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Genome Sequence of the Repetitive-Sequence-Rich *Mycoplasma fermentans* Strain M64▽

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***Mycoplasma fermentans* is a microorganism commonly found in the genitourinary and respiratory tracts of healthy individuals and AIDS patients. The complete genome of the repetitive-sequence-rich *M. fermentans* strain M64 is reported here. Comparative genomics analysis revealed dramatic differences in genome size between this strain and the recently completely sequenced JER strain.**

Mycoplasma fermentans is a fastidious Gram-negative bacterium commonly isolated from human genitourinary and respiratory tracts. It has been implicated in HIV pathogenesis, sexually transmitted genital tract infections, systemic infections, rheumatic disorders, chronic fatigue syndrome, and other diseases (1, 2, 8, 9, 12, 14, 15, 18, 25, 27, 28). Notwithstanding the clinical significance, the roles it plays and the molecular mechanisms involved in various diseases have yet to be elucidated.

In this study, we have determined the repetitive-sequence-rich genome sequence of *M. fermentans* strain M64 isolated from a non-AIDS patient (16). *Escherichia coli* DH5α and pUC18 vector were used in the construction of whole-genome shotgun libraries (2 to 3 kb and 5 to 7 kb) as previously described (7, 30). The whole-genome shotgun sequences (BigDye Terminator 3.1 kit and ABI3730XL DNA analyzer; both from Applied Biosystems, Foster City, CA) were assembled with the Phredphrap software using the "Hide-and-Seek" sequence assembly strategy (4, 10, 11, 13, 22). The assembled genome sequence was validated by restriction mapping and Southern hybridization analyses of BglI, KpnI, MluI, and AhdI fragments resolved by pulsed-field gel electrophoresis (CHEF Mapper XA system; Bio-Rad, Hercules, CA) (26). The gene prediction program GeneMark.hmm (version 2.4; http://exon.gatech.edu/gmhmm2_prok.cgi) was applied to predict the protein-coding genes using *Mycoplasma synoviae*, the phylogenetically closest species, as the training model. The functions of predicted proteins (translation table 4) were analyzed by Blastp (3) searches against the proteomes of sequenced prokaryotic organisms in the Integr8 database (www.ebi.ac.uk/integr8/). The protein motifs and COG (clusters of orthologous groups) functional categories were assigned by RPSBLAST searches (3) against the NCBI Conserved Domain Database (20, 21). The rRNA and tRNA genes were identified by blastn (3) and tRNAscan-SE (19), respectively.

M. fermentans M64 has a 1,118,751-bp circular chromosome encoding 1,050 putative protein genes, two 16S-23S rRNA operons, one 5S rRNA gene, and 35 tRNA genes. Like *Mycoplasma mycoides* subsp. *mycoides* (29), the *M. fermentans* M64 genome also bears a high density of transposable elements. The genome contained nine copies of two families of large repeat sequences (integrative conjugal elements of *M. fermentans* [ICEF] and ϕMFV1 prophage) and many insertion sequence elements, including IS*1550*, IS*1630*, and ISMf*1* (5, 6, 17, 24). These sequences and elements accounted for approximately 21.6% of the genome. Among these, a new family of ICEF, designated ICEF-III, was discovered in this study. Perusal of the integrity of the IS elements and ICEFs and their flanking sequences suggested that the current genome architecture of *M. fermentans* M64 might be partly associated with the transposition and/or recombination of these elements in the past. Interestingly, despite the fact that the organizations of the two genomes are highly similar, the *M. fermentans* M64 genome is approximately 141 kb bigger than the recently completely sequenced *M. fermentans* strain JER (977,524 bp) (23). The size difference is mainly attributed to the differences in the copy numbers of ICEF and ϕMFV1 prophage which are by and large absent in the JER genomes (6, 24). The transposable-element-rich region in the middle of the genome exhibited chaotic arrangement probably due to transposition events. After all, unveiling this special reduced genome which is rich in transposable elements may help to elucidate the elusive rela-

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tionships between *M. fermentans* M64 and the implicated diseases.

Nucleotide sequence accession number. The genome sequence with annotations of *Mycoplasma fermentans* strain M64 reported in this paper has been deposited in the GenBank database under accession number NC_014921.

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