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Mini Review

A description of genes of *Corynebacterium pseudotuberculosis* useful in diagnostics and vaccine applications

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ABSTRACT. *Corynebacterium pseudotuberculosis*, a Gram-positive intracellular pathogen, is the etiological agent of caseous lymphadenitis or CLA. This bacterium infects goats and sheep and causes great economic losses worldwide annually, mainly for goat producers. Despite its importance, CLA is still poorly characterized. However, with advances in the genomic field, many *C. pseudotuberculosis* genes have already been characterized, mainly those related to virulence such as phospholipase D. Here, we examined the use of the several available genes of *C. pseudotuberculosis* and reviewed their applications in vaccine construction, more efficient diagnostics for CLA, and control of this disease, among other applications.

Key words: Phospholipase D; RecA; Caseous lymphadenitis

INTRODUCTION

The genus *Corynebacterium* belongs to the group of actinomycetes that includes the CMN group (*Corynebacterium*, *Mycobacterium*, *Nocardia* and *Rhodococcus* species) (Hard, 1969; Songer et al., 1988; Songer, 1997; Paule et al., 2004; Dorella et al., 2006a). These Gram-positive bacteria constitute a very heterogeneous group that shares particular characteristics, such as a specific cell wall organization (Hard, 1975; Funke et al., 1995; Collins et al., 1982, 1998; Connor et al., 2000; Bayan et al., 2003; Hall et al., 2003; Dorella et al., 2006a) and high G + C content (47-74%) (Garg et al., 1985; Goodfellow, 1989; Funke et al., 1995; Navas, 1996; Dorella et al., 2006a). Belonging to this group is *Corynebacterium pseudotuberculosis*, an important animal pathogen and the etiological agent of a disease called caseous lymphadenitis (CLA) (Williamson, 2001).

This disease is spread worldwide, and its considerable economic importance (Williamson, 2001; Paton et al., 2003) has prompted investigation of its pathogenesis. However, the genetic determinants of *C. pseudotuberculosis* virulence are still poorly characterized (Dorella et al., 2006a); moreover, this species has only 19 proteins identified in the GenPept database as shown in Table 1 and about 1230 genomic survey sequences (Dorella et al., 2006b) already deposited in the GenBank database.

Table 1. Genes of *Corynebacterium pseudotuberculosis* deposited in the GenPept, National Center for Biotechnology Information (NCBI).

Accession number (GenPept)	Putative protein	Reference
ABI29892	10-kDa chaperonin GroES	Coelho KS and Azevedo V (unpublished results)
AAV48830	60-kDa chaperonin 1	Estevam E, Miyoshi A and Azevedo V (unpublished results)
ABI75067	65-kDa heat shock protein	Flandrois J-P and Fardel G (unpublished results)
AAB71614	AroB (3-dehydroquinate synthase)	Simmons et al., 1997
AAB71615	AroB (3-dehydroquinase)	Simmons et al., 1997
P96749	AroB (3-dehydroquinate synthase)	Simmons et al., 1997
P96750	AroQ (3-dehydroquinate dehydratase)	Simmons et al., 1997
AAL79811	FagA (integral membrane protein)	Billington et al., 2002
AAL79810	FagB (iron-enterobactin transporter)	Billington et al., 2002
AAL79809	FagC (ATP-binding cytoplasmic membrane protein)	Billington et al., 2002
AAL79812	FagD (iron-siderophore binding protein)	Billington et al., 2002
P20626	Phospholipase D precursor	Hodgson et al., 1990
AAA64910	Phospholipase D	Cuevas and Songer, 1993
AAA99867	Phospholipase D	McNamara et al., 1994
CAA01541	Phospholipase D	–
AAA82608	Protein recA	Pogson et al., 1996
P48288	Protein recA	Pogson et al., 1996
AAS89201	RpoB (RNA polymerase β subunit)	Khamis et al., 2004
AAA67924	Serine proteinase precursor	Wilson et al., 1995

The majority of these genes belongs to virulence factors, or modulate positively virulence genes or encode virulence factors that confer pathogenic characteristics to *C. pseudotuberculosis*. For this reason, virulence genes are targets for the development of new vaccines and more efficient therapies and diagnostics in the control of illnesses, mainly CLA which is still managed by rudimentary prophylaxis. In this study, we conducted an overview of the cited genes, as well as their application in helping to control CLA.

GENES AND THEIR APPLICATIONS

Phospholipase D: virulence factor

After the report of Hodgson and colleagues (1990) about the nucleotide sequence, cloning and expression of *C. pseudotuberculosis* phospholipase D (*pld*) gene, several studies about this gene were conducted. Phospholipase D (PLD) has for some years been implicated as the major virulence factor of *C. pseudotuberculosis* (Hodgson et al., 1999). The *pld* gene encodes an exotoxin (Burrel, 1983; Hodgson et al., 1994) that probably promotes bacterial dissemination, increasing vascular permeability (Egen et al., 1989; Cardenas and Clements, 1992; Hodgson et al., 1994) through the hydrolysis of ester bonds in sphingomyelin in mammalian cell membranes following infection (Carne and Onon, 1978; Lipsky et al., 1982; Coyle and Lipsky, 1990; Tachedjian et al., 1995; Navas, 1996; Tambourgi et al., 2002; Dorella et al., 2006a), beyond enabling the bacteria to escape from neutrophils, and impairing neutrophil chemotaxis toward the site of infection (Yozwiak and Songer, 1993).

Several studies have been carried out involving the biological activities of *C. pseudotuberculosis* PLD, as well as its molecular structures, and results have shown similarities with sphingomyelinases present in the venom of the medically important spider genus *Loxosceles* (Bernheimer et al., 1985; Songer, 1997; Tambourgi et al., 2002; van Meeteren et al., 2004; Binford et al., 2005). Unlike diphtheria toxin, which occurs in about half of the isolates of *C. diphtheria* (Saragea et al., 1966; Toshach et al., 1977), PLD probably is characteristic of most or almost all strains of *C. pseudotuberculosis* and *C. ulcerans* and have not been found in any other Corynebacteria (Barksdale et al., 1981).

Due to the immunogenic characteristic of the PLD, it has often been used as a tool in vaccine development against CLA. The vaccines that are currently produced for control of CLA generally use formalin-inactivated PLD-rich *C. pseudotuberculosis* culture supernatants because PLD is considered to be the major protective antigen (Pizza et al., 1989; Johnson and Nicholls, 1994; Hodgson et al., 1999), or they use DNA as vaccine (De Rose, 2002). Although a commercial vaccine is already available, it is thought that conventional attenuated vaccines are still more advantageous as they offer better long-lived humoral and cytotoxic T-lymphocyte responses (Davis et al., 1996; Chaplin et al., 1999) with only a single dose in mice (Chaplin et al., 1999). Hodgson and colleagues (1994) used a strain of *C. pseudotuberculosis* with the *pld* gene deleted from the chromosome and showed that a single subcutaneous vaccination of this attenuated strain, Toxminus, protected sheep against wild-type challenge (Hodgson et al., 1992, 1994).

Pacheco and colleagues (2007) used the *pld* gene to develop a multiplex PCR assay that also included the *rpo*B and 16S rRNA genes. This methodology allowed to differentiate *C. pseudotuberculosis* from other closely related species of Corynebacteria such as *C. ulcerans* and *C. diphtheriae* (Dorella et al., 2006a), thus supplying an accurate diagnostic method for CLA directly for clinical use.

AroB and aroQ: amino acid biosynthesis

Dehydroquinases are enzymes involved in the shikimate biosynthetic pathway. Amino acid biosynthesis was described mainly in *Escherichia coli* (Kleanthous et al., 1990) from which the dehydroquinases have already been purified (Chaudhuri et al., 1986), cloned, sequenced, and overexpressed (Maskell et al., 1988). These enzymes consist of three constitutively expressed

subtypes: a monofunctional form (Berlyn and Giles, 1969; Chaudhuri et al., 1987), a bifunctional form found in plants and a multifunctional form of dehydroquinase, which is a large polypeptide (Kleanthous et al., 1990).

Kim et al. (2006) reported the presence in *Mycobacterium tuberculosis* of such enzymes which are essential for the biosynthesis of phenylalanine, tyrosine, tryptophan (Garbe et al., 1991), and other aromatic compounds in bacteria and other organisms such as fungi, algae and plants, but they are absent in mammals (Haslam, 1993; Kim et al., 2006). An advantage of the non-occurrence of these enzymes in mammals is the possibility of the development of new drugs (Cole et al., 1998; Camus et al., 2002; Kim et al., 2006).

Several pathogens have been attenuated by mutations in the aromatic amino acid biosynthetic pathway such as some *Salmonella* ssp (Hoiseth and Stocker, 1981; Levine et al., 1987; Schijns et al., 1994) and other pathogenic bacteria. Thus, Simmons and colleagues (1997) obtained *C. pseudotuberculosis* strains that lack virulence through attenuation of *aro*B and *aro*Q gene, using allelic exchange. Such strains were unable to cause CLA in murine models, suggesting its use as vaccine vectors and a potential attenuated vaccine in the control of CLA (Simmons et al., 1998).

FagA, fagB, fagC and fagD: iron uptake

Iron acquisition is one of the most important factors for bacterial survival during infection in the host environment (Brown and Holden, 2002). This mechanism has been widely described in Gram-negative bacteria and also in Gram-positive bacteria, where several papers have been published on the molecular basis of iron uptake (Heinrichs et al., 1999; Brown et al., 2001a,b).

Studies in *Staphylococcus aureus* have reported some genes and pathways involving iron uptake and the search for the presence of siderophores (Courcol et al., 1997) and systems using ABC transporters of three compounds (Heinrichs et al., 1999; Sebulsky et al., 2000; Vera-Cabrera et al., 2001; Sebulsky and Heinrichs, 2001). A common characteristic of the occurrence of genes encoding iron transporters is their location in pathogenicity islands (PAIs), where there is evidence of horizontal transfer (Brown and Holden, 2002). PAIs have also been described in Gram-positive organisms such as *C. diphtheriae* and *S. pneumoniae*, where *C. diphtheriae* possesses a gene (*IRP1*) homologous to the ABC transporter gene (*pia*) involved in iron uptake in *S. pneumoniae*, located inside a PAI (Schmitt et al., 1997).

Wennerhold and colleagues (2005) identified a protein DtxR, which is involved in the regulation of iron uptake, mainly in high GC Gram-positive genera [e.g., *Corynebacterium*, *Mycobacterium* and *Rhodococcus* (Tao et al., 1994; Rodriguez and Smith, 2003)], and modulates the expression of siderophores and other compounds in the iron acquisition pathway.

However, in *C. pseudotuberculosis*, Billington and colleagues (2002) described an operon (fagABC) with 32-47% identity to proteins involved in bacterial iron uptake systems such as ABC transporters which confer to *C. pseudotuberculosis* persistence in a goat infection model. Present in this operon are four genes: *fag*A, *fag*B, *fag*C, *fag*D, located downstream from the *pld* gene. An approach used to study such genes was the fusion of the operon to *lac*Z gene to evaluate expression. It was observed that the operon was poorly expressed in iron-rich media, and in iron-limited media, the expression of β-galactosidase activity was increased about 3-fold, suggesting that the expression of this operon appears to contribute to the virulence of bacteria.

Heat shock proteins: chaperonin immunogenicity and vaccine development

Heat shock proteins (HSPs) are one of the most highly conserved group of proteins among organisms (Qamra et al., 2005). They were initially described as being involved in thermal stress, but it is currently known that HSPs participate in other events such as nutrient depletion, hypotaxia, metabolic disruption, viral infection, transformation (Young and Elliot, 1989; Morimoto and Milarski, 1990; Young, 1990; Kultz, 2005), and other cellular processes. Chaperonin proteins are found in almost all organisms (if not all) (Braig, 1998) and are classified into six families: *Hsp*10, *Hsp*40, *Hsp*60, *Hsp*70, *Hsp*90, and *Hsp*100. However, in prokaryotes, the HSPs most characterized are *hsp*60 (*gro*EL), hsp70 (*dna*K) and *hsp*10 (*gro*ES*)* (Eom et al., 2005), and in the *Corynebacterium* genus two paralogue genes of *hsp*60 have been described (Barreiro et al., 2004).

Studies conducted by Coelho KS, Miyoshi A and Azevedo V (unpublished data) characterized and isolated for the first time the *hsp*10 and *hps*60 genes of *C. pseudotuberculosis*. It is known that the majority of HSPs are able to promote a humoral and cellular response, and therefore, they have immunogenic properties. Based on this information, the same research group tested an *hsp*60 DNA vaccine and a recombinant protein subunit vaccine in challenges in mice and obtained a significant production of specific IgG.

RecA and rpoB of *Corynebacterium pseudotuberculosis*: diagnostics and phylogeny

The protein RecA is present in eubacteria in general and is a highly conserved protein among bacterial organisms (Roca and Cox, 1990; Clark and Sandler, 1994; Kowalczykowski et al., 1994). It participates in homologous recombination, DNA repair, and the SOS response. Specifically, it binds stretches of single-stranded DNA and unwinds duplex DNA (Karlim et al., 1995). In this context, Pogson et al. (1996) generated isogenic mutants of *C. pseudotuberculosis* where the *rec*A gene was mutated. As a result, a 10- to 12-fold reduction in recombination was obtained in the mutants compared with the parental strain, suggesting its use as a vaccine vector.

The *rpo*B gene is responsible for encoding the β-subunit of DNA-dependent RNA polymerase (Severinov et al., 1996; Ko et al., 2002); however, there are several lines of evidence suggesting its relation with rifampin resistance (Kim et al., 1999). Recently, *rpoB* sequences were used as an alternative tool for determining the phylogeny of some enteric bacteria (Mollet et al., 1997), *Borrelia* (Lee et al., 2000; Renesto et al., 2000), *Mycobacterium* (Kim et al., 1999), and *Bartonella* (Renesto et al., 2001). Dorella et al. (2006a) used the *rpo*B and 16S rRNA genes to build a phylogenetic tree of the *Corynebacterium* genus. This study showed that *C. pseudotuberculosis* is more closely related to *C. ulcerans* than to *C. diphtheriae*, suggesting that *C. diphtheriae* diverged from both at some time.

Novel protein of *Corynebacterium pseudotuberculosis* - CP40: other secreted toxin?

A novel gene of *C. pseudotuberculosis* discovered by Wilson et al. (1995), CP40, encodes a protein with a molecular weight of 40 kDa and length of about 351 amino acids. Preliminary characterization of the CP40 protein revealed that it is probably a serine protease, although it did not have any homology with motifs of other serine proteases in database searched. Perhaps this absence of homology has occurred because CP40 is intrinsic to *C. pseudotuberculosis*.

This protein showed biochemical similarities with the protein PLD. Both are hydrophobic (von Heijine, 1983, 1985) and have a leader sequence containing an Ala-x-Ala cleavage motif at the C terminus. This protein was found in *C. pseudotuberculosis* culture supernatant, indicating that it is probably secreted (Walker et al., 1994).

Approach of the gene content of *Corynebacterium pseudotuberculosis*: perspectives

Currently, there are great advances in genomics, mainly in the optimization of sequencing techniques, which means that sequencing has become less expensive and more accurate. A massive deposit of sequences such as genomic sequence survey in public databases has facilitated searches for homology and prediction of novel genes. Through these advances in the genomic field, we aim at using such tool for the discovery of new genes, new diagnostics and more efficient vaccines for CLA control. Moreover, genomes of several *Corynebacteria* ssp are widely available, where this information can be used in comparative genomics to help gain better insight into gene content and genomic organization of *C. pseudotuberculosis*.

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REFERENCES

Barksdale L, Linder R, Sulea IT and Pollice M (1981). Phospholipase D activity of *Corynebacterium pseudotuberculosis* (*Corynebacterium ovis*) and *Corynebacterium ulcerans*, a distinctive marker within the genus *Corynebacterium*. *J. Clin. Microbiol.* 13: 335-343.

Barreiro C, Gonzalez-Lavado E, Patek M and Martin JF (2004). Transcriptional analysis of the gro*ES*-gro*EL1*, gro*EL2*, and dnaK genes in *Corynebacterium glutamicum*: characterization of heat shock-induced promoters. *J. Bacteriol.* 186: 4813-4817.

Bayan N, Houssin C, Chami M and Leblon G (2003). Mycomembrane and S-layer: two important structures of *Corynebacterium glutamicum* cell envelope with promising biotechnology applications. *J. Biotechnol.* 104: 55-67.

Berlyn MB and Giles NH (1969). Organization of enzymes in the polyaromatic synthetic pathway: separability in bacteria. *J. Bacteriol.* 99: 222-230.

Bernheimer AW, Campbell BJ and Forrester LJ (1985). Comparative toxinology of *Loxosceles reclusa* and *Corynebacterium pseudotuberculosis*. *Science* 228: 590-591.

Billington SJ, Esmay PA, Songer JG and Jost BH (2002). Identification and role in virulence of putative iron acquisition genes from *Corynebacterium pseudotuberculosis*. *FEMS Microbiol. Lett.* 208: 41-45.

Binford GJ, Cordes MH and Wells MA (2005). Sphingomyelinase D from venoms of *Loxosceles* spiders: evolutionary insights from cDNA sequences and gene structure. *Toxicon* 45: 547-560.

Braig K (1998). Chaperonins. *Curr. Opin. Struct. Biol.* 8: 159-165.

Brown JS and Holden DW (2002). Iron acquisition by Gram-positive bacterial pathogens. *Microbes Infect.* 4: 1149-1156.

Brown JS, Gilliland SM and Holden DW (2001a). A *Streptococcus pneumoniae* pathogenicity island encoding an ABC transporter involved in iron uptake and virulence. *Mol. Microbiol.* 40: 572-585.

Brown JS, Ogunniyi AD, Woodrow MC, Holden DW, et al. (2001b). Immunization with components of two iron uptake ABC transporters protects mice against systemic *Streptococcus pneumoniae* infection. *Infect. Immun.* 69: 6702-6706.

Burrel DH (1983). Caseous lymphadenitis vaccine. *N. S. W. Vet. Proc.* 19: 53-57.

Camus JC, Pryor MJ, Medigue C and Cole ST (2002). Re-annotation of the genome sequence of *Mycobacterium tuberculosis* H37Rv. *Microbiology* 148: 2967-2973.

Cardenas L and Clements JD (1992). Oral immunization using live attenuated *Salmonella* spp. as carriers of foreign antigens. *Clin. Microbiol. Rev.* 5: 328-342.

Carne HR and Onon EO (1978). Action of *Corynebacterium ovis* exotoxin on endothelial cells of blood vessels. *Nature* 271: 246-248.

Chaplin PJ, De Rose R, Boyle JS, McWaters P, et al. (1999). Targeting improves the efficacy of a DNA vaccine against *Corynebacterium pseudotuberculosis* in sheep. *Infect. Immun.* 67: 6434-6438.

Chaudhuri S, Lambert JM, McColl LA and Coggins JR (1986). Purification and characterization of 3-dehydroquinase from *Escherichia coli*. *Biochem. J.* 239: 699-704.

Chaudhuri S, Duncan K and Coggins JR (1987). 3-Dehydroquinate dehydratase from *Escherichia coli*. *Methods Enzymol.* 142: 320-324.

Clark AJ and Sandler SJ (1994). Homologous genetic recombination: the pieces begin to fall into place. *Crit. Rev. Microbiol.* 20: 125-142.

Cole ST, Brosch R, Parkhill J, Garnier T, et al. (1998). Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 393: 537-544.

Collins MD, Goodfellow M and Minnikin DE (1982). Fatty acid composition of some mycolic acid-containing coryneform bacteria. *J. Gen. Microbiol.* 128: 2503-2509.

Collins MD, Falsen E, Akervall E, Sjoden B, et al. (1998). *Corynebacterium kroppenstedtii* sp. nov., a novel *Corynebacterium* that does not contain mycolic acids. *Int. J. Syst. Bacteriol.* 48 (Pt 4): 1449-1454.

Connor KM, Quirie MM, Baird G and Donachie W (2000). Characterization of United Kingdom isolates of *Corynebacterium pseudotuberculosis* using pulsed-field gel electrophoresis. *J. Clin. Microbiol.* 38: 2633-2637.

Courcol RJ, Trivier D, Bissinger MC, Martin GR, et al. (1997). Siderophore production by *Staphylococcus aureus* and identification of iron-regulated proteins. *Infect. Immun.* 65: 1944-1948.

Coyle MB and Lipsky BA (1990). Coryneform bacteria in infectious diseases: clinical and laboratory aspects. *Clin. Microbiol. Rev.* 3: 227-246.

Cuevas WA and Songer JG (1993). *Arcanobacterium haemolyticum* phospholipase D is genetically and functionally similar to *Corynebacterium pseudotuberculosis* phospholipase D. *Infect. Immun.* 61: 4310-4316.

Davis HL, Mancini M, Michel ML and Whalen RG (1996). DNA-mediated immunization to hepatitis B surface antigen: longevity of primary response and effect of boost. *Vaccine* 14: 910-915.

De Rose R, Tennent J, McWaters P, Chaplin PJ, et al. (2002). Efficacy of DNA vaccination by different routes of immunisation in sheep. *Vet. Immunol. Immunopathol.* 90: 55-63.

Dorella FA, Pacheco LG, Oliveira SC, Miyoshi A, et al. (2006a). *Corynebacterium pseudotuberculosis*: microbiology, biochemical properties, pathogenesis and molecular studies of virulence. *Vet. Res.* 37: 201-218.

Dorella FA, Fachin MS, Billault A, Dias NE, et al. (2006b). Construction and partial characterization of a *Corynebacterium pseudotuberculosis* bacterial artificial chromosome library through genomic survey sequencing. *Genet. Mol. Res.* 5: 653-663.

Egen NB, Cuevas WA, McNamara PJ, Sammons DW, et al. (1989). Purification of the phospholipase D of *Corynebacterium pseudotuberculosis* by recycling isoelectric focusing. *Am. J. Vet. Res.* 50: 1319-1322.

Eom CY, Kim E, Ro YT, Kim SW, et al. (2005). Cloning and molecular characterization of GroESL heat-shock operon in methylotrophic bacterium *Methylovorus* sp. strain SS1 DSM 11726. *J. Biochem. Mol. Biol.* 38: 695-702.

Funke G, Lawson PA and Collins MD (1995). Heterogeneity within human-derived centers for disease control and prevention (CDC) coryneform group ANF-1-like bacteria and description of *Corynebacterium auris* sp. nov. *Int. J. Syst. Bacteriol.* 45: 735-739.

Garbe T, Servos S, Hawkins A, Dimitriadis G, et al. (1991). The *Mycobacterium tuberculosis* shikimate pathway genes: evolutionary relationship between biosynthetic and catabolic 3-dehydroquinases. *Mol. Gen. Genet.* 228: 385-392.

Garg DN, Nain SPS and Chandiramani NK (1985). Isolation and characterisation of *Corynebacterium ovis* from sheep and goats. *Indian Vet. J.* 62: 805-808.

Goodfellow M (1989). Suprageneric classification of actinomycetes. In: Bergey's manual of systematic bacteriology (Williams ST, Sharpe ME and Holt JG, eds.). Williams and Wilkins, Baltimore, 2333-2339.

Hall V, Collins MD, Hutson RA, Lawson PA, et al. (2003). *Corynebacterium atypicum* sp. nov., from a human clinical source, does not contain corynomycolic acids. *Int. J. Syst. Evol. Microbiol.* 53: 1065-1068.

Hard GC (1969). Electron microscopic examination of *Corynebacterium ovis*. *J. Bacteriol.* 97: 1480-1485.

Hard GC (1975). Comparative toxic effect of the surface lipid of *Corynebacterium ovis* on peritoneal macrophages. *Infect. Immun.* 12: 1439-1449.

Haslam E (1993). Shikimic acid: metabolism and metabolites. John Wiley and Sons Ltd., Chichester.

Heinrichs JH, Gatlin LE, Kunsch C, Choi GH, et al. (1999). Identification and characterization of SirA, an iron-regulated protein from *Staphylococcus aureus*. *J. Bacteriol.* 181: 1436-1443.

Hodgson AL, Bird P and Nisbet IT (1990). Cloning, nucleotide sequence, and expression in *Escherichia coli* of the phospholipase D gene from *Corynebacterium pseudotuberculosis*. *J. Bacteriol.* 172: 1256-1261.

Hodgson AL, Krywult J, Corner LA, Rothel JS, et al. (1992). Rational attenuation of *Corynebacterium pseudotuberculosis*: potential cheesy gland vaccine and live delivery vehicle. *Infect. Immun.* 60: 2900-2905.

Hodgson AL, Tachedjian M, Corner LA and Radford AJ (1994). Protection of sheep against caseous lymphadenitis by use of a single oral dose of live recombinant *Corynebacterium pseudotuberculosis*. *Infect. Immun.* 62: 5275-5280.

Hodgson AL, Carter K, Tachedjian M, Krywult J, et al. (1999). Efficacy of an ovine caseous lymphadenitis vaccine formulated using a genetically inactive form of the *Corynebacterium pseudotuberculosis* phospholipase D. *Vaccine* 17: 802-808.

Hoiseth SK and Stocker BA (1981). Aromatic-dependent *Salmonella typhimurium* are non-virulent and effective as live vaccines. *Nature* 291: 238-239.

Johnson VG and Nicholls PJ (1994). Identification of a single amino acid substitution in the diphtheria toxin A chain of CRM 228 responsible for the loss of enzymatic activity. *J. Bacteriol.* 176: 4766-4769.

Karlin S, Weinstock GM and Brendel V (1995). Bacterial classifications derived from recA protein sequence comparisons. *J. Bacteriol.* 177: 6881-6893.

Khamis A, Raoult D and La Scola B (2004). *rpo*B gene sequencing for identification of *Corynebacterium* species. *J. Clin. Microbiol.* 42: 3925-3931.

Kim BJ, Lee SH, Lyu MA, Kim SJ, et al. (1999). Identification of mycobacterial species by comparative sequence analysis of the RNA polymerase gene (rpoB). *J. Clin. Microbiol.* 37: 1714-1720.

Kim SK, Reddy SK, Nelson BC, Vasquez GB, et al. (2006). Biochemical and structural characterization of the secreted chorismate mutase (Rv1885c) from *Mycobacterium tuberculosis* H37Rv: an *AroQ enzyme not regulated by the aromatic amino acids. *J. Bacteriol.* 188: 8638-8648.

Kleanthous C, Campbell DG and Coggins JR (1990). Active site labeling of the shikimate pathway enzyme, dehydroquinase. Evidence for a common substrate binding site within dehydroquinase and dehydroquinate synthase. *J. Biol. Chem.* 265: 10929-10934.

Ko KS, Lee HK, Park MY, Park MS, et al. (2002). Population genetic structure of *Legionella pneumophila* inferred from RNA polymerase gene (*rpo*B) and DotA gene (*dot*A) sequences. *J. Bacteriol.* 184: 2123-2130.

Kowalczykowski SC, Dixon DA, Eggleston AK, Lauder SD, et al. (1994). Biochemistry of homologous recombination in *Escherichia coli*. *Microbiol. Rev.* 58: 401-465.

Kultz D (2005). Molecular and evolutionary basis of the cellular stress response. *Annu. Rev. Physiol* 67: 225-257.

Lee SH, Kim BJ, Kim JH, Park KH, et al. (2000). Differentiation of *Borrelia burgdorferi* sensu lato on the basis of RNA polymerase gene (rpoB) sequences. *J. Clin. Microbiol.* 38: 2557-2562.

Levine MM, Herrington D, Murphy JR, Morris JG, et al. (1987). Safety, infectivity, immunogenicity, and *in vivo* stability of two attenuated auxotrophic mutant strains of *Salmonella typhi*, 541Ty and 543Ty, as live oral vaccines in humans. *J. Clin. Invest.* 79: 888-902.

Lipsky BA, Goldberger AC, Tompkins LS and Plorde JJ (1982). Infections caused by nondiphtheria corynebacteria. *Rev. Infect. Dis.* 4: 1220-1235.

Maskell DJ, Morrissey P and Dougan G (1988). Cloning and nucleotide sequence of the aroA gene of *Bordetella pertussis*. *J. Bacteriol.* 170: 2467-2471.

McNamara PJ, Bradley GA and Songer JG (1994). Targeted mutagenesis of the phospholipase D gene results in decreased virulence of *Corynebacterium pseudotuberculosis*. *Mol. Microbiol.* 12: 921-930.

Mollet C, Drancourt M and Raoult D (1997). rpoB sequence analysis as a novel basis for bacterial identification. *Mol. Microbiol.* 26: 1005-1011.

Morimoto RI and Milarski KL (1990). Expression and function of vertebrate Hsp70 genes. In: Stress proteins in biology and medicine (Morimoto RI, Tissieres A and Georgopoulos C, eds.). Cold Spring Harbor Laboratory, Cold Spring Harbor, 323-359.

Navas J (1996). Genetic tools in pathogenic nocardioform actinomycetes. *Microbiologia* 12: 297-304.

Pacheco LG, Pena RR, Castro TL, Dorella FA, et al. (2007). Multiplex PCR assay for identification of *Corynebacterium pseudotuberculosis* from pure cultures and for rapid detection of this pathogen in clinical samples. *J. Med. Microbiol.* 56: 480-486.

Paton MW, Walker SB, Rose IR and Watt GF (2003). Prevalence of caseous lymphadenitis and usage of caseous lymphadenitis vaccines in sheep flocks. *Aust. Vet. J.* 81: 91-95.

Paule BJ, Meyer R, Moura-Costa LF, Bahia RC, et al. (2004). Three-phase partitioning as an efficient method for extraction/concentration of immunoreactive excreted-secreted proteins of *Corynebacterium pseudotuberculosis*. *Protein Expr. Purif.* 34: 311-316.

Pizza M, Covacci A, Bartoloni A, Perugini M, et al. (1989). Mutants of *pertussis* toxin suitable for vaccine development. *Science* 246: 497-500.

Pogson CA, Simmons CP, Strugnell RA and Hodgson AL (1996). Cloning and manipulation of the *Corynebacterium pseudotuberculosis* recA gene for live vaccine vector development. *FEMS Microbiol. Lett.* 142: 139-145.

Qamra R, Mande SC, Coates AR and Henderson B (2005). The unusual chaperonins of *Mycobacterium tuberculosis*. *Tuberculosis* 85: 385-394.

Renesto P, Lorvellec-Guillon K, Drancourt M and Raoult D (2000). *rpoB* gene analysis as a novel strategy for identification of spirochetes from the genera *Borrelia*, *Treponema*, and *Leptospira*. *J. Clin. Microbiol.* 38: 2200-2203.

Renesto P, Gouvernet J, Drancourt M, Roux V, et al. (2001). Use of *rpoB* gene analysis for detection and identification of *Bartonella* species. *J. Clin. Microbiol.* 39: 430-437.

Roca AI and Cox MM (1990). The RecA protein: structure and function. *Crit. Rev. Biochem. Mol. Biol.* 25: 415-456.

Rodriguez GM and Smith I (2003). Mechanisms of iron regulation in mycobacteria: role in physiology and virulence. *Mol. Microbiol.* 47: 1485-1494.

Saragea A, Maximescu P, Meitert E, Stuparu I, et al. (1966). Incidence and geographical distribution of phage types of *Corynebacterium diphtheriae* in the dynamics of the epidemic process of diphtheria in the Rumanian Socialist Republic. *Microbiol. Parazitol. Epidemiol.* 11: 351-362.

Schijns VE, Haagmans BL, Rijke EO, Huang S, et al. (1994). IFN-gamma receptor-deficient mice generate antiviral Th1-characteristic cytokine profiles but altered antibody responses. *J. Immunol.* 153: 2029-2037.

Schmitt MP, Talley BG and Holmes RK (1997). Characterization of lipoprotein IRP1 from *Corynebacterium diphtheriae*, which is regulated by the diphtheria toxin repressor (DtxR) and iron. *Infect. Immun.* 65: 5364-5367.

Sebulsky MT and Heinrichs DE (2001). Identification and characterization of *fhuD1* and *fhuD2*, two genes involved in iron-hydroxamate uptake in *Staphylococcus aureus*. *J. Bacteriol.* 183: 4994-5000.

Sebulsky MT, Hohnstein D, Hunter MD and Heinrichs DE (2000). Identification and characterization of a membrane permease involved in iron-hydroxamate transport in *Staphylococcus aureus*. *J. Bacteriol.* 182: 4394-4400.

Severinov K, Mustaev A, Kukarin A, Muzzin O, et al. (1996). Structural modules of the large subunits of RNA polymerase. Introducing archaebacterial and chloroplast split sites in the beta and beta' subunits of *Escherichia coli* RNA polymerase. *J. Biol. Chem.* 271: 27969-27974.

Simmons CP, Hodgson AL and Strugnell RA (1997). Attenuation and vaccine potential of *aroQ* mutants of *Corynebacterium pseudotuberculosis*. *Infect. Immun.* 65: 3048-3056.

Simmons CP, Dunstan SJ, Tachedjian M, Krywult J, et al. (1998). Vaccine potential of attenuated mutants of *Corynebacterium pseudotuberculosis* in sheep. *Infect. Immun.* 66: 474-479.

Songer JG (1997). Bacterial phospholipases and their role in virulence. *Trends Microbiol.* 5: 156-161.

Songer JG, Beckenbach K, Marshall MM, Olson GB, et al. (1988). Biochemical and genetic characterization of *Corynebacterium pseudotuberculosis*. *Am. J. Vet. Res.* 49: 223-226.

Tachedjian M, Krywult J, Moore RJ and Hodgson AL (1995). Caseous lymphadenitis vaccine development: site-specific inactivation of the *Corynebacterium pseudotuberculosis* phospholipase D gene. *Vaccine* 13: 1785-1792.

Tambourgi DV, De Sousa Da SM, Billington SJ, Goncalves De Andrade RM, et al. (2002). Mechanism of induction of complement susceptibility of erythrocytes by spider and bacterial sphingomyelinases. *Immunology* 107: 93-101.

Tao X, Schiering N, Zeng HY, Ringe D, et al. (1994). Iron, DtxR, and the regulation of diphtheria toxin expression. *Mol. Microbiol.* 14: 191-197.

Toshach S, Valentine A and Sigurdson S (1977). Bacteriophage typing of *Corynebacterium diphtheriae*. *J. Infect. Dis.* 136: 655-660.

van Meeteren LA, Frederiks F, Giepmans BN, Pedrosa MF, et al. (2004). Spider and bacterial sphingomyelinases D target cellular lysophosphatidic acid receptors by hydrolyzing lysophosphatidylcholine. *J. Biol. Chem.* 279: 10833-10836.

Vera-Cabrera L, Hernandez-Vera MA, Welsh O, Johnson WM, et al. (2001). Phospholipase region of *Mycobacterium tuberculosis* is a preferential locus for IS6110 transposition. *J. Clin. Microbiol.* 39: 3499-3504.

von Heijine G (1983). Patterns of amino acids near signal-sequence cleavage sites. *Eur. J. Biochem.* 133: 17-21.

von Heijine G (1985). Signal sequences. The limits of variation. *J. Mol. Biol.* 184: 99-105.

Walker J, Jackson HJ, Eggleton DG, Meeusen EN, et al. (1994). Identification of a novel antigen from *Corynebacterium pseudotuberculosis* that protects sheep against caseous lymphadenitis. *Infect. Immun.* 62: 2562-2567.

Wennerhold J, Krug A and Bott M (2005). The AraC-type regulator RipA represses aconitase and other iron proteins from *Corynebacterium* under iron limitation and is itself repressed by DtxR. *J. Biol. Chem.* 280: 40500-40508.

Williamson LH (2001). Caseous lymphadenitis in small ruminants. *Vet. Clin. North Am. Food Anim. Pract.* 17: 359-71, vii.

Wilson MJ, Brandon MR and Walker J (1995). Molecular and biochemical characterization of a protective 40-kilodalton antigen from *Corynebacterium pseudotuberculosis*. *Infect. Immun.* 63: 206-211.

Young RA (1990). Stress proteins and immunology. *Annu. Rev. Immunol.* 8: 401-420.

Young RA and Elliott TJ (1989). Stress proteins, infection, and immune surveillance. *Cell* 59: 5-8.

Yozwiak ML and Songer JG (1993). Effect of *Corynebacterium pseudotuberculosis* phospholipase D on viability and chemotactic responses of ovine neutrophils. *Am. J. Vet. Res.* 54: 392-397.