Facultad de Odontología
Universidad de Chile

Laboratorio de
Bioquímica
Y Biología Oral

Enero-2008

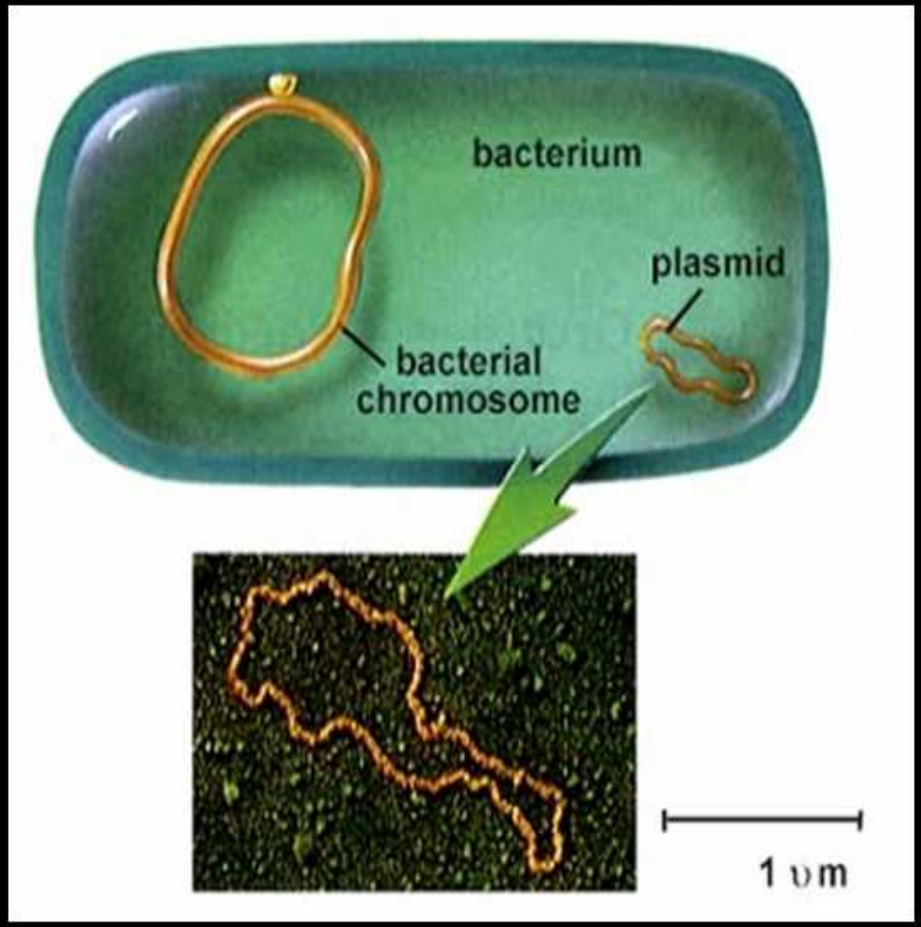
"Mecanismos Moleculares de la Patogenicidad Microbiana. ¿Que son los Factores de Virulencia?" ¿Qué hay dentro de la caja de Pandora?

Escuela de verano Universidad de Chile

Dr. Patricio Retamales M.

Factores de virulencia

- La patogénesis esta asociada con genes codificados
  - En el cromosoma
  - En el plásmido de virulencia.

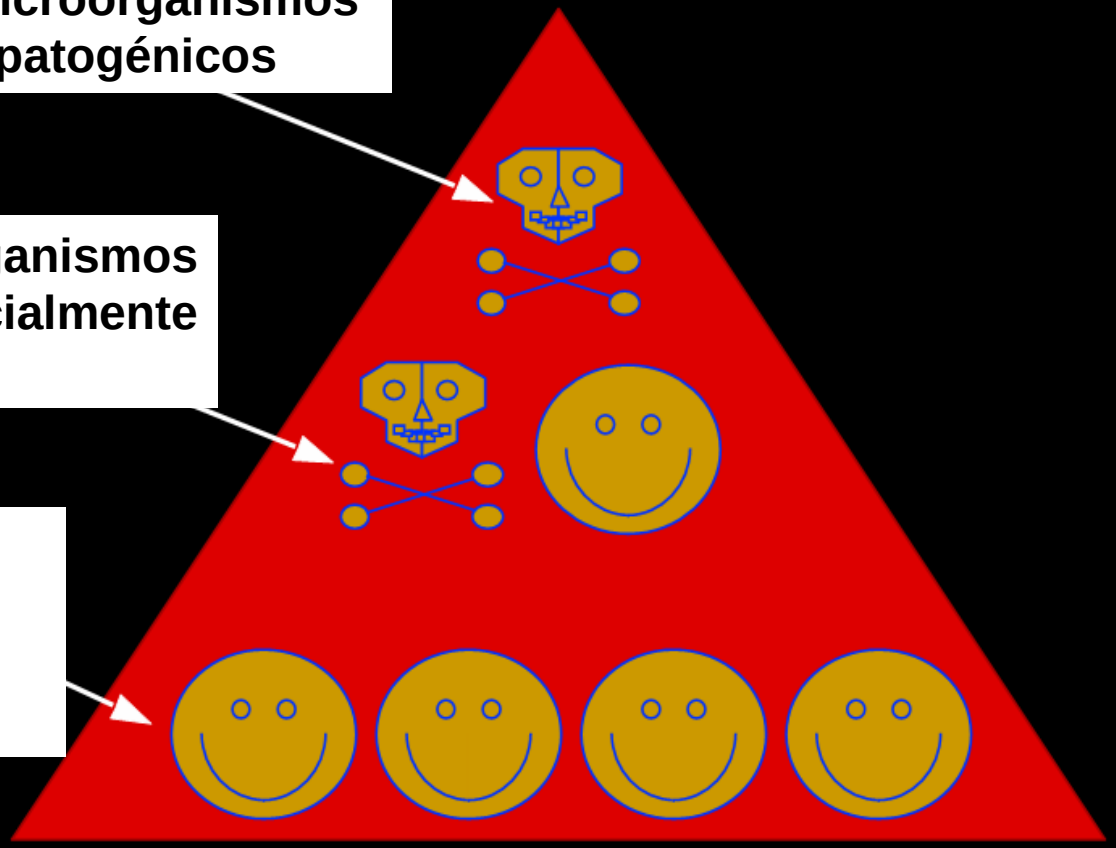


Microorganismos y humanos

Muy pocos microorganismos
Son siempre patogénicos

Muchos microorganismos son sólo potencialmente patogénicos

La mayoría de los microorganismos nunca son patogénicos



Veamos algunas definiciones básicas de entrada:

Infección: Cuando un agente patógeno (bacteria) capaz de causar una enfermedad y llega a establecerse en el hospedante por sobre una dosis de infección mínima (**M**inimum **I**nfectious **D**ose-**MID**).

Enfermedad: Es una infección que causa síntomas que llevan a una alteración de estructuras y/o funciones en el hospedante.

Virulencia: Habilidad de un agente patógeno para causar una infección (Es una medición cuantitativa de la patogenicidad).

Patogenicidad: Capacidad de un agente infeccioso de producir enfermedad en un huésped susceptible.

Nota: La virulencia del agente infeccioso y la resistencia del hospedante están en constante cambio

Patógeno: **Agente productor o causante de una enfermedad**

Patógeno Primario

Infección por sobre el MID por un patógeno primario resultara en enfermedad

Patógeno Oportunista

Infección resultará en enfermedad solamente cuando las defensas del hospedante estén comprometidas

Infectividad: Es la capacidad que tiene un agente patógeno para introducirse en nuestro organismo y sobrevivir en su interior, multiplicándose.

Factor de Virulencia: Es una propiedad, producto bacterial o estrategia que contribuye a virulencia.

***Colonización**

***Evitar defensas del hospedante**

***Multiplicación y diseminación**

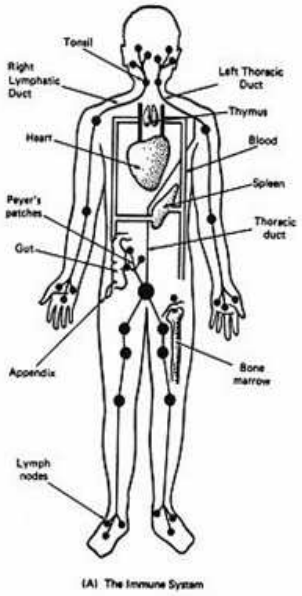
***Invasión de tejidos del hospedante**

***Destrucción de los tejidos del hospedante**

***Adaptación a la condición “Hospedante”**

Bacterias patogénicas utilizan un amplio rango de factores de virulencia para establecer una infección

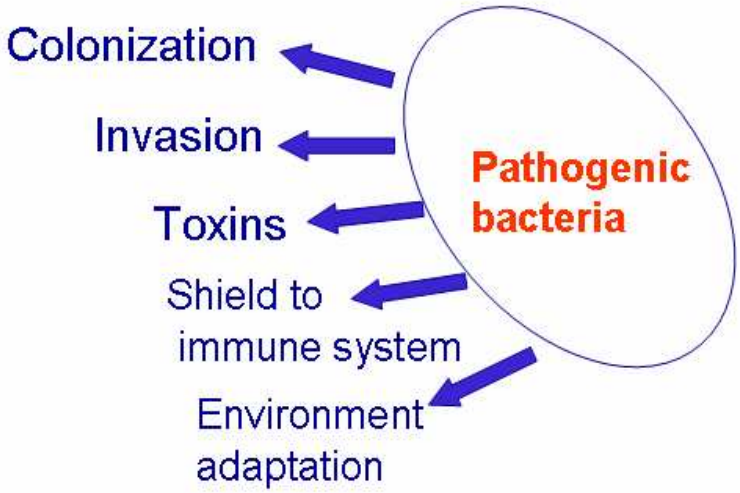
Host defenses

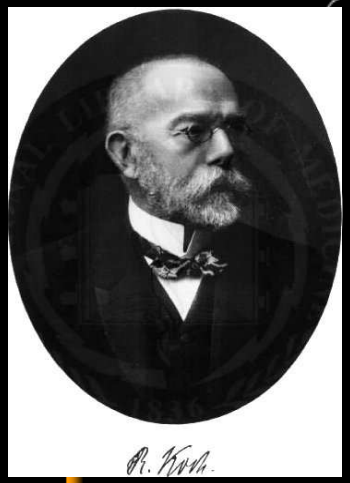


Specific Immunity

Innate Immunity

Virulence mechanisms





¿Como podemos saber si un micoorganismo determinado causa una enfermedad específica?

* Postulados de Koch- 1882 (*Bacillus*) PNM 1905

1.- El microorganismo debe estar presente en todos los individuos con la misma enfermedad.

2.-El microorganismo debe ser recuperado del individuo enfermo y poder ser aislado en medio de cultivo.

3.-El microorganismo proveniente de ese cultivo debe causar la misma enfermedad cuando se lo inocula a otro huésped.

4.-El individuo experimentalmente infectado debe contener el microorganismo.

Algunas limitaciones de los postulados de Koch's

Patógeno no puede ser cultivado *in vitro* (cultivo puro)

Animal modelo disponible y válido (Ej: el patógeno no genera la enf. en animales de experimentación).

Asume que la susceptibilidad del hospedante es irrelevante

Portadores asintomático y "sanos" (ej: Amebiasis, meningococo)

Clonalidad

Solamente una simple especie causa enfermedad (etiología multifactorial de la enfermedad)

limitaciones al medir procesos causales y desconocimiento acerca del papel de otros factores.

Virulence Factors

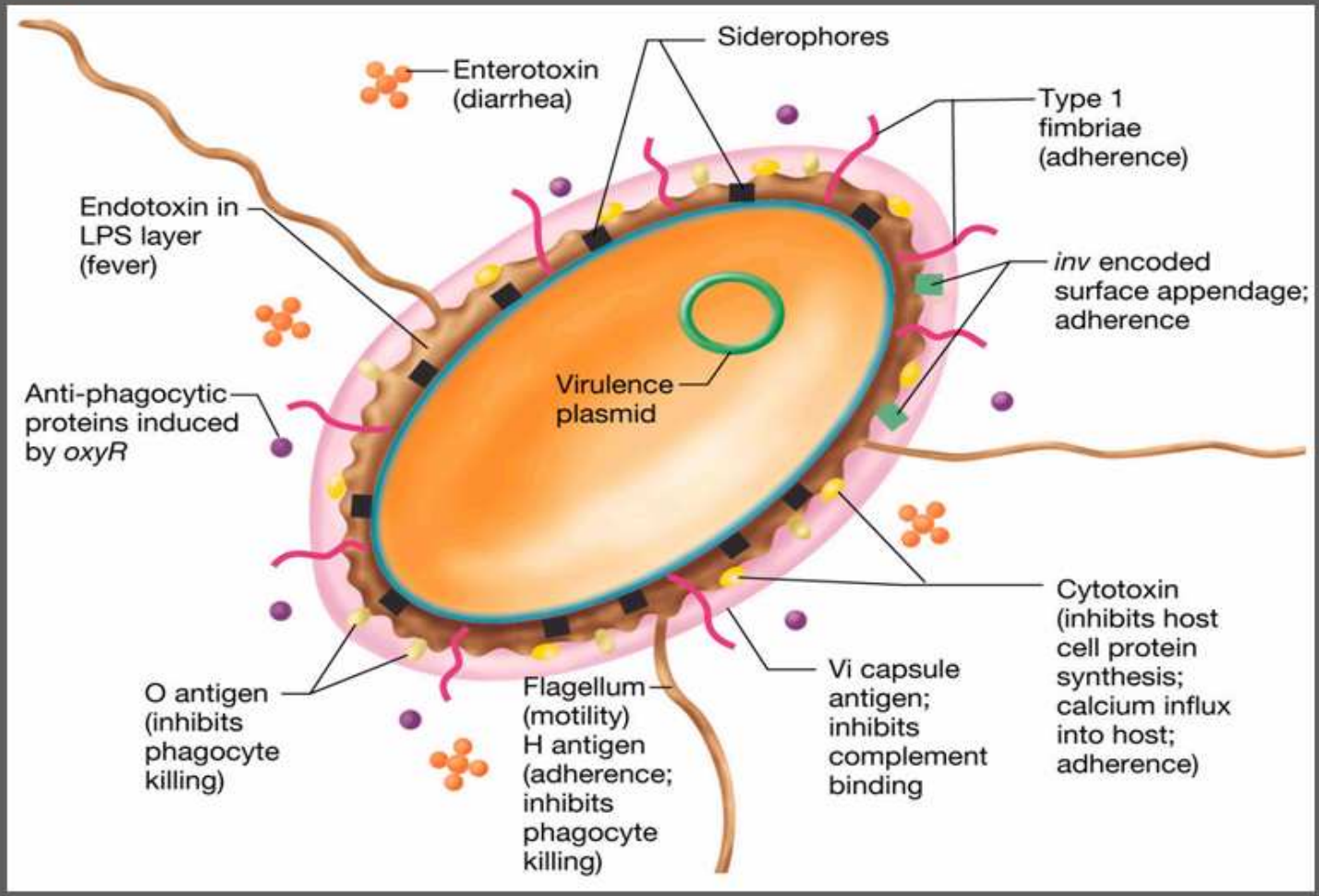


Tabla 6-1: Factores de virulencia que promueven la colonización y sobrevida de la bacteria infectante.

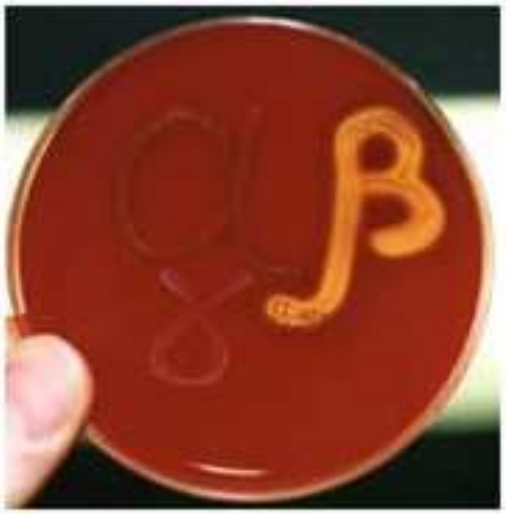
FACTOR DE VIRULENCIA	FUNCIÓN
Pili	Adherencia superficies mucosas
Adhesinas no fimbriales	Permiten unión firme a células del hospedero
Moléculas que inducen el reordenamiento del citoesqueleto de la célula hospedera	Fagocitosis forzada de la bacteria por células que normalmente no son fagocíticas; movimiento de la bacteria dentro del hospedero
Motilidad y quimiotaxis	Permite llegada a las superficies mucosas
sIgA proteasas	Previenen el atrapamiento de la bacteria en el mucus
Cápsulas	Previenen la ingesta de fagocitos; reducen la activación del complemento

Tabla 6-2: Enzimas que dañan las células del hospedero facilitando la diseminación del patógeno.

ENZIMA	AGENTE PRODUCTOR	FUNCIÓN
Hialuronidasas	*Streptococcus* *Staphylococccus* *Clostridium*	Atacan el cemento intersticial del tejido conectivo depolimerizando el ácido hialurónico.
Colagenasas	*Clostridium hystoliticum* *Clostridium perfringens*	Rompen el colágeno del tejido muscular, lo cual facilita la gangrena gaseosa.
Neuraminidasas	*Vibrio cholerae* *Shigella dysenteria*	Degradan el ácido neuramínico, un cemento intercelular del tejido epitelial de la mucosa intestinal.
Estreptoquinasa y Estafiloquinasa	*Streptococcus* *Staphylococcus*	Convierten el plasminógeno inactivo a plasmina, la cual digiere la fibrina, previniendo la coagulación de la sangre. La ausencia de fibrina en el sitio de la lesión, permite que el patógeno difunda rápidamente desde el sitio de la infección.

ENZIMAS QUE PRODUCEN HEMÓLISIS O LEUCÓLISIS:

Fosfolipasas	*Clostridium perfringens*	Hidrolizan fosfolípidos en las membranas celulares (membranas celulares polares).
Lecitinasas	*Clostridium perfringens*	Destruyen la lecitina (fosfatidil) en las membranas celulares.
Hemolisinas	*Staphylococcus* *Streptococcus* (estreptolisina) *Clostridium*	Proteínas formadoras de canales que destruyen glóbulos rojos y otras células por lisis.



Dirección http://mgc.ac.cn/VFs/main.htm

Virulence Factors of Pathogenic Bacteria

HOME < SEARCH < STATUS < FEEDBACK < LINK < CONTACT <

- **Bacteria**
  - Bacillus
  - Bordetella
  - Campylobacter
  - Escherichia
  - Haemophilus
  - Helicobacter
  - Legionella
  - Listeria
  - Mycobacterium
  - Neisseria
  - Pseudomonas
  - Salmonella
  - Shigella
  - Staphylococcus
  - Streptococcus
  - Vibrio
  - Yersinia
- **Chlamydia**
- **Mycoplasma**

Definitions:

» A **bacterial pathogen** is usually defined as any bacterium that has the capacity to cause disease. Its ability to cause disease is called **pathogenicity**.

» **Virulence** provides a quantitative measure of the pathogenicity or the likelihood of causing disease.

» **Virulence factors** refers to the properties (i.e., gene products) that enable a microorganism to establish itself on or within a host of a particular species and enhance its potential to cause disease. Virulence factors include bacterial toxins, cell surface proteins that mediate bacterial attachment, cell surface carbohydrates and proteins that protect a bacterium, and hydrolytic enzymes that may contribute to the pathogenicity of the bacterium.

About VFDB:

VFDB is an integrated and comprehensive database of virulence factors for bacterial pathogens (It's only the current status, we do plan to include more pathogens, such as *Chlamydia* and *Mycoplasm*, in the next release.). You can visit the status page to know the current statistics of the entire database.

The motivation for constructing VFDB was twofold:

» First, to provide in-depth coverage major virulence factors of the best-characterized bacterial pathogens, with the structure features, functions and mechanisms used by these pathogens to allow them to conquer new niches and to circumvent host defense mechanisms, and cause disease.

» Second, to provide current knowledge of the wide variety of mechanisms used by bacterial pathogens for researchers to elucidate pathogenic mechanisms in bacterial diseases that are not yet well characterized and to develop new rational approaches to the treatment and prevention of infectious diseases.

Query page

* ***Text Search***

Select one field: Virulence factors

Query field: actin

Search

* ***Blast Search***

Regular BLAST without client-server support
Regular BLAST with client-server support

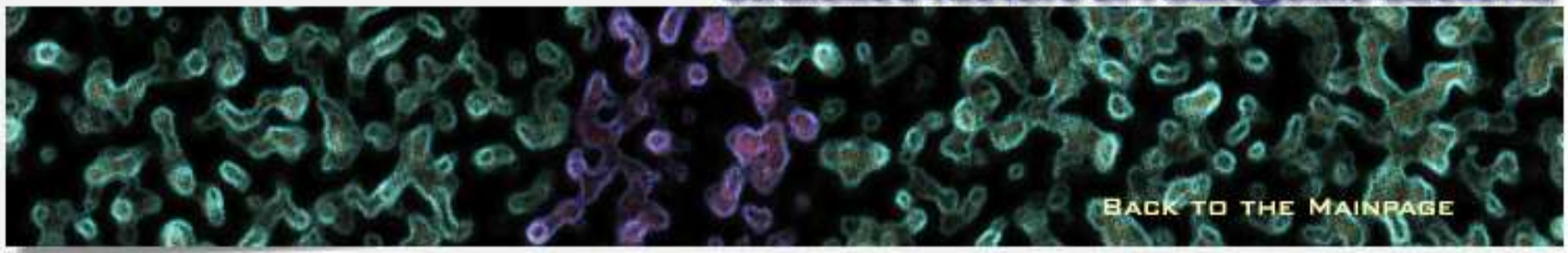
PSI/PHI BLAST without client-server support
PSI/PHI BLAST with client-server support

BLAST 2 sequences without client-server support
BLAST 2 sequences with client-server support

* ***Choose from Keyword list***

Offensive virulence factors
1) Adherence
2) Invasion
3) Toxin

Virulence Factors of Pathogenic Bacteria



Search Result - *Query text = 'actin'*

VFs	Origin	Function
α-C protein	*S.agalactiae*	Interacts with host cell glycosaminoglycan (GAG) and mediates bacterial internalization by a mechanism that involves Rho GTPase-dependent actin rearrangements;
ActA	*L.monocytogenes*	Factor responsible for actin-based motility and cell-to-cell spread
CNF-1	UPEC	Induces the formation of actin stress fibers and membrane ruffling, necrosis
CNF-1	MNEC	Induces the formation of actin stress fibers and membrane ruffling, necrosis
Dnt	*B.pertussis*	Dermonecrosis-inducing toxin stimulates the assembly of actin stress fibers and focal adhesions by deamidating or polyaminating small GTPase Rho;
ExoT	*P.aeruginosa*	Contributes to actin cytoskeleton disruption and inhibition of internalization of the bacteria
IcsA (VirG)	*S.flexneri (serotype 2a)*	Mediates intracellular movement and formation of actin tails
InlA	*L.monocytogenes*	Promotes entry into host cells. Mediates the crossing of the intestinal and placental barriers, and invasion of the central nervous system (CNS) may also be mediated by the interaction between InlA and E-cadherin.
		Binding of intimin to Tir triggers dramatic intracellular changes including

- **Bacteria**
  - Bacillus
  - Bordetella
  - Campylobacter
  - Escherichia
  - Haemophilus
  - Helicobacter
  - Legionella
  - Listeria
  - Mycobacterium
  - Neisseria
  - Pseudomonas
  - Salmonella
  - Shigella
  - Staphylococcus
  - Streptococcus
  - Vibrio
  - Yersinia
- **Chlamydia**
- **Mycoplasma**

Identified Virulence Factors of Listeria : Actin-based motility

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ActA

Related genes: *actA*;

Keywords: Actin-based motility;

Structure features:

» 639-amino acid protein with a 610-residue mature form that is attached to the bacterial cell wall via its C-terminal

» Mature ActA can be divided into three distinct domains:

(1)N-terminal domain rich in positively charged residues, amino acids 121 to 170 contain an Arp2/3 complex-binding and activation domain, amino acids 60 to 101 is the monomeric **actin** binding region

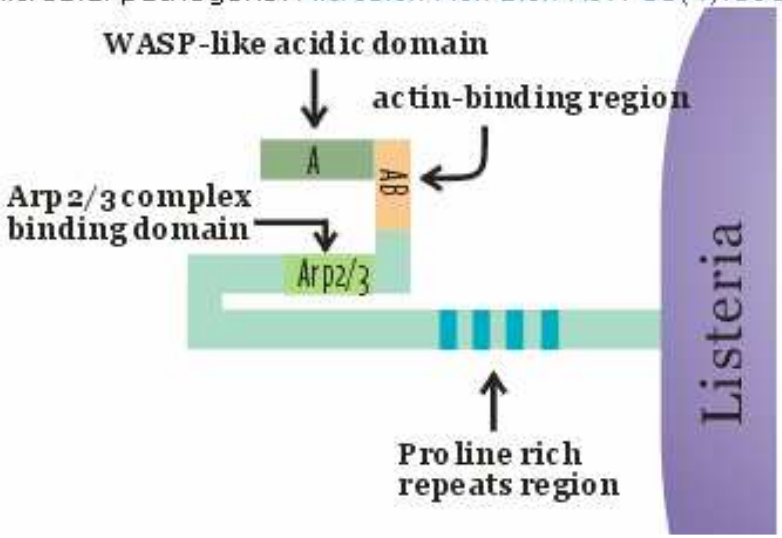
(2) central region of proline-rich repeats required for binding of VASP and Mena, VASP binds profilin to recuit monomeric **actin** to sites of **actin** reorganizaition or by bundling of **actin** filaments into a tail to accelerate the **actin** nucleation

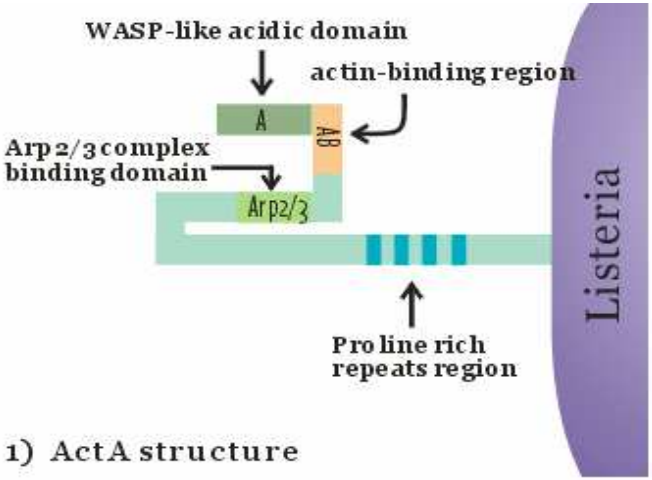
(3) C-terminal domain with a highly hydrophobic stretch that anchors the protein to the bacterial surface

Figures:

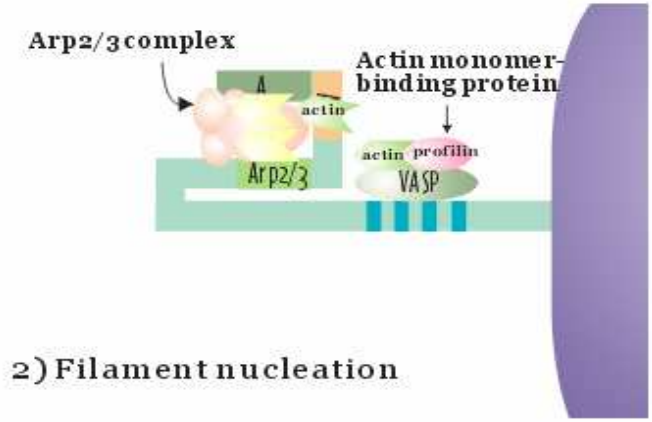
» Domains of ActA and model of *Listeria* actin tail assembly

(Reproduced from: Goldberg MB, 2001. Actin-based motility of intracellular microbial pathogens. *Microbiol. Mol. Biol. Rev.* 65(4):595-626.)

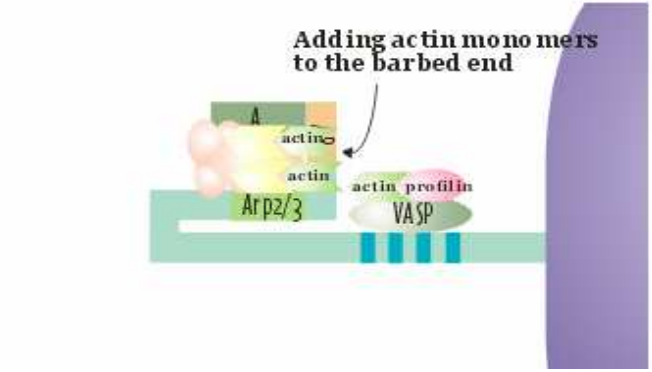




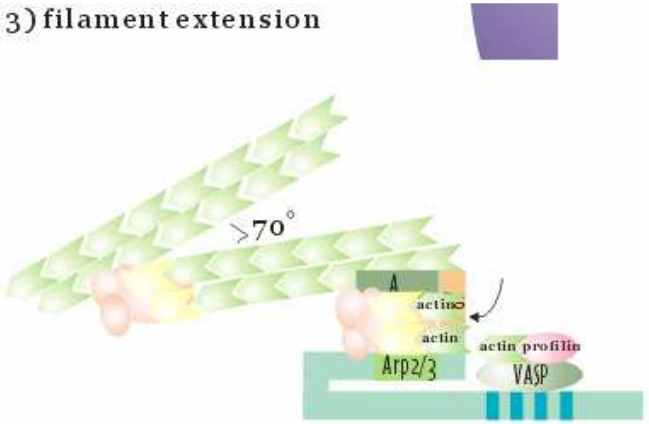
1) ActA structure



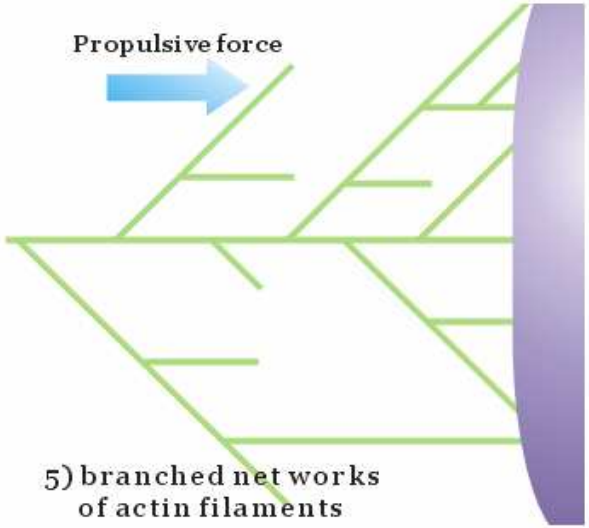
2) Filament nucleation



3) filament extension



4) Arp2/3 complex release, links filaments at 70° angles



5) branched networks of actin filaments

Functions:

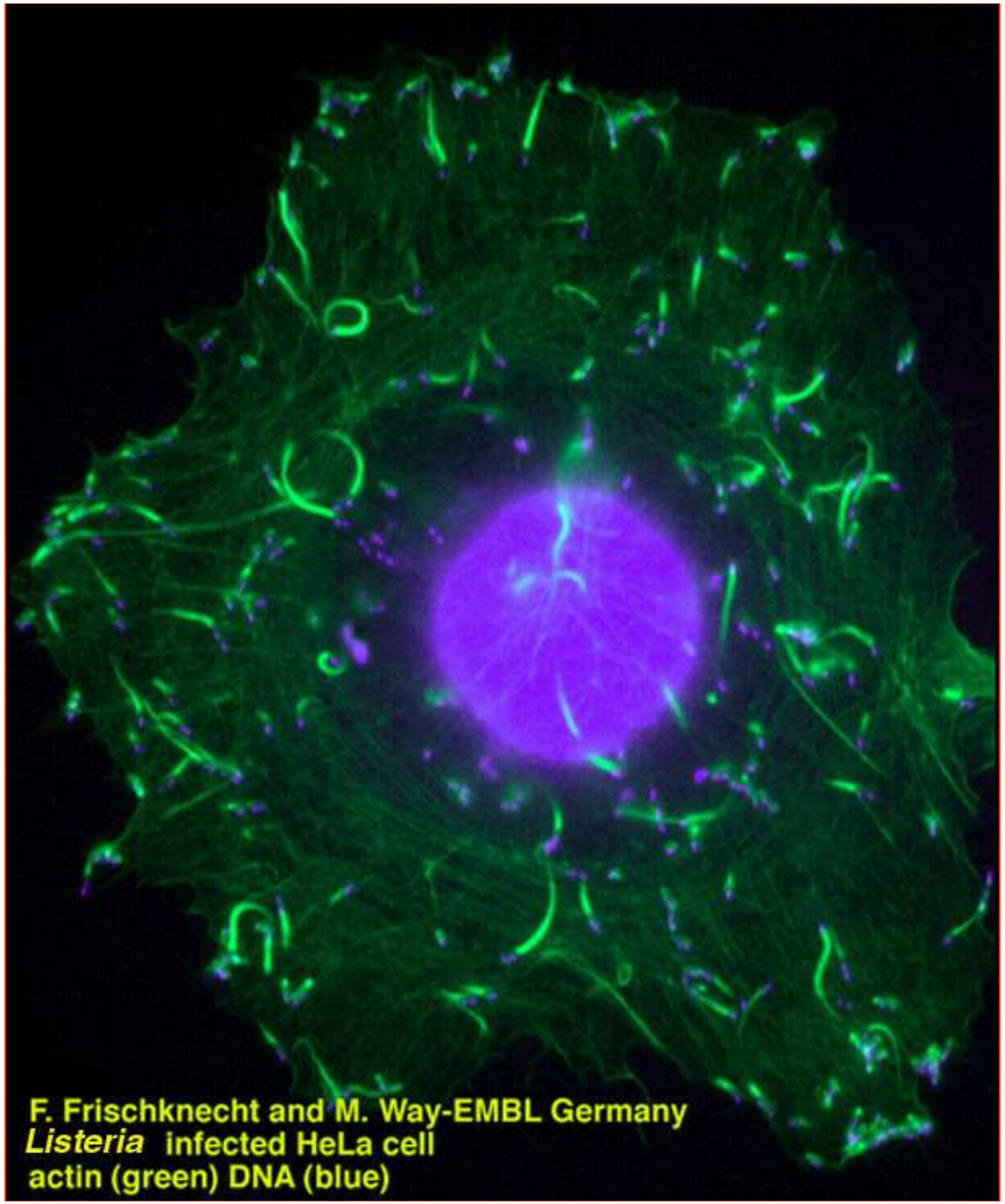
» Factor responsible for **actin**-based motility and cell-to-cell spread

Mechanism:

» ActA recruits and activates Arp2/3 complex, hence generating a dendritic network of branched **actin** filaments. Modulation and control of **actin**-based movements also involve several other proteins such as cofilin (**actin**-depolymerizing factor, ADF), capping protein, profilin, VASP and α-actinin.

References:

↗ Kocks C, et al., 1992. *L. monocytogenes*-induced actin assembly requires the actA gene product, a surface protein. *Cell* 68(3):521-531.



Bacterias del género *Listeria* se mueven dentro de la célula hospedante, utilizando la fuerza propulsora del citoesqueleto de actina mediada por un "Complejo-Motor" conducido por actA

ELSEVIER

FEMS Microbiology Letters 201 (2001) 1–7

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

MiniReview

Bacterial virulence: can we draw the line?

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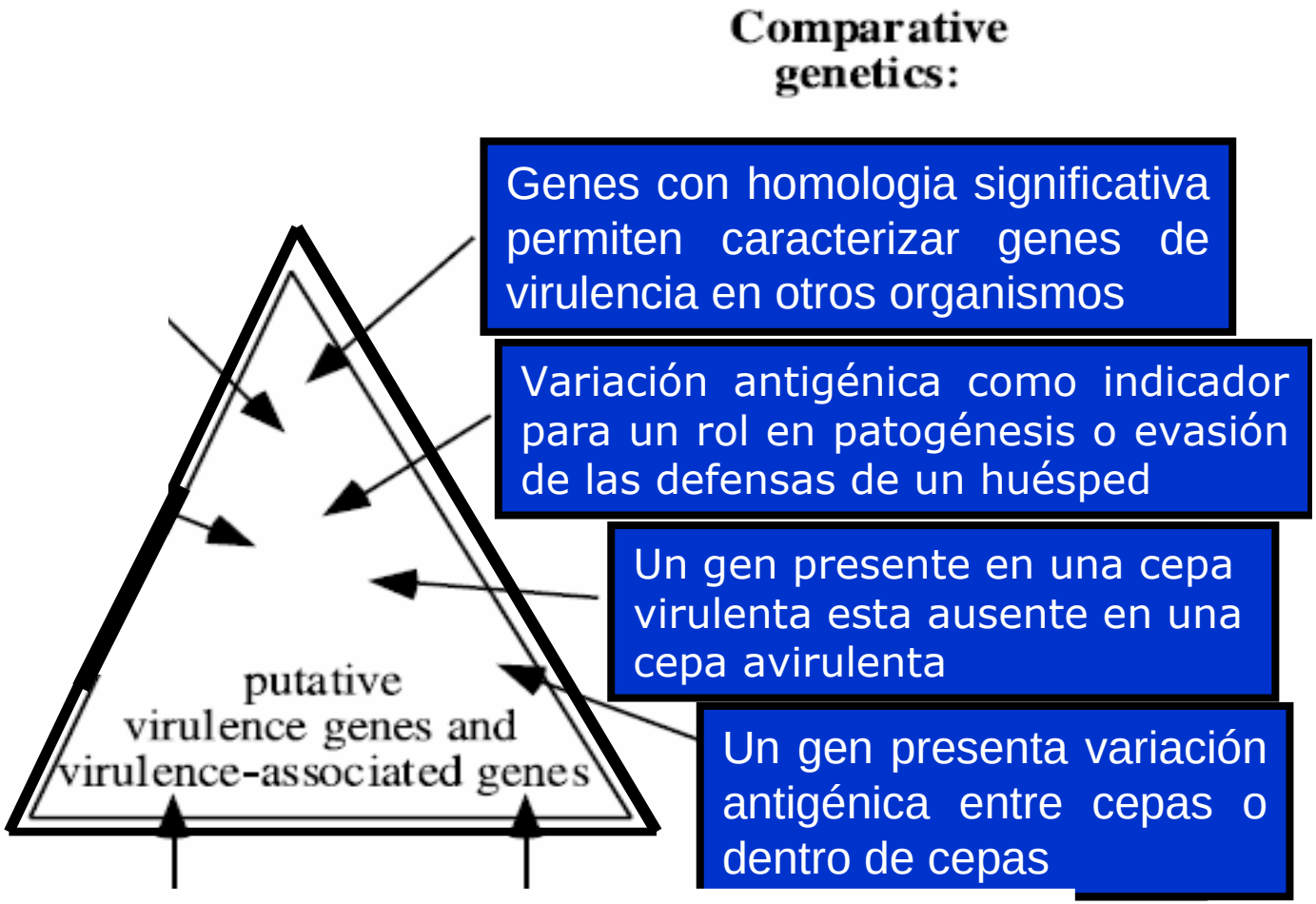
Abstract

The molecular approach to microbial pathogenesis has resulted in an impressive amount of data on bacterial virulence genes. Bacterial genome sequences rapidly add candidate virulence genes to electronic databases. The interpretation of this overwhelming information is obscured because every gene involved in pathogenicity is called a virulence gene, regardless of its function in the complex process of virulence. This review summarizes the changing concept of bacterial virulence and the detection and identification strategies followed to recognize virulence genes. A refined definition of virulence genes is proposed in which the function of the gene in the virulence process is incorporated. We propose to include the life-style of bacteria in the assessment of their putative virulence genes. A universal nomenclature in analogy to the EC enzyme numbering system is proposed. These recommendations would lead to a better insight into bacterial virulence and a more precise annotation of (putative) virulence genes, which would enable more efficient use of electronic databases.

Keywords: Bacterial virulence; Virulence gene nomenclature; Standardization; Comparative genomics



Diferentes enfoques para identificar genes de virulencia y genes asociados a virulencia

Fue la primera mujer, hecha por orden de Zeus como parte de un castigo a Prometeo por haber revelado a la humanidad el secreto del fuego.

“La caja de Pandora”

Virulence life-style genes: all genes essential for a pathogenic life-style

A pathogenic life-style requires survival, multiplication, and causing damage in a host; this includes to gain a foothold and to compete with other bacteria in the host, and to avoid normal host defense mechanisms

True virulence genes

True virulence genes: all genes whose products are involved in interactions with the host and are responsible for pathological damage; absent in apathogens

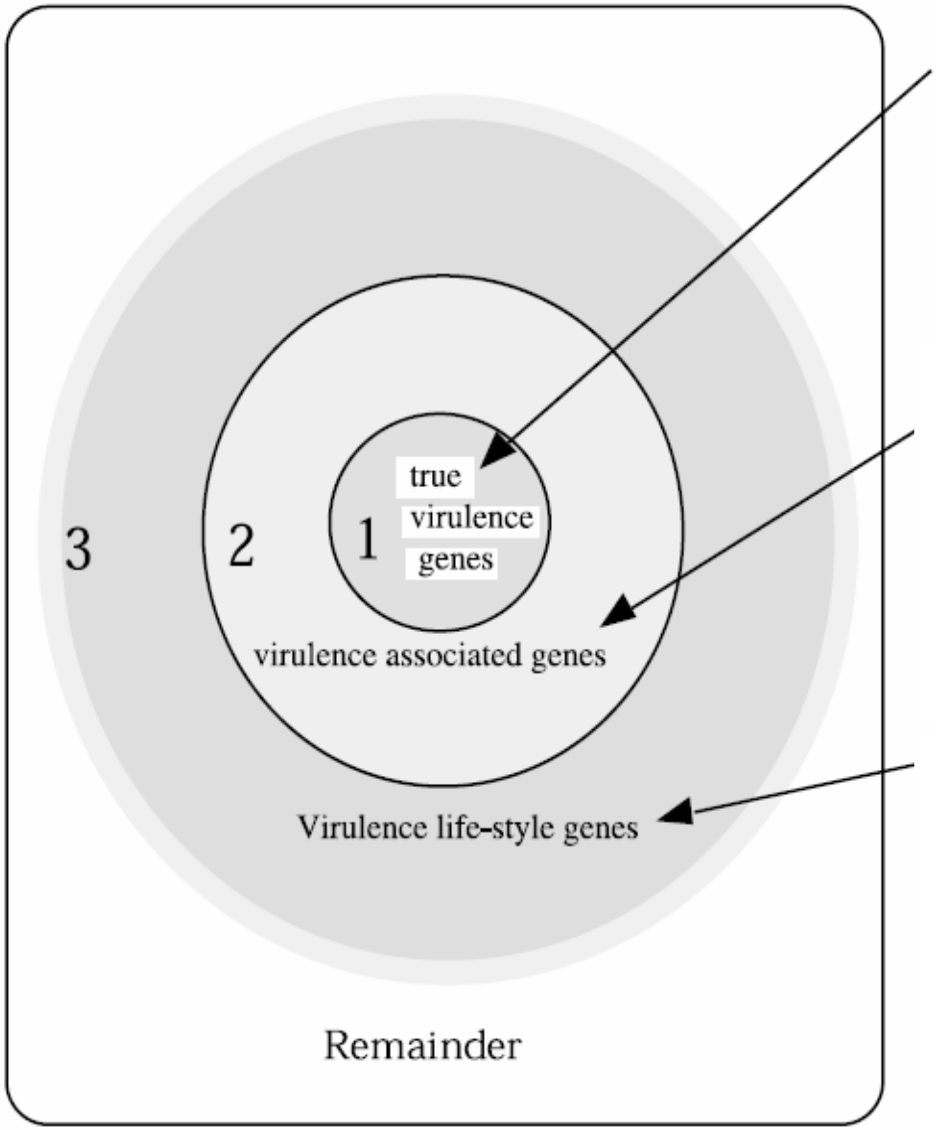
Virulence-associated genes

Processing virulence genes

Auxiliary virulence genes

Regulatory virulence genes

Pathogenic housekeeping genes



True virulence genes are genes coding for factors or for enzymes producing factors that are

- involved in interactions with the host, AND
- directly responsible for the pathological damage during infection, AND
- absent in apathogens.

Dependiendo del nivel en la definición de virulencia, más o menos genes pueden ser llamados genes de virulencia

Table 1
Definitions for subclasses of virulence life-style genes

First digit: life-style of organism	Second digit: gene class	Definition	Examples of this class
PA: virulence genes from bacteria that are exclusively pathogenic	1. True virulence genes	Their gene products are directly involved in interactions with the host and are directly responsible for the pathological damage. These genes are exclusively expressed in pathogens.	Cholera toxin, anthrax toxin, botulin toxin, shiga toxin, *Bordetella* adenylate cyclase toxin, etc.
HS: virulence genes from bacteria displaying host-dependent pathogenicity	2. Colonization genes	Their gene products enable colonization of a host and determine the localization of the infection.	Adhesins, fimbriae, intimin, invasins.
	3. Defense system evasion genes	Their gene products are involved in evasion of the host immune system.	Immunoglobulin-specific proteases, cytotoxins directed against immune cells, surface layers, slime polysaccharide.

OP: virulence genes from opportunistic pathogens	4. Processing virulence genes	Their gene products are involved in the biosynthesis of virulence life-style factors by enzymatic processing.	Specific proteases, methylases, chaperonins, glycosyltransferases, with virulence life-style genes as a substrate.
	5. Secretory virulence genes	Their gene products are responsible for secretion of virulence life-style factors.	Type III secretion machinery, type I secretion machinery.
	6. Virulence housekeeping genes	Their gene products provide nutrients during colonization, improve competition with other microbes, or provide the proper microenvironment.	Urease, catalase, superoxide dismutase, siderophores, proteinase inhibitors. Flagella could also belong to this class although they are strictly speaking structural components of the organism.
	7. Regulatory genes	Their gene products are involved in regulation of virulence life-style gene expression.	Alternative sigma factors, global regulators, specific transcription activators, regulators of phase variation by gene/promoter inversion.

Patogenia: factores bacterianos

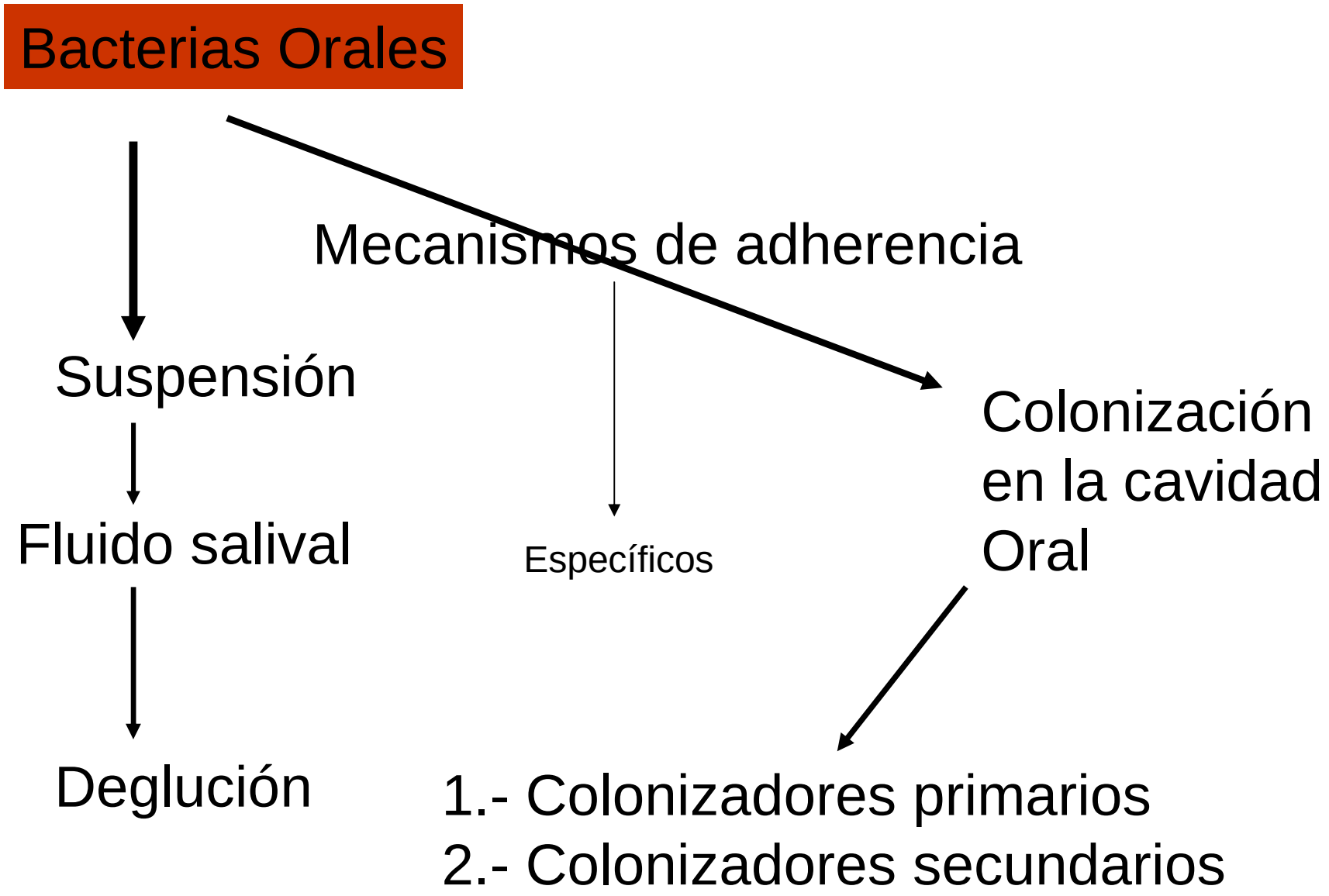
- Colonización
  - Adhesinas
  - Coagregación
  - Crecimiento en biocapa y anaerobiosis
- Evasión de las defensas
  - Proteasas
  - Inhibición de la fagocitosis
- Destrucción tisular
  - endotoxinas
  - Proteasas y colagenasas

Existen diferentes tipos de interacción:

Aglutinación: Interacción entre microorganismos que son genéticamente idénticos

Co-agregación: Referida a la formación de redes de interacciones entre dos o más tipos bacterianos. Aplicable también a dos microorganismos en suspensión

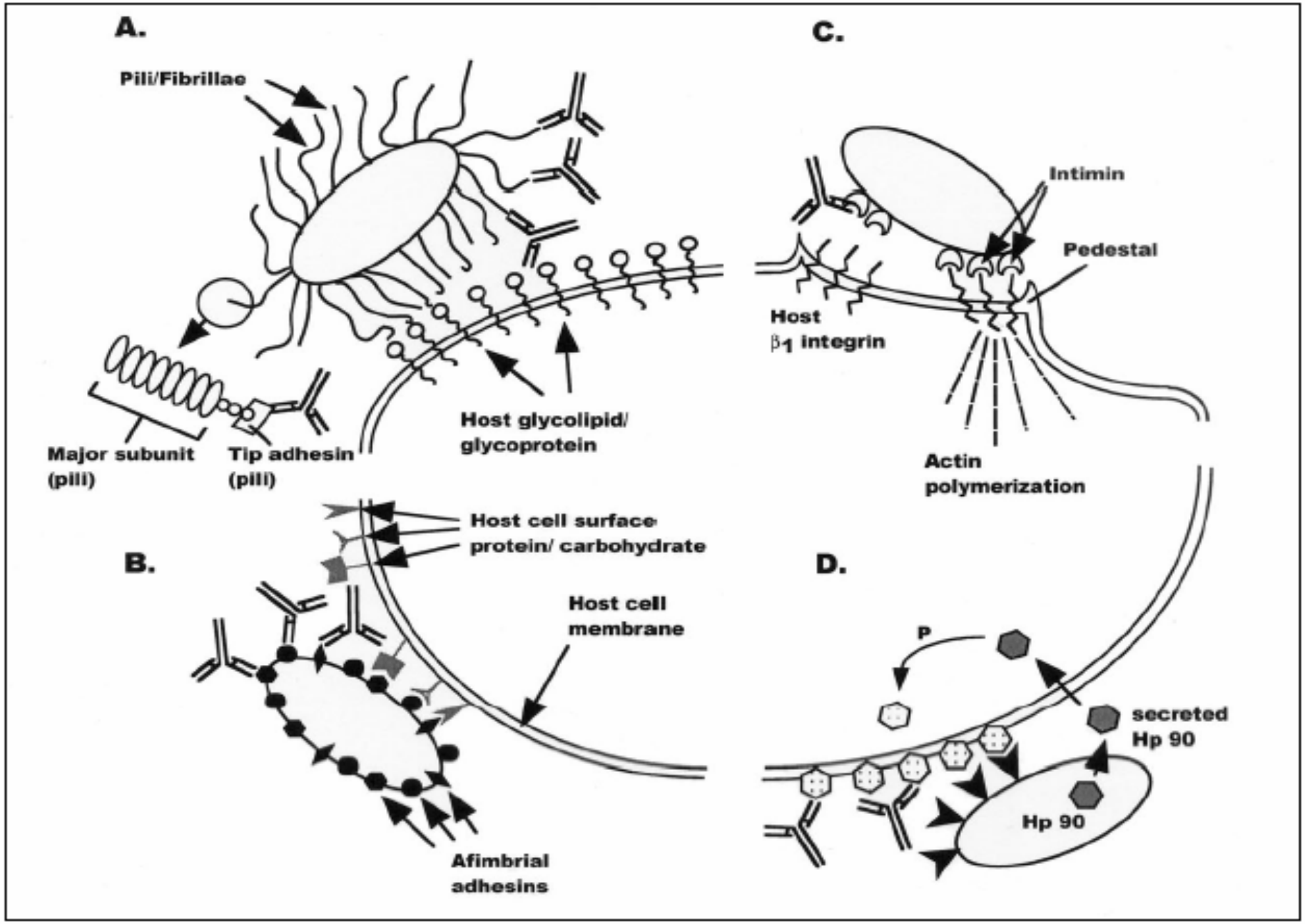
Co-adhesión: Interacción entre microorganismos iguales o distintos, donde uno se encuentra fijo y el otro en suspensión


Bacterias Orales
Mecanismos de adherencia
Suspensión
Fluido salival
Deglución
Específicos
Colonización
en la cavidad
Oral
1.- Colonizadores primarios
2.- Colonizadores secundarios

Adhesinas: Amplio grupo de moléculas, en su mayoría de origen proteico que se encuentran en la superficie de los microorganismos mediando la adhesión a superficies, bacterias y otras moléculas.

Específicas: Reconocen un tipo de molécula “blanco” en la superficie donde se adhiere el microorganismo

Inespecíficas: Reconocen un amplio espectro de moléculas “blanco”



Mecanismos de adherencia donde es posible teóricamente diseñar vacunas anti-adhesinas, los cuales podrían bloquear la colonización e infección.

Review 1

GLUCAN-BINDING PROTEINS OF THE ORAL STREPTOCOCCI

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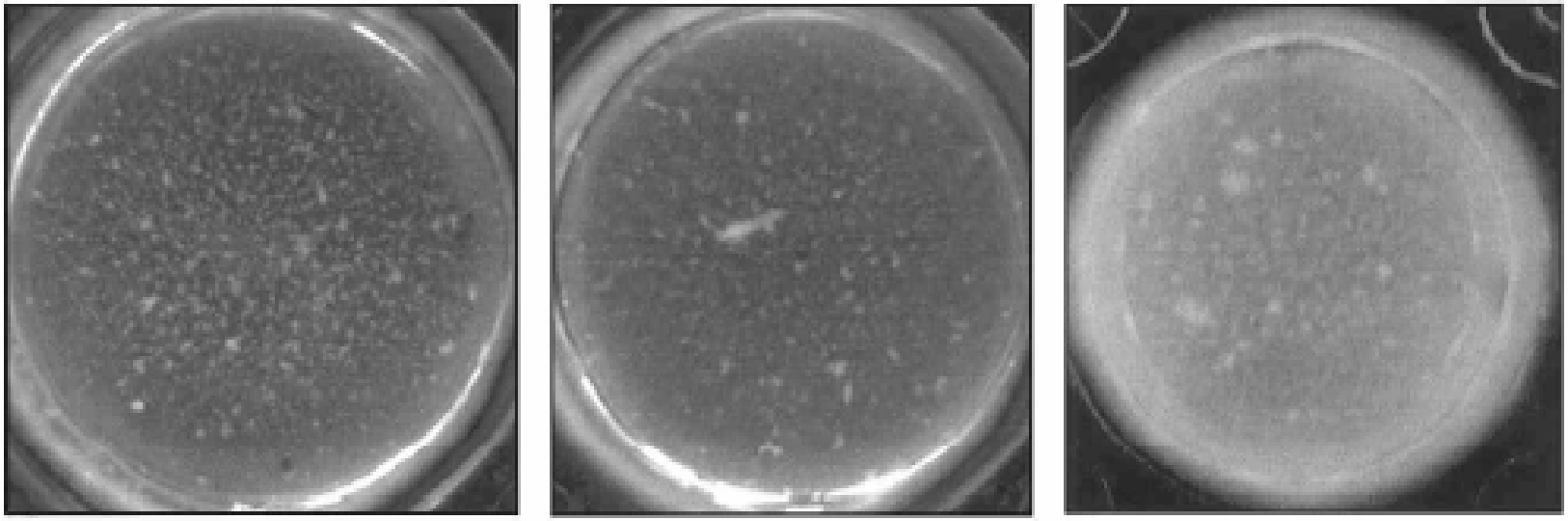
ABSTRACT: The synthesis of extracellular glucan is an integral component of the sucrose-dependent colonization of tooth surfaces by species of the mutans streptococci. In investigators' attempts to understand the mechanisms of plaque biofilm development, several glucan-binding proteins (GBPs) have been discovered. Some of these, the glucosyltransferases, catalyze the synthesis of glucan, whereas others, designated only as glucan-binding proteins, have affinities for different forms of glucan and contribute to aspects of the biology of their host organisms. The functions of these latter glucan-binding proteins include dextran-dependent aggregation, dextranase inhibition, plaque cohesion, and perhaps cell wall synthesis. In some instances, their glucan-binding domains share common features, whereas in others the mechanism for glucan binding remains unknown. Recent studies indicate that at least some of the glucan-binding proteins modulate virulence and some can act as protective immunogens within animal models. Overall, the multiplicity of GBPs and their aforementioned properties are testimonies to their importance. Future studies will greatly advance the understanding of the distribution, function, and regulation of the GBPs and place into perspective the facets of their contributions to the biology of the oral streptococci.

Key words. Glucan, glucosyltransferase, GBP, streptococci, plaque.

A



WT GbpA⁻ GbpC⁻

B

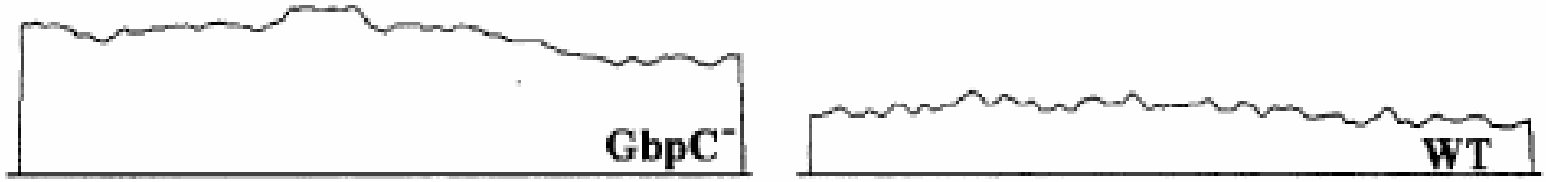


TABLE
Glucan-binding Proteins among the Mutans Streptococci

Host	Protein	Gene	Size (kDa)*	Function	Reference
S. mutans Ingbritt	GbpA	*gbpA*	58	Biofilm morphology	Banas *et al.* (1990)
S. mutans SJ32	GbpB	*gbpB*	41	Peptidoglycan hydrolase	Mattos-Graner *et al.* (2001)
S. mutans 109c	GbpC	*gbpC*	58	Aggregation	Sato *et al.* (1997)
S. mutans UA159	GbpD	*gbpD*	75	Cohesion/enzyme	Shah and Russell (2002)
S. sobrinus 6715-49	GBP-1	NC	15-16	Unknown	Landale and McCabe (1987)
S. sobrinus B13	GBP-2	NC	71-87	Aggregation	Ma *et al.* (1996), Smith *et al.* (1998), Wu-Yuan and Gill (1992)
S. sobrinus 6715	GBP-3	NC	52-68	Unknown	Smith *et al.* (1998)
S. sobrinus 6715	GBP-4/GBL	NC	58-60	Aggregation	Ma *et al.* (1996), Smith *et al.* (1998)
S. sobrinus 6715	GBP-5	NC	41-49	Unknown	Ma *et al.* (1996), Smith *et al.* (1998)
S. sobrinus UAB108	Dei	*dei*	31	Dextranase inhibitor	Sun *et al.* (1994)

* Sizes are based on the amino acid composition of the mature protein derived from the nucleotide sequence when known, and the predicted signal peptide cleavage site when applicable. Other sizes are based on SDS-PAGE migration. The designation NC indicates that the gene has not been cloned. The *dei* gene also hybridizes to *S. downei* and *S. criceti* (Sun *et al.*, 1994). Original work with the GBL was done in *S. sobrinus* and *S. criceti* (Lu *et al.*, 1992). It is not known if the GBL activity is due to an analogous protein in both species. The 87-kDa *S. sobrinus* GBP-2 cross-reacts weakly with antibody to *S. mutans* GbpA (Wu-Yuan and Gill, 1992).

Resumen Review 1:

-Las GBP de estreptococos orales representan un grupo heterogéneo de proteínas con roles en la biología de la placa dental.

-Glucanos protegen a las bacterias de compuestos tóxicos

-Expresión y actividad de GTF puede ser influenciada por diferentes condiciones ambientales (pH, concentración de iones, disponibilidad de moléculas receptoras, potencial de oxido-reducción)

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Functional Analysis of Glucan Binding Protein B from *Streptococcus mutans*

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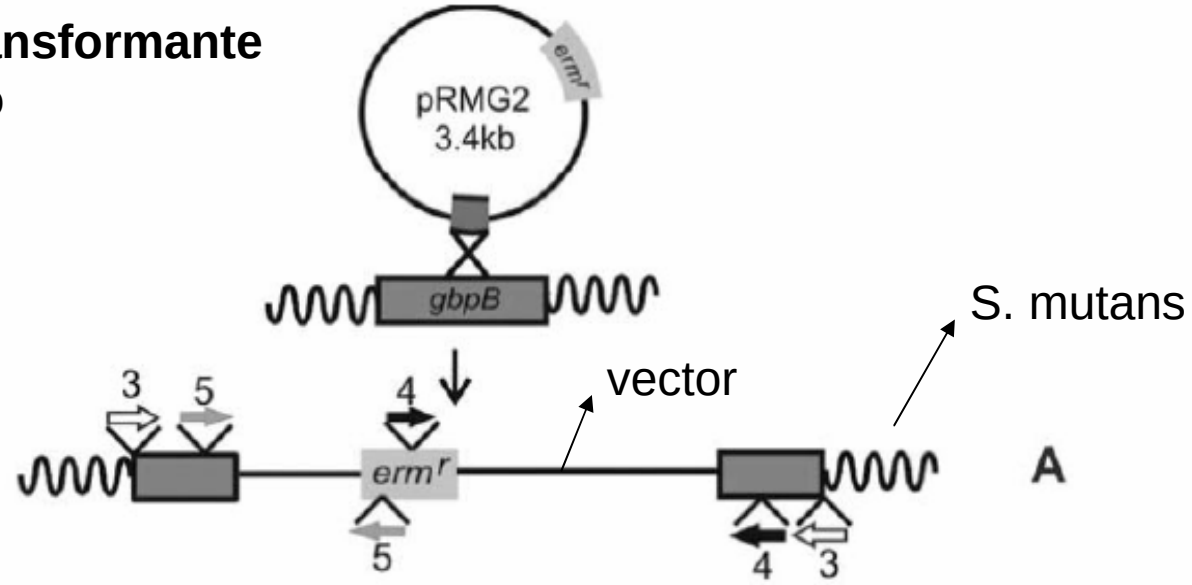
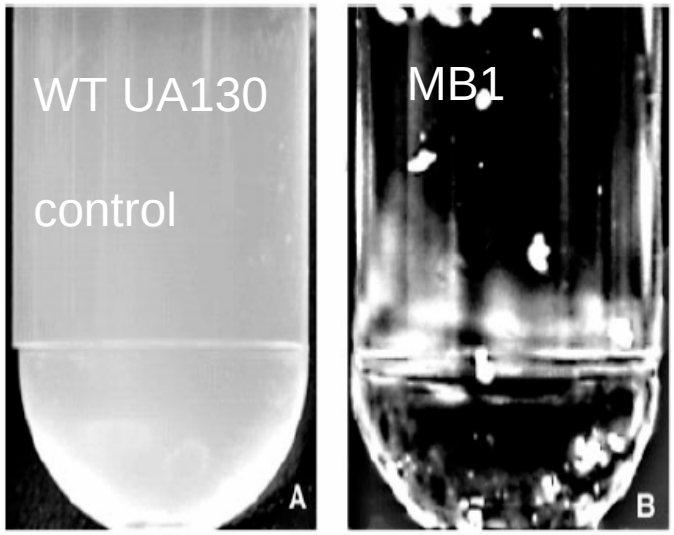
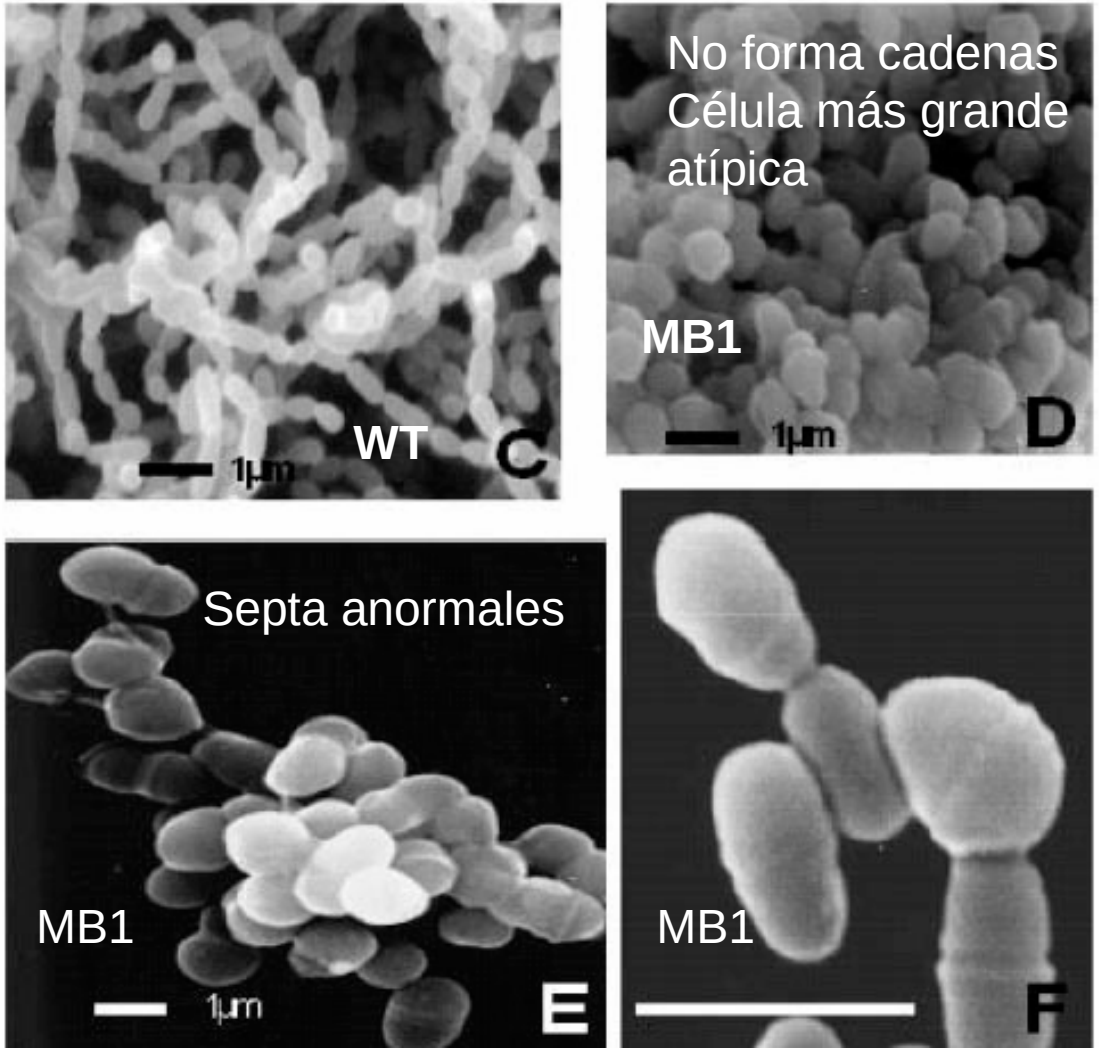
Mutans streptococci are major etiological agents of dental caries, and several of their secreted products contribute to bacterial accumulation on teeth. Of these, *Streptococcus mutans* glucan binding protein B (GbpB) is a novel, immunologically dominant protein. Its biological function is unclear, although GbpB shares homology with a putative peptidoglycan hydrolase from *S. agalactiae* and *S. pneumoniae*, indicative of a role in murein biosynthesis. To determine the cellular function of GbpB, we used several approaches to inactivate the gene, analyze its expression, and identify interacting proteins. None of the transformants analyzed were true *gbpB* mutants, since they all contained both disrupted and wild-type gene copies, and expression of functional GbpB was always conserved. Thus, the inability to obtain viable *gbpB* null mutants supports the notion that *gbpB* is an essential gene. Northern blot and real-time PCR analyses suggested that induction of *gbpB* expression in response to stress was a strain-dependent phenomenon. Proteins that interacted with GbpB were identified in pull-down and coimmunoprecipitation assays, and these data suggest that GbpB interacts with ribosomal protein L7/L12, possibly as part of a protein complex involved in peptidoglycan synthesis and cell division.

Análisis de transformante representativo



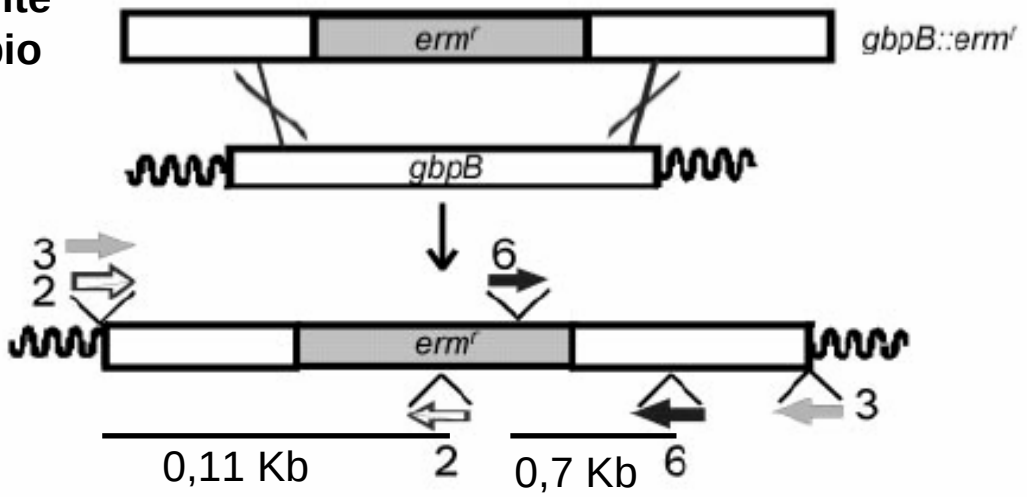


S. mutans. 9-10 días de incubación

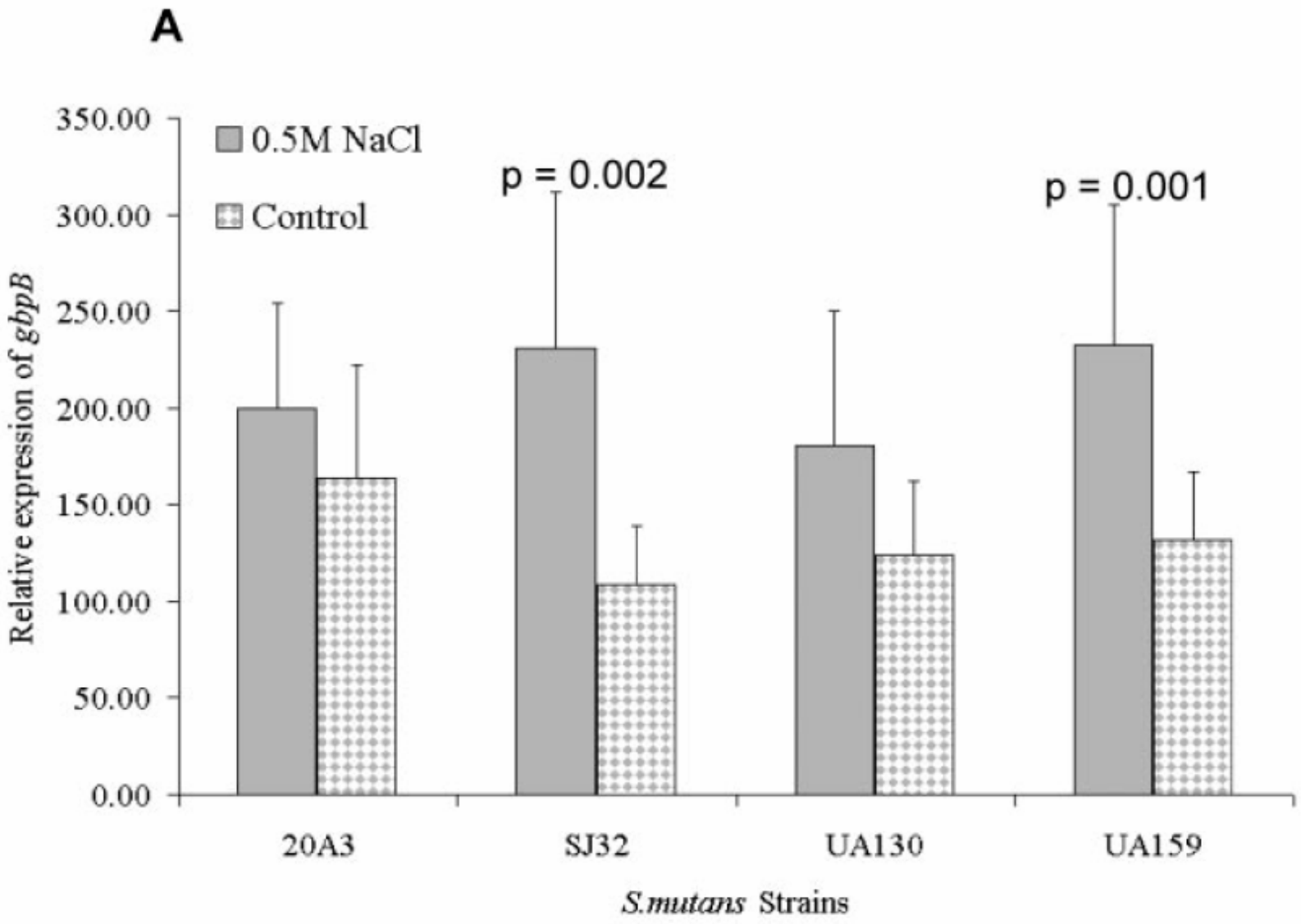


Fenotipos de transformantes de *S. mutans* UA130 obtenidos por integración de pRMG2 que genera disrupción del gen *gbpB.*

Análisis de transformante obtenido por intercambio alélico



A



Aumento de la expresión de *gbpB* en condiciones de estrés salino

TABLE 3. Expression of *gbpB* and control genes under stress conditions measured by QRT-PCR

Gene	Fold increase in expression of the following *S. mutans* strain under the indicated growth condition[a]			
	SJ32		UA159	
	0.5 M NaCl	pH 5.5	0.5 M NaCl	pH 5.5
gbpB	2.3	1.25	−1.87	−2.12
groEL	3.46	1.89	0.82	−1.82
dnaK	3.61	2.07	0.78	−2.89
ldh[b]	1.0	1.0	1.0	1.0

[a] Expression levels were calculated using a Pfaffl equation (32) as described in Materials and Methods.

[b] The lactate dehydrogenase gene (*ldh*) was used as a reference gene for normalization.

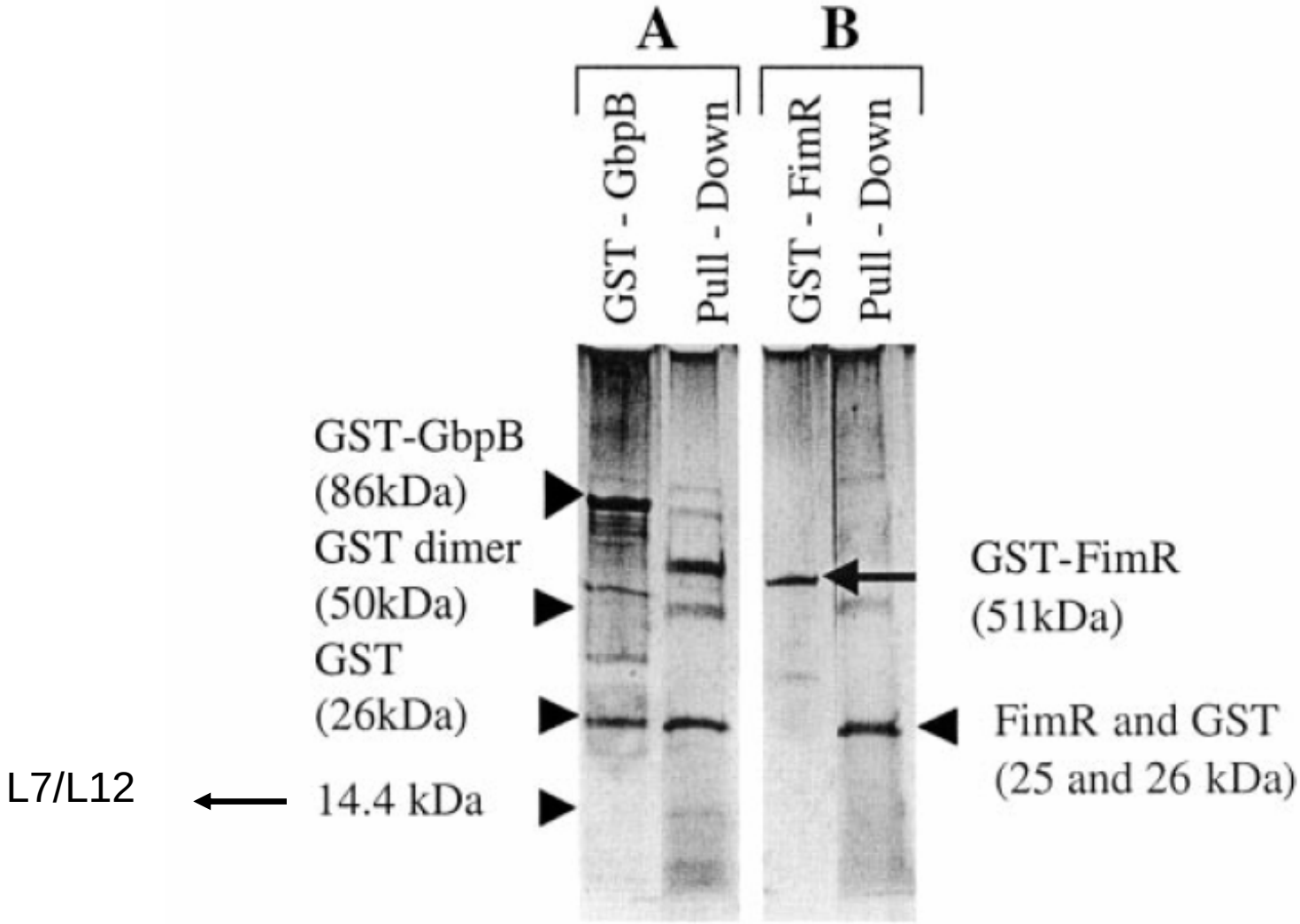


FIG. 6. Pull-down assay with GbpB. (A) Silver-stained SDS-PAGE gel showing purified GST-GbpB that was used in the assay and the proteins recovered from the pull-down fraction. The indicated 14.4-kDa protein was excised and sequenced. (B) Control pull-down assay carried out with GST-FimR from *P. gingivalis*.

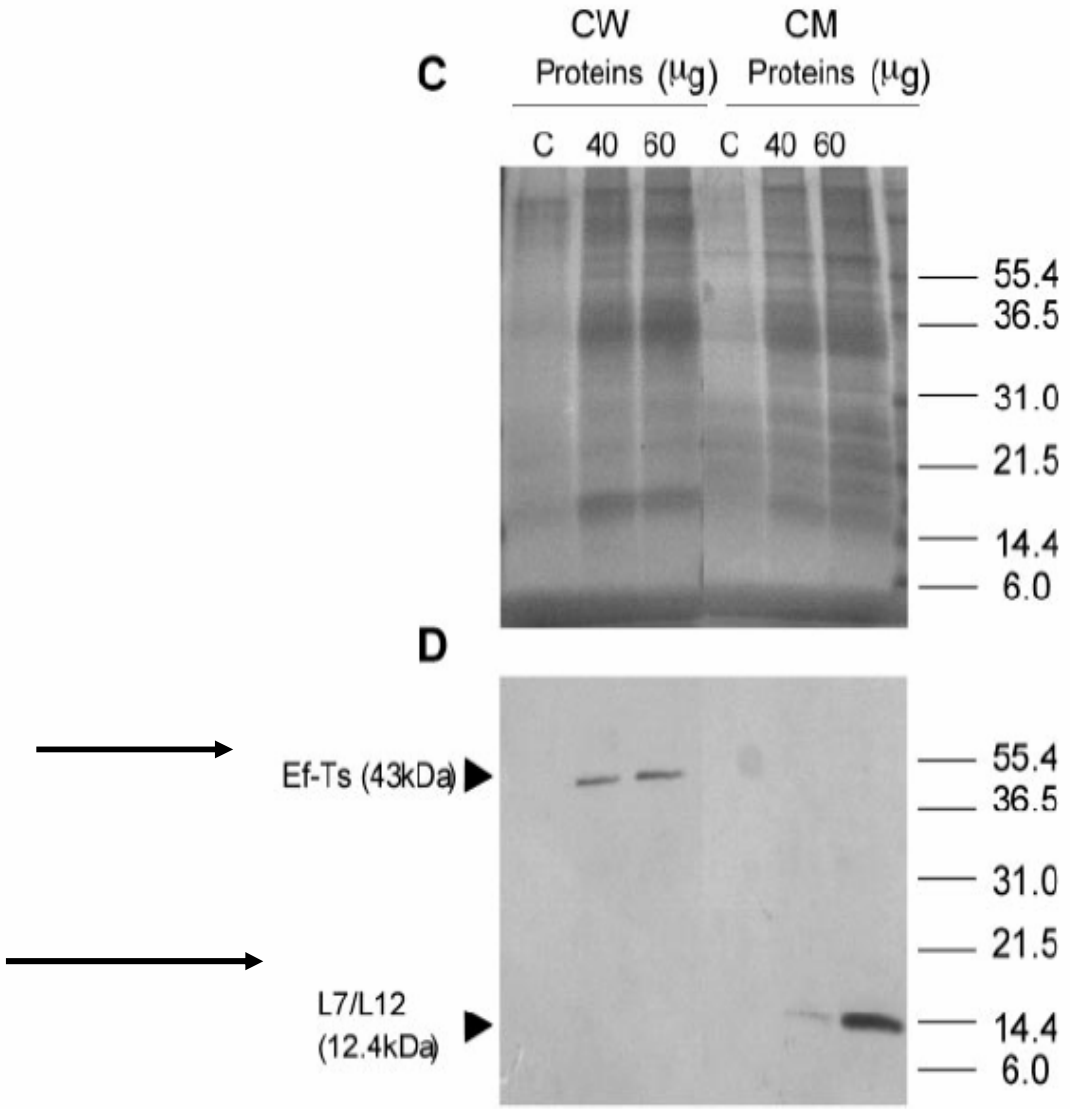


FIG. 7. Coimmunoprecipitation assays. (A) Western blot of *S. mutans* cell wall (CW) and cell membrane (CM) proteins probed with anti-GbpB antibody. (B) Western blot of the same fractions probed with anti-L7/L12 antibody. (C) Zinc-stained SDS-PAGE of either cell wall or cell membrane proteins dissociated from uncoupled columns (lanes C [control]) and from anti-GbpB antibody-coupled columns. (D) Western blot of dissociated proteins after probing with anti-L7/L12 antibody.

Conclusiones Paper 1.

-GbpB tiene una función esencial para el desarrollo de la célula en *S. mutans*, y *gbpB* es un gen vital (Todos los transformantes “disruption” tienen una copia wt de *gbpB* en otra parte del genoma)

-GbpB interactúa con L7/L12 y EF-Ts como parte de un sistema involucrado en la división celular, la síntesis de pared celular y expansión celular.

-Mutantes en *gbpB* fueron inestables, reafirmando que el producto génico tiene un rol esencial (todos merodiploides).


Muchas gracias…