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Complete Genome Sequence and Analysis of the Multiresistant Nosocomial Pathogen *Corynebacterium jeikeium* K411, a Lipid-Requiring Bacterium of the Human Skin Flora

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***Corynebacterium jeikeium* is a "lipophilic" and multidrug-resistant bacterial species of the human skin flora that has been recognized with increasing frequency as a serious nosocomial pathogen. Here we report the genome sequence of the clinical isolate *C. jeikeium* K411, which was initially recovered from the axilla of a bone marrow transplant patient. The genome of *C. jeikeium* K411 consists of a circular chromosome of 2,462,499 bp and the 14,323-bp bacteriocin-producing plasmid pKW4. The chromosome of *C. jeikeium* K411 contains 2,104 predicted coding sequences, 52% of which were considered to be orthologous with genes in the *Corynebacterium glutamicum*, *Corynebacterium efficiens*, and *Corynebacterium diphtheriae* genomes. These genes apparently represent the chromosomal backbone that is conserved between the four corynebacteria. Among the genes that lack an ortholog in the known corynebacterial genomes, many are located close to transposable elements or revealed an atypical G+C content, indicating that horizontal gene transfer played an important role in the acquisition of genes involved in iron and manganese homeostasis, in multidrug resistance, in bacterium-host interaction, and in virulence. Metabolic analyses of the genome sequence indicated that the "lipophilic" phenotype of *C. jeikeium* most likely originates from the absence of fatty acid synthase and thus represents a fatty acid auxotrophy. Accordingly, both the complete gene repertoire and the deduced lifestyle of *C. jeikeium* K411 largely reflect the strict dependence of growth on the presence of exogenous fatty acids. The predicted virulence factors of *C. jeikeium* K411 are apparently involved in ensuring the availability of exogenous fatty acids by damaging the host tissue.**

During the last few years, there have been an increasing number of scientific publications related to the clinical microbiology and antimicrobial susceptibility of pathogenic corynebacteria. At least two different features have contributed to this development: (i) there are a large number of patients with immunosuppressive diseases or other risk factors, whose diagnosis and therapy have become ever more intensive and invasive, resulting in better growth conditions for nosocomial pathogens, and (ii) so-called nondiphtherial corynebacteria, whose pathogenic potential was initially underestimated, are now recognized with increasing frequency as opportunistic human pathogens (23). The most notable human pathogen of the genus *Corynebacterium* is obviously exotoxin-producing *Corynebacterium diphtheriae*, the causative agent of the acute, communicable disease diphtheria (42). Additionally, *Corynebacterium jeikeium*, the most frequently recovered medically significant corynebacterial species at intensive care facilities, has been recognized as a serious nosocomial pathogen (23).

Johnson and Kaye (30) first drew attention to the association of bacterial endocarditis following cardiac surgery with the isolation from blood cultures of nondiphtherial corynebacteria, which were typically multiresistant against antimicrobial agents. These bacteria were tentatively designated "group JK corynebacteria" by the Centers for Disease Control and Prevention in Atlanta, Ga. (63), and later were taxonomically delineated as *C. jeikeium* (27). Subsequent reports have established that *C. jeikeium* is the causative agent of a variety of severe nosocomial infections, most frequently associated with immunocompromised patients with malignancies, in-place medical devices, breaks in the skin barrier, and therapy with broad-spectrum antibiotics (23). A high mortality rate was documented in the case of *C. jeikeium* sepsis in hematological patients (80). A new trend, however, has been the increasing recognition of *C. jeikeium* infections in immunocompetent hosts. *C. jeikeium* is considered part of the normal flora of the human skin, and colonization is predominantly found in the

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axillary, inguinal, and perineal areas, particularly of inpatients (18, 81).

Antimicrobial susceptibility studies revealed that a preponderant proportion of the reported *C. jeikeium* isolates are substantially multiresistant against clinically relevant antibiotics and that only glycopeptides, such as vancomycin and teicoplanin, remain universally active against this species (4, 38, 79). This emergence of multiresistant phenotypes largely limits the therapeutic options and has thus tremendous consequences for successful treatment of *C. jeikeium* infections, especially in immunocompromised patients. Up to now, the molecular basis for multiresistance of *C. jeikeium* against antimicrobial agents remained unexplained. A few studies, however, have investigated the presence of plasmids in *C. jeikeium*, indicating that the multiresistant phenotype is encoded by the bacterial chromosome rather than associated with extrachromosomal DNA (36, 58, 77). Accordingly, it was concluded that the multiresistance of *C. jeikeium* is a consequence of the accumulation of specific genetic events and/or may involve a set of nonspecific mechanisms, such as increased antibiotic efflux or changes in the permeability of the corynebacterial cell wall.

In this report, we present the complete genome sequence and bioinformatics analysis of the multiresistant clinical isolate *C. jeikeium* K411, which was originally recovered from the axilla of a bone marrow transplant patient who received immunosuppressive therapy and broad-spectrum antibiotics (34). The knowledge on the genome architecture of *C. jeikeium* and the characterization of its complete gene repertoire provide a fundamental step in understanding not only the cellular physiology and lifestyle but also the molecular and biochemical basis for multiresistance as well as the pathogenic potential of this clinically important species. For comparative genomic analysis, we took advantage of the availability of the complete genome sequence of the human pathogen *C. diphtheriae* (16) and those of the nonpathogenic species *Corynebacterium glutamicum* (32) and *Corynebacterium efficiens* (54).

MATERIALS AND METHODS

Genome cloning and whole-genome shotgun sequencing. *C. jeikeium* K411 was obtained as a lyophilized culture from the National Collection of Type Cultures (London, United Kingdom) and was routinely cultured on BYT complex medium containing 1% (vol/vol) Tween 80 (77). The genome of *C. jeikeium* K411 was sequenced by a shotgun strategy using large-insert DNA libraries as scaffolds (75). Two genomic shotgun libraries of *C. jeikeium* K411 with insert sizes ranging from 1.5 to 3 kb and from 5 to 6 kb were constructed in the pGEM-T Easy vector system (Promega, Mannheim, Germany) by MWG (Ebersberg, Germany). A fosmid library of *C. jeikeium* K411 was constructed in the pCC1FOS vector by means of the Fosmid Library Production Kit (Epicentre, Madison, WI), and additionally, a bacterial artificial chromosome (BAC) library of the purified 70-kb size fraction of the *C. jeikeium* K411 chromosome was established in pBeloBAC11 as described previously (75). Subsequently, a total of 24,454 terminal sequences of the shotgun libraries (giving a sevenfold genome coverage) were determined with an ABI 3730xl DNA analyzer and dye terminator chemistry, using template DNA previously amplified with the TempliPhi DNA sequencing template amplification kit (Amersham Biosciences, Freiberg, Germany). Moreover, 634 terminal fosmid sequences and 950 terminal BAC sequences were determined and combined with the shotgun reads. Remaining gaps in the assembled genome sequence of *C. jeikeium* K411 were closed by primer walking on shotgun clones, fosmids, and BACs (IIT GmbH, Bielefeld, Germany).

Genome assembly and bioinformatics analysis. Assembly of the nucleotide sequence data of *C. jeikeium* K411 was performed by means of the recently described bioinformatics pipeline along with the new software tool BioMake (31). The accuracy of the final DNA sequence assembly was confirmed by automatically generating high-resolution fosmid and BAC maps of the *C. jeikeium* K411 chromosome. Annotation of the assembled genome sequence was carried out with the genome annotation system GenDB (44). A combined gene prediction strategy was applied by means of the GLIMMER 2.0 system and the CRITICA program suite (43) along with postprocessing by the RBSfinder tool (71). The deduced proteins were functionally characterized by automated searches in public databases, including SWISS-PROT and TrEMBL (10), Pfam (5), KEGG (33), and COG (72). Proteins orthologous between *C. jeikeium* and *C. glutamicum* (32), *C. efficiens* (54), and *C. diphtheriae* (16) were identified as best BLASTP matches by using a $1 \times e^{-50}$ E-value cutoff (2).

Nucleotide sequence accession numbers. The annotated genome sequence of *C. jeikeium* K411 was deposited in the EMBL database with accession number CR931997. The annotated pKW4 plasmid sequence is available under accession number AF401314.

TABLE 1. General features of the *Corynebacterium jeikeium* K411 genome

Feature	Chromosome	pKW4
Total size (bp)	2,462,499	14,323
G+C content (%)	61.4	53.8
No. of coding sequences	2,104	16
No. of pseudogene coding sequences	68	1
Coding density (%)	89.2	71.2
Average gene length (bp)	1,030	639
No. of rRNAs	3 × (16S-23S-5S)	
No. of tRNAs	50 (43 different)	
No. of other stable RNAs	2	
No. of insertion sequences	92	1
No. of transposons	6	
No. of *iap* repeats[a]	61	

[a] The *iap* repeat signature of *C. jeikeium* K411 is similar to that of *Escherichia coli* K-12 (48).

RESULTS AND DISCUSSION

General features of the *C. jeikeium* K411 genome and its architecture. The genome of *C. jeikeium* K411 consists of a circular chromosome of 2,462,499 bp and of the circular bacteriocin-producing plasmid pKW4 (34) with a size of 14,323 bp. General features deduced from the genome sequence of *C. jeikeium* K411 are summarized and graphically depicted in Table 1 and Fig. 1, respectively. The mean G+C content of the *C. jeikeium* K411 genome is 61.4%, which is close to the value of 60.7% that was previously determined by means of a thermal denaturation method (27). Analysis of the GC skew (25) of the *C. jeikeium* K411 chromosome clearly indicated a bidirectional replication mechanism (Fig. 1). The putative origin of replication was predicted downstream of the *dnaA* gene (*jk0001*) by similarity to the location of *oriC* in *Mycobacterium smegmatis* (66). The bioinformatics identification of a potential *dif* locus involved in chromosome dimer resolution by the site-specific recombinases XerC (*jk1177* [*xerC*]) and XerD (*jk0873* [*xerD*]) allowed us to predict the region of termination of replication at around kb 1109 of the genomic map. This location of the replication terminus appears to be skewed from the expected position of 180° from *oriC*, resulting in a 244-kb size difference between the replichores of the *C. jeikeium* K411 chromosome.

Three sets of 16S, 23S, and 5S rRNA operons were identified, all of which are located on the leading strand of the shorter right replichore of the *C. jeikeium* K411 chromosome (Fig. 1). In comparison, the genomes of *C. glutamicum*, *C. efficiens*, and *C. diphtheriae* contain six and five rRNA operons,

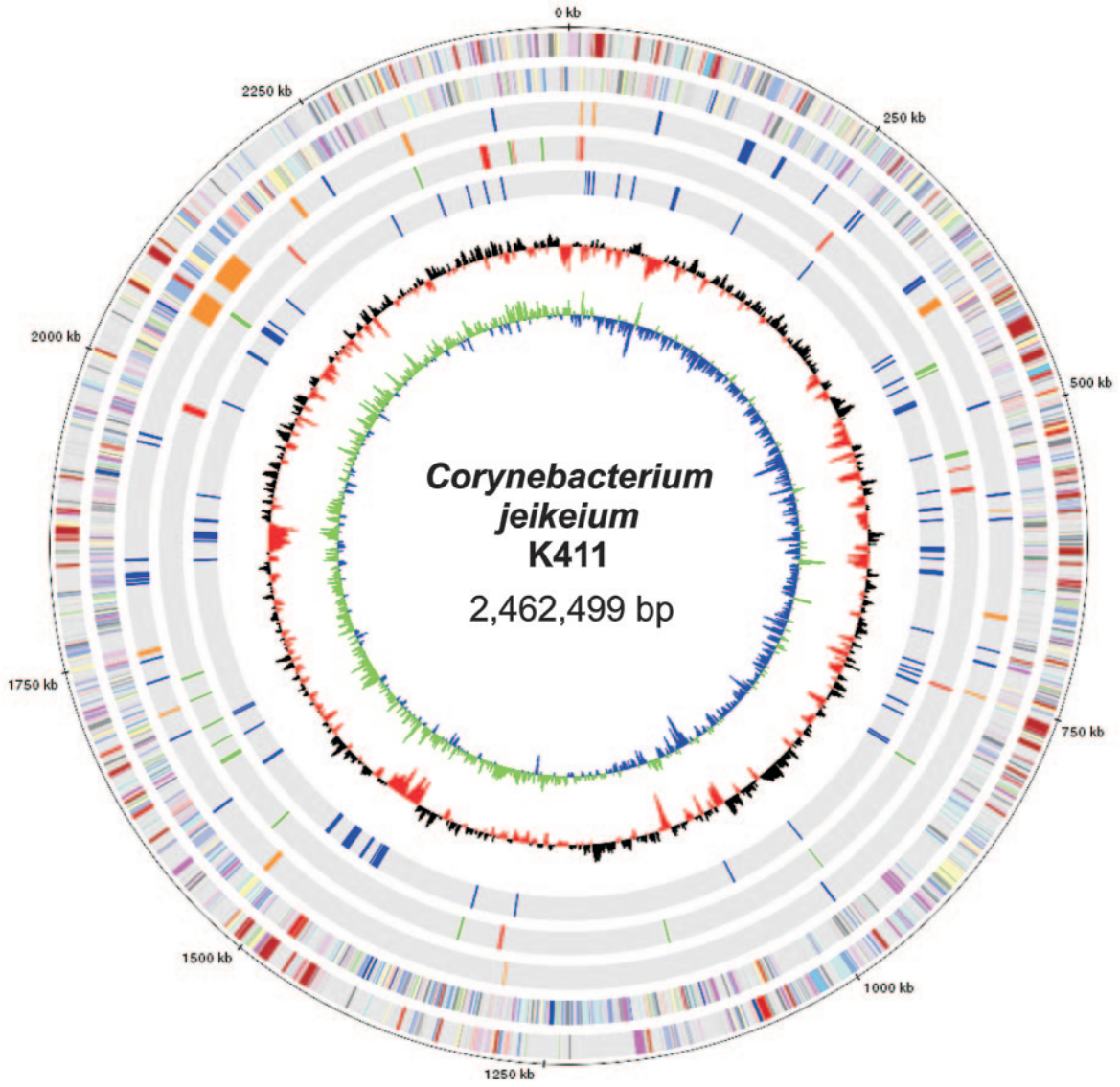

FIG. 1. Circular representation of the *C. jeikeium* K411 chromosome. From the outer circle to the inner circle: circle 1, DNA base pair numbers; circles 2 and 3, genes transcribed clockwise and counterclockwise, respectively (genes are marked according to the assigned COG classes [72]); circle 4, genes involved in fatty acid metabolism (blue) and in iron and manganese homeostasis (orange); circle 5, genes potentially involved in multidrug resistance (green) and predicted virulence factors (red); circle 6, insertion sequences; circle 7, G+C content with values positively deviating from the median shown in black; circle 8, GC skew. The G+C content and GC skew were calculated within a 3,000-bp window along with a sliding window of 1,000 bp.

respectively, which are distributed on the leading strands of both replichores. A total of 50 tRNA genes were detected in *C. jeikeium* K411, recognizing 43 out of the 61 possible sense codons. The number of tRNA genes in *C. jeikeium* K411 is thus slightly smaller than those deduced from the genome sequences of *C. glutamicum* and *C. diphtheriae* (16, 32). Furthermore, 2,104 coding sequences (CDS), of which 68 represent pseudogenes, were identified in the chromosome of *C. jeikeium* K411. The shorter right replichore carries 931 CDS (44.2%), whereas 1,173 CDS (55.8%) are present on the left replichore of the chromosome. A significant bias in gene orientation was observed, since 63% of the CDS are carried on the leading strand and thus transcribed in the same orientation as the movement of the replication fork. This value is identical to that calculated for the *C. diphtheriae* chromosome but significantly higher than those deduced from the genome sequences of the nonpathogenic species *C. glutamicum* (59%) and *C. efficiens* (54%).

The predicted proteins of the *C. jeikeium* K411 genome were compared by best BLASTP matches (2) with the complete sets of proteins encoded by *C. glutamicum*, *C. efficiens*, and *C. diphtheriae* (Fig. 2). Accordingly, 1,089 genes (52%) of the *C. jeikeium* K411 chromosome were considered orthologous with genes from *C. glutamicum*, *C. efficiens*, and *C. diphtheriae*, apparently representing the conserved chromosomal backbone in these four species, whereas 367 genes (17%) were considered orthologous with genes in only one or two of the other corynebacteria. Further genome comparisons revealed a highly

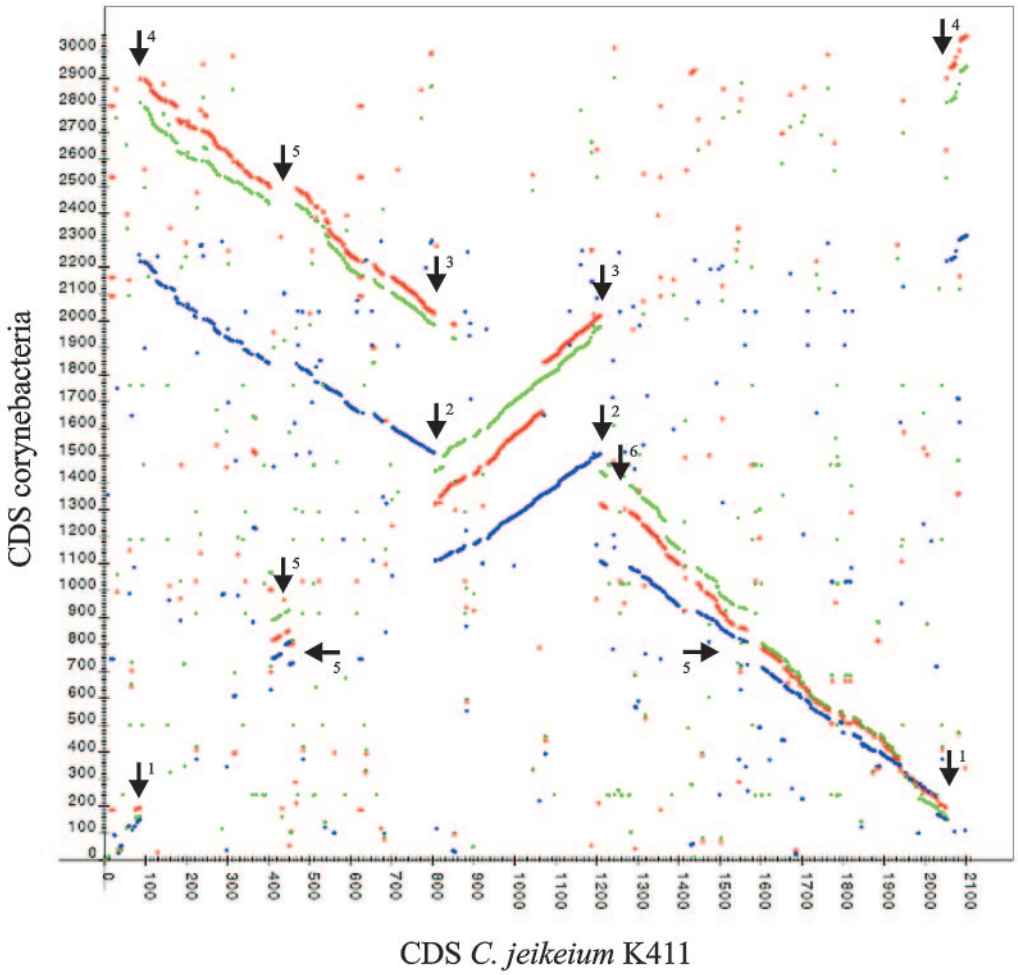

FIG. 2. Synteny between the *C. jeikeium* K411 genome and the *C. glutamicum* ATCC 13032, *C. efficiens* YS-314, and *C. diphtheriae* NCTC 13129 genomes. The diagram shows *x-y* plots of dots forming syntenic regions between the corynebacterial genomes. Each dot represents a *C. jeikeium* CDS having an ortholog in another corynebacterial genome, with coordinates corresponding to the CDS number in each genome. The orthologs were identified by best BLASTP matches of amino acid sequences deduced from CDS 2104 of the *C. jeikeium* K411 chromosome with proteins encoded by *C. glutamicum* (CDS 3002; red dots), *C. efficiens* (CDS 2950; green), and *C. diphtheriae* (CDS 2320, blue). Apparent breakpoints in synteny revealing genomic inversions in the *C. jeikeium* K411 chromosome are specifically marked by arrows 1 to 4. Differences in synteny originating from DNA translocation are indicated by pairs of vertical and horizontal arrows (arrows 5). Additionally, the position of an apparent insertion of DNA in the *C. jeikeium* K411 genome (*jk1226* to *jk1265*) is shown (arrow 6).

conserved order of the orthologous genes between the sequenced corynebacteria and only 10 apparent breakpoints of synteny, indicating the rarity of genome rearrangements in these species (Fig. 2). The apparent breakpoints are indicative of the insertion of a DNA segment (*jk1226* to *jk1265*) that is flanked by clustered insertion sequences, of the translocation of a small DNA region to kb 489 of the genomic map of *C. jeikeium* K411, and of two distinct inversion events. Whereas no clearly defined boundaries (*~jk0408* to *jk0458*) could be identified in the case of the translocated DNA region (Fig. 2), one breakpoint of synteny regarding the inverted genomic segments was localized in close proximity to clusters of insertion sequences and copy A of the rRNA operons. The detection of rearrangements in the *C. jeikeium* K411 chromosome is interesting since the hitherto-sequenced corynebacteria lack any detectable inversion between the three genome sequences. The only remarkable difference in overall synteny is the presence of a large putative prophage region in the *C. glutamicum* chromosome (32, 54). Although little is known about the mechanisms for the inversion of genomic fragments, it was suggested recently that the absence of the *recBCD* recombination pathway might contribute to the genome stability in corynebacteria or, conversely, to the lack of extensive genome rearrangements (47). The different types of genome rearrangements detected in *C. jeikeium* K411 indicate that a moderate reorganization of the chromosomal architecture can still occur in corynebacteria by means of recombinational mechanisms other than those of the *recBCD* pathway.

Analysis of the locations of the 648 genes (31%) without an ortholog in any of the other sequenced corynebacterial species revealed additional architectural features of the *C. jeikeium* K411 chromosome. The respective genes are more or less evenly dispersed around the *C. jeikeium* K411 chromosome, resulting in approximately 350 interspersed gene regions rang-

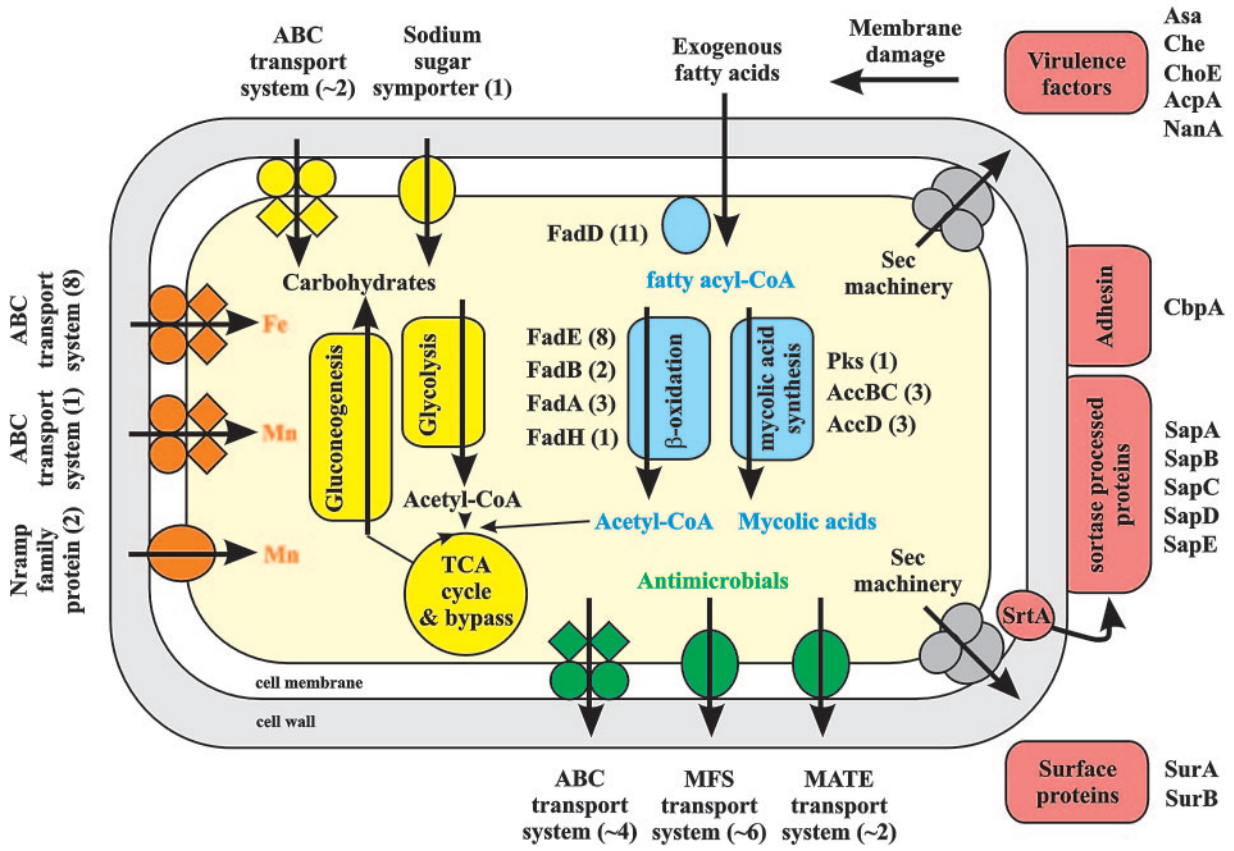

FIG. 3. Overview of prominent metabolic and medically relevant features of *C. jeikeium* K411 deduced from the complete genome sequence. Processes and relevant proteins associated primarily with carbohydrate metabolism (yellow), fatty acid metabolism (blue), iron and manganese uptake (orange), antibiotic efflux (green), and virulence (red) are indicated. Putative transport systems are shown within the cell membrane; the numbers in parentheses indicate the approximate numbers of each type. Structural elements of ABC transporters are depicted as circles for membrane-spanning permeases and diamonds for nucleotide-binding proteins; other transporters are drawn as ovals. Major metabolic pathways are shown in the center of the cell; the numbers in parentheses indicate the numbers of paralogous genes present in the *C. jeikeium* K411 genome. Prominent proteins most likely secreted by the machinery of the general secretory (sec) pathway are shown on the right, along with sortase (SrtA) processed proteins and a predicted collagen adhesin (CbpA), which are anchored to the cell wall. The putative membrane-damaging role of the predicted virulence factors, ensuring the availability of exogenous fatty acids for growth of *C. jeikeium*, is indicated.

ing from 1 to 14 CDS. It should be noted that 194 genes are unique hypothetical CDS which can be considered specific for *C. jeikeium* K411. Moreover, the base compositions of 132 CDS deviate significantly from the mean G+C content of *C. jeikeium* K411, which becomes obvious when inspecting the large variability of the genomic G+C content, which ranges from 42.9 to 71.5% (Fig. 1). Furthermore, many of these genes are located close to insertion sequences, of which 92 were identified in the chromosome of *C. jeikeium* K411 (Table 1). These insertion sequences can be classified into four different groups, including the IS*256*, IS*3*, IS*30*, and IS*110* families (41). It is remarkable that insertion sequences with a high copy number in the *C. jeikeium* K411 chromosome, such as IS*1249*, IS*1513*, IS*3503*, and IS*3504*, were detected previously during nucleotide sequence analysis of corynebacterial plasmids (73, 76, 77). Consequently, the *C. jeikeium* K411 chromosome has retained roughly two-thirds of the ancestral gene equipment of corynebacteria during speciation and microevolution, which moreover includes gene acquisition by horizontal gene transfer. These horizontally transferred genes are mostly organized as genomic islets and apparently represent the flexible gene pool of *C. jeikeium* K411. The flexible gene pools of pathogenic species often include genetic information that may be advantageous under specific growth conditions, such as colonization or infection (26). The genetic and functional compositions of the flexible gene pool along with a characteristic gene loss during speciation and microevolution may therefore be responsible for both the specific metabolic and medically relevant features of *C. jeikeium* K411. Figure 3 schematically depicts the most prominent characteristics of the *C. jeikeium* K411 genome, which are presented in detail below.

Metabolic features deduced from the *C. jeikeium* K411 genome sequence. Early taxonomic studies regarding the carbohydrate utilization of *C. jeikeium* already indicated that only glucose and galactose are substantially catabolized by most isolates (27, 63). Genes necessary for the conversion of glucose (*jk0728* [*glk*]) and galactose (*jk0290* [*galT*], *jk0291* [*galK*], *jk1098* [*galE*], *jk1525* [*galU*], and *jk0468* [*pgm*] into glucose 6-phosphate are apparently present in the genome sequence of *C. jeikeium* K411. On the other hand, components of phosphoenolpyruvate:carbohydrate phosphotransferase systems (PTSs) are not encoded by *C. jeikeium* K411. PTSs play a key

role in the uptake and phosphorylation of numerous carbohydrates in many bacteria and are also present in the genomes of *C. glutamicum* (56), *C. efficiens* (54), and *C. diphtheriae* (57). PTSs are involved, moreover, in monitoring the bacterial environment to choose between various carbon sources and their substrates, and components are known to operate in the regulation of a number of metabolic pathways (56). The absence of PTSs in *C. jeikeium* K411 obviously implies that its metabolism is devoid of such a type of global carbon regulation. Carbohydrate uptake in *C. jeikeium* K411 might be mediated by a putative sodium:solute (sugar) symporter (*jk0288*) or by putative sugar-specific ATP-binding cassette (ABC) transporters (*jk2036*, *jk2037*, and *jk1684* to *jk1686*). In principle, the restricted carbohydrate utilization pattern and the lack of PTSs might reflect an adaptation of *C. jeikeium* to the availability of nutrients in the predominantly colonized areas of the human skin.

Further analyses of carbohydrate metabolism in *C. jeikeium* K411 revealed a complete set of genes involved in glycolysis and the pentose phosphate pathway. The *C. jeikeium* K411 genome encodes phosphoenolpyruvate carboxylase (*jk0998* [*ppc*]), which obviously fulfills the sole anaplerotic reaction by the irreversible carboxylation of phosphoenolpyruvate to oxaloacetate, whereas gluconeogenesis is accomplished by phosphoenolpyruvate carboxykinase (*jk0151* [*pck*]) and fructose-1,6-bisphosphatase (*jk1453* [*fbp*]). Enzymatic conversions around the pyruvate node of the *C. jeikeium* K411 metabolism are thus less complex than those present in the soil bacterium *C. glutamicum* (32).

The tricarboxylic acid cycle and the glyoxylate bypass of *C. jeikeium* K411 appear to be complete, with the exception of the *sucCD* genes encoding α and β subunits of the succinyl coenzyme A (succinyl-CoA) synthetase. In *C. jeikeium* K411, the conversion between succinyl-CoA and succinate might be catalyzed by a putative succinyl-CoA:CoA transferase (*jk0384* [*cat1*]) that showed similarity to the Cat1 protein of *Clostridium kluyveri*. The Cat1 protein was proposed to catalyze the reversible transfer of the CoA moiety from several acyl-CoAs to acetate and revealed significant succinyl-CoA:CoA transferase activity in *Escherichia coli* (68). Thus, it seems possible that *C. jeikeium* K411 is able to compensate for the substrate-level phosphorylation reaction mediated by succinyl-CoA synthetase by utilizing an acyl-CoA:CoA transfer to form succinate and acetyl-CoA.

Since *C. jeikeium* is an aerobically growing bacterium, with no detectable growth occurring under strictly anaerobic conditions (27), it requires oxygen as exogenous electron acceptor for respiration. Components constituting a nonbranched respiratory chain are apparently present in *C. jeikeium* K411 along with complete menaquinone and heme biosynthesis pathways. The terminal oxidase of the respiratory chain of *C. jeikeium* K411 most likely consists of both cytochrome bc_1 (*jk0720* to *jk0722* [*qcrCAB*]) and cytochrome aa_3 oxidases (*jk0716* [*ctaC*], *jk0717* [*ctaF*], *jk0719* [*ctaE*], and *jk0481* [*ctaD*]) that may form an exceptional cytochrome bc_1-aa_3 supercomplex, as shown recently for *C. glutamicum* (11). The genes encoding the eight subunits of the F_1F_0-ATP synthase that is essential for ATP generation by oxidative phosphorylation are present (*jk1334* to *jk1341* [*atpBEFHAGDC*]) and are organized in a putative operon, whereas the conserved *atpI* gene of unknown function is apparently not carried by the *C. jeikeium* K411 genome.

Furthermore, all de novo biosynthesis pathways for amino acids are completely present in the *C. jeikeium* K411 genome, as are the anabolic pathways for purine and pyrimidine nucleotides. The biosynthesis pathways for most vitamins and cofactors are also complete. Conversely, biotin biosynthesis of *C. jeikeium* K411 is apparently incomplete, since only the *bioB* gene (*jk0682*) is present in the genome sequence. Biotin uptake in *C. jeikeium* K411 might be mediated by a member of the BioY protein family (*jk1118*) that was recently suggested to represent transporters of biotin or some biotin precursors (64). On the other hand, the *C. jeikeium* K411 genome encodes the components of a glycine cleavage enzyme system (*jk0209* [*gcvP*], *jk0210* [*gcvT*], *jk0211* [*gcvH*], and *jk1936* [*lpd*]) that is not present in the genomes of *C. glutamicum*, *C. efficiens*, and *C. diphtheriae*. The presence of these genes strongly suggests that *C. jeikeium* K411 utilizes a GCV enzyme complex for the important balancing of the cells' glycine and one-carbon unit requirements by converting the excess glycine to C_1 units.

Fatty acid metabolism and the "lipophilic" phenotype of *C. jeikeium* K411. *C. jeikeium* belongs taxonomically to the group of "lipophilic" corynebacteria whose growth is generally enhanced by the addition of lipids to the culture medium (23). Good growth of *C. jeikeium* isolates was observed, for instance, in BYT medium containing 1% Tween 80, in comparison to no detectable growth in nonsupplemented medium (77). Metabolic analysis of the *C. jeikeium* K411 genome sequence provides a molecular genetic explanation for the "lipophilic" phenotype, which most likely originates from the lack of a type I fatty acid synthase gene, such as *fasI-A*, which is responsible for bulk fatty acid synthesis in *Corynebacterium ammoniagenes* (67). The absence of fatty acid synthase apparently results in a strict nutritional requirement of *C. jeikeium* that has to be supplemented by the availability of exogenous fatty acids in its natural habitats. It was shown recently that *C. jeikeium* has a cellular fatty acid composition with the majority of these compounds being of the straight-chain, monounsaturated types (83), and it is worth mentioning that the chemical composition of Tween 80 almost exactly meets this requirement of *C. jeikeium*. Thus, either of the terms "lipid-requiring" or "lipid auxotroph" seems to be more appropriate for the taxonomic description of *C. jeikeium* isolates than the term "lipophilic." However, it remains to be elucidated whether this explanation is also applicable for the phenotypic description of other pathogenic corynebacterial species that exhibit a "lipophilic" phenotype.

Fatty acids are, moreover, building blocks for the synthesis of corynomycolic acids, which are major constituents of the cell envelopes of most corynebacterial species, including *C. jeikeium* (27). Corynomycolic acids attached to the cell wall are organized with other lipids to form a permeability barrier that contributes to the natural resistance of corynebacteria to various antimicrobials (6). The synthesis of corynomycolic acids in *C. jeikeium* K411 is apparently ensured by the presence of a unique polyketide synthase (*jk0138* [*pks13*]). It was demonstrated very recently that the polyketide synthase Pks13 of *C. glutamicum* represents a condensase that catalyzes key steps in the biosynthesis of corynomycolic acids, along with acyl-CoA carboxylases and a distinct fatty acyl-CoA synthetase (24, 59).

TABLE 2. Characteristic features of transposons identified in the *Corynebacterium jeikeium* K411 genome

Transposon	Position in genome sequence	Size (bp)	Target site duplication (bp)	G+C (%)	Internal CDS	Prominent gene products or predicted physiological function
Tn*3595*	967583–970045	2,463	6	64.2	*jk0815-jk0817*	Glyoxylase/bleomycin resistance protein family member
Tn*3596*	2058425–2092031	33,607	8	60.1	*jk1767-jk1791*	Siderophore synthesis and iron acquisition
Tn*3597a*	20428–26970	6,543	3	58.4	*jk0015-jk0021*	High-affinity manganese uptake system MntH1 and Nudix hydrolase Utp1
Tn*3597b*	749407–755947	6,541	3	58.4	*jk0621-jk0627*	High-affinity manganese uptake system MntH2 and Nudix hydrolase Utp2
Tn*3598*	432471–444491	12,021	8	65.2	*jk0360-jk0370*	TetAB-type ABC transporter and undecaprenyl pyrophosphate phosphatase Upp2
Tn*3599*	1663190–1666938	3,749	6	59.1	*jk1402-jk1405*	Chloramphenicol exporter Cmx

The *C. jeikeium* K411 genome contains three genes specifying α subunits of acyl-CoA carboxylases (*jk1550* [*accBC1*], *jk1669* [*accBC2*], and *jk0405* [*accBC3*]), as well as three others coding for β subunits of this enzyme (*jk1551* [*accD1*], *jk1662* [*accD2*]), and *jk0139* [*accD3*]). In addition, 11 *fadD* genes encoding fatty acyl-CoA synthetases were identified in the *C. jeikeium* K411 genome. FadD enzymes are involved in activating fatty acids of various chain lengths concomitant with transport into the cell, with the highest specificity for long-chain fatty acid substrates (9). In respect to the apparent fatty acid auxotrophy of *C. jeikeium* and the necessity to synthesize corynomycolic acids, the *fadD* genes are likely to play a pivotal role in the growth of *C. jeikeium*.

On the other hand, the activation of exogenous fatty acids to acyl-CoA thioesters by fatty acyl-CoA synthetases is an initial step in fatty acid degradation. The genome of *C. jeikeium* K411 encodes a complete β-oxidation pathway for fatty acid degradation, comprising a set of *fadA* (three paralogs), *fadB* (two paralogs), and *fadE* (eight paralogs) genes. Furthermore, a *fadH* gene (*jk0075*) encodes 2,4-dienoyl-CoA reductase, which is generally required for the degradation of unsaturated fatty acids, whose double bond extends from an even-numbered carbon atom. Degradation of fatty acids results in their gradual conversion to acetyl-CoA and in the concomitant production of one equivalent each of $FADH_2$ and NADH, which may be used in ATP generation. In principle, *C. jeikeium* K411 may use exogenous fatty acids as carbon and energy sources, since the glyoxylate bypass and gluconeogenesis are complete. In this context, the strict dependence of growth of *C. jeikeium* K411 on the availability of exogenous fatty acids might explain the limited repertoire of genes involved in carbohydrate utilization. Additionally, the absence of PTSs and the concomitant lack of PTS-mediated global carbon regulation seem to be more or less insignificant for metabolism, since exogenous fatty acids are apparently the dominant constraint of growth of *C. jeikeium*.

Iron and manganese acquisition by *C. jeikeium* K411. The majority of pathogenic bacteria need specialized mechanisms for acquiring iron because its limitation is a common strategy by which host organisms suppress bacterial growth (3). In many cases, iron acquisition involves the synthesis and secretion of high-affinity iron sequestration molecules, termed siderophores, which are often synthesized by nonribosomal peptide synthetases (13). The chromosome of *C. jeikeium* K411 contains two genes (*jk1779* [*pstX*] and *jk1811* [*dhbF*]) whose deduced proteins showed striking similarities to a number of predicted nonribosomal peptide synthetases. The respective genes of *C. jeikeium* K411 are part of two gene clusters (*jk1772* to *jk1783* and *jk1805* to *jk1821*) apparently involved in siderophore synthesis and iron acquisition. Analysis of the flanking DNA regions revealed that cluster I (*jk1772* to *jk1783*) is an integral part of the 33.6-kb transposable element Tn*3596* (Table 2) This composite transposon encodes additional proteins involved in siderophore biosynthesis (*jk1777* [*fxbA*] and *jk1780* [*pvdA*]), a putative enterochelin esterase (*jk1778* [*fes*]), and an ABC transport system (*jk1772* to *jk1774* [*fhuABC*]) most likely required for iron uptake. Further analysis of the genome sequence of *C. jeikeium* K411 revealed in all eight putative iron ABC transport systems, two of which are similar to the siderophore-dependent iron uptake system Irp6 (60) and to the hemin utilization system HmuTUV of *C. diphtheriae* (19), respectively. *C. jeikeium* K411 also encodes a transcriptional repressor of the DtxR type (*jk1097* [*dtxR*]) that is probably involved in global regulation of gene expression in response to iron. It would be interesting to elucidate how the horizontally transferred iron acquisition gene cluster of Tn*3596* is integrated into the iron-dependent regulatory network controlled by the DtxR repressor of *C. jeikeium* K411.

Furthermore, manganese acquisition plays an important role in growth and survival of pathogenic bacteria, since the conversion of oxygen radicals is often ensured by manganese-dependent superoxide dismutases (70). *C. jeikeium* K411 encodes a superoxide dismutase of the manganese type (*jk0112* [*sodA*]) along with a catalase (*jk1994* [*katA*]) to remove superoxides and to provide protection against toxic H_2O_2. Three additional enzymes that may detoxify reactive oxygen species were identified: a putative iron-dependent peroxidase (*jk0008*), an organic hydroperoxide resistance protein (*jk1993* [*ohrA*]), and the two subunits of an alkyl hydroperoxide reductase (*jk0154* [*ahpF*] and *jk0155* [*ahpC*]). The uptake of manganese in *C. jeikeium* K411 might be mediated by an ABC transport system (*jk1486* to *jk1488* [*mntABC*]) that is apparently under negative transcriptional control by a DtxR-type regulator (*jk1485* [*mntR*]). Moreover, the *C. jeikeium* K411 genome contains two genes (*jk0016* [*mntH1*]) and *jk0626* [*mntH2*]) that encode members of the widespread Nramp (natural resistance-associated macrophage protein) family. Nramp proteins form a class of secondary active transporters that are involved in high-affinity manganese uptake into the bacterial cell and are especially known to ensure the survival of intracellular pathogens

(1). The two *mntH* genes of *C. jeikeium* K411 are identical, and a closer inspection of the surrounding DNA regions revealed that they are integral parts of the 6.5-kb composite transposons Tn*3597a* and Tn*3597b*, which are slightly different in their genetic organization due to the inversion of the terminal insertion sequence (Table 2). Another interesting aspect of these transposons is the presence of genes encoding Nudix (nucleoside diphosphates linked to some other moiety x) family proteins (*jk0017* [*utp1*] and *jk0625* [*utp2*]), which generally hydrolyze nucleoside diphosphate derivatives (8). The *utp* genes of the Nudix hydrolase superfamily were recently shown to function in the hydrolysis of UTP and 5-methyl-UTP (82). The clustering of Nramp and Nudix genes in the *C. jeikeium* K411 chromosome is similar to that observed on the pNG2 plasmid of *C. diphtheriae* (76), suggesting a common functional context of these genes. A primary role for the Nramp proteins may be to help protect the bacterial cell against reactive oxygen species by manganese sequestration, a function that is particularly important during infection of the host (28), whereas the Nudix hydrolases may be thought of as surveillance system (8), hydrolyzing toxic or deleterious compounds possibly arising through DNA damage. Consequently, both protein families may be involved in the overall bacterial defense against reactive oxygen species. The corresponding transposons carrying Nramp and Nudix genes may therefore be considered to represent pathogenicity islets of *C. jeikeium* K411.

The gene repertoire of *C. jeikeium* K411 potentially involved in multidrug resistance. Multidrug resistance is one of the most prominent features of clinical isolates of *C. jeikeium* (4, 23). Analysis of the *C. jeikeium* K411 genome sequence led to the identification of an *erm*(X) gene (*jk1436*) encoding a 23S rRNA adenine *N*-6-methyltransferase. This gene is highly similar to *erm*(X) variants present on the *C. diphtheriae* plasmid pNG2 (76) and in the central region of *Corynebacterium striatum* transposon Tn*5432* (73), but it is apparently nonmobile. The *erm*(X) resistance determinant was shown previously to confer resistance against a wide spectrum of macrolide, lincosamide, and streptogramin B antibiotics as well as against the novel ketolide telithromycin (65, 73). Another source of resistance by 23S rRNA modification might be the *rrmA* gene (*jk1386*) of *C. jeikeium* K411, the product of which revealed similarity to the mycinamycin resistance protein $RmlA^{II}$ encoded by the *C. glutamicum* plasmid pAG1 (74). The $RmlA^{II}$ protein acts in synergy with the adenine *N*-6-methyltransferase Erm(N) by single methylations at specific nucleotides of the 23S rRNA conferring resistance to the 16-member-ring macrolides tylosin and mycinamycin (39). An ortholog of an *erm*(N) gene is, however, not present in the genome sequence of *C. jeikeium* K411.

Furthermore, a set of novel transposons may play an important role in antibiotic resistance of *C. jeikeium* K411 (Table 2). The 2.5-kb transposon Tn*3595* encodes a protein probably belonging to the glyoxylase/bleomycin resistance protein family (*jk0816*). Whether this gene is indeed involved in bleomycin resistance of *C. jeikeium* K411 remains to be elucidated. The 12-kb class II transposon Tn*3598* is equipped with the *tetA-tetB* gene pair (*jk0361* and *jk0362*), which is known to mediate resistance against tetracycline and the structurally unrelated β-lactam antibiotic oxacillin in corynebacteria (73). The TetAB proteins represent archetypes of a specific group of tetracycline resistance determinants that use ATP rather than the proton gradient as an energy source (17). The *C. jeikeium* K411 genome contains three additional ABC transport systems (*jk0437* and *jk0438*, *jk1781* and *jk1782*, and *jk1372*) that showed similarity to the exceptional TetAB resistance determinant and to ATP-dependent multidrug transporters. Tn*3598* also contains the *uppP2* gene (*jk0367*), encoding an undecaprenyl pyrophosphate phosphatase that might confer bacitracin resistance upon overexpression (20). A substantial expression level of undecaprenyl pyrophosphate phosphatase activity might be achieved in *C. jeikeium* K411, since a paralog (*jk0957* [*uppP1*]) of this gene is also present in the chromosome. Additionally, the 3.7-kb transposon Tn*3599* carries the *cmx* gene (*jk1404*), specifying a 12-spanner membrane protein of the drug:H^+ antiporter (DHA12) drug efflux family (55) that mediates chloramphenicol resistance in corynebacteria, for instance, as integral part of the transposons Tn*45* and Tn*5564* (73).

The genome of *C. jeikeium* K411 is equipped with five further genes (*jk0701*, *jk1309*, *jk1456*, *jk2051*, and *jk2082*) that encode 14-spanner membrane proteins of the drug:H^+ antiporter (DHA14) drug efflux family (55). The *C. glutamicum* gene considered orthologous with *jk2051* (*lmrB*) was already shown to confer lincomycin resistance in this species (37). It would be interesting to identify by targeted genetic experiments the transport capabilities and the spectrum of antimicrobial resistance that is mediated by this class of putative drug efflux proteins in *C. jeikeium* K411. Moreover, *C. jeikeium* K411 encodes a membrane protein (*jk1982* [*matE*]) that belongs to the multidrug and toxic compound extrusion (MATE) family (14). The MatE protein resembles a Na^+-driven multidrug efflux pump and shows similarity to proteins of the NorM cluster of the MATE family that appear to protect cells from drugs and other toxic compounds (14). Efflux proteins of the NorM cluster are involved primarily in resistance to fluoroquinolones, such as norfloxacin and ciprofloxacin, but they can also confer resistance to aminoglycosides and to a number of structurally unrelated antimicrobial compounds (12, 46). A member of the DinF cluster of the MATE family was also identified in the *C. jeikeium* K411 genome sequence (*jk1140* [*dinF*]). The physiological role of DinF membrane proteins is unknown, but expression of them has been demonstrated to be DNA damage inducible (14). Obviously, it requires experimental data to precisely define the role of the predicted antimicrobial resistance genes in *C. jeikeium* K411, but the overall repertoire of potential resistance determinants already suggests that the combination of both intrinsic properties and protein functions acquired by transpositional DNA transfer might be responsible for the development of multidrug resistance in *C. jeikeium* K411.

Bacterium-host interaction and putative virulence factors of *C. jeikeium* K411. Further analysis of the genome sequence revealed that *C. jeikeium* K411 expresses a variety of extracellular proteins, which might be implicated in virulence. Among these are surface-anchored and secreted proteins (Table 3). *C. jeikeium* K411 encodes two putative surface proteins (*jk2032* [*surA*] and *jk0220* [*surB*]) that display internal repeats in their amino acid sequences. Variations in the number of repeating units of the *C. jeikeium* K411 surface proteins may change their antigenicity, which is a general mechanism to generate pheno-

TABLE 3. Coding sequences potentially involved in bacterium-host interaction and virulence of *Corynebacterium jeikeium* K411

Coding sequence			Predicted function and characteristics	Similar characterized protein	
No.	Gene	G+C (%)		Microorganism	GenBank accession no.
jk2032	*surA*	58.3	Cell surface protein	*Streptococcus pyogenes*	AAD39085
jk0220	*surB*	59.3	Cell surface protein	*Streptococcus agalactiae*	Q02192
jk0007	*sapE*	66.3	Surface-anchored protein; LANTG sortase motif		
jk1699	*sapC*	60.3	Surface-anchored protein; LAQTG sortase motif	*Corynebacterium diphtheriae*	CAE48742
jk1700	*srtA*	55.5	Fimbrial-associated sortase-like protein	*Corynebacterium diphtheriae*	CAE48741
jk1701	*sapB*	57.5	Surface-anchored protein; LPNTG sortase motif	*Corynebacterium diphtheriae*	CAE48740
jk1702	*sapA*	56.9	Surface-anchored protein; MPKTG sortase motif	*Corynebacterium diphtheriae*	CAE50592
jk1856	*sapD*	64.3	Surface-anchored protein; LADTG sortase motif	*Corynebacterium diphtheriae*	CAE48948
jk0461	*cbpA*	54.9	Surface-anchored collagen adhesin; LALTG sortase motif	*Arcanobacterium pyogenes*	AA043108
jk0448	*nanA*	62.1	Secreted neuraminidase	*Streptococcus pneumoniae*	P62575
jk1103	*asa*	61.3	Secreted alkaline ceramidase	*Pseudomonas aeruginosa*	BAA88409
jk0629	*choE*	64.6	Secreted cholesterol oxidase	*Rhodococcus equi*	CAC44897
jk2054	*che*	62.8	Secreted cholesterol esterase	*Streptomyces lavendulae*	AAC60485
jk0010	*acpA*	61.7	Secreted acid phosphatase	*Francisella tularensis*	AAB06624

typic diversity and to escape host immunity. The SurA and SurB proteins of *C. jeikeium* K411 are similar to surface proteins of group A and group B *Streptococcus* species that promote binding to human epithelial cells and confer protective immunity (45, 69). The SurA and SurB proteins may thus play an important role during an infectious process of *C. jeikeium* by mediating interactions between the pathogen and the host cell or by allowing evasion from the host defense. The supposed activity of these proteins in eliciting protective immunity implies that they may be used for vaccine development.

Furthermore, genes encoding putative surface-anchored proteins bearing a cell wall sorting signal motif were identified (Table 3). These proteins are covalently linked to the bacterial cell wall by a transpeptidylation mechanism, requiring a C-terminal sorting signal with a conserved LPXTG motif (50). The enzyme that catalyzes transpeptidylation is a membrane-associated protein termed sortase. The genome of *C. jeikeium* K411 contains two genes encoding putative sortases (*jk1700* [*srtA*] and *jk0103* [*srtB*]). The *srtB* gene seems to be part of the backbone of the corynebacterial chromosome, since it is also present in the genomes of *C. glutamicum*, *C. efficiens*, and *C. diphtheriae*, and hence it may encode the housekeeping sortase. On the other hand, the *srtA* gene is part of a gene cluster also comprising the *sapABCD* genes, which encode surface-anchored proteins (Table 3). These proteins resemble factors in the *C. diphtheriae* genome required for sortase-mediated formation of adhesive pili that are anchored to the bacterial cell wall (78). According to this similarity, it is likely that *C. jeikeium* K411 expresses proteinaceous pili and that the SapB protein represents the major pilin subunit. Proteinaceous filaments on the bacterial cell surface provide adhesive functions during attachment and colonization and may therefore be involved in the initial stages of bacterial infection (78).

Another surface-anchored protein of *C. jeikeium* K411 revealed similarity to a collagen-binding protein of the animal pathogen *Arcanobacterium pyogenes* (21) and is encoded by the *cbpA* gene (Table 3). The CbpA protein of *C. jeikeium* K411 belongs to the MSCRAMM (microbial surface components recognizing adhesive matrix molecules) family, a class of surface-anchored proteins that adhere to components of the host extracellular matrix to initiate colonization (29). In particular, collagen adhesins are thought to act as virulence factors, specifically by promoting adhesion to collagen-rich tissues (22). Adhesion to collagen might be promoted further by the enzymatic action of a neuraminidase, which is also encoded by *C. jeikeium* K411 (*jk0448* [*nanA*]). The deduced NanA protein sequence showed similarity to neuraminidases from *Streptococcus pneumoniae* (7, 15). Neuraminidases cleave terminal sialic acid residues from a variety of glycolipids, glycoproteins, and oligosaccharides on the cell surface. Such activity not only might unmask potential cell surface receptors for bacterial adhesins but also has the potential to cause great damage to the host tissue.

The *C. jeikeium* K411 genome also encodes a putative alkaline ceramidase (*jk1103* [*asa*]) with similarity to the respective enzyme from *Pseudomonas aeruginosa* (52). Ceramidases are a class of lipolytic enzymes that hydrolyze the amide bond in ceramides to yield free fatty acids and sphingosine. The latter lipid is known to downregulate the activity of macrophages that are stimulated by bacterial surface structures, and hence the bacterium may escape the host defense system. Accordingly, the ceramidase gene *asa* may represent a virulence factor in *C. jeikeium* K411 infections. Another important physiological role of *asa*, however, might be the release for bacterial growth of exogenous fatty acids originating from sphingolipids, which are significant constituents of the eukaryotic cell and mainly occur in the plasma membrane. Another membrane-damaging enzyme might be the putative cholesterol oxidase of *C. jeikeium* K411 (*jk0629* [*choE*]), which revealed similarity to a cytotoxic and macrophage-destroying factor of the facultative intracellular parasite *Rhodococcus equi* (51). It was suggested that cholesterol oxidase acts in cooperative lytic processes along with sphingomyelin degradation, allowing the enzyme to reach its target substrate upon sublytic damage of the membrane. Cholesterol is thus oxidized, leading to the formation of 4-cholesten-3-one and to the total disorganization of the eukaryotic plasma membrane (40). It is interesting that *C. jeikeium* K411 also encodes a secreted cholesterol esterase (*jk2054* [*che*]) that apparently catalyzes the hydrolysis of long-chain fatty acid esters from cholesterol (53) and may thus be involved in both virulence and fatty acid metabolism of *C. jeikeium* K411. Accordingly, it would be interesting to analyze the expression

pattern of the putative virulence genes with respect to the availability of exogenous fatty acids.

Finally, the *C. jeikeium* K411 genome encodes an exceptional acid phosphatase (*jk0010* [*acpA*]) with similarity to the AcpA protein from the facultative intracellular pathogen *Francisella tularensis* (61). Purified AcpA protein of *F. tularensis* revealed a broad substrate specificity, including phospholipase C activity that releases diglycerides by hydrolyzing phosphatidylcholine. Acid phosphatases of intracellular pathogens have been implicated as virulence factors by suppressing the respiratory burst of human neutrophils (61). However, whether the burst-inhibiting activity of acid phosphatases is also relevant for the pathogenicity of bacteria that are primarily extracellular remains to be determined.

Deduced lifestyle of the nosocomial pathogen *C. jeikeium* K411. The genome sequence data also provided interesting information on the lifestyle of *C. jeikeium* K411, which was originally identified as part of the axilla flora of an inpatient. *C. jeikeium* K411 harbors plasmid pKW4, which was shown to produce a bacteriocin-like substance with a narrow spectrum of bactericidal activity mainly limited to other corynebacteria (35). This microbial weapon is most likely encoded by the *bls* gene cluster of pKW4, which is, however, characterized by a low G+C content. The human axilla is known as a skin region whose bacterial flora is dominated either by *Staphylococcus* species or by a dense population of corynebacteria (49). In the latter case, even the narrow killing spectrum of a bacteriocin-like substance provides a great advantage during colonization of the human skin (62). Additionally, the bacteriocin-like substance may play a defensive role and act to prohibit the invasion of other corynebacterial species into the habitat occupied by *C. jeikeium* K411.

Very recently, it was demonstrated that *C. jeikeium* is involved in axillary odor formation from odorant precursors secreted in the human axilla (49). These nonodorous molecules that are present in apocrine secretions have to be transformed by specific bacterial enzymes, such as Zn^{2+}-dependent aminoacylases, to generate odorous compounds. Interestingly, the *C. jeikeium* K411 genome contains three genes (*jk1673* [*amiA*], *jk0802* [*amiB*], and *jk0500* [*amiC*]) that encode putative metal-dependent aminoacylases. The potential participation of *C. jeikeium* in odor formation with specialized enzymes that recognize odorant precursors might indicate an adaptation of this species to the specific axilla secretions. Another apparent adaptation to the natural habitats is the "lipid-requiring" phenotype of *C. jeikeium*. Microbiological studies clearly indicated that *C. jeikeium* is recovered predominantly from the axillary, inguinal, and perineal areas of the human skin (18). These areas are intertriginous sites of the human body that are characterized by an elevated moisture of the skin along with a substantial formation of hydrolipid films, which are composed of triglycerides, fatty acids, ceramides, cholesterol, and cholesterol esters. Accordingly, it is apparent that *C. jeikeium* is preferably recovered from those sites of the human body that provide an appropriate amount of exogenous fatty acids for growth. Furthermore, it seems likely that some of the putative virulence factors of *C. jeikeium* K411, such as alkaline ceramidase and cholesterol esterase, are involved in the release of additional exogenous fatty acids by damaging the plasma membrane of host cells. Consequently, the analysis of the genome sequence of *C. jeikeium* K411 sheds light on its pathogenic potential in such a way that the enzymatic functions of the predicted virulence factors are closely linked to the exceptional fatty acid metabolism of this species (Fig. 3). Although the role of *C. jeikeium* as part of the skin flora has to be studied in more detail, these observations also indicate a very close interaction of this bacterium with humans (and their apocrine secretions) in the preferably colonized areas of the skin. However, once getting access to and colonizing otherwise sterile sites of the human body, such as the endocardium, *C. jeikeium* can utilize its gene repertoire to cause serious nosocomial infections.

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