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A Putative Monofunctional Glycosyltransferase Is Expressed in *Ralstonia eutropha*

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A gene, *mgt*, encoding a protein homologous to the N-terminal module of class A high-molecular-mass penicillin-binding proteins was identified in *Ralstonia eutropha*. By using specific antibodies, the corresponding Mgt protein was detected in association with the membrane, confirming that the N-terminal hydrophobic segment functioned as a membrane anchor. A derivative in which the hydrophobic sequence was deleted was overexpressed as a maltose-binding fusion protein in *Escherichia coli*. Cleavage of the product resulted in substantial amounts of soluble Mgt derivative, indicating that folding occurs independently on other proteins or on homologous domains of penicillin-binding proteins.

Penicillin-binding proteins (PBPs) are members of the superfamily of bacterial penicillin-interacting serine D,D-peptidases that include β-lactamases of classes A and C (5). PBPs are typical modular proteins, characterized by a (central) penicillin-binding domain of approximately 320 amino acids that contains three conserved amino acid motifs: SXXK with the active site serine, SXN, and K(H)T(S)G. A few PBPs, such as the *Streptomyces* R61 enzyme, and all β-lactamases consist essentially of this domain and, after processing of an N-terminal signal peptide, represent hydrophilic, soluble enzymes.

PBPs are grouped into three classes, low-molecular-mass PBPs and high-molecular-mass (HMM) PBPs of classes A and B, according to size and overall amino acid sequence relatedness. In several PBPs, the penicillin-binding module functions as a penicillin-sensitive transpeptidase. The majority of the PBPs contain at least two other domains: an N-terminal domain and a C-terminal extension. The three-dimensional structure of *Streptococcus pneumoniae* class B HMM PBP2x clearly confirmed its postulated arrangement in three domains (17).

The only non-penicillin-binding module of known function is the approximately 300-amino-acid N-terminal domain of class A HMM PBPs. In *Escherichia coli* PBP1b, this domain clearly possesses biosynthetic glycosyltransferase activity (formerly addressed as transglycosylase) which is penicillin insensitive. It is, however, sensitive to the antimicrobial drug moenomycin (flavomycin), which is not used therapeutically (16, 23–25), demonstrating that this domain principally constitutes a target for nonpenicillin antibiotics. This aspect is particularly interesting, since PBP variants with a highly reduced affinity to penicillin that occur in resistant bacteria still represent important target proteins for novel antimicrobial agents (15). Investigations of the transglycosylase activity are difficult, however, for several reasons. Soluble, active HMM PBPs of class A have not yet been obtained, nor has it been possible to construct active monofunctional derivatives from HMM PBPs that act as transglycosylase (or act as a transpeptidase or show penicillin binding).

Open reading frames (ORFs) encoding peptides with a high degree of similarity to the N-terminal domain of class A HMM PBPs were found in the *Haemophilus influenzae* (6) and *E. coli* genomic sequences and were reported in *Klebsiella* and *Neisseria* spp. as well (22). Such proteins are of special interest since they provide potentially easier access to the structure of a transglycosylase domain, and enzymatic studies, such as those done for the *E. coli* enzyme, which was demonstrated to represent a monofunctional glycosyltransferase (Mgt) (4), are also possible.

An *mgt* gene in *Ralstonia eutropha*. Previously, a DNA sequence of the 5′ end of ORF5, an ORF in *R. eutropha* (formerly *Alcaligenes eutrophus* [27]) that encoded a putative protein with high homology to the N-terminal part of class A HMM PBPs, was reported (11). Further sequencing revealed that ORF5 terminated without association to genes encoding other PBP-related domains, thus representing a separate non-penicillin-binding module of a class A HMM PBP (13). ORF5 terminated after 696 bp with a T4317AA stop codon, which was followed by a short palindromic sequence. Another ORF, ORF6, started at A4392TG, but no recognizable promoter sequences were found upstream of it. The GC content of ORF5 (63%) was slightly less than that of ORF3 or ORF4 (68 and 70%, respectively). DNA sequencing of cloned DNA fragments was performed using the dideoxy method (20) with the T7 sequencing kit (Pharmacia, Freiburg, Germany).

Sequence comparison. The deduced 231-amino-acid sequence of ORF5 was highly related to the N-terminal domain of class A HMM PBPs. It contained an N-terminal hydrophobic peptide (amino acids 11 to 30), suggesting that it is membrane bound, similar to HMM PBPs. The *R. eutropha* gene was named according to the *E. coli* homolog (4) *mgt*. The deduced peptide of the 5′ end of ORF6 had no homology to PBP domains or other proteins.

The family of biosynthetic glycosyltransferases. Amino acid alignment of homologous proteins and domains identified several clusters of highly conserved amino acid sequences. Conserved in all sequences analyzed were the amino acids E67DXXFXXXXXG, G97, Q104, R119KXXE, Y143XN, A177, and L206 (numbers of the *R. eutropha* Mgt; Fig. 1A). Some motifs are common to the N-terminal domains of HMM class A and class B PBPs as well, corresponding to box 1 and box 4 as defined by Ghuysen and Dive (7). Only box 1 is located within the sequence of Mgt proteins (Fig. 1A). The three-dimensional structure of the HMM class B PBP of *S. pneumoniae*, PBP2x, is known (17). Its N-terminal domain,

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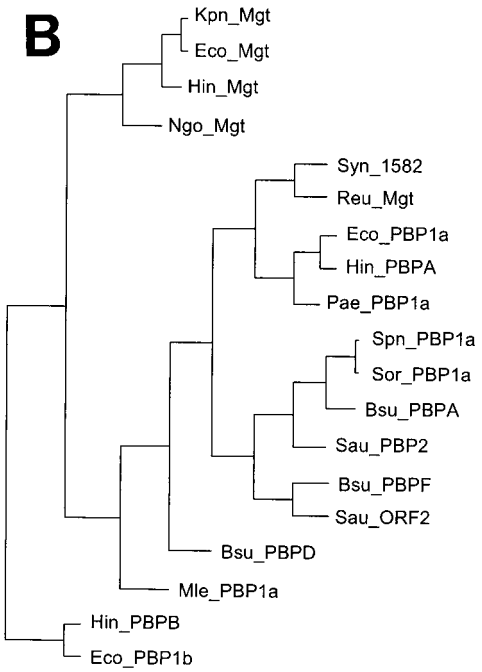

FIG. 1. Comparison of the N-terminal domains of class A HMM PBPs and monofunctional glycosyltransferases. (A) Sequence alignment. Deduced proteins and protein domains related to the *R. eutropha* Mgt were identified by using the BLAST program (1). Amino acid residues common to more than 50% of the sequences are indicated on top; bold letters indicate residues conserved in all—or all but one—of the sequences. *S. pneumoniae* PBP2x, an HMM class B PBP, is included, and underlined sequences refer to boxes 1, 2, and 3, as defined by Ghuysen and Dive (7). (B) Protein sequence relationship. The dendrogram was created using the Pileup and Growtree program which is part of the GCG sequence analysis software package, version 8.0, of the University of Wisconsin Department of Genetics (3). Species and nucleotide sequence accession numbers are as follows: Reu_Mgt, *R. eutropha* Y10537; Bsu_PBPA, *Bacillus subtilis* M97208; Bsu_PBPD, *B. subtilis* P40750; Bsu_PBPF, *B. subtilis* P38050; Eco_PBP1a, *E. coli* P02918; Eco_PBP1b, *E. coli* X02163; Eco_Mgt, *E. coli* P46022; Hin_PBPA, *H. influenzae* P31776; Hin_PBPB, *H. influenzae* P45345; Hin_Mgt, *H. influenzae* B64159; Kpn_Mgt, *Klebsiella pneumoniae* Z54198; Mle_PBP1a, *Mycobacterium leprae* L39923; Ngo_Mgt, *Neisseria gonorrhoeae* L47159; Pae_PBP1a, *Pseudomonas aeruginosa* L13867; Sau_PBP2, *S. aureus* L25426; Sau_ORF2, *S. aureus* L19300; Spn_PBP1a, *S. pneumoniae* M90527; Spn_2x, *S. pneumoniae* PBP2x P14677; Sor_PBP1a, *Streptococcus oralis* M90528; Syn_1582, *Synechosystis* sp. strain D64000.

which is formed like a "sugar tongue," is in close spatial proximity to the penicillin-binding domain. Box 1 (R70 to G76 in the *R. eutropha* protein) corresponds to the first beta strand defined in the N-terminal domain of PBP2x and could play a role in the interaction with the transpeptidase domain (17). The two residues K120 and E123, a region which also has homology to PBP2x, correspond to amino acids in helix H1 of PBP2x (K121 and E124). Box 4 of HMM PBPs is not present in the Mgt proteins (it would be approximately 25 amino acids distal to their C termini). In PBP2x, this box is located at the transition between the N-terminal domain and the penicillin-binding domain and may represent the linker between the two PBP domains.

Cluster analysis positioned the *R. eutropha* protein within a group of N-terminal domains of HMM PBPs of gram-negative bacteria, including *E. coli* PBP1a (Fig. 1B), whereas the other four Mgt proteins clustered separately. The PBP domains of gram-positive organisms and the two PBP1b proteins of *E. coli* and *H. influenzae* defined further groups. In this context it is interesting to note that, e.g., the N-terminal domain of *S. pneumoniae* class A PBP1a has 45% amino acid identity with that of *E. coli* PBP1a or -1b, whereas the penicillin-binding domain has only 22 to 24% identity, suggesting that the domains have evolved independently from each other (14).

Expression of Mgt in *E. coli*. For overexpression of the Mgt protein in *E. coli*, either the entire gene or a shorter soluble derivative in which the hydrophobic N-terminal peptide had been deleted was cloned into the pQE vector system. *E. coli* SG13009 containing the repressor plasmid pREP4 (8) and pQE derivatives were obtained from Qiagen (Hilden, Germany). *mgt* sequences were amplified by PCR using the oligonucleotide primers CGGGATCCA3628AAAGACTCTGGTCC GCCTTC3648 (primer a) and CCCCAAG4322CTTACCTCA GCCTCAACGGCTCCA4297 (primer b) for amplification of the entire *mgt* gene; for a derivative in which amino acids Met1 to Ala30 were deleted, the primer CGGGATC3713CAACCG GCAGCTGCCGTCGCTC3735 (primer c) and primer b, described above, were used. The oligonucleotides were designed to engineer *Bam*HI and *Hin*dIII sites, respectively, at the ends of the PCR products which were used for cloning of the DNA fragments into pQE8, resulting in six N-terminal consecutive histidine residues (His tag), or pQE12, with no His tag. PCRs were carried out in a Biomed thermocycler for 30 cycles with denaturation for 30 s at 96°C, annealing for 1 min at 52°C, and extension for 1 min at 72°C, which was followed by a 5-min extension period at 72°C. A 100-μl reaction mixture contained 10 pmol of each oligonucleotide primer, 0.8 mM deoxyribonucleoside triphosphates, 5 to 6 mM $MgCl_2$, 2.5 U of *Taq* polymerase (Perkin Elmer, Norwalk, Conn.), and buffer according to the manufacturer. The plasmids encoding the membrane-bound protein were named pPEM8 and pPEM12, and those encoding the cytoplasmic derivatives were named pPEC8 and pPEC12.

When expression was induced by isopropyl-β-D-thiogalactopyranoside (IPTG), *E. coli* strains harboring the intact, presumably membrane-associated protein stopped growing immediately, whereas those expressing the presumed soluble derivative in the cytoplasm continued to grow for a period of at least 1.5 h (data not shown). Whereas the cytoplasmic protein was overexpressed to a high degree but as inclusion bodies, the intact protein was associated with the membrane fraction, confirming the role of the hydrophobic N-terminal peptide as a membrane anchor; the expression of the protein, however, was much lower (Fig. 2 and 3). The presence of the His tag resulted in a slightly slower electrophoretic mobility (data not shown).

Overexpression of the *E. coli* Mgt protein resulted in increased peptidoglycan synthesis activity when solubilized membrane fractions of the cells were assayed (4). To date, attempts to detect corresponding activity of the *R. eutropha* enzyme in *E. coli* membranes overexpressing the intact Mgt have failed (3a), suggesting that the requirements for the *R. eutropha* Mgt enzyme are distinct from those for the *E. coli* enzyme.

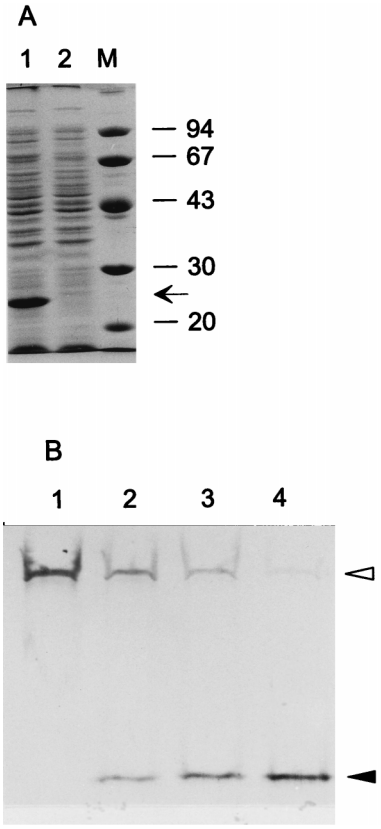

FIG. 2. (A) Expression of *mgt* in *E. coli*. Exponentially growing cells carrying the plasmid pPEC12 were treated with IPTG (2 mM) at an OD_{600} of 0.7 to 0.8 and harvested after 3 h. Cells were disrupted by sonication, and total cellular protein was separated on SDS-polyacrylamide gels and stained with Coomassie blue. Lane 1, *E. coli* pPEC12; lane 2, control cells harboring the plasmid without the *mgt* insert; lane M, molecular weight markers. Numbers to the right indicate molecular weights (in thousands). The arrow depicts the position of the Mgt derivative. (B) A soluble Mgt derivative. Overexpression of the maltose-binding protein—Mgt fusion protein was induced in *E. coli* TB1 containing pMPE at 28°C. The maltose-binding protein—Mgt fusion protein was purified from *E. coli* TB1 containing pMPE after induction at 28°C by affinity chromatography and treated with factor Xa as described in the text. Mgt was detected after SDS-polyacrylamide gel electrophoresis, Western blotting, and immunostaining with specific antiserum. Fifty micrograms of fusion protein and 1 μg of factor Xa were used; samples correspond to approximately 12 μg. Incubation time: lane 1, 0 min; lane 2, 6 h; lane 3, 12 h; lane 4, 24 h.

Mgt-specific antiserum and immunological detection of Mgt. Mgt was prepared from *E. coli* cells after induction of pPEC8. Proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, and the Mgt fragment was purified by electroelution. For immunization, 100 μg of protein in complete Freund adjuvant was injected into rabbits, which was followed by booster injections of approximately 40 μg of protein in each case in incomplete Freund adjuvant. Proteins were separated on 15% SDS-polyacrylamide gels. Western blotting was performed as described previously (10), and Mgt was visualized using a 1:20,000 dilution of the antiserum in phosphate-buffered saline-Tween and alkaline phos-

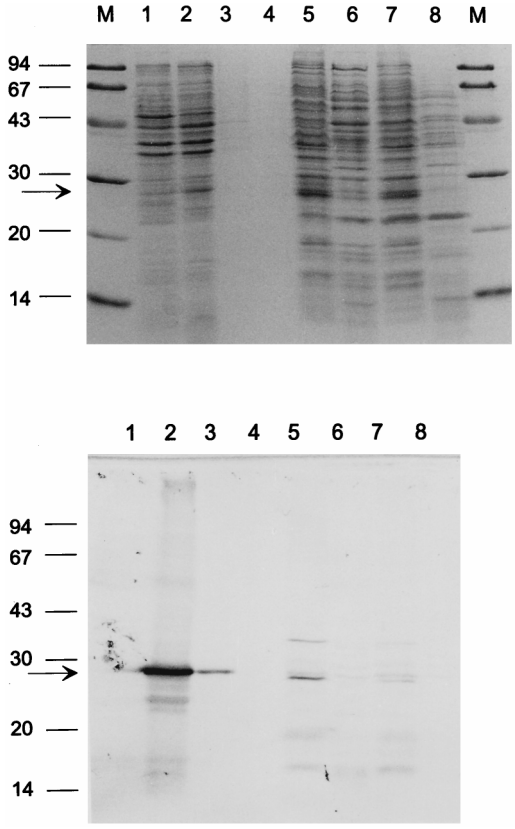

FIG. 3. Mgt in *R. eutropha*. Cells of *R. eutropha* were grown under aerobic conditions up to an OD_{436} of 10, as described in the text (lanes 5 and 6) and then shifted to conditions of restricted oxygen supply for 14 h (lanes 7 and 8). Solubilized cytoplasmic membrane proteins were used (lanes 5 and 7, membranes; lanes 6 and 8, soluble proteins). As controls, whole cell lysates of *E. coli* harboring pPEM8 (expressing the membrane-bound Mgt) were used. Lane 1, *E. coli* SG13009 containing plasmid pQE8 without the insert; lanes 2 through 4, cell lysates of *E. coli*/pPEM8 (lane 3, 1:20 of lane 2; lane 4, 1:100 of lane 2); lane M, molecular weight marker proteins. The arrow on the left side marks the position of Mgt-His. Numbers to the left indicate molecular weights (in thousands). (A) Coomassie blue-stained SDS-polyacrylamide gel. (B) Immunoblot of gel used in panel A stained with specific anti-Mgt antiserum.

phatase-conjugated anti-rabbit immunoglobulin G (Sigma Chemical Co., St. Louis, Mo.) by the method of Blake et al. (2).

A soluble Mgt derivative. The *R. eutropha* Mgt protein excluding the first 30 amino acids was expressed as a fusion to the maltose-binding protein MBP. *E. coli* TB1 (9) grown in Luria-Bertani medium was used for propagation of the pMAL plasmids (12). A DNA segment encoding amino acids 31 to 231 of *mgt* was amplified with the oligonucleotide primer A3715AC CGGCAGCTGCCGTCGCTCGACGCCCTCA3745 (primer d) and primer c, described above. After ligation with pMALc2 digested with *Xmn*I/*Hin*dIII, the resulting plasmid pMPE was transformed into *E. coli* TB1. Protein expression was induced overnight by using 0.3 mM IPTG. Again, inclusion bodies were formed when the protein was induced at 37°C; however, at 28°C, the fusion protein was recovered to over 50% in the soluble fraction. Curiously, cellular growth of the *E. coli* strain stopped immediately upon induction of the pMal-Mgt fusion protein when grown at 28°C, and at 37°C, cells continued to grow but with a reduced generation time, suggesting that the native form of Mgt interferes with some cellular functions, even if it is not translocated through the membrane.

The fusion protein was purified after disruption of the cells in a French pressure cell and centrifugation of the cell lysate from the supernatant by using amylose-affinity chromatography as described by the manufacturer (New England Biolabs, Schwalbach, Germany). Soluble Mgt was obtained after incubation of the fusion protein for 24 h with factor Xa (2%, wt/wt) at 4°C, resulting in complete cleavage as shown in Fig. 2B.

Expression of Mgt in *R. eutropha*. *R. eutropha* (27) H16 (DSM 428) was grown aerobically in a mineral salt medium (21) with 1.5% sodium gluconate as the carbon source with an air flow of 500 ml/min. Cells were disrupted in a French pressure cell, and membranes were separated from the cytoplasmic fraction by centrifugation (60 min, 18,000 rpm, Sorvall centrifuge). Cytoplasmic membrane proteins were solubilized with 2% sarcosyl for 20 min at room temperature, and unsolubilized outer membrane proteins and cell wall material were sedimented at 18,000 rpm for 40 min in a Sorvall centrifuge. Mgt was determined on Western blots by using the anti-Mgt antiserum. The antiserum reacted with a membrane protein of a size similar to that of the membrane-bound Mgt protein expressed in *E. coli*, which is slightly larger than the cytoplasmic derivative (Fig. 3), clearly documenting that the Mgt protein is indeed expressed and associated with the membrane in *R. eutropha.*

The *mgt* gene (ORF5) was suggested to be the third gene of an operon that included ORF3 and the lactate dehydrogenase (LDH) gene, *ldh* (ORF4). Promoter sequences were detected only upstream of ORF3 (11). Membranes were prepared from cells grown under aerobic conditions in which no LDH activity was detected and from cells grown under restricted oxygen supply in which LDH activity was high (61 U/mg of protein). For growth under semianaerobic conditions, air flow was reduced to 50 ml/min at an optical density at 436 nm (OD_{436}) of 10, 1.5% sodium gluconate was added as a supplement, and cells were harvested after 14 h. Mgt was determined immunologically with specific antibodies. As shown in Fig. 3, no difference in the amounts of Mgt was detected, suggesting that the LDH activity may not be regulated via expression of the enzyme or that *mgt* is expressed independently of *ldh.*

Concluding remarks. In a variety of bacterial species, genes encoding monodomain glycosyltransferases were detected on the basis of sequence homology to PBP genes encoding class A high-molecular-weight PBPs. Peptidoglycan-synthesizing activity was associated with *E. coli mgt* (4). In the present report, we demonstrate that the *R. eutropha* protein is expressed under normal growth conditions. Both data indicate that *mgt* genes are functional genes. A soluble and therefore presumably native *R. eutropha* Mgt derivative was obtained. This may suggest that structural information can be obtained from these one-domain proteins more easily than from the HMM PBPs of class A.

Sequence alignments will help to define the Mgt-like domain of class A HMM PBPs. In fact, the definition of a soluble N-terminal domain is not straightforward in the case of PBPs; it was suggested that in addition to the hydrophobic N-terminal peptide, another membrane attachment site exists in *E. coli* PBP1b within the first 163 periplasmic amino acids (Asp88 to Ala251) (26). This attachment site must not necessarily be present in Mgt proteins, since significant homology of *E. coli* PBP1b to Mgt proteins does not start before residue 200 and since the *R. eutropha* derivative was obtained in a soluble form. The presence of amino acid extensions may also be responsible for some differences in enzymatic activities of the PBPs and the Mgt proteins, such as the flavomycin sensitivity of *E. coli* PBP1b, which is not characteristic of the *E. coli* Mgt (4).

To date, the database has revealed Mgt-like proteins only in gram-negative bacteria. PBP-independent biosynthetic glyco-

syltransferase activities in the gram-positive species *Staphylococcus aureus*, *Micrococcus luteus*, and *S. pneumoniae* have been described (18, 19), suggesting that upcoming sequence information of these organisms may also include ORFs that represent *mgt* genes.

Nucleotide sequence accession number. The nucleotide sequence data have been deposited in the EMBL nucleotide sequence database under the accession number Y10537.

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REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Blake, M. S., K. H. Johnson, G. Russell-Jones, and E. C. Gotschlich.** 1984. A rapid sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. Anal. Biochem. **136:**175–179.
3. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.

3a. **Di Berardino, M.** Unpublished data.

4. **Di Berardino, M., A. Dijkstra, D. Stüber, W. Keck, and M. Gubler.** 1996. The monofunctional glycosyltransferase of *Escherichia coli* is a member of a new class of peptidoglycan-synthesising enzymes: overexpression and determination of the glycan-polymerising activity. FEBS Lett. **392:**184–188.
5. **Englebert, S., A. El Kharroubi, G. Piras, B. Joris, J. Coyette, M. Nguyen-Distèche, and J.-M. Ghuysen.** 1993. Molecular design of the bi(multi?)functional penicillin-binding proteins, p. 319–333. *In* M. A. de Pedro, J.-V. Höltje, and W. Löffelhardt (ed.), Bacterial growth and lysis: metabolism and structure of the bacterial sacculus. Plenum Publishing Co., New York, N.Y.
6. **Fleischmann, R. D., M. D. Adams, O. White, R. A. Clayton, E. F. Kirkness, A. R. Kerlavage, C. J. Bult, J.-F. Tomb, B. A. Dougherty, J. M. Merrick, K. McKenney, G. Sutton, W. FitzHugh, C. Fields, J. D. Gocayne, J. Scott, R. Shirley, L.-I. Liu, A. Glodek, J. M. Kelley, J. F. Weidman, C. A. Phillips, T. Spriggs, E. Hedblom, M. D. Cotton, T. R. Utterback, M. C. Hanna, D. T. Nguyen, D. M. Saudek, R. C. Brandon, L. D. Fine, J. L. Fritchman, J. L. Furhmann, N. S. M. Geoghagen, C. L. Gnehm, L. A. McDonald, K. V. Small, C. M. Fraser, H. O. Smith, and J. C. Venter.** 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science **269:**496–512.
7. **Ghuysen, J.-M., and G. Dive.** 1994. Biochemistry of the penicilloyl-serine transferases, p. 103–129. *In* J.-M. Ghuysen and R. Hakenbeck (ed.), Bacterial cell wall. Elsevier Sciences BV, Amsterdam, The Netherlands.
8. **Gottesmann, S., E. Halpern, and P. Trisler.** 1981. Role of *sulA* and *sulB* in filamentation by Lon mutants of *Escherichia coli* K-12. J. Bacteriol. **148:**265–273.
9. **Guan, C., P. D. Li, and M. Inouye.** 1987. Vectors that facilitate the expression and purification of foreign peptides in *Escherichia coli* by fusion to maltose-binding protein. Gene **67:**21–30.
10. **Hakenbeck, R., H. Ellerbrok, T. Briese, S. Handwerger, and A. Tomasz.** 1986. Penicillin-binding proteins of penicillin-susceptible and -resistant pneumococci: immunological relatedness of altered proteins and changes in peptides carrying the β-lactam binding site. Antimicrob. Agents Chemother. **30:**553–558.
11. **Jendrossek, D., H. D. Kratzin, and A. Steinbüchel.** 1993. The *Alcaligenes eutrophus ldh* structural gene encodes a novel type of lactate dehydrogenase. FEMS Microbiol. Lett. **112:**229–236.
12. **Maina, C. V., P. D. Riggs, A. G. I. Grandea, B. E. Slatko, L. S. Moran, J. A. Tagliamonte, L. A. McReynolds, and C. Guan.** 1988. A vector to express and purify foreign protein in *Escherichia coli* by fusion to, and separation from, maltose binding protein. Gene **74:**365–373.
13. **Martin, C.** 1992. Molekulargenetische Untersuchungen des Penicillin-bindenden Proteins (PBP) 1a von *Streptococcus pneumoniae*: Verwandtschaft von PBP 1a Mosaikgenen in Penicillin resistenten klinischen Stämmen. Dissertation. Freie Universität Berlin, Berlin, Germany.
14. **Martin, C., T. Briese, and R. Hakenbeck.** 1992. Nucleotide sequences of genes encoding penicillin-binding proteins from *Streptococcus pneumoniae* and *Streptococcus oralis* with high homology to *Escherichia coli* penicillin-binding protein 1A and 1B. J. Bacteriol. **174:**4517–4523.
15. **Martin, C., C. Sibold, and R. Hakenbeck.** 1992. Relatedness of penicillin-binding protein 1a genes from different clones of penicillin-resistant *Streptococcus pneumoniae* isolated in South Africa and Spain. EMBO J. **11:**3831–3836.
16. **Nakagawa, J., S. Tamaki, S. Tomioka, and M. Matsuhashi.** 1984. Functional biosynthesis of cell wall peptidoglycan by polymorphic bifunctional polypeptides. J. Biol. Chem. **259:**13937–13946.
17. **Pares, S., N. Mouz, Y. Pétillot, R. Hakenbeck, and O. Dideberg.** 1996. X-ray structure of *Streptococcus pneumoniae* PBP2x, a primary penicillin target enzyme. Nature Struct. Biol. **3:**284–289.
18. **Park, W., and M. Matsuhashi.** 1984. *Staphylococcus aureus* and *Micrococcus luteus* peptidoglycan transglycosylases that are not penicillin-binding proteins. J. Bacteriol. **157:**538–544.
19. **Park, W., H. Seto, R. Hakenbeck, and M. Matsuhashi.** 1985. Major peptidoglycan transglycosylase activity in *Streptococcus pneumoniae* that is not a penicillin-binding protein. FEMS Microbiol. Lett. **27:**45–48.
20. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
21. **Schlegel, H. G., H. Kaltwasser, and G. Gottschalk.** 1961. Ein submersverfahren zur Kultur wasserstoffoxidierender Bakterien: Wachstumsphysiologische Untersuchungen. Arch. Mikrobiol. **38:**119–128.
22. **Spratt, B. G., J. Zhou, M. Taylor, and M. J. Merrick.** 1996. Monofunctional biosynthetic peptidoglycan transglycosylases. Mol. Microbiol. **19:**639–640.
23. **Suzuki, H., Y. van Heijenoort, T. Tamura, J. Mizoguchi, Y. Hirota, and J. van Heijenoort.** 1980. In vitro peptidoglycan polymerization catalysed by penicillin-binding protein 1b of *Escherichia coli* K 12. FEBS Lett. **110:**245–249.
24. **van Heijenoort, Y., M. Derrien, and J. van Heijenoort.** 1979. Polymerization by transglycosylation in the biosynthesis of the peptidoglycan of *Escherichia coli* K 12 and its inhibition by antibiotics. FEBS Lett. **89:**141–144.
25. **van Heijenoort, Y., and J. van Heijenoort.** 1980. Biosynthesis of the peptidoglycan of *Escherichia coli* K 12. Properties of the in vitro polymerization by transglycosylation. FEBS Lett. **110:**241–244.
26. **Wand, C. C., D. E. Schultz, and R. A. Nicholas.** 1996. Localization of a putative second membrane association site in penicillin-binding protein 1B of *Escherichia coli*. Biochem. J. **316:**149–156.
27. **Yabuuchi, E., Y. Kosako, I. Yano, H. Hotta, and Y. Nishiuchi.** 1995. Transfer of two *Burkholderia* and one *Alcaligenes* species to *Ralstonia* gen. nov.: proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1986) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. Microbiol. Immunol. **39:**897–904.