Identification of a Terminal Rhamnopyranosyltransferase (RptA) Involved in Corynebacterium glutamicum Cell Wall Biosynthesis


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A bioinformatics approach identified a putative integral membrane protein, NCgl0543, in Corynebacterium glutamicum, with 13 predicted transmembrane domains and a glycosyltransferase motif (RXRDE), features that are common to the glycosyltransferase C superfamily of glycosyltransferases. The deletion of C. glutamicum NCgl0543 resulted in a viable mutant. Further glycosyl linkage analyses of the mycolyl-arabinogalactan-peptidoglycan complex revealed a reduction of terminal rhamnopyranosyl-linked residues and, as a result, a corresponding loss of branched 2,5-linked arabinofuranosyl residues, which was fully restored upon the complementation of the deletion mutant by NCgl0543. As a result, we have now termed this previously uncharacterized open reading frame, rhamnopyranosyltransferase A (rptA). Furthermore, an analysis of base-extractable lipids from C. glutamicum revealed the presence of decaprenyl-monophosphorylhamnose, a putative substrate for the cognate cell wall transferase.

A common feature of members of the Corynebacterineae is that they possess an unusual cell wall dominated by a heteropolysaccharide termed an arabinogalactan (AG), which is linked to both mycolic acids and peptidoglycan, forming the mycolyl-arabinogalactan-peptidoglycan (mAGP) complex (5, 10, 12, 15, 24, 25, 34). The formation of the arabinan domain of AG (4) is the common feature of the corynebacterial cell wall, and the biosynthesis of these residues remains to be clarified; nevertheless, they are a feature of the corynebacterial cell wall, and the biosynthesis of which needs to be addressed. The current paradigm of AG biosynthesis follows a linear pathway which is built upon a decaprenyl pyrophosphate lipid carrier. The unique saccharide linker and galactan domain is synthesized by a variety of GT-A and GT-B family glycosyltransferases, all of which utilizing a nucleotide diphosphate-activated sugar substrate for transferase activity. It has been hypothesized by us (3, 5) and others (8) that a major shift in the biosynthetic machinery takes place upon the initiation of arabinan polymerization. AftA, Emb, AftC, and AftB all belong to the GT-C family of glycosyltransferases, all of which utilize DPA as the sole lipid-activated sugar donor for arabinose transfer into the cell wall. Since t-Rhap residues are present in the arabinan component of the cell wall, the enzyme(s) responsible for its addition is likely to belong to the GT-C family of glycosyltransferases and, as determined through deduction, is one which utilizes a lipid-phosphate-derived rhamnose substrate similar to DPA. Herein, we present the putative protein NCgl0543 as a distinct t-Rhap of the GT-C superfamily, which is responsible for the transfer of t-Rhap residues to the arabinan domain to form the branched 2,5-linked Araf motifs of C. glutamicum. In addition, we have identified a novel decaprenyl-monophosphorylhamnose.
and discuss its role in substrate presentation for AG biosynthesis in *C. glutamicum*.

### MATERIALS AND METHODS

**Bacterial strains and growth conditions.** *C. glutamicum* ATCC 13032 and *Escherichia coli* DH5α were grown in Luria-Bertani broth (Difco) at 30°C and 37°C, respectively. The recombinant strains generated in this study were grown on rich brain heart infusion (BHI; Difco) or brain heart infusion sorbitol (BHIS) medium, the latter containing 0.5 mM sorbitol and the salt medium CGXII as described previously (17). *M. tuberculosis* H37Rv DNA was obtained from the NIH Tuberculosis Research Materials and Vaccine Testing Contract at Colorado State University. Samples for lipid analyses were prepared by harvesting cells at an optical density of 10 to 15 followed by a saline wash and freeze-drying.

**Construction of plasmids and strains.** The vectors made were pVWEx-Cg-petI, pVWEx-Rv3779, and pK19nosbceBΔpetI. To construct the latter primer vector, crossover PCR was applied with primer pairs AB (GTTGCGCTGCGGTACCGTCGCGGGGGAGAGG; C, CCCCACCCACACAACTACCATAGTCTGTCGACATC) and CD (CTTGGTAAGTTGCCATCGGATATGCGAGCCGAACG; D, GCTTTCTAGAATGCGGGCAAGTGG). Amplified products were ligated into pMD18T vector and sequenced prior to transformation into *E. coli*. The obtained sequence was used to design primers for amplification of the genomic DNA of *C. glutamicum*.

**Extraction and analysis of cell wall-bound mycolic acids.** C. glutamicum cultures (5 ml) were grown and metabolically labeled at mid-logarithmic phase of growth using 1 μCi/ml [1,2,4-14C]acetate (50 to 62 mCi/mmol; GE Healthcare, Amersham Bioscience) for 4 h at 38°C with gentle shaking, harvested, washed, and freeze-dried. Cells were then extracted by two consecutive extractions with 2 ml of CHCl3/CH3OH/H2O (10:10:3, vol/vol/vol) and CHCl3/CH3OH (100 g; 5 mg/ml stored in ethanol [10 μl]) was dried under nitrogen and was resuspended by the addition of 50 μl of a 1% IgGel CA-630 (Sigma-Aldrich) solution in buffer A and sonicated. The basic assay mix consisted of 2 mg of membranes and 0.5 mg cell wall “P60” from either *C. glutamicum* or *C. glutamicum* ΔpetA, 1 mM ATP, and 1 mM NADP in a final volume of 160 μl of buffer A and initiated by the addition of 100,000 cpm [1,2-14C]acetate (50 μg; 5 mg/ml stored in ethanol [10 μl]). Reaction mixtures were incubated at 37°C for 2 h, quenched by the addition of CHCl3/CH3OH (1:0.66), dried, and resuspended for autoradiography and scintillation counting.

**Isolation of the mAGP complex, glycosyl composition, and linkage analysis of glycolipids.** Cells (10 g) from *C. glutamicum* cultures were treated with 2 ml of CHCl3/CH3OH/H2O (10:10:3, vol/vol/vol) for 4 h at 50°C to provide cell wall-bound mycolic acids. The thawed cells were resuspended in CHCl3/CH3OH/H2O (1:0.66) and centrifuged at 27,000 × g for 10 min. The supernatant was dried under nitrogen and was resuspended by the addition of 10 μl of a 1% IgGel CA-630 (Sigma-Aldrich) solution in buffer A and sonicated. The basic assay mix consisted of 2 mg of membranes, 0.5 mg cell wall “P60”, and 1 mM ATP, and 1 mM NADP in a final volume of 160 μl of buffer A and initiated by the addition of 100,000 cpm [1,2-14C]acetate (50 μg; 5 mg/ml stored in ethanol [10 μl]) was dried under nitrogen and was resuspended by the addition of 50 μl of a 1% IgGel CA-630 (Sigma-Aldrich) solution in buffer A and sonicated. The basic assay mix consisted of 2 mg of membranes, 0.5 mg cell wall “P60”, and 1 mM ATP, and 1 mM NADP in a final volume of 160 μl of buffer A and initiated by the addition of 100,000 cpm [1,2-14C]acetate (50 μg; 5 mg/ml stored in ethanol [10 μl]). Reaction mixtures were incubated at 37°C for 2 h, quenched by the addition of CHCl3/CH3OH (1:0.66), dried, and resuspended for autoradiography and scintillation counting.

**Chemical identification of decaprenyl-1-monophosphoryl-romanophorane.** Membranes from *C. glutamicum* or *C. glutamicum* ΔpetA were assayed as described above but with or without the addition of 50 μg/ml tunicamycin. Decaprenyl monophosphoryl (50 μg; 5 mg/ml stored in CHCl3/CH3OH [2:1, vol/vol]) was dried under nitrogen and resuspended by the addition of 107 μl of a 1% IgGel CA-630 (Sigma-Aldrich) solution in buffer A and sonicated. The basic assay mix consisted of 2 mg of membranes, 0.5 mg cell wall “P60”, and 1 mM ATP, and 1 mM NADP in a final volume of 160 μl of buffer A and initiated by the addition of 50 μg; 5 mg/ml stored in ethanol [10 μl]) was dried under nitrogen and was resuspended by the addition of 50 μl of a 1% IgGel CA-630 (Sigma-Aldrich) solution in buffer A and sonicated. The basic assay mix consisted of 2 mg of membranes, 0.5 mg cell wall “P60”, and 1 mM ATP, and 1 mM NADP in a final volume of 160 μl of buffer A and initiated by the addition of 50 μg; 5 mg/ml stored in ethanol [10 μl]) was dried under nitrogen and was resuspended by the addition of 50 μl of a 1% IgGel CA-630 (Sigma-Aldrich) solution in buffer A and sonicated. The basic assay mix consisted of 2 mg of membranes, 0.5 mg cell wall “P60”, and 1 mM ATP, and 1 mM NADP in a final volume of 160 μl of buffer A and initiated by the addition of 50 μg; 5 mg/ml stored in ethanol [10 μl]) was dried under nitrogen and was resuspended by the addition of 50 μl of a 1% IgGel CA-630 (Sigma-Aldrich) solution in buffer A and sonicated. The basic assay mix consisted of 2 mg of membranes, 0.5 mg cell wall “P60”, and 1 mM ATP, and 1 mM NADP in a final volume of 160 μl of buffer A and initiated by the addition of 50 μg; 5 mg/ml stored in ethanol [10 μl]) was dried under nitrogen and was resuspended by the addition of 50 μl of a 1% IgGel CA-630 (Sigma-Aldrich) solution in buffer A and sonicated. The basic assay mix consisted of 2 mg of membranes, 0.5 mg cell wall “P60”, and 1 mM ATP, and 1 mM NADP in a final volume of 160 μl of buffer A and initiated by the addition of 50 μg; 5 mg/ml stored in ethanol [10 μl]).
and analyzed by electrospray MS (ES-MS) in the negative mode on a Micromass LCT mass spectrometer as described previously (20).

RESULTS

Genome comparison of the NCgl0543 locus. An examination of the genome sequence of \textit{C. glutamicum} revealed that there are 14 glycosyltransferases of the GT-C family. To date, we have already identified the functions of AftA (5), AftB (34), AftC (12), MptA (29), and MptB (30), which act as membrane-bound glycosyltransferases using polyisoprenylated phosphoarabinosyl and -mannosyl sugar donors, respectively, in the synthesis of AG and lipoarabinomannan. The above-mentioned proteins are also present in \textit{M. tuberculosis}, illustrating that they are required to build the elementary cell wall structure of the \textit{Corynebacterineae} (16).

An as-yet-uncharacterized GT-C glycosyltransferase of \textit{C. glutamicum} is encoded by the putative gene NCgl0543. Orthologues of this gene, with identities exceeding 34\%, are present in \textit{C. glutamicum} strain R, \textit{Corynebacterium efficiens}, \textit{Corynebacterium glucuronolyticum} (ATCC 51867), and \textit{Corynebacterium amycolatum} SK46, the latter two saprophytic organisms being human skin pathogens (21, 37). The genomic organization surrounding NCgl0543 is largely syntenic and is preceded by a putative tRNA pseudouridine synthase, and downstream of NCgl0543 is a membrane protein of unknown function ( Pfam class DUF690). There are no orthologues of NCgl0543 and its orthologs are somewhat different than that of the corynebacterial proteins, the loop region is strongly conserved in the \textit{Corynebacterineae}, and this is indicated for selected mycobacterial species underneath the sequence alignment. Shown are aa 127 to 188 of the \textit{M. tuberculosis} sequence and aa 184 to 235 of the \textit{C. glutamicum} sequence. On top of the sequence comparison, the predicted secondary structure is given, with L indicating a loop region, H a helical structure, and E an extended sheet structure. The abbreviations are as follows: C. glu., \textit{Corynebacterium glutamicum} ATCC 13032; C. glR, \textit{Corynebacterium glutamicum} strain R; C. eff., \textit{Corynebacterium efficiens}; C. gly., \textit{Corynebacterium glucuronolyticum}; C. amy., \textit{Corynebacterium amycolatum}; M. mar., \textit{Mycobacterium marinum} (ATCC BAA-535); M. tub., \textit{Mycobacterium tuberculosis}; M. par., \textit{Mycobacterium paratuberculosis}; M. lep., \textit{Mycobacterium leprae}.
present in Corynebacterium diphtheriae, Corynebacterium jeikeium, or Corynebacterium urealyticum DSM 7109 (13, 36, 37).

NCgl0543 of C. glutamicum is a large polytopic membrane protein of 799 amino acid residues and is predicted to possess 13 transmembrane-spanning helices (TMH) (Fig. 1A). It is further characterized by a periplasmic carboxy-terminal extension of 237 amino acids (aa) similar to the C-terminal features of AftA (5), AftB (34), and the Emb proteins (4, 9, 35, 38). A particularly highly conserved region is present at the end of the long loop connecting TMH 3 and TMH 4. This region is schematically shown in Fig. 1A, as is part of its sequence (Fig. 1B). This sequence resembles the glycosyltransferase GT-C family DXD motif (22), as it contains a number of basic and acidic residues, with the latter being shown in mutational studies to be essential for glycosyltransferase activity using polyprenylated phosphosugar donors (9, 35).

**Construction and growth of mutants.** In an attempt to delete NCgl0543 in C. glutamicum, the nonreplicative vector pK19mobsacBΔNCgl0543 was constructed. This was introduced into C. glutamicum via electroporation and the kanamycin-resistant clones obtained, indicating integration in the chromosome by heterologous recombination (31). Using the sucrose resistance provoked by the sacB gene, a second heterologous recombination event was selected. A total of nine clones were analyzed by PCR, and in two of them, a wild-type reversion at the NCgl0543 locus was restored, whereas a deletion of NCgl0543 was obtained in the seven remaining clones. These numbers indicate that the loss of NCgl0543 is not detrimental to cell growth or viability. As a result, and based on the results described below, one clone was subsequently termed C. glutamicum ΔrptA and confirmed by PCR to have rptaCg deleted, whereas controls with the C. glutamicum wild type resulted in the expected larger amplification product (data not shown).

The growth of wild-type C. glutamicum and C. glutamicum Δrpta in liquid mineral salt medium CGXII and rich BHIS medium was compared (17). Both strains exhibited comparable growth rates of 0.36 ± 0.03 h⁻¹ and 0.60 ± 0.05 h⁻¹ for the respective media. Thus, C. glutamicum Δrpta does not exhibit an apparent growth defect, indicating some degree of tolerance to the deletion of rptaCg. For further analyses, C. glutamicum Δrpta was transformed with a plasmid bearing rptaCg, as well as with a plasmid encoding Rv3779 of M. tuberculosis to result in C. glutamicum Δrpta/pVWEx-Cg-rpta and C. glutamicum Δrpta/pVWEx-Rv3779.

**mAGP complex analyses from C. glutamicum, C. glutamicum Δrpta, C. glutamicum Δrpta/pVWEx-Cg-rpta, and C. glutamicum Δrpta/pVWEx-Rv3779.** To study the function of a corynebacterial rpta deletion mutant, defatted cells were analyzed quantitatively for AG-esterified corynomycolic acids. Wild-type C. glutamicum exhibited the known profile of CMAMEs (data not shown) as previously described (4). Furthermore, cell wall-bound CMAMEs were not significantly altered in C. glutamicum Δrpta (data not shown), which is contrary to the deletion of other GT-C glycosyltransferases in C. glutamicum (4, 5). An analysis of cell wall-associated lipids in several independent experiments highlighted no change in the lipid profiles of the rptaCg deletion mutant compared to that of C. glutamicum (data not shown). This was confirmed quantitatively through [14C]acetate labeling of cultures and equal loading of the radioactivity of extractable free lipids from C. glutamicum, C. glutamicum Δrpta, and the complemented C. glutamicum Δrpta strain using plasmid pVWEx-Cg-rpta. These results demonstrate that, unlike embCg or aftACg (4, 5), rptaCg has little or no involvement in the structure or biosynthesis of cell wall-bound/extractable lipids in C. glutamicum.

The cell wall core (the mAGP complex) was prepared from C. glutamicum and C. glutamicum Δrpta as described previously (4, 10, 15), and the ratio of Rha/Ara/Gal in the mAGP
complex was determined by gas chromatography (GC) analysis of alditol acetates (4, 10, 15) (Fig. 2). The glycosyl compositional analysis of wild-type \emph{C. glutamicum} revealed a relative molar ratio of Rha/Ara/Gal of 21:71:31, which is in accordance with previous data (4) (Fig. 2). The \emph{C. glutamicum} \(\Delta rptA\) mutant yielded AG with a significant reduction in Rha content with no relative change in the molar ratio of Rha-Ara-Gal (1:71:31) (Fig. 2). The complementation of \emph{C. glutamicum} \(\Delta rptA\) with pVWEEx-Cg-\(rptA\) restored the Rha/Ara/Gal ratio to that of wild-type \emph{C. glutamicum} (Fig. 2). Interestingly, complementation with pVWEEx-Rv3779 did not complement and yielded a phenotype identical to that of \emph{C. glutamicum} \(\Delta rptA\) (data not shown).

GC/MS analysis of per-O-methylated alditol acetate derivatives prepared from \emph{C. glutamicum} and \emph{C. glutamicum} \(\Delta rptA\) indicated a loss of \(t\)-linked Rhap residues with a corresponding loss of 2,5-linked Araf residues (Fig. 3). The complementation of \emph{C. glutamicum} \(\Delta rptA\) with pVWEEx-Cg-\(rptA\) restored the glycosyl linkage profile to that of wild-type \emph{C. glutamicum} (Fig. 3). Furthermore, as demonstrated by our total sugar analysis and cell wall sugar linkage analysis, \(\Delta rptA\) restored the glycosyl linkage profile to that of wild-type \emph{C. glutamicum} (Fig. 3). These results demonstrate that the putative protein NCgl0543 is involved in the biosynthesis of \emph{C. glutamicum} AG through the addition of \(t\)-Rhap residues to the C2 position of the five-linked backbone domain of specific Araf residues.

Recognition of a rhamnose lipid-linked sugar donor, decaprenyl-P-rhamnose. Initial assays involved wild-type membranes from \emph{C. glutamicum} and UDP-[\(^{14}\)C]GlcNAc, dTDP-[\(^{14}\)C]Rha, UDP-[\(^{14}\)C]Gal, or p[\(^{14}\)C]Rpp as the sugar donor for AG biosynthesis. Samples of the radioactive lipids from each assay were applied to TLC plates which were then developed in CHCl\(_3\)/CH\(_3\)OH/NH\(_4\)OH/H\(_2\)O (65:25:0.5:3.6) and autoradiograms obtained (Fig. 4). As expected, a series of polar glycolipids were observed, including GlcNAc-P-polypropenyl (GL-1), Rha-GlcNAc-P-polypropenyl (GL-2), Gal-Rha-GlcNAc-P-polypropenyl (GL-3), Gal-Gal-Rha-GlcNAc-P-polypropenyl (GL-4), and DPA/DPR (\(\beta\)-d-ribofuranosyl-\(\alpha\)-monophosphoryldecaprenyl) consistent with our previous studies utilizing \emph{Mycobacterium smegmatis} membranes (27). Interestingly, the inclusion of dTDP-[\(^{14}\)C]Rha also resulted in the synthesis of a more apolar rhamnose-labeled lipid X product, in comparison to that of GL-2 to -4 (Fig. 4). This lipid X was sensitive to acid and resistant to mild-base treatment, indicating that this product was also a polypropenyl-P-based lipid (data not shown). Importantly, an increase in the synthesis of lipid X, based on TLC and densitometry, was found when assays were repeated with membranes prepared from \emph{C. glutamicum} \(\Delta rptA\) (lipid X, 4,300 cpm) compared to \emph{C. glutamicum} (lipid X, 3,057 cpm), indicating that lipid X was probably the lipid-linked sugar donor for the GT-C glycosyltransferase RptA in this study (Fig. 5). Furthermore, the synthesis of lipid X was unaffected by the addition of tunicamycin (\emph{C. glutamicum} lipid X, 3,222 cpm; C.}

FIG. 3. GC/MS analysis of cell walls of \emph{C. glutamicum}, \emph{C. glutamicum} \(\Delta rptA\), and \emph{C. glutamicum} \(\Delta rptA/pVWEEx-Cg-rptA\). Samples of per-O-methylated cell walls were hydrolyzed with 2 M TFA, reduced, per-O-acetylated, and analyzed as described in Materials and Methods (4, 10).
glutamicum ΔrptA lipid X, 4,203 cpm) which inhibits the GlcNAc phosphotransferase activity of Rv1302, thus leading to a marked decrease in GL-1 and higher GLs (e.g., higher than GL-2) (Fig. 5). The basic assay mixture was scaled up containing unlabeled dTDP-Rha and the lipid X extracted for subsequent characterization by treatment with a mild base. Negative-ion ES-MS revealed a major signal [M-H]⁻ at m/z 777 for decaprenyl phosphate (Fig. 6A, inset), and a product ion at m/z 924 corresponding to decaprenyl-P-rhamnose (Fig. 6A). It has not escaped our attention that we also observe mass ions at m/z 934 and m/z 936, which could indicate five and six fully saturated isoprene units out of a possible 10. Similar observations have previously been reported in other Corynebacteriaceae glycolipids, such as staphylococci, streptococci, bacilli, and pseudomonades (40). In Pseudomonas aeruginosa, L-rhamnose is present in the form of mono- or disubstituted rhamnolipids, and these virulence factors are thought to act by disrupting lipid membranes by acting as a surfactant (39). In other gram-positive bacteria, rhamnose is present in the cell wall as part of the carbohydrate moieties of teichoic acids and other cell wall glycopolymers (6). However, since C. glutamicum is nonpathogenic, the introduction of t-Rhap residues is probably related to either (i) structural integrity or (ii) a mechanism to cap and end arabinan synthesis and provide a control point for the extent of mycolation of the “terminal” arabinan units.

DISCUSSION

It is clear from this present study that rptA<sub>Cg</sub> is nonessential and is involved in the addition of t-Rhap residues to a five-linked Ara<sub>f</sub> backbone giving rise to branched 2,5-linked Ara<sub>f</sub> residues. We also report a Rha/Ara molar ratio of 21:71 in C. glutamicum AG (Fig. 2), which equates to approximately seven t-Rhap residues per arabinan triscosamer. This significant amount of rhamnose in the cell wall must not be dismissed, and more so, the functional significance must be elucidated. Rhamnose is present in the cell walls of many gram-positive bacteria such as staphylococci, streptococci, bacilli, and pseudomonades (40). In Pseudomonas aeruginosa, t-rhamnose is present in the form of mono- or disubstituted rhamnolipids, and these virulence factors are thought to act by disrupting lipid membranes by acting as a surfactant (39). In other gram-positive bacteria, rhamnose is present in the cell wall as part of the carbohydrate moieties of teichoic acids and other cell wall glycopolymers (6). However, since C. glutamicum is nonpathogenic, the introduction of t-Rhap residues is probably related to either (i) structural integrity or (ii) a mechanism to cap and end arabinan synthesis and provide a control point for the extent of mycolation of the “terminal” arabinan units.

rptA<sub>Cg</sub> shares approximately 40% sequence similarity with the putative M. tuberculosis protein Rv3779. Although, the total lengths of the proteins differ by 133 aa, there are remarkable structural similarities, such as the high identity at the sequence level in the GT-C loop, which is located between TMH 3 and 4 in C. glutamicum (Fig. 1) and TMH 4 and 5 in M. tuberculosis Rv3779. This loop is followed by 10 TMH in C. glutamicum, which are present and similarly arranged in the M. tuberculosis ortholog. Interestingly, within the final periplasmic region, there is also a similar stretch of amino acid residues (Fig. 1A, white star). Since the carboxy-terminal periplasmic domain is suggested to play a role in substrate recognition, which in the case of the GT-C glycosyltransferases is a growing polysaccharide, we speculate that both RptA<sub>Cg</sub> and the putative Rv3779 protein recognize a related substrate, such as an arabinan oligosaccharide and distinct sugar donors, since the
putative protein Rv3779 failed to complement C. glutamicum ΔrptA.

NCgl0543 is part of the rfb locus, and its genomic organization is well retained in Corynebacterineae. Orthologues of this locus, which include rfbE, NCgl0197, NCgl0195, and rfbD (NCgl0198), are present in C. glutamicum. RfbE has similarity to an ATP-dependent export carrier and is tentatively annotated as being a polysaccharide export ATP-binding protein, and rfbD is tentatively annotated as being a polysaccharide export ABC transporter permease gene (14). We have found that the galactosyltransferase NCgl0195 (2, 26) is essential in C. glutamicum (data not shown) and is involved in GL-3 and GL-4 biosynthesis. Due to the function of RptA_Cg and its similarity to the putative protein Rv3779, we speculate that the rfb locus is essential for polysaccharide biosynthesis and the resultant export to the periplasm. Finally, the discovery of rfbA_Cg has shed new light on the glycosyltransferases which are key to building the cell wall AG of Corynebacterineae.

The biosynthesis of high-energy nucleotide-derived sugar substrates of Corynebacteriaceae glycosyltransferases, such as

FIG. 6. MS analysis and identification of decaprenyl-1-monophosphorylrhamnose. (A) ES-MS (in the negative mode) of decaprenyl-1-monophosphorylrhamnose. (B) Structure of decaprenyl-1-monophosphorylrhamnose.
UDP-α-D-galactofuranose (41) and dTDP-Rhap (23), has been well characterized. These substrates are utilized by GT-A and -B glycosyltransferases in the cytoplasm of the cell to form a preliminary linear galactan polysaccharide before being exported to the periplasm, by an as-yet-unidentified flippase. At this point, further polysaccharide biosynthesis employs glycosyltransferases belonging to the GT-C family which make use of prenylated phosphosugar substrates (8, 22). In Corynebacterineae, decaprenyl (C50), and to a lesser extent octahydroheptaprenyl (C35), are the predominant lipid carriers of UDP-[3H]/H9251-D-galactofuranose (41) and dTDP-Rha, which is the substrate for RptA and novel with respect to DPA, the first report of a rhamnosylated monophosphodecaprenyl (GPM) (33). To date, this is the first report of a GT-C glycosyltransferase belonging to the GT-C family which transports to the periplasm, by an as-yet-unidentified flippase. At preliminary linear galactan polysaccharide before being ex-

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