Characterization of the role of LtgB, a putative lytic transglycosylase in Neisseria gonorrhoeae

Petra L. Kohler, Karen A. Cloud, Kathleen T. Hackett, Eric T. Beck and Joseph P. Dillard

Neisseria gonorrhoeae releases monomeric peptidoglycan (PG) fragments during growth. These PG fragments affect pathogenesis-related phenotypes including induction of inflammatory cytokines and killing of ciliated fallopian tube cells. Although the biological activities of these molecules have been established in multiple systems, the genes and gene products responsible for their production in N. gonorrhoeae have not been determined. The authors previously identified genes for three lytic transglycosylase homologues (ltgA, ltgB and ltgC) in the N. gonorrhoeae genome sequence. Mutation of ltgA was found to affect PG fragment release, and mutation of ltgC affected cell separation. In this study the effects of complete deletion or point mutations in ltgB were characterized. Point mutations were introduced by a combination of insertion-duplication mutagenesis and positive and negative selection, thereby generating selectable marker-less mutations. The ltgB deletion mutant had normal growth characteristics and was not affected in PG fragment release. When expressed in Escherichia coli, gonococcal LtgB was able to substitute for lambda endolysin to cause cell lysis. Mutation of the predicted catalytic-site glutamic acid residue did not decrease lysis in this system. However, mutation of a nearby glutamic acid residue eliminated lysis activity.

INTRODUCTION

Most bacteria encode multiple lytic peptidoglycan (PG) transglycosylases. Although the function of several of these enzymes produced by Escherichia coli or bacteriophages has been characterized biochemically (reviewed by Höltje, 1998), the importance of this reaction to the bacterial cell and the need for multiple enzymes remain unclear. Lytic transglycosylases cleave the N-acetylglucosamine–β-1,4-N-acetylmuramic acid linkage in PG and catalyse the formation of a 1,6-anhydro bond on the N-acetylglucosamine acid (Höltje et al., 1975). Ostensibly, the biological function of these enzymes is to remove PG strands to allow for cell wall expansion during growth and for remodelling during cell division. However, E. coli mutants lacking several of the lytic transglycosylases are not affected in growth or division, and growth phenotypes are only seen in the presence of cell wall synthesis inhibitors or in strains containing mutations affecting PG-synthesizing enzymes (Lommatzsch et al., 1997). Recently, an E. coli mutant lacking all six lytic transglycosylases was shown to be slightly affected in cell separation and grew in short chains (Heidrich et al., 2002).

Liberated PG fragments contribute to virulence in infections caused by the bacterium Neisseria gonorrhoeae. Unlike most other Gram-negative bacteria, gonococci release soluble PG fragments into the surrounding milieu during growth. The major fragments released are the 1,6-anhydrodisaccharide PG monomers, the type of fragments produced by lytic transglycosylases (Sinha & Rosenthal, 1980). These PG fragments have been shown to cause the sloughing of ciliated fallopian tube cells in the organ culture model of gonococcal pelvic inflammatory disease (PID) (Melly et al., 1984). PG fragments have also been shown to induce arthritis in a rat model, producing symptoms similar to the arthritis seen in patients with disseminated gonococcal infection (DGI) (Fleming et al., 1986). PG monomers have been shown to induce production of the inflammatory cytokines IL-1 and IL-6 in human monocytes (Dokter et al., 1994). Similar PG fragments are also involved in the pathogenesis of infections caused by Bordetella pertussis and Haemophilus influenzae. In B. pertussis, released monomeric PG fragments kill ciliated cells of the trachea (Luiker et al., 1995). In a rabbit model of H. influenzae meningitis, various soluble PG fragments were shown to contribute to brain oedema and leukocytosis (Burroughs et al., 1993). These results suggest that PG fragments are involved in the pathogenesis of PID and DGI, and possibly the large inflammatory response characteristic of symptomatic, uncomplicated gonorrhoea.

Although the effects of PG fragments on biological systems have been well characterized, the genes involved in
production and release of these fragments have not been identified in any pathogen known to produce them. It might be hypothesized that the lytic PG transglycosylases generate the fragments, but it is unclear why the fragments are not efficiently recycled as occurs in *E. coli*. (For a review of PG recycling, see Park, 1995.) Do gonococci simply produce more PG monomers than can be handled by the recycling apparatus, or does production occur in some manner that favours release rather than uptake? To better understand the production and release of PG fragments by *N. gonorrhoeae*, we have produced mutations in lytic transglycosylase genes. These mutations will allow us to assess the role and relative contribution of each gene product to PG monomer production and release of PG fragments by *N. gonorrhoeae*.

**METHODS**

**Bacterial strains and growth conditions.** Bacterial strains and plasmids used in this study are listed in Table 1. All experiments except transformations were performed with nonpiliated variants. Gonococci were grown with aeration in GC base liquid (GCBL) medium (1% proteose peptone no. 3, 0.4% K2HPO4, 0.1% KH2PO4, 0.1% NaCl; pH 7-2) containing Kellogg’s supplements and 0.042% NaHCO3 or on GCB agar plates (Difco) in 5% CO2 at 37 °C (Kellogg et al., 1963; Morse & Bartenstein, 1974). *E. coli* was grown in Luria broth or on Luria agar plates (Sambrook et al., 1989). Antibiotics were used at the following concentrations: for *N. gonorrhoeae*, 10 µg erythromycin (ErmR) ml−1 and 100 µg streptomycin (Str) ml−1; and for *E. coli*, 500 µg ErmR ml−1 and 100 µg ampicillin (Amp) ml−1.

**Cloning and mutagenesis of *ltgB***. For cloning of *ltgB*, the following specific primers were designed based on the Gonococcal Genome Project sequence: *ltgBF* (5'-GACGACGGATAACCGGT-GAA-3') and *ltgBR* (5'-TCAGAACCTCGGATTTTCCGTTGC-3'). *ltgB* was amplified by PCR from MS11 chromosomal DNA (Tm 59 °C). The PCR product was digested with *Sma* I and EcoRI and ligated with T4 DNA ligase into pKC1. The ligation reaction was transformed into chemically competent *E. coli* (Active Motif, strain TAM1) following the manufacturer’s instructions, and ErmR transformants were screened for a plasmid of the expected 3.5 kb size (pEB33). To construct a plasmid encoding *ltgBE117A*, pEB33 was amplified by PCR using mutagenic primers 5'-AAGTGGATCTAGGC-GTCCGAGC-3' and 5'-AAGCCTGATCAGGTCATCGACG-3'. The PCR product was gel-purified (GeneClean, Bio 101), digested with *Nhe* I and ligated into *E. coli*. The resulting plasmid pEB4 included an *Nhe* I site at the site of the mutation. Plasmid construction was verified by restriction digest and DNA sequencing. Plasmid clones were sequenced at the DNA Sequence Laboratory of the University of Wisconsin Biotechnology Center using the BigDye fluorescent method as described by the manufacturer (Perkin-Elmer).

The plasmid encoding *ltgBE117N* (pPK44) was constructed by PCR amplifying pEB33 with mutagenic primers *ltgBetoN1* (5'-GGGATTACCGCGAAACATGCTCGGATC-3') and *ltgBetoN2* (5'-GCGGATTAAGGGGAGAGGGTTGTCGCGGATC-3') (Tm 63-5 °C, extension time 1-5 min). An *Ase* I site was introduced at the site of the mutation. The resulting PCR product was digested with *Ase* I and self-ligated using T4 DNA ligase. The ligation reaction was transformed into competent *E. coli* (TAM1), ErmR isolates were screened by PCR and digestion with *Ase* I for the mutation. Isolation of a strain containing the expected plasmid was confirmed by restriction mapping.

A plasmid for the deletion of *ltgB* was created by PCR amplifying *ltgB* and surrounding sequence with primers *ltgB1* (5'-GGGATTACCGCGAAACATGCTCGGATC-3') and *ltgB2* (5'-GCGGATTAAGGGGAGAGGGTTGTCGCGGATC-3')

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**Rz**

**beginning of pPK20** was PCR amplified from the end of SCCGACCGATACC-3

**ltgB colonies** with primers ltgBlysisF (5′-CGGAGCTCTCAGCG-3′) and ltgBlysisR (5′-CGGCTGCTGGACGGCATTAT-3′) to create a vector with the lambda genes.

**Nru**

amplification of the plasmid using primers ltgBmut1 (5′-CGCGGTACCTTATTGATTTCTACCATCTTCTACTCC-3′) and ltgBmut2 (5′-AGTCGCTAGCTGAGGAGGCTGCTGCTGCTG-3′) to create pKH58. The coding region of **ltgB** and then diluted to an OD 550 of 0.2% L-arabinose is not likely to be a membrane-bound lipoprotein as is MltC. In addition to the similarity to MltC, LtgB also shares 31% identical and 47% similar to MltC over a 100 amino acid region, and the sequence motifs common to family 1 lytic transglycosylases are conserved in LtgB (Fig. 1) (Blackburn & Clarke, 2001). One difference between LtgB and MltC is that the LtgB predicted amino acid sequence does not contain a lipoprotein-processing site, and thus LtgB is likely to be a membrane-bound lipoprotein as is MltC. In addition to the similarity to MltC, LtgB also shares over 90% amino acid identity with putative lytic transglycosylases in the two sequenced strains of *Neisseria meningitidis*. Examination of the *N. gonorrhoeae* genome sequence in the **ltgB** region shows that just upstream of **ltgB** is a copy of the insertion sequence IS1106, and immediately downstream of **ltgB** is a gene for a hypothetical transporter of the major facilitator family.

**LtgB can functionally replace the lytic transglycosylase in the bacteriophage lambda lysis system**

The homology of LtgB with MltC suggests that LtgB is a PG lytic transglycosylase. We investigated the ability of LtgB to function in PG degradation by using LtgB to replace the PG transglycosylase in the bacteriophage lambda lysis system. Lambda encodes three gene products necessary for lysis of the host cell during the lytic stage of the bacteriophage lifecycle, R, S and Rz (Young, 1992). R is the PG transglycosylase necessary for degradation of the cell wall during lysis (Bienkowska-Szewczyk et al., 1981). S is a holin, a small transmembrane protein necessary to allow entry of R into the periplasm (Young, 1992). The function of Rz is unclear, although the locus encodes two gene products, Rz and Rz1 (Hanych et al., 1993). We cloned S, R and Rz from lambda
and placed them under the control of an arabinose-inducible promoter to create the plasmid pPK20. Upon induction with arabinose, E. coli carrying pPK20 lysed rapidly (Fig. 2). We next constructed a plasmid containing \( \text{ltgB} \) instead of \( \text{R} \) in order to determine whether LtgB could functionally replace R in the lambda lysis system. The strain of E. coli carrying the plasmid containing \( \text{S} \), \( \text{ltgB} \) and \( \text{Rz} \) (pPK33) lysed when arabinose was added to the culture medium, though not as quickly as the strain carrying the plasmid encoding the lambda lysis genes, pPK20 (Fig. 2). This result suggests that LtgB degrades PG and may function as an autolysin.

The similarity of LtgB to MltC suggests that LtgB, like MltC, is a PG transglycosylase, i.e. it is predicted to cleave the N-acetylmuramic acid–\( \beta \)-1,4-N-acetylglucosamine bond and create a 1,6-anhydro bond on the N-acetylmuramic acid (Dijkstra & Keck, 1996). Residues important in this reaction have been characterized in the E. coli lytic transglycosylases Slt70 and Slt35 and in the lambda lytic transglycosylase R (Jespers et al., 1992; Leung et al., 2001; Thunnissen et al., 1994, 1995; van Asselt et al., 1999a, b, 2000). In order to examine the function of LtgB as a putative PG transglycosylase we made a point mutation in the putative active site of LtgB. The PG transglycosylases contain a conserved acidic residue necessary for enzymic activity. The family 1 PG transglycosylases, which include MltC, contain an invariant glutamic acid positioned adjacent to an invariant serine (Blackburn & Clarke, 2001). We identified a putative catalytic glutamic acid in LtgB by alignment with MltC (Fig. 1). A point mutation was made at this residue, changing glutamic acid 117 to alanine (LtgB \( E117A \)). LtgB \( E117A \) was substituted for \( \text{R} \) in pPK20, resulting in pPK36. Surprisingly, when E. coli cells carrying pPK36 were induced with arabinose they displayed lysis, though lysis was not as rapid as seen with pPK33 carrying the wild-type \( \text{ltgB} \) (Fig. 2).

The amino acid sequence of LtgB revealed another glutamic acid at position 115, very close to the predicted catalytic residue, E117 (Fig. 1). Because glutamic acid 115 is only three residues away from the conserved serine, it was a candidate for the essential glutamic acid necessary for enzymic activity. The family 1 PG transglycosylases, which include MltC, contain an invariant glutamic acid positioned adjacent to an invariant serine (Blackburn & Clarke, 2001). We identified a putative catalytic glutamic acid in LtgB by alignment with MltC (Fig. 1). A point mutation was made at this residue, changing glutamic acid 117 to alanine (LtgB \( E117A \)). LtgB \( E117A \) was substituted for \( \text{R} \) in pPK20, resulting in pPK36. Surprisingly, when E. coli cells carrying pPK36 were induced with arabinose they displayed lysis, though lysis was not as rapid as seen with pPK33 carrying the wild-type \( \text{ltgB} \) (Fig. 2).

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![Fig. 1. Multiple sequence alignment of N. gonorrhoeae LtgB with E. coli MltC and Slt70. Only a portion of each protein sequence is shown. Amino acids in bold are conserved in all three proteins. Motif boundaries for the family 1 lytic transglycosylases, as defined by Blackburn & Clarke (2001), are indicated below the alignment. Residues marked with a star were changed by mutation; the black star indicates loss of PG degradation activity.](image1)

![Fig. 2. Induced lysis of E. coli. An arabinose-inducible promoter was used to drive expression of the bacteriophage lambda genes necessary for lysis. The gene encoding the lytic transglycosylase from lambda (R) was replaced with \( \text{ltgB} \) and \( \text{ltgB} \) point mutants. Expression of \( \text{ltgB} \) compensates for the loss of \( \text{R} \), and a point mutation at glutamic acid 115 eliminates enzymic activity of LtgB. The values are the means from three separate experiments, and the error bars indicate the standard error of the mean.](image2)
whether LtgB truly functions as a PG transglycosylase or may function as a peptidoglycanase with a different specificity.

**Creation of a point mutation in *ltgB* in the gonococcal chromosome using an insertional plasmid and positive and negative selection**

We have sought to create marker-less mutations in the lytic transglycosylase genes as we are attempting to make gonococcal mutants with multiple disruptions. In our mutagenesis of *ltgA*, we used a cassette containing *ermC*, an Erm resistance marker, and *rpsL*, a Str sensitivity marker, to interrupt *ltgA* in the gonococcal chromosome (Cloud & Dillard, 2002). The insertion was subsequently replaced in a second transformation using a plasmid carrying an *in vitro*-constructed deletion, a method developed for gonococci by Johnston & Cannon (1999). We devised a shortened method for introduction of a point mutation into *ltgB* (Fig. 3). A plasmid containing *ltgBE115N* was constructed using pKCI, the plasmid carrying *ermC* and *rpsL*, as the parent vector. The resulting plasmid, pPK44, was transformed into *N. gonorrhoeae* MS11, and ErmR StrS transformants were obtained. Strain MS11, like many other gonococcal strains, is naturally StrS. However, Str sensitivity is dominant over Str resistance when both alleles of the gene are expressed (Lederberg, 1951). In order to select for isolates that had lost the inserted plasmid, ErmR transformants were grown without antibiotics in liquid culture and plated to medium containing Str. These isolates were replica-plated to medium containing Str or Erm to confirm Str resistance and to test for Erm sensitivity. Of 20 StrEermS transformants isolated screened for the mutation by PCR and restriction digestion, 18 had retained the point mutation. The resulting gonococcal strain encoding LtgB*E115N* was designated PK106.

Several elements of the mutagenesis were surprising. The frequency of StrR isolates was much higher than expected for resolution resulting in loss of the plasmid from the chromosome. In our previous experiments with inserted plasmids, we were unable to detect loss of the insertion in 10^5 cells (Hamilton *et al.*, 2001). However, in the *ltgB* mutagenesis, StrR colonies were obtained at a frequency of 8\*10^-2 ± 2\*10^-2. Also, Str resistance did not always indicate loss of the inserted plasmid. When StrR colonies were plated to medium containing Erm, 11.5% of StrR colonies were also ErmR, indicating that the original plasmid we had introduced, pPK44, was still inserted in the chromosome. To further investigate the appearance of StrR ErmR gonococci, we PCR amplified and sequenced *rpsL* from the inserted plasmid in four StrR ErmR isolates. Three of these isolates contained the same point mutation as is found in the original chromosomal copy of *rpsL*. The fourth contained a nonsense mutation in *rpsL*.

**Characterization of *ltgB* mutants of *N. gonorrhoeae***

In order to examine the function of *ltgB* in *N. gonorrhoeae*, an in-frame deletion was created in *ltgB*. A plasmid containing the region surrounding *ltgB* and an in-frame deletion of the *ltgB* coding sequence was used to transform wild-type gonococcal strain MS11. We screened by PCR for homologous recombination and the loss of *ltgB*. The strain carrying the deletion of *ltgB* was designated KH539. We characterized this strain and PK106, carrying the *ltgBE115N* mutation, for effects on PG fragment release.

To characterize PG turnover, gonococcal strains were grown in medium containing [6-^3^H]glucosamine and lacking glucose, to metabolically label the PG. Macromolecular PG was collected during a chase period and the amount of radioactivity present was determined to quantify the amount of the original PG that remained in the cell wall and the rate at which fragments were released into the culture supernatant. If LtgB contributes to PG fragment release, then the *ltgB* mutants would be expected to show a reduced rate of PG turnover. However, no significant difference in PG turnover was observed between the wild-type and mutant strains (Fig. 4).

To determine whether the same types of soluble PG fragments were being released during growth of the mutant and

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**Fig. 3.** Schematic of insertion-duplication, positive/negative selection mutagenesis. Plasmids containing *ermC* (Erm resistance), *rpsL* (Str sensitivity) and *ltgB* with the mutation of interest were transformed into MS11, and ErmR transformants selected. ErmR transformants were transferred to medium containing Str. StrR colonies were screened for mutations in *ltgB* (represented by a star) by PCR and restriction endonuclease digestion.
and the 3H content measured at different times. The values were obtained from culture. Aliquots were taken, the macromolecular PG harvested, and the 3H content measured at different times. The values shown are the arithmetic means of the percentages of original radioactivity remaining from at least three trials. Error bars indicate the standard error of the mean.

![Fig. 4. Mutation of ltgB does not affect PG turnover. MS11 (wild-type), KH539 (ltgB deletion), and PK106 (ltgB\textsubscript{E117N}) were each pulse-labelled with \([6^\text{3H}]\)glucosamine and grown in liquid culture. Aliquots were taken, the macromolecular PG harvested, and the 3H content measured at different times. The values shown are the arithmetic means of the percentages of original radioactivity remaining from at least three trials. Error bars indicate the standard error of the mean.](image1)

wild-type strains, the PG fragments in culture supernatants from KH539 and MS11 were analysed by size-exclusion chromatography. KH539 exhibited a fragment profile similar to that of the wild-type strain (Fig. 5). The amounts of PG multimers, PG monomers and free disaccharide released by the ltgB mutant were not different from those of the wild-type. This result indicates that ltgB is not involved in the release of cytotoxic PG monomers by gonococci.

![Fig. 5. An ltgB mutant is able to release PG monomers as well as wild-type gonococci. Supernatants from each strain, containing \(^{3}\text{H}\)-labelled PG fragments, were separated by passage over gel-filtration columns. Fractions were collected and their radioactivity measured. Data are presented as percentage of counts per minute in the included volume versus the volume passed through the columns. PG fragments were identified by comparison with chromatographs of known standards.](image2)

**DISCUSSION**

The data presented here indicate that LtgB functions in PG degradation. LtgB was able to function in place of the lytic transglycosylase of the bacteriophage lambda lysis system. This activity and the sequence similarity with known lytic transglycosylases suggest that LtgB is a lytic transglycosylase, but further characterization of its biochemical activity would be necessary to demonstrate this function conclusively. Unfortunately, expression of ltgB in *E. coli* resulted in insoluble protein, and we were not able to assay the biochemical function of LtgB directly. Sequence analysis has shown that a glutamic acid directly adjacent to a serine is conserved among lytic transglycosylases (Blackburn & Clarke, 2001; Dijkstra & Thunnissen, 1994; Koonin & Rudd, 1994). This conserved glutamic acid is hypothesized to be the catalytic residue in lytic transglycosylases as it has been demonstrated to be for Slt70, Slt35 and lambda R (Jespers et al., 1992; Leung et al., 2001; Thunnissen et al., 1994, 1995; van Asselt et al., 1999a, b, 2000). The predicted amino acid sequence of LtgB shows a conserved glutamic acid residue at amino acid 117 adjacent to a serine. This group of residues aligns well with the predicted catalytic site of MltC from *E. coli* (Fig. 1) and the catalytic glutamic acid of *E. coli* Slt70. However, mutation of the putative active-site glutamic acid (E117) did not attenuate the enzymic activity of LtgB as measured by activity in the lambda lysis system. Further mutational analysis revealed that glutamic acid 115 (E115) is essential for LtgB function because a mutation changing E115 to an asparagine eliminated the ability of LtgB to lyse *E. coli* in the lambda lysis system. It is unclear how mutation of E115 might affect the function of LtgB. Replacement of E115 in LtgB with asparagine may not allow for proper conformation for substrate binding. Examination of the alignment of LtgB and MltC from *E. coli* (Fig. 1) reveals that in MltC there is a glutamine residue in the position occupied by E115 in LtgB. Thus, mutation of LtgB at E115 makes LtgB\textsubscript{E115N} more similar to MltC at position 115 (position 216 in MltC) than wild-type LtgB. It is possible that E115 is the catalytic residue and, even though not in the expected position in the primary sequence, it may be in the correct position in the secondary structure to carry out the lytic transglycosylase reaction. Alternatively, this result may indicate that LtgB cleaves PG at a different bond from lytic transglycosylases and may have a different specificity from the related *E. coli* enzymes. Database searches using LtgB as the query showed that there are at least 17 more examples of lytic transglycosylase homologues with the ExES pattern that we observe in LtgB.

The studies presented here demonstrate the utility of combining insertion-duplication mutagenesis and positive and negative selection for the introduction of mutations into the gonococcal chromosome. Mutants were obtained at an appreciable frequency, and the undesired isolates that became Str sensitive but did not resolve out the duplication could be screened out by replica plating on medium containing Str and Erm. The reason for the high rate of Str\textsuperscript{R} Erm\textsuperscript{R} isolates is not completely clear, but we found two
different types of mutations in the introduced rpsL that resulted in Srr. Since three of the four mutations we found were identical to the sequence in the original Srr rpsL, it appears that recombination between the resident rpsL (Srr) and the rpsL (Str) on the inserted plasmid resulted in these mutations through a gene-conversion event. However, we also found a nonsense mutation in one of the Srr Erms isolates. This result, together with the high frequency of Erms Srr isolates, suggests that the production of both RpsL proteins may be detrimental. A similar phenomenon was observed when rpsL was used as a counter-selectable marker in Streptococcus pneumoniae (Sung et al., 2001).

Examination of PG turnover in the ltgB deletion mutant shows that LtgB does not significantly affect the amount of PG released. In addition, the ltgB deletion mutant still produced PG monomers in amounts no different from the wild-type. Thus it is clear that LtgB does not substantially contribute to PG monomer production. This result raises the question of what the function of LtgB may be in N. gonorrhoeae. It is clear from E. coli lysis assays that LtgB can degrade PG. Thus it might function in the removal of PG strands during growth or division, or for breaking down fragments for PG recycling. However, we did not observe any defects in growth or division in the ltgB mutants, and we would expect mutants defective in recycling to show higher rates of PG turnover. A lack of phenotypes is also observed for E. coli lytic transglycosylase mutants. A triple lytic transglycosylase mutant in E. coli did not show any growth phenotypes (Lommatzsch et al., 1997), and a mutant lacking six lytic transglycosylases was only slightly affected in cell separation (Heidrich et al., 2002). It has been proposed that there is functional redundancy in PG hydrolases in E. coli, i.e. one of the many other PG-degrading enzymes may substitute for the lost function (Heidrich et al., 2002). This could be the case in N. gonorrhoeae as well. The common N. gonorrhoeae chromosome encodes five lytic transglycosylase homologues (Blackburn & Clarke, 2001; Cloud & Dillard, 2002, 2004) (GenBank accession AE004969), and the gonococcal genetic island present in most gonococcal strains encodes two additional lytic transglycosylase homologues (Dillard & Seifert, 1997; Hamilton et al., 2005). One of these enzymes or the PG-degrading amidase, endopeptidase or glucosaminidase (Chapman & Perkins, 1983; Hebeler & Young, 1976; Stefanova et al., 2003) of gonococci may substitute for LtgB function.

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