UNIVERSIDADE FEDERAL DE PELOTAS
CENTRO DE BIOTECNOLOGIA_CDTec

Construção de vacinas recombinantes contra leptospirose

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Bióloga

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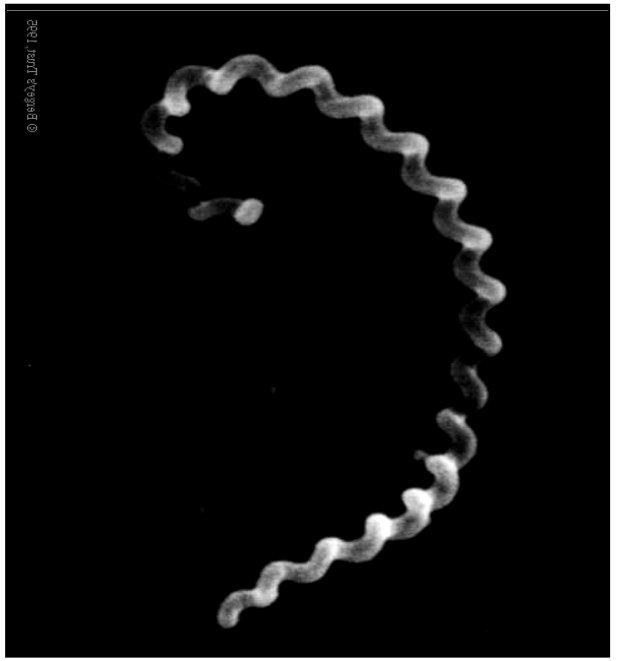
A LEPTOSPIROSE

A leptospirose é uma doença infecciosa causada por espécies patogênicas de bactérias do gênero *Leptospira.*

Leptospira interrogans

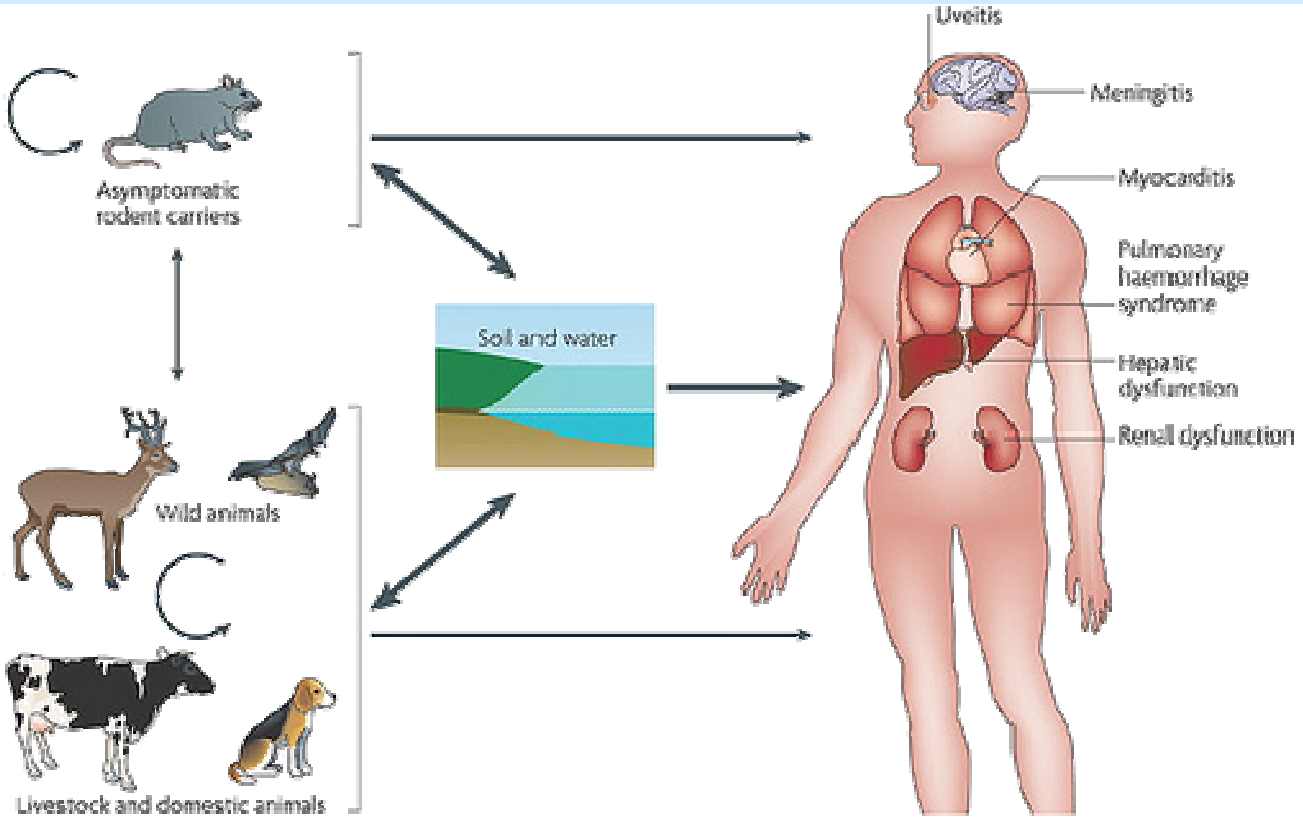
Agente etiológico

0,1-0,2 µm de diâmetro
6 a 20 µm de comprimento
Tempo de geração: 4 a 6 horas



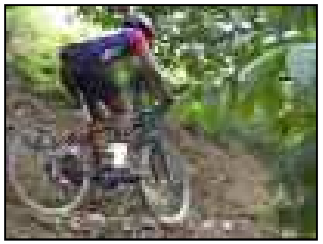
EPIDEMIOLOGIA

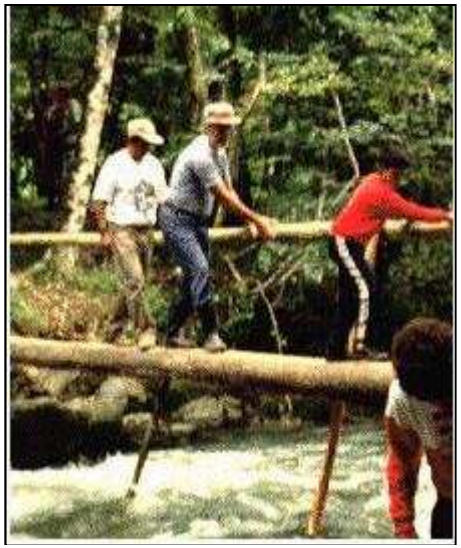
O ciclo de transmissão envolve a interação entre reservatórios animais, um ambiente favorável e grupos humanos/animais susceptíveis



Países desenvolvidos

Ocupacionais e recreacionais





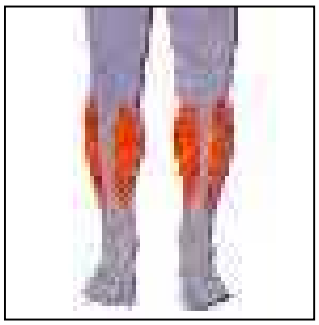
Países em desenvolvimento

Saneamento básico

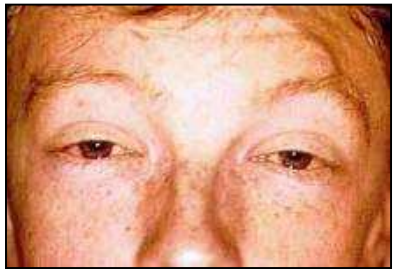
1 bilhão de pessoas vivem em favelas
(15% da população mundial)

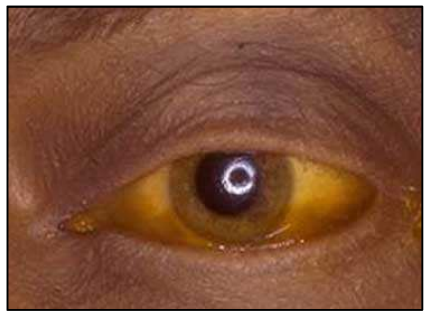
SINTOMATOLOGIA (Enfermidade bifásica)

- **Fase aguda e convalescente**
  - Corrente sanguínea ➢ multiplicam-se ➢ BACTEREMIA
  - Em 90% dos casos são benignos e autolimitantes
  - Estado febril, dores de cabeça, musculares e abdominais, vômitos, anemia, icterícia...



- **Fase imune**
  - Desaparem da circulação
  - Produção de Ac
  - Eliminação de leptospiras na urina





- **Leptospirose severa**
  - Síndrome de *Weil* e síndrome hemorrágica pulmonar grave (SPHS) com taxa de mortalidade de >50%.

A leptospirose no Brasil

- Cerca de 13.000 casos notificados por ano
- Destes 3.000 são confirmados
- 300 óbitos/ano

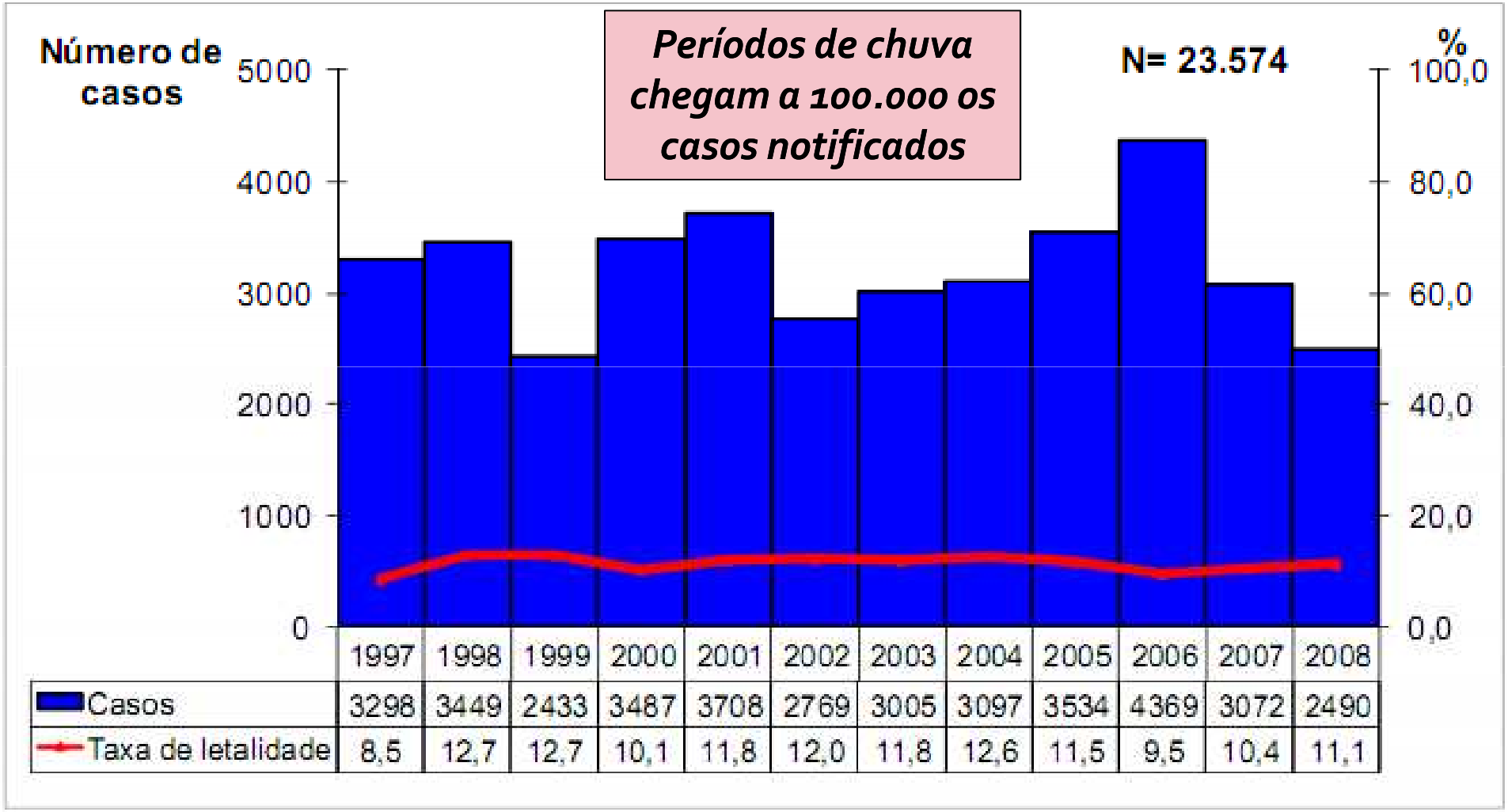
Fonte:

Ministério da Saúde

BRASIL UM PAÍS DE TODOS GOVERNO FEDERAL

SVS Secretaria de Vigilância em Saúde

Casos e letalidade da Leptospirose no Brasil, 1997 a 2008

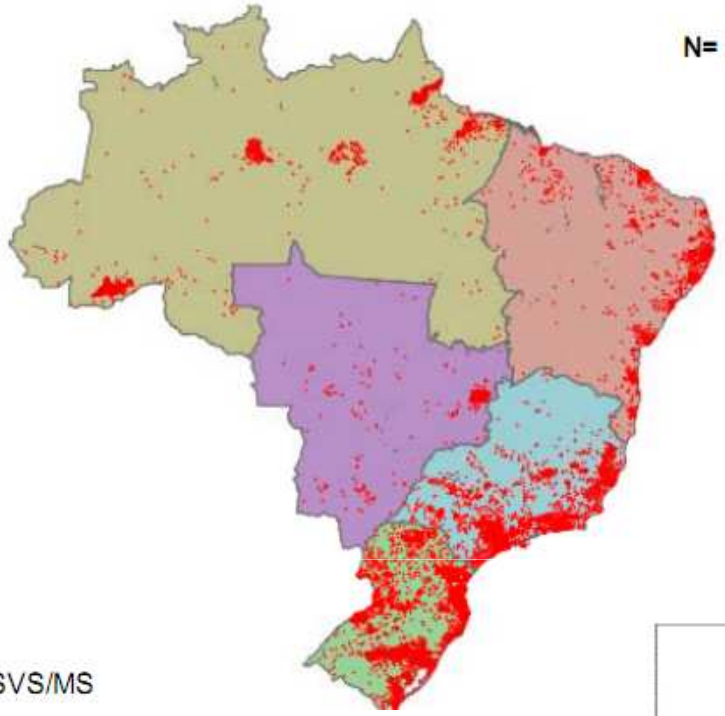


	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Casos	3298	3449	2433	3487	3708	2769	3005	3097	3534	4369	3072	2490
Taxa de letalidade	8,5	12,7	12,7	10,1	11,8	12,0	11,8	12,6	11,5	9,5	10,4	11,1

Fonte: SINAN/SVS/MS

Ministério

Casos de Leptospirose por municípios do país, Brasil, 2001 a 2007

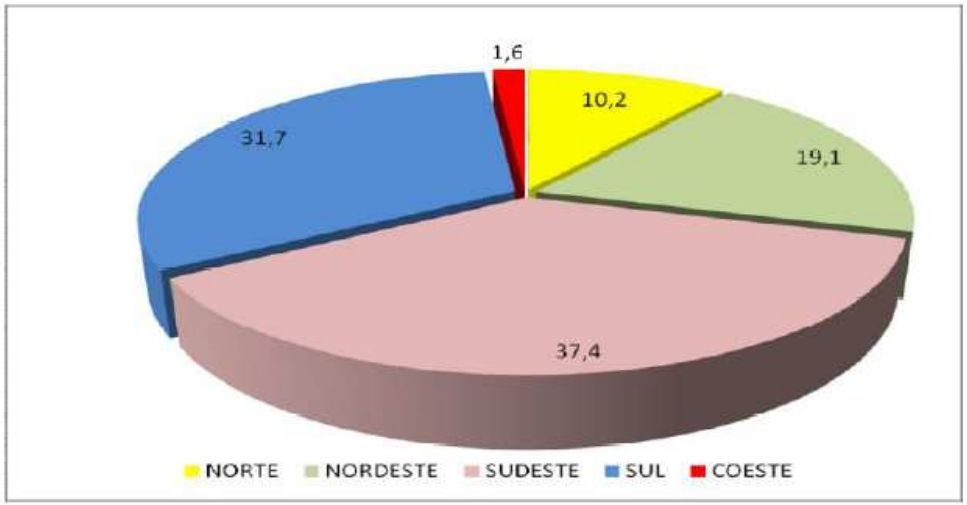


N= 23.574

Fonte: SINAN/SVS/MS

60% dos casos de leptospirose no Brasil (2001 – 2007) estão associados a zona urbana

Casos de Leptospirose segundo região do Brasil, 2004 a 2008



Fonte: SINAN/SVS/MS

PREVENÇÃO

Vacinas

Convencionais

Bacterina

- Específicas
- Imunidade curta
- LPS

Recombinantes

Estas vacinas podem ser desenvolvidas de diversas maneiras, dependendo do antígeno em questão e do tipo de resposta imune que se busca desencadear contra ele.

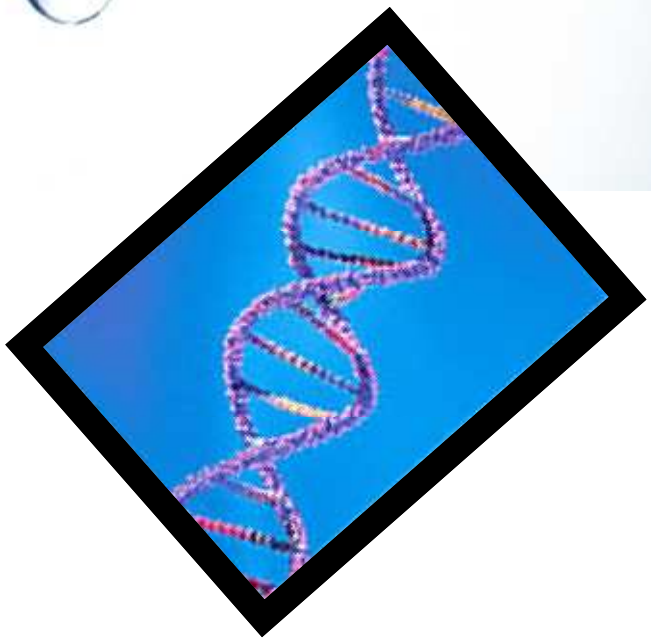
Vacinas de DNA

Vacinas Vetorizadas

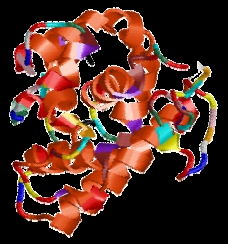
Vacinas de Subunidade

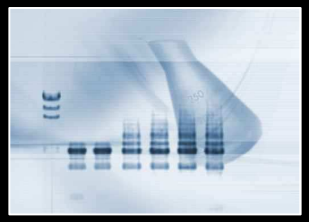
Tipos de vacinas recombinantes

- Subunidade Recombinante
- Vacina de DNA
- Vetorizadas



Vacina de subunidade recombinante





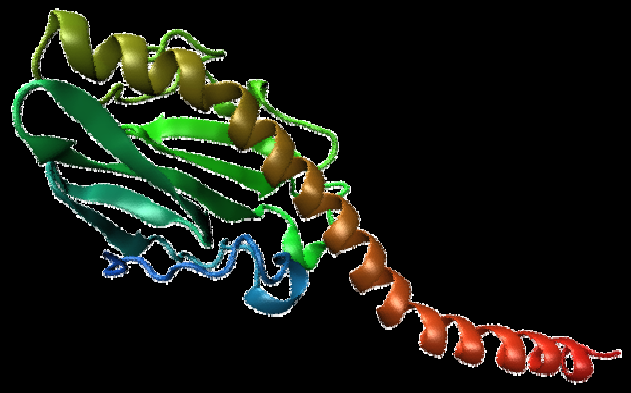
Usam fragmentos antigênicos de um microrganismo que melhor estimulam uma resposta imune. As vacinas de subunidades produzidas por técnicas de engenharia genética, onde outros microrganismos são programados para produzir a fração antigênica desejada, são chamadas de **vacinas recombinantes.**

- Mais produzidas e administradas
- São licenciadas
- Pouco ou nenhum efeito colateral
- Induz imunidade humoral

Escherichia coli

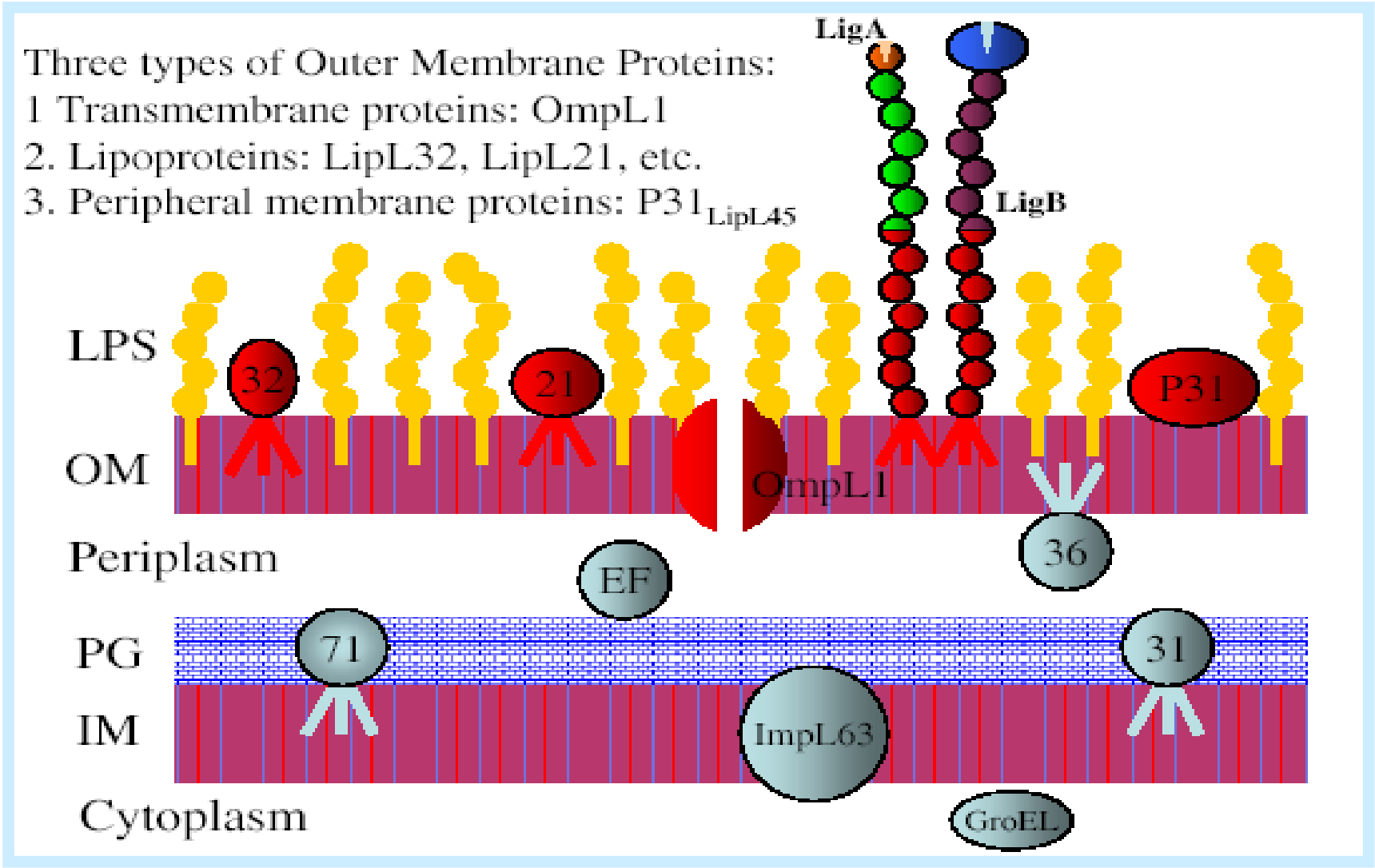
Pichia pastoris

Subprojeto 1

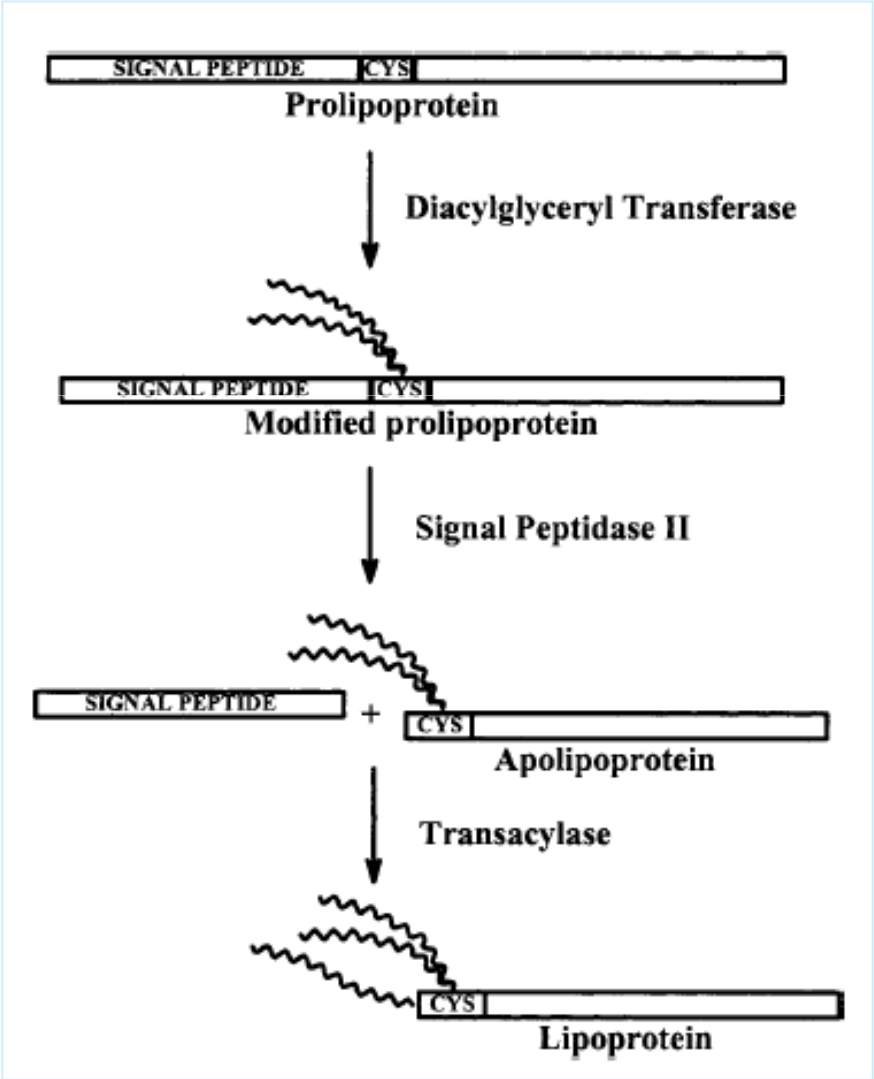


Clonagem, expressão e avaliação do potencial imunoprotetor de lipoproteínas de *L. interrogans*

Antígenos Potenciais



Biossíntese de lipoproteínas



PROLIPOPROTEÍNA

↓

Transferência de um grupo diacilglicerídeo para a cisteína

↓

PROLIPOPROTEÍNA MODIFICADA

↓

Clivagem da seqüência sinal

↓

APOLIPOPROTEÍNA

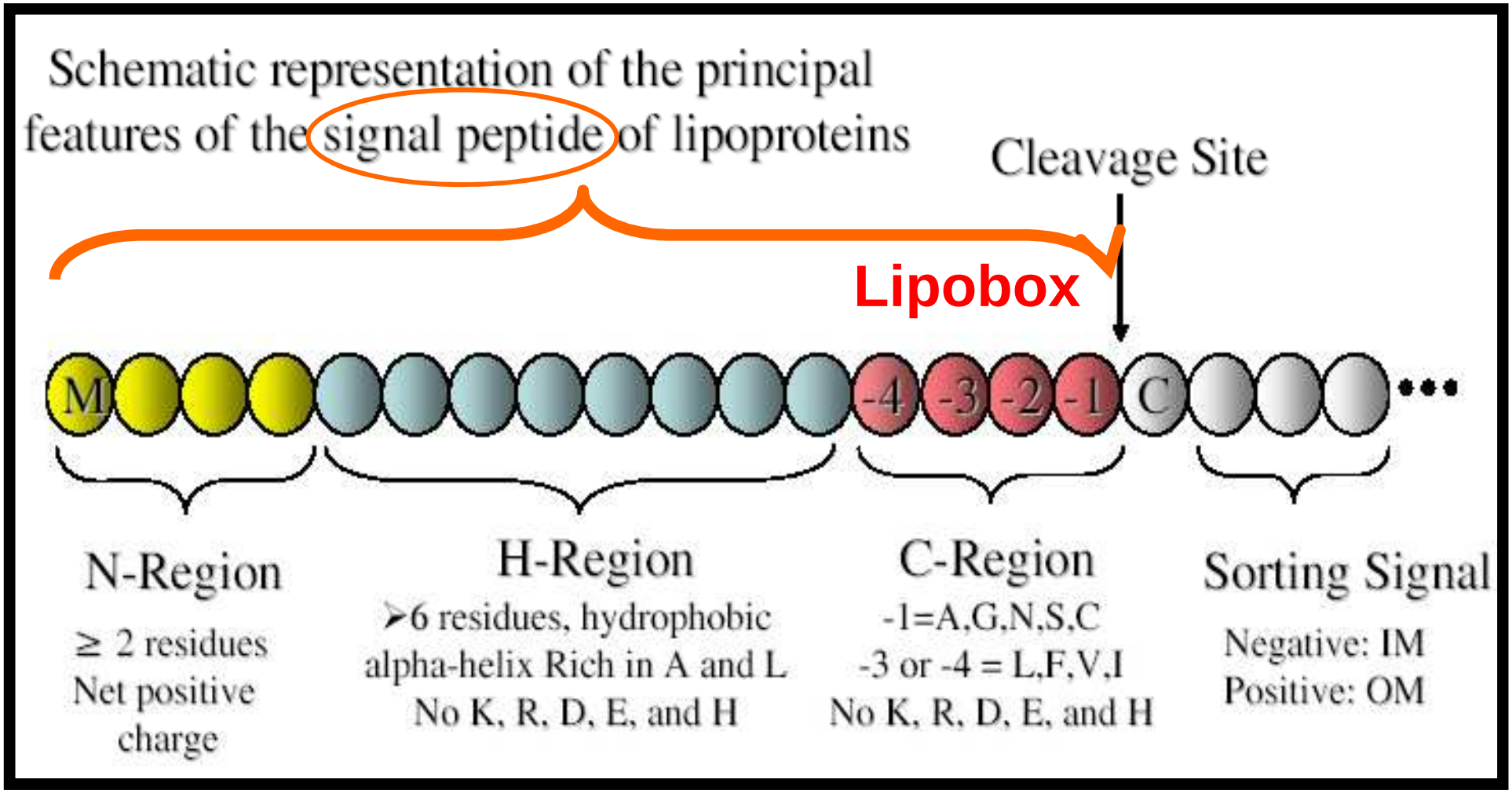
↓

Acetilação grupo amino na porção N-terminal da cisteína

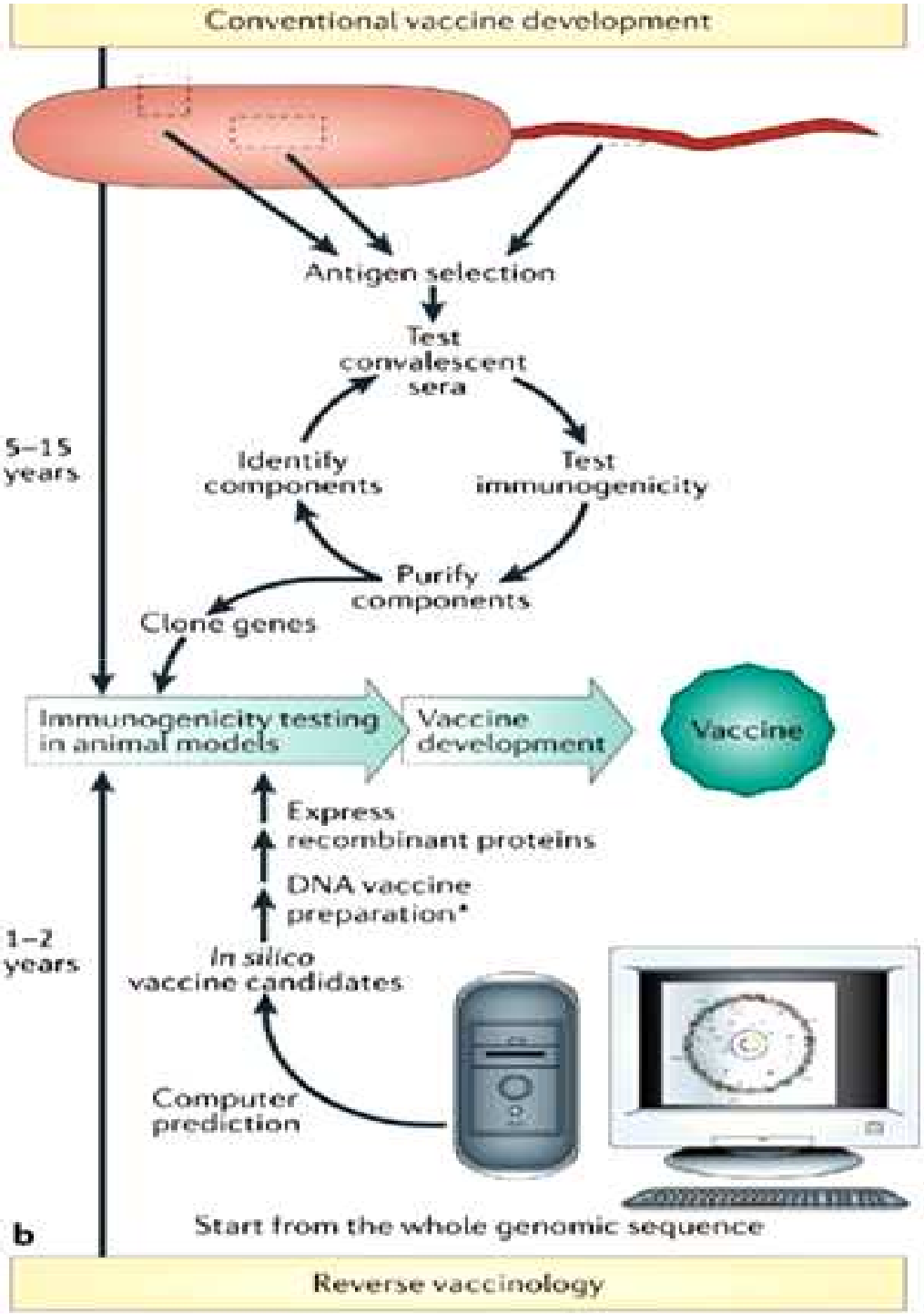
↓

LIPOPROTEÍNA

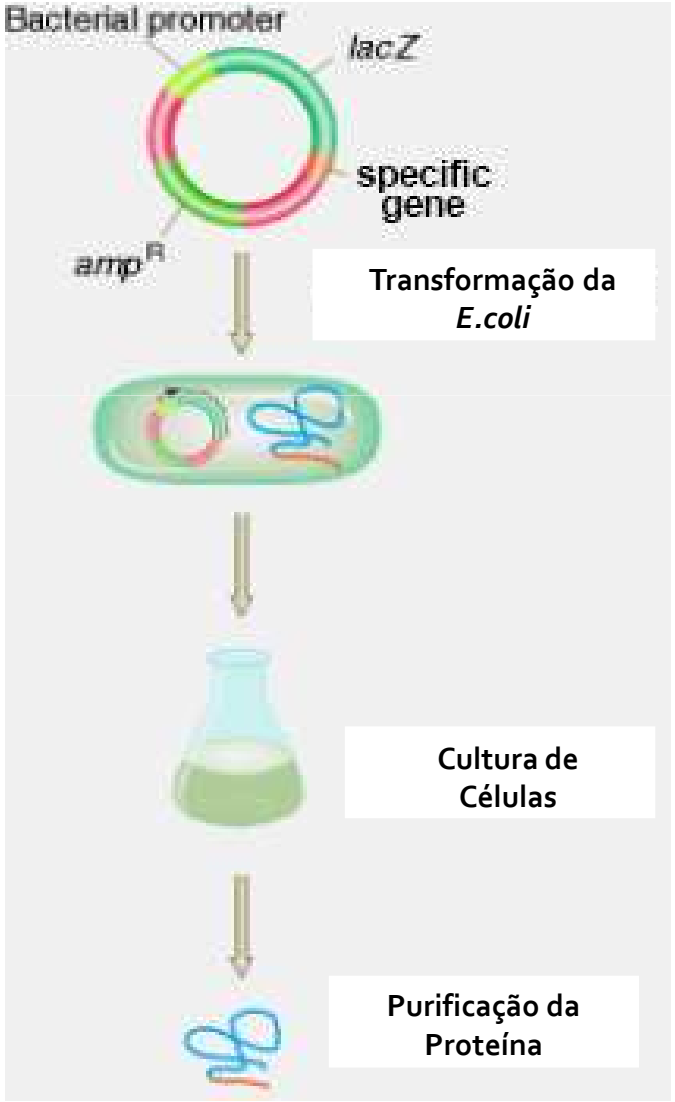
A região **lipobox** do peptídeo sinal das lipoproteínas das espiroquetas as diferencia das lipoproteínas de outras bactérias



Vacinologia Reversa



Diferentes sistemas de expressão



Bactérias- ***Escherichia coli***

Vantagens:

. Facilidade de crescimento

. Purificação (secreção, fusionadas)

. Várias cepas com o genótipo conhecido

. Vários plasmídeos comercialmente disponíveis

. Menor custo

Desvantagens:

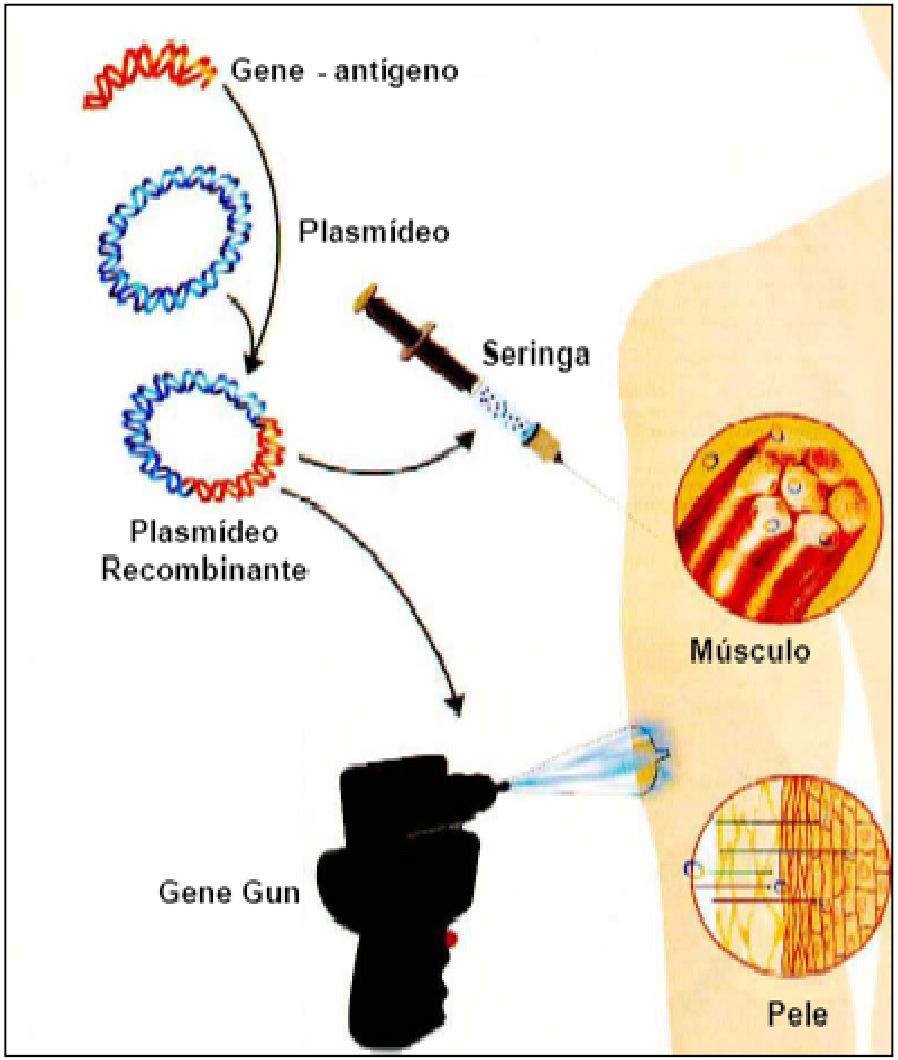
. Sem modificações pós-tradução (ex:glicosilações)

Vacina de DNA

Lipoproteína de *L interrogans* LIC11058

É uma vacina composta de DNA plasmidial capaz de expressar uma proteína antigênica no interior de células transfectadas, induzindo uma resposta imune.

Vacinas de DNA



Vantagens:

-Estabilidade

-Resposta imune de amplo espectro (humoral e celular)

-Resposta imune de longa duração

-Possibilidade de utilização de vários genes simultaneamente

-Busca de candidatos a vacinas (rapidez)

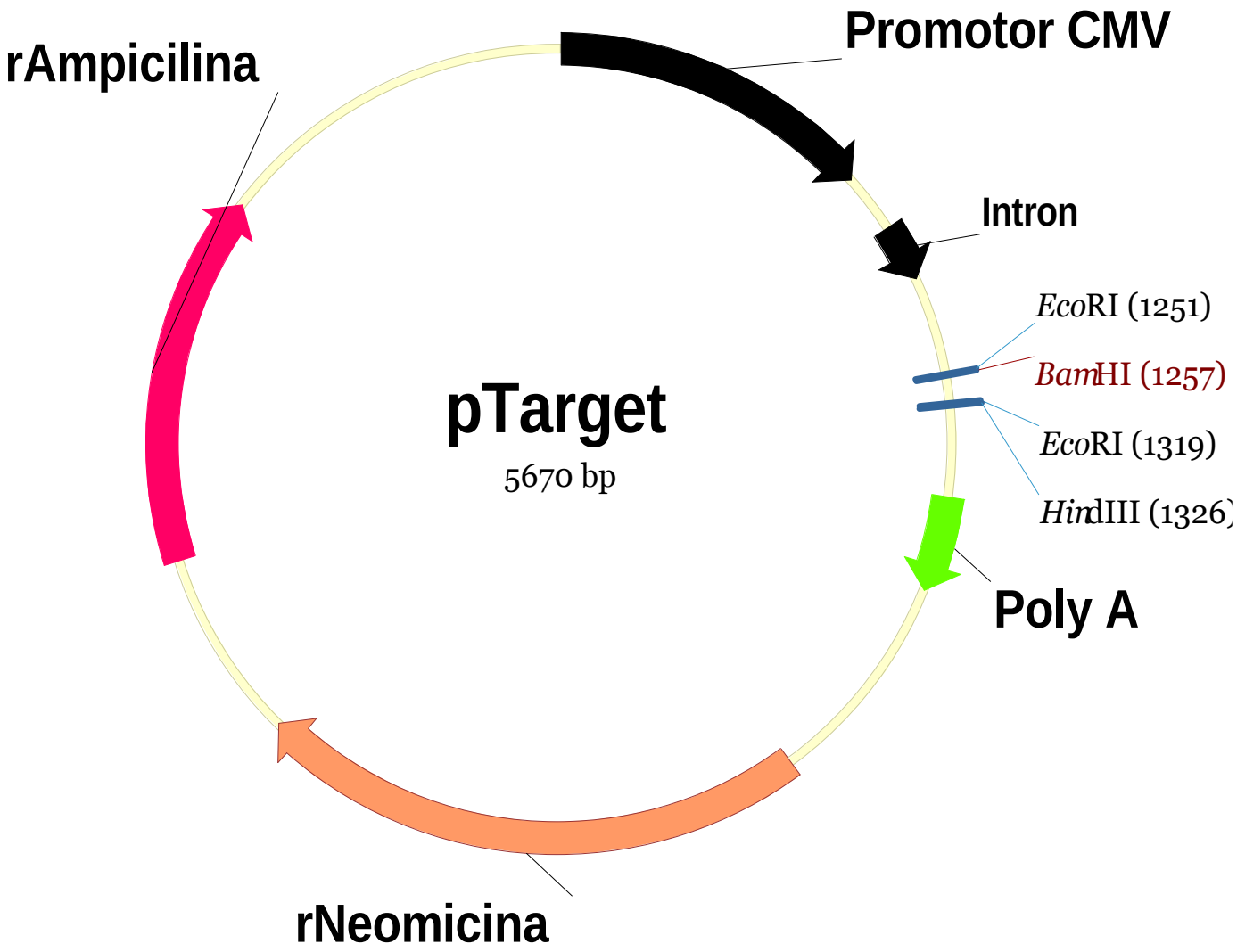
-Sem risco infeccioso

-Fácil preparo, menor custo

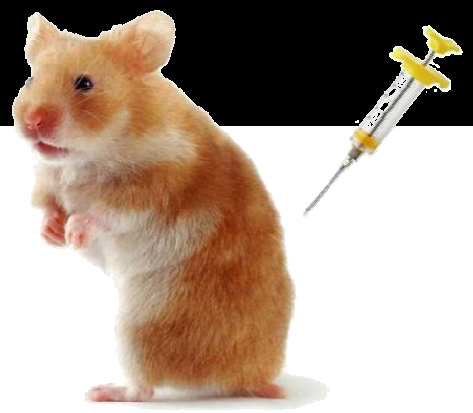
Desvantagens:

- Não licenciadas

Vetor de Expressão em Eucariotos

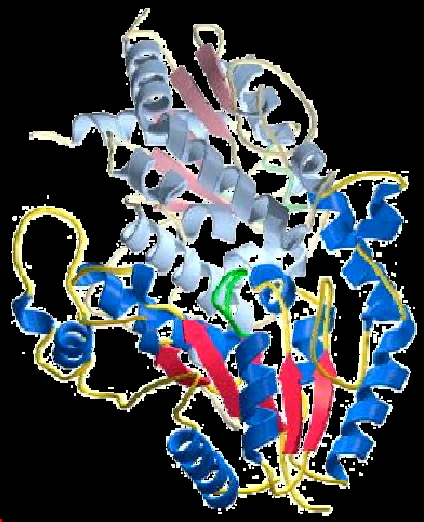


Experimento desafio...



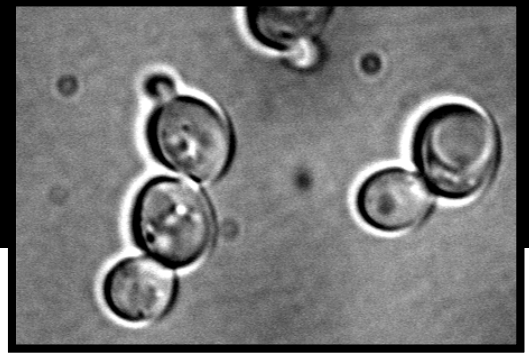
Grupos	Imunógeno	Número de animais	Dose	Adjuvante	Número de doses	Via	Desafio (21 dias após 2ª dose)
A	DNA + DNA	8	100 µg	-	1 (dia 0) + 1 (dia 21)	IM	5 *x* DL50 *L. interrogans* L1-130
B	DNA + proteína	8	100 µg	$Al(OH)_3$ 15%	1 (dia 0) + 1 (dia 21)	IM	5 *x* DL50 *L. interrogans* L1-130
C	proteína + proteína	8	100 µg	$Al(OH)_3$ 15%	1 (dia 0) + 1 (dia 21)	IM	5 *x* DL50 *L. interrogans* L1-130
D	PBS	8	100 µL	$Al(OH)_3$ 15%	1 (dia 0) + 1 (dia 21)	IM	5 *x* DL50 *L. interrogans* L1-130
E	Bacterina	6	10^9 cél.mL^{-1}	$Al(OH)_3$ 15%	1 (dia 0) + 1 (dia 21)	IM	5 *x* DL50 *L. interrogans* L1-130

Subprojeto 2



Clonagem e expressão das proteínas LigAni e LipL32 de *L. interrogans* em *P. pastoris*

Pichia pastoris



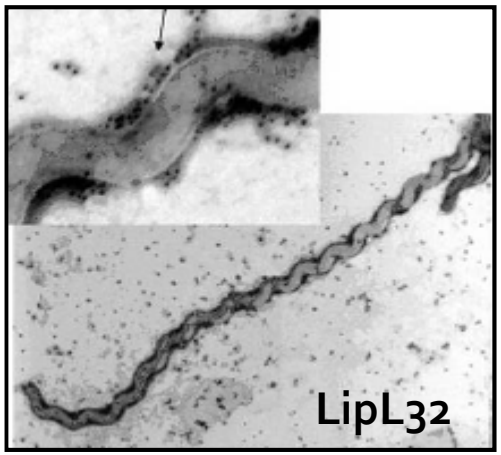
- levedura metilotrófica
  - utiliza metanol como única fonte de carbono

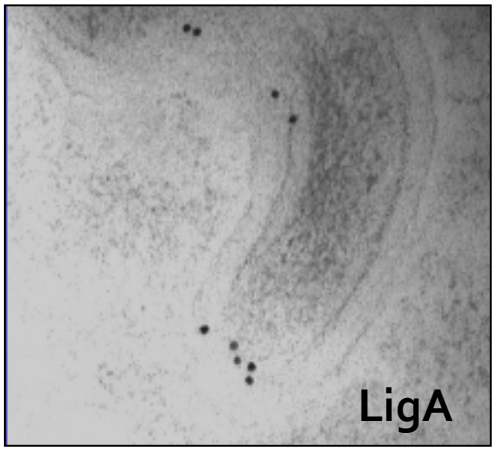
- adequada para a expressão de proteínas heterólogas
  - fácil manipulação genética
  - crescimento rápido em meios relativamente simples
  - promotor *AOX* induzível por metanol
  - alta taxa de expressão
  - capacidade de fazer modificações pós-traducionais
  - permite expansão para produção de proteínas

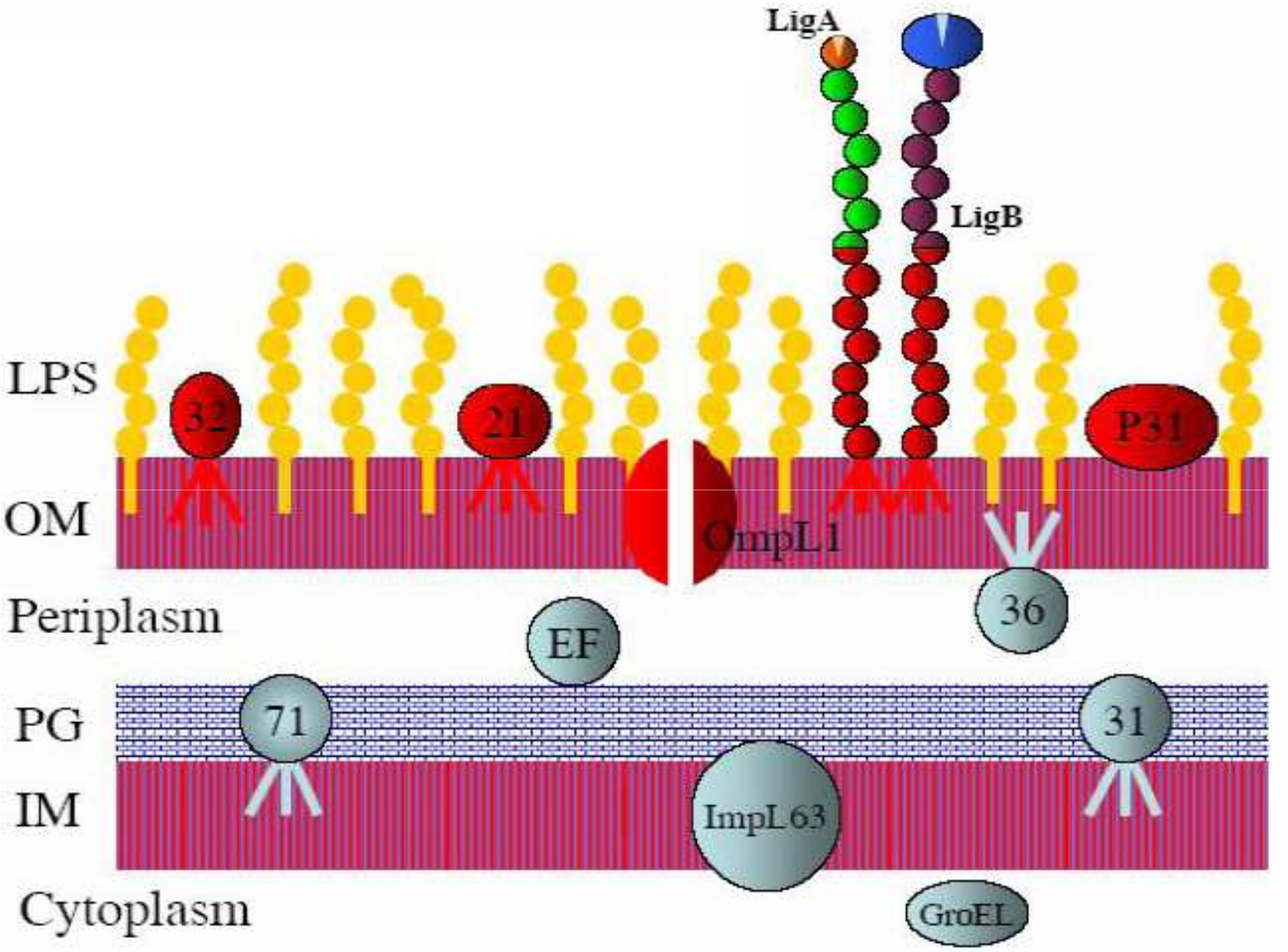
em escalas industriais

OMPs de *Leptospira* (LigA e LipL32)

- Expostas na superfície celular
- Expressas durante a infecção em mamíferos
- Conservadas em sorovares patogênicos
- Ausentes em saprófitas
- Ligantes de componentes da matriz extracelular

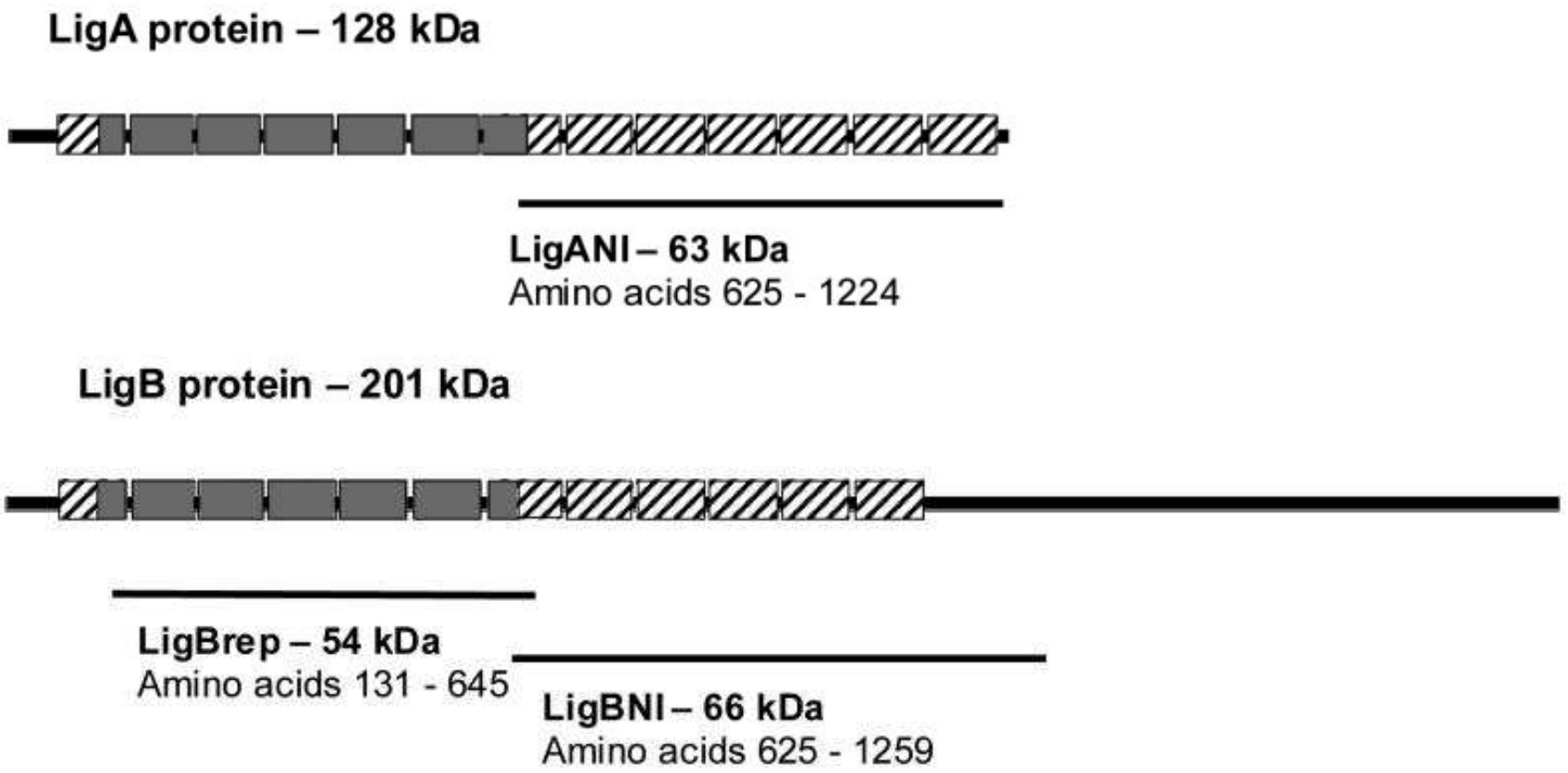






Haake , 2005. ILS meeting (Tailândia)

Antígenos



Schematic representation of LigA and LigB proteins and recombinant fragments. Boxes represent bacterial immunoglobulin-like (Big) tandem repeat domains (~90 amino acids). Amino acids 102 to 630 (Big domains 2-6 and part of 7) of LigA and LigB, the region with 100% amino acid sequence identity between these two proteins, are represented as grey boxes. The C-terminal Big domains of LigA (amino acid position 631-1,224) and LigB (amino acid position 631-1,119) have lower amino acid sequence identity (38%) and are represented as hatched boxes. Lines represent the three recombinant fragments, LigANI, LigBrep and LigBNI that were cloned and expressed.

Antígenos

67 – 100% de imunoproteção em hamsters

- LigAni

Published in final edited form as:
Vaccine. 2007 August 14; 25(33): 6277–6286.

The terminal portion of leptospiral immunoglobulin-like protein LigA confers protective immunity against lethal infection in the hamster model of leptospirosis

Éverton F. Silva[a,b], Marco A. Medeiros[c], Alan J. A. McBride[a], Jim Matsunaga[d,e], Gabriela S. Esteves[c], João G. R. Ramos[a], Cleiton S. Santos[a], Júlio Croda[a], Akira Homma[c], Odir A. Dellagostin[b], David A. Haake[d,e], Mitermayer G. Reis[a], and Albert I. Ko[a,f]

a*Gonçalo Moniz Research Center, Oswaldo Cruz Foundation, Brazilian Ministry of Health, Salvador, Brazil*

b*Biotechnology Centre, Federal University of Pelotas, Pelotas, Brazil*

c*Bio-Manguinhos, Oswaldo Cruz Foundation, Brazilian Ministry of Health, Rio de Janeiro, Brazil*

d*Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, California*

e*Department of Medicine, the David Geffen School of Medicine at UCLA, Los Angeles, California*

f*Division of International Medicine and Infectious Disease, Weill Medical College of Cornell University, New York, USA*

Antígenos

- LipL32

rHap1 produced in E. coli was tested in vaccination trials but showed no evidence of direct protection (data not shown).

INFECTION AND IMMUNITY, Nov. 2001, p. 6831–6838 Vol. 69, No.
0019-9567/01/$04.00+0 DOI: 10.1128/IAI.69.11.6831–6838.2001

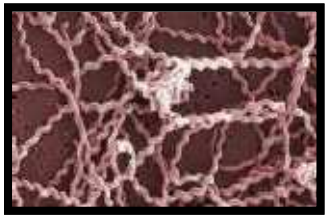
Identification of the Hemolysis-Associated Protein 1 as a Cross-Protective Immunogen of *Leptospira interrogans* by Adenovirus-Mediated Vaccination

C. BRANGER,[1] C. SONRIER,[1] B. CHATRENET,[2] B. KLONJKOWSKI,[3] N. RUVOEN-CLOUET,[1] A. AUBERT,[2] G. ANDRÉ-FONTAINE,[1]* AND M. ELOIT[3]

Unité de Bactériologie Médicale et Moléculaire des Leptospires, Ecole Nationale Vétérinaire de Nantes, 44307 Nantes Cedex 03,[1] Virbac Laboratories, 06511 Carros Cedex,[2] and UMR ENVA-INRA 955 de Génétique Moléculaire et Cellulaire, Génétique Virale, 94704 Maisons Alfort,[3] France

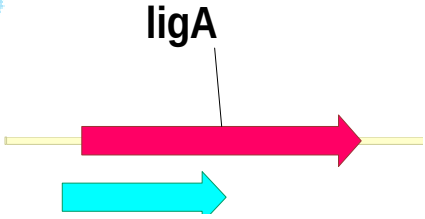
Received 16 April 2001 /Returned for modification 5 June 2001/Accepted 19 July 2001

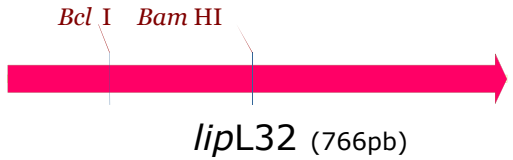
Metodologia

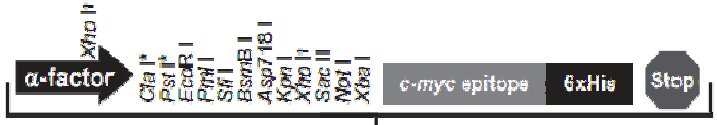


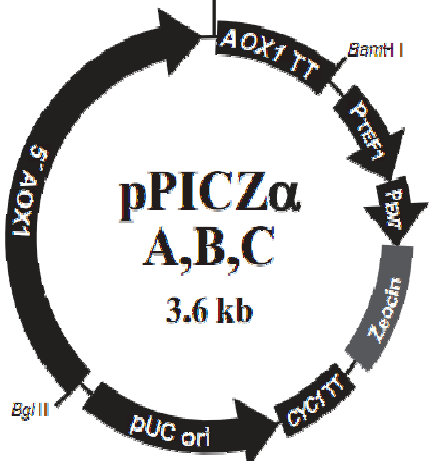
Extração de DNA genômico

Amplificação dos genes alvos



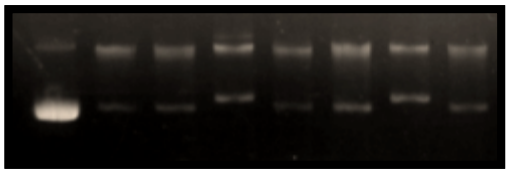






Ligação

Transformação em *E.coli* TOP10

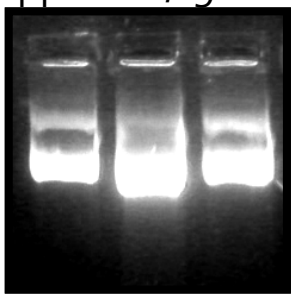


Screening

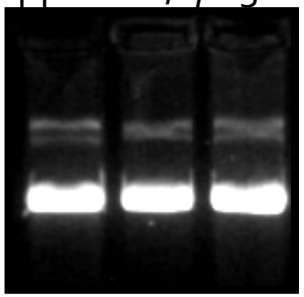
Digestão para checar liberação do inserto

Metodologia

ppicZαB/*lig*Ani

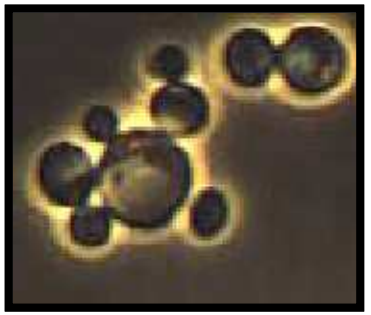


ppicZαB/*lip*L32



Extração de plasmídeos em larga escala

Linearização dos plasmídeos (*Pme*I)



Transformação em *Pichia pastoris* (5 – 10 µg de DNA)

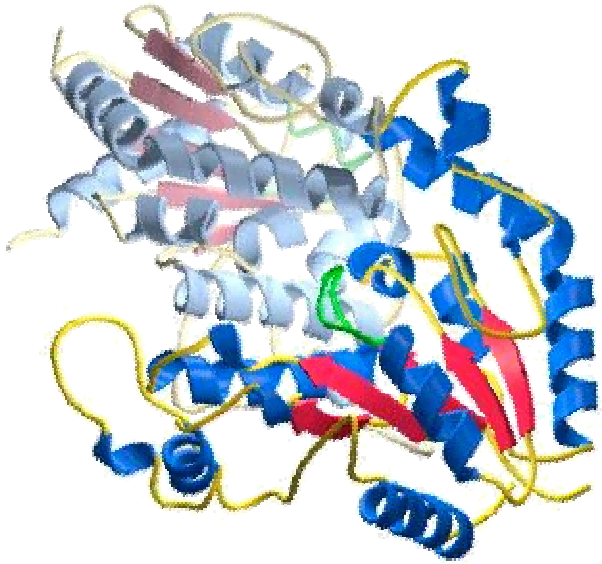
Avaliação da expressão em meio líquido

PCR de colônia

Colony blotting

Metodologia

- **Purificação/Concentração das proteínas**
  - Precipitação com sulfato de amônia
  - Ultrafiltração
  - Liofilização



Metodologia (Precipitação sulfato de amônia)

Solução saturada de sulfato de amônia 85%

90 mL de sobrenadante contendo as proteínas recombinantes

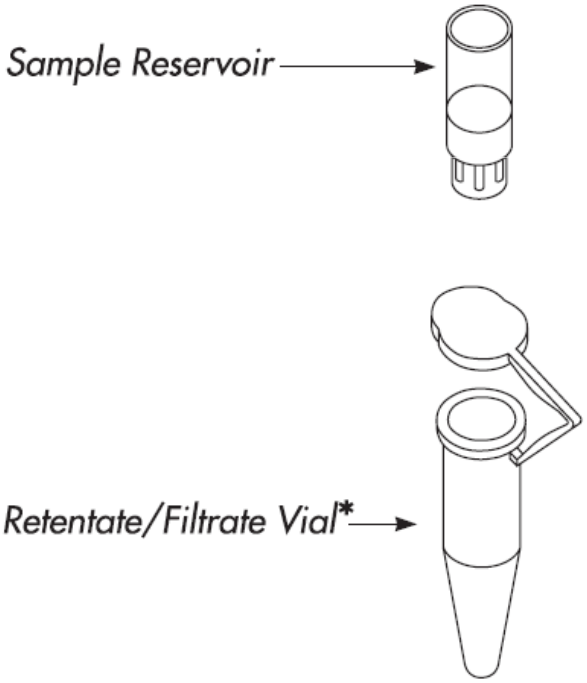
Concentração do sal

- 25%
- 35%
- 45%
- 60%
- 70%
- 80%

- Incubadas sob agitação por 1 h à 4 °C
- Centrifugadas 10.000 *x g* por 15 min à 4 °C
- Pellet ressuspendido em PBS pH 7.4
- Dialisadas por 48 h em PBS
- Analisadas e quantificadas

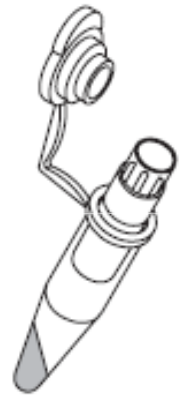
Metodologia (Ultrafiltração)

- Colunas Microcon YM-30 (Millipore)

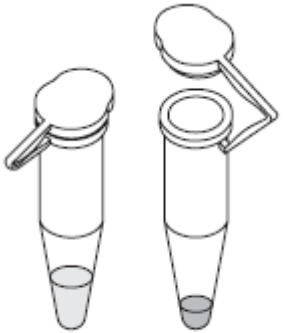


*2 identical vials are supplied with each Microcon unit for use in concentration and recovery spins.

Yellow — Ultracel YM-3 membrane
Green — Ultracel YM-10 membrane
Clear — Ultracel YM-30 membrane
Rose — Ultracel YM-50 membrane
Blue — Ultracel YM-100 membrane

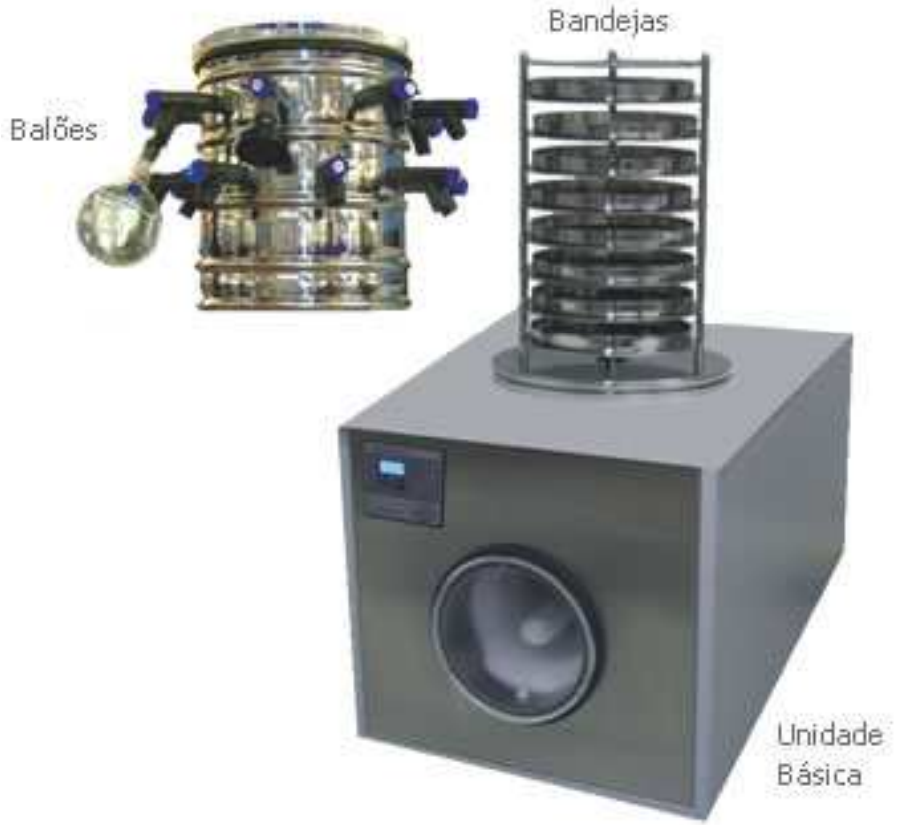


Retentate transfer during recovery spin



Filtrate and retentate in sealable storage vials

Metodologia (Liofilização)



- 2 mL sobrenadante cultivo
- Liofilizadas durante 28 h
- Ressuspendidas em PBS
  - mesmo volume inicial
  - 10 x concentrada
- Analisadas e quantificadas

Glicosilação em *P. pastoris*

- N-glicosilação (preferencial) – de 8 a 14 resíduos de manose adicionados.

  (Asn-X-Ser/Thr)

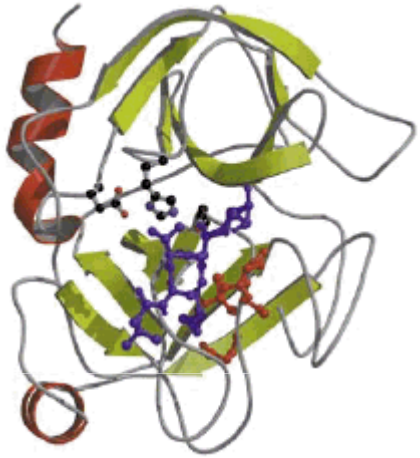
- O-glicosilação (pouco conhecida)

- LipL32 (Asn-Glu-Thr) – 1 sítio

- LigAni (Asn-Ile-Thr) – (Asn-Val-Ser) – (Asn-Ser-Thr) – (Asn-Ala-Thr) - (Asn-Ala-Thr) – (Asn-Ile-Thr) – (Asn-Ile-Thr) – 7 sítios

Metodologia (Glicosilação)

Digestão com Endo H e PNGase F



1 – 20 µg das proteínas recombinantes

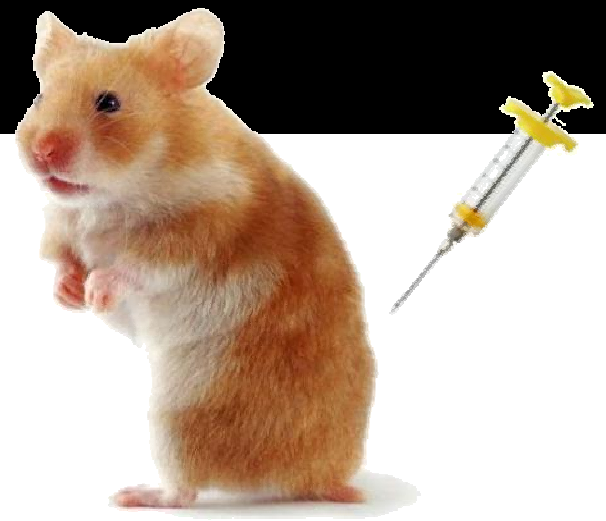
Incubadas com tampão de desnaturação de proteínas 1x (100 °C por 10 min)

1 – 5 µL Endo H
Tampão de reação G5 5x
(37 °C por 1 h)

1 – 5 µL PNGase F
Tampão de reação G7 10x
(37 °C por 1 h)

SDS-page
WB

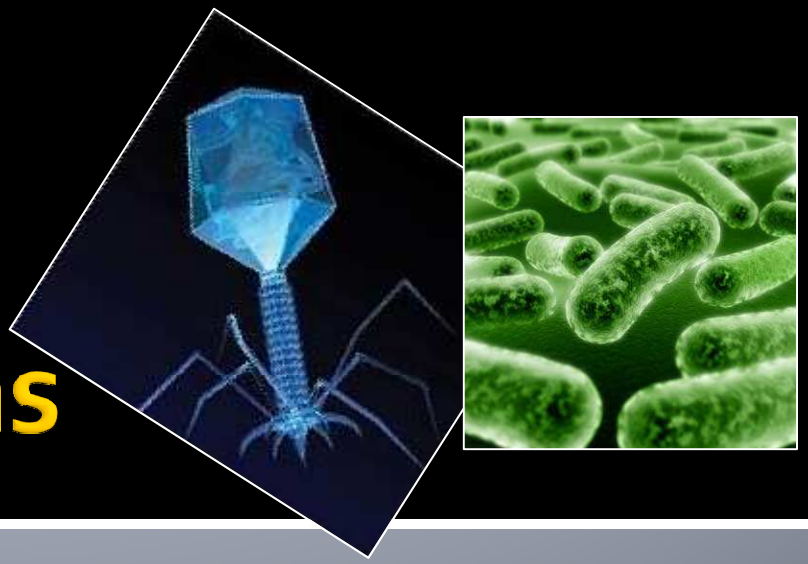
Perspectivas futuras



- Avaliação do potencial imunoprotetor em hamsters das proteínas LigAni e LipL32 expressas em *Pichia pastoris*.

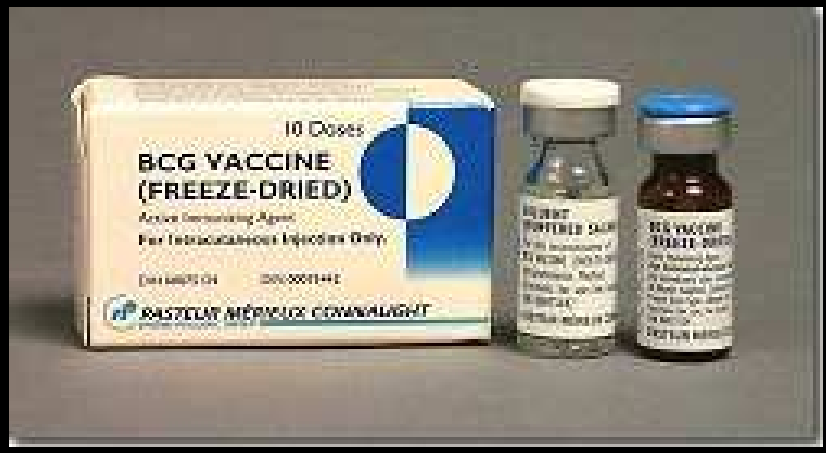
Grupos	Imunógeno	Número de animais	Dose	Adjuvante	Número de doses	Via	Desafio (21 dias após 2ª dose)
A	rLigAni *P. pastoris*	12	80 µg	$Al(OH)_3$ 15%	1 (dia 0) + 1 (dia 21)	IM	5 *x* DL50 *L. interrogans* L1-130
B	rLipL32 *P. pastoris*	12	80 µg	$Al(OH)_3$ 15%	1 (dia 0) + 1 (dia 21)	IM	5 *x* DL50 *L. interrogans* L1-130
C	PBS	12	100 µL	$Al(OH)_3$ 15%	1 (dia 0) + 1 (dia 21)	IM	5 *x* DL50 *L. interrogans* L1-130
D	Bacterina	06	10^9 cél.mL^{-1}	$Al(OH)_3$ 15%	1 (dia 0) + 1 (dia 21)	IP	5 *x* DL50 *L. interrogans* L1-130

Vacinas Vetorizadas



Nas vacinas vetorizadas, bactérias ou vírus são os carreadores de genes de patógenos, que serão expressos dentro do organismo a ser imunizado.

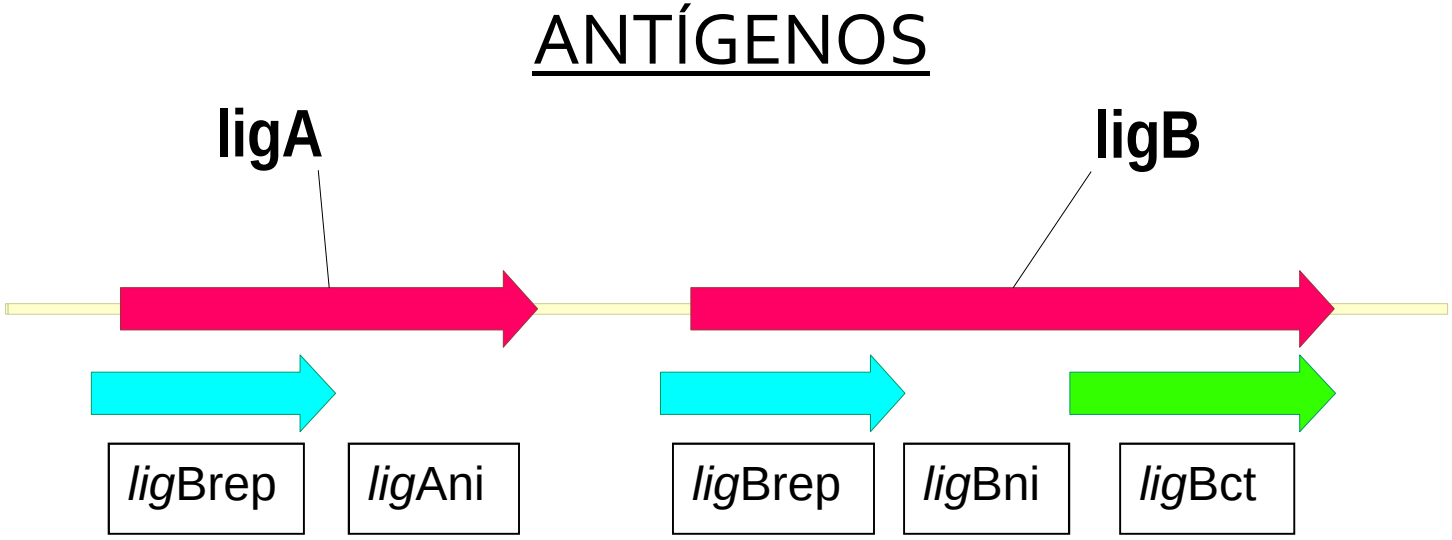
Subprojeto 3



Expressão de antígenos em BCGr

Experimentos

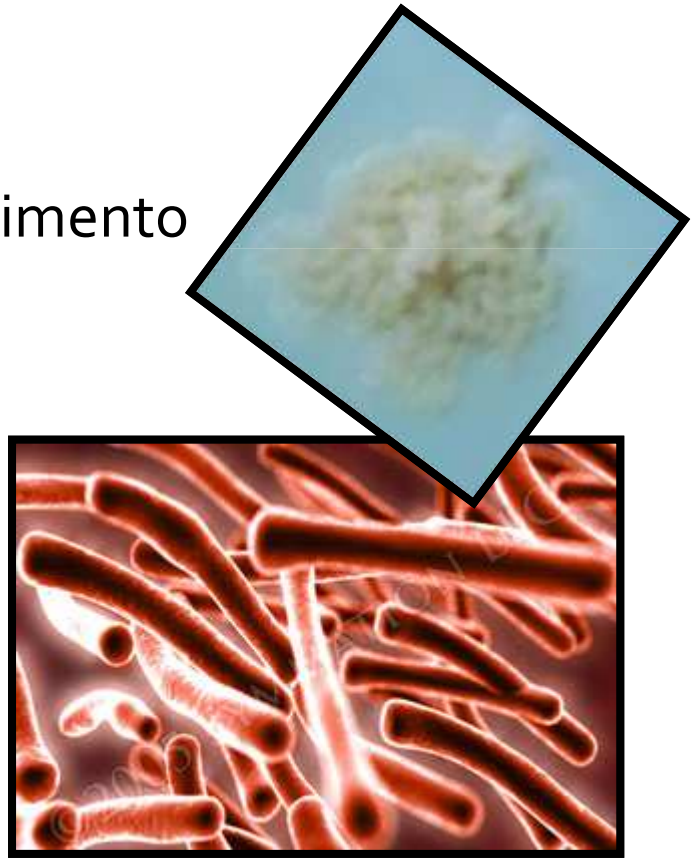
Experimento: Expressão das proteínas Ligs em BCG Pasteur e avaliação da imunoproteção em hamsters



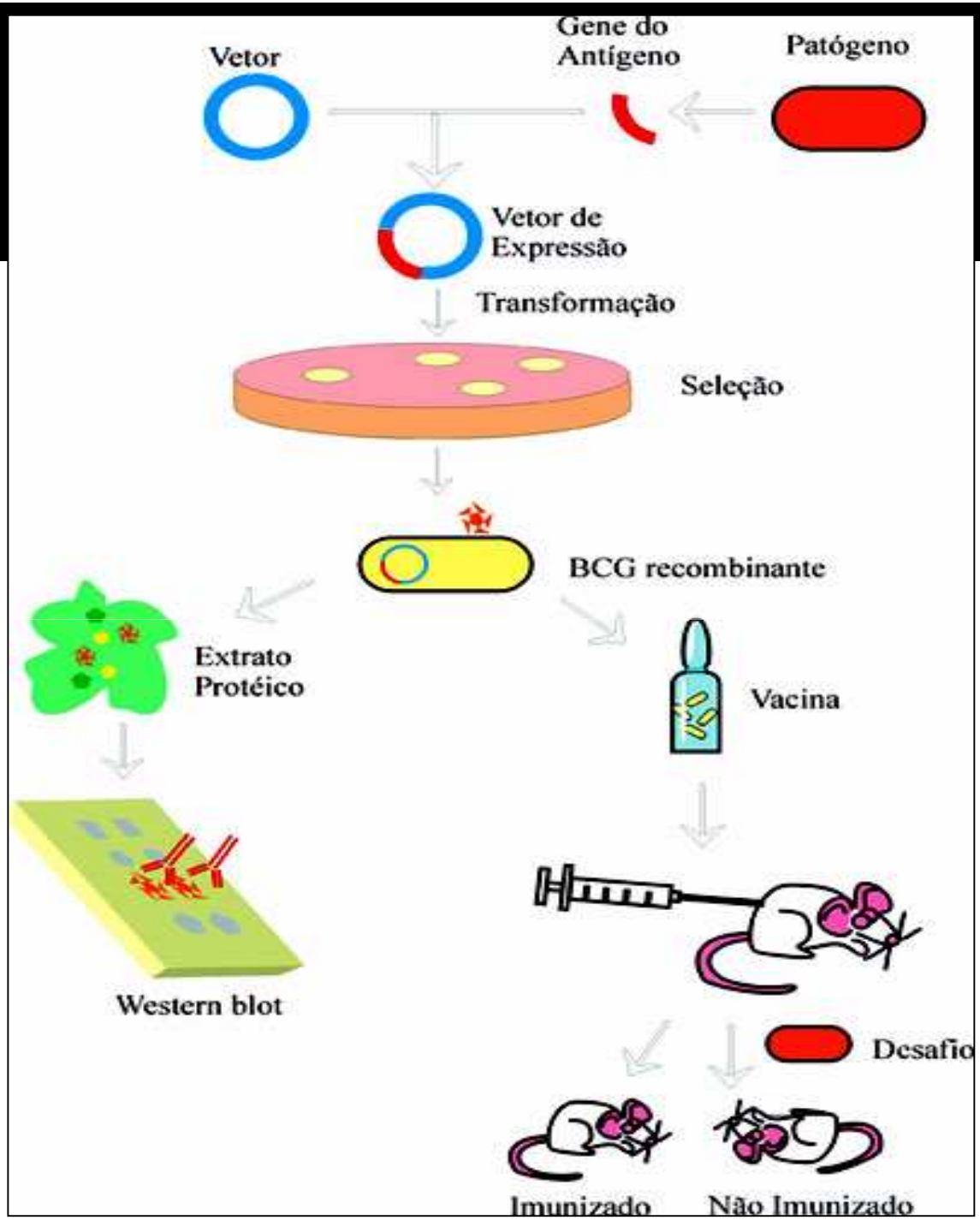
Mycobacterium bovis BCG Pasteur

- Vantagens

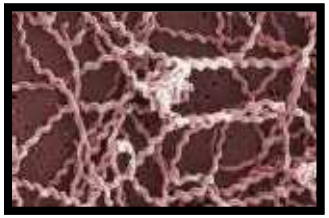
  - Vacina mais utilizada no mundo
  - Administrada em dose única após o nascimento
  - Importante adjuvante
  - Pode ser administrada via oral
  - Estável ao calor
  - Baixo custo de produção
  - Induz imunidade celular e humoral



Construção de um BCGr

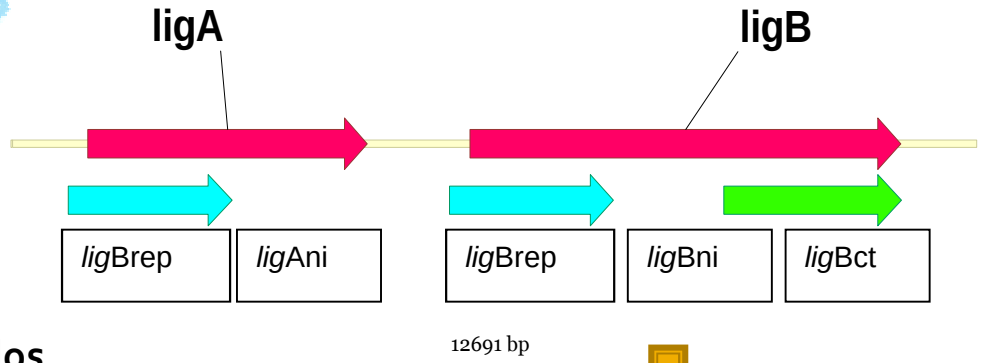


Metodologia

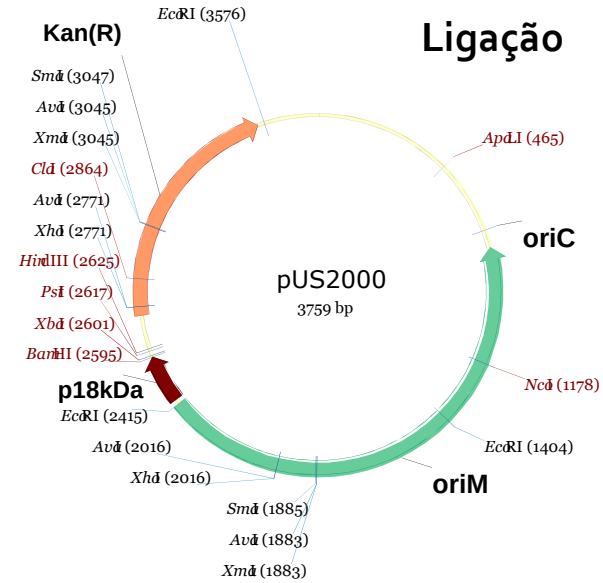


Extração de DNA genômico

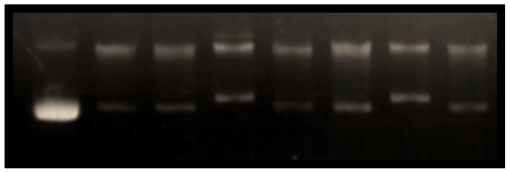
Amplificação dos genes alvos



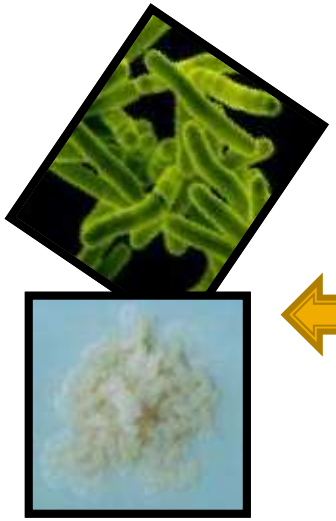
Ligação



Transformação em *E.coli* TOP10



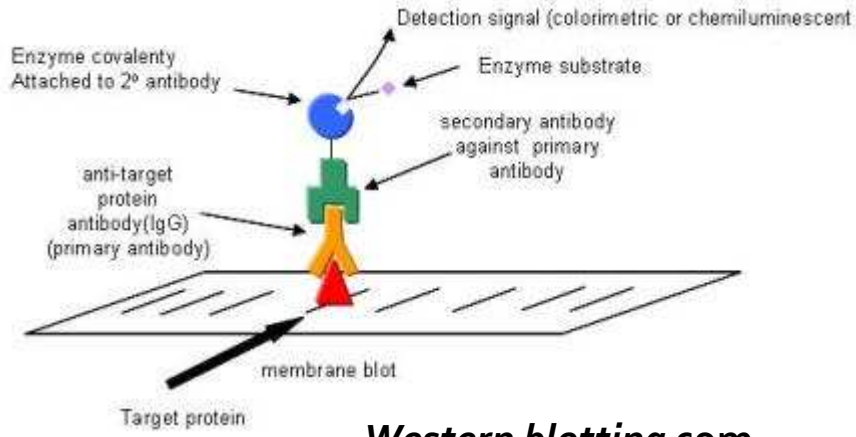
- *Screening*
- Digestão com enzimas de restrição



Transformação em BCG Pasteur

Metodologia

Teste de expressão em BCG Pasteur



Western blotting **com anticorpos policlonais**

Imunoproteção em hamsters
Histopatologia
Cultura

Evaluation of different ways of presenting LipL32 to the immune system with the aim of developing a recombinant vaccine against leptospirosis

Fabiana Kömmling Seixas, Claudia Hartleben Fernandes, Daiane Drawanz Hartwig, Fabricio Rochedo Conceição, José Antônio Guimarães Aleixo, and Odir Antônio Dellagostin

Abstract: Leptospirosis, caused by bacteria of the genus *Leptospira*, is a direct zoonosis with wide geographical distribution. The implications in terms of public health and the economical losses caused by leptospirosis justify the use of a vaccine against *Leptospira* in human or animal populations at risk. In this study, we used the external membrane protein LipL32 as a model antigen, as it is highly immunogenic. The LipL32 coding sequence was cloned into several expression vectors: (*i*) pTarget, to create a DNA vaccine; (*ii*) pUS973, pUS974, and pUS977 for expression in BCG (rBCG); and (*iii*) pAE, to express the recombinant protein in *Escherichia coli*, for a subunit vaccine. Mice were immunized with the various constructs, and the immune response was evaluated. The highest humoral immune response was elicited by the subunit vaccine (rLipL32). However, with rBCG, the titer was still rising at the end of the experiment. The serum of vaccinated animals was able to recognize LipL32 on the membrane of the *Leptospira*, detected by indirect immunofluorescence. A monoclonal antibody anti-LipL32 was shown to inhibit the growth of *Leptospira* in vitro, indicating potential protection induced by the LipL32 antigen.

Key words: Leptospira, LipL32, recombinant BCG, subunit vaccine, DNA vaccine.

Canadian Journal of Microbiology_2007

Table 1. Bacterial strains, plasmids, and primers used in this study.

Strain, plasmid, or primer	Relevant information	Source or reference
Strain		
Escherichia coli DH5α	F⁻, *lacZ* ΔM15, *end*A1, *rec*A1, *sup*E44, *rel*A1	Invitrogen, USA
E. coli BL21(DE3) pLysS	F⁻ *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm* Δ(*srl-recA*)*306*::Tn*10*(TcR) (DE3) pLysS(CmR)	Novagen, USA
Mycobacterium bovis BCG Pasteur	Vaccine strain	FIOCRUZ-RJ
Leptospira interrogans	Strain Fiocruz L1-130 was isolated from a patient during an outbreak of leptospirosis in Salvador, Brazil	Ko et al. 1999
Plasmid		
pTARGET	Mammalian expression vector, Amp^r, CMV promoter	Promega, USA
pAE	Cloning and expression vector, Amp^r, T7 promoter	Ramos et al. 2004
pUS973	*E. coli* – mycobacteria shuttle vector, Kan^r, oriM, promoter *hsp60* from *Mycobacterium tuberculosis*	Medeiros et al. 2002
pUS974	*E. coli* – mycobacteria shuttle vector, Kan^r, oriM, *hsp60* promoter and signal sequence of *M. tuberculosis* antigen 19 (MT19)	Medeiros et al. 2002
pUS977	*E. coli* – mycobacteria shuttle vector, Kan^r, oriM, promoter P_{AN} from *Mycobacterium paratuberculosis*	Medeiros et al. 2002
Primer		
LipPTF	5′-ATGGGTGGTCTGCCAAGCCTAAAAAGCTC-3′	This work
LipPTR	5′-TTACTTAGTCGCGTCAGAAGCAGC-3′	This work
LippAEF	5′-ccg**CTCGAG**GGTGGTCTGCCAAGCCT-3′	This work
LippAER	5′-g**GAATTC**TTACTTAGTCGCGTCAGAAGC-3′	This work
LipBCGF	5′-ta**TCTAGA**GGGTGGTCTGCCAAG-3′	This work
LipBCGR	5′-cgg**AAGCTT**TTACTTAGTCGCG-3′	This work

Note: In primer sequences, lowercase letters denote nucleotides added or modified to facilitate incorporation of restriction sites, marked in bold.

Fig. 1. (A) Sodium dodecyl sulfate – polyacrylamide gel electrophoresis of purified rLipL32. Lane 1, protein ladder (Invitrogen); lanes 2–4, purified rLipL32 fractions. (B) Western blot with monoclonal antibody 1D9 demonstrating LipL32 expression in BCG. Lane 1, rBCG transformed with pUS973/*lipL32*; lane 2, rBCG transformed with pUS974/*lipL32*; lane 3, rBCG transformed with pUS977/*lipL32*; lane 4, BCG (control).

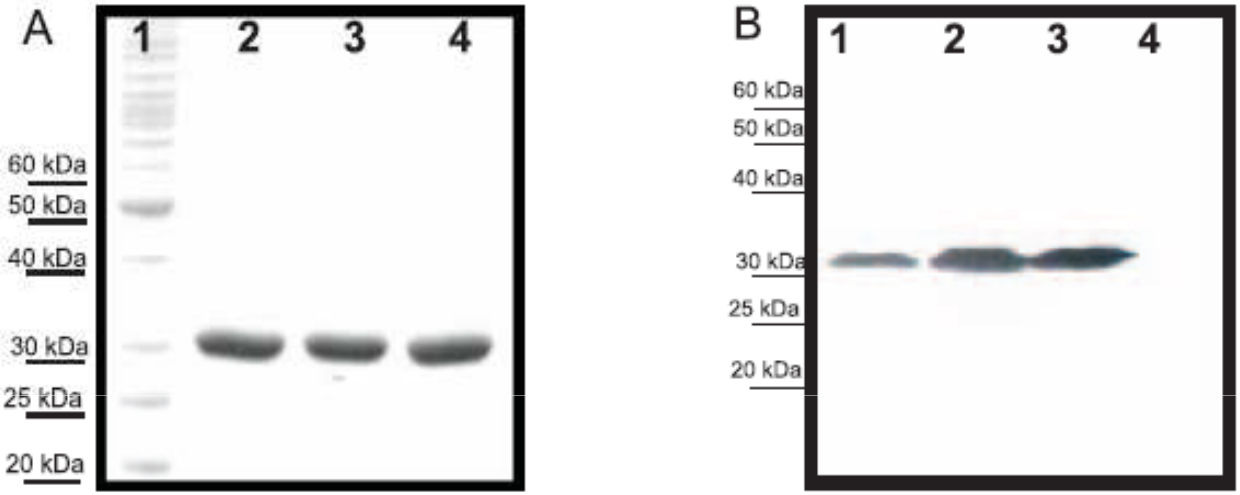
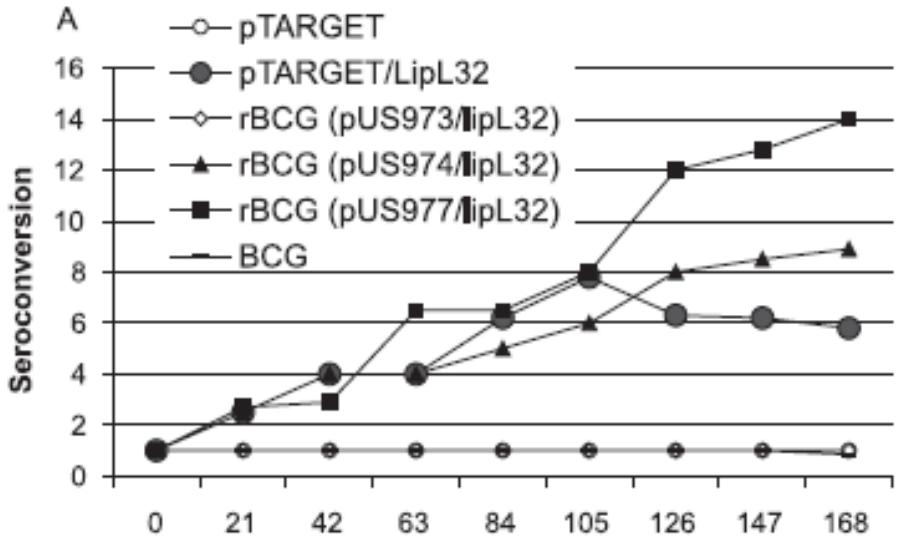


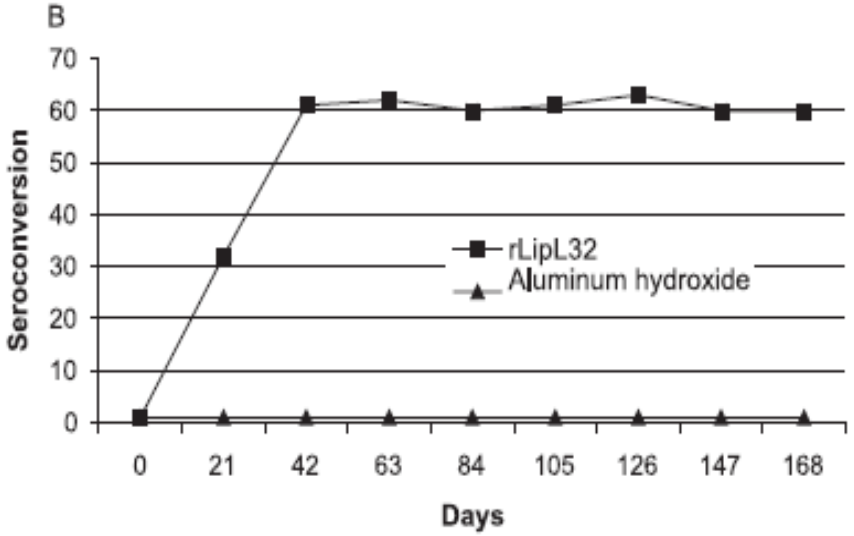
Table 2. Groups of mice and vaccine preparations used in the experiment.

Group	Immunogen	Dose	Route
A	pTARGET (control)	100 µg of DNA	IM
B	pTARGET/*lipL32*	100 µg of DNA	IM
C	Aluminum hydroxide	15% Aluminum hydroxide	IM
D	Recombinant LipL32	100 µg of rLipL32 + 15% aluminum hydroxide	IM
E	BCG (control)	10^6 CFU of BCG	IP
F	rBCG (pUS973/*lipL32*)	10^6 CFU of BCG	IP
G	rBCG (pUS974/*lipL32*)	10^6 CFU of BCG	IP
H	rBCG (pUS977/*lipL32*)	10^6 CFU of BCG	IP

Note: CFU, colony-forming units; IP, intraperitoneal injection; IM, intramuscular.

Fig. 2. Mean seroconversion, determined by ELISA, of anti-LipL32 systemic antibodies from mice inoculated with different vaccine preparations. Mice were inoculated at days 0 and 21 of the experiment. (A) Evaluation of the immune response elicited by the DNA vaccine pTARGET/*lipL32* and the rBCG constructs. (B) Evaluation of the immune response elicited by the subunit vaccine (purified rLipL32).





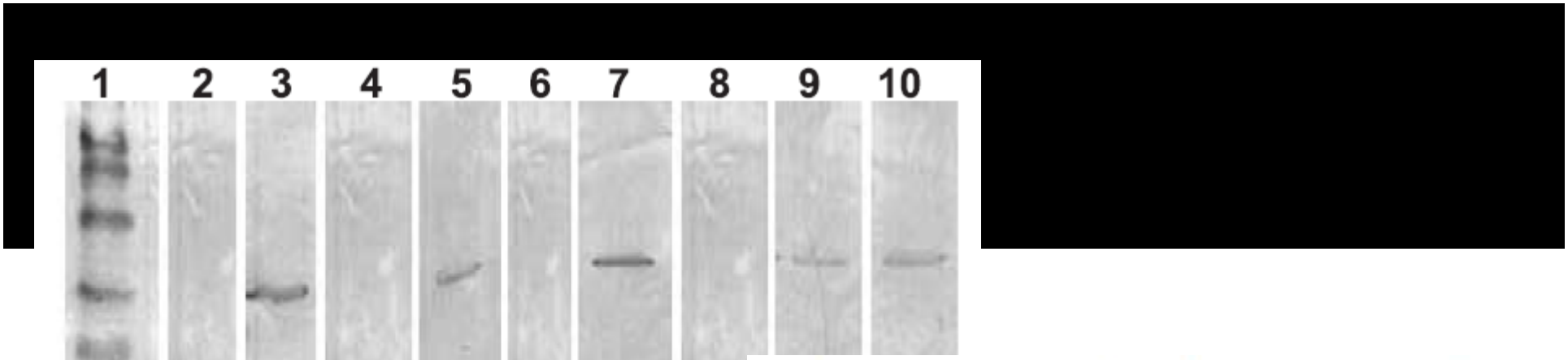
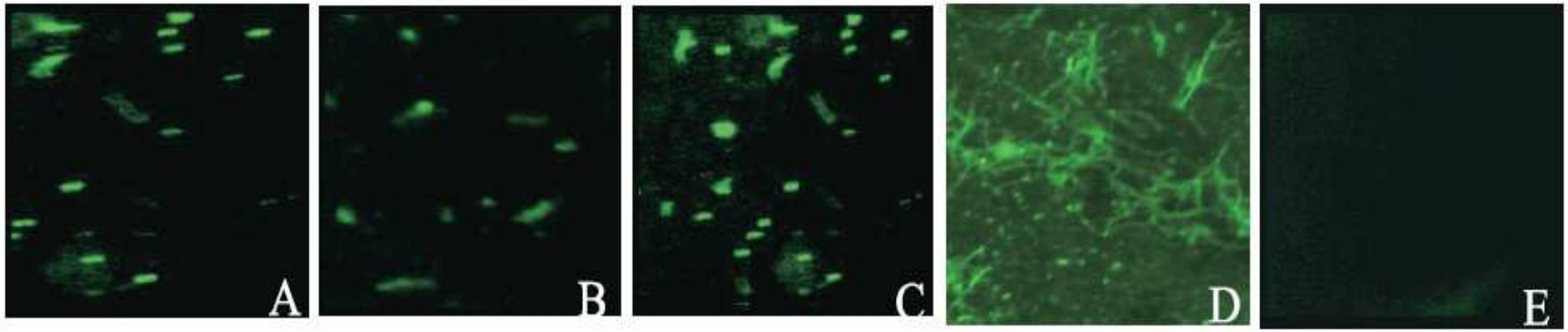


Fig. 3. Western blot analysis of pooled sera from mice inoculated with different vaccines against crude extract of *Leptospira interrogans*. Lane 1, Benchmarker™ prestained protein ladder (Invitrogen); lane 2, pTARGET/*lipL32* (day 0); lane 3, pTARGET/*lipL32* (day 168); lane 4, rBCG (pUS974/*lipL32*) (day 0); lane 5, rBCG (pUS974/*lipL32*) (day 168); lane 6, rBCG (pUS977/*lipL32*) (day 0); lane 7, rBCG (pUS977/*lipL32*) (day 168); lane 8, rLipL32 (day 0); lane 9, rLipL32 (day 168); lane 10, monoclonal antibody 1D9.

Fig. 4. Indirect immunofluorescence with intact *Leptospira interrogans*. (A) Pooled sera from animals vaccinated with the DNA vaccine (pTARGET/*lipL32*), (B) rBCG (pUS974/*lipL32*), (C) rBCG (pUS977/*lipL32*), (D) rLipL32, (E) pooled sera from the saline group.



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Vaccine (2007) xxx, xxx–xxx

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journal homepage: www.elsevier.com/locate/vaccine

Recombinant *Mycobacterium bovis* BCG expressing the LipL32 antigen of *Leptospira interrogans* protects hamsters from challenge

Fabiana Kömmling Seixas[a], Éverton Fagonde da Silva[a], Daiane Drawanz Hartwig[a,b], Gustavo Maia Cerqueira[a], Marta Amaral[a,b], Michel Quevedo Fagundes[a], Robson Grando Dossa[a], Odir Antônio Dellagostin[a,b,*]

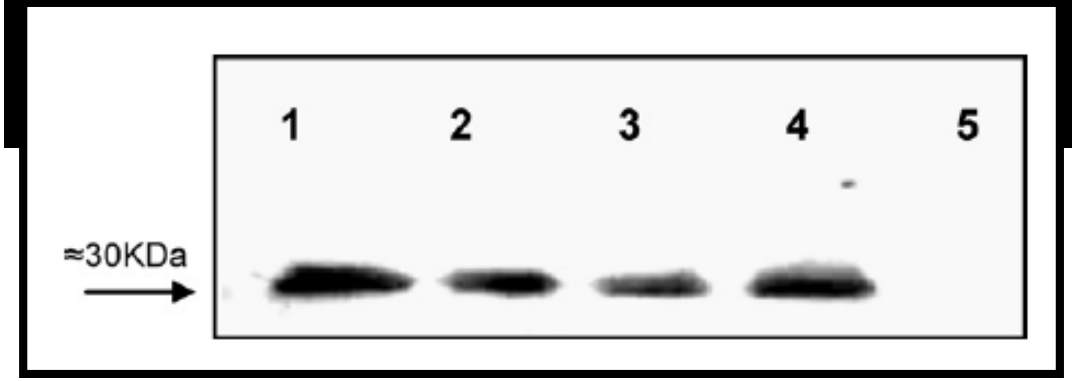

Fig. 1 Western blot developed with MAb 1D9 demonstrating LipL32 expression in BCG. Lane 1, rBCG transformed with pUS973/*lipL32; lane 2, rBCG transformed with pUS974/lipL32;* lane 3, rBCG transformed with pUS977/*lipL32; lane 4, rBCG* transformed with pUS2000/*lipL32 and lane 5, wtBCG (control).*

Table 1 Groups of hamster and vaccine preparations used in the experiment

Group	Immunogen	Dose	Route
Group A	wtBCG (control)	10^6 CFU of BCG	i.p.
Group B	rBCG (pUS973*lipL32*)	10^6 CFU of BCG	i.p.
Group C	rBCG (pUS974/*lipL32*)	10^6 CFU of BCG	i.p.
Group D	rBCG (pUS977/*lipL32*)	10^6 CFU of BCG	i.p.
Group E	rBCG (pUS2000/*lipL32*)	10^6 CFU of BCG	i.p.
Group F	Killed whole-leptospires	10^9 Leptospires	i.p.

i.p., intraperitoneal injection; CFU, colony-forming units.

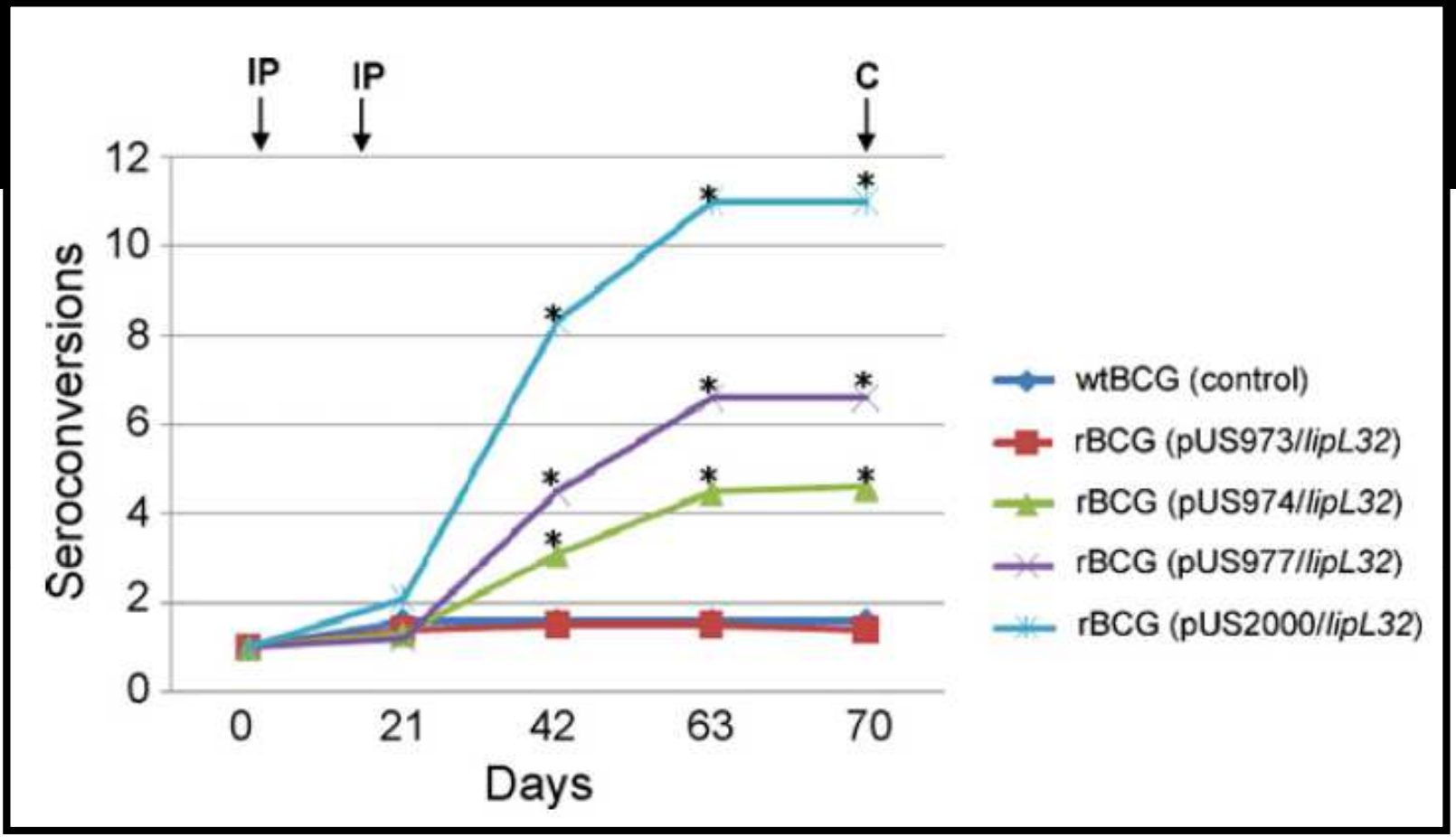

Fig. 2 Seroconversion of total antibodies anti-LipL32 of hamsters inoculated with wtBCG, rBCG (pUS973/*lipL32*), rBCG (pUS974/*lipL32*), *rBCG (pUS977/lipL32) or rBCG* (pUS2000/*lipL32*), *expressed in ELISA units. Recombinant* LipL32 was used as antigen in the ELISA. Results are expressed as mean seroconversion, for pool serum samples. *$p < 0.05$ in comparison to the control groups. (IP) Intraperitoneally immunized animals. (C) Intraperitoneally challenged. Each point corresponds to the pool of sera of the corresponding group of animals from the first experiment.

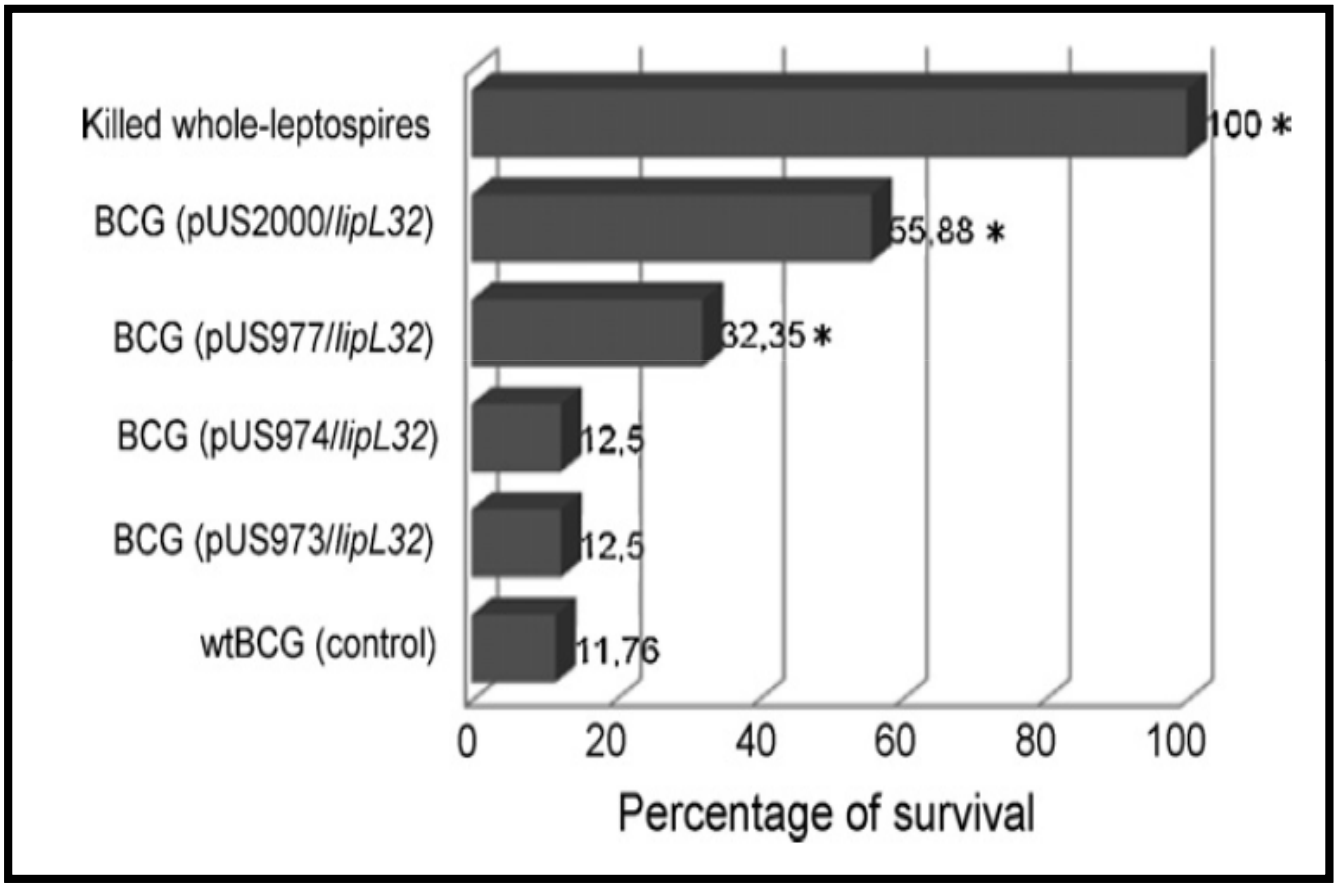

Fig. 3 Percentage of survival of hamsters challenged with *L. interrogans L1130. Asterisks denote a significant difference in* survival rate when compared to the control group ($p < 0.05$). Data represent all experiments summarized.

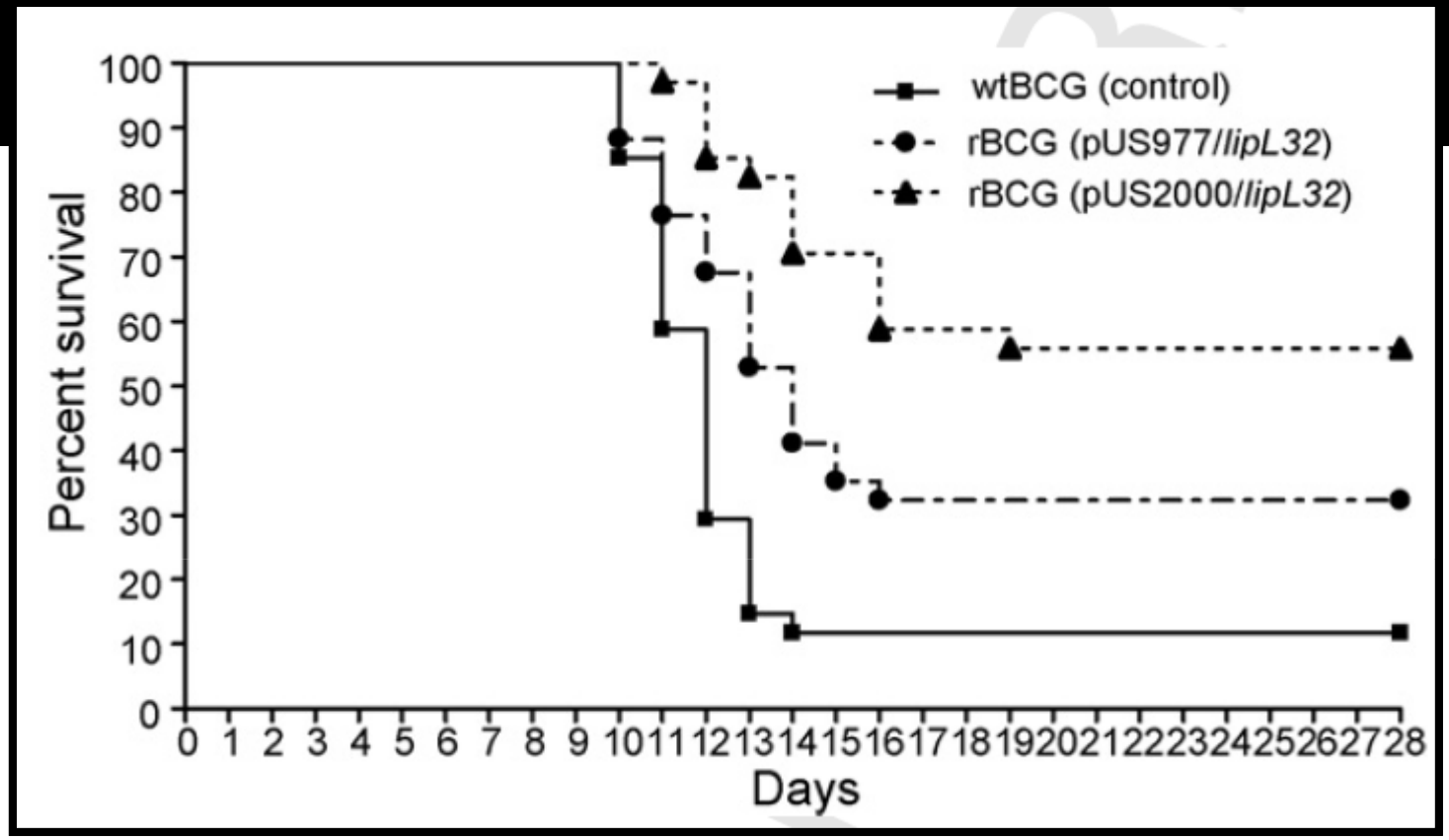

Fig. 4 Survival of hamsters challenged with *L. interrogans* L1130 after immunization with rBCG. Hamsters were immunized with wtBCG (control), rBCG (pUS977/*lipL32) and rBCG* (pUS2000/*lipL32). The log-rank sum test was used to determine* significant differences for survival, between the groups immunized with rBCG and the negative control group (*p≤0.05). Data* represent the combined results of three separate experiments.

Grupo de pesquisa em leptospirose

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UFPel – CCZ
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UFF
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OBRIGADA PELA ATENÇÃO!!!

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