Complete genome sequence and comparative genomic analysis of an emerging human pathogen, serotype V Streptococcus agalactiae

Hervé Tettelin*, Vega Masignani†, Michael J. Cieslewicz§¶, Jonathan A. Eisen§¶, Scott Peterson†‡, Michael R. Wessels§¶††, Ian T. Paulsen†, Karen E. Nelson†, Immaculada Margarit‡, Timothy D. Read†, Lawrence C. Madoff§¶, Alex M. Wolf†, Maureen J. Beanan†, Lauren M. Brinkac†, Sean C. Daugherty†, Robert T. DeBoy†, A. Scott Durkin†, James F. Kolonay†, Ramana Madupu†, Matthew R. Lewis†, Diana Radune‡, Nadezhda B. Fedorova†, David Scanlan†, Hoda Khouri†, Communicated by Elliott D. Kieff, Harvard University, Boston, MA, June 26, 2002 (received for review May 6, 2002)

The 2,160,267 bp genome sequence of Streptococcus agalactiae, the leading cause of bacterial sepsis, pneumonia, and meningitis in neonates in the U.S. and Europe, is predicted to encode 2,175 genes. Genome comparisons among S. agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, and the other completely sequenced genomes identified genes specific to the streptococci and to S. agalactiae. These in silico analyses, combined with comparative genome hybridization experiments between the sequenced serotype V strain 2603 V and 19 S. agalactiae strains from several serotypes using whole-genome microarrays, revealed the genetic heterogeneity among S. agalactiae strains, even of the same serotype, and provided insights into the evolution of virulence mechanisms.

Streptococcus agalactiae, or group B Streptococcus, is the leading cause of bacterial sepsis, pneumonia, and meningitis in neonates in the U.S. and Europe. Although S. agalactiae usually behaves as a commensal organism that colonizes the gastrointestinal or genital tract of 25–40% of healthy women, it can cause life-threatening invasive infection in susceptible hosts: newborn infants, pregnant women, and nonpregnant adults with underlying chronic illnesses (1, 2). Since guidelines recommending intrapartum antibiotic prophylaxis for high-risk or colonized women were issued in 1996 (3), the incidence of neonatal infections has decreased, and invasive S. agalactiae infections in immunocompromised adults have become more common. Adult disease now accounts for the majority of serious S. agalactiae infections. First recognized as a pathogen in bovine mastitis, S. agalactiae is distinguished from other pathogenic streptococci by the cell wall-associated group B carbohydrate.

Another polysaccharide constitutes the organism’s capsule, an important S. agalactiae virulence determinant. S. agalactiae strains of capsular serotype V were rarely isolated before the mid-1980s but now account for approximately one-third of clinical isolates in the U.S. (4–6). Type V is the most common capsular serotype associated with invasive infection in nonpregnant adults, and the emergence of type V strains over the past decade has been temporally linked to an increase in S. agalactiae disease in this population (7). As a species, S. agalactiae shares certain features with other pathogenic streptococci; however, the precise repertoire of shared and unique attributes that account for the emergence of S. agalactiae as a major pathogen for specific human populations remains undefined. To elucidate the molecular basis for S. agalactiae virulence, we determined the complete genome sequence (8) of the recent clinical type V isolate 2603 V/R (www.tigr.org) and performed comparative analyses with other S. agalactiae strains and with other species of pathogenic streptococci.

Methods

ORF Prediction and Gene Identification. ORFs were predicted by GLIMMER (9, 10) trained with ORFs larger than 600 bp from the genomic sequence and S. agalactiae genes available in GenBank. All predicted proteins larger than 30 aa were searched against a nonredundant protein database (11). Frame-shifts and point mutations were detected and corrected where appropriate; those remaining were annotated as “authentic frame-shift” or “authentic point mutation.” Protein membrane-spanning domains were identified by TOPRED (12). Candidate lipoprotein signal peptides (13) were flagged by N-terminal exact matches to the pattern {DERK} (6)-[LIVMFWSTAG] (2)-[LIVMFYSTAGCQ]-[AGS]-C. Putative signal peptides were identified by using SIGNALP (14). Two sets of hidden Markov models were used to determine ORF membership in families and superfamilies: PFAM version 5.5 (15) and TIGRFAMS 1.0 (16). Domain-based paralogous families were built by performing all-versus-all searches on the protein sequences by using a modified version of a previously described method (17). Potential lineage-specific gene duplications were estimated by identification of ORFs more similar to other ORFs within the S. agalactiae genome than to ORFs from other complete genomes. All ORFs were searched with FASTA3 (18) against all ORFs from the complete genomes and matches with a FASTA P value of 10−15 were considered significant.

Western Blot. S. agalactiae 2603 V/R strain cells were grown in Todd–Hewitt broth (Difco) to OD600 nm = 0.5. The culture was centrifuged for 20 min at 3,000 × g. The supernatant was discarded, and bacteria were washed once with PBS, resuspended in 2 ml of 50 mM Tris-HCl pH 6.8, containing 400 units of Mutanolysin (Sigma), and incubated at 37°C. After three cycles of freeze and thaw, cellular debris was removed by centrifugation at 13,000 × g for 10 min, and the protein concentration of the supernatant was measured by the Bio-Rad Protein assay with BSA as a standard. Purified recombinant proteins (50 ng) (see purification method in ref. 19), and total cell extracts (25 μg) derived from S. agalactiae

Abbreviation: CGH, comparative genome hybridizations.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AE009948).

†To whom reprint requests should be addressed. E-mail: cmfraser@tigr.org.
serotype V 2603 V/R strain were separated by SDS/PAGE and electroblotted onto nitrocellulose membranes for 1 hr at 100 V. The membranes were saturated by overnight incubation at 4°C in 5% skimmed milk and 0.1% Tween 20 in PBS and incubated for 1 hr at room temperature with sera from immunized mice diluted 1:500–1:1,000 in saturation buffer. To reduce background due to antibodies raised against contaminating Escherichia coli proteins, sera were preincubated with E. coli protein extracts adsorbed on nitrocellulose strips. The membranes were washed twice in 3% skimmed milk and 0.1% Tween 20 in PBS and incubated for 1 hr with a 1:1,000 dilution of horseradish peroxidase-conjugated anti-mouse Ig (DAKO). After washing with 0.1% Tween 20 in PBS, the membranes were developed with the Opti-4CN Substrate kit (Bio-Rad).

Fluorescence-Activated Cell Sorter (FACS). S. agalactiae 2603 V/R strain cells were grown in Todd–Hewitt broth (Difco) to OD_{600} nm = 0.5. The culture was centrifuged for 20 min at 3,000 × g, and bacteria were washed once with PBS, resuspended in PBS containing 0.05% paraformaldehyde, and incubated for 1 hr at 37°C and then overnight at 4°C. Fifty microliters of fixed bacteria (OD_{600} nm 0.1) was washed once with PBS, resuspended in 20 μl of newborn calf serum (Sigma), and incubated for 20 min at room temperature. The cells were then incubated for 1 hr at 4°C in 100 μl of preimmune or immune sera and diluted 1:200 in dilution buffer (PBS, 20% newborn calf serum, 0.1% BSA). After centrifugation and washing with 200 μl of washing buffer (0.1% BSA in PBS), samples were incubated for 1 hr at 4°C with 50 μl of R-phycocerythrin-conjugated F(ab)2 goat anti-mouse IgG (Jackson ImmunoResearch) diluted 1:100 in dilution buffer. Cells were washed with 200 μl of washing buffer and resuspended in 200 μl of PBS. Samples were analyzed by using a FACS calibur apparatus (Becton Dickinson), and data were analyzed by using CELL QUEST (Becton Dickinson). A shift in mean fluorescence intensity of >75 channels compared with pre-immune sera from the same mice was considered positive. This cutoff was determined from the mean plus two standard deviations of shifts obtained with control sera raised against mock purified recombinant proteins from cultures of S. agalactiae strains. A total of 339 of 571 proteins were confirmed by Western blot analysis, and the proteins were annotated as “proteins of unknown function.” A total of 339 paralogous protein families were identified in strain 2603, containing 941 predicted proteins (43% of the total).

Comparative Genome Hybridizations (CGH). Predicted genes from strain 2603 V/R were amplified by PCR and arrayed on glass microscope slides (20). Genomic DNA was labeled according to protocols provided by J. DeRisi (www.microarrays.org/pdfs/ GenomicDNALabel.B.pdf), except that the DNA was not digested or sheared before labeling. Arrays were scanned with a GENEPIX 4000 B scanner (Axon Instruments, Foster City, CA), and individual hybridization signals were quantitated with TIGR SPOTFINDER (21). Cy3/Cy5 (2603 V/R signal/test strain) ratio cutoffs were defined arbitrarily as Cy3/Cy5 = 1.0–3.0, gene present in test strain; 3.0–10.0, ambiguous result; >10.0, gene absent. For ambiguous results, the gene may be divergent in the test strain relative to 2603 V/R, or the gene may be absent in the test strain but still produces a hybridization signal because the 2603 V/R gene is part of a paralogous gene family or a repetitive element. Although cutoffs are arbitrary, they fit nicely the results for the variation of the capsule locus in the strains tested (see region 9 on Fig. 1) where most genes are slightly divergent and only a few are completely different.

Profile Clustering. The information on presence and absence of genes based on the CGH results was used to group genes based on their distribution patterns. The analysis used was essentially identical to that used for phylogenomic profile analysis (22). Each gene was assigned a binary profile based on its presence/absence across the different strains, with presence determined by a Cy3/Cy5 ratio <3.0 and absence ≥3.0. The gene profiles were then clustered by using the single-linkage clustering algorithm with column weighting (all with default settings) of CLUSTER (http://rana.lbl.gov). The CLUSTER program also groups the strains (columns) based on similarity of gene profiles. Clusters of genes and strains were viewed by using TREEVIEW (http://rana.lbl.gov).

Phylogeny. The sequences of genes from the different strains were aligned by using CLUSTALW (23) and trimmed to remove ambiguously aligned regions. Phylogenetic trees of individual genes and of concatenated alignments of multiple genes were inferred by using maximum likelihood methods of PAUP* 4.0 b10 (Sinauer, Sunderland, MA). Bootstrap analysis was carried out by using PAUP* as well. The possibility of recombination among strains was examined by using analysis of sequence variation using SIMPLOT (http://sray.med.som.jhmi.edu/RaySoft/SimPlot/) and analysis of phylogenetic heterogeneity by using MACCLADE (Sinauer).

Results

General Genome Features. The genome consists of a circular chromosome of 2,160,267 bp with a G+C content of 35.7%. Base pair one of the chromosome was assigned within the putative origin of replication. The genome contains 80 tRNAs, 7 rRNAs, and 3 sRNAs. Approximately 78% of the 2,175 predicted genes (Table 1, which is published as supporting information on the PNAS web site, www.pnas.org) are transcribed in the same direction as that of DNA replication, a feature also observed in S. pneumoniae and other low-GC Gram-positive organisms (8) (Fig. 1 and Fig. 4, which is published as supporting information on the PNAS web site). Biological roles were assigned to 1,333 (61%) of the predicted proteins according to the classification scheme adapted from Riley (24). Another 623 predicted proteins (29%) matched proteins of unknown function, and the remaining 219 (10%) had no database match. The expression of 50 of these hypothetical proteins was confirmed by Western blot analysis, and the proteins were annotated as “proteins of unknown function.” A total of 339 paralogous protein families were identified in strain 2603, containing 941 predicted proteins (43% of the total).

Polysaccharides. S. agalactiae produces two surface polysaccharides: the cell wall-associated group B carbohydrate common to all S. agalactiae strains and the type-specific capsular polysaccharide. A
cluster of 13 adjacent genes (SAG1410-SAG1424) was tentatively identified as encoding enzymes required for synthesis of the group B carbohydrate, a complex multiantennary structure of rhamnose, glucitol phosphate, N-acetylglucosamine, and galactose. Predicted proteins encoded within this cluster include seven putative glycosyltransferases, four of which are similar to rhamnosyltransferases in other streptococcal species; a putative dTDP-L-rhamnose synthase; and proteins involved in glucitol synthesis. All nine recognized *S. agalactiae* capsular polysaccharide types contain sialic acid residues as part of their repeating unit structure, a feature that contributes to virulence by inhibiting activation of the alternative complement pathway (25). The type V capsular polysaccharide gene cluster consists of 18 genes. A region of glycosyltransferases and related proteins (SAG1162-SAG1170) that direct the synthesis of the type V polysaccharide repeat unit is flanked on either side by genes that are conserved in all known *S. agalactiae* capsule serotypes. Downstream of this region are genes that encode enzymes for the biosynthesis and activation of sialic acid (SAG1158-SAG1161). Upstream of the serotype-specific region are genes (SAG1171-SAG1175) found not only in all nine *S. agalactiae*...
Capsular serotypes but also in a variety of other polysaccharide-producing streptococci. The sequence of the capsule gene cluster in strain 2603 V/R is consistent with that in type V strain CNCTC 1/82 previously deposited in GenBank (AF349539).

**Surface Proteins.** Several genes identified as encoding *S. agalactiae* surface proteins and secreted products are potential virulence factors or targets of protective immunity: *Sip* (SAG0032) (26), CAMP factor (SAG2043), R5 protein (SAG1331), streptococcal enolase (SAG0628), hyaluronidase (SAG1197) (27) and hemolysin/cytolysin (clyE, SAG0669) (28). Two genes, *lmb* encoding laminin-binding protein (SAG1234) and *scpB* encoding Csa peptidase (SAG1236), are adjacent to a group II intron as previously described (29, 30), but the peptidase is frame-shifted near its C terminus in strain 2603 V/R. A putative protease similar to the Csa peptidase (SAG0416) is located elsewhere in this genome. Surface protein Rib (SAG0433), a member of the tandem-repeat-containing family of *S. agalactiae* surface proteins (31), was identified in this genome. The 14 tandem repeats in the gene here encodes 67 amino acids, 12 fewer than those in the published sequence. The Rib protein has previously been detected predominantly in *S. agalactiae* strains of serotypes II and III, whereas type V *S. agalactiae* strains generally express a related member of the protein family, Alp3 (32).

Prediction algorithms (see Methods and ref. 19) estimate that approximately 650 proteins are membrane-associated or secreted, some of which are potential adhesion factors or virulence-associated determinants. These include 24 proteins predicted to be anchored on the cell wall through their LPxTG motif, 51 lipoproteins, and 177 proteins carrying a signal peptide (Table 2, which is published as supporting information on the PNAS web site). Two novel enzymes are related to metabolism of host sugars or hexosamines, a putative pullulanase (SAG1216) and a neuraminidase-related protein (SAG1932).

The expression of the 650 predicted surface-exposed proteins was tested experimentally. The 291 recombinant proteins successfully expressed were used to immunize mice and the corresponding antibodies were used in Western blot analysis against the total protein extract of the homologous type V strain 2603 V/R. One hundred thirty-nine sera revealed a predominant protein of the expected molecular weight, and all but seven of the proteins were expected in any given intersection are slightly different due to gene duplications in some species.

**Comparative Genomics.** The complete genome sequence is available for two other important human pathogens of the genus *Streptococcus*, *S. pneumoniae* (8) and *S. pyogenes* (36). Although these species colonize different regions of the body, they are all capable of causing severe invasive disease. Hence, it is likely that these species will share some virulence factors, whereas other factors will determine specific colonization, invasion, and disease characteristics. One thousand sixty genes (50%) have homologs in all three species (Fig. 2). *S. agalactiae* shares 176 genes with *S. pneumoniae* and 225 genes with *S. pyogenes*, whereas *S. pneumoniae* and *S. pyogenes* share only 74 genes. Finally, 683 genes are unique to *S. agalactiae*. Conservation of gene synteny is more pronounced between *S. agalactiae* and *S. pyogenes* (828 genes in 35 regions spanning ~1,104 kb) than between *S. agalactiae* and *S. pneumoniae* (128 genes in 9 regions spanning ~131 kb).

To place the variation in a wider context, the 1,060 genes shared by the three streptococcal species were compared with the gene complements of all of the completely sequenced genomes. Using a more stringent cutoff, 12 *Streptococcus*-specific genes were identified (Table 3, which is published as supporting information on the PNAS web site). Using the same approach, 315 of the 683 genes that *S. agalactiae* did not share with the other two streptococci were not similar to genes from other completely sequenced genomes. These include six proteins predicted to be anchored on the cell wall (SAG0677, SAG0771, SAG1052, SAG1331, SAG1473, and SAG1996), three of the capsule-related genes (SAG1163, SAG1167, and SAG1168), six transcriptional regulators, and four genes of the *cyl* operon (SAG0663-SAG0673) essential for *S. agalactiae* hemolytic activity and production of pigment (28). The rest of the 315 proteins include 219 hypothetical proteins with no similarity to other proteins in databases.

Metabolic differences between the streptococci inferred from genome analysis may reflect the availability of compounds in their respective microenvironments in the human host. *S. agalactiae* has genes required for the synthesis of arginine, aspartate, and citrulline, whereas it apparently lacks the systems the other two streptococci have for the metabolism of fucose, lactose, mannitol, raffinose, lycine, and threonine. *S. pneumoniae* was notable for its large array of sugar phosphoenolpyruvate-dependent phospho-
transferase system (PTS) transporters, which may be important for utilization of host-derived carbohydrates and for recycling of capsule constituents (8). Although *S. agalactiae* has uptake and metabolism systems for ribose and gluconate and a complete operon for the metabolism of glucuronic acid, it lacks the uptake systems for lactose, cellobiose, mannitol, and the putative fucose-related system present in *S. pneumoniae*, and it also lacks a raffinose ABC transporter. Instead, *S. agalactiae* has a galactitol PTS, a ribose ABC transporter, a gluconate transporter, and a sodium ion-driven glucuronide uptake system, correlating with the novel pathways present in *S. agalactiae*. *S. agalactiae* also possesses two additional peptidase ABC uptake systems not present in *S. pneumoniae*, which together with the presence of many secreted peptidases may imply that host proteins/peptides are an important nutrient source for this organism. *S. agalactiae* lacks two of the ABC ferric chelate transporters present in *S. pneumoniae*, but instead encodes two Nramp transporters for manganese or iron uptake, suggesting distinct mechanisms for acquisition of these ions that may be important in virulence. These metabolic and transport systems specific to *S. agalactiae* probably relate to adaptation to distinct niches in its human and animal hosts.

Seventeen two-component signaling systems are identified in the *S. agalactiae* genome, one of which (SAG2127-SAG2128) is absent from the other two streptococci. Another one (SAG1921-SAG1922) is shared with *S. pyogenes* only.

Many of the 315 genes specific to *S. agalactiae* are located in regions likely to constitute mobile genetic elements. Two of these regions resemble prophages (SAG0545-SAG0610 and SAG1835-SAG1885) displaying a mosaic structure with segments most similar to different bacteriophages, a pattern that suggests frequent recombination events. PblA and PblB are adhesins from a *Streptococcus mitis* prophage where they contribute to endocarditis by binding to human platelets (37, 38). Their orthologs in *S. agalactiae* are located on separate prophages and display a different protein structure. Another region (SAG1247-SAG1299) encodes a putative conjugative transposon that carries genes for cadmium efflux and mercury resistance.

Finally, 130 genes in *S. agalactiae* (Table 4, which is published as supporting information on the PNAS web site) were probably duplicated after the divergence of this species from other lineages for which complete genomes are available. Such lineage-specific gene duplications may reveal species-specific adaptations because gene duplication is frequently accompanied by functional diversification and divergence. These duplications include glycosyl transferases, sortases, proteins anchored on the cell wall, β-lactam resistance factors, and many hypothetical proteins.

**CGH and Phylogeny.** Little is known of the genome differences between the serotypes or of the genome variation within serotypes. To shed light on this variation, CGH (Fig. 1) using DNA microarrays were performed between the sequenced type V strain 2603 V/R and 19 other *S. agalactiae* strains of multiple serotypes (Table 5, which is published as supporting information on the PNAS web site). The CGH detected 1,698 genes in all of the strains, whereas 401 genes from strain 2603 V/R (18% of the gene complement) were not detected in at least one other strain, suggesting that they are absent or significantly divergent in those strains. Two hundred sixty (38%) of the 683 genes specific to *S. agalactiae* when compared with the other two streptococci (Fig. 2), including virulence determinants and surface proteins, vary among *S. agalactiae* strains, whereas only 47 (4%) of the genes common to all three streptococcal species, including 5 of the 6 sortases identified in the genome, vary among strains. Thus, the *in silico* analysis of genes shared by the streptococci that are not expected to vary among this genus is consistent with the CGH analysis. Forty-four (25%) of the genes shared by *S. agalactiae* and *S. pneumoniae* and 44 (20%) of those shared by *S. agalactiae* and *S. pyogenes* vary in the CGH analysis. The first set contains many glycosyl transferases and proteins carrying a cell-wall anchor, whereas the second set displays many phase-related genes. One hundred thirty-six of the 315 genes unique to *S. agalactiae* when compared with all sequenced genomes vary among strains. These include R5, three capsular genes, two cell wall-anchored proteins, and three transcriptional regulators.

Three hundred sixty-four (91%) of the 401 varying genes correspond to 15 regions containing 5 or more contiguous genes. Ten of these regions display an atypical nucleotide composition in strain 2603 V/R (Fig. 1), consistent with the possibility that they were laterally transferred into this strain. Two of the largest regions (region 4, a prophage and region 7, similar to Tn916 from *Enterococcus faecalis*) are flanked by insertion sequence elements. The 15 regions contain many proteins predicted to be anchored on the cell wall or surface exposed, including Rib (region 3), sortases, glycosyl transferases, the capsule locus (region 9, divergent in all strains but the other type V strain CJB111), and phage-related genes. Region 14 is unique to *S. agalactiae* and spans 33 genes (SAG1989-SAG2021), including 25 proteins of unknown function, some of which carry a cell-wall anchor. It is flanked by an ISL3 transposase and displays an atypical nucleotide composition. Region 1, unique to *S. agalactiae*, is a possible plasmid or remnant of a phage (SAG0218-SAG0238), contains mostly hypothetical proteins, and is flanked by a site-specific recombinase. Region 8 is specific to *S. agalactiae*, comprises 20 proteins of unknown function (SAG1018-SAG1037), most of which are predicted to be membrane associated or secreted, and displays an atypical nucleotide composition.

The CGH results were analyzed by profile clustering where genes are grouped based on their distribution patterns (Fig. 5, which is published as supporting information on the PNAS web site). Sixteen clusters of five or more contiguous and noncontiguous genes comprising a total of 300 genes were identified (Table 6, which is published as supporting information on the PNAS web site). Several clusters correspond to regions of contiguous genes described above. Some clusters of genes that do not share sequence
some similarity and are located at different loci in the genome display an identical profile. For instance, a cluster of genes containing a surface antigen (SAG0674-SAG0681) follows the same distribution as another cluster containing only hypothetical proteins (SAG0247-SAG0249). A putative pathogenicity protein (SAG2063) also clusters with a region containing several glycosyl transferases and Sec proteins (SAG1447-SAG1462).

Profile clustering was also used to group strains based on similarity of gene content (Fig. 5). This method is not ideal for inferring relationships among strains, because the genes specific to strains other than 2603 V/R were not represented on the microarray and were therefore not interrogated. To generate a more accurate picture of the strain relationships, the sequences of 19 genes from each of 11 S. agalactiae strains were determined after PCR amplification and used for phylogenetic analyses. The set comprised 8 housekeeping genes and 11 genes coding for proteins predicted to be surface-exposed (Table 7, which is published as supporting information on the PNAS web site). Phylogenetic trees were inferred for the complete set of 19 genes and for the subsets of housekeeping and surface-exposed genes. Because the branching patterns in all three trees were identical, only the tree of the 19 genes is shown in Fig. 3. The degree of polymorphism of the housekeeping and the surface-exposed genes is similar (≈1 variable site among all of the strains per 100 bp). Analysis of this variation showed no evidence for major recombination events between the strains. There were no long stretches of polymorphic sites that strongly supported other trees (analysis with MACCLADE), and there were no crossover events in plots of sequence similarity between strains (analysis with SIMPLOT).

Some strain groupings (clades) generated by phylogenetic analysis were similar to clusters from the profile analysis (type III strains M781, M732 and COH1; type Ia strain 909 and nontypable strain CJ8110), whereas others were different, possibly because of the aforementioned problems with the profile clustering. In both the phylogenetic analysis and the profile clustering, there is serotype-dependent and -independent clustering (Figs. 3 and 5). The presence of strains of the same serotype in different clades or clusters could be due to lateral gene transfer. Previous studies showed that a single gene in the capsule gene locus converts serotype Ia to III (39). Serotype switching during natural infection was shown in S. pneumoniae (40–42) and in Neisseria meningitidis (43–45), which may result from selective pressure of host immunity and could lead to convergent or parallel evolution. Serotype-independent clustering supports the possibility of capsule switching, although such events have not yet been demonstrated to occur spontaneously in S. agalactiae.

Conclusion

The S. agalactiae genome reveals substantial similarity with those of the related human pathogens S. pyogenes and S. pneumoniae. S. agalactiae differs from the other streptococci in several metabolic pathways and related membrane transport systems that probably relate to adaptation to distinct niches in its human and animal hosts. Comparison with all characterized bacterial genomes identified genes unique to S. agalactiae that are expected to play a role in colonization or disease: surface proteins, capsule synthesis genes, hemolysin, and several transcriptional regulators. Many of these are associated with mobile elements, including bacteriophages, transposons, and insertion sequences, an observation that supports acquisition of virulence traits from other species. The presence of more than 100 genes probably duplicated recently suggests evolution of additional species-specific functions. Genetic heterogeneity among S. agalactiae strains, even of the same serotype, provides evidence that these mechanisms of acquisition, duplication, and reassortment have produced the genetic diversity within the species that has permitted S. agalactiae to adapt to new environmental niches and to emerge as a major human pathogen.

We thank O. White, S. Angiuli, L. Umayam, J. Peterson, M. Wu, M. Heaney, S. Lo, M. Holmes, B. Lee, R. Karamchedu, and V. Sapiro for informatics support at The Institute for Genomic Research (TIGR) and the TIGR faculty and sequencing core for expert advice and assistance. We also thank G. Orefici and M. Oggioni for isolation and characterization of the sequenced strain. This work was supported by Chiron Corporation and National Institutes of Health Grants AI23339, AI38424, and AI24940, and Contract AI75536.