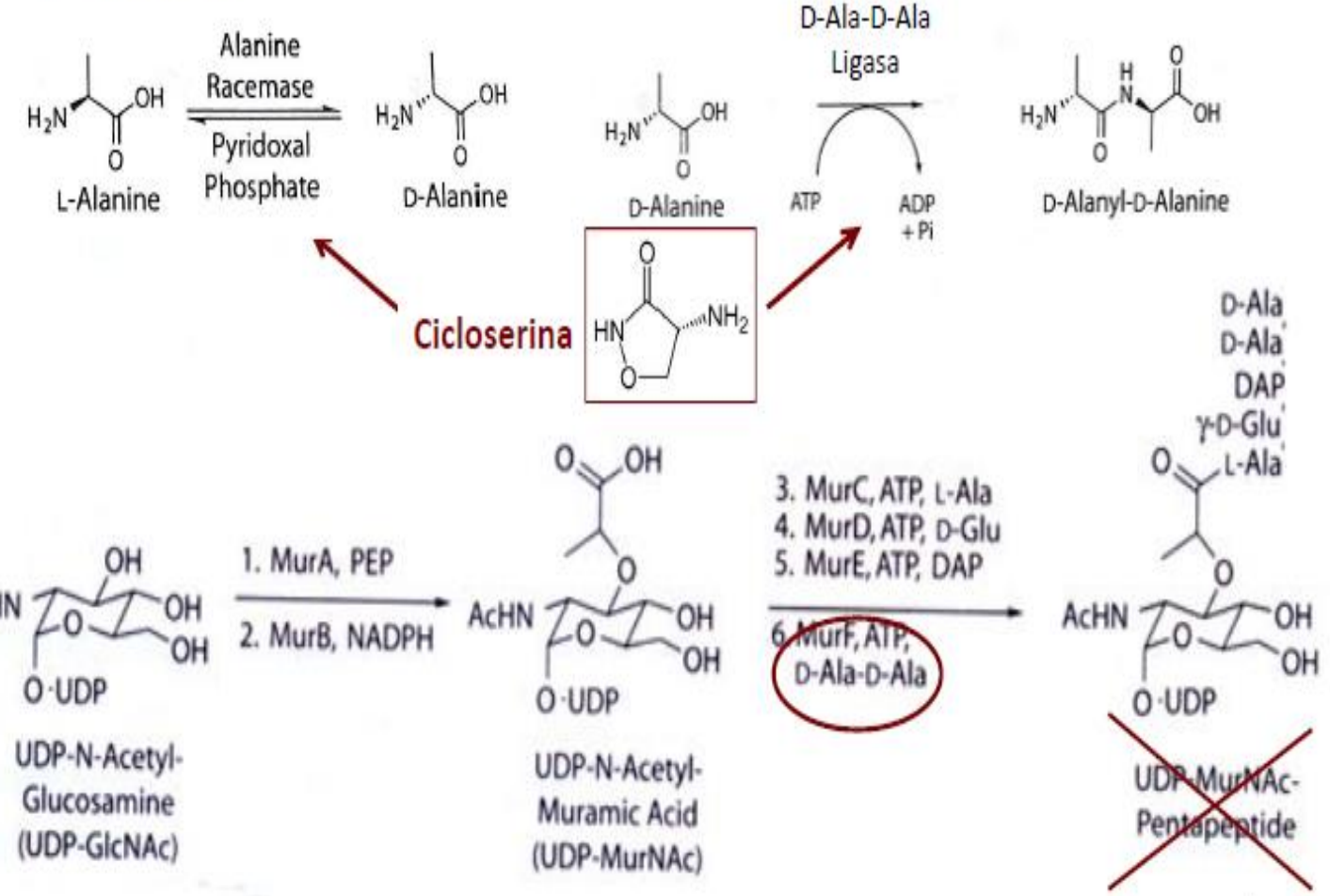
- Inhiben enzimas biosintéticas
  Fosfomicina, Cicloserina *
- Se combinan con moléculas transportadoras
  Bacitracina
- Secuestro de sustratos de la pared
  Vancomicina
- Inhiben las reacciones de entrecruzamiento del peptidoglicano
  Penicilinas, Cefalosporinas *

* Análogos de sustratos

Antibióticos que inhiben la síntesis de pared celular

Inhibición de enzimas biosintéticas

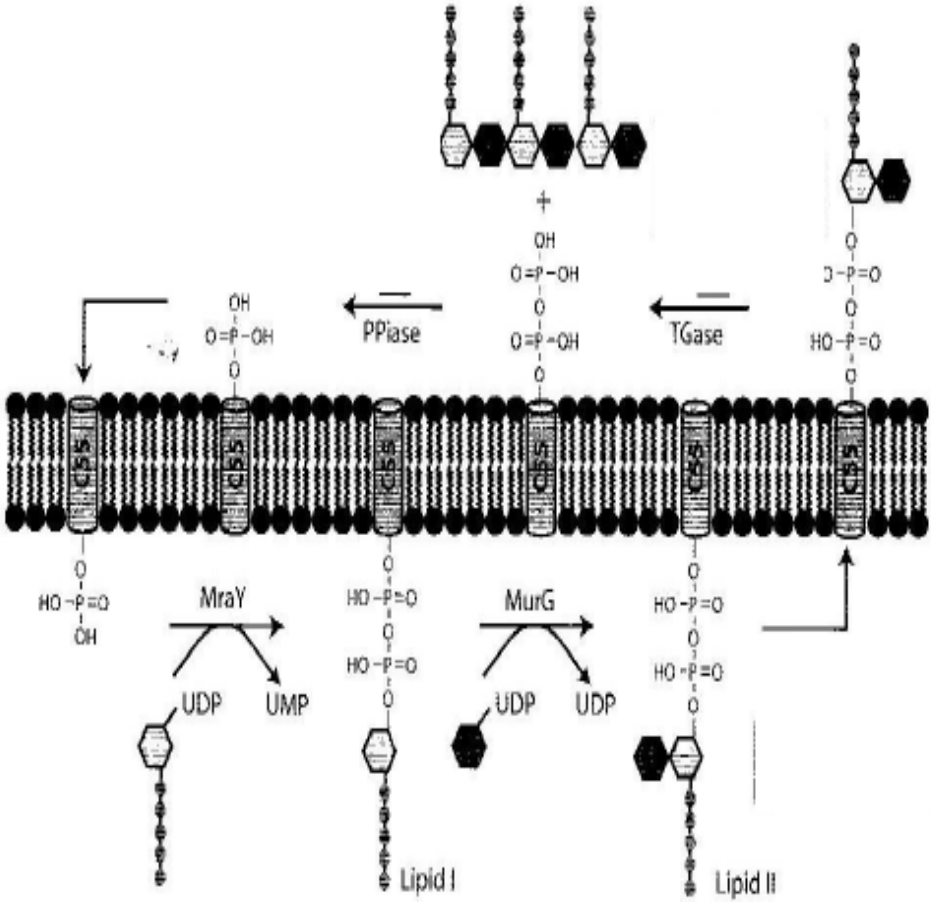
Cicloserina: Se comporta como análogo estructural de la D-alanina, por lo que inhibe la acción de la racemasa que convierte la L-ala a D-ala, así como de la reacción de unión de dos D-ala.



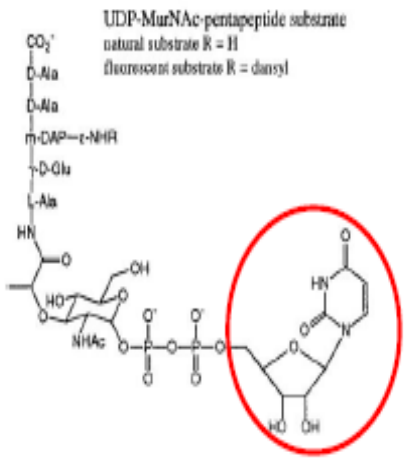
Antibióticos que inhiben la síntesis de pared celular

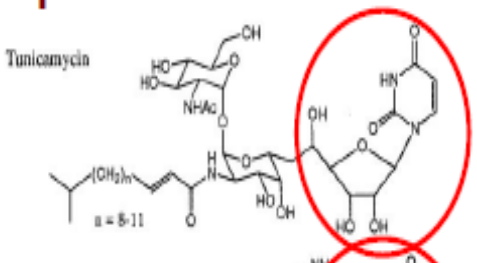
Interacción con moléculas transportadoras

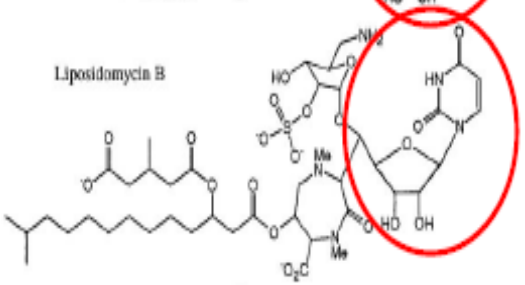
Bactoprenol: poliisoprenoide de 55 á tomos de C que transporta las subunidades de peptidoglicano hacia la cara externa de la membrana.

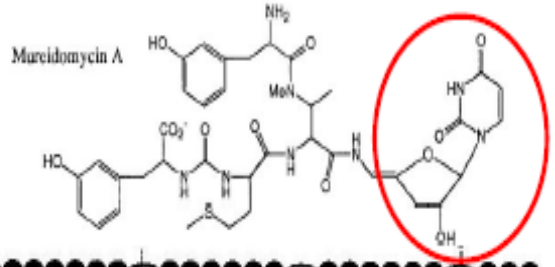


Antibióticos que inhiben la síntesis de pared celular

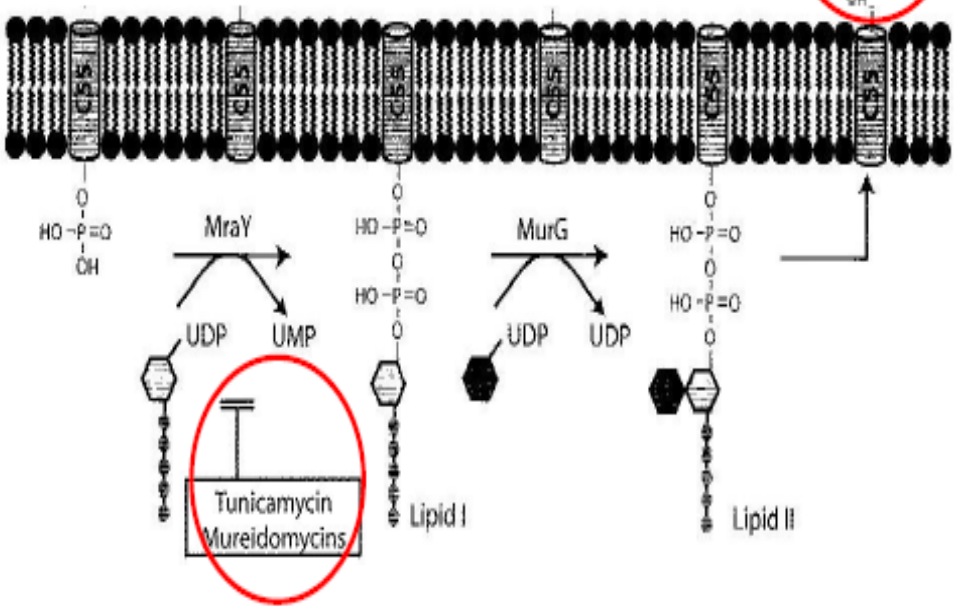




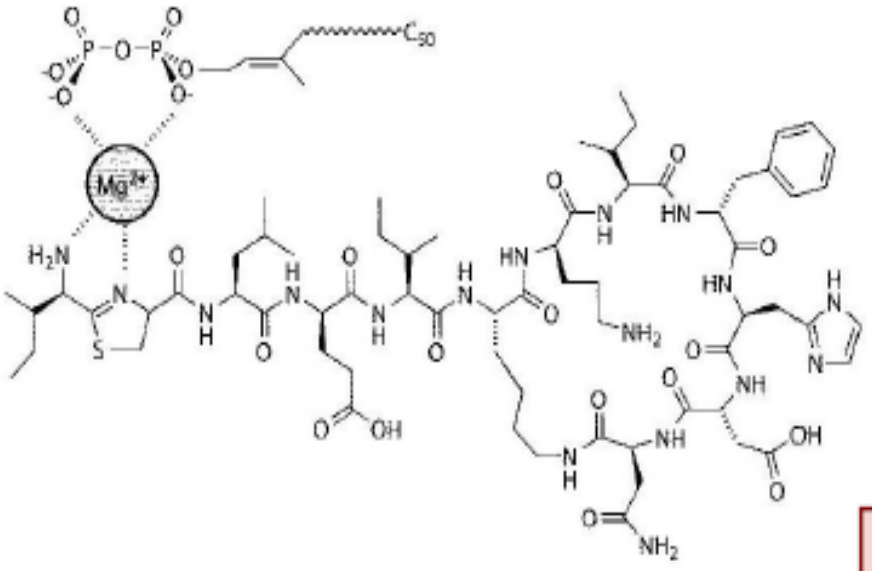




Tunicamicinas y Mureidomicinas son uridil péptidos que actúan como análogos de sustrato

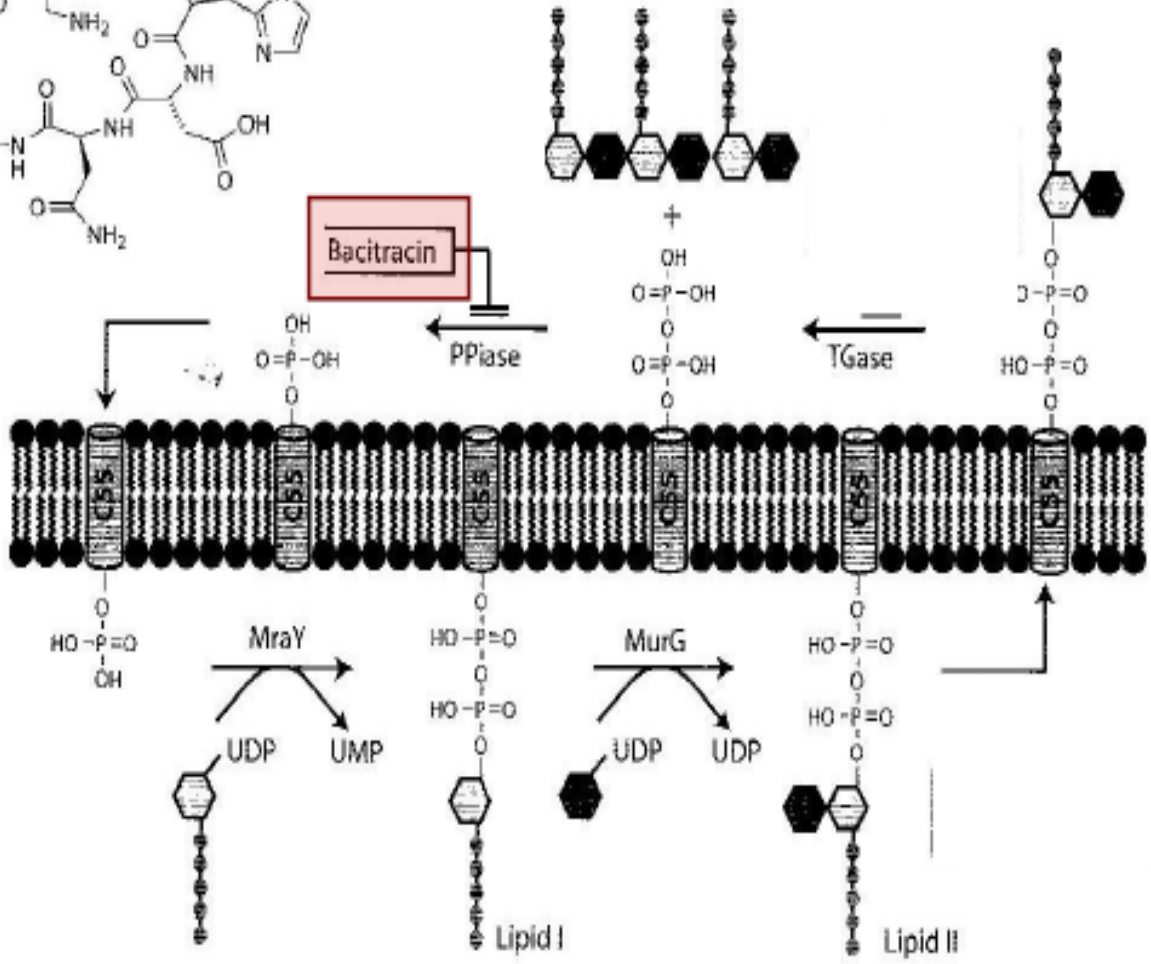


Antibióticos que inhiben la síntesis de pared celular



Bacitracina: péptido no ribosómico

Se une al undecaprenol-pirofosfato en presencia de Mg^{+2}, bloqueando su desfosforilación, impidiendo la regeneración del transportador de membrana



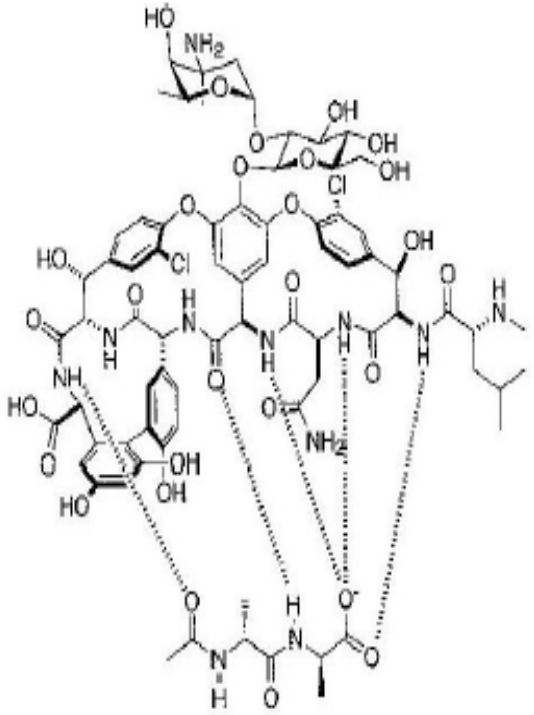
Antibióticos que inhiben la síntesis de pared celular

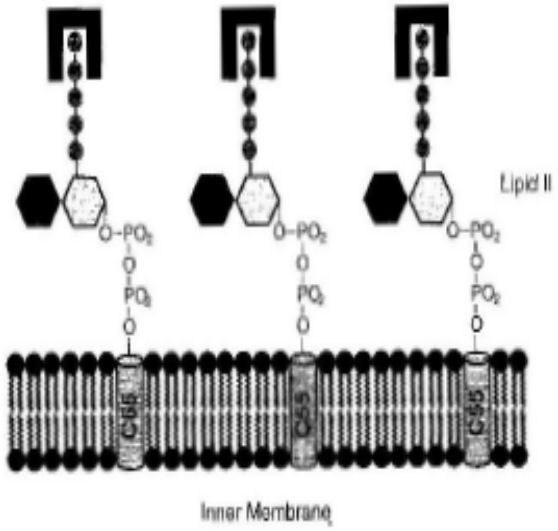
Secuestro del sustrato de la pared

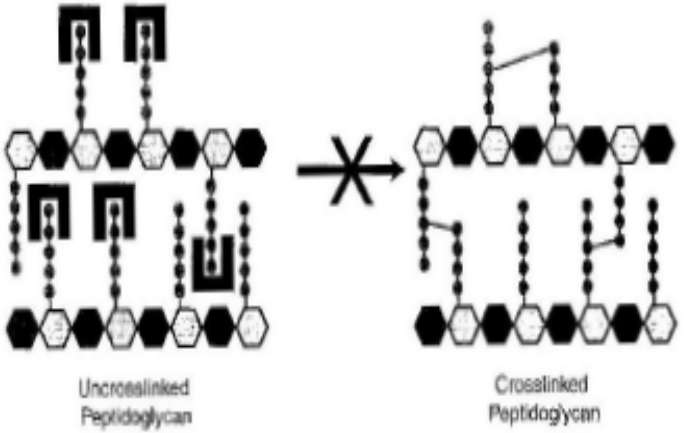
***Vancomicina*: glicopéptido**

Forma un complejo con los residuos de D-alanina del muramilpentapéptido, estabilizado por cinco puentes de hidrógeno.

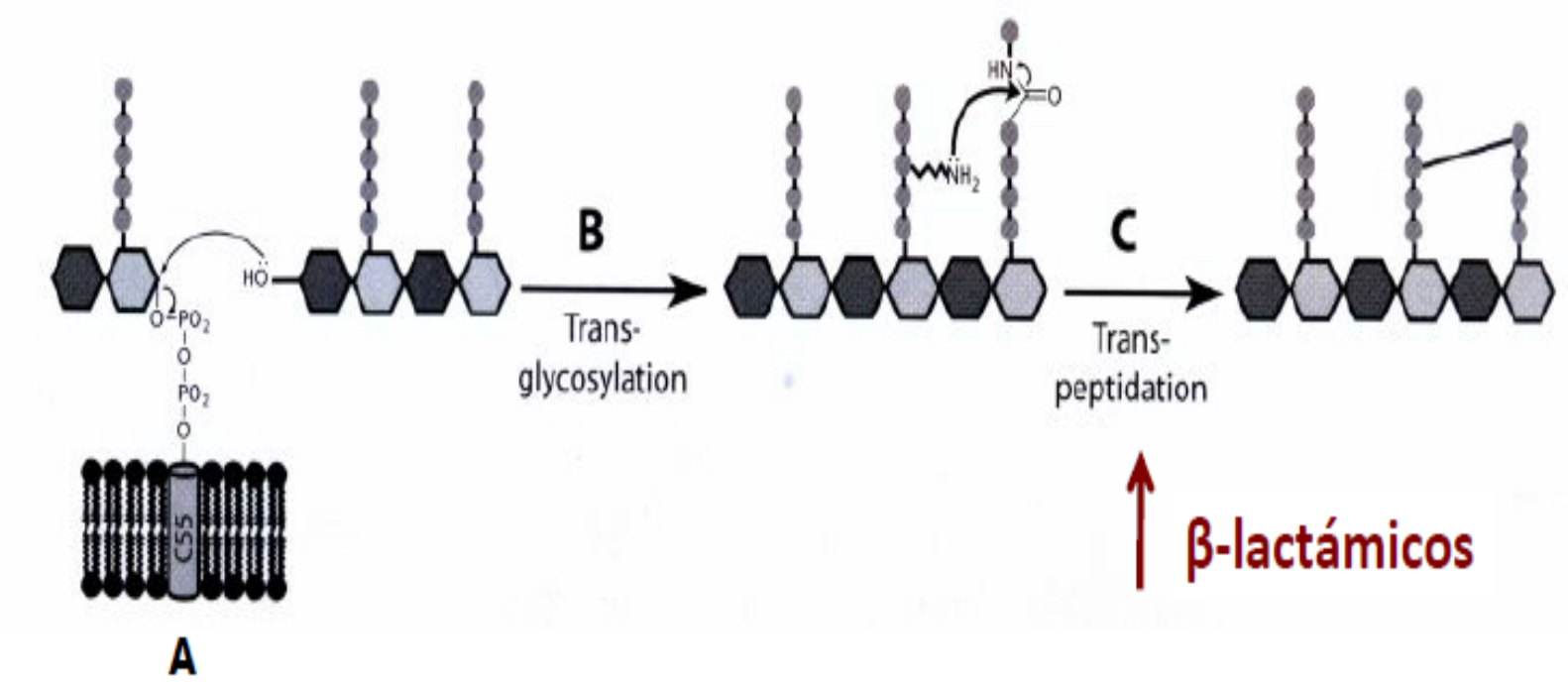
Impide la transferencia de los precursores desde el transportador lipídico y la transpeptidación.







Etapas finales de la síntesis de pared celular



A- Bactoprenol: transporta los monómeros de peptidoglicano sobre la membrana citoplasmatica

B- Transglicosilasa: forma la unión entre la unidades β-(1-4)-disacaridicas

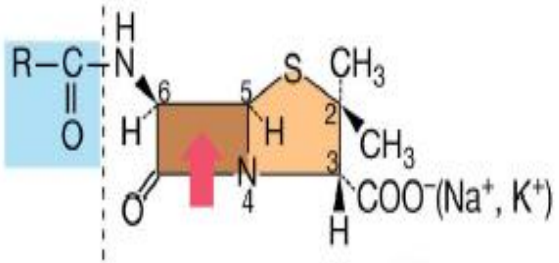
C- Transpeptidasa: enlaces interpeptídicos

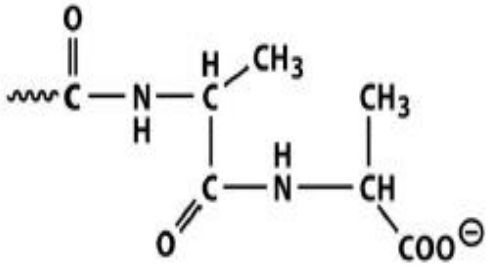
D- Carboxipeptidasas: eliminan el extremo D-Ala de las cadenas que van a entrecruzarse

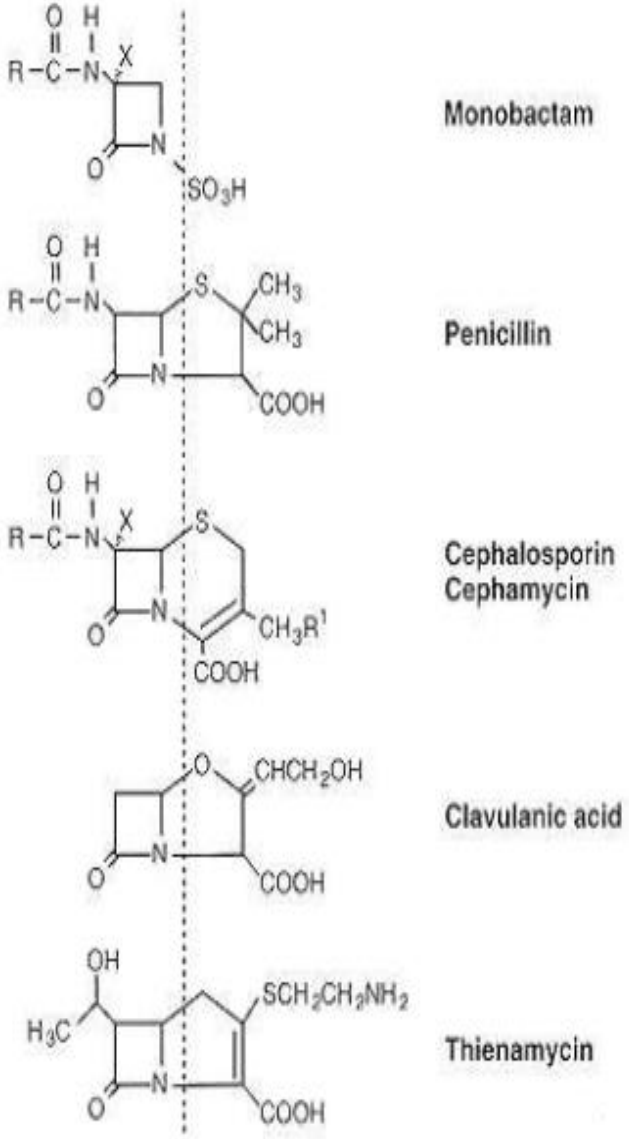
Antibióticos que inhiben la síntesis de pared celular

Inhibición de las reacciones de entrecruzamiento (transpeptidación) del peptidoglicano

β-lactámicos







Antibióticos que inhiben la síntesis de pared celular

Inhibición de las reacciones de entrecruzamiento (transpeptidación) del peptidoglicano

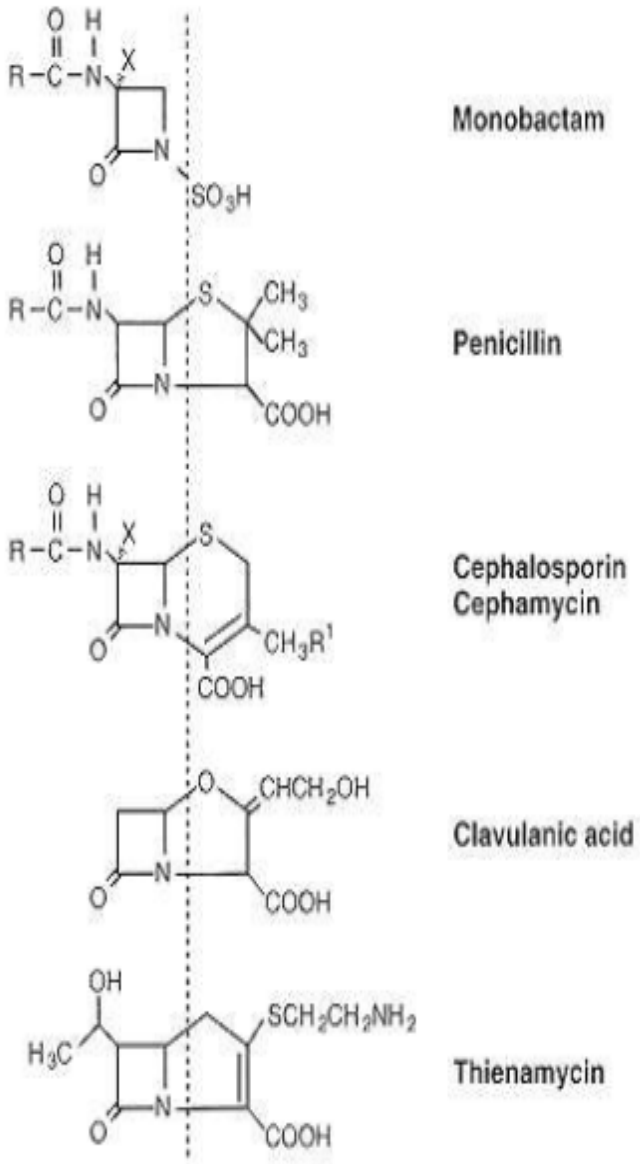
Monobactamas: ej. Aztreonam. Activos sólo frente a Gram negativos.

Penicilinas naturales: ej. Penicilina G, Penicilina V: espectro reducido, activas contra Gram positivos. Sensibles pH ácido

Cefalosporinas: ej. Cefazolina, Cefalexina. Espectro reducido a Gram positivos.

Carbapenemas: ej. Imipenem. Amplio espectro. Resistentes a β-lactamasas al igual que las Monobactamas.

Ac. Clavulámico: Inhibe las β -lactamasas



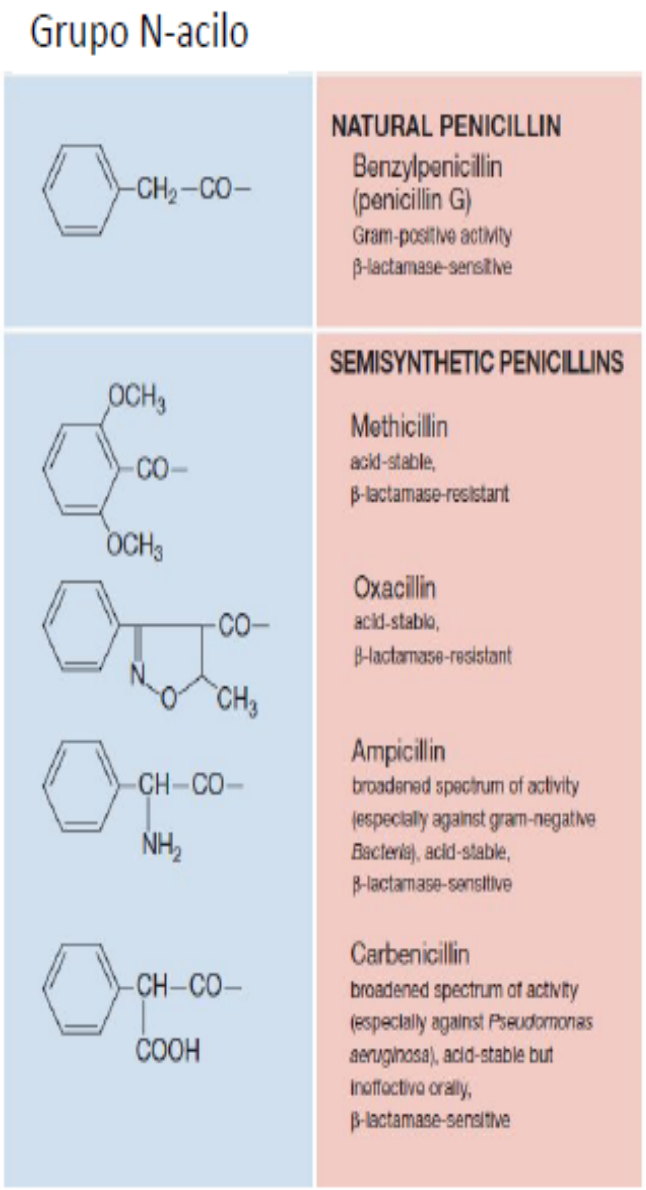
Antibióticos que inhiben la síntesis de pared celular

β-lactámicos de segunda generación

Penicilinas naturales: ej. Penicilina G, Penicilina V: espectro reducido, activas contra Gram positivos. *Sensibles pH ácido*

Aminopenicilinas: ej. Ampicilina, Amoxicilina. Amplio espectro. Activas contra Gram positivos y Gram negativos. *Más resistentes al pH ácido.*

Carboxipenicilinas: ej. Carbenicilina, Ticarcilina. Actividad anti *Pseudomonas*

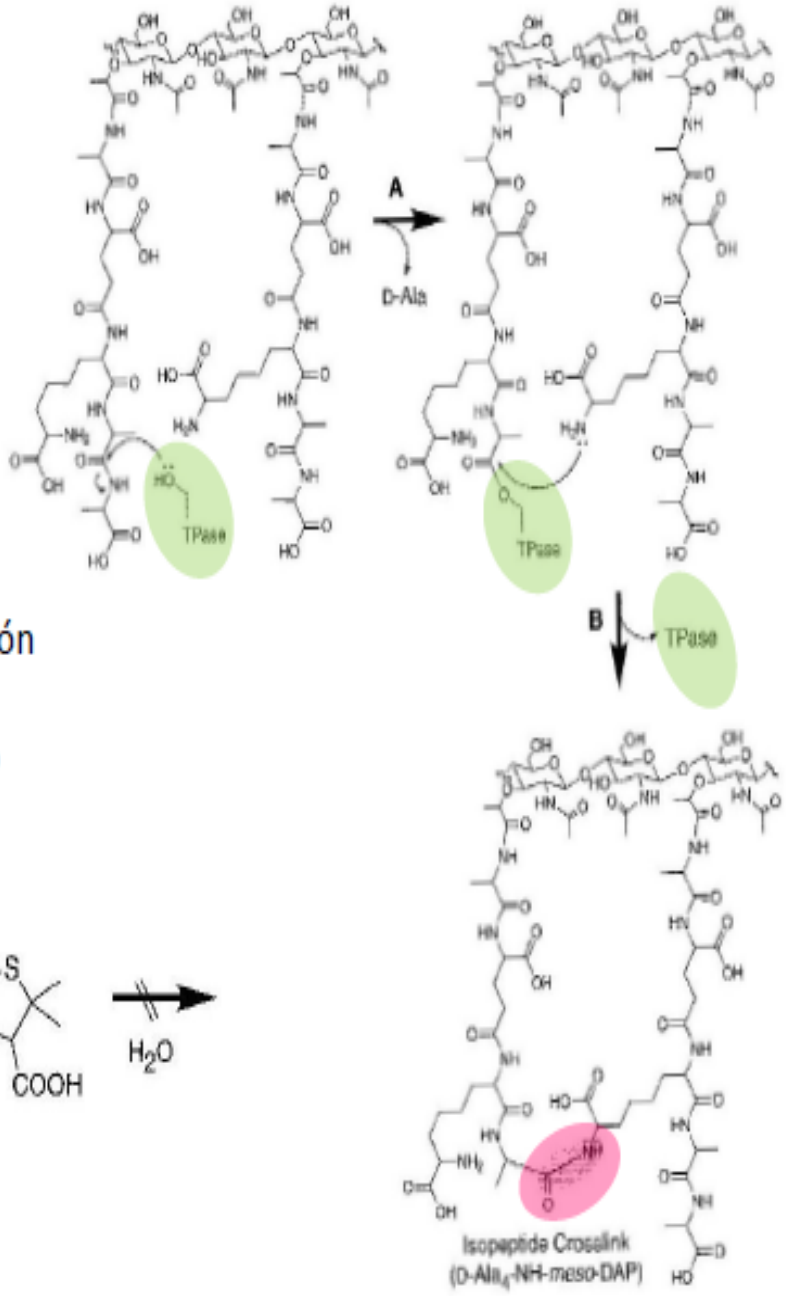


Antibióticos que inhiben la síntesis de pared celular

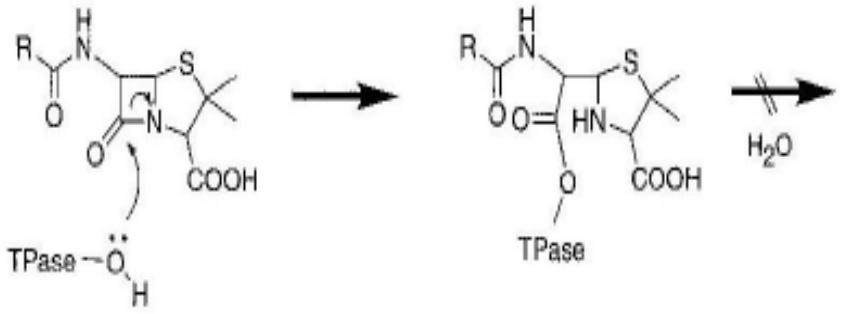
ß-lactámicos: Mecanismo de acción

Transpeptidasas

PBP (*Penicillin Binding Proteins*)



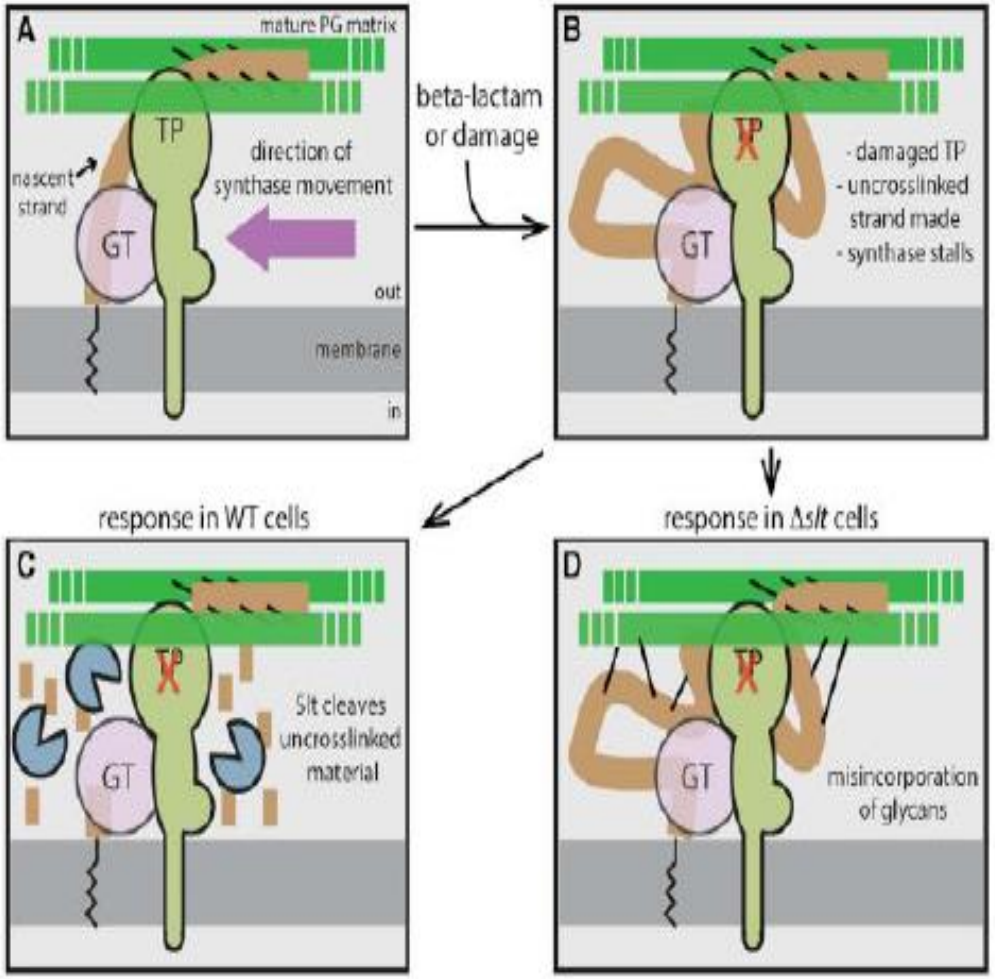
Inhibición de la reacción de transpeptidación y del entrecruzamiento del peptidoglicano
Activan el mecanismo autolítico endógeno bacteriano



Acción bactericida de los β-lactámicos

Acción bactericida de β-lactámicos:

Inhiben la TP, acumulación de precursores del PG, activación de autolisinas. Si esta en medio hipotónico la bacteria se lisa. Cuando no está creciendo no tienen efecto



Proteínas de unión a β-lactámicos (PBP)

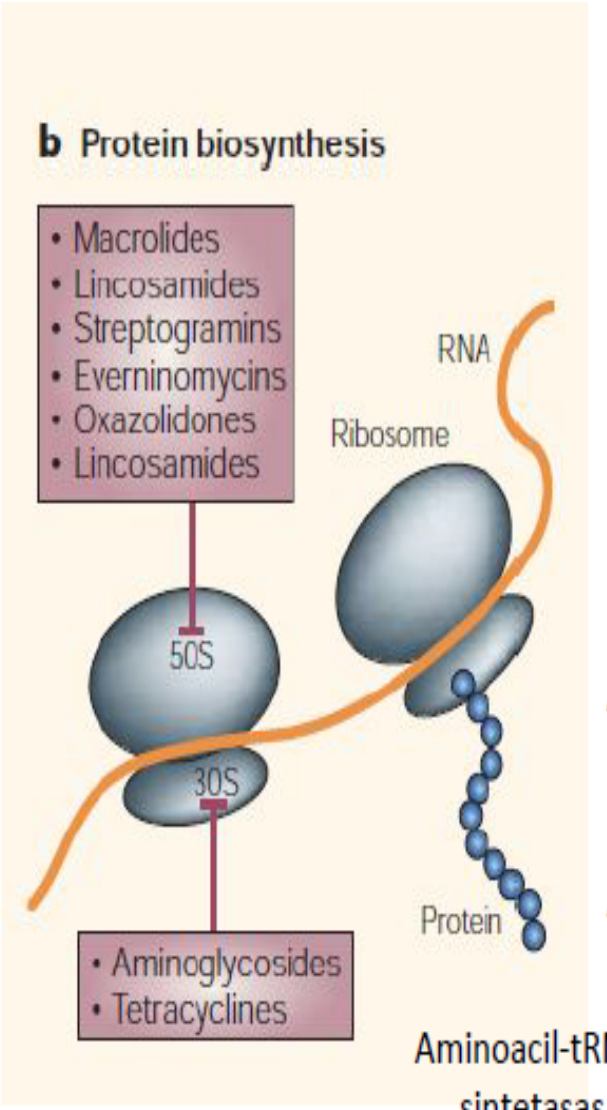
- ✓ Enzimas sensibles a penicilina o cefalosporinas
- ✓ Distinto grado de afinidad frente a los distintos β-lactámicos
- ✓ Existen distintas PBPs con diferentes actividades.
- ✓ Intervienen en elongación y en la forma de la bacteria
- ✓ No todas las especies bacterianas presentan idéntico perfil de PBPs.
- ✓ Algunas tienen actividad carboxipeptidasa (CP) que liberan la alanina terminal
- ✓ Algunas son bifuncionales (TP y CP)

SDS-PAGE
^{14}C-peniciloil-proteinas

PBPs con actividad transglucosidasa y transpeptidasa:	Función natural	Acción de la penicilina
PBP 1a y PBP 1b	Elongación del cilindro celular	lisis rápida
PBP 2	Condiciona la forma de la célula	la célula se redondea y muere
PBP 3	Formación del septo transversal	Filamentación y muerte
PBPs con actividad carboxipeptidasa (endopeptidasa)	**Función natural**	**Acción penicilina**
PBP 4, PBP 5, PBP 6	Eliminan la D-ala terminal delpentapéptido (maduración PG)	no letal

Inhibición de la síntesis de proteínas

Antibióticos que inhiben la síntesis de proteínas



Actúan sobre la subunidad 30S del ribosoma

Aminoglicósidos: Estreptomicina, Neomicina, Kanamicina, Gentamicina, Tobramicina, Amikacina

Tetraciclinas: Doxiciclina, Tetraciclina

Actúan sobre la unidad 50S del ribosoma

Cloranfenicol

Macrólidos: Eritromicina, Azitromicina, Claritromicina

Lincosamidas: Clindamicina

Actúan sobre el ensamblado del ribosoma

2-Oxazolidona: Linezolida

Actúan sobre las aminoacil-tRNA sintetasas

Mupirocina

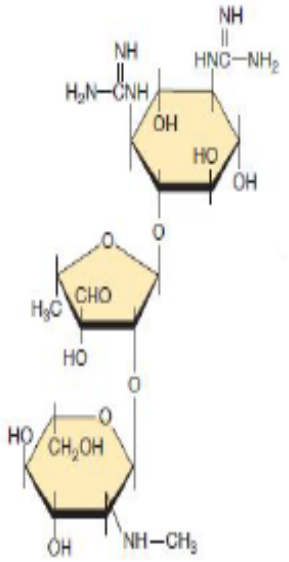
Antibióticos que inhiben la síntesis de proteínas

Actúan sobre la subunidad 30S del ribosoma

Aminoglicósidos

Son azúcares complejos unidos por enlaces glicosídicos. Atraviesan la membrana citoplasmática por un mecanismo oxígeno-dependiente. Los grupos $-NH_2$ y -OH interactúan con proteínas del ribosoma.

Dr. Selman A. Waksman

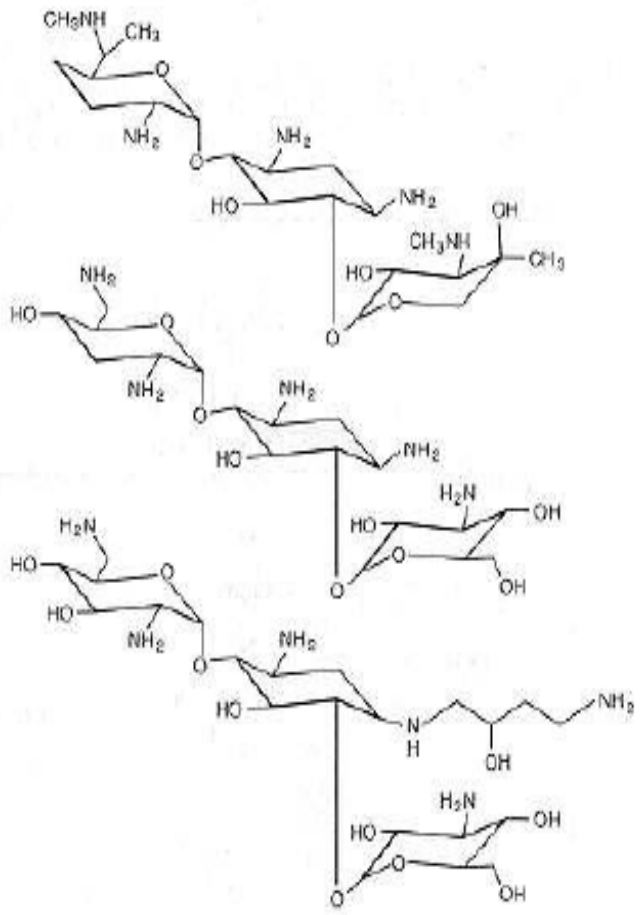


La **estreptomicina** fue aislada en 1944 por el Dr. Selman Waksman a partir de *Streptomyces griseus*

Gentamicin

Tobramycin

Amikacin



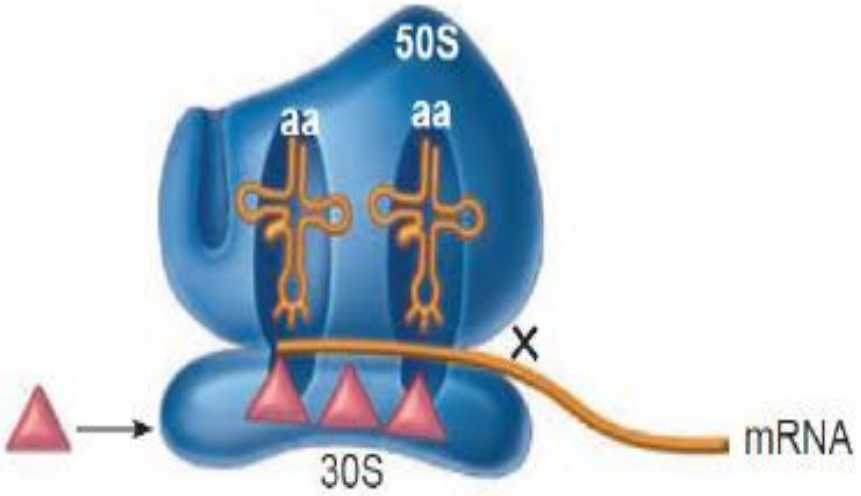
Antibióticos que inhiben la síntesis de proteínas

Que actúan sobre la subunidad 30S del ribosoma

Aminoglicósidos: Mecanismo de Acción

Se unen a la subunidad 30S del ribosoma:

- Bloquea la formación del complejo de iniciación
- Produce lectura errónea del mensaje: proteína defectuosa
- El resultado final es la muerte de la bacteria, son bactericidas



Antibióticos que inhiben la síntesis de proteínas

Que actúan sobre la subunidad 30S del ribosoma

Aminoglicósidos: Mecanismo de Acción

Se unen a la subunidad 30S del ribosoma:

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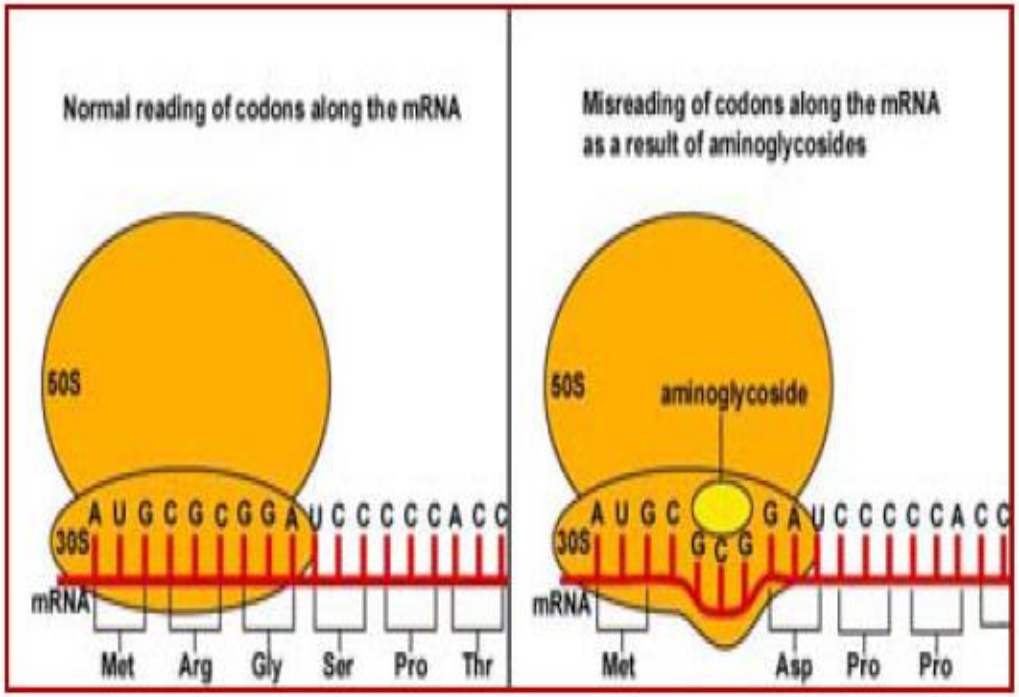
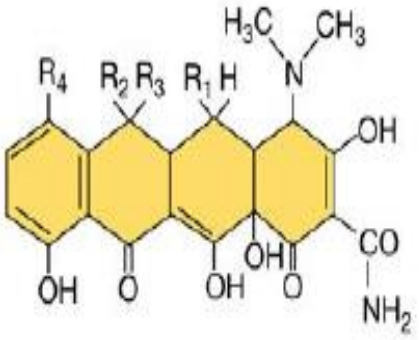


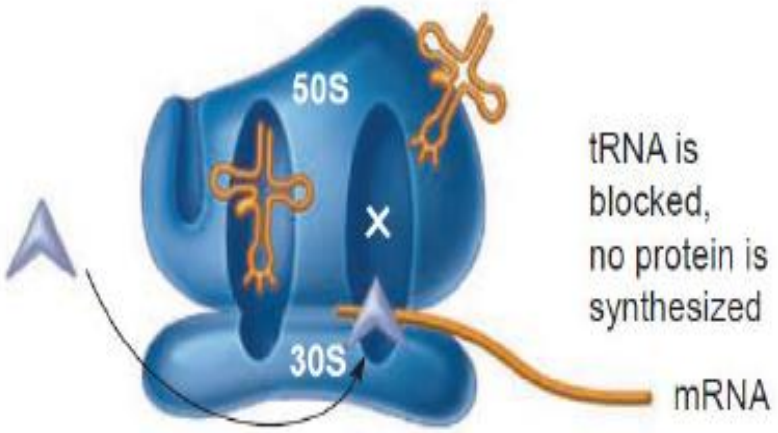
Antibióticos que inhiben la síntesis de proteínas

Que actúan sobre la subunidad 30S del ribosoma

Tetraciclinas: Estructura química y Mecanismo de acción



Tetracycline analog	R_1	R_2	R_3	R_4
Tetracycline	H	OH	CH_3	H
7-Chlortetracycline (aureomycin)	H	OH	CH_3	Cl
5-Oxytetracycline (terramycin)	OH	OH	CH_3	H



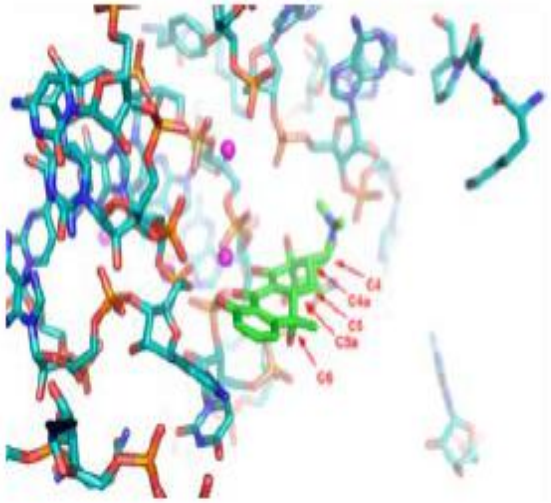
Bloquean la inserción del aminoacil-tRNA

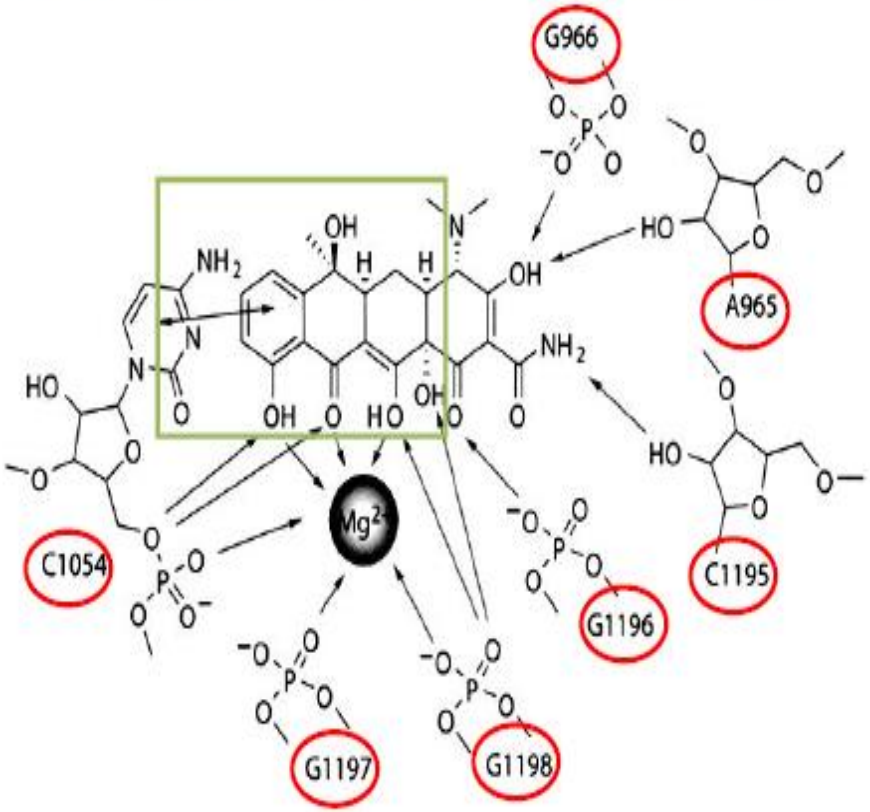
La unión es transitoria, por lo que su efecto es reversible: son bacteriostáticos.

Antibióticos que inhiben la síntesis de proteínas

Que actúan sobre la subunidad 30S del ribosoma

Tetraciclinas: Unión de la tetraciclina al ribosoma





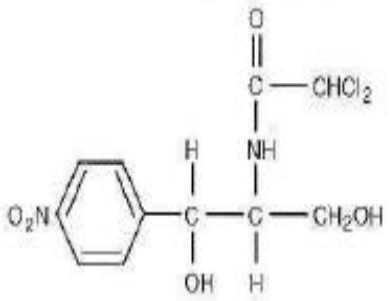
El conocimiento del sitio de unión e interacción del ATB con el blanco permite el diseño racional de nuevos ATB. Es activa en presencia de Mg^{+2}

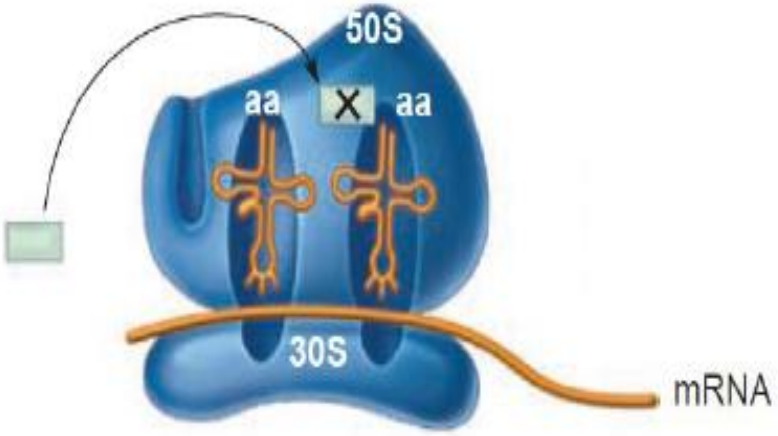
Antibióticos que inhiben la síntesis de proteínas

Que actúan sobre la subunidad 50S del ribosoma

Cloranfenicol: *Estructura química y Mecanismo de acción*

Originalmente producido por *Streptomyces venezuelae,* actualmente se sintetiza químicamente.





Se une a la enzima peptidil transferasa

en la subunidad 50S

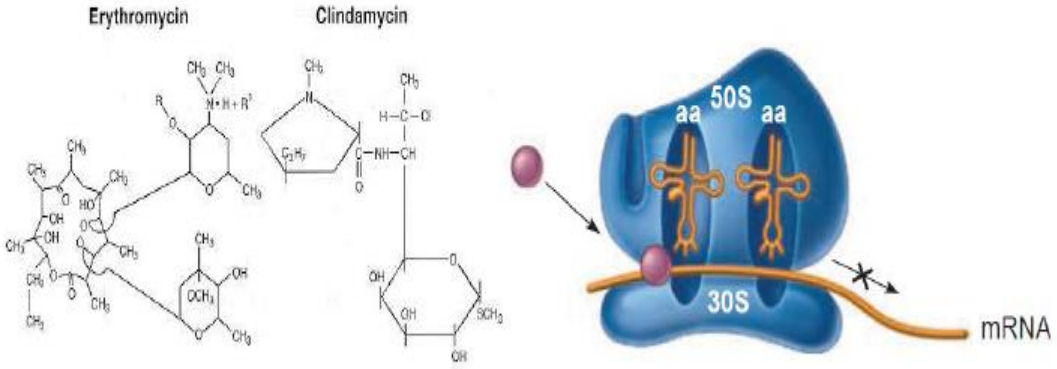
Inhibe la formación del enlace peptídico. Detiene la síntesis de proteínas.

Es un agente bacteriostático.

Antibióticos que inhiben la síntesis de proteínas

Que actúan sobre la subunidad 50S del ribosoma

Macrólidos y Lincosamidas: Estructura y Mecanismo de acción



Inhiben la peptidil transferasa y la translocación

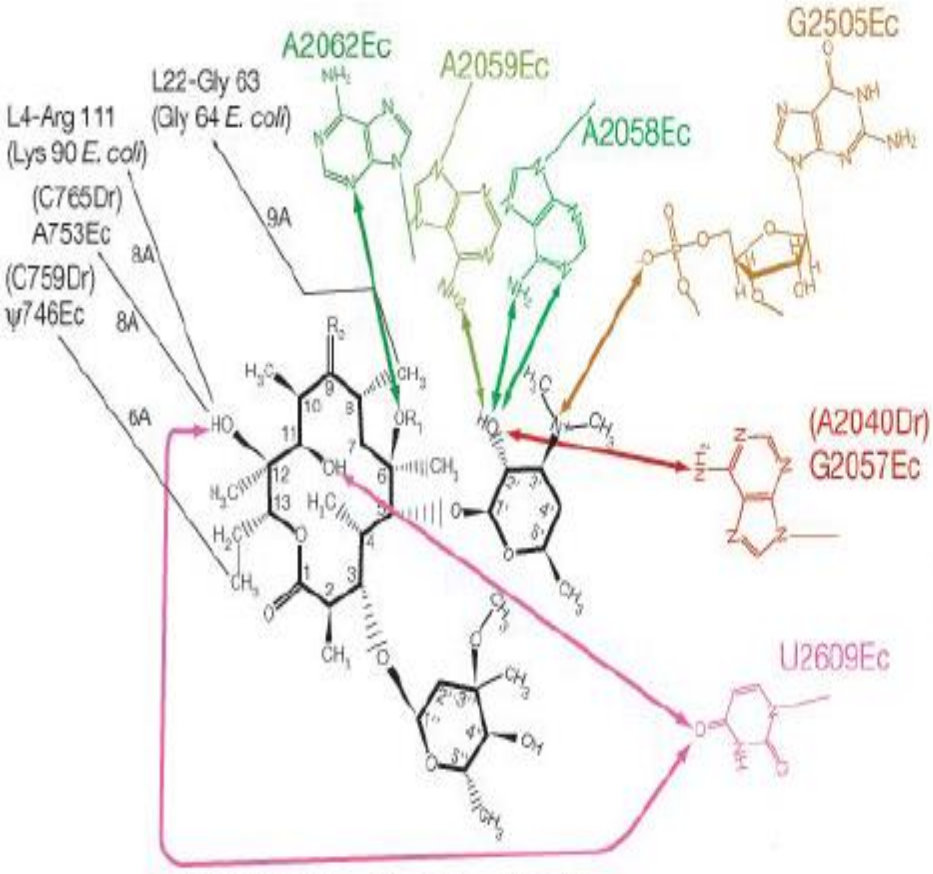
Se detiene la síntesis de proteínas liberando prematuramente el peptidil tRNA.

Son bacteriostáticos.

Antibióticos que inhiben la síntesis de proteínas

Que actúan sobre la subunidad 50S del ribosoma

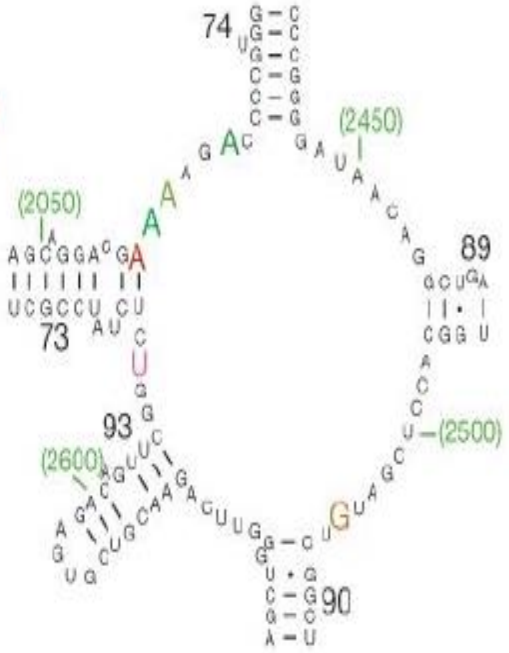
Macrólidos: Unión al ribosoma



Erythromycin R_1 = H; R_2 = O

Clarithromycin R_1 = CH_3; R_2 = O

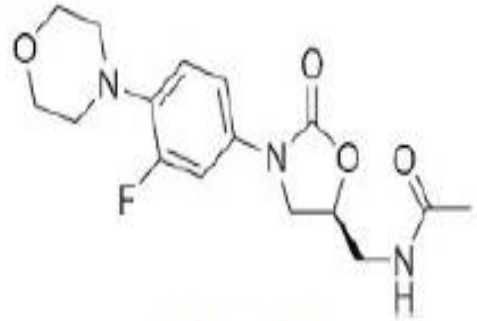
Roxithromycin R_1 = H; R_2 = N-O-CH2-O-CH2-CH2-O-CH3



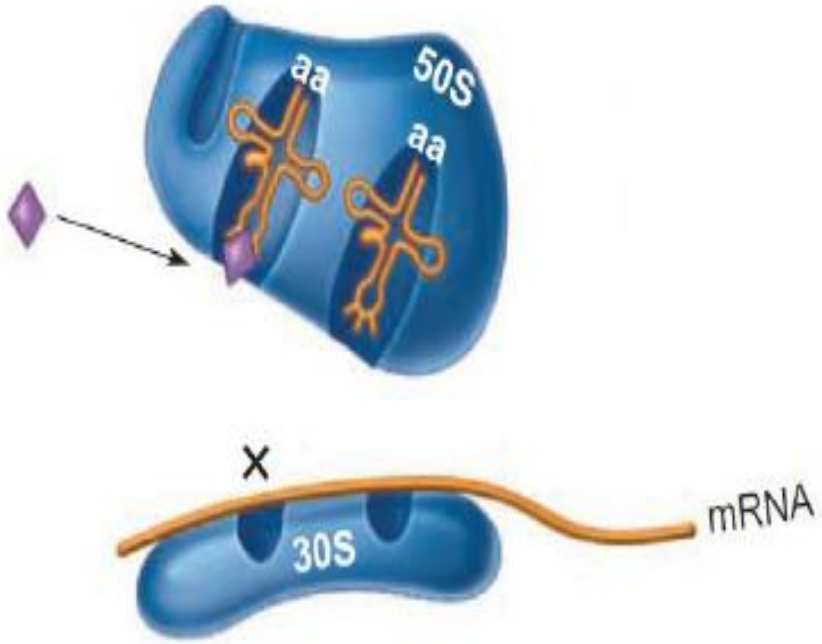
Antibióticos que inhiben la síntesis de proteínas

Inhibición del ensamblado de los Ribosomas

2-Oxazolidona: Estructura y Mecanismo de acción



Linezolida

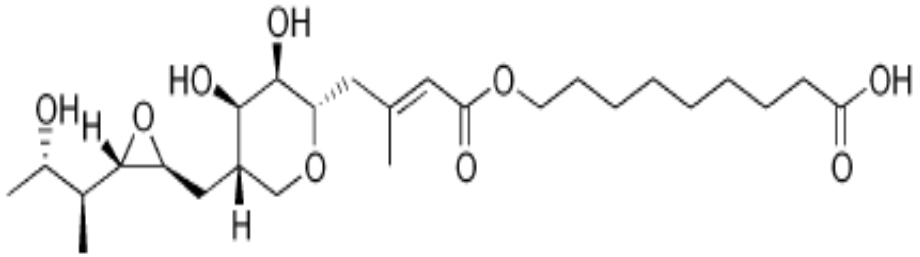


Previenen la iniciación y bloquean el ensablado del ribosoma

Antibióticos que inhiben la síntesis de proteínas

Inhibición de aminoacil-tRNA sintetasas

Mupirocina: Estructura y Mecanismo de acción



Se une a la isoleucil-tRNA sintetasa del microorganismo, de manera que impide la incorporación de la isoleucina a las proteínas.

Bacteriostático a bajas concentraciones y bactericida a altas concentraciones.

Inhibición de la síntesis de ácidos nucleicos

Antibióticos que inhiben la síntesis de ácidos nucleicos

- Inhibición de la DNA girasa

  Quinolonas

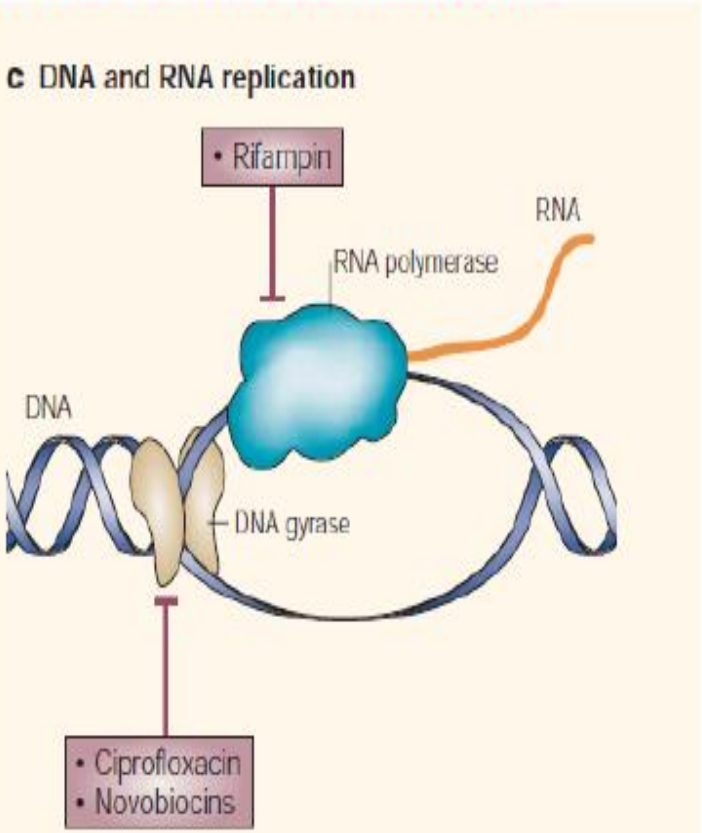
- Inhibición de la RNA polimerasa

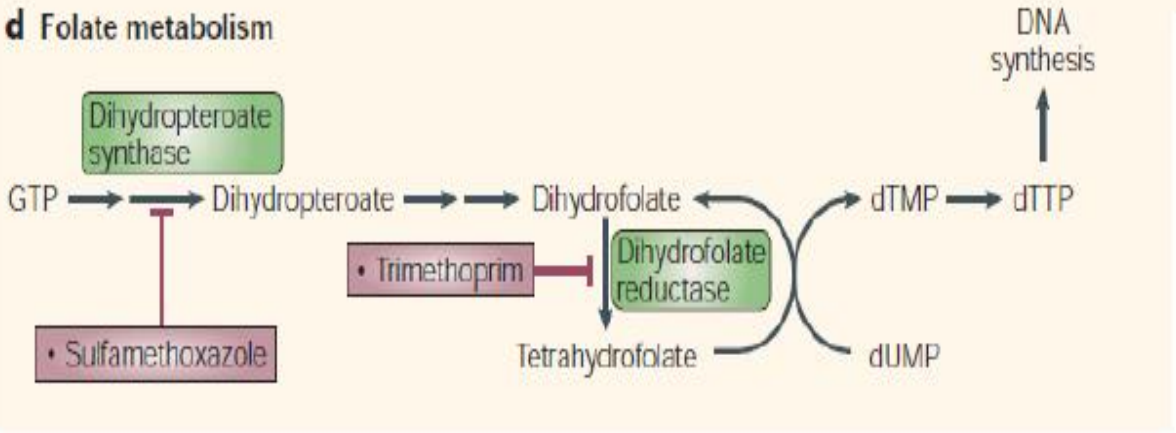
  Rifampicina

- Inhibición de la síntesis de precursores

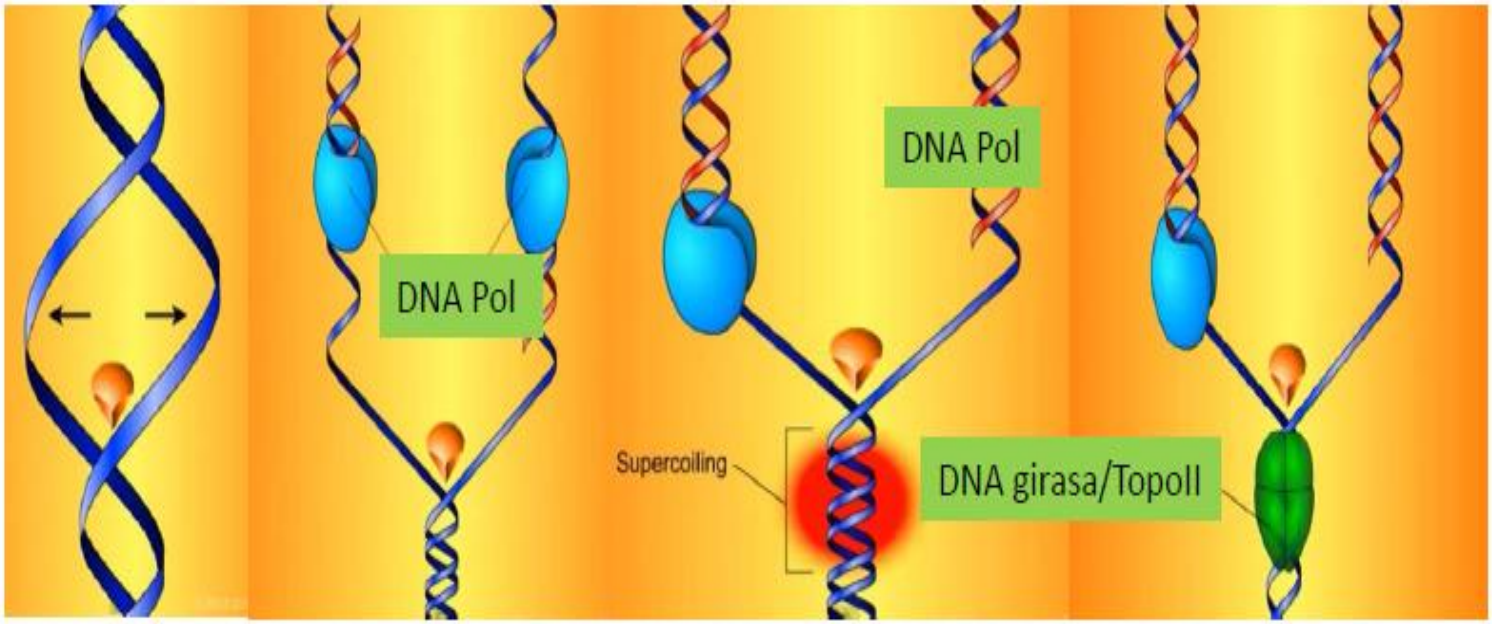
  Sulfamidas

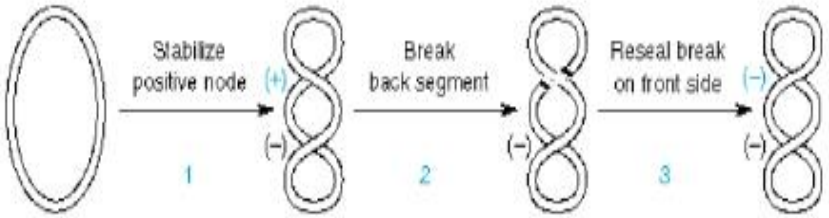
  Trimetoprima

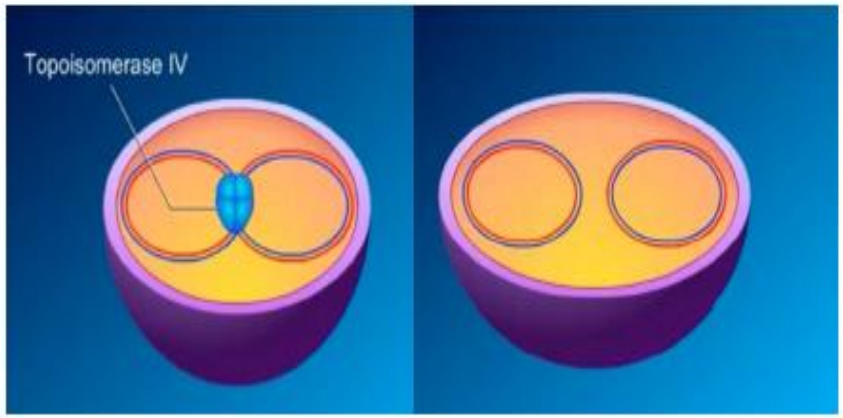




Replicación del DNA

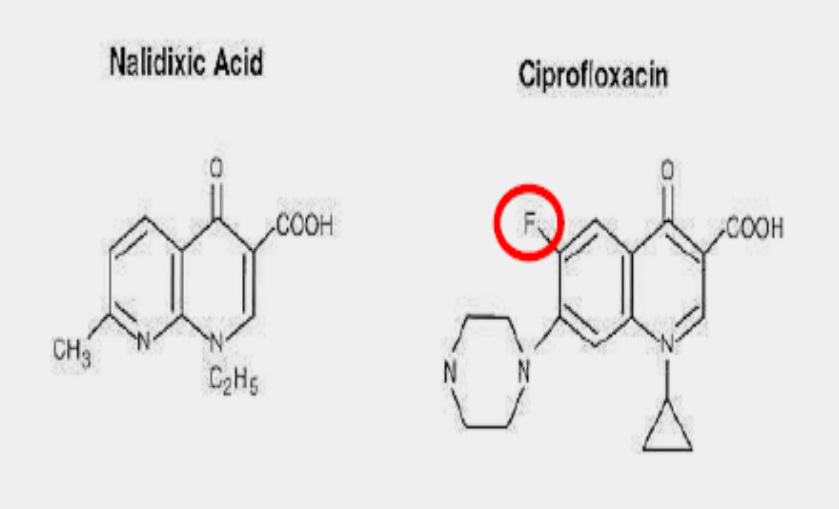






Antibióticos que inhiben la síntesis de ácidos nucleicos

Quinolonas



Interfieren la síntesis de ADN, bloquean la reacción de superenrollamiento dependiente de ATP catalizada por la girasa.

En altas concentraciones pueden inhibir la Topoisomerasa II (enzima que presenta homología con la girasa).

En bacterias G(+) actúan sobre Topoisomerasa IV

No afectan la estructura de cromosomas humanos.

Tienen un efecto bactericida

Antibióticos que inhiben la síntesis de ácidos nucleicos

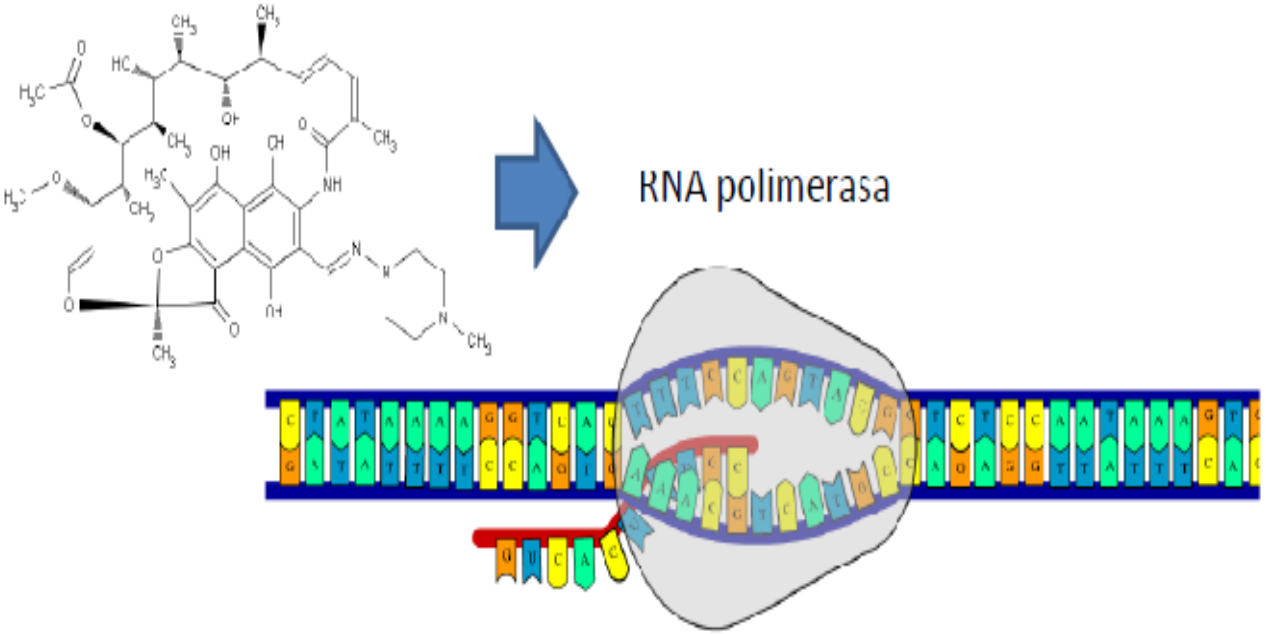
Rifampicina:

Es un antibiótico semisintético derivado de *Amycolatopsis rifamycinica* (previamente conocido como *Streptomyces mediterranei*).

Se une de modo no covalente a la subunidad ß de la ARN polimerasa RNA polimerasa bloqueando la síntesis del mRNA.

Es útil en el tratamiento de la Tuberculosis, en combinación con drogas antituberculosas, como Isoniazida (inhibe la síntesis de lípidos de *Mycobacterium tuberculosis*) y Etambutol.

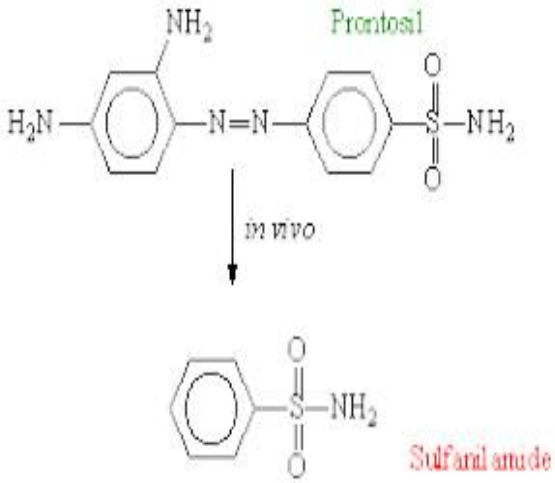
También se usa en combinación con esta drogas para el tratamiento de la Lepra.



Antibióticos que inhiben la síntesis de ácidos nucleicos

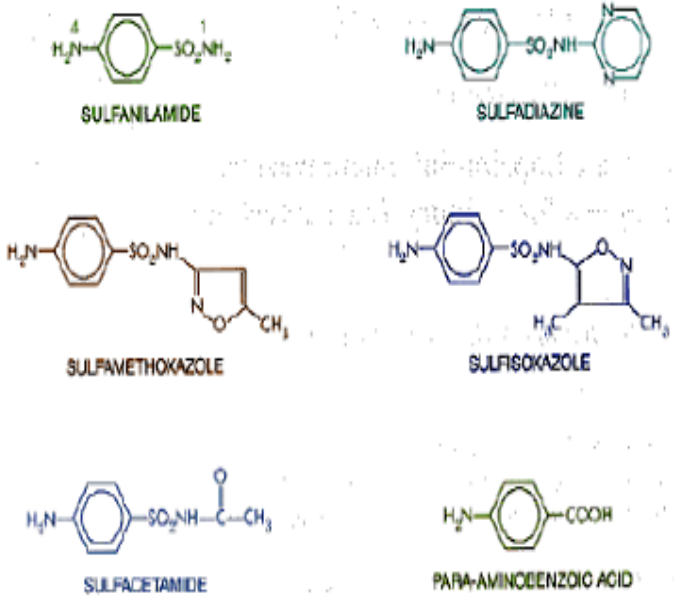
Sulfamidas

Descubiertas por Gerhard Domagk (1932) mientras buscaba colorantes para teñir *S. aureus*. Un colorante rojo (Prontosil Rubrum) protegía a ratones y conejos contra dosis letales de estáfilos y estreptococos hemolíticos.



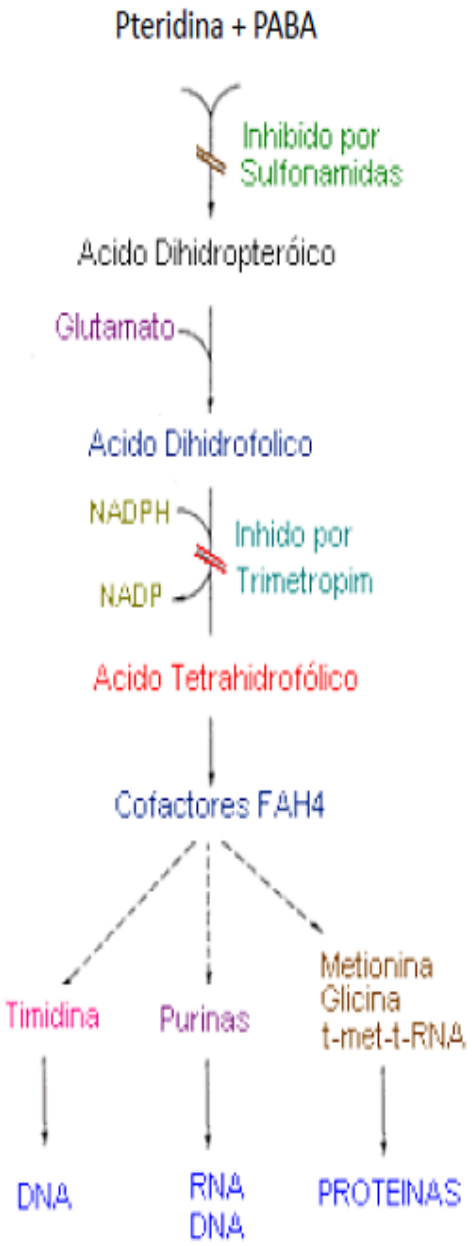
Antibióticos que inhiben la síntesis de ácidos nucleicos

Sulfamidas



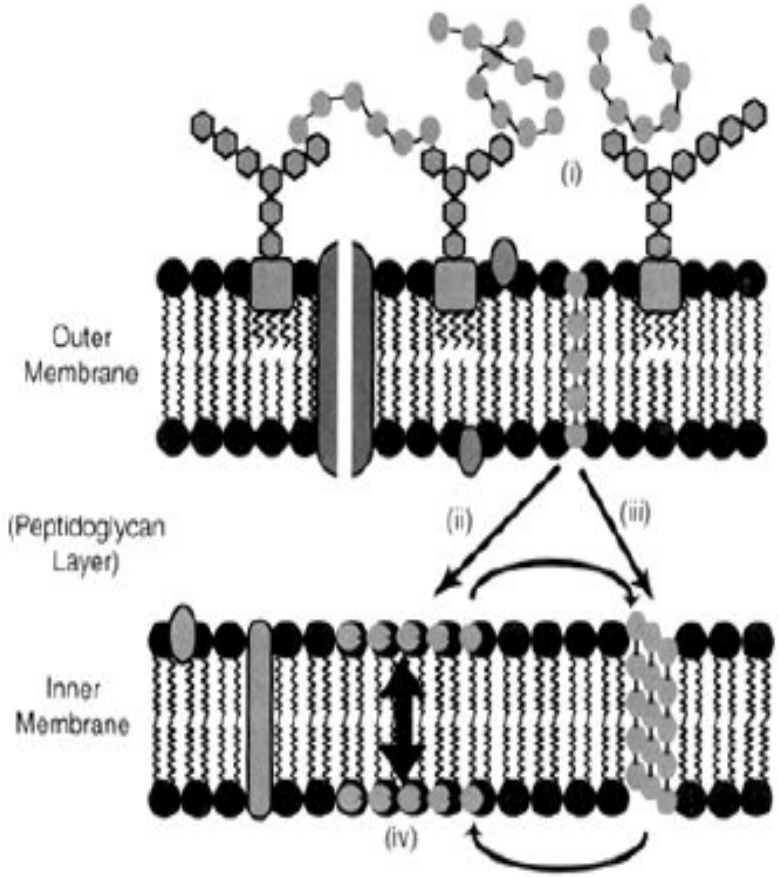
Análogos del PABA, antagonistas competitivos en la síntesis del ácido fólico bacteriano.

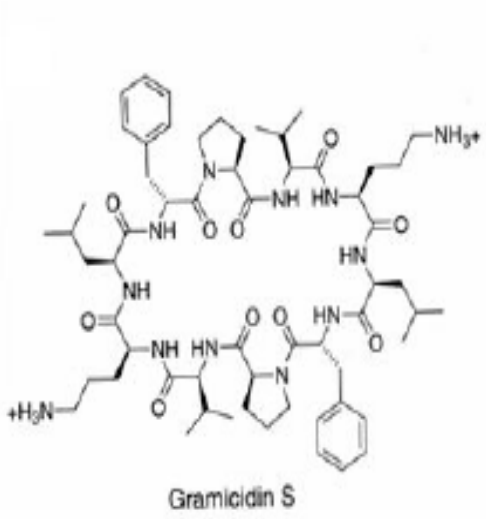
También inhiben la dihidropteroato sintetasa, necesaria para la incorporación del PABA al ácido dihidropteroico (precursor del ácido fólico).

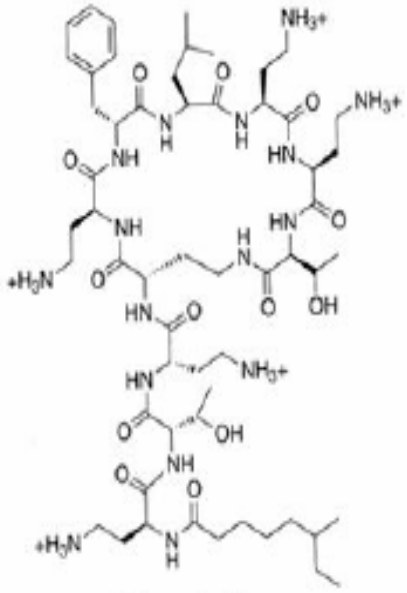


Antibióticos que interfieren con la membrana celular

Polimixina B: Péptidos catiónicos que se insertan en la membrana. ATB se une al LPS o a las cargas negativas de la membrana, desorganizando la MC y generando permeabilidad de la misma y despolarización de la membrana. Relativamente tóxicos, poca utilidad terapéutica.

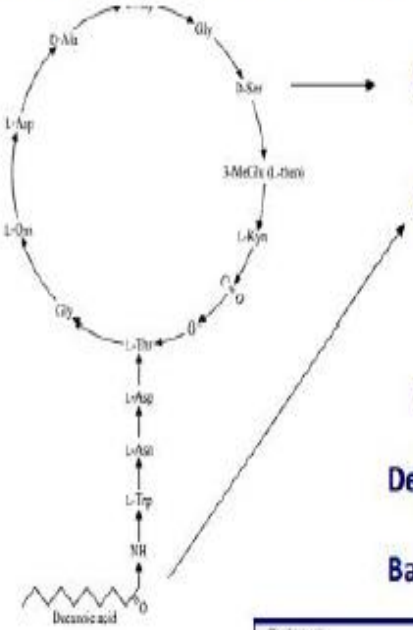






Antibióticos que interfieren con la membrana celular

Daptomicina: Antibiótico lipopéptido cíclico, producido por *Streptomyces roseosporus*. Es bactericida por unión a la membrana celular de Gram (+). En presencia de Ca^{+2} produce una rápida despolarización de la membrana y muerte bacteriana, sin lisis celular.



Cadena cíclica de 13 péptidos

Cadena lateral altamente lipofílica (Acido decanoico)

↓

Se inserta en la membrana plasmática e induce la salida de K+

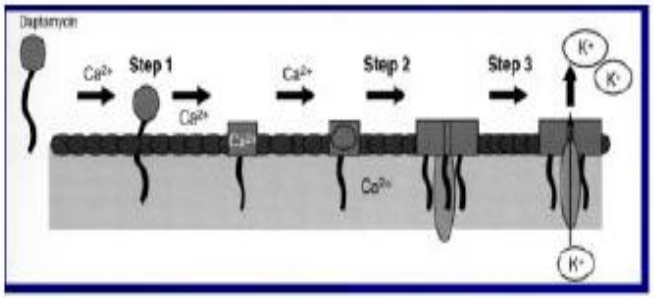
↓

Depolarización de la membrana

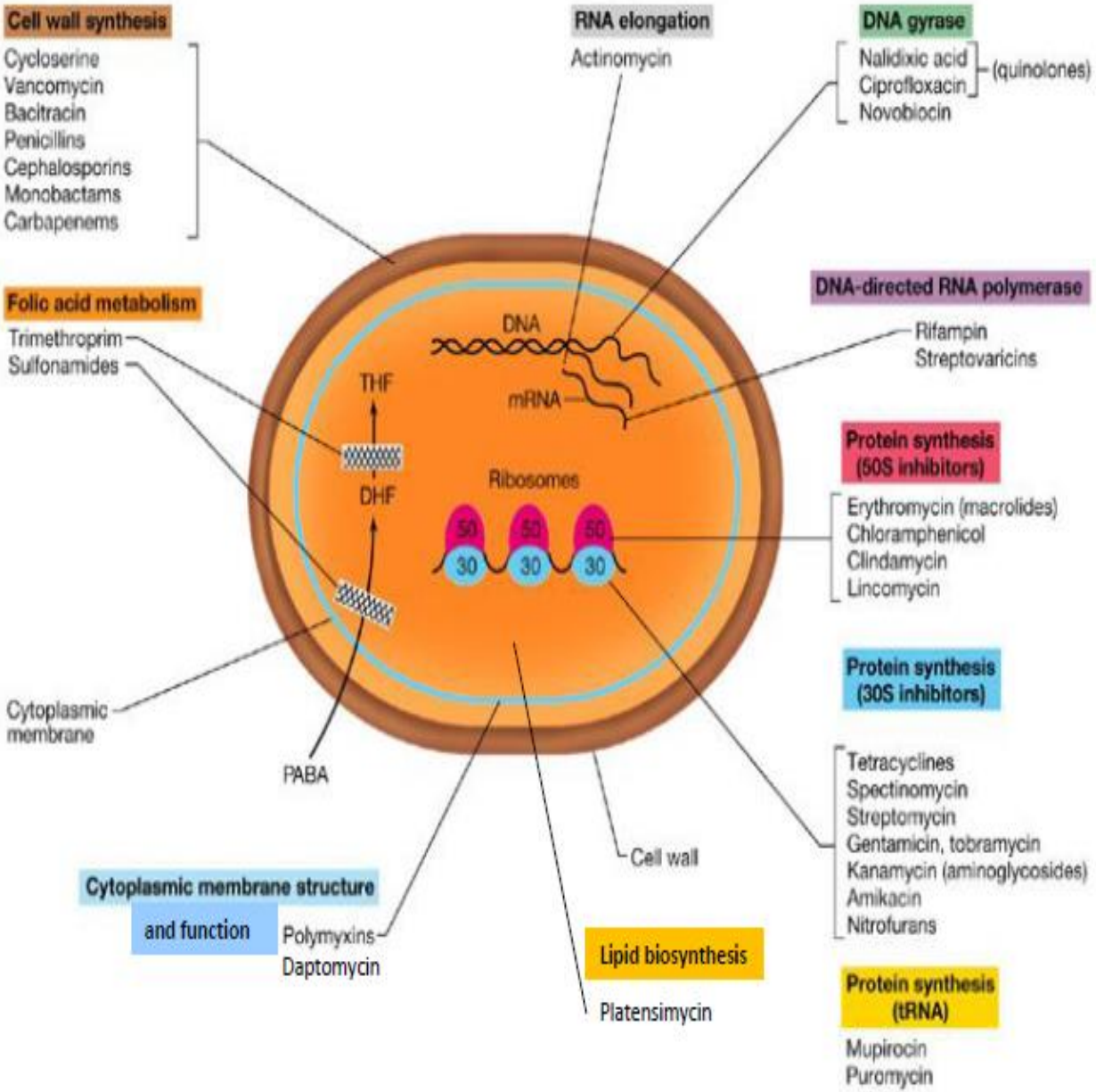
↓

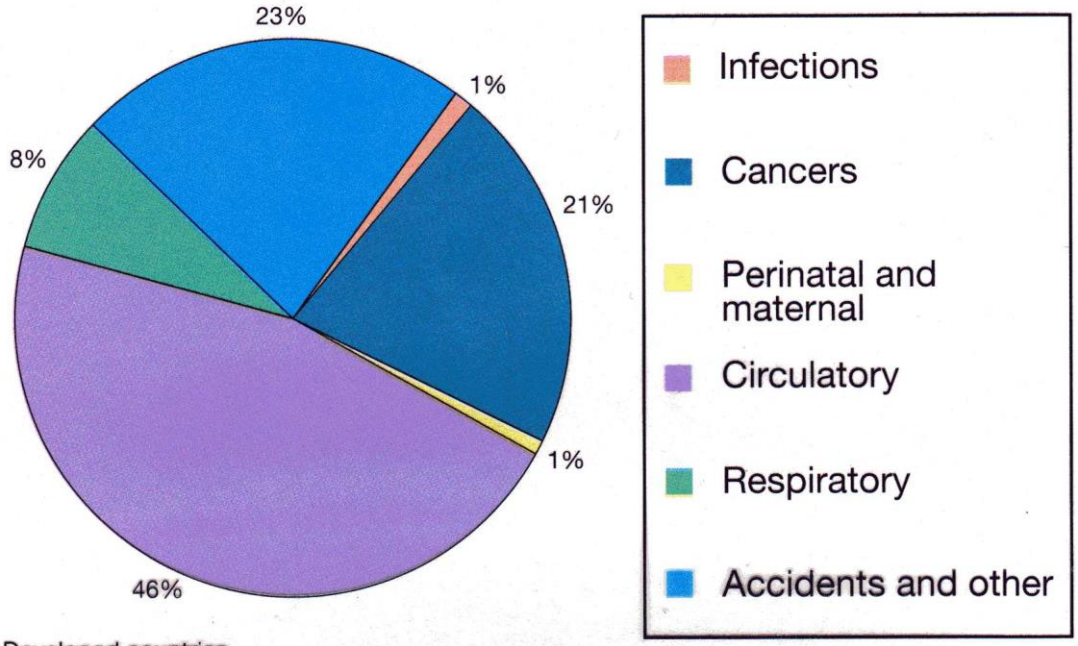
Bacteria no puede sintetiza ATP

Requiere formar un complejo con Ca++

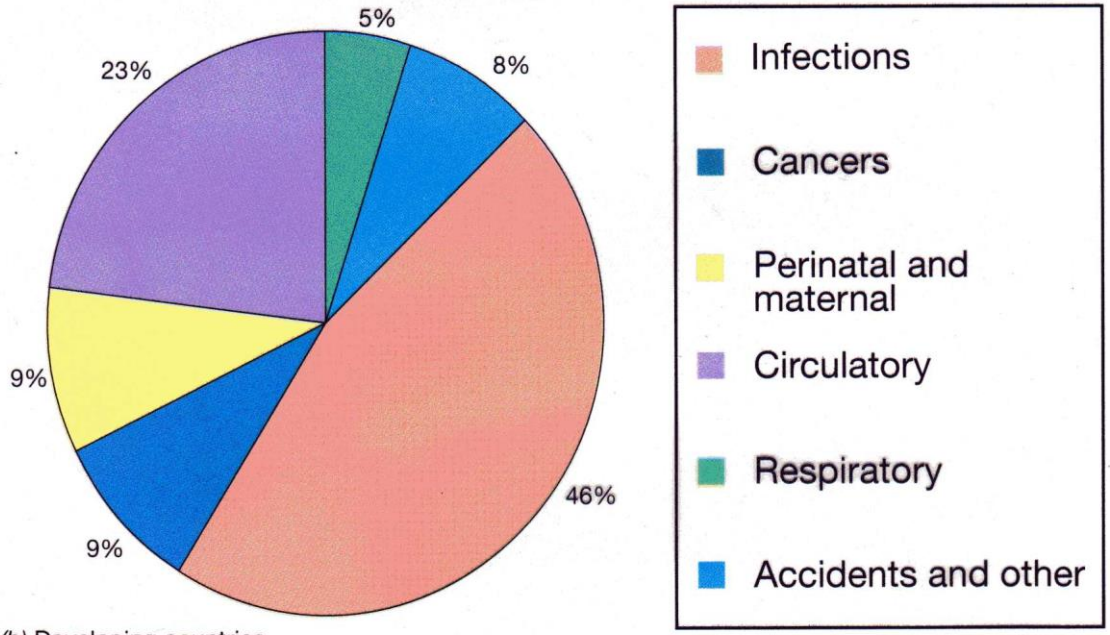


Sitios blanco de acción de los antimicrobianos

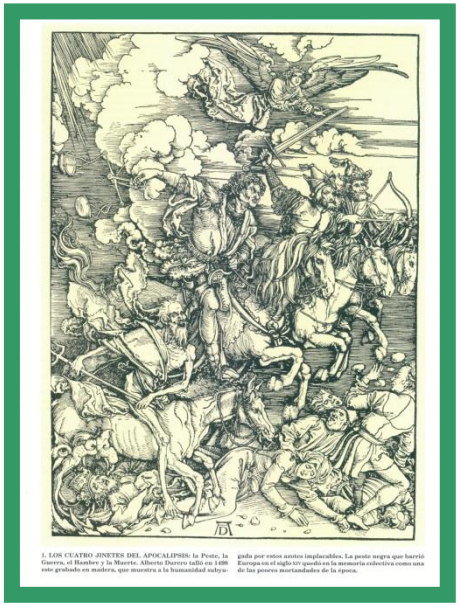


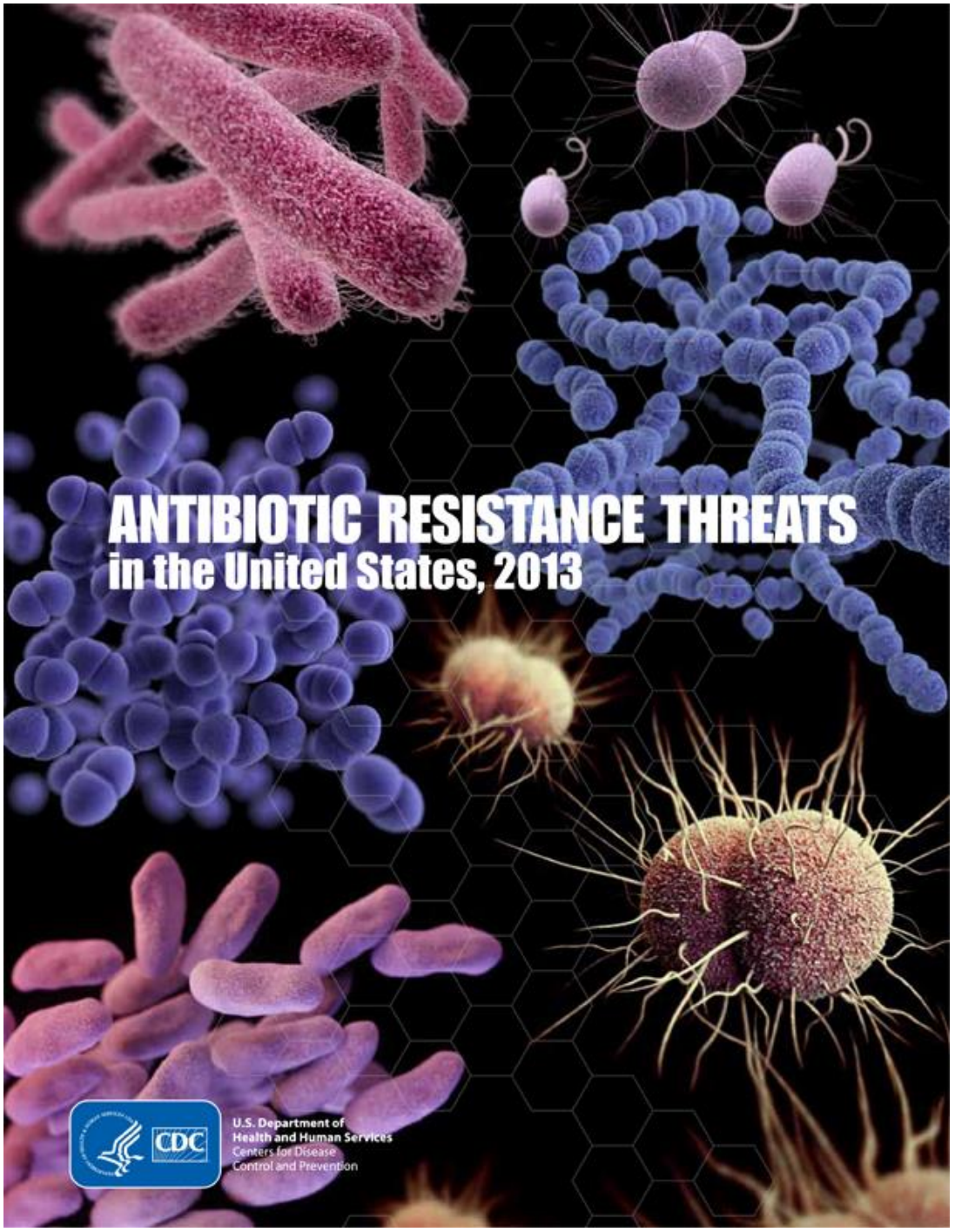


(a) Developed countries



(b) Developing countries




ANTIBIOTIC RESISTANCE THREATS
in the United States, 2013
CDC
U.S. Department of
Health and Human Services
Centers for Disease
Control and Prevention

NATIONAL SUMMARY DATA

Estimated minimum number of illnesses and deaths caused by antibiotic resistance*:

At least **2,049,442** illnesses,

23,000 deaths

**bacteria and fungus included in this report*

+

Estimated minimum number of illnesses and death due to *Clostridium difficile* (*C. difficile*), a unique bacterial infection that, although not significantly resistant to the drugs used to treat it, is directly related to antibiotic use and resistance:

At least **250,000** illnesses,

14,000 deaths

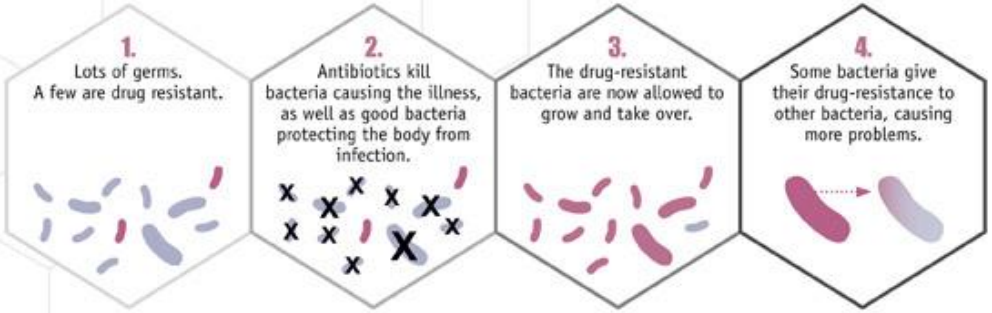
WHERE DO INFECTIONS HAPPEN?

Antibiotic-resistant infections can happen anywhere. Data show that most happen in the general community; however, most deaths related to antibiotic resistance happen in healthcare settings, such as hospitals and nursing homes.

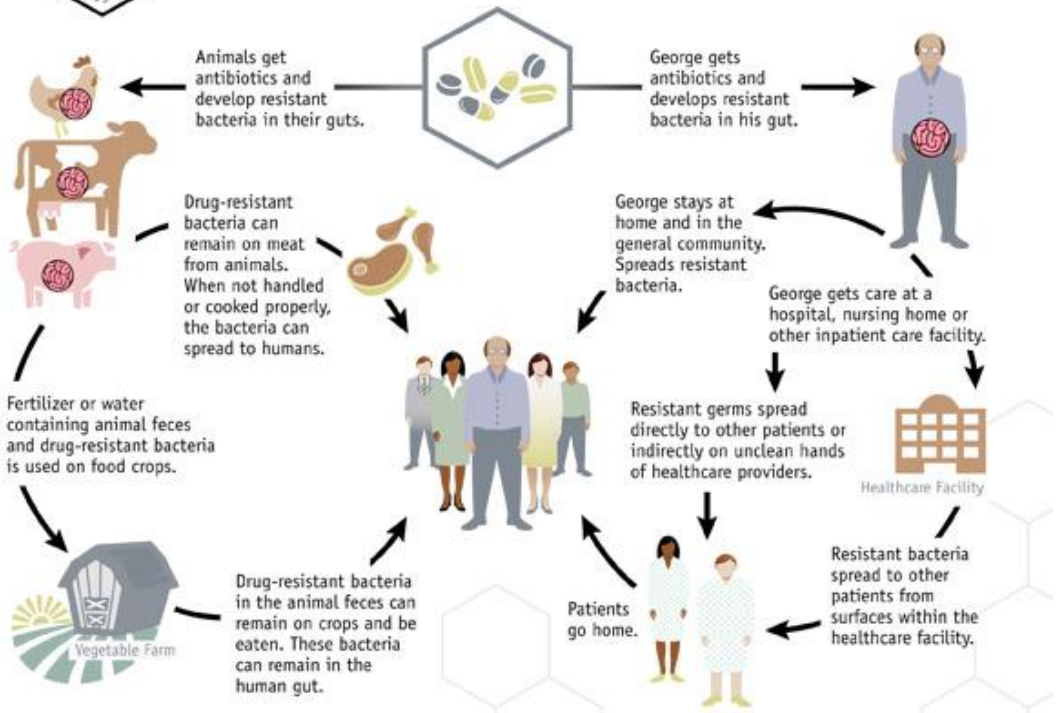
U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

CS239559

How Antibiotic Resistance Happens



Examples of How Antibiotic Resistance Spreads



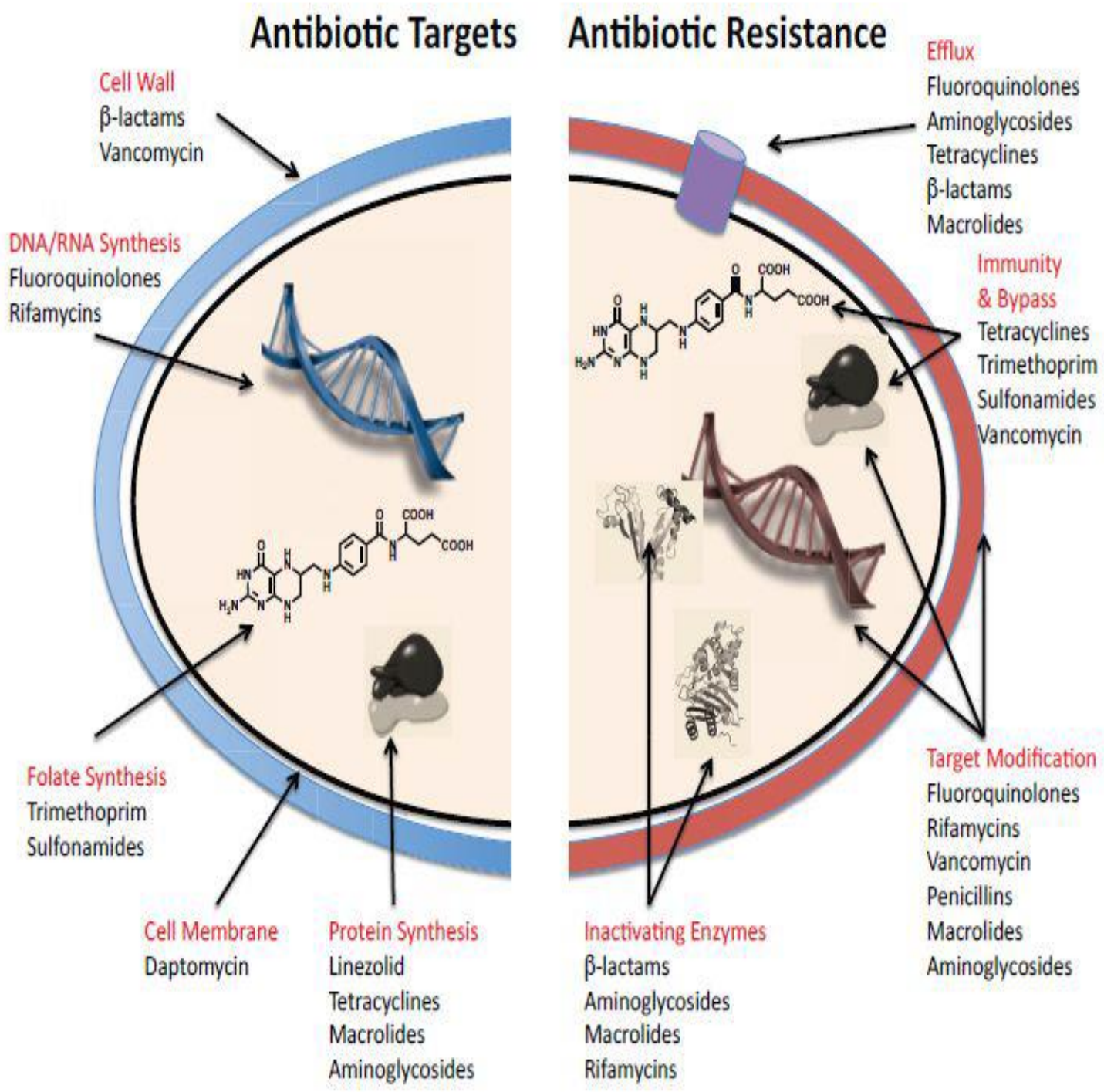
Simply using antibiotics creates resistance. These drugs should only be used to treat infections.

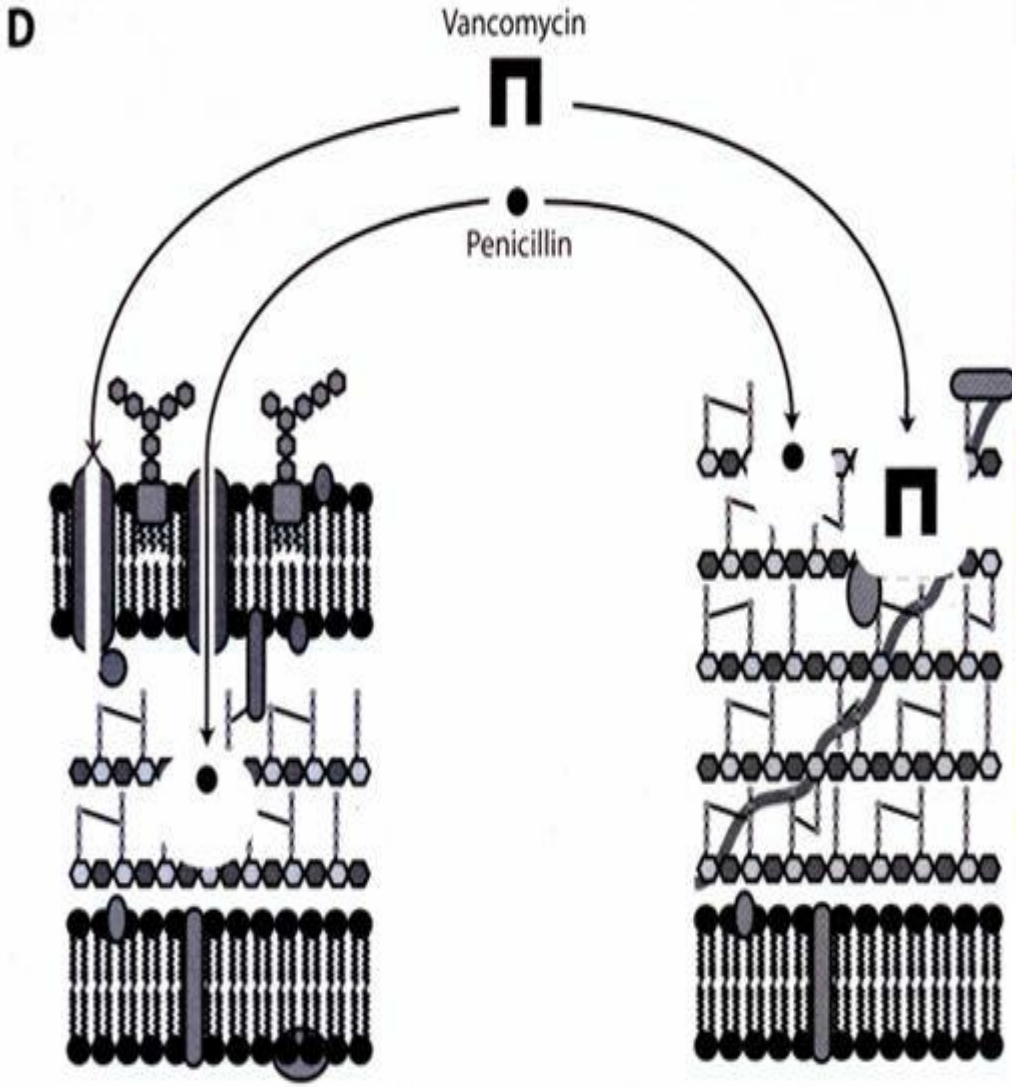
CS239559

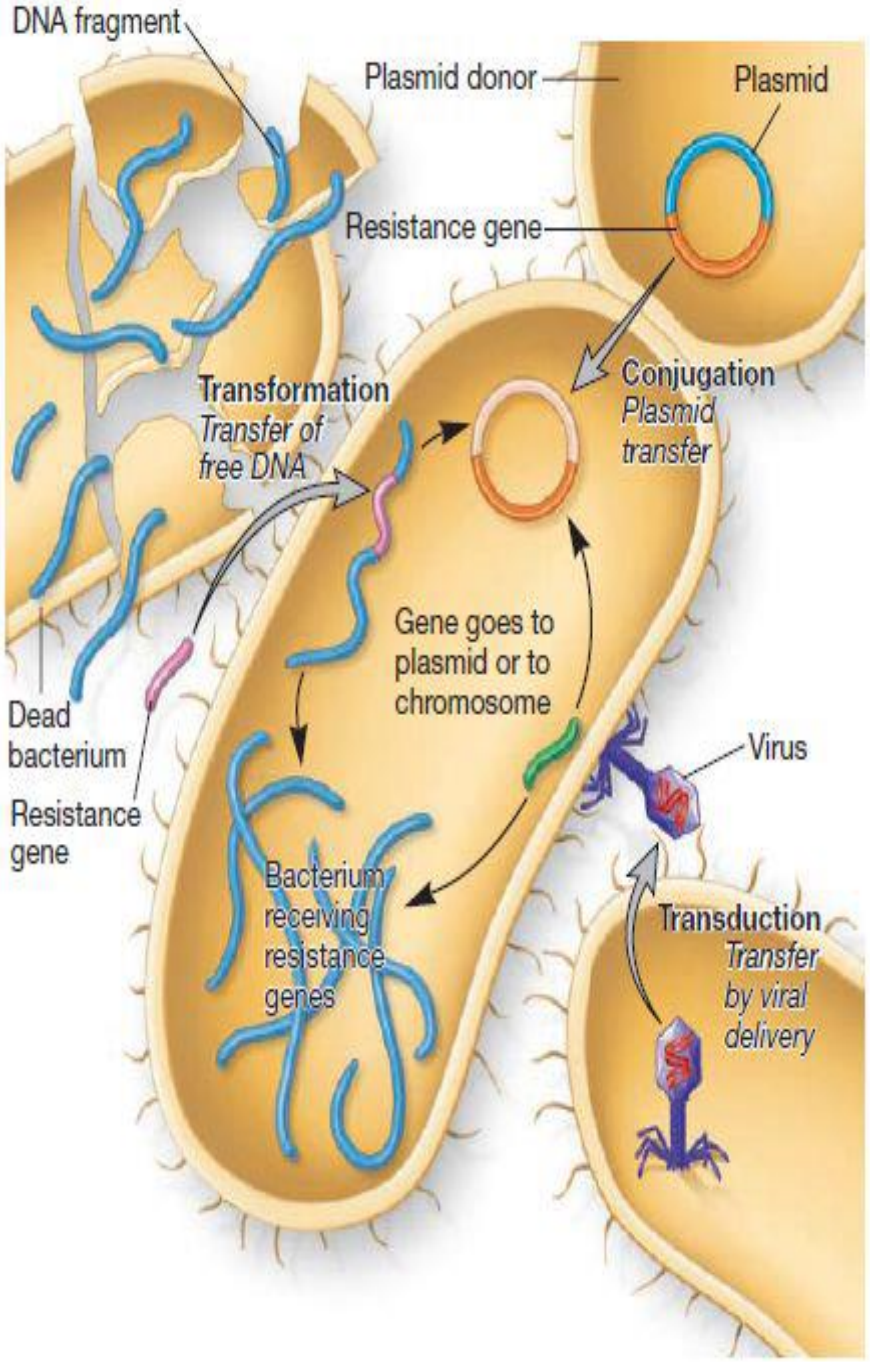
Antibiótico	Uso Clínico	Resistencia observada
Sulfonamidas	1930	1940
Penicilina	1943	1946
Estreptomicina	1945	1959
Cloramfenicol	1947	1959
Tetracycline	1948	1953
Erythromycin	1952	1988
Vancomicina	1956	1988
Cefalosporinas	1960	Fines de 1960
Meticilina	1960	1961
Ampicilina	1961	1973

3 años

36 años


Antibiotic Targets
Antibiotic Resistance
Cell Wall
β-lactams
Vancomycin
DNA/RNA Synthesis
Fluoroquinolones
Rifamycins
Folate Synthesis
Trimethoprim
Sulfonamides
Cell Membrane
Daptomycin
Protein Synthesis
Linezolid
Tetracyclines
Macrolides
Aminoglycosides
Efflux
Fluoroquinolones
Aminoglycosides
Tetracyclines
β-lactams
Macrolides
Immunity
& Bypass
Tetracyclines
Trimethoprim
Sulfonamides
Vancomycin
Target Modification
Fluoroquinolones
Rifamycins
Vancomycin
Penicillins
Macrolides
Aminoglycosides
Inactivating Enzymes
β-lactams
Aminoglycosides
Macrolides
Rifamycins


D
Vancomycin
Penicillin


DNA fragment
Plasmid donor
Plasmid
Resistance gene
Transformation
Transfer of free DNA
Conjugation
Plasmid transfer
Gene goes to plasmid or to chromosome
Dead bacterium
Resistance gene
Virus
Bacterium receiving resistance genes
Transduction
Transfer by viral delivery

Antibiótico	Uso Clínico	Resistencia observada
Sulfonamidas	1930	1940
Penicilina	1943	1946
Estreptomicina	1945	1959
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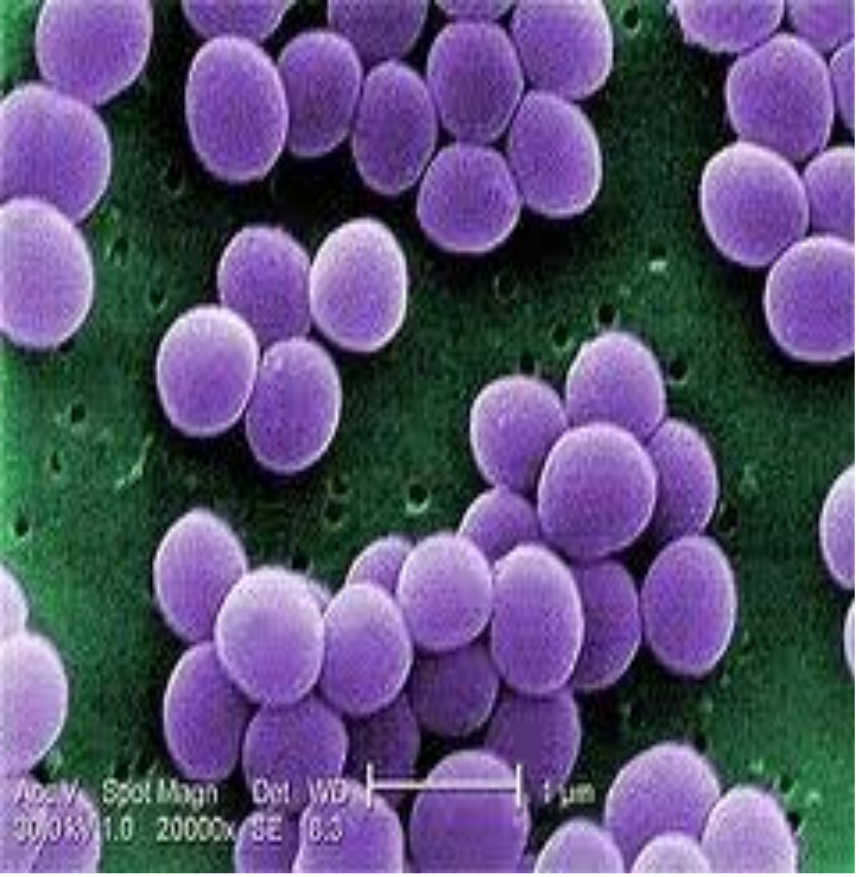
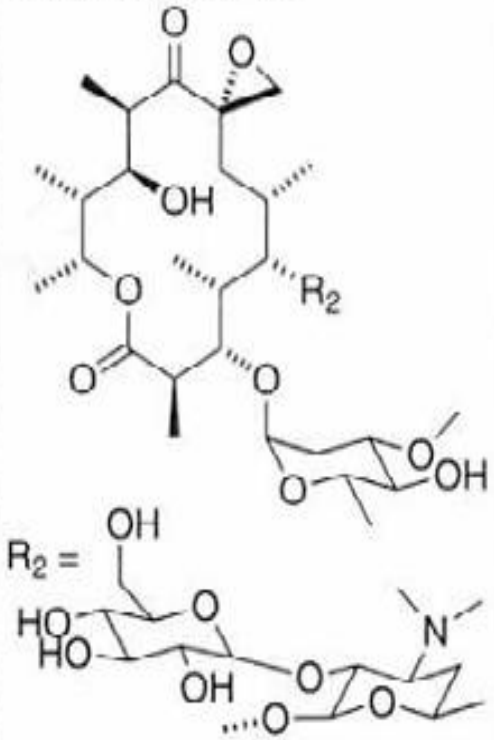
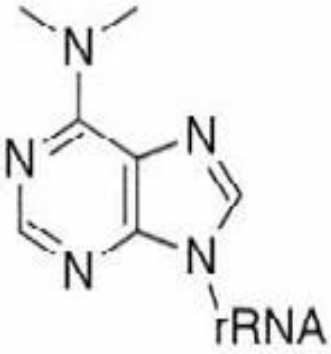
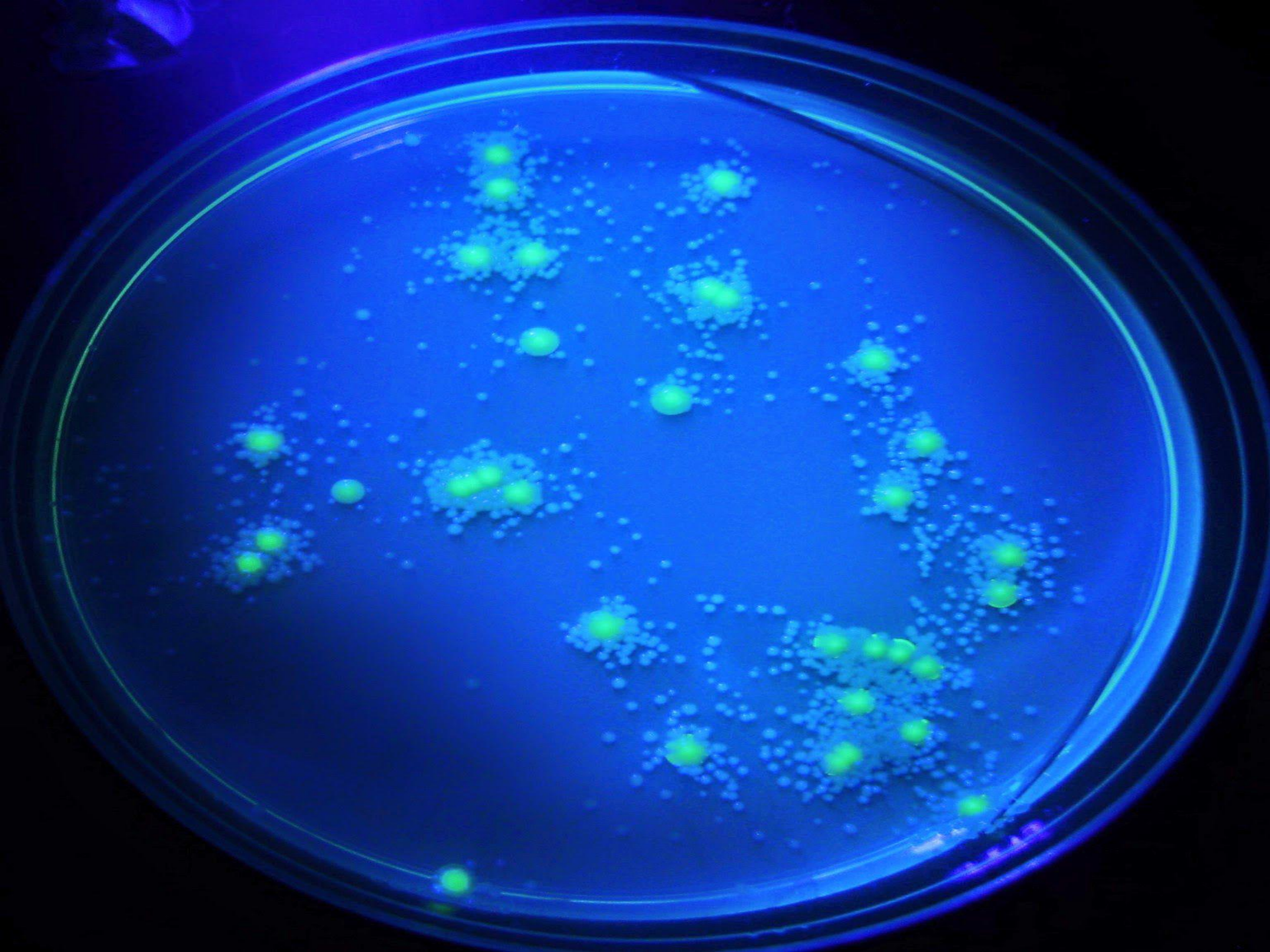
3 años

36 años


Penicillin 1946
Methicillin 1961
Vancomycin 1986
Zyvox 1999

Method of Producer Self-Protection	Temporary Intracellular Inactivation of Antibiotic	Efflux of Produced Antibiotic	Modification of Target in Producer
Example of Macrolide Producer Self-Protection	Glycosylation of Oleandomycin 	Export of Oleandomycin by OleB 	Dimethylation of Adenine in 23S rRNA  23S rRNA:m_2A2085
Analogous Mode of Clinically Observed Bacterial Resistance	Inactivation of Antibiotic	Efflux of Antibiotic	Modification of Target

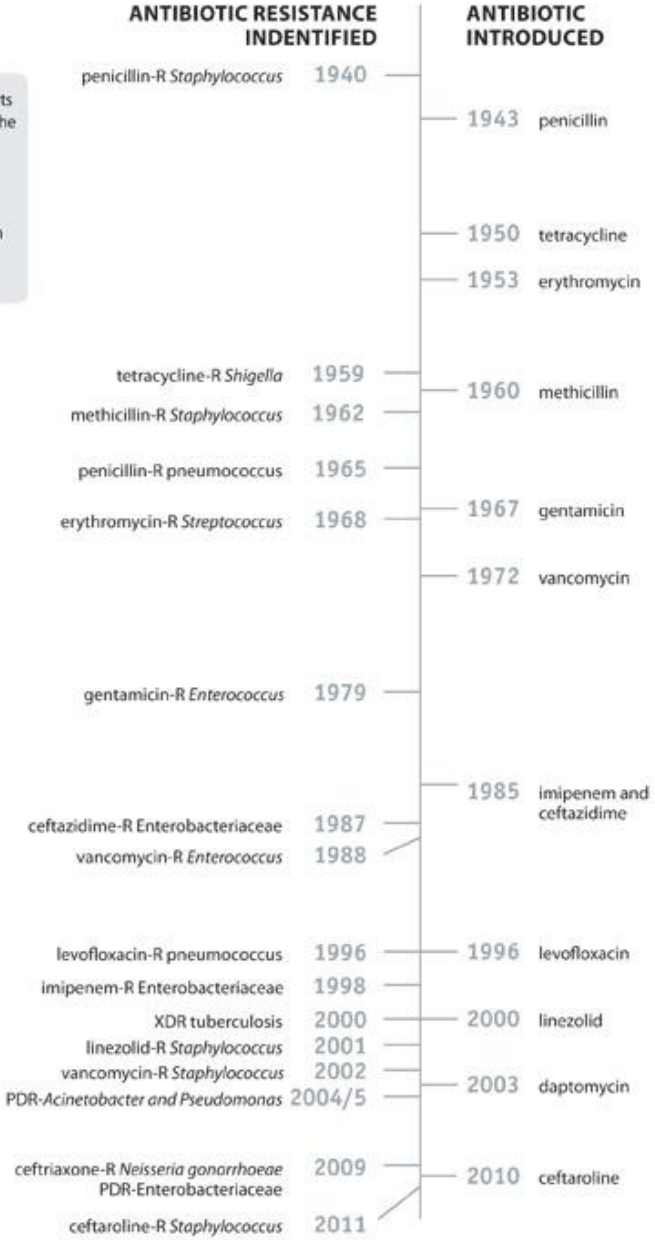

Amoxicilina / Ácido clavulánico MERCK
875/125 mg comprimidos recubiertos EFG
Amoxicilina / Ácido clavulánico MERCK
875/125 mg comprimidos recubiertos EFG
24 comprimidos recubiertos
999830.1
Vía oral
MERCK genéricos



Developing Resistance

Timeline of Key Antibiotic Resistance Events

Dates are based upon early reports of resistance in the literature. In the case of pan drug-resistant (PDR)-*Acinetobacter* and *Pseudomonas*, the date is based upon reports of healthcare transmission or outbreaks. Note: penicillin was in limited use prior to widespread population usage in 1943.



Examples of Recently Approved Drugs

Drug Name	Year Approved	Key Targeted Pathogens	Drug's Use and Resistance Trends
Quinupristin/ Dalfoprisitin	1999	*Staphylococcus* *Streptococcus*	This is a combination of two drugs that can be used to treat gram-positive infections. Because side effects are common, this drug is usually not a first choice for therapy. Resistance in target pathogens has been described, but the percentage in the United States is still low.
Moxifloxacin	1999	Enterobacteriaceae *Staphylococcus* *Streptococcus*	Moxifloxacin, like other fluoroquinolones, demonstrates broad spectrum activity, and it can be used to treat a range of infections. Unfortunately, there is cross-resistance among the fluoroquinolones, and resistance is increasing in all targeted pathogens, especially Enterobacteriaceae.
Linezolid	2000	*Staphylococcus* *Enterococcus*	Linezolid can be used to treat serious gram-positive infections. Resistance has occurred but it is still uncommon.
Ertapenem	2001	Enterobacteriaceae *Staphylococcus* *Streptococcus*	Ertapenem is a carbapenem that can be used to treat a wide range of infections. Dissemination of carbapenem-resistant Enterobacteriaceae (CRE) is impacting the drug's overall effectiveness.
Gemifloxacin	2003	Enterobacteriaceae *Streptococcus*	Gemifloxacin is a fluoroquinolone that can be used to treat mild to moderate community-associated respiratory disease. Like moxifloxacin, there is cross-resistance with other fluoroquinolone drugs so resistance is increasing.
Daptomycin	2003	*Staphylococcus* *Streptococcus* *Enterococcus*	Daptomycin is often used for treatment of serious gram-positive infections. Resistance is emerging in all of the targeted pathogens, but the resistance rates are currently low.
Tigecycline	2005	Enterobacteriaceae *Staphylococcus* *Streptococcus* *Enterococcus*	Tigecycline is often one of the only active agents for carbapenem-resistant gram-negative infections, and resistance is emerging. However, even in the absence of resistance, the effectiveness of this agent for treatment of the most serious infections is a concern.
Doripenem	2007	Enterobacteriaceae *Pseudomonas aeruginosa* *Acinetobacter spp.* *Streptococcus spp.*	Doripenem is a carbapenem drug most commonly used to treat serious gram-negative infections. Dissemination of carbapenem-resistant gram-negative pathogens like CRE is reducing the overall effectiveness of this drug.
Telavancin	2008	*Staphylococcus* *Streptococcus* *Enterococcus*	Telavancin is approved for treatment of gram-positive skin and soft tissue infections. Use is limited because it is administered intravenously and is therefore difficult to use in an outpatient setting. In addition, it should not be used in a woman of childbearing age without a pregnancy test.

Drug Name	Year Approved	Key Targeted Pathogens	Drug's Use and Resistance Trends
Ceftaroline	2010	Enterobacteriaceae *Staphylococcus* *Streptococcus*	Ceftaroline is a cephalosporin drug, but unlike other cephalosporins, this one can be used to treat MRSA infections. Resistance has been identified but is rare. Ceftaroline does not demonstrate any enhanced activity compared to other cephalosporins for Enterobacteriaceae. ESBL-producing isolates and CRE isolates are resistant to this drug as well. ESBL (extended-spectrum β-lactamase) is an enzyme that allows bacteria to become resistant to a wide variety of penicillins and cephalosporins .Bacteria that contain this enzyme are known as ESBLs or ESBL-producing bacteria.

Resistencia frente a ATB

Antibiótico	Uso Clínico	Resistencia observada
Sulfonamidas	1930	1940
Penicilina	1943	1946
Estreptomicina	1943	1959
Cloramfenicol	1947	1959
Vancomicina	1956	1988
Cefalosporinas	1960	Fines de 1960
Meticilina	1960	1961

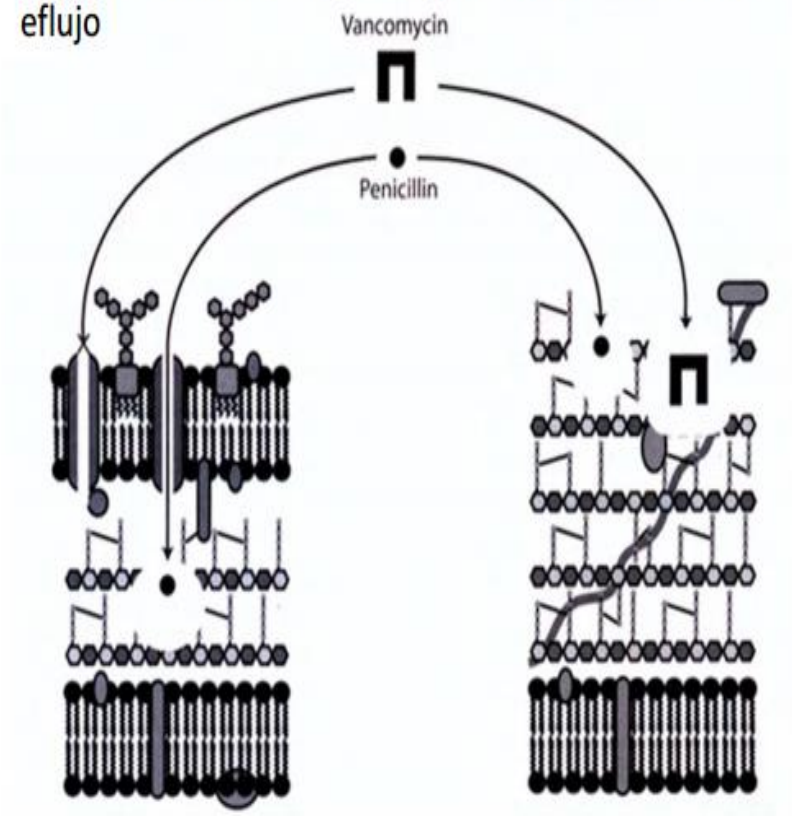
Necesidad constante de encontrar o desarrollar nuevos antibióticos

Resistencia microbiana a los antibióticos

Perdida de sensibilidad de un microorganismo a un ATB

Intrínseca

- La bacteria no tiene la molécula/reacción enzimática que es el blanco del antibiótico
- Diferencias en la permeabilidad
- El ATB es expulsado eficientemente por sistemas de eflujo

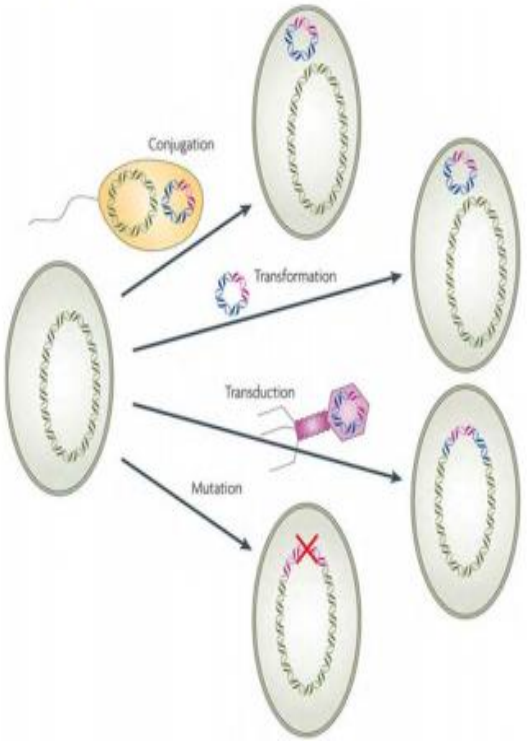


Gram -

Gram +

Adquirida

- Adquisición de genes por transferencia horizontal:
  - Conjugación
  - Transformación
  - Transducción
  - Mutaciones



Autoprotección de Bacterias productoras de Productos Naturales

Table 3. Resistance to some clinically useful antibiotics in their producing organisms. In a number of cases, strains have multiple resistance mechanisms for the antibiotic formed (*70*). *Str.*, *Streptomyces*.

Producing strain	Antibiotic	Resistance gene	Mechanism
Str. capreolus	Capreomycin	*cph, cac*	Phosphotransferase, acetyltransferase
Nocardia lactamdurans	Cephamycin	—	β-lactamase (?)*†
Str. venezuelae	Chloramphenicol	—	Hydrolase‡
Str. erythreus	Erythromycin	*erm*	Target modification (ribosome)
Str. fradiae	Fosfomycin	*fos*	Glutathione adduct (?)†
Micromonospora purpurea	Gentamicin	*grm*	Target modification (ribosome)
Str. kanamyceticus	Kanamycin	*aac*	*N*-Acetyltransferase
Str. antibioticus	Oleandomycin	*mgt*	Glycosyltransferase
Str. griseus	Streptomycin	*aph*	*O*-Phosphotransferase
Str. rimosus	Tetracycline	*otr*A, otrB	Efflux, resistant translation system
Str. tenebrarius	Tobramycin	*aac, kgm, kam*	Acetyltransferase, target modification (ribosome)
Str. vinaceus	Viomycin	*vph*	Phosphotransferase

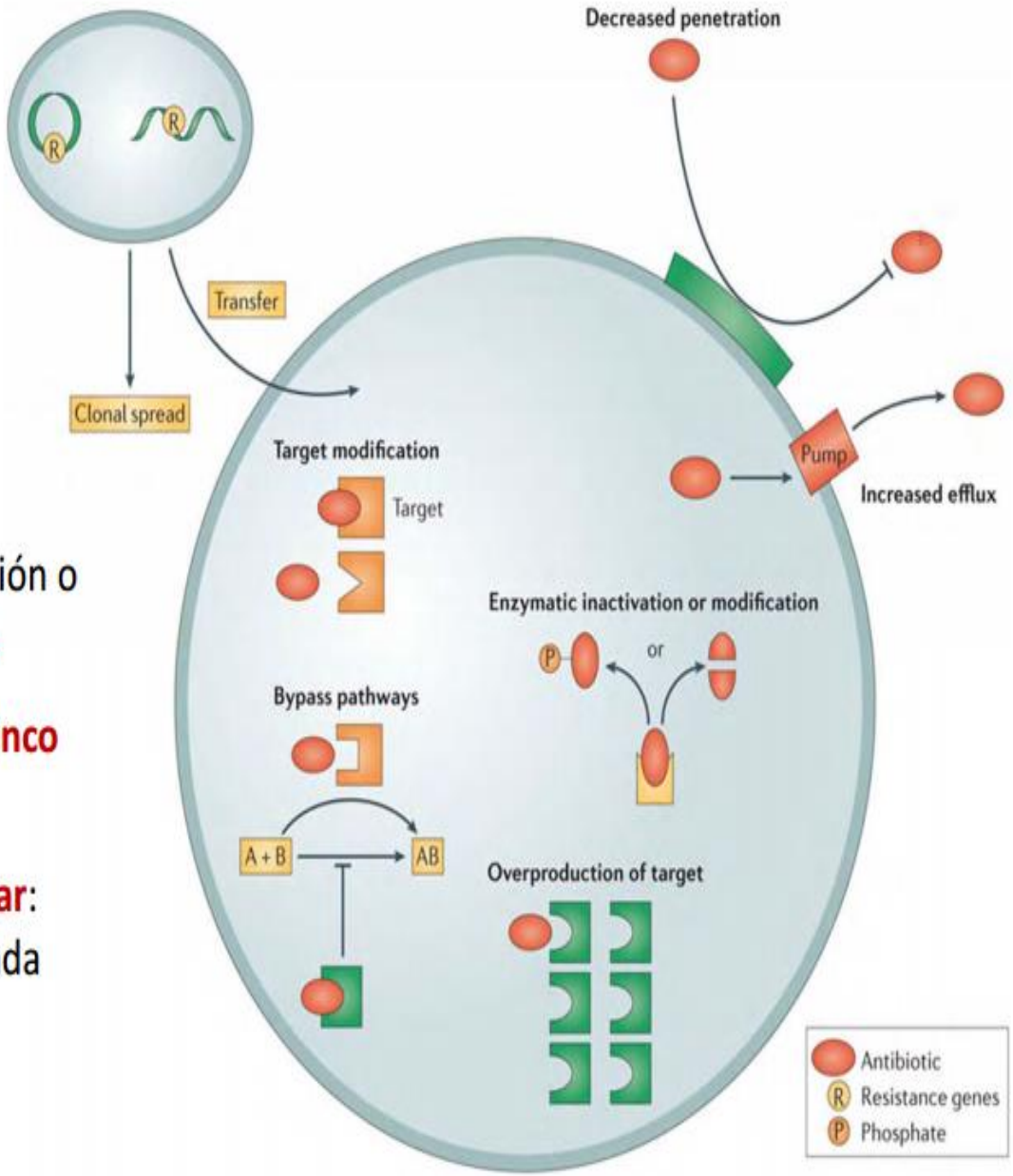
"Los productores de antibióticos desarrollaron mecanismos de protección e inmunidad contra sus propias armas, son un reservorio natural de genes de defensa anteriores al uso clínico de los antibióticos ..."

Mecanismos de Resistencia frente a ATB

1- Inactivación por destrucción o modificación de la **droga**

2- Modificación del **sitio blanco**

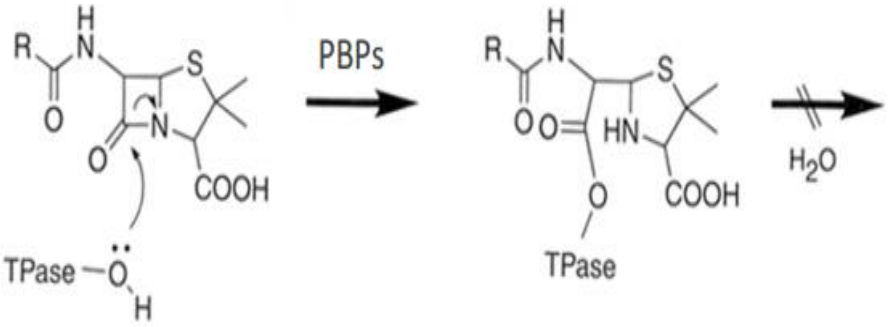
3- Disminución de la **concentración intracelular**:
- Disminución de la entrada
- Aumento de la salida



Destrucción o modificación de la droga

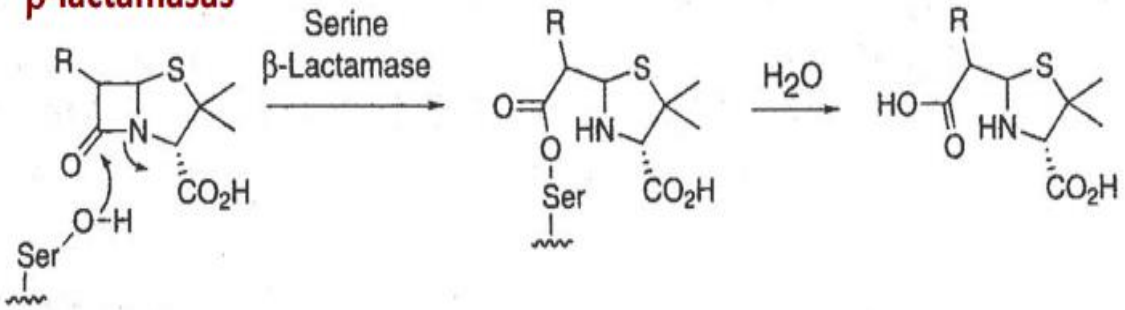
Destrucción de la droga

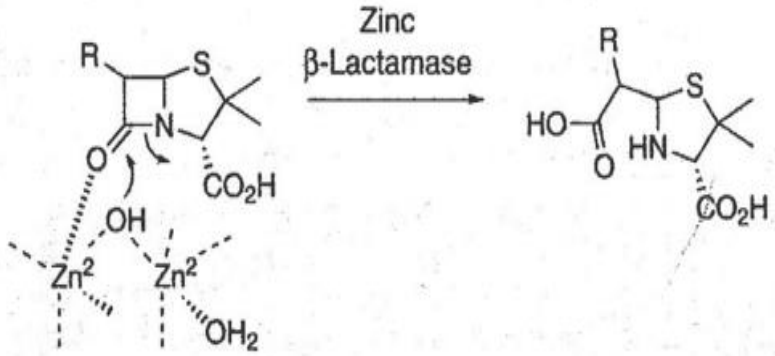
Transpeptidasas



Inhibición de la reacción de transpeptidación y del entrecruzamiento del peptidoglicano
Activan el mecanismo autolítico endógeno bacteriano

β-lactamasas

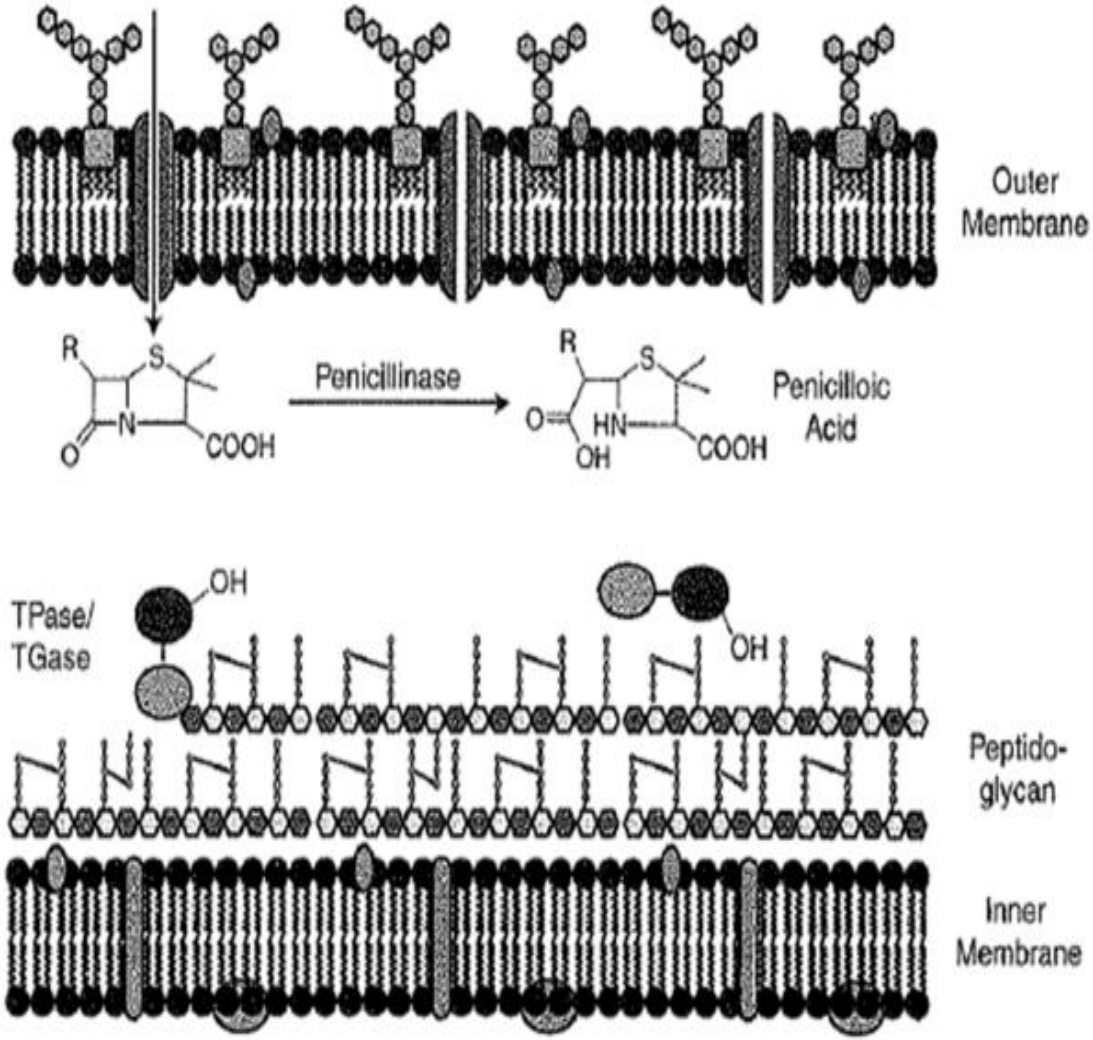




Existe enzimas de diferentes tipo:

- Serin-β-lactamasas (Tipo A, C y D)
- Metalo-β-lactamasas (Zn^{+2})
- Periplasmicas o unidas a membrana interna (Gram -) o extracelulares (Gram +)
- Constitutivas o inducibles

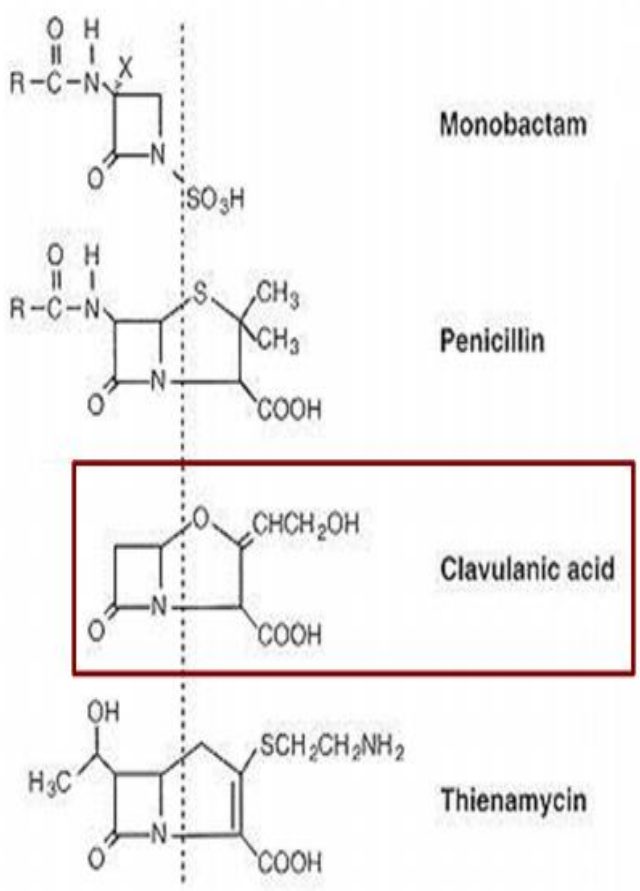
Las β-lactamasas en el periplasma evitan que los ATB lleguen a sus blancos

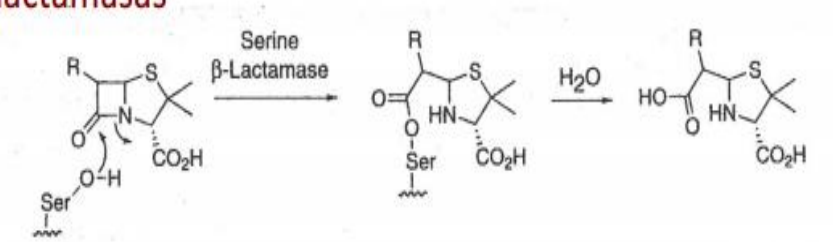


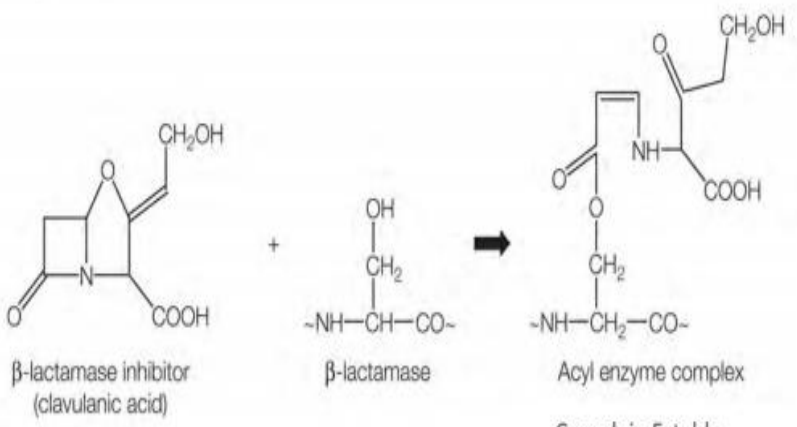
Estrategias para neutralizar las β-lactamasas

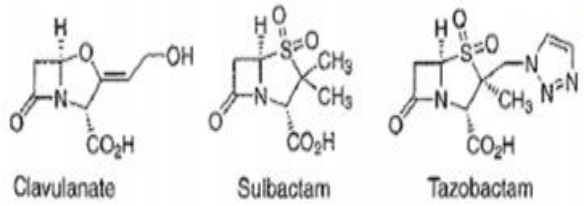
Desarrollo de β-lactámicos semisintéticos que sean hidrolizados lentamente (ej: carbapenem, monobactam)

Inhibidores (competitivos) de β-lactamasas





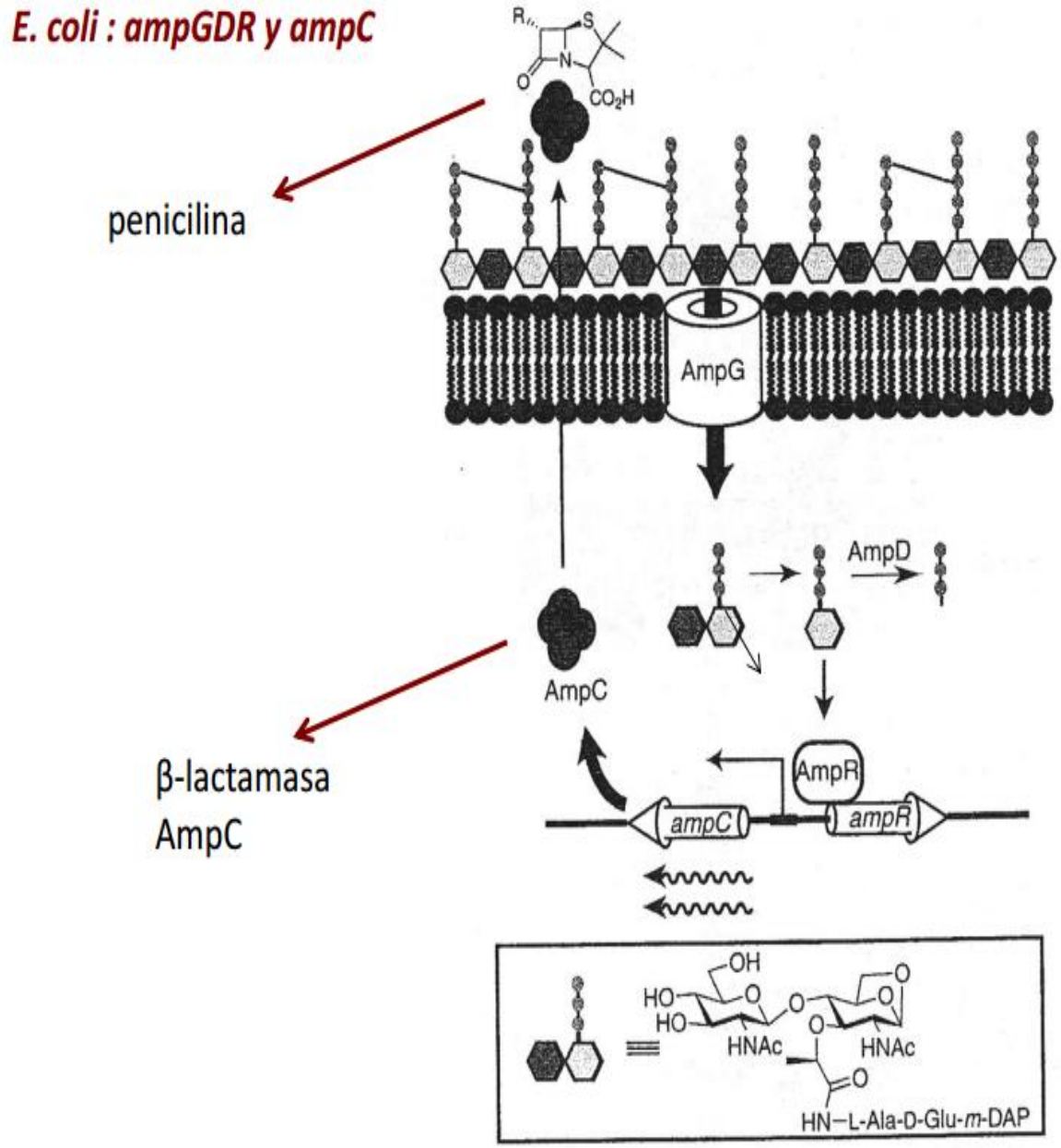




Clavulanate-Amoxicillin → Augmentin
Clavulanate-Ticarcillin → Timentin
Sulbactam-Ampicillin → Unasyn
Tazobactam-Piperacillin → Zocin

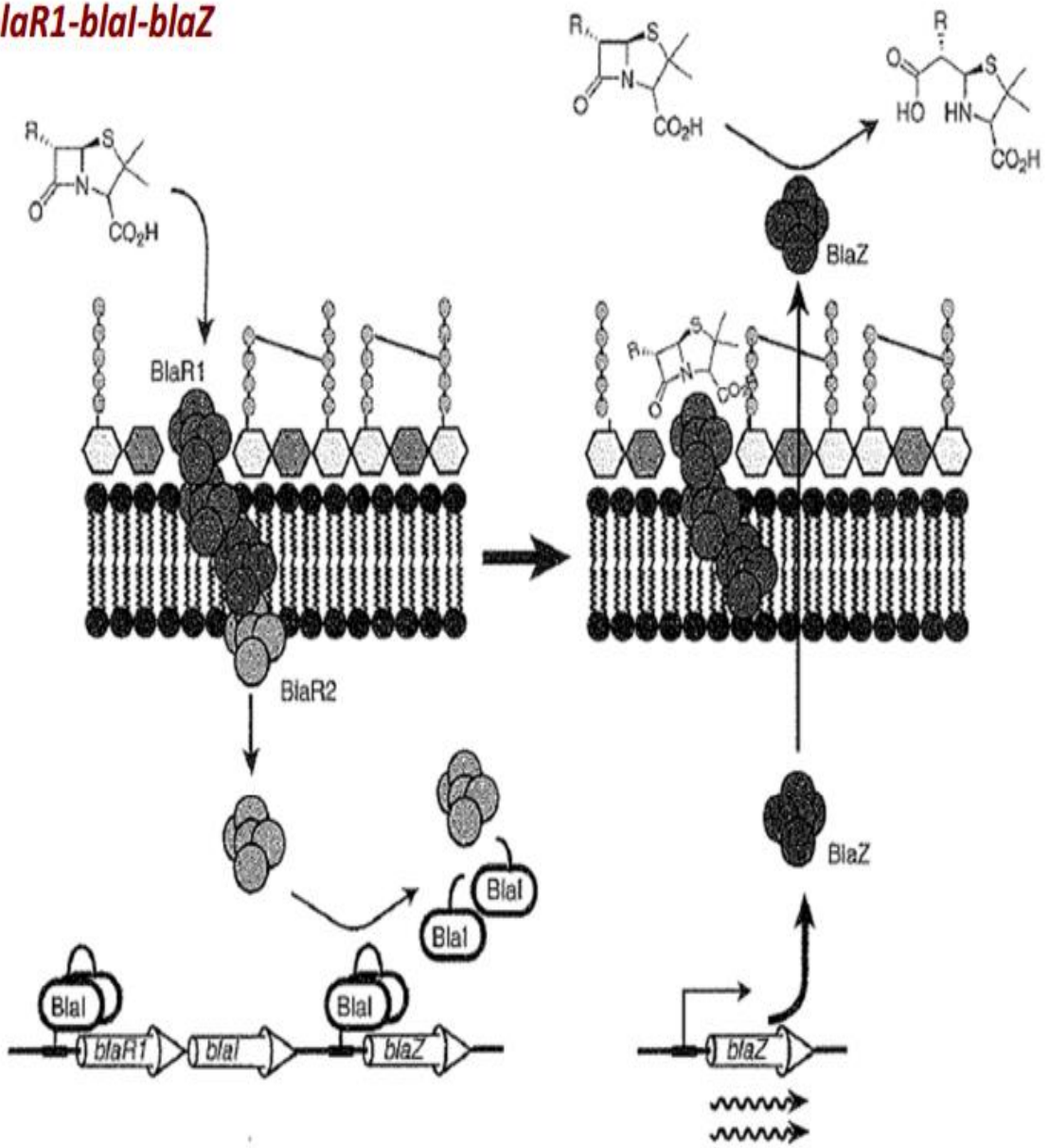
Regulación de la expresión de los genes de las β-lactamasas

E. coli : ampGDR y ampC

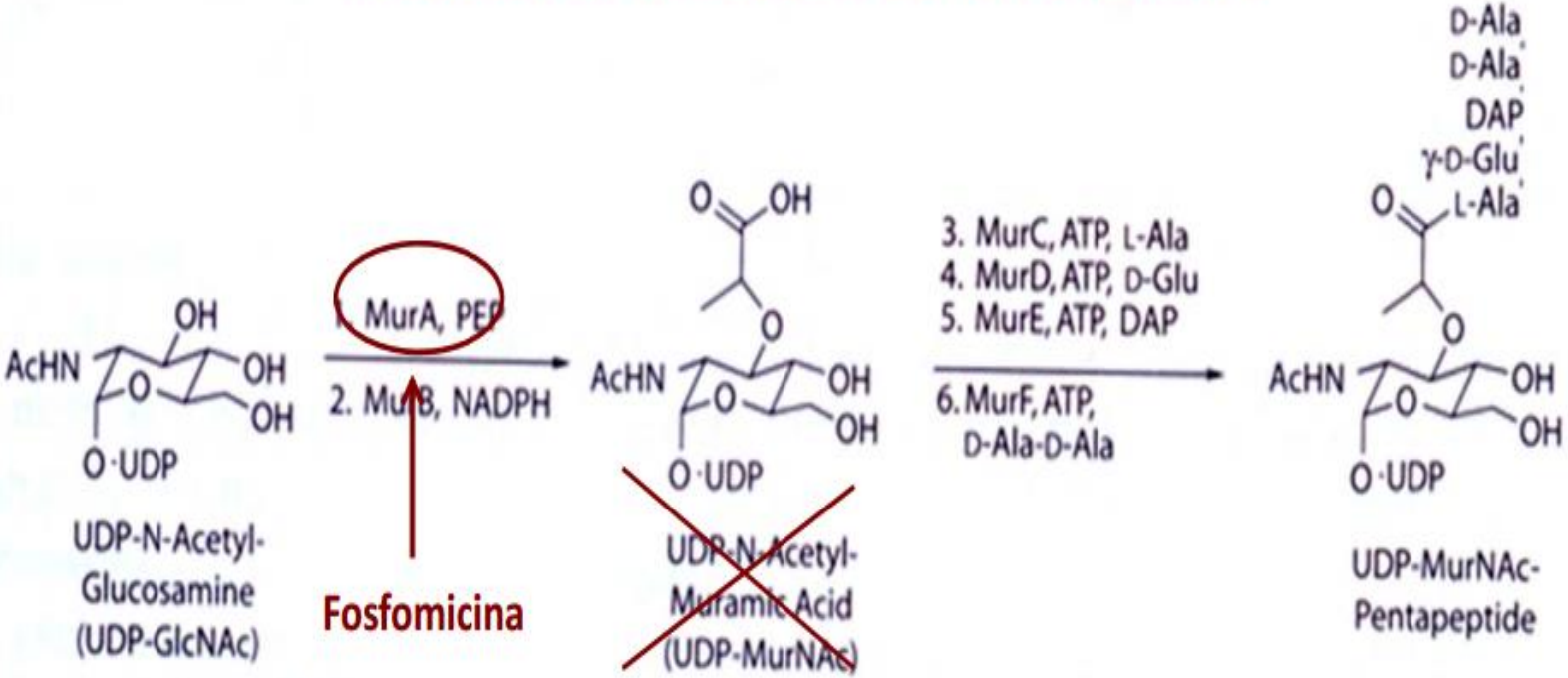


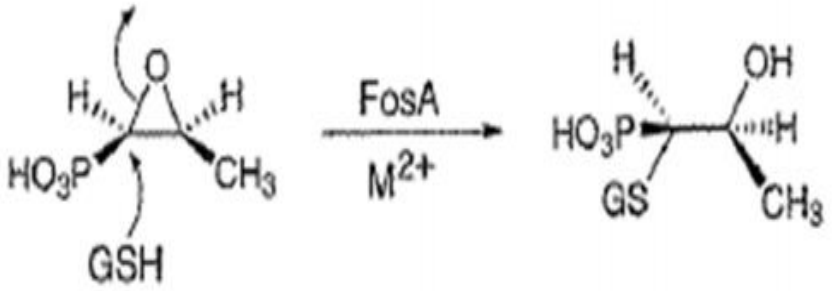
Regulación de la expresión de los genes de las β-lactamasas

S. aureus : blaR1-blaI-blaZ



Inactivación enzimática de la fosfomicina

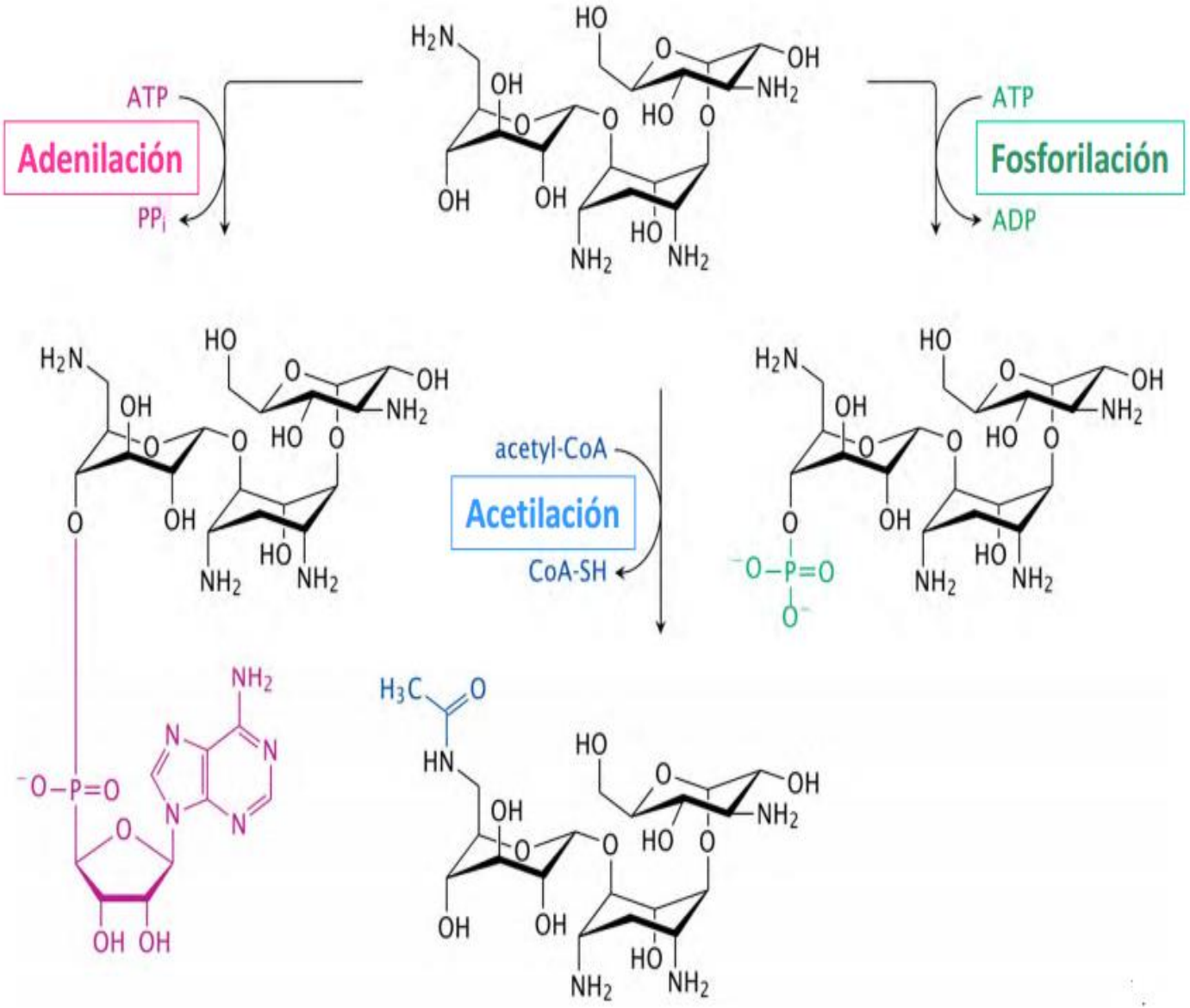




FosA: fosfomicin-glutatión S-transferasa

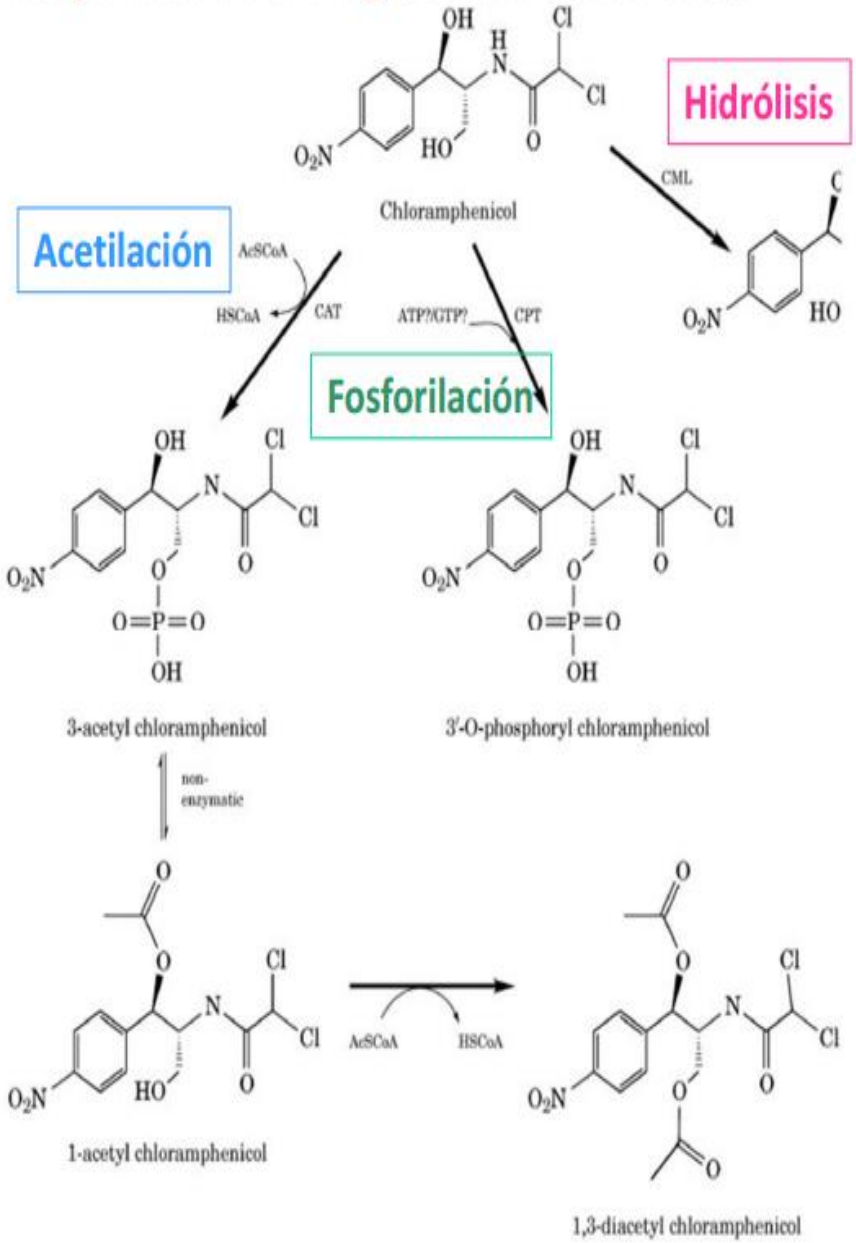
Modificación de la droga

Modificaciones químicas de aminoglicosidos: **Kanamicina**



Modificación de la droga

Modificaciones químicas de aminoglicósidos: **Cloranfenicol**

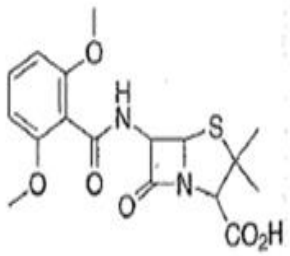


Modificación del blanco

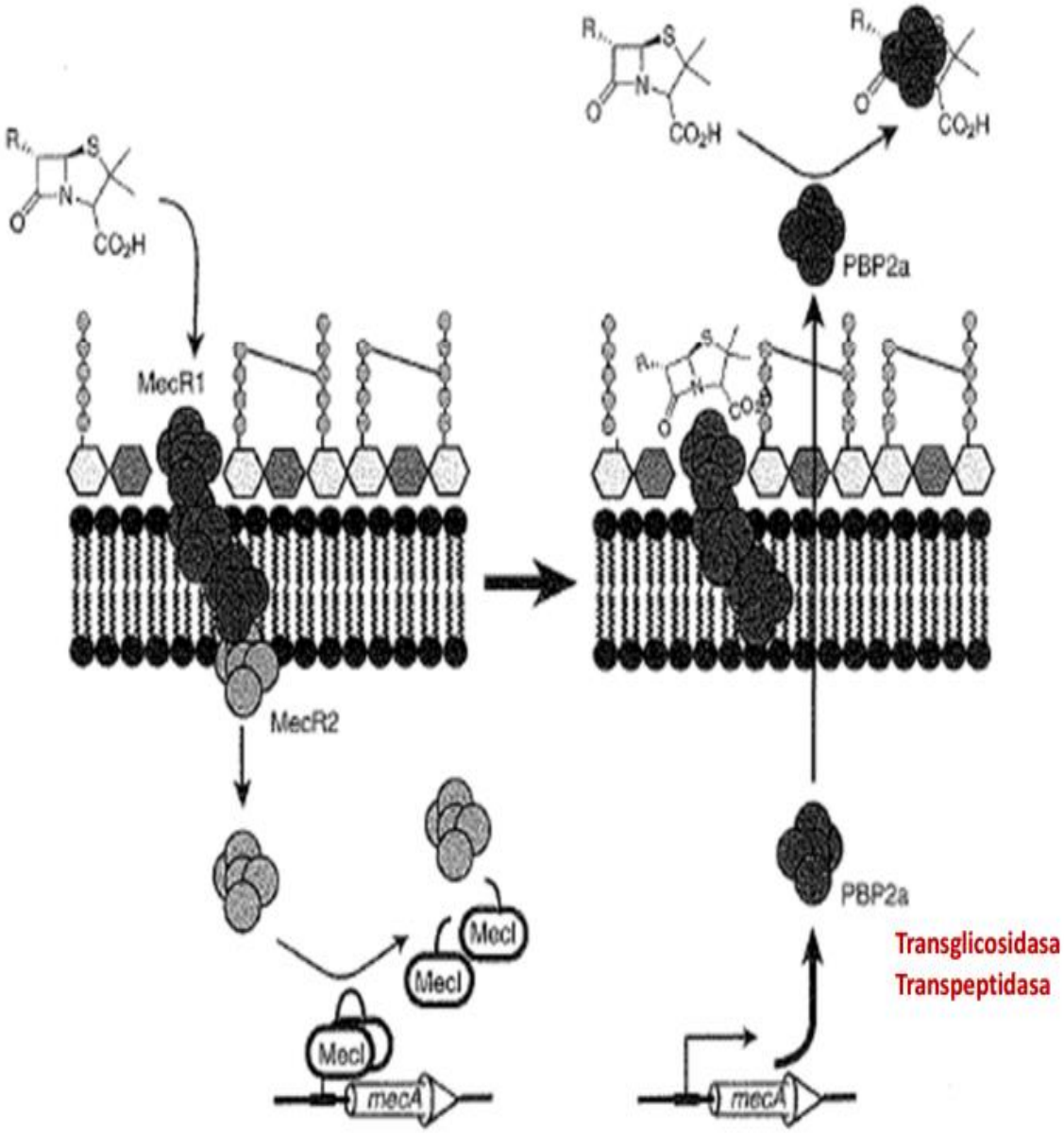
Reemplazo del sitio blanco

Resistencia a β-lactámicos: Síntesis de PBP2a insensible a β-lactámicos

***S. aureus* MRSA**

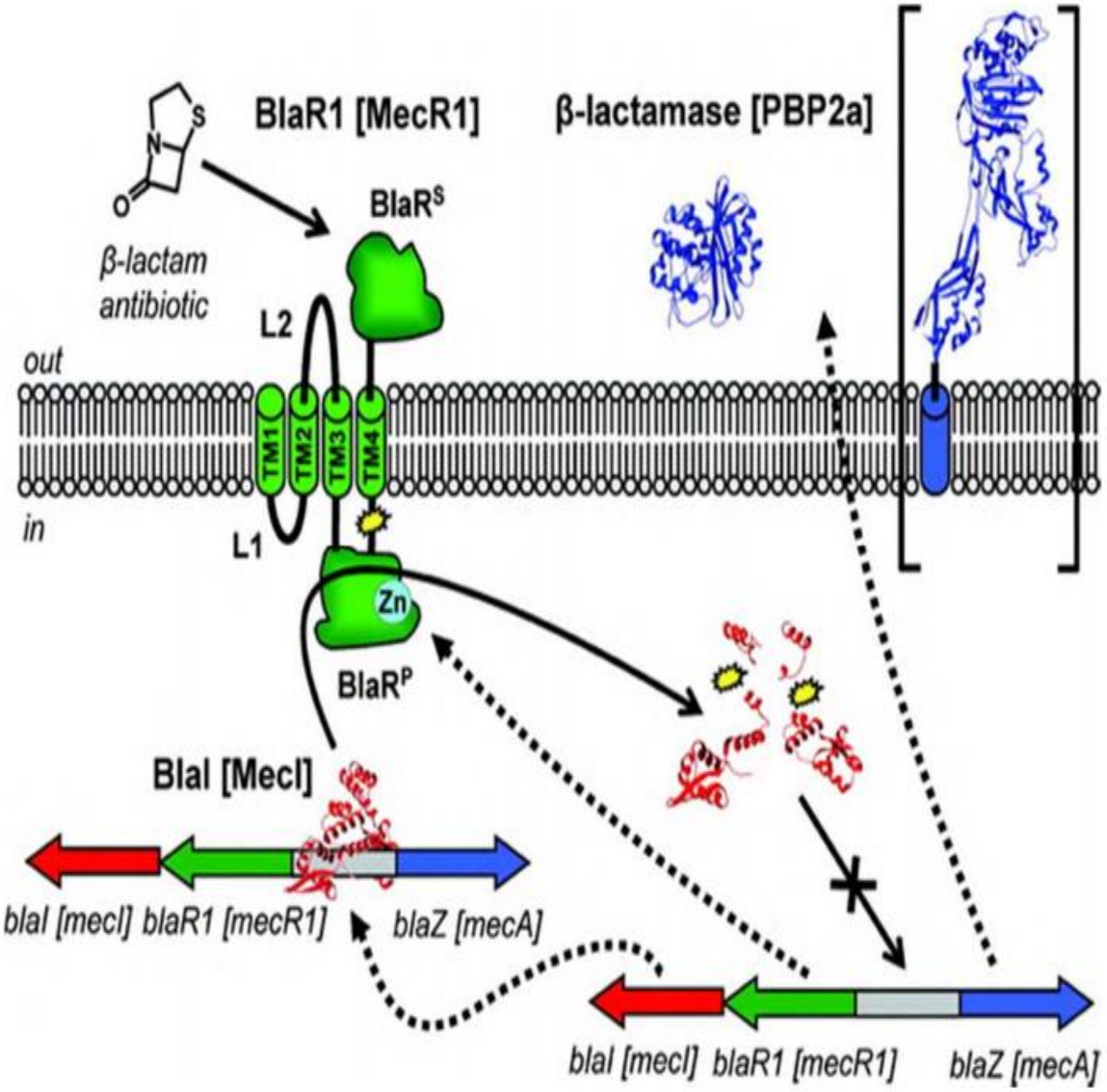


Methicillin



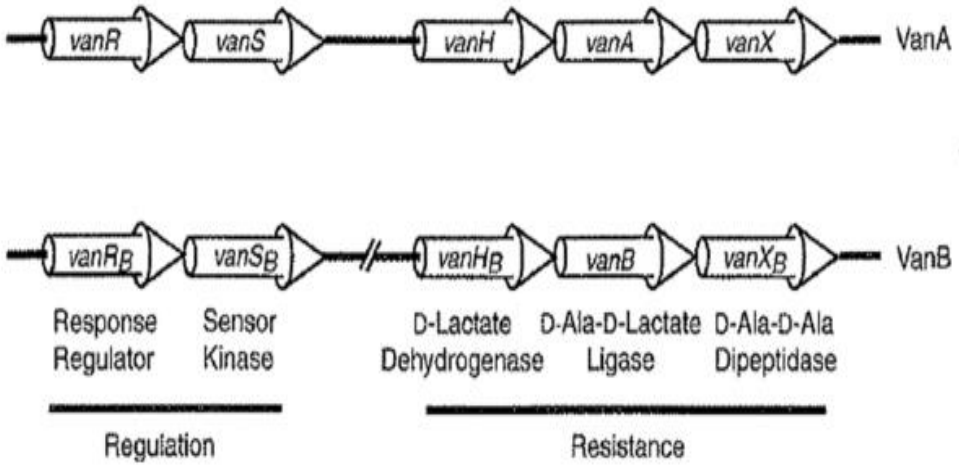
Comparación de mecanismos de regulación de resistencia a β-lactámicos

S. aureus MRSA



Modificación del sitio blanco

Resistencia a Vancomicina: *vanR, vanS, vanH, vanA,, vanX, vanY* y *vanZ*



VanS: Proteina sensora

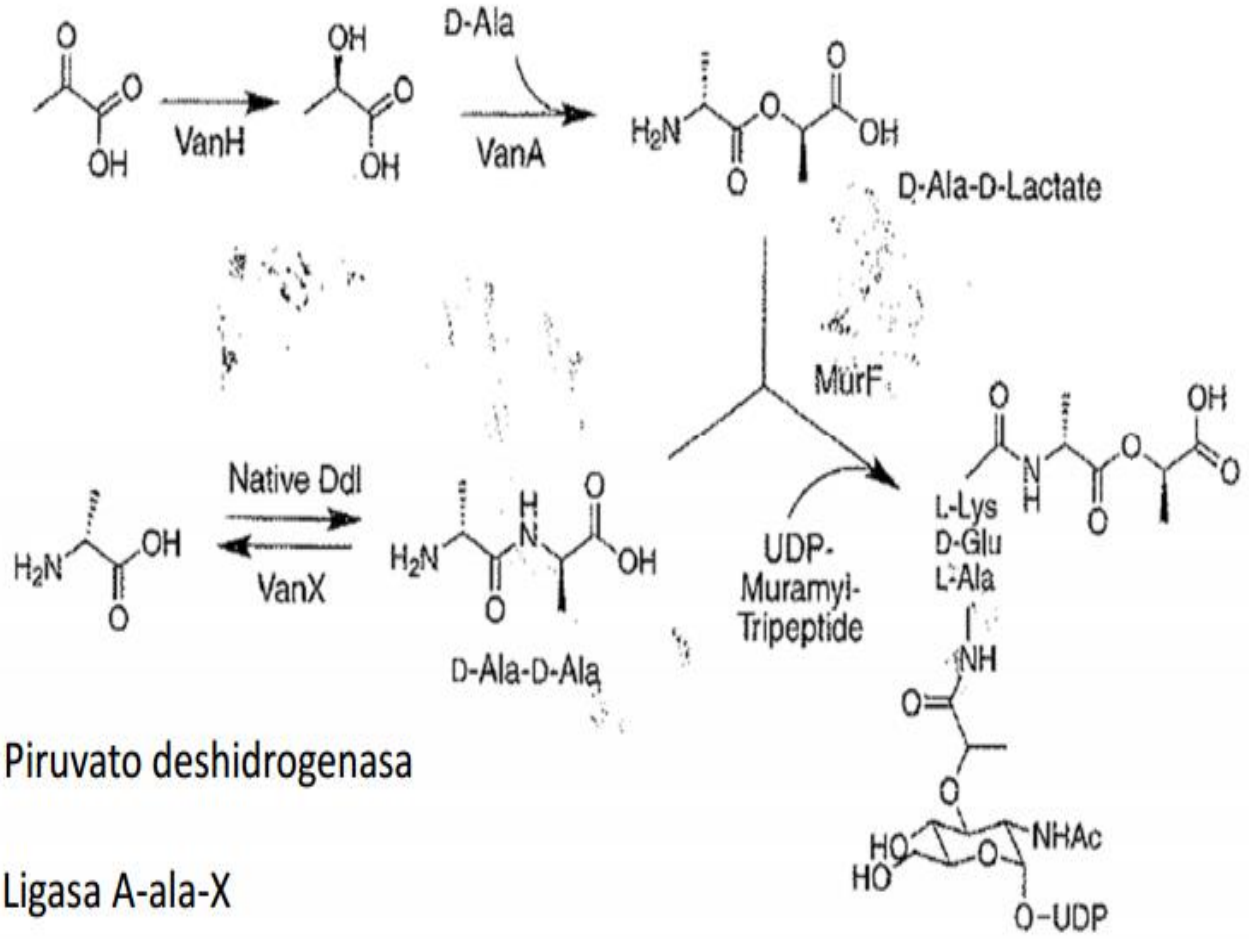
VanR: Activador transcripcional

VanH: Piruvato deshidrogenasa

VanA: Ligasa A-ala-X

VanX: D-ala-D-ala dipeptidasa

VanY: D-D Carboxipeptidasa



VanH: Piruvato deshidrogenasa

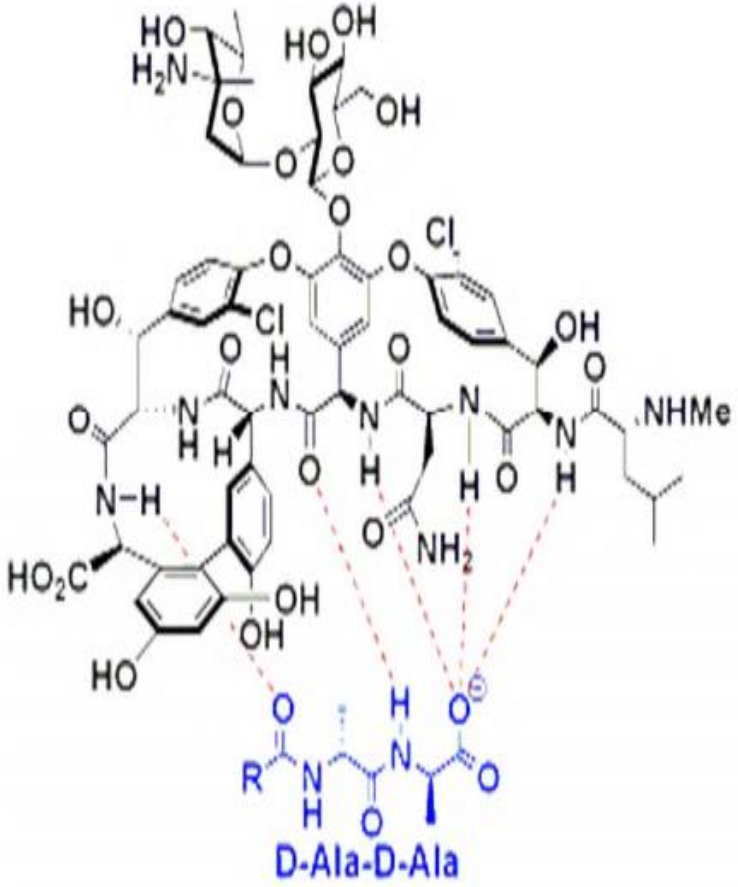
VanA: Ligasa A-ala-X

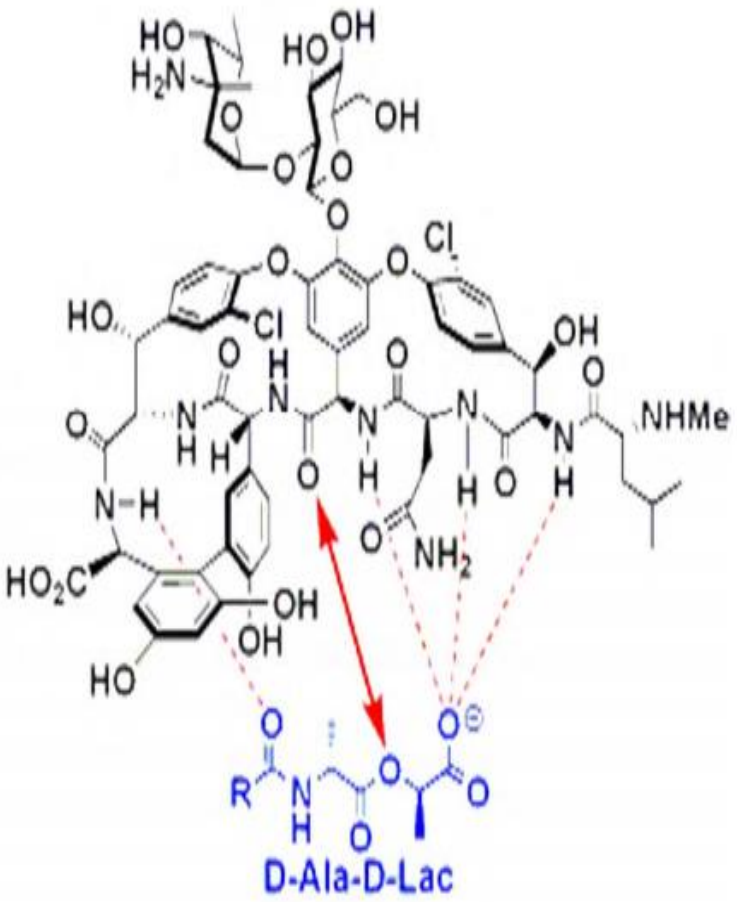
VanX: D-ala-D-ala dipeptidasa

VanY: D-D Carboxipeptidasa

Modificación del sitio blanco

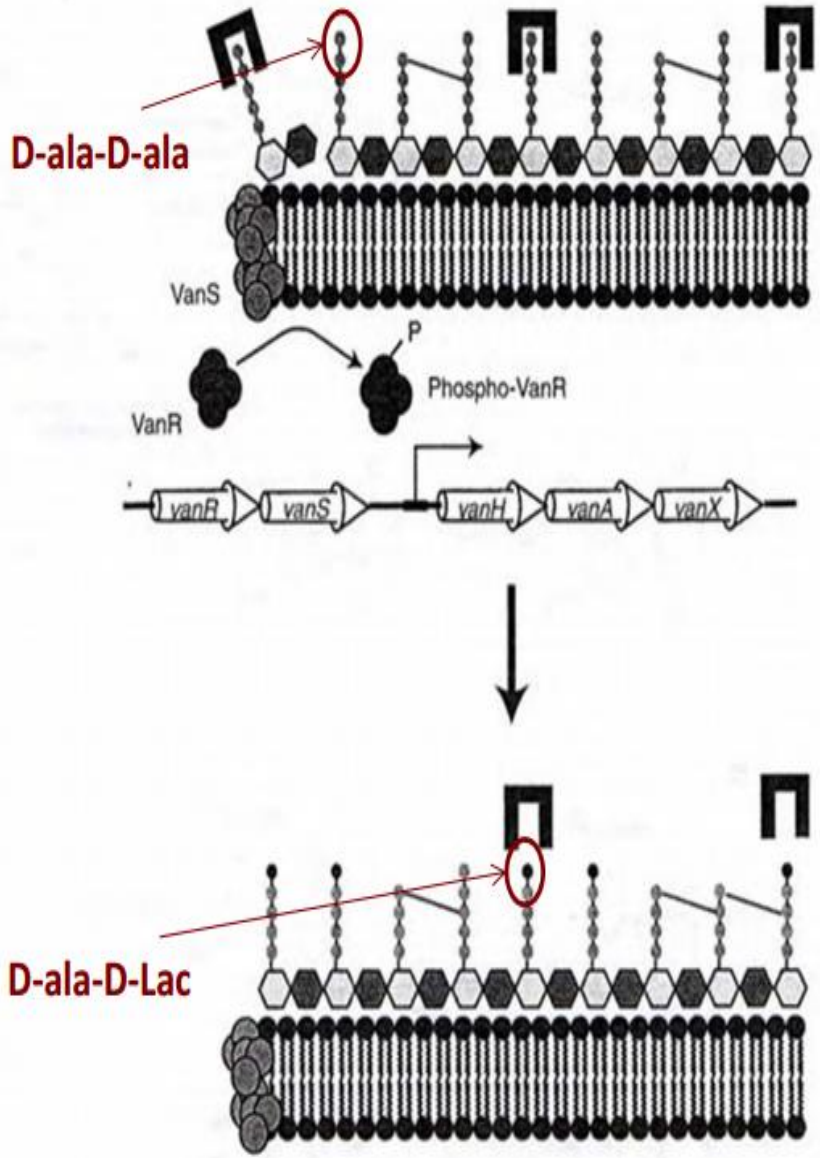
Resistencia a Vancomicina por modificación de D-Ala por D-Lac





Modificación del sitio blanco

Resistencia a Vancomicina: *vanR, vanS, vanH, vanA,, vanX, vanY* y *vanZ*



VanS: Proteina sensora

VanR: Activador transcripcional

VanH: Piruvato deshidrogenasa

VanA: Ligasa A-ala-X

VanX: D-ala-D-ala dipeptidasa

VanY: D-D Carboxipeptidasa

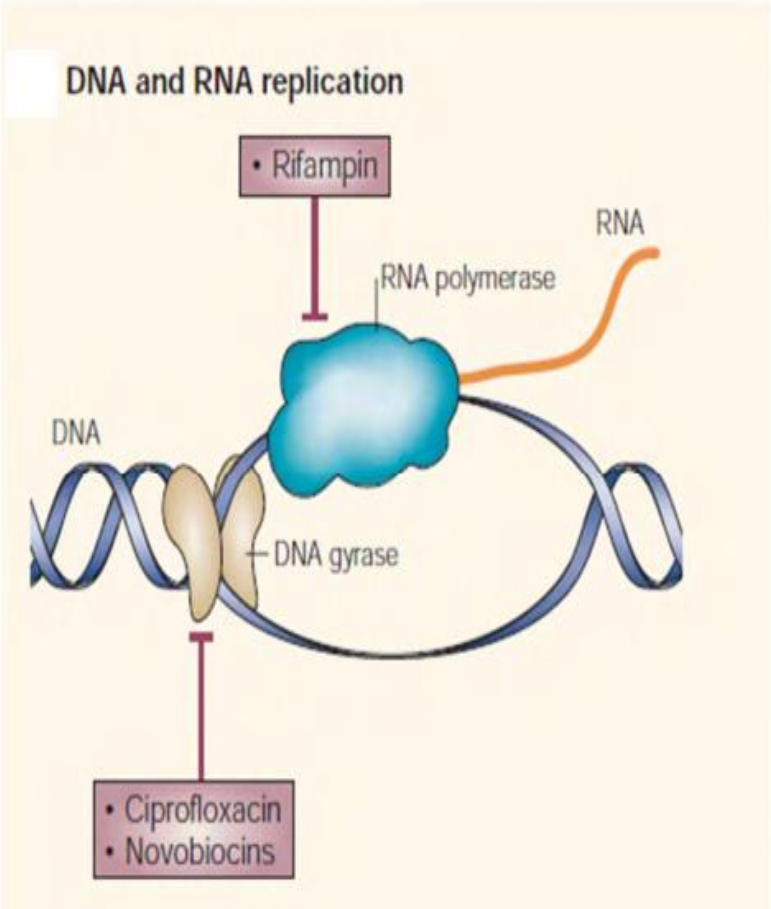
Modificación del sitio blanco

Resistencia a Rifampicina:

Mutaciones puntuales de subunidad β RNA Pol

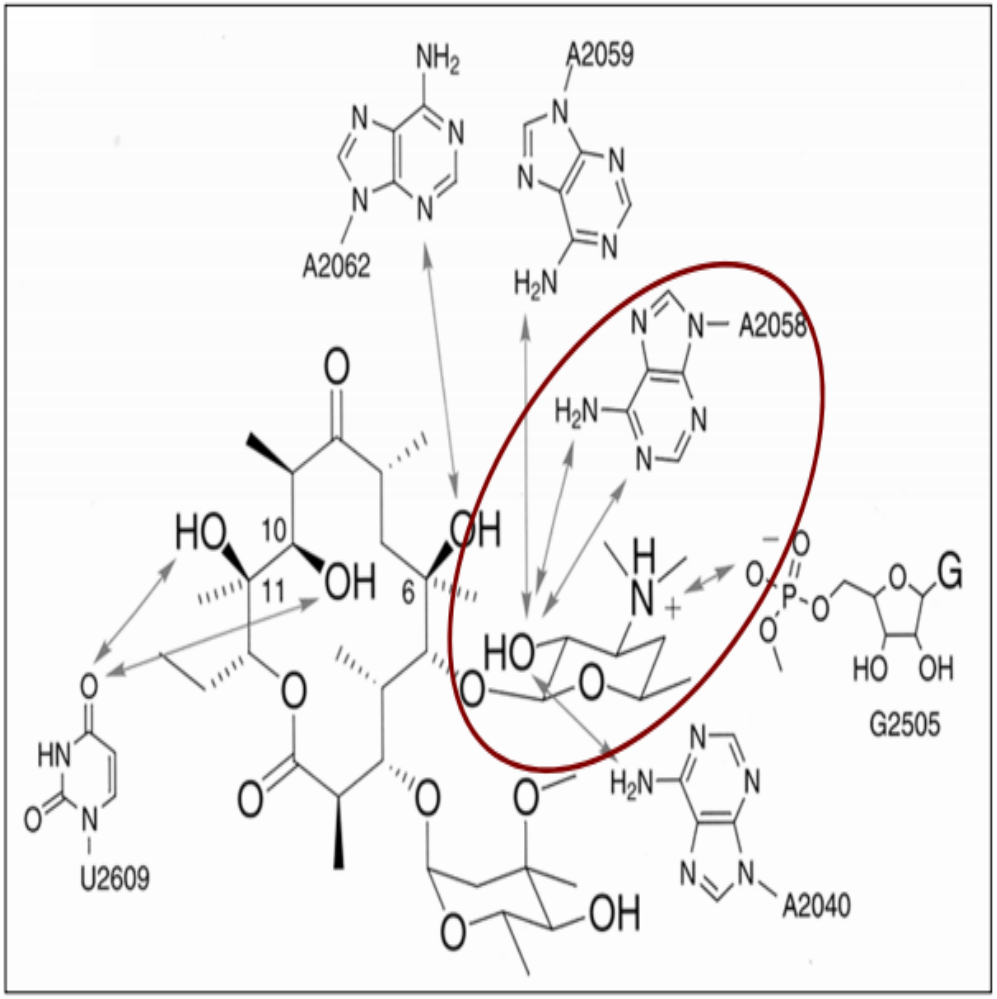
Resistencia a Quinolonas:

Mutaciones puntuales de subunidad A de DNA Girasa



Modificación del sitio blanco

Unión de **Macrólidos** al ribosoma



Dimetilación de residuo Adenina rRNA 23S

Mutaciones puntuales de rRNA o proteínas ribosomales

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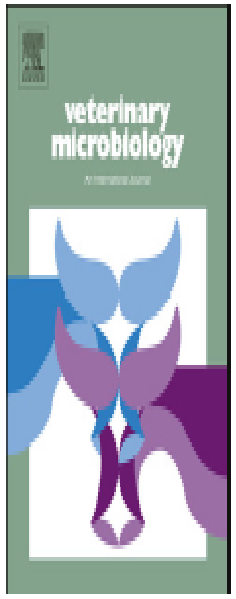

Functional metagenomic analysis reveals rivers are a reservoir for diverse antibiotic resistance genes

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Waste water
Environmental resistance
Sewage

ABSTRACT

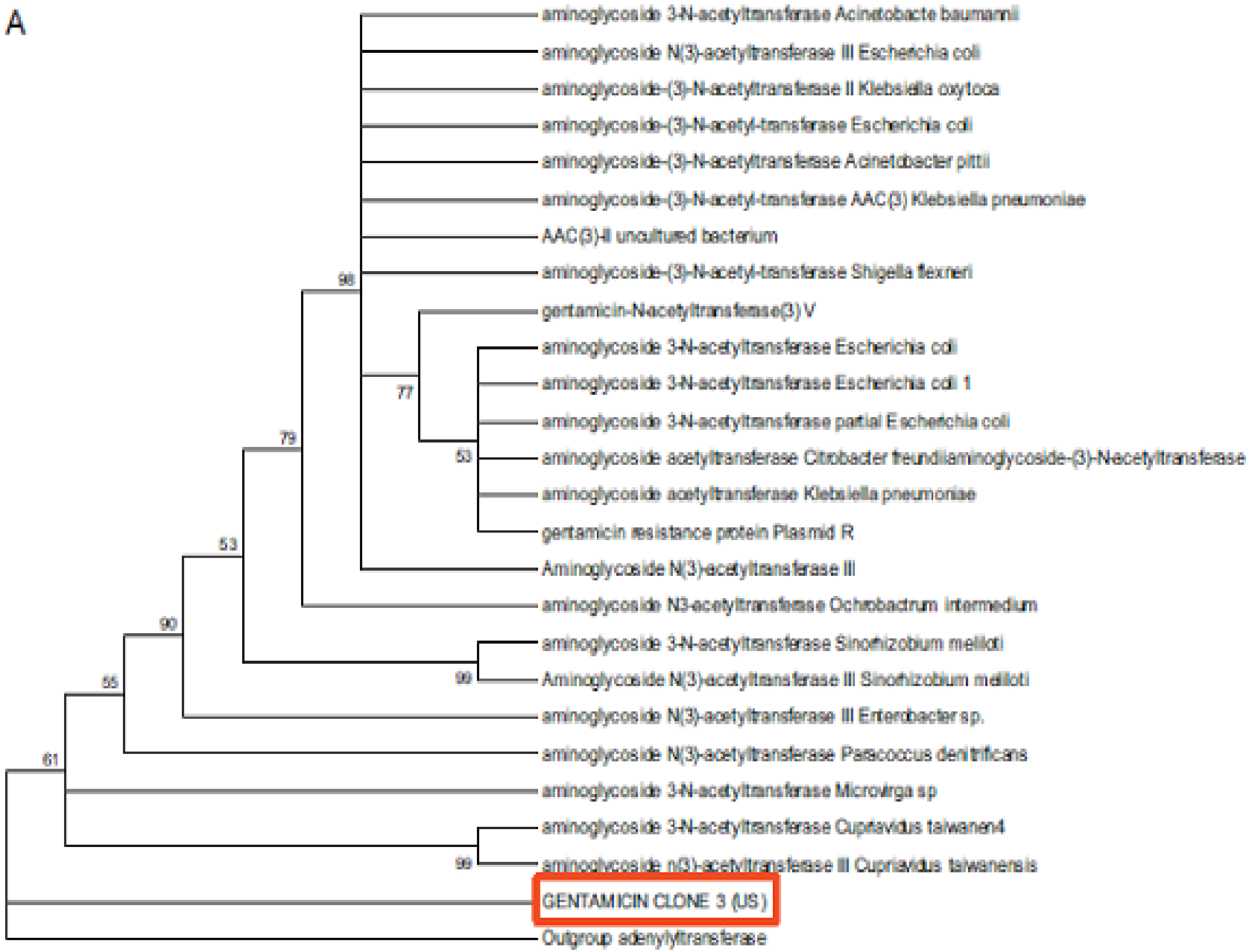
The environment harbours a significant diversity of uncultured bacteria and a potential source of novel and extant resistance genes which may recombine with clinically important bacteria disseminated into environmental reservoirs. There is evidence that pollution can select for resistance due to the aggregation of adaptive genes on mobile elements. The aim of this study was to establish the impact of waste water treatment plant (WWTP) effluent disposal to a river by using culture independent methods to study diversity of resistance genes downstream of the WWTP in comparison to upstream. Metagenomic libraries were constructed in *Escherichia coli* and screened for phenotypic resistance to amikacin, gentamicin, neomycin, ampicillin and ciprofloxacin. Resistance genes were identified by using transposon mutagenesis. A significant increase downstream of the WWTP was observed in the number of phenotypic resistant clones recovered in metagenomic libraries. Common β-lactamases such as bla_{TEM} were recovered as well as a diverse range of acetyltransferases and unusual transporter genes, with evidence for newly emerging resistance mechanisms. The similarities of the predicted proteins to known sequences suggested origins of genes from a very diverse range of bacteria. The study suggests that waste water disposal increases the reservoir of resistance mechanisms in the environment either by addition of resistance genes or by input of agents selective for resistant phenotypes.

Table 1
Summary of metagenomic libraries made from DS and US samples.

Sample	Average insert size	Average number of clones	Library coverage
DS	4.2 kb	2×10^6	8.4 Gb
US	4.7 kb	2×10^6	9.4 Gb

Table 2
Analysis of resistant clones in DS and US libraries.

Antibiotic	MIC of *Epi*300 strain ($mg\,L^{-1}$)	Number of resistant clones DS	Number of resistant clones US	Proportion of bacteria carrying resistant gene downsteam (%)	Proportion of bacteria carrying resistant gene upstream (%)	MIC tested for resistant clones ($mg\,L^{-1}$)
Ampicillin	8	>50	>50	2.74	2.45	16
Gentamicin	3	15	9	0.82	0.44	6
Neomycin	8	14	6	0.76	0.29	16
Amikacin	4	4	0	0.22	0	16
Ciprofloxacin	0.25	4	1	0.22	0.05	1


A
aminoglycoside 3-N-acetyltransferase Acinetobacte baumannii
aminoglycoside N(3)-acetyltransferase III Escherichia coli
aminoglycoside-(3)-N-acetyltransferase II Klebsiella oxytoca
aminoglycoside-(3)-N-acetyl-transferase Escherichia coli
aminoglycoside-(3)-N-acetyltransferase Acinetobacter pittii
aminoglycoside-(3)-N-acetyl-transferase AAC(3) Klebsiella pneumoniae
AAC(3)-II uncultured bacterium
aminoglycoside-(3)-N-acetyl-transferase Shigella flexneri
gentamicin-Nacetyltransferase(3) V
aminoglycoside 3-N-acetyltransferase Escherichia coli
aminoglycoside 3-N-acetyltransferase Escherichia coli 1
aminoglycoside 3-N-acetyltransferase partial Escherichia coli
aminoglycoside acetyltransferase Citrobacter freundiaminoglycoside-(3)-Nacetyltransferase
aminoglycoside acetyltransferase Klebsiella pneumoniae
gentamicin resistance protein Plasmid R
Aminoglycoside N(3)-acetyltransferase III
aminoglycoside N3-acetyltransferase Ochrobactrum intermedium
aminoglycoside 3-N-acetyltransferase Sinorhizobium meliloti
Aminoglycoside N(3)-acetyltransferase III Sinorhizobium meliloti
aminoglycoside N(3)-acetyltransferase III Enterobacter sp.
aminoglycoside N(3)-acetyltransferase Paracoccus denitrificans
aminoglycoside 3-N-acetyltransferase Microvirga sp
aminoglycoside 3-N-acetyltransferase Cupriavidus taiwanen4
aminoglycoside n(3)-acetyltransferase III Cupriavidus taiwanensis
GENTAMICIN CLONE 3 (US)
Outgroup adenylyltransferase
98
77
79
53
53
90
55
99
99
61

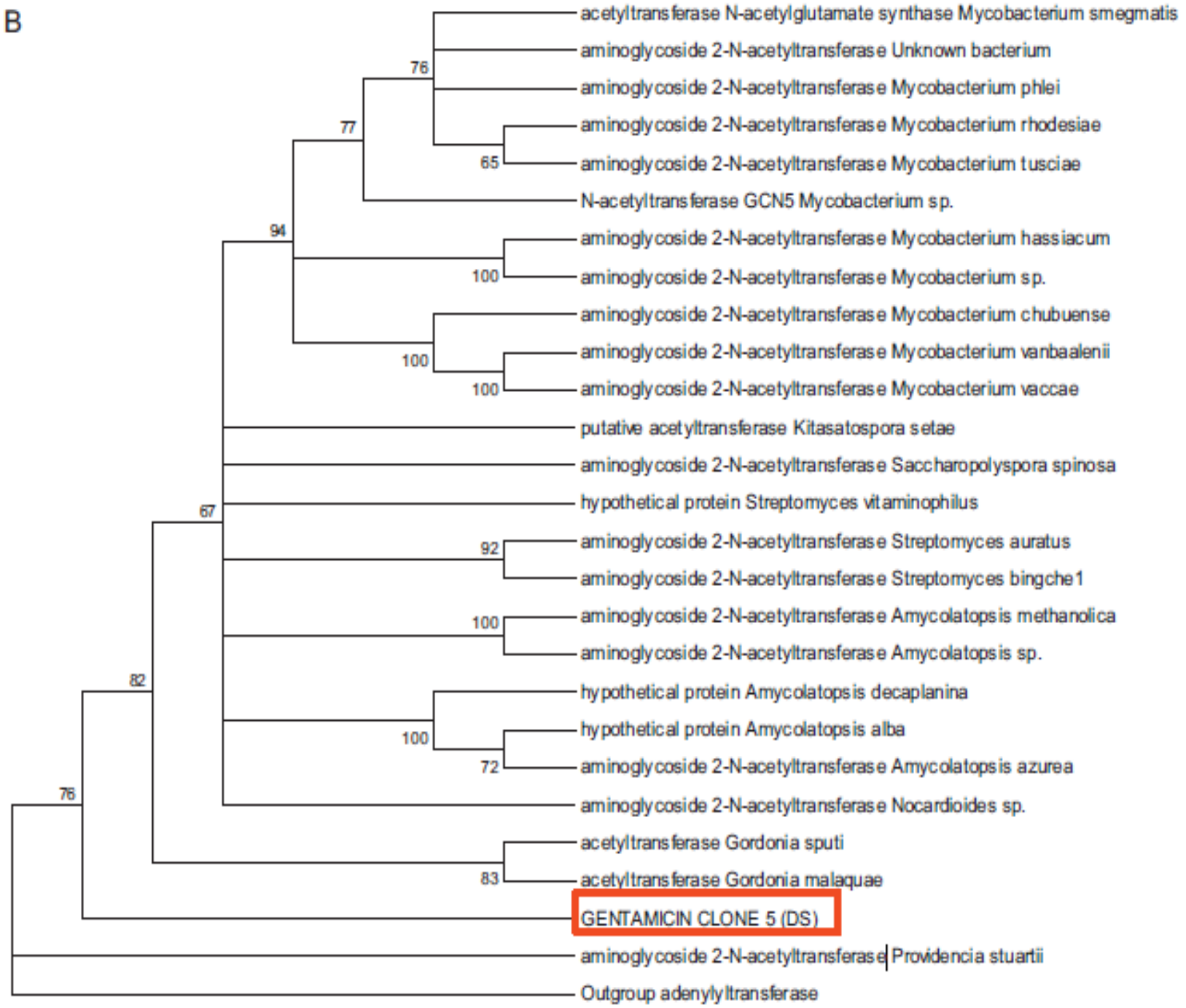

B
acetyltransferase N-acetylglutamate synthase Mycobacterium smegmatis
aminoglycoside 2-N-acetyltransferase Unknown bacterium
aminoglycoside 2-N-acetyltransferase Mycobacterium phlei
aminoglycoside 2-N-acetyltransferase Mycobacterium rhodesiae
aminoglycoside 2-N-acetyltransferase Mycobacterium tusciae
N-acetyltransferase GCN5 Mycobacterium sp.
aminoglycoside 2-N-acetyltransferase Mycobacterium hassiacum
aminoglycoside 2-N-acetyltransferase Mycobacterium sp.
aminoglycoside 2-N-acetyltransferase Mycobacterium chubuense
aminoglycoside 2-N-acetyltransferase Mycobacterium vanbaalenii
aminoglycoside 2-N-acetyltransferase Mycobacterium vaccae
putative acetyltransferase Kitasatospora setae
aminoglycoside 2-N-acetyltransferase Saccharopolyspora spinosa
hypothetical protein Streptomyces vitaminophilus
aminoglycoside 2-N-acetyltransferase Streptomyces auratus
aminoglycoside 2-N-acetyltransferase Streptomyces bingche1
aminoglycoside 2-N-acetyltransferase Amycolatopsis methanolica
aminoglycoside 2-N-acetyltransferase Amycolatopsis sp.
hypothetical protein Amycolatopsis decaplanina
hypothetical protein Amycolatopsis alba
aminoglycoside 2-N-acetyltransferase Amycolatopsis azurea
aminoglycoside 2-N-acetyltransferase Nocardioides sp.
acetyltransferase Gordonia sputi
acetyltransferase Gordonia malaquae
GENTAMICIN CLONE 5 (DS)
aminoglycoside 2-N-acetyltransferase Providencia stuartii
Outgroup adenylyltransferase
76
77
65
94
100
100
100
67
92
100
82
100
72
76
83

Table 3
Identities of resistance genes and predicted proteins from clones analysed by transposon mutagenesis.

Antibiotic resistance conferred (library)	Predicted size of protein (amino acids)	Predicted domains	Nearest sequence identity (bacteria identity)
Gentamicin clone 1 (US library)	420	Potassium transporter superfamily	77% Potassium transport protein (*Janthinobacterium* sp.)
Gentamicin clone 2 (US library)	58	None	75% Hypothetical protein (*Escherichia coli*)
Gentamicin clone 3 (US library)	264	Aminoglycoside 3-N-acetyltransferase	59% Aminoglycoside-(3)-N-acetyltransferase (*Escherichia coli*)
Gentamicin clone 4 (US library)	329	Thiamine pyrophosphate family	88% Pyruvate dehydrogenase subunit E1 (*Janthinobacterium* sp.)
Gentamicin clone 5 (DS library)	178	Aminoglycoside 3-N-acetyltransferase	36% Acetyltransferase (GNAT) family protein (*Providencia rettgeri*)
Gentamicin clone 6 (DS library	77	DUF4111	90% Aminoglycoside 3′-adenylytransferase (*Yersinia pestis*)
Gentamicin clone 7 (DS library)	88	Aminoglycoside 3′-phosphotransferase (APH)	100% Aminoglycoside 3′-phosphotransferase (*Pseudomonas putida*)
Amikacin clone 1 (DS library	185	Aminoglycoside 3-N-acetyltransferase	58% Aminoglycoside N(6′)-acetyltransferase (*Gloeocapsa* sp.)
Amikacin 2 (DS library)	119	Nucleotidyl transferase superfamily	62% Methionyl-tRNA synthetase (*Haliscomenobacter hydrossis*)
Ampicillin clone 1 (US library	289	Beta-lactamase2 superfamily	99% Beta-lactamase TEM (*Bacillus subtilis*)
Ampicillin clone 2 (DS library)	289	Beta-lactamase2 superfamily	99% Beta-lactamase TEM (*Bacillus subtilis*)
Ampicillin clone 3 (DS library)	289	Beta-lactamase2 superfamily	99% Beta-lactamase TEM (*Bacillus subtilis*)
Neomycin clone 1 (DS library)	107	TroA like superfamily FepB BC-type Fe3+ -hydroxamate transport system, periplasmic component	48% Hypothetical protein (*Streptomyces* sp.)
Neomycin clone 2 (DS library)	162	Glycosyltransferase family 25	36% Glycosyltransferase 25 family member 1 (*Agrobacterium* sp.)
Ciprofloxacin clone 1 (DS library)	145 154	RecX (recombination regulator) RecA (bacterial DNA recombination protein)	33% Regulatory protein RecX (*Listeria seeligeri*) 74% Recombinase A (*Geobacter lovleyi*)

Wastewater Treatment Plants Release Large Amounts of Extended-Spectrum β-Lactamase–Producing *Escherichia coli* Into the Environment

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(See the Editorial Commentary by Griffiths and Barza on pages 1666–7.)

Background. The determinants of the spread of extended-spectrum β-lactamase–producing *Escherichia coli* (ESBLEC) in the community remain unclear. To evaluate its dissemination in the environment, we analyzed the ESBLEC population throughout an urban wastewater network.

Methods. Samples were collected weekly, over a 10-week period, from 11 sites throughout the wastewater network of Besançon city (France). Total *E. coli* and ESBLEC loads were determined for each sample. As a control, we analyzed 51 clinical ESBLEC isolates collected at our hospital. We genotyped both environmental and clinical ESBLEC by pulsed-field gel electrophoresis and multilocus sequence typing and identified their bla_{ESBL} genes by sequencing.

Results. The *E. coli* load was higher in urban wastewater than in hospital wastewater (7.5×10^5 vs 3.5×10^5 CFU/mL, respectively). ESBLEC was recovered from almost all the environmental samples and accounted for 0.3% of total *E. coli* in the untreated water upstream from the wastewater treatment plant (WWTP). The ESBLEC load was higher in hospital wastewater than in community wastewater (27×10^3 vs 0.8×10^3 CFU/mL, respectively). Treatment by the WWTP eliminated 98% and 94% of total *E. coli* and ESBLEC, respectively. The genotyping revealed considerable diversity within both environmental and clinical ESBLEC and the overrepresentation of some clonal complexes. Most of the sequence types displayed by the clinical isolates were also found in the environment. CTX-M enzymes were the most common enzymes whatever the origin of the isolates.

Conclusions. The treatment at the WWTP led to the relative enrichment of ESBLEC. We estimated that >600 billion of ESBLEC are released into the river Doubs daily and the sludge produced by the WWTP, used as fertilizer, contains 2.6×10^5 ESBLEC per gram.

Keywords. sequence types; WWTP; multidrug-resistant bacteria; environmental risk; sludge.

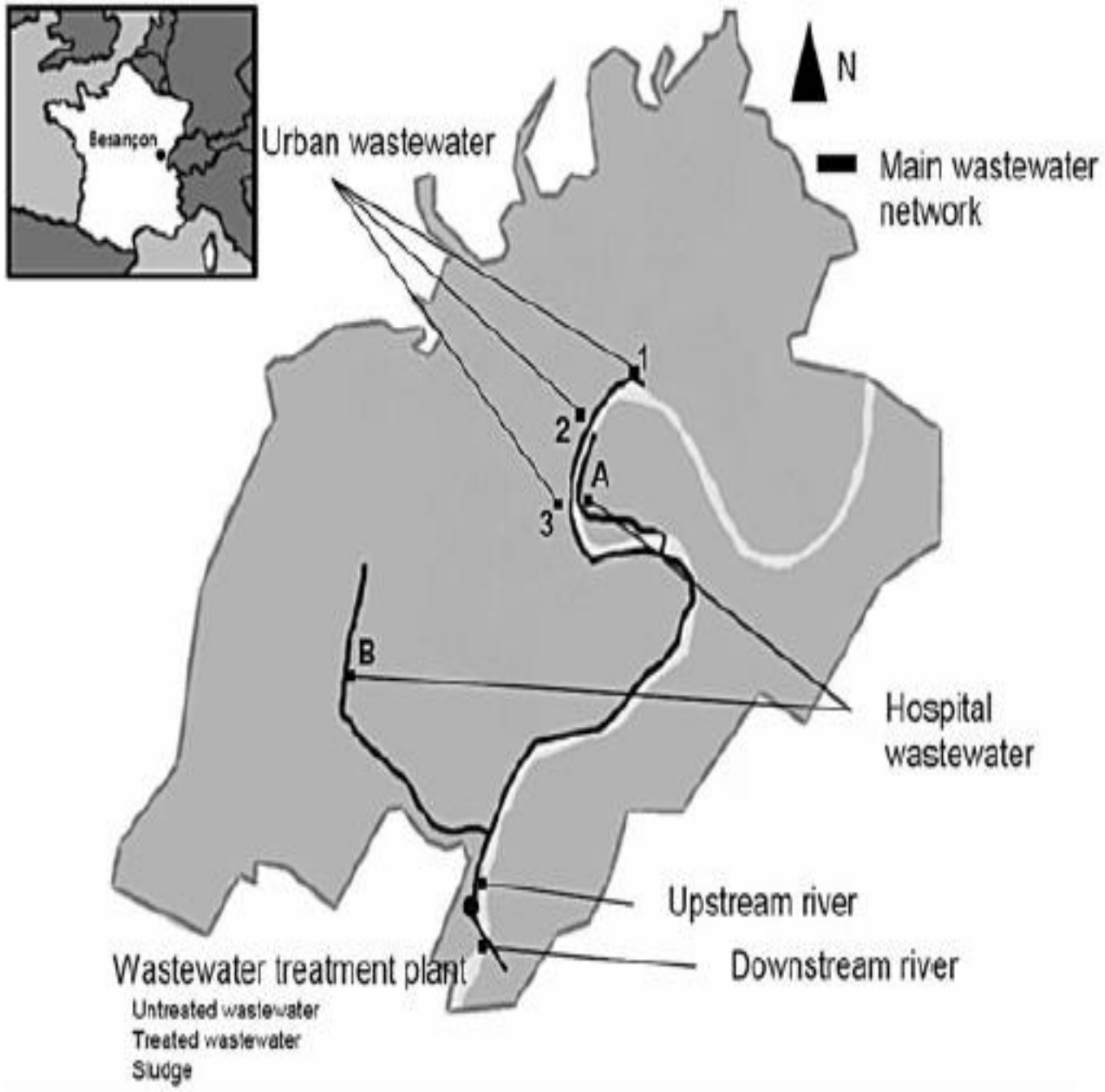
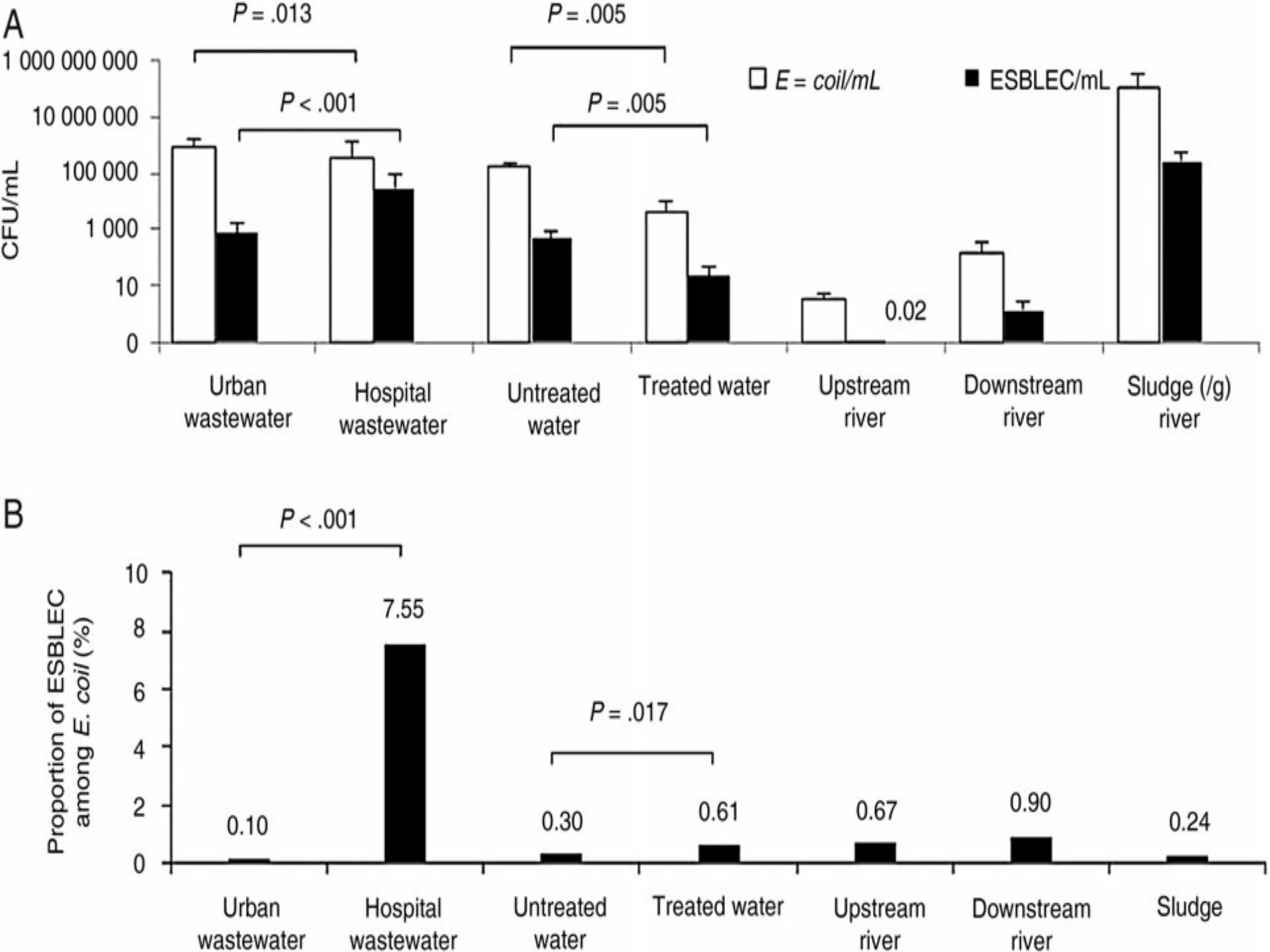
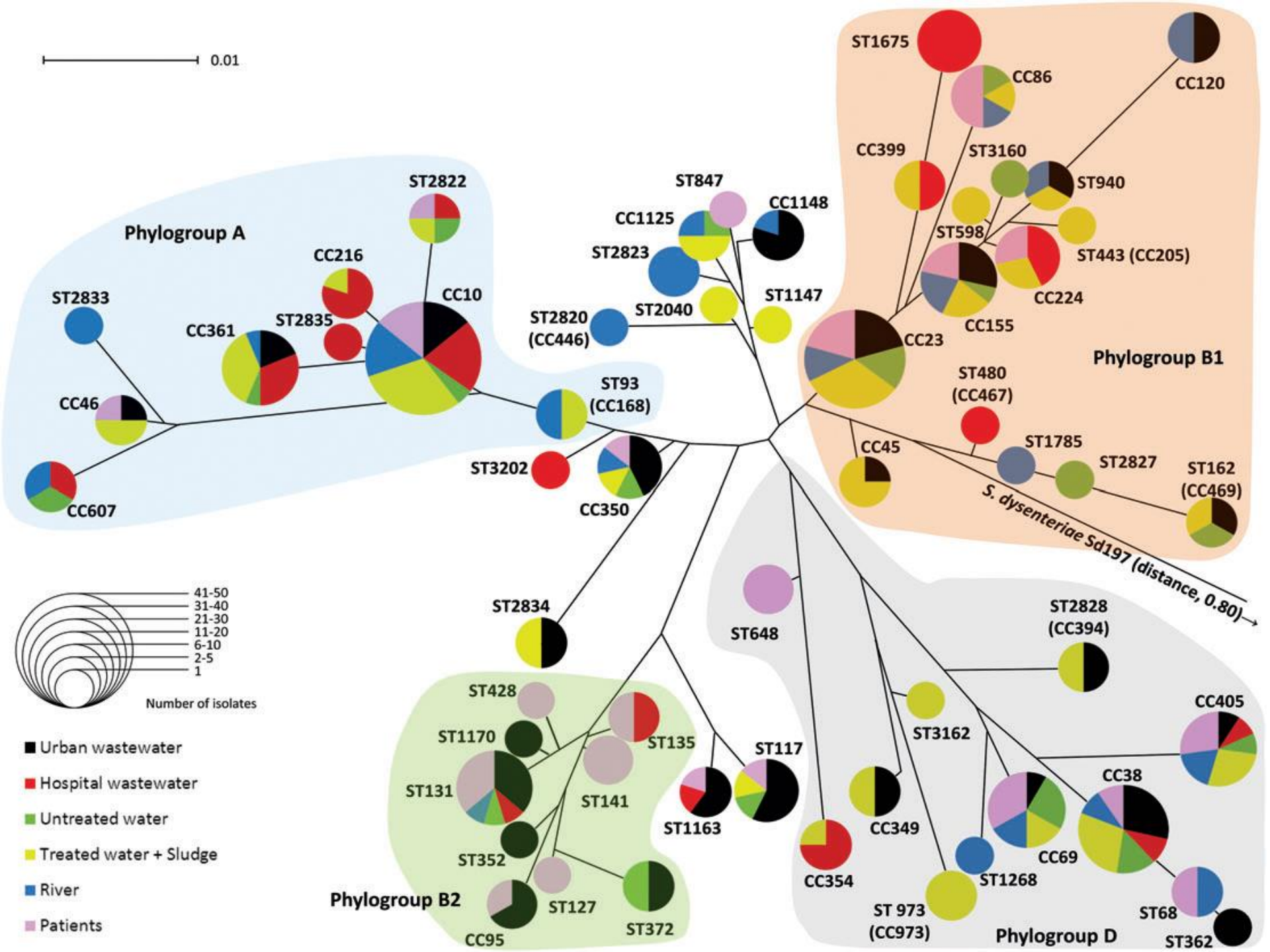
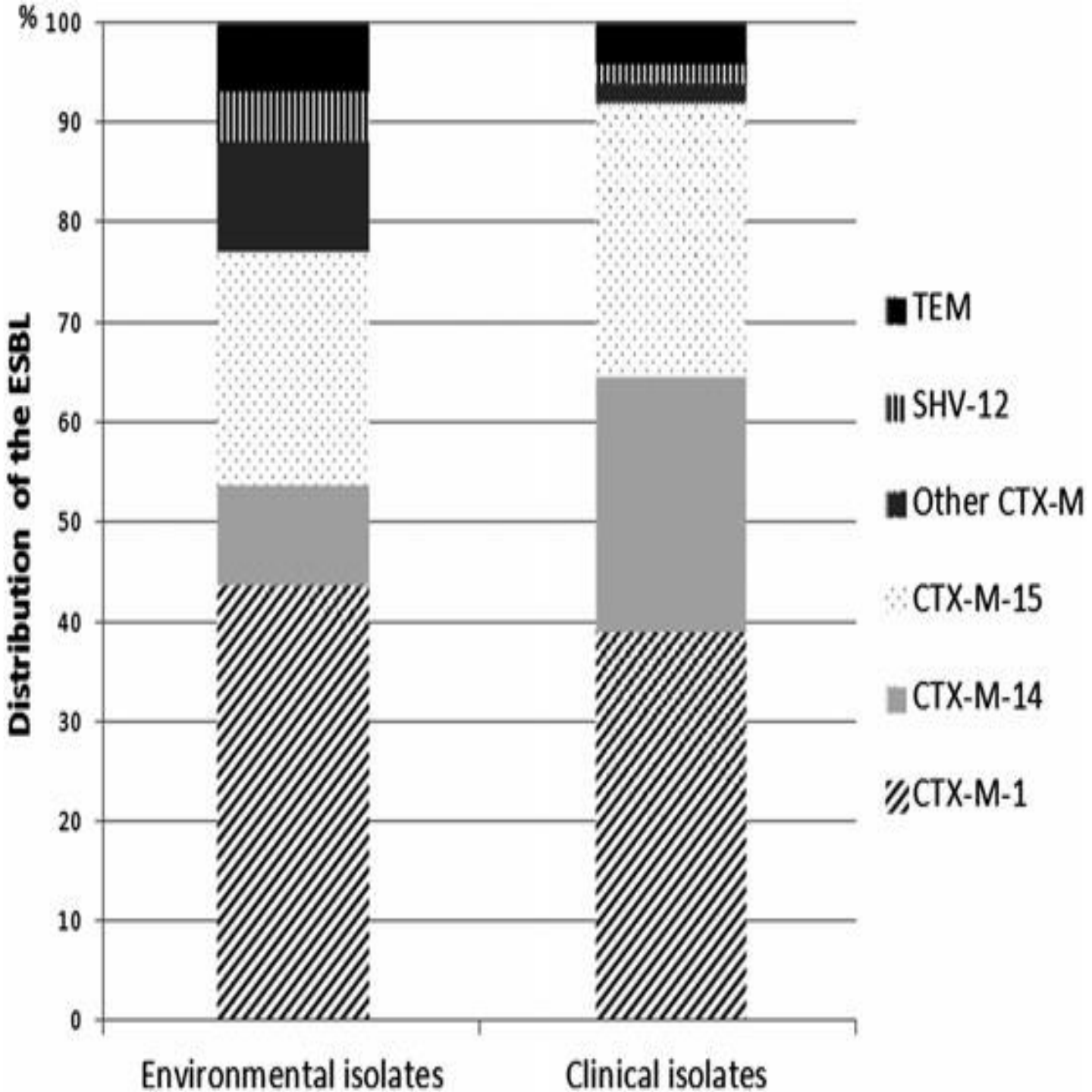

Besançon
Urban wastewater
N
Main wastewater network
1
2
3
A
B
Hospital wastewater
Upstream river
Downstream river
Wastewater treatment plant
Untreated wastewater
Treated wastewater
Sludge

Table 1. Antimicrobial Susceptibility of Extended-Spectrum β-Lactamase–Producing *Escherichia coli* Found in the Wastewater Network of Besançon, France, and in Patients Hospitalized at the University Hospital of Besançon During the Same Time Period (January–April 2011)

Sampling Sites or Origin	No. of Isolates	Susceptibility Rate, %[a]								
		Caz	Ctx	Tzp	Fox	Ipm	Nal	Oflo	Amk	Fos
Urban wastewater	59	**46**[b]	12	97	100	100	53	**66**[b]	100	93
Hospital wastewater	45	**11**[b]	20	91	98	100	33	**33**[b]	100	87
Wastewater treatment plant										
Untreated inflow	26	42	8	100	96	100	69	73	100	92
Treated outflow	58	31	2	91	93	100	55	59	100	90
River										
Upstream	10	80	0	100	100	100	70	70	90	100
Downstream	27	30	9	96	85	100	70	74	100	85
Sludge	17	53	18	94	94	100	71	76	100	71
Inpatients	51	24	4	88	80	100	41	39	100	94


A
P = .013
P = .005
P < .001
P = .005
E = coil/mL
ESBLEC/mL
1 000 000 000
10 000 000
100 000
1 000
10
0
CFU/mL
0.02
Urban wastewater
Hospital wastewater
Untreated water
Treated water
Upstream river
Downstream river
Sludge (/g) river
B
P < .001
P = .017
Proportion of ESBLEC among E. coil (%)
10
8
6
4
2
0
0.10
7.55
0.30
0.61
0.67
0.90
0.24
Urban wastewater
Hospital wastewater
Untreated water
Treated water
Upstream river
Downstream river
Sludge


0.01
Phylogroup A
Phylogroup B1
Phylogroup B2
Phylogroup D
ST1675
CC86
CC120
CC399
ST3160
ST940
ST598
ST443 (CC205)
CC224
CC155
CC23
ST480 (CC467)
CC45
ST1785
ST2827
ST162 (CC469)
S. dysenteriae Sd197 (distance, 0.80)
ST847
CC1148
CC1125
ST2823
ST1147
ST2040
ST2820 (CC446)
ST2822
CC216
CC10
ST2833
ST2835
CC361
CC46
CC607
ST93 (CC168)
ST3202
CC350
ST2834
ST648
ST2828 (CC394)
CC405
ST3162
CC38
CC69
ST1268
CC349
CC354
ST117
ST1163
ST428
ST1170
ST135
ST131
ST141
ST352
ST127
CC95
ST372
ST 973 (CC973)
ST68
ST362
41-50
31-40
21-30
11-20
6-10
2-5
1
Number of isolates
Urban wastewater
Hospital wastewater
Untreated water
Treated water + Sludge
River
Patients


%
100
90
80
70
60
50
40
30
20
10
0
Distribution of the ESBL
TEM
SHV-12
Other CTX-M
CTX-M-15
CTX-M-14
CTX-M-1
Environmental isolates
Clinical isolates

Insects Represent a Link between Food Animal Farms and the Urban Environment for Antibiotic Resistance Traits

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Antibiotic-resistant bacterial infections result in higher patient mortality rates, prolonged hospitalizations, and increased health care costs. Extensive use of antibiotics as growth promoters in the animal industry represents great pressure for evolution and selection of antibiotic-resistant bacteria on farms. Despite growing evidence showing that antibiotic use and bacterial resistance in food animals correlate with resistance in human pathogens, the proof for direct transmission of antibiotic resistance is difficult to provide. In this review, we make a case that insects commonly associated with food animals likely represent a direct and important link between animal farms and urban communities for antibiotic resistance traits. Houseflies and cockroaches have been shown to carry multidrug-resistant clonal lineages of bacteria identical to those found in animal manure. Furthermore, several studies have demonstrated proliferation of bacteria and horizontal transfer of resistance genes in the insect digestive tract as well as transmission of resistant bacteria by insects to new substrates. We propose that insect management should be an integral part of pre- and postharvest food safety strategies to minimize spread of zoonotic pathogens and antibiotic resistance traits from animal farms. Furthermore, the insect link between the agricultural and urban environment presents an additional argument for adopting prudent use of antibiotics in the food animal industry.

TABLE 1 Insects with antibiotic-resistant bacteria from food animal production farms and surrounding urban environments

Insect	Bacterial species	Antibiotic resistance profile[a]	Environment(s)	Reference
Cockroaches (Dictyoptera)				
German cockroach (*Blattella germanica*)	*Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Enterococcus casseliflavus*	AMP, CHL, CIP, ERY, KAN, STR, TET	Swine farms	56
Flies (Diptera)				
Housefly (*Musca domestica*)	*Enterococcus faecalis, Enterococcus faecium, Enterococcus casseliflavus*	CIP, ERY, KAN, STR, TET	Fast-food restaurants	59
Housefly (*Musca domestica*) Blowfly (*Lucilia* spp.) Bottle fly (*Phaenicia* spp.)	*Enterococcus faecalis, Enterococcus faecium, Staphylococcus* spp.	CLN, ERY, PEN, SYN, TET	Poultry farms	55
Housefly (*Musca domestica*)	*Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Enterococcus casseliflavus*	AMP, CHL, CIP, ERY, KAN, STR, TET	Swine farms	56
Housefly (*Musca domestica*)	*Enterococcus faecalis, Enterococcus faecium*	DOX, ERY, GEN, STR, TET	Wastewater treatment facilities	61
Housefly (*Musca domestica*)	*Escherichia coli* O157:H7	AMP, CER, CTE, GEN, NEO, OXY, SPC, SXT	Cattle farm	46
Housefly (*Musca domestica*)	*Escherichia coli*	AMP, STR, SUL, TET	Swine farms	51
Housefly (*Musca domestica*) Stable fly (*Stomoxys calcitrans*)	*Escherichia coli*	AMP, AMX, CHL, CEP, CIP, GEN, NAL, SUL, STR, SXT, TET	Dairy cattle farm	52
Housefly (*Musca domestica*) False stable fly (*Muscina stabulans*)	*Escherichia coli*	AMP, CED, CEZ, STR, TET, TRM	Cattle farm	53
Housefly (*Musca domestica*) Blowfly (*Lucilia* spp.)	*Escherichia coli*	CAZ, CEF	Poultry farms	54
Australian bush fly (*Musca vetustissima*)	*Escherichia coli, Salmonella* spp., *Shigella* spp.	AMX, CLR, ROX	Cattle farm, urban area, outdoor eateries	50

Muchas gracias!

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