The *Listeria monocytogenes* Sortase-B Recognizes Varied Amino Acids at Position 2 of the Sorting Motif*□

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Sortases are bacterial enzymes that anchor surface proteins covalently to the peptidoglycan upon cleavage of a motif located at their C-terminal end. Motifs recognized by sortases of the class-B (SrtB) are defined by the consensus sequence NP(Q/K)(T/S)(N/G/S)(D/A). Evidence supporting this consensus is limited to IsdC of *Staphylococcus aureus* and *Bacillus anthracis*, cleaved at motifs NPQTN and NPKTG, respectively. In *Listeria monocytogenes*, SrtB has two substrates, Lmo2185 and Lmo2186, containing NAKTN and NKVTN (or the overlapping sequence NPKSS) as putative sorting motifs. Some of these motifs do not match the consensus, because they lack either proline (P) at position 2 or glutamine/lysine (Q/K) at position 3. Here, we identified NPKSS as a sorting motif of Lmo2186 by monitoring anchoring to peptidoglycan of chimeras lacking each of its two predicted motifs. Motif-swapping experiments confirmed that NPKSS, but not NKVTN, could replace NAKTN for anchoring of an Lmo2185 chimera. Residue substitutions in the NPKSS sequence revealed the essentiality of proline at position 2 for recognition of this particular motif. Lysine at position 3 was however dispensable. Deletion of NAKTN, on the other hand, abrogated SrtB-mediated anchoring of the Lmo2185 chimera. NAKTN, therefore, represents an exception to the rule of a conserved proline in position 2 of the sorting motif. Taken together, our data indicate that proline is not absolutely required for substrate recognition by sortases of the class-B. In addition, they prove the capacity of a single sortase, as SrtB of *L. monocytogenes*, to recognize varied amino acids at position 2 of the sorting motif.

The cell wall of Gram-positive bacteria is formed by a thick layer of peptidoglycan decorated with anionic polymers such as teichoic and lipoteichoic acids, and a myriad of surface proteins (1–3). Surface proteins can be partially embedded in the membrane via lipid modification in their N termini or hydrophobic C-terminal anchoring domains. Noncovalent association of certain surface proteins with outermost cell wall components such as teichoic acid, lipoteichoic acid, and peptidoglycan is also known (1, 3). Another type of association is the covalent attachment of the surface protein to the peptidoglycan, exemplified by protein A of *Staphylococcus aureus* (2, 4, 5).

Sortases are enzymes conserved in Gram-positive bacteria that promote covalent anchoring of surface proteins to the peptidoglycan (2). Sortases anchor to the peptidoglycan individual proteins and pili. In this latter case, a specialized pili-dedicated sortase together with the housekeeping sortase are involved in the anchoring of polymerized pilin (6). In all cases known, sortases catalyze a transpeptidation reaction that occurs upon cleavage at a specific motif present in the C-terminal sorting signal of the protein substrate (2). The sorting signal is composed of this motif followed by a hydrophobic domain and a positively charged tail (7, 8).

Recent genomic analyses have unveiled the existence of sortase homologs, all sharing a conserved TLXTGC motif and a histidine (H) involved in catalysis (9, 10). These sortases are classified in five classes or subfamilies, according to their homology in primary sequence, tridimensional structure, clustering with genes encoding protein substrates, location of the membrane anchor domain of the sortase, and sorting motifs predicted to be recognized by the enzyme (2, 9, 10). Thus, sortases (Srt)* of the class A (SrtA, subfamily 1) recognize motifs with the consensus sequence LPXG and are encoded by genes often nonclustered with those encoding their substrates (2, 10). Proteomic analysis in srtA mutants of diverse Gram-positive bacteria, as well as *in vivo* assays with purified SrtA enzyme and peptides covering the predicted motifs, have unequivocally demonstrated the recognition of the LPXTG motif by SrtA and the cleavage of this motif between the threonine (T) and glycine (G) residues (11–16). Sortases of the class B (SrtB, subfamily 2) are generally expressed in operons containing genes encoding their substrates and recognize motifs with the consensus sequence NP(Q/K)(T/S)(N/G/S)(D/A) (9, 10). Noteworthy, the sorting motif recognized by SrtB has been experimentally tested only with the surface protein IsdC involved in iron acquisition. IsdC of *S. aureus* and *Bacillus anthracis* are recognized by SrtB at NPQTN and NPKTG motifs, respectively (17, 18). Bioinformatic analyses performed on available bacterial genome sequences have predicted different sorting motifs for sortases of other subfamilies and new putative sortase substrates (9, 10, 19, 20).

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§ The on-line version of this article (available at http://www.jbc.org) contains supplemental Table S1.

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2 The abbreviations used are: Srt, sortase; Inl, internalin.
for LPXTG, NPQTN, and NPKTG motifs, experimental data supporting the recognition of other sorting motifs are lacking. 

*L. monocytogenes* is a bacterial pathogen that contains two sortases, SrtA and SrtB (12, 21). *L. monocytogenes*, as well as other species of the genus *Listeria*, are among the Gram-positive bacteria containing the highest number of surface proteins, in the range of 40–45, with predicted covalent association to peptidoglycan (1, 20, 22, 23). Proteomic analyses of cell wall material isolated from *L. monocytogenes* srtA and srtB mutants revealed that SrtA anchors proteins bearing LPXTG motifs, while two surface proteins, Lmo2185 and Lmo2186, are anchored to the peptidoglycan in a StrB-dependent manner (12, 24). Both Lmo2185 and Lmo2186 genes are clustered in the same operon containing srtB (21). This feature is also observed in *S. aureus* for srtB and isdC (17).

Like IsdC of *S. aureus* and *B. anthracis*, Lmo2185 and Lmo2186 contain NEAT domains, implicated in iron metabolism, and are encoded by genes forming part of a large operon that encodes components of a putative iron transporter (18, 23). This *L. monocytogenes* operon is induced under iron deprivation (25), although no evidence for a role of Lmo2185 or Lmo2186 in withstanding low iron stress has been found. Another aspect undefined is the exact motif recognized by *L. monocytogenes* SrtB in each of these two surface proteins. We have previously shown that SrtB anchors to the peptidoglycan chimeras consisting of an N-fragment from Internalin-B (lacking the GW domains that promote attachment to the cell envelope (26)) fused in its C-end to the putative sorting signals of Lmo2185 or Lmo2186 (24). These sorting signals of Lmo2185 and Lmo2186 initiate with the NAKTN and NKVTNPKSS sequences, respectively. Given that the Lmo2186 sequence used in this work contained two putative SrtB motifs, NKVTN or NPKSS, our study did not formally identify the motif recognized by StrB in this surface protein. Of interest, neither the putative NAKTN motif of Lmo2185 nor NKVTN of Lmo2186 contain the invariant proline (P) at position 2 of the consensus NP(Q/K)(T/S)(N/G/S)(D/A) (9). Noteworthy, proteomic analyses reported the presence of a Lmo2186 peptide with the sequence SDSSNKVTNPK in peptidoglycan material of *L. monocytogenes* containing strongly associated mature proteins (24). This finding provided an indirect evidence for NPKSS as the motif recognized and cleaved by SrtB.

Considering that the predicted motif of Lmo2185 lacks proline (P) at position 2 (NAKTN) and that two putative motifs can be assigned in Lmo2816, we were interested in defining the identity of the motifs recognized by SrtB of *L. monocytogenes*. Here, we provide the identity of these motifs and uncover the capacity of this sortase for naturally recognizing motifs with varied amino acid at position 2 of the sorting motif.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions—**The *L. monocytogenes* strains used in this study are isogenic derivatives of the wild-type virulent strain EGD-e (serotype 1/2a), with the complete genome sequence known (22). These strains are listed in Table 1. Bacteria were routinely grown in Brain-Heart-Infusion (BHI) medium (Difco) at 37 °C under shaking conditions (175 rpm). Erythromycin (5 µg/ml) was added to the medium to grow bacteria carrying chimera-expressing plasmids (see below).

**Construction of Plasmids Expressing Variants of the InlB-Cter-Lmo2185 and InlB-Cter-Lmo2186 Chimeras—**Plasmids expressing the InlB-Cter-Lmo2185 and InlB-Cter-Lmo2186 protein fusions have been previously described (24). Both fusions contain amino acids 1–398 of InlB (lacking GW domains) followed by residues 536–569 of Lmo2185 (InlB-Cter-Lmo2185) or residues 170–207 of Lmo2186 (InlB-Cter-Lmo2186). These plasmids were used as template to generate variants in which putative motifs were deleted or mutated at specific sites. Table 2 lists the complete set of plasmids constructed for this work. These variants were generated by amplification of two regions encompassing the InlB and C-terminal sorting signal sequences, respectively, followed by overlapping PCR and cloning of the resulting product in pP1 plasmid (27) using the Sacl and Sphl restriction sites. The ExSite PCR-based site-directed mutagenesis kit (Stratagene) was used in the case of the plasmid pJM021, expressing a InlB-Cter-Lmo2186-variant bearing only the putative motif NKVTN (Table 2). The modification(s)
introduced in every variant were verified by sequencing. Oligonucleotides used are listed in supplemental Table S1.

**Bacterial Fractionation**—The method described by Jonquieres et al. (28) was used to prepare cell wall extracts. Briefly, bacteria grown overnight in BHI medium (4°C) and containing in 1 ml of culture were collected by centrifugation (15,000 × g, 10 min, 4°C), washed once in phosphate-buffered saline, and once in TS buffer (10 mM Tris-HCl pH 6.9, 10 mM MgCl₂, 0.5 M sucrose). The pellets were resuspended in lysis buffer (TS buffer containing 60 μg/ml mutanolysin (Sigma, catalog no. M9901), 250 μg/ml RNase, and complete EDTA-free protease inhibitor mixture (Roche Applied Science, catalog no. 1873580)) and incubated with slow-rotating agitation at 37°C for 1 h. Protoplast formation was monitored using optical microscopy and plating. Protoplasts were recovered by centrifugation at 15,000 × g, 10 min, 4°C, and resuspended in phosphate-buffered saline. The supernatants (cell wall fractions) were filtered and precipitated on ice in 16% trichloroacetic acid for 45 min. Proteins present in the cell wall fraction were recovered by centrifugation at 21,000 × g, 10 min, 4°C, washed in cold-acetone and resuspended in phosphate-buffered saline. An appropriate volume of Laemmli sample buffer was added to both protoplast and cell wall fractions. The extracellular medium fraction was prepared from the supernatant collected after the centrifugation of the bacterial culture, which was passed through 0.22-μm pore size Millipore filters. Proteins were precipitated using a 10% trichloroacetic acid and acetone washing procedure as described (21). Unless otherwise indicated, the amount of each fraction loaded into the gels corresponded to a 6:4:1 ratio in the number of bacteria for the cell wall:extracellular medium:protoplast fractions, respectively. Proteins were resolved by Tris-glycine polyacrylamide electrophoresis, using 8% gels. The experiments were repeated a minimum of three times.

**Immunoblot Analyses**—The different chimeras were detected with mouse monoclonal anti-InlB antibody (29). Mouse monoclonal anti-InLA (30) was also used as control proteins for the cell wall fractions. Goat anti-mouse or anti-rabbit conjugated to horseradish peroxidase (Bio-Rad) was used as secondary antibodies. Proteins recognized by the antibodies were visualized by chemoluminescence using the luciferin-luminal reagents. In the case of requiring more sensitivity, the ECL Western blotting detection system was used (Amersham Biosciences, catalog: RPN2209). In some cases, the relative amount of protein anchored to the peptidoglycan was assessed by quantification of specific bands corresponding to the chimeras obtained in cell wall versus protoplast fractions using the Quantity One 1-D Software, v. 4.6.5, from Bio-Rad.

**RESULTS**

**NPKSS Is a Motif Recognized by Sortase-B (SrtB) of L. monocytogenes in Lmo2186**—The surface protein Lmo2186 (207 amino acids) contains a C-terminal sorting region predicted to initiate with the motif NKVTNPKSS, followed by a stretch of 22 hydrophobic amino acids and a positively charged tail (Fig. 1). We have previously shown that an InlB-CterLmo2186 chimera containing this sorting signal is anchored to the peptidoglycan in a StrB-dependent manner (24). However, the exact identity of the motif was not investigated. To determine this, new InlB-Cter-Lmo2186 chimeras were constructed bearing either NKVTN or NPKSS as the only motif (Fig. 2A). These variants were expressed in wild-type (EGD-e) and isogenic ΔsrtA or ΔsrtB bacteria, which were grown to stationary phase in BHI nutrient medium. Upon mutanolysin digestion of peptidoglycan, bacteria were fractionated into cell wall extracts and protoplasts containing cytosol and membrane material. Proteins secreted to the growth medium were also examined as an additional fraction. InlB-CterLmo2186 chimeras containing as motifs NKVTNPKSS, NKTVN, or NPKSS were examined in these three fractions. Control assays with antibodies detecting Internalin-A (InLA), a SrtA substrate, verified the purity of the peptidoglycan fractions (Fig. 2B). Our results showed that the variants containing either NKVTNPKSS or NPKSS were efficiently anchored to the peptidoglycan (Fig. 2B). The NPKSS variant was however detected in the peptidoglycan at slightly lesser amounts than the one containing the entire NKVTNPKSS sequence (Fig. 2B). The presence of these two chimeras in the peptidoglycan was shown to require a functional SrtB and to be independent of SrtA (Fig. 2C). Interestingly, the variant bearing only the NKTVN sequence was not anchored to the peptidoglycan in any of the three genetic backgrounds tested (Fig. 2C). This variant was detected in large amounts in the growth medium (data not shown). Because NPKSS shares amino acids at positions 1 and 2 with the previously validated motifs NPQTN and NPKTG, our data support a prominent role of these two positions for substrate recognition by SrtB sortases.

FIGURE 1. Sorting signals of peptidoglycan-anchored proteins in which experimental evidence has been demonstrated to be recognized by sortases of the class B (SrtB)/IsdC of *S. aureus*; IsdC of *B. anthracis*; and, *Lmo2186* and Lmo2186 of *L. monocytogenes*. Indicated are the different regions of the protein substrate, including the N-terminal signal peptide and the three regions that compose the C-terminal sorting signal: the sorting motif, followed by the hydrophobic retention domain, and the positively charged tail. Highlighted are the sorting motifs predicted to be recognized by SrtB in each substrate protein. In the case of Lmo2186, the two putative motifs are overlapped (NKVTNPKSS). Asterisks denote residues in each sorting motif that are conserved with NPQTN, the first SrtB motif demonstrated experimentally. The length of the hydrophobic domain is also indicated for each substrate.
NPKSS, but Not NKVTN, Can Mediate SrtB-dependent Anchoring of an InlB-Cter-Lmo2185 Fusion—To unequivocally demonstrate the identity of the sorting motif recognized by SrtB in Lmo2186, we generated a new series of InlB-Cter-Lmo2185 chimeras in which the endogenous NAKTN motif was exchanged to either NKVTN or NPKSS, the two putative motifs of Lmo2186 (Fig. 3A). These chimeras were expressed in wild-type, \( \texttt{H9004} \) srtA, and \( \texttt{H9004} \) srtB strains. Analysis of peptidoglycan material obtained from these strains showed that NPKSS, but not NKVTN, was recognized by SrtB to promote anchoring of the InlB-Cter-Lmo2185 chimera (Fig. 3B). An InlB-Cter-Lmo2185 variant lacking the NAKTN motif was also generated for these assays. Importantly, the analysis of protoplast, cell wall, and extracellular medium fractions showed that the lack of NAKTN totally abrogated the recognition and anchoring to peptidoglycan of the chimera by SrtB, resulting in its secretion to the extracellular medium (Fig. 3B). Taken together, these results confirmed the recognition of NPKSS by SrtB, even when placed in the context of the heterologous sorting signal of Lmo2185. Likewise, the data unveiled the unique capacity of \( L. \) monocytogenes SrtB for recognizing a sorting motif lacking the invariant proline at position (2), exemplified by NAKTN.

Proline (P) at Position 2, and Not Lysine (K) at Position 3, Is Essential for the Recognition of NPKSS by SrtB—The above experiments demonstrated that NAKTN (Lmo2185) and NPKSS (Lmo2186), but not NKVTN, are recognized as sorting motifs by \( L. \) monocytogenes SrtB. These data suggested that lysine (K) at position 3 could play an important role for SrtB recognition. To test this hypothesis, we generated new variants using the NPKSS sequence as template and the C-terminal sorting signal of Lmo2185. These NPKSS variants carried changes at position 2 (NAKSS); 3 (NPQSS); and 3-4-5 (NPQTN) (Fig. 4A). The latter variant was designed to recreate the NPQTN motif recognized in IsdC by the \( S. \) aureus SrtB, which lacks lysine (K) at position 3. Analysis of cell wall, protoplast, and extracellular fractions revealed that NPKSS and NPQSS were
recognized as sorting motifs since the respective chimeras were detected in the cell wall (Fig. 4B). Although the chimera harboring the NPQTN motif was expressed at lower levels than the others chimeras used, it was also detected in cell wall extracts. A densitometry analysis confirmed the recognition of the NPQTN motif to an extent comparable to those of the motif NPKSS and the NAKTNPKSS sequence present in Lmo2186 (Fig. 4, C and D). Therefore, we concluded that NPQTN is recognized by the L. monocytogenes sortase SrtB.

On the other hand, a P→A change in the position 2 of the NPKSS motif of Lmo2186 (NAKSS variant) abrogated anchoring to the peptidoglycan (Fig. 4, B and C). Control experiments using a ΔsrtB strain revealed that in those cases in which the anchoring to peptidoglycan was detected for a particular chimera, this was SrtB-dependent (data not shown). Taken together, these results demonstrated that, unlike lysine (K) at position 3, proline (P) at position 2 is essential for recognition of the NPKSS sorting motif by the L. monocytogenes SrtB sortase.

**DISCUSSION**

In this work, we have identified the sorting motifs of Lmo2185 and Lmo2186, two L. monocytogenes surface proteins that are anchored to the peptidoglycan in an SrtB-dependent manner (24). Our previous proteomic study identified the SDSSNKTNPK peptide, corresponding to Lmo2186, in material obtained from cell wall extracts (24). The detection of such peptide in the cell wall-derived extract suggested, although not proved, that NPKSS could be the sorting motif recognized by SrtB in Lmo2186. These proteomic analyses also provided evidence for a marked presence of peptides from Lmo2185 and Lmo2186 within the pool of peptides obtained upon trypsin digestion of the cell wall material (24). In fact, Lmo2185 and Lmo2186 peptides were identified at a higher rate that those of other surface proteins with similar molecular weight. Lmo2185 and Lmo2186 seem therefore to be abundant among the set of proteins covalently bound to the peptidoglycan, which implies that SrtB of L. monocytogenes must be a sortase displaying high activity in vivo.

Taking in account these observations, we focused on the unequivocal identification of sorting motifs recognized by SrtB in Lmo2185 and Lmo2186. To this aim, chimeras harboring natural and recombinant sequences expected to be recognized by this sortase were analyzed. This experimental design relied on predictions considering that the sequences NAKTN in Lmo2185, and NKVTN (or the overlapping NPKSS) in Lmo2186, could be recognized as sorting motifs (2, 9, 10, 21). Our experiments showed that NPKSS, but not the upstream sequence NKVTN, is absolutely required for SrtB-mediated anchoring of a chimera containing the C-terminal end of Lmo2186. This result was in agreement with the consensus motif N(P/Q)/K(T/S)(N/G/S)(D/A), proposed by Comfort and Clubb (9) for the class-B sortases (StrB) upon comparison of multiple bacterial genome sequences.

Next, we further explored the essentiality of the residues composing the NPKSS sorting motif of Lmo2186. The P→A substitution was shown to abrogate anchoring of the corresponding chimera bearing the NAKSS variant. This result, to
of their respective motifs. Interestingly, sortases classified in the subfamily 5 (class-D) are predicted to recognize motifs with a consensus sequence NA(E/A/S/H)TG (9), which contains an invariant alanine (A) at position 2. Sortases of subfamily 5 are found in Streptomyces spp., Corynebacterium spp., Tropheryma whipplei, Thermofibida fusca, and Bifidobacterium longum. None of these Gram-positive bacteria have either SrtA or SrtB homologs. In fact, it has been proposed that sortases of the subfamily 5 could play a housekeeping role equivalent to that assigned to SrtA (9). Based on these observations, it is tempting to speculate that SrtB could have evolved by acquiring some properties inherent to sortases of the subfamily 5 and the class-A (SrtA), which recognize alanine (A) or proline (P) at position 2 of the sorting motif, respectively. Such assumption could explain the flexibility of the L. monocytogenes SrtB for recognizing varied amino acids at the position 2 of the sorting motif. To our knowledge, this property had not been shown for any sortase before this study. Data validating SrtB substrates of Bacillus species containing serine (S) at position 2 of the motif could be of high value to further support the capacity of sortases of the class B for recognizing varied amino acids at this position.

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REFERENCES

1. Pucciarelli, M. G., Bierne, H., and García-del Portillo, F. (2007) in Listeria monocytogenes: Pathogenesis and Host Response (Shen, H. G. H., ed) pp. 81–110, Springer, New York
2. Marraffini, L. A., Dedent, A. C., and Schneewind, O. (2006) Microbiol. Mol. Biol. Rev. 70, 192–221
3. Weidenmaier, C., and Peschel, A. (2008) Nat. Rev. Microbiol. 6, 276–287
4. Dramsi, S., Magnet, S., Davison, S., and Arthur, M. (2008) FEMS Microbiol. Rev. 32, 307–320
5. DeDent, A. C., McAdow, M., and Schneewind, O. (2007) J. Bacteriol. 189, 4473–4484
6. Mandlik, A., Swierczynski, A., Das, A., and Ton-That, H. (2008) Trends Microbiol. 16, 33–40
7. Schneewind, O., Model, P., and Fischetti, V. A. (1992) Cell 70, 267–281
8. Schneewind, O., Mihaylova-Petkov, D., and Model, P. (1993) EMBO J. 12, 4803–4811
9. Comfort, D., and Clubb, R. T. (2004) Infect. Immun. 72, 2710–2722
10. Dramsi, S., Trieu-Cuot, P., and Bierne, H. (2005) Res. Microbiol. 156, 289–297
11. Ton-That, H., Liu, G., Mazmanian, S. K., Faull, K. F., and Schneewind, O. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 12424–12429
12. Bierne, H., Mazmanian, S. K., Trot, M., Pucciarelli, M. G., Liu, G., De-houx, P., Jansch, L., Garcia-del Portillo, F., Schneewind, O., and Cossart, P. (2002) Mol. Microbiol. 43, 869–881
13. Laloui, L., Pellegrini, E., Dramsi, S., Baptista, M., Bourgeois, N., Doucet-Populaire, F., Rusniok, C., Zouine, M., Glaser, P., Kunst, F., Poyart, C., and Trieu-Cuot, P. (2005) Infect. Immun. 73, 3342–3350
14. Garandeau, C., Reglier-Poupet, H., Dubail, I., Beretti, J. L., Berche, P., and Charbit, A. (2002) Infect. Immun. 70, 1382–1390
15. Kruger, R. G., Otvos, B., Frankel, B. A., Bentley, M., Dontel, P., and McCafferty, D. G. (2004) Biochemistry 43, 1541–1551
16. Gaspar, A. H., Marraffini, L. A., Glass, E. M., Debord, K. L., Ton-That, H., and Schneewind, O. (2005) J. Bacteriol. 187, 4646–4655
17. Mazmanian, S. K., Ton-That, H., Su, K., and Schneewind, O. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 2293–2298
18. Maresso, A. W., Chapa, T. J., and Schneewind, O. (2006) J. Bacteriol. 188, 6145
Sorting Motifs Recognized by L. monocytogenes SrtB

8145–8152
19. Litou, Z. I., Bagos, P. G., Tsirigos, K. D., Liakopoulos, T. D., and Hamodrakas, S. J. (2008) J. Bioinform. Comput. Biol. 6, 387–401
20. Boekhorst, J., de Been, M. W., Kleerebezem, M., and Siezen, R. J. (2005) J. Bacteriol. 187, 4928–4934
21. Bierne, H., Garandeau, C., Pucciarelli, M. G., Sabet, C., Newton, S., Garcia-del Portillo, F., Cossart, P., and Charbit, A. (2004) J. Bacteriol. 186, 1972–1982
22. Glaser, P., Frangeul, L., Buchrieser, C., Rusniok, C., Amend, A., Baquero, F., Berche, P., Bloecher, H., Brandt, P., Chakraborty, T., Charbit, A., Chetouani, F., Couve, E., de Daruvar, A., Dehoux, P., Domann, E., Dominguez-Bernal, G., Duchaud, E., Durant, L., Dussurget, O., Entian, K. D., Fsihi, H., Garcia-del Portillo, F., Garrido, L., Goebel, W., Gomez-Lopez, N., Hain, T., Hauf, J., Jackson, D., Jones, L. M., Kaerst, U., Kreft, J., Kuhn, M., Kunst, F., Kurapkat, G., Madueno, E., Maitournam, A., Vicente, J. M., Ng, E., Nedjar, H., Nordsiek, G., Novella, S., de Pablos, B., Perez-Diaz, J. C., Purcell, R., Remmel, B., Rose, M., Schlueter, T., Simoes, N., Tierrez, A., Vazquez-Boland, J. A., Voss, H., Wehland, J., and Cossart, P. (2001) Science 294, 849–852
23. Bierne, H., and Cossart, P. (2007) Microbiol. Mol. Biol. Rev. 71, 377–397
24. Pucciarelli, M. G., Calvo, E., Sabet, C., Bierne, H., Cossart, P., and Garcia-del Portillo, F. (2005) Proteomics 5, 4808–4817
25. Newton, S. M., Klebba, P. E., Raynaud, C., Shao, Y., Jiang, X., Dubail, J., Archer, C., Frehel, C., and Charbit, A. (2005) Mol. Microbiol. 55, 927–940
26. Braun, L., Dramsi, S., Dehoux, P., Bierne, H., Lindahl, G., and Cossart, P. (1997) Mol. Microbiol. 25, 285–294
27. Trieu-Cuot, P., Carlier, C., Poyart-Salmeron, C., and Courvalin, P. (1991) Gene 102, 99–104
28. Jonquieres, R., Bierne, H., Fiedler, F., Gounon, P., and Cossart, P. (1999) Mol. Microbiol. 34, 902–914
29. Braun, L., Ohayon, H., and Cossart, P. (1998) Mol. Microbiol. 27, 1077–1087
30. Mengaud, J., Ohayon, H., Gounon, P., Mege, R. M., and Cossart, P. (1996) Cell 84, 923–932