Genome Sequence and Analysis of *Escherichia coli* MRE600, a Colicinogenic, Nonmotile Strain that Lacks RNase I and the Type I Methyltransferase, EcoKI

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Abstract

Escherichia coli strain MRE600 was originally identified for its low RNase I activity and has therefore been widely adopted by the biomedical research community as a preferred source for the expression and purification of transfer RNAs and ribosomes. Despite its widespread use, surprisingly little information about its genome or genetic content exists. Here, we present the first de novo assembly and description of the MRE600 genome and epigenome. To provide context to these studies of MRE600, we include comparative analyses with *E. coli* K-12 MG1655 (K12). Pacific Biosciences Single Molecule, Real-Time sequencing reads were assembled into one large chromosome (4.83 Mb) and three smaller plasmids (89.1, 56.9, and 7.1 kb). Interestingly, the 7.1-kb plasmid possesses genes encoding a colicin E1 protein and its associated immunity protein. The MRE600 genome has a G + C content of 50.8% and contains a total of 5,181 genes, including 4,913 protein-encoding genes and 268 RNA genes. We identified 41,469 modified DNA bases (0.83% of total) and found that MRE600 lacks the gene for type I methyltransferase, EcoKI. Phylogenetic, taxonomic, and genetic analyses demonstrate that MRE600 is a divergent *E. coli* strain that displays features of the closely related genus, *Shigella*. Nevertheless, comparative analyses between MRE600 and *E. coli* K12 show that these two strains exhibit nearly identical ribosomal proteins, ribosomal RNAs, and highly homologous tRNA species. Substantiating prior suggestions that MRE600 lacks RNase I activity, the RNase I-encoding gene, *rna*, contains a single premature stop codon early in its open-reading frame.

Key words: *Escherichia coli*, *Shigella*, MRE600, epigenetics, ribonuclease I, colicin.

Introduction

Escherichia coli is a Gram-negative, nonsporulating, rod-shaped, facultative anaerobe that inhabits the intestines of warm-blooded animals and reptiles (Gordon and Cowling 2003). *Escherichia coli* is both a widespread gut commensal in vertebrates and a versatile and virulent pathogen that affects millions of humans each year (Kosek et al. 2003). Due to its ability to grow rapidly in chemically defined media, its metabolic versatility, and its ease of genetic manipulation, *E. coli* is also one of biology's most important model organisms (Casali and Preston 2003). *Escherichia coli* has therefore become one of the most highly characterized organisms on Earth and, as an experimental model system, has been integral to our ability to investigate and understand many fundamental biological processes.

As a species, *E. coli* is exceptionally diverse and is comprised of innumerable strains that are differentiated by their genetic content and physiological properties. The first published *E. coli* genome assembly was of strain K-12 MG1655 (K12) which was chosen because it had been maintained in the lab with minimal genetic manipulation (Blattner et al. 1997). Subsequent analyses have revealed substantial genomic heterogeneity between different *E. coli* strains. For example, finished genomes listed in the Joint Genome Institute's Integrated Microbial Genomes (IMG) database show that *E. coli* genome size can range from 3.98 Mb (strain K-12 subMDS42) to 5.86 Mb (strain O26:H11 11368), and can contain between 3,696 genes (strain K-12 subMDS42) and 5,919 genes (strain O157:H7 str. EDL933) (Markowitz et al. 2012). A recent study investigating the genomes of 20 *E. coli* strains identified a total of 17,838 distinct genes, with only 1,976 being common to all (Touchon et al. 2009). Such genomic variation contributes to each strain's distinct physiological properties, such as their varied abilities to metabolize sugars, resistance to particular antibiotics, and growth rate-temperature profiles (Gordon 2004). The *E. coli* MRE600 strain has become a key workhorse for the RNA research community as the source for isolating RNA species such as mRNAs, tRNAs, and ribosomes due to its reported lack of RNAse I activity (Cammack and Wade 1965). The molecular basis of this distinction, however, has yet to be shown.

Although many of the details regarding the initial isolation of *E. coli* MRE600 (MRE600) (ATCC #29417, NCTC #8164, NCIB #10115, original strain reference C6) are not well documented, it is believed that this strain was derived from an environmental sample taken in 1950 by E. Windle Taylor of the Metropolitan Water Board of London (Public Health England). This strain was subsequently deposited into the Culture Collections of Public Health England, and in 1962, a personal communication written by A. Rogers described this strain as being RNase I deficient (Public Health England). Specifically, RNase I activity refers to a latent enzymatic degradation of the 30S ribosomal subunit upon exposure to denaturing conditions, such as urea, high salt, or ethylenediaminetetraacetic acid (Elson 1959). The first mention of MRE600 in the published literature came from the Microbial Research Establishment at Porton Down (UK). Cammack and Wade (1965) screened 13 bacterial strains for ribonuclease content using assays developed by Wade and Robinson (1963) and found that MRE600 exhibited negligible ribonuclease activity (Wade and Robinson 1963; Cammack and Wade 1965). Then, using a biochemical assay for ribonuclease activity, Wade and Robinson (1965) found that MRE600 lysate displayed a similar level of ribonuclease activity to known ribonuclease-deficient strains (Wade and Robinson 1965). As a result of these studies, MRE600 became the strain of choice for expressing and purifying stable RNA species and, as such, has played a key role in the field of translation biology.

In this report, we describe the 4.98 Mb *E. coli* MRE600 genome and epigenome for the first time. To provide context to the MRE600 genome, we provide comparative analyses with *E. coli* K12, a common lab strain, and perform additional phylogenetic and taxonomic studies to gain insight into its evolutionary history. Due to the importance of MRE600 to the translation biology field, we describe the genes encoding ribosomal components (ribosomal RNAs [rRNAs], ribosomal proteins [RPs]), while also highlighting other key characteristics of this organism. These key characteristics include the identification of a premature stop codon in the RNase I-encoding gene, *rna*, the possession of a colicin E1-expressing plasmid, which was previously predicted (Salaj-Smic 1978), and the lack of an *hsdM* gene, which encodes the type I methyltransferase, EcoKI.

Materials and Methods

Cell Growth Conditions and Genomic DNA Preparation

MRE600 was grown in lysogeny broth, Miller (EMD) at 37 °C in a rotating culture tube. Genomic DNA was extracted from cells using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. DNA quality and quantity were determined using an Agilent 2200 TapeStation and Qubit dsDNA BR Assay (Life Technologies), respectively.

Genome Sequencing, Assembly, and Epigenome Characterization

Single Molecule, Real-Time (SMRT) sequencing libraries were prepared using the SMRTbell Template Prep Kit 1.0 (Pacific Biosciences) and 20 kb Template Preparation Using BluePippin Size-Selection System protocol (Pacific Biosciences). Library quality and quantity were determined using an Agilent 2200 TapeStation and Qubit dsDNA BR Assay (Life Technologies), respectively. Sequencing was conducted using P5-C3 chemistry and a v3 SMRT Cell (Pacific Biosciences) at Weill Cornell Medical College. Genome assembly was conducted using the Hierarchical Genome Assembly Process 2.0 (HGAP 2.0) (Chin et al. 2013). Raw sequencing reads were filtered for length and quality such that the minimum polymerase read score was 0.8, the minimum subread length was 500 bp, and the minimum polymerase read score was greater than 100. The assembly was generated using CeleraAssembler v1 with the default parameters and was polished using the Quiver algorithm (Chin et al. 2013). To identify methylated DNA bases, we used the RS Modification and Motif Analysis module of Pacific Biosciences' SMRT Analysis 2.3 with a Quality Value (QV) threshold of 30. This analysis uses an in silico kinetic reference and a *t*-test-based kinetic score to detect modified bases (Flusberg et al. 2010).

Genome Annotation

Genes were identified using Prodigal (Hyatt et al. 2010) as part of the Joint Genome Institute's genome annotation pipeline (Mavromatis et al. 2009). In this pipeline, predicted coding sequences are translated and used to search the National Center for Biotechnology Information nonredundant database, as well as the UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro databases. RNA genes were identified using HMMer 3.0rc1 (Finn et al. 2011), tRNAscan-SE (Lowe and Eddy 1997), and INFERNAL 1.0.2 (Nawrocki et al. 2009). Further gene prediction analysis and annotation were performed within the Integrated Microbial Genomes—Expert Review (IMG-ER) platform (Markowitz et al. 2009).

Electron Microscopy

Cells were grown to mid-log phase before being prepared for electron microscopy. For negative staining, cells were placed on a formvar/carbon-coated copper 400-mesh grid and treated with 1.5% uranyl acetate solution. For transmission electron microscopy (TEM), cells were pelleted and fixed overnight at +4 °C in 0.1 M sodium cacodylate buffer (pH = 7.3) containing 2.5% glutaraldehyde and 4% paraformaldehyde. Cells were then washed with 0.1 M sodium cacodylate buffer (pH = 7.3) and postfixed in reduced osmium tetroxide (1% OsO_4, 1.5% potassium ferrocyanide) for 1 h. Cells were washed again and then treated with 1.5% uranyl acetate solution for 1 h in the dark. Samples were dehydrated into ethanol, embedded in resin, and sectioned to 65–70 nm using a diamond knife (Diatome) on a Leica Ultracut S ultramicrotome. All imaging was performed using a JEOL JEM-1400 transmission electron microscope at Weill Cornell Medical College.

Results

The MRE600 Genome Includes a Colicin E1-Expressing Plasmid

The genome sequence of MRE600 was determined at an average coverage of 184× and our assembly generated four contigs, including a 4.83 Mb chromosome and three plasmids of 89.1, 56.9, and 7.1 kb, respectively (fig. 1). The 7.1-kb plasmid, which we are calling pColE1-MRE600, contains a colicin E1 gene as well as the associated immunity protein. The identification of these genes was predicted by a previous study that showed MRE600 to possess colicinogenic activity (Salaj-Smic 1978). Our annotation identified a total of 5,181 genes, 4,913 of which are predicted to encode proteins, whereas 268 are classified as RNA genes (table 1). Of the identified genes, 3,566 (68.83%) could be categorized into Clusters of Orthologous Groups of proteins (COGs) (Tatusov et al. 2000); an overview of which can be seen in table 2.

The MRE600 Epigenome Reveals the Absence of EcoKI Type I Methyltransferase Activity

Our use of Pacific Biosciences SMRT sequencing also enabled the identification of 41,469 methylated DNA bases (0.83% of all bases) in the MRE600 genome. The majority of detected modifications were 6-methyladenine (m^6A) at G$\underline{A}$TC motifs, but we were also able to detect putative 5-methylcytosine (m^5C) modifications (table 3). Interestingly, we did not detect any m^6A modifications at the well-known GC$\underline{A}$C(N_6)GTT motif in MRE600, although it was clearly identified in K12 (table 4). In bacteria, this particular modification is generated by the type I methyltransferase, EcoKI, which is encoded by the *hsdM* gene (Clark et al. 2013). Consistent with these epigenetic data, we were unable to identify the *hsdM* gene, or any close homologs, in our MRE600 assembly. Matching fragments were also absent in any of our sequencing reads, indicating that MRE600 is a knockout for the *hsdM* gene.

MRE600 Lacks Flagella and Exhibits Similarities to Shigella

Images of MRE600 taken using TEM failed to identify flagella (fig. 2), which are commonly observed in *E. coli*. A lack of flagella, and consequently motility, is common, however, to species within the closely related genus, *Shigella*. To gain greater insight into the evolutionary relationship between MRE600, other strains of *E. coli*, and species within *Shigella*, we performed phylogenetic, taxonomic, and genetic analyses. First, we generated a phylogenetic tree using multilocus sequencing typing based on seven housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA*, and *recA*) (Wirth et al. 2006). This analysis found MRE600 to be more similar to species of *Shigella* than to other strains of *E. coli* (fig. 3A). We then performed a taxonomic analysis of MRE600 using Kraken (Wood and Salzberg 2014) and found that MRE600 exhibited 31% and 9% matches with *Escherichia* and *Shigella*, respectively (fig. 3B). As a comparison, we performed the same analysis using data from an *E. coli* K-12 MG1655 assembly (125× average coverage) that we generated. This showed a single major match of 66% with *Escherichia* followed by a minor match with *Shigella* (0.9%) (fig. 3C). These results prompted us to consider additional features that would help classify MRE600. To further investigate the appropriate taxonomic assignment of MRE600, we examined other documented genetic factors that differentiate shigellae from *E. coli*. Previously, it has been shown that shigellae differ from *E. coli* through certain genomic deletions that contain the *cadA* and *ompT* genes (Nakata et al. 1993; Maurelli et al. 1998). Neither of these genes was identified in MRE600, supporting its similarity to species of *Shigella*.

The Architecture of the MRE600 Genome Is Distinct from That of E. coli K12

MRE600 has frequently been used as a model strain for understanding *E. coli* biology; however, our analysis suggests that it is distantly related to common lab strains. As genomes evolve, large-scale changes to genome architecture occur that are not easily resolvable by the comparison of short stretches of DNA. For this reason, we wanted to compare the genome structures of MRE600 and K12. To do this, we aligned the MRE600 and *E. coli* K12 chromosomes using MAUVE (Darling et al. 2010) to identify structural rearrangements, single nucleotide polymorphisms (SNPs), and gaps (fig. 4).

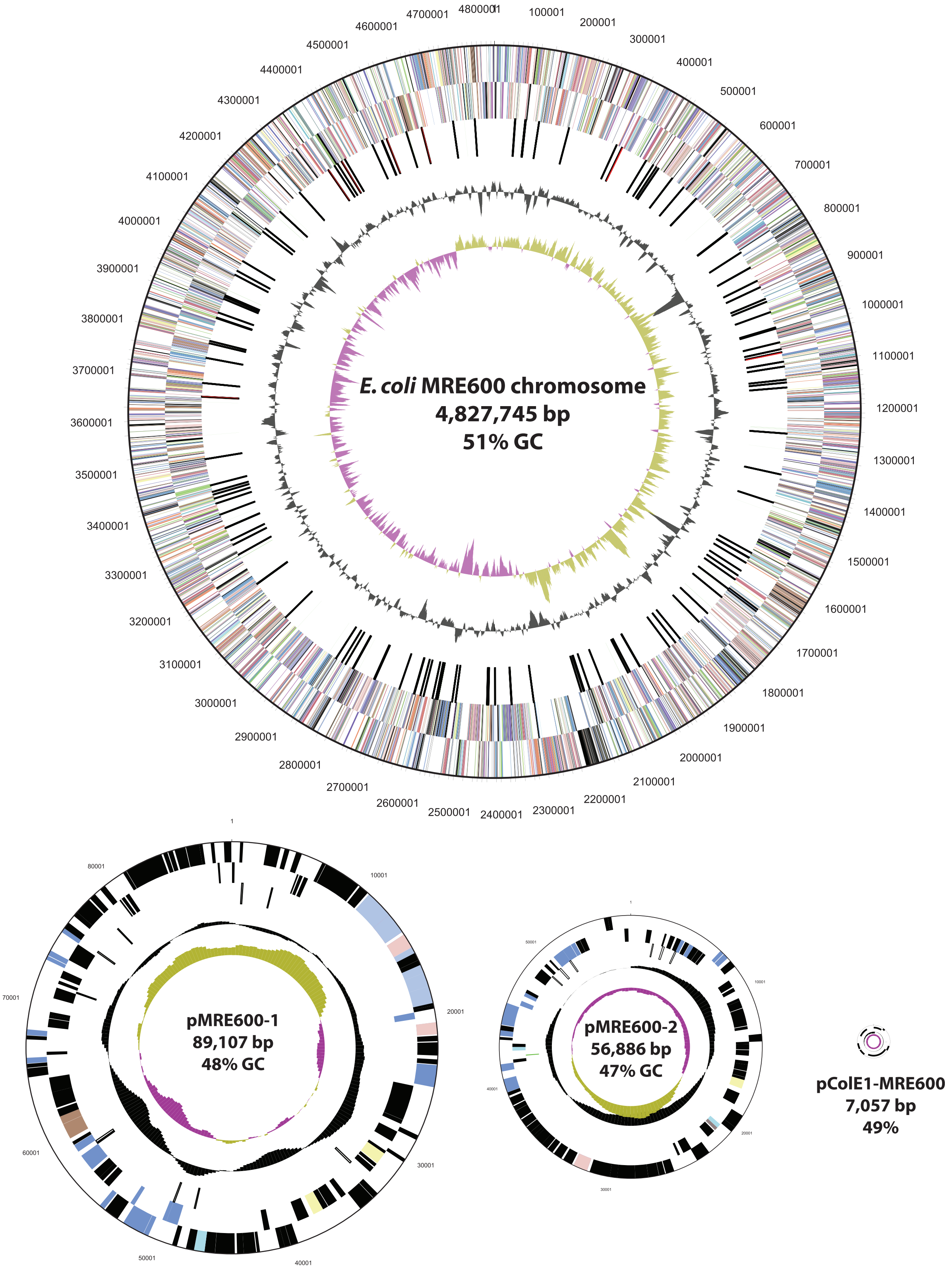


Fig. 1.—Graphical map of the *E. coli* MRE600 genome. Plasmids sizes are relative to pMRE600-1. The maps are annotated from bottom to top as follows: Genes on forward strand (colored by COG categories; Tatusov et al. 2000), genes on reverse strand (colored by COG categories; Tatusov et al. 2000), RNA genes (tRNAs = green, rRNAs = red, other RNAs = black), G + C content (black), G + C skew (purple/olive).

Table 1
Comparing the General Genome Statistics of MRE600 and K12

Attribute	MRE600 Value (% of total)	K12[a] Value (% of total)
Genome size (bp)	4,980,795 (100)	4,639,675 (100)
DNA coding (bp)	4,357,764 (87.5)	4,137,898 (89.2)
DNA G+C (bp)	2,529,115 (50.8)	2,356,477 (50.8)
DNA scaffolds	4	1
Total genes	5,181 (100)	4,497 (100)
Protein coding genes	4,913 (94.8)	4,321 (96.1)
RNA genes	268 (5.2)	179 (3.9)
rRNA genes	22 (0.4)	22 (0.5)
tRNA genes	91 (1.8)	89 (2.0)
Genes with function prediction	4,254 (82.1)	3,897 (86.7)
Genes assigned to COGs[b]	3,566 (68.8)	3,398 (75.6)
Genes with Pfam domains[c]	4,472 (86.3)	3,932 (87.4)
Genes with signal peptides	402 (7.8)	426 (9.5)
Genes with transmembrane helices	1,089 (21.0)	1,038 (23.1)

[a] IMG Genome ID: 646311926.
[b] As described by Tatusov et al. (2000).
[c] As described by Finn et al. (2010).

Table 2
The Number of MRE600 and K12 Genes Associated with Clusters of Orthologous Groups of Proteins Functional Categories (Tatusov et al. 2000)

Code	MRE600 Value (%)	K12[a] Value (%)	Description
J	241 (6.08)	236 (6.19)	Translation, ribosomal structure, and biogenesis
A	2 (0.05)	2 (0.05)	RNA processing and modification
K	285 (7.18)	294 (7.71)	Transcription
L	149 (3.76)	139 (3.64)	Replication, recombination, and repair
D	40 (1.01)	39 (1.02)	Cell cycle control, Cell division, chromosome partitioning
V	87 (2.19)	91 (2.39)	Defense mechanisms
T	189 (4.76)	191 (5.01)	Signal transduction mechanisms
M	245 (6.18)	242 (6.34)	Cell wall/membrane biogenesis
N	133 (3.35)	102 (2.67)	Cell motility
U	60 (1.51)	50 (1.31)	Intracellular trafficking and secretion
O	157 (3.96)	156 (4.09)	Posttranslational modification, protein turnover, chaperones
C	265 (6.68)	284 (7.44)	Energy production and conversion
G	354 (8.92)	381 (9.99)	Carbohydrate transport and metabolism
E	341 (8.6)	355 (9.31)	Amino acid transport and metabolism
F	96 (2.42)	107 (2.8)	Nucleotide transport and metabolism
H	182 (4.59)	179 (4.69)	Coenzyme transport and metabolism
I	114 (2.87)	121 (3.17)	Lipid transport and metabolism
P	214 (5.39)	223 (5.85)	Inorganic ion transport and metabolism
Q	65 (1.64)	68 (1.78)	Secondary metabolites biosynthesis, transport, and catabolism
R	252 (6.35)	261 (6.84)	General function prediction only
S	202 (5.09)	203 (5.32)	Function unknown
—	1615 (31.17)	1099 (24.44)	Not in COGs

Note.—The percentage is relative to the total number of protein-coding genes.
[a] IMG Genome ID: 646311926.

We found 17 regions of high homology, called Local Collinear Blocks (LCBs), and identified a small number of structural rearrangements (fig. 4). According to our analysis, MRE600 and *E. coli* K12 have 63,880 SNPs and 1,850 gaps between their chromosomes. As a control, we performed the same comparative analysis on *E. coli* K12 and *E. coli* BL21 (accession number: NZ_CP010816.1), another strain in phylogenetic group A of *E. coli* (fig. 3) (Meier-Kolthoff et al. 2014). This analysis identified a single LCB, no rearrangements, 39,241 SNPs, and 978 gaps (data not shown). Collectively, these data show that *E. coli* K12 and *E. coli* BL21, which are two closely related strains, have similar genome structures, whereas MRE600 has a much different genome in terms of genomic variants and structure.

Table 3

Summary of DNA Methylation Motifs Discovered across the *Escherichia coli* MRE600 Genome

Motif	Modified Position	Modification Type	% Motifs Detected	No. Motifs Detected	No. of Motifs in the Genome	Mean Modification QV	Mean Motif Coverage
G**A**TC	2	m^{6}A	99.65	39,329	39,468	143.35	91.11
R**C**CGGCRYD	2	m^{5}C*	31.58	378	1,197	46.18	97.90
C**C**AGGVDH	2	m^{5}C*	17.36	912	5,254	42.76	82.98
RC**C**GGY	3	m^{5}C*	6.67	850	12,752	41.29	83.67

Note.—The modified base within each motif is shown underlined and in bold. The QV refers to the level of confidence that a particular base is methylated, and a score of >30 is considered significant. Modification types highlighted with an asterisk (*) were initially classified as either "unknown" or "m^{4}C"; however, their low signal at this coverage suggests that they are more likely CCWGG, CCGG, or RCCGGY motifs containing the m^{5}C modification.

Table 4

Summary of DNA Methylation Motifs Discovered across the *Escherichia coli* K-12 MG1655 Genome

Motif	Modified Position	Modification Type	% Motifs Detected	No. Motifs Detected	No. of Motifs in the Genome	Mean Modification QV	Mean Motif Coverage
GC**A**C(N_6)GTT	3	m^{6}A	100	595	595	87.00	61.85
A**A**C(N_6)GTGC	2	m^{6}A	99.83	594	595	83.35	57.91
G**A**TC	2	m^{6}A	99.94	38,218	38,240	102.46	62.57
C**C**AGGAVH	2	m^{5}C*	33.79	418	1,240	43.10	53.90

Note.—The modified base within each motif is shown underlined and in bold. The QV refers to the level of confidence that a particular base is methylated, and a score of >30 is considered significant. The GCAC(N6)GTT and AAC(N6)GTGC motifs are reverse complements of one another. The m^{5}C modification (*) was initially called an m^{4}C modification by the software; however, its low signal at this coverage suggests that it is more likely a CCWGG motif containing an m^{5}C modification.

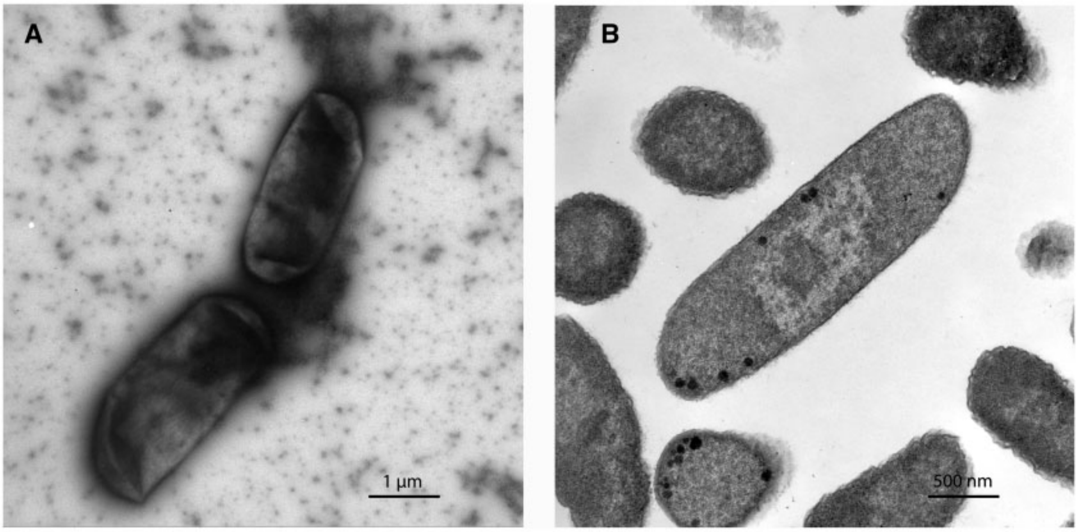


Fig. 2.—Electron micrographs of *E. coli* MRE600 highlighting a lack of flagella. (*A*) MRE600 imaged using a negative stain to improve contrast. (*B*) MRE600 imaged using TEM. For more detail on the procedures used, see Materials and Methods.

The MRE600 Translational Machinery Is Similar to That of K12

Given that MRE600 has been widely used in the field of translation biology, we used our genome assembly to compare the ribosome and tRNA genes of MRE600 and *E. coli* K12. First, we compared all of the 16S rRNA sequences from MRE600 with those in *E. coli* K12. Both strains have seven rRNA operons and all 16S rRNAs share greater than 99% identity (fig. 5A). We also found that MRE600 has the same 55 RP genes as *E. coli* K12 and that for all such proteins, their amino acid sequences share greater than 99% sequence identity (supplementary table S1, Supplementary Material online). Also, MRE600 is frequently used for the expression and purification of tRNAs. To compare the tRNAs of MRE600 with those of K12, we performed pairwise sequence alignments (supplementary figs. S1–S3, Supplementary Material online).

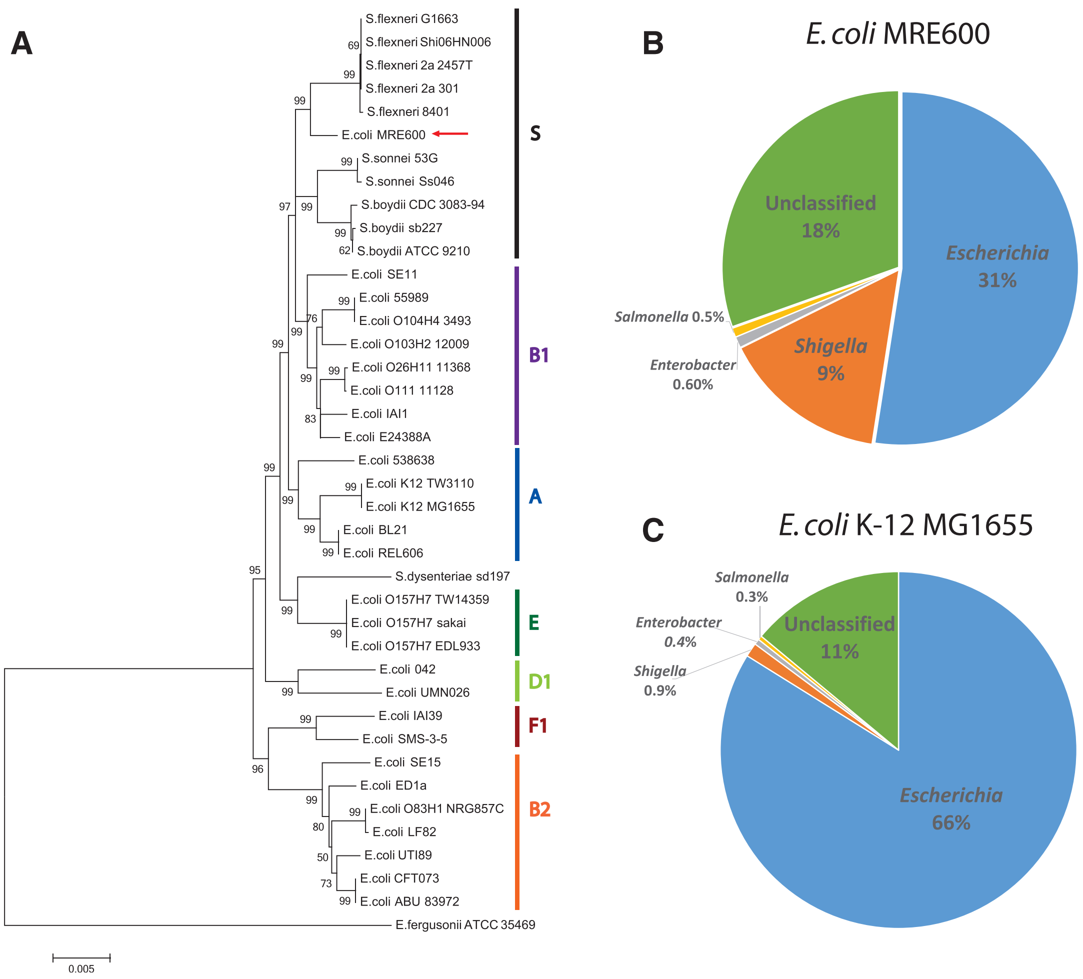


Fig. 3.—Phylogenetic and taxonomic analysis of *E. coli* MRE600. (*A*) Phylogenetic tree showing the relationship of *E. coli* MRE600 with various strains of *E. coli* and *Shigella* based on multilocus sequence typing using seven housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA*, and *recA*) (Wirth et al. 2006). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). *Escherichia coli* phylogenetic groups (A, B1, B2, D1, E, F1) as well as a *Shigella* group (S) are indicated. *Escherichia fergusonii* ATCC 35469 was used as the outgroup. The optimal tree with the sum of branch length = 0.17149911 is shown. The confidence probability (multiplied by 100) that the interior branch length is greater than 0 was estimated using the bootstrap test (1,000 replicates) and is shown next to the branches (Dopazo 1994; Rzhetsky and Nei 1992). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. The analysis involved 40 sequences (9,015 positions each), and positions containing gaps and missing data were eliminated. All analyses were conducted in MEGA6 (Tamura et al. 2013). (*B*, *C*) Sequence classification of MRE600 and *E. coli* K12 using Kraken (Wood and Salzberg 2014). The filtered subreads used for this K12 analysis were from a 125× coverage assembly of *E. coli* K-12 MG1655, which we generated in the same manner as that for MRE600. Pie charts highlight the top four genera associated with the indicated strains. (*B*) MRE600 shows greater similarity to *Escherichia* (31% matches) than *Shigella* (9% matches). (*C*) *Escherichia coli* K12 also showed the greatest similarity to *Escherichia* (66%), but its match with *Shigella* was much lower (0.9%).

Between MRE600 and K12, tRNAs containing the same anticodon generally showed very high sequence identity (>95%).

The RNase I Gene in MRE600 Bears a Premature Stop Codon

MRE600 became a widely used lab strain due to observations that it lacked ribonuclease I activity (Cammack and Wade 1965). We found that MRE600 possesses the RNase I-encoding *rna* gene, but that the gene's fifth codon is a premature stop codon. Thus, as predicted, MRE600 should be unable to produce a full-length RNase I protein.

Discussion

Although *E. coli* MRE600 has served as a key strain for many laboratories focused on the isolation of stable RNAs prominent in translation biology, this study is the first to examine its

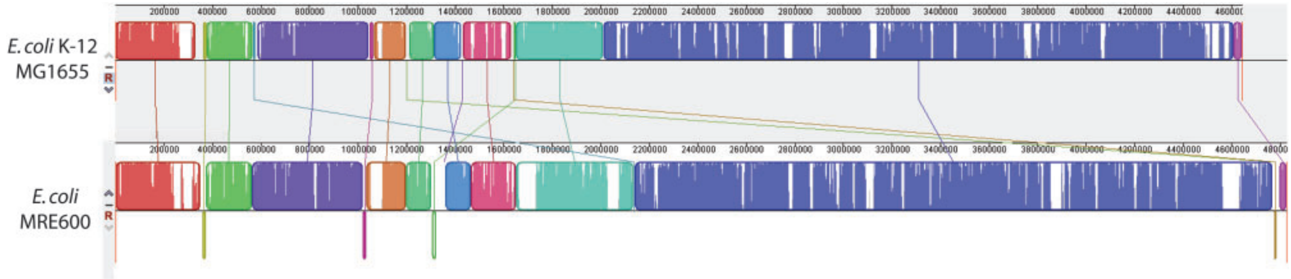


Fig. 4.—Structural analysis of aligned *E. coli* MRE600 and *E. coli* K12[a] chromosomes. Sequences were aligned using the MAUVE aligner version 20150226 build 10 (Darling et al. 2010) and this figure was generated using the MAUVE viewer. LCBs are represented by blocks of different colors and the degree of similarity is indicated using white areas, that is, areas that are completely white within an LCB failed to align and likely contain genome-specific sequence elements. Rearrangements are highlighted using intergenome lines connecting similarly colored LCBs. This alignment identified a total of 63,880 SNPs and 1,850 gaps. The identified gaps had an average length of 722 ± 3,973 bp (SD). [a]Accession #U00096.3.

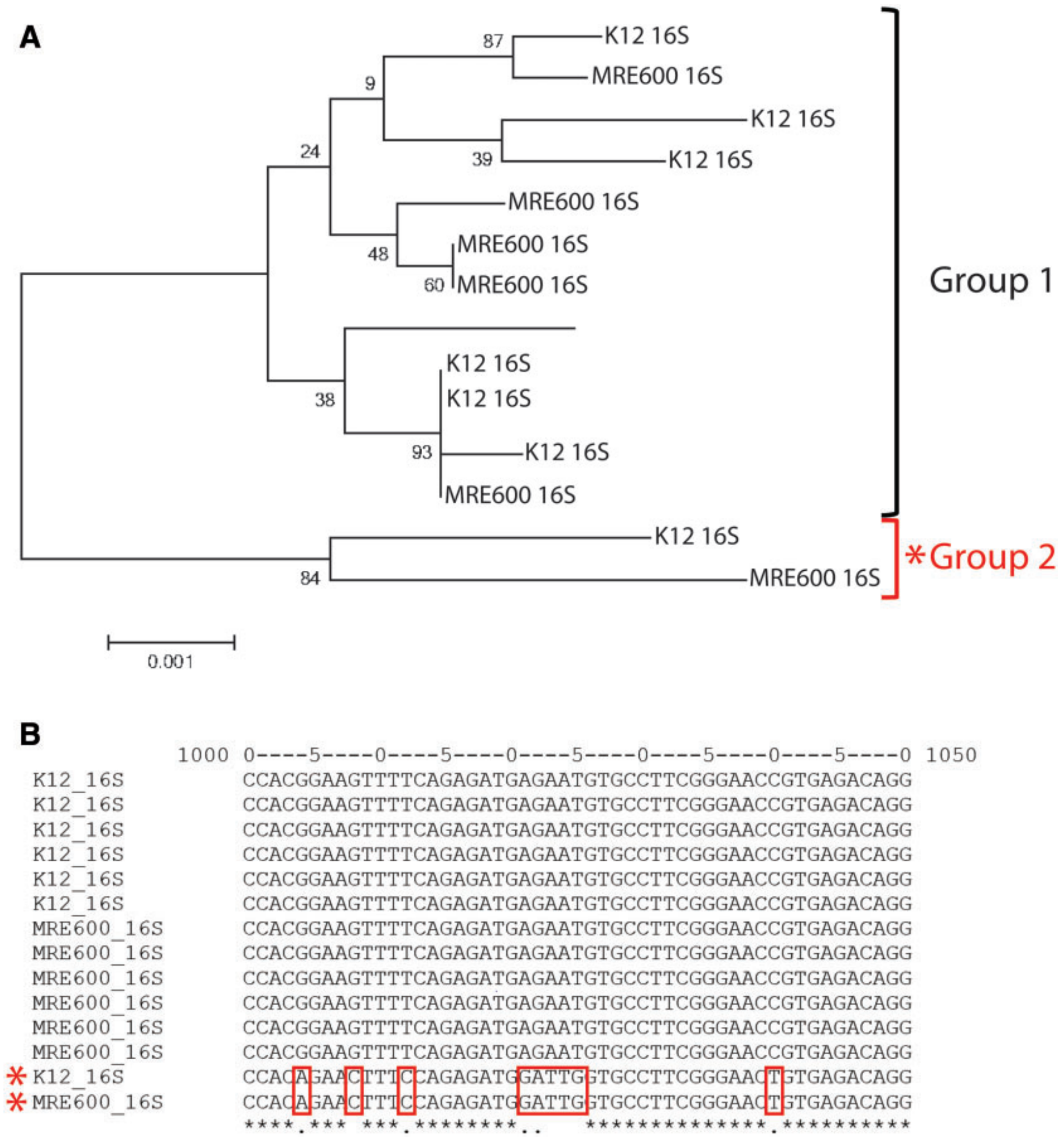
genomic and epigenomic contents in detail. In addition to providing vital context to the myriad studies that have utilized this strain, we have identified MRE600 as a unique organism that possesses qualities similar to other strains of *E. coli*, but also to species of *Shigella*. Difficulties differentiating *E. coli* and *Shigella* have been well documented as numerous studies have suggested that *Shigella* should be regarded as a single species along with *E. coli* (Goullet 1980; Ochman et al. 1983; Whittam et al. 1983; Hartl and Dykhuizen 1984; Pupo et al. 1997). At this time, however, we refrain from suggesting that MRE600 should be reclassified, but note the intermediate location of this strain within the phylogeny (fig. 3).

One factor that prompted our investigation was the observation of endogenous plasmids within MRE600. The presence of plasmids is uncommon among typical lab strains of *E. coli* and we therefore rationalized that a genomic examination of this strain should be performed to assess the potential consequences of using MRE600 alongside other lab strains. Interestingly, we found a 7.1-kb plasmid that contains the genes for a colicin E1 protein as well as its associated immunity protein (fig. 1). The existence of a colicin-expressing plasmid in this organism was predicted by a previous study which identified colicinogenic activity in MRE600 (Salaj-Smic 1978). Colicin E1 expression is toxic to neighboring bacteria that do not express the appropriate immunity protein. Using BtuB as a receptor, colicin E1 proteins kill affected bacteria by forming depolarizing pores in their membranes (Cascales et al. 2007). Colicin-expressing strains are often found in the guts of animals, and although the ecological function of colicin expression is unclear, it is believed to play a role in anticompetition by enabling the invasion of a strain into an established ecological niche (Cascales et al. 2007). Alternatively, it has been proposed that colicin expression can play a defensive role in protecting an established ecological niche against invading strains (Cascales et al. 2007). At this time, we cannot speculate upon the function of colicin expression in MRE600 as we do not currently know the ecological niche in which it evolved.

Given the importance of this strain to the field of translation biology, our assembly and analysis is notable in that it predicts that the composition of *E. coli* K12 and MRE600 ribosomes is nearly identical at both the RNA and protein level (fig. 5 and supplementary table S1, Supplementary Material online). Similarities in the protein synthesis machineries of these two strains also extend to distinctions in the primary sequences of individual rRNA operons (fig. 5), including one operon in particular (the *rrnH* operon in *E. coli* K12). This operon, as well as a homologous operon in MRE600, contains a distinct and varied stretch of nucleotides between positions 1000–1050 within the helix 33 region of 16S rRNA (fig. 5B).

Substantiating reports that MRE600 lacks RNase I activity (Cammack and Wade 1965), our investigation identified a premature stop codon in the RNase I-encoding gene, *rna*. *Escherichia coli* RNase I is a nonspecific endoribonuclease that belongs to the T2 family (Meador and Kennell 1990). RNase I activity was first identified in ribonucleoprotein fractions from *E. coli* in 1958 (Elson 1958) and it was subsequently shown to be specifically associated with 30S ribosomal subunits (Elson and Tal 1959). Mechanistically, RNase I attacks the 3′ phosphates of RNA to yield ribonucleoside 3′-phosphates through a 2′, 3′-cyclic phosphate intermediate (Elson 1959; Spahr and Hollingworth 1961). The name RNase I was introduced in 1964 (Spahr 1964). We conclude that MRE600 does, in fact, lack the ability to express a functional RNase I protein due to an encoded termination site close to the N-terminus of the gene sequence.

Our epigenetic analysis identified a lack of EcoKI-mediated type I methyltransferase activity in MRE600. A subsequent genetic investigation failed to identify the EcoKI-encoding gene, *hsdM*, in MRE600. EcoKI methylates adenine residues within the 5′-GC$\underline{A}$C(N_6)GTT-3′ motif as well as its reverse complement 5′-A$\underline{A}$C(N_6)GTGC-3′ motif (modified adenines are underlined) (Clark et al. 2013). In bacteria, DNA base methylation has been shown to play important roles in a variety of biological processes, including virulence in *E. coli* (Rasko et al. 2011; Kozdon et al. 2013; Beaulaurier et al.



```
           1000 0----5----0----5----0----5----0----5----0----5----0 1050
  K12_16S       CCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGG
  K12_16S       CCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGG
  K12_16S       CCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGG
  K12_16S       CCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGG
  K12_16S       CCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGG
  K12_16S       CCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGG
  MRE600_16S    CCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGG
  MRE600_16S    CCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGG
  MRE600_16S    CCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGG
  MRE600_16S    CCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGG
  MRE600_16S    CCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGG
  MRE600_16S    CCACGGAAGTTTTCAGAGATGAGAATGTGCCTTCGGGAACCGTGAGACAGG
* K12_16S       CCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGG
* MRE600_16S    CCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGG
                ****.*** ***.********..     **************.**********
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Fig. 5.—The relationship between MRE600 and *E. coli* K12 16S rRNAs. (*A*) Phylogenetic tree showing the relationship between MRE600 and *E. coli* K12 16S rRNAs. Two groups of rRNAs were identified, which we named "Group 1" and "Group 2." These relationships were inferred using the Neighbor-Joining method (Saitou and Nei 1987). The percentage of replicate trees in which the associated sequences clustered together in the bootstrap test (1,000 replicates) is shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. The analysis involved 14 sequences (1,543 positions each), and all positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013). (*B*) The two groups of 16S rRNAs are identified by distinct sequences in the region encompassing nucleotides 1000–1050. This multiple sequence alignment was generated using MAFFT (Katoh and Standley 2013).

2015). Thus, as a natural EcoKI knockout strain, MRE600 may become an important model organism for further investigations of this particular modification.

Although the genome structure as well as epigenetic and genetic content of MRE600 shows that it is markedly distinct from common laboratory strains of *E. coli*, such as K12, targeted comparisons of the translational machinery from these two strains show that they are remarkable similar at the primary RNA and amino acid sequence level. These analyses validate the interchangeability of protein synthesis components derived from K12 and MRE600 strains for continued investigations of the translation mechanism.

Supplementary Material

Supplementary figures S1–S3 and table S1 are available at *Genome Biology and Evolution* online (http://www.gbe.oxfordjournals.org/).

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