ISSN 0026-2617, Microbiology, 2019, Vol. 88, No. 3, pp. 309–315. © Pleiades Publishing, Ltd., 2019.
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EXPERIMENTAL ARTICLES

# Genetic Analysis of the Probiotic Strain *Escherichia coli* M-17 and the Potentially Probiotic Strain *Escherichia coli* BM

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Received October 16, 2018; revised January 17, 2019; accepted February 1, 2019

**Abstract**—Genomic sequences were studied for two *Escherichia coli* strains: an industrial strain *E. coli* M-17 and a newly isolated high-technology strain *E. coli* BM. *E. coli* M-17 was confirmed to possess the species-specific biochemical profile; transmissive genes of antibiotic resistance, pathogenicity, and virulence, as well as integrated plasmids were not revealed. The sequence type of the strain was found to be ST141. *E. coli* BM was found to possess decreased enzymatic activity. Its genome was found to contain determinants for antibiotic resistance (*strA*, *strB*, *sul2*, *aadA1*), several pathogenicity and virulence determinants, the *Tn7* transposon, and fragments of incorporated plasmids (*IncFIB* and *Col156*). The strain was found to belong to the tenth sequence type, ST-10. These results indicate that *E. coli* strain M-17 satisfies all modern requirements to probiotic-producing strains, while due to genomic properties of *E. coli* BM this strain may not be considered industrially promising for production of probiotic preparations, in spite of its good technological characteristics. Complete genome sequences of the strains *E. coli* M-17 and *E. coli* BM were deposited to GenBank under accession nos. NZ_LBDD00000000 and NZ_LBDC00000000, respectively.

*Keywords: Escherichia coli* M-17, full genome sequencing, genome annotation, probiotic strains

**DOI:** 10.1134/S0026261719030032

The strain *Escherichia coli* M-17, introduced by Peretts (1955), is used for producing coli-containing probiotics in Russia. This strain is characterized by a broad spectrum of useful properties and has been used in biotechnology for over seventy years; however, its technological efficiency, is relatively low due to a low biomass accumulation rate. Search for alternative industrially promising strains of this species suitable to be used for technological purposes is therefore an urgent issue. This work represents the results of the study of the genomes of two *Escherichia* strains, M-17 conventionally used for production of probiotics and the new strain *E. coli* BM isolated from the intestines of a healthy human and characterized by good technological characteristics.

According to the present-day normative documents, the safety of probiotic producer strains must be confirmed by the absence in them of transmissive genes of antibiotic resistance, virulence and pathogenicity genes, as well as of incorporated plasmids and transposons (*Metodicheskie ukazaniya no. 2.3.2.2789-10*, 2010; *Metodicheskie ukazaniya no. 4.2.2602-10*, 2011). The biological properties and technological characteristics of both strains were studied earlier; however, no in-depth study of their genomes was conducted (Gaiderov, 2007; Ladygina et al., 2009). Within the framework of this work, we studied whole-genome sequences of the strains using the following resources available online: the Warwick University EnteroBase International *Escherichia* and *Shigella* genomic sequence database (Warwick Medical School, UK, http://enterobase.warwick.ac.uk) and the services available at the Center for Genomic Epidemiology website (Denmark, http://www. genomicepidemiology.org/). Thus, analysis of genomes for the presence of the pathogenicity, antibiotic resistance, and virulence determinants was carried out, as well as their molecular typing using the multilocus sequence typing (MLST).

The goal of the present work was to study the genomes of the strains *E. coli* M-17 and *E. coli* BM for the presence of the antibiotic resistance, pathogenicity, and virulence determinants, as well as of incorporated plasmids and transposons.

## MATERIALS AND METHODS

**Study subjects.** The work used the strains *Escherichia coli* M-17 and *E. coli* BM. The strain *E. coli* M-17 was obtained from the NPO Mikrogen Stock Cultures Collection, Ministry of Health of the Russian Federation, Nizhny Novgorod, ImBio Enter-

prise for Manufacture of Bacterial Preparations. The strain is registered in the Collection of the Scientific Center of Expert Evaluation of Medical Products (SCPM State Collection of Pathogenic Microorganisms, no. 240418). The strain *E. coli* BM obtained from the collection of the Laboratory for Human Microbiome and the Means of Its Correction, Blokhina Nizhny Novgorod Research Institute of Epidemiology and Microbiology, Federal Service for Supervision of Consumer Rights Protection and Human Wellbeing, was isolated from intestine of a healthy human and was characterized as a high-technology one. The strains were obtained in the liophilically dry state.

Strain recovery was performed using the HiMedia nutrient broth (India). The grown culture was titrated in the $10^{-1}$–$10^{-7}$ dilution range, and Endo medium (nutrient medium for isolation of enterobacteria–GRM Endo agar, Obolensk, Russia) was inoculated with 0.05 mL of the culture; the inocula were incubated at 37 ± 1°C for 24 h. The colonies of grown microorganisms were assessed by morphology and selected for extensive study of the biochemical features using Diagnostic Systems Biochemical Enterobacterium-Differentiating Plates (BEDP) (Russia), Paper Indicator Systems (PIS) for identification of microorganisms—kit no. 2 for intergeneric and species differentiation of enterobacteria (NPO Mikrogen, Ministry of Health of the Russian Federation, Russia), as well as HiMedia media (India).

**Genomic DNA** was isolated using the commercial QIAamp DNA Mini Kit (Qiagen, Germany); fragmentation was carried out using the Covaris E210 ultrasonic fragmentation system (Applied Biosystems, United States) according to the manufacturer's instructions. The mixture was purified and 300–500-bp fragments were selected using Agencourt AMPure magnetic beads (Beckman Coulter, United States). The TrueSeq Kit (Illumina, United States) was used for library preparation; sequencing was performed on the MiSeq platform (Illumina, United States). Short reads were collected into contigs using the CLC Bio v. 8.0 software package (Aarhus, Denmark).

**Genome annotation** was performed using the Prokka v. 1.11 utility (Seemann, 2014) and the RAST genomic server (http://rast.nmpdr.org). For detailed genome studies, specialized software products available at the Center for Genomic Epidemiology website (https://cge.cbs.dtu.dk/services/) were used. The study of the CRISPR region was carried out with the CrisprFinder software package (Grissa et al., 2017); search for the antibiotic resistance and pathogenicity determinants, with ResFinder 2.1 and PathogenFinder (Cosentino et al., 2013; Zankari et al., 2012). The VirulenceFinder package was used to search for virulence determinants; PlasmidFinder 1.3, to detect incorporated plasmids; SerotypeFinder 1.1 was used for serotype determination; the FimHTyper service, to study the genovariant of FimH adhesin (Joensen et al., 2014; Carattoli et al., 2014; Joensen et al., 2015; Roer et al., 2017).

MLST typing was carried out using the MLST-1.8 Server software package (Larsen et al., 2012) and the Warwick University MLST database (Mark Achtman Database, http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/) by analyzing the sequences of seven constitutive housekeeping genes (Alikhan et al., 2018).

## RESULTS AND DISCUSSION

Investigation of the biochemical properties of *E. coli* strains using the standard BEDP and PIS test-systems, as well as additional tests (xylose, rhamnose, dulcitol, and gelatinase activity) using HiMedia media, revealed differences in the biochemical profiles of the strains. *E. coli* M-17 was capable of fermenting sucrose, maltose, sorbitol, glucose, lactose, arabinose, mannitol, xylose, and rhamnose; it formed indole, possessed β-galactosidase activity, utilized sodium citrate with glucose, and possessed lysine and ornithine decarboxylases. The strain was unable to liquefy gelatin, utilize citrate and sodium malonate, did not have arginine dehydrolase and phenylalanine deaminase, did not form acetylmethylcarbinol, did not ferment inositol and dulcitol, did not hydrolyze urea and did not form hydrogen sulfide. It is this biochemical profile that is characteristic of the strain *E. coli* M-17 proposed by Peretts in 1930 and declared in the pharmacopoeial monograph for dry colibacterin (Peretts, 1955). The strain *E. coli* BM differed in that it was incapable of fermenting sucrose, malose, and sorbate and of decarboxylating ornithine.

When whole-genome sequencing of *E. coli* M-17 and *E. coli* BM was performed, 42 (N50 = 465.806, mean coverage 250) and 59 (N50 = 205.755, mean coverage 250) contigs, respectively, were collected. Further analysis allowed us to determine the main characteristics of the strain genomes (Table 1).

It is evident that both strains possessed similar genome sizes and both genomes include colicin V synthesis operons, while *E. coli* M-17 additionally contained colicin B synthesis genes.

CRISPR regions were present in the genomes of both strains, *E. coli* M-17 CRISPR locus was within contig 57 (LBDD01000002); that of *E. coli* BM was within contig 68 (LBDC01000001). In terms of structural organization, these determinants of both strains can be assigned to type I-E, which is typical of members of the genus *Escherichia* (Makarova et al., 2015). A distinctive feature of this type is the presence of the *cas3* gene, which encodes single-stranded DNA stimulating helicase that untwists double-stranded DNA (Fig. 1).

In a number of cases, the CRISPR cassettes of microorganisms are used for strain typing as this genomic region does not correlate with phylogeny and

**Table 1.** Phenotypic and genetic characteristics of *E. coli* strains M-17 and BM

| Strain | | *E. coli* M-17 | *E. coli* BM |
|---|---|---|---|
| Biochemical activity | SUC* | + | – |
| | MAL | + | – |
| | SOR | + | – |
| | ORN | + | – |
| Genome size, bp | | 5105186 | 5224908 |
| GC, % | | 50.7 | 50.7 |
| Gene number | | 5.107 | 5.559 |
| CDS (protein-encoding sequences) | | 4.930 | 5.094 |
| CRISPR region | | 1 | 1 |
| Colicin synthesis operons | | Colicin V<br>Contig_14 LBDD01000032<br>3025_2210<br>Colicin B<br>Contig_72 NZ_LBDD01000012<br>40387_41076 | Colicin V<br>Contig_107 NZ_LBDC01000009<br>94132_94620 |
| Antibiotic resistance determinants | *strB* | – | Contig_22 NZ_LBDC01000022.1<br>5610_6446 |
| | *aadA1* | – | Contig_61 NZ_LBDC01000028.1<br>29677_30465 |
| | *strA* | – | Contig_22 NZ_LBDC01000022.1<br>4807_5610 |
| | *sul2* | – | Contig_22 NZ_LBDC01000022.1<br>3931_4746 |
| | *DfrA1* | – | Contig_61 NZ_LBDC01000028.1<br>31142_31615 |
| | mdtABCD cluster | Contig_55 LBDD01000001<br>1044601_1048971 | Contig_78 LBDC01000013.1<br>73875_69381 |
| | MAR locus | Contig_66 LBDD01000004<br>415943_417243 | Contig_103 NZ_LBDC01000020<br>25244_23280 |
| | Efflux pumps | CmeA CmeB<br>Contig_63 LBDD01000005<br>431924_436197<br>TolC Contig_63 LBDD01000005<br>202366_203847<br>AcrR Contig_63 LBDD01000005<br>431525_430863<br>MacA MacB<br>Contig_57 LBDD01000002<br>117495_114479<br>MATE MDR Pump<br>Contig_65 LBDD01000007<br>225090_223717<br>MFS Contig_76 LBDD01000003<br>414925_416151 | CmeA Contig_32 LBDC01000025<br>10318_9161<br>CmeB Contig_56 LBDC01000005<br>102713_105862<br>TolC Contig_99 LBDC01000002<br>186357_184876<br>MacA Contig_60 NZ_LBDC01000016<br>47123_46008<br>MATE MDR pump<br>Contig_100 LBDC01000003<br>292755_291382<br>MFS Contig_72 NZ_LBDC01000015<br>92555_93781 |

**Table 1.** (Contd.)

| Strain | | *E. coli* M-17 | *E. coli* BM |
|---|---|---|---|
| Virulence and pathogen city genes | *TieB* | – | Contig_73 LBDC01000014 73595.74770 |
| | *iha* | – | Contig_51 LBDC01000012 2522.4612 |
| | *gad* | – | Contig_99 LBDC01000002 522096.523496 |
| | *exc1* | – | Contig_22 NZ_LBDC01000022.1 1397.1837 |
| Incorporated plasmids and mobile elements | Transposon *Tn7* | – | Contig_61 NZ_LBDC01000028 19928_27710 |
| | *IncFIB* | – | Contig_46 LBDC01000004 186596_217956 |
| | *Col156* | – | Contig_73 LBDC01000014 68539_69105 |
| Serotype | | O2:H6 | O21:H4 |
| FimH type | | 14 | 215 |
| ST | | 141 | 10 |
| Number in the GenBank DB | | NZ_LBDD00000000.1 | NZ_LBDC00000000.1 |
| Number in the EnteroBase DB | | ESC_GA8607AA | ESC_IA6791AA |

* SUC, sucrose; MAL, maltose; SOR, sorbitol; ORN, ornithine.

its structure may differ even in different strains of the same species (Makarova et al., 2015). However, we established in the course of the work that, despite the fact that the CRISPR loci of both strains differed in the arrangement of the Cas proteins and had different sequences of spacers and repeats (Fig. 1), their spacers were not strain-specific and had 100% identity with the sequences of a number of other *E. coli* strains represented in the GenBank database.

The genomic context of strain metabolism was studied with RAST. Several pathways of central carbohydrate metabolism were found to be determined in the genomes: glycolysis, the Entner–Doudoroff pathway, and the pentose phosphate pathway. Using the KEGG map algorithm (http://rast.nmpdr.org/seedviewer.cgi), it was shown that the metabolic pathways of the strains had no specific features. However, the genome of *E. coli* M-17 had the determinants responsible for sucrose utilization: the sucrose-6-phosphate hydrolase (*scrB*) and phosphofructokinase (*fruK*) genes, and the corresponding transportation system (*PTS scrA*). At the same time, *E. coli* BM did not possess these determinants, which was consistent with the specific features of its biochemical profile—the strain was unable to ferment sucrose. Moreover, *E. coli* BM did not decarboxylate ornithine and did not ferment maltose and sorbitol, this being its distinctive feature, because over 90% of *E. coli* strains utilize maltose and sorbitol, and the ability to utilize sucrose and ornithine is variable within the species (De Vos et al., 2009). It was noted that the determinants of maltose, sorbitol, and ornithine metabolism were present in the strain

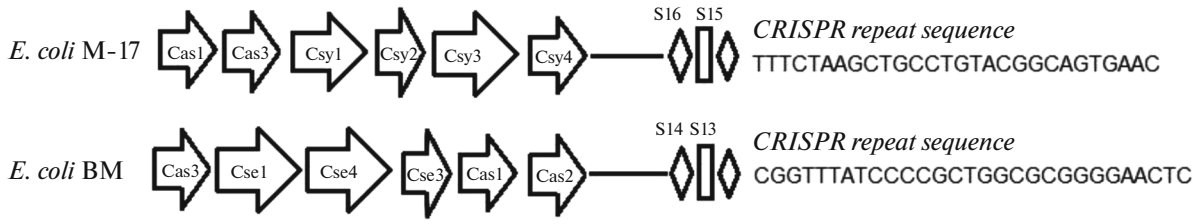


**Fig. 1.** Organization of CRISPR cassettes of the two *E. coli* strains. The arrows designate the corresponding Cas proteins, the diamonds, repeats; the triangles, unique spacers.

genome, but for some reason, they did not reveal themselves phenotypically. Such a loss of individual determinants or their inactivation may be associated with the adaptation of a microorganism to a specialized ecological niche—human intestine (Ravin and Shestakov, 2013). On the whole, both strains possessed a marked metabolic potential, being capable of synthesizing colicins and utilizing a broad spectrum of substrates. The whole-genome sequences of the strains were analyzed for the presence of the antibiotic resistance, pathogenicity, and virulence determinants, as well as for the presence of incorporated plasmids and transposons. In the process, it was established that both genomes shared the transporter gene cluster (*mdtABCD*) responsible for resistance to novobiocin and doxycholate, the MAR locus genes responsible for the system of efflux of pharmaceutical preparations, including antibacterials, as well as other forms of molecular efflux pumps: *cmeA*, *cmeB*, *tolC*, *acrR*, *macA*, *macB*, the MATE and MFS families. All the above determinants have chromosomal localization, are typical of this genus, and do not pose threat in terms of transmissive spread (Baranova and Nikaido, 2002; Alekshun and Levy, 2004).

Investigation of *E. coli* M-17 did not reveal the antibiotic resistance determinants capable of transmissive transfer, whereas the *sul2* sulfanilamide resitance gene in the genome of *E. coli* BM was found to encode sulfanilamide-resistant dehydropteroate synthase, as well as the aminoglycoside resistance genes: the *aadA*1 gene encoding aminoglycoside-adenylyl transferase, the genes *strB* and *strA* encoding aminoglycoside phosphotransferases, and the *dfrA1* trimethoprim resitance determinant. In the studied genome, the determinants *sul2*, *strB,* and *strA* found in the twenty-second contig were flanked by proteins from the family of transposases (WP_001120891.1), and the genes *aadA*1 and *dfrA1* located within the sixty-first contig were the components of transposon *Tn7* that plays an important role in genetic information transfer and the spread of antibiotic resistance genes (Petrova, 2008).

When studying the virulence and pathogenicity determinants, we did not reveal in the genomes of either strain the determinants encoding enterotoxins *Stx1* and *Stx2*, thermostable toxin *St*, intimins *Eae*, and enterohemolysins *Ehx*, which are linked to the clinically significant diarrheagenic strains of *E. coli* (Kartsev et al., 2018). However, the genome of *E. coli* BM was found to have other significant determinants: the *tieB* gene of plasmid origin also encoding enterotoxin, the bifunctional adhesion gene *iha*, the glutamate decarboxylase gene *gad,* and the gene encoding the *Exc1* mobile transfer protein (Table 1). According to the PathogenFinder service, *E. coli* BM was assigned to the strains pathogenic for humans (score = 0.868). No pathogenicity and virulence determinants were revealed in the genome of *E. coli* M-17; the strain was characterized as nonpathogenic (score = 2.0).

The strain genomes were tested for the presence of incorporated plasmids and their fragments using the PlasmidFinder-1.3 software. It was found that the genome of *E. coli* M-17 did not contain incorporated plasmids, while the genome of *E. coli* BM contained the fragments of two plasmids—IncFIB typical of uropathogenic *Escherichia* strains and Col156. The genes of plasmid origin were located within contigs 46 and 73 and encoded a complex of the conjugative transfer proteins *TraA*, *TraM*, *TraY,* etc. (Table 1).

The presence and genovariants of the *fimH* gene determining type I mannose-sensitive pili responsible for adhesion of *E. coli* cells (Markova et al., 2018) were then studied. It was established that both strains carried this gene, *E. coli* M-17 *fimH* gene being identified with type 14; *fimH* of *E. coli* BM belonged to type 215. Some researchers consider the presence of *fimH* as a property determining potential virulence of a microorganism due to high adhesion values and ability to form biofilms and, hence, unsuitability for industrial purposes (Nechaeva et al., 2016; Slukin et al., 2017). However, according to the present-day data, the presence of type I pili and of adhesin *fimH* is not always associated with virulence and occurs in *E. coli* strains not possessing the adhesive–invasive phenotype. Note that the authors link an increased capacity for adhesion and invasion to point mutations of the *fimH* gene at hot points (Dreux et al., 2013). No mutations determining the G73A/E/R/W and T158A/P substitutions and contributing to increased adhesion of a bacterial cell on intestinal epithelial cells, which is characteristic of adhesive–invasive *E. coli* (AIEC), were found in the *fimH* genes of the strains studied. *Fim*-types 14 and 215, the identity with which was revealed in the course of the study, are not inherent in pathogenic strains and occur rather rarely among the population of *E. coli* (Roer et al., 2017).

Using the SerotypeFinder-1.1 software product, we analyzed the determinants of *E. coli* antigens: the genes encoding the structural protein flagellin *FliC* (KLD44527.1; KLD52182.1) and the O-antigen proteins: *Wzx* (KLD49178.1; KLD50213.1) and *Wzy* (KLD49266.1; KLD50216.1). It was established that *E. coli* M-17 and *E. coli* BM belonged to serotypes O2:H6 and O21:H4, respectively.

In the framework of the study, MLST typing of the strains was carried out using the scheme proposed by Worwick University. This scheme uses the fragments of seven housekeeping genes: the adenylate kinase (*adk*), fumarate hydratase (*fumC*), DNA gyrase (*gyrB*), isocitrate dehydrogenase (*icd*), malate dehydrogenase (*mdh*), adenylosuccinate dehydrogenase (*purA*), and recombinase A (*recA*) genes. It was established that the strain *E. coli* M-17 belonged to sequence type 141 (ST-141), and *E. coli* BM was attributed to sequence type 10 (ST-10) (Table 2).

According to the EnteroBase International Database information (accession date April 28, 2018), 12

**Table 2.** Allelic profiles of the studied *Escherichia* strains

| Strain | Alleles | | | | | | | ST |
|---|---|---|---|---|---|---|---|---|
| | *adk* | *fumC* | *gyrB* | *icd* | *mdh* | *purA* | *recA* | |
| *E. coli* M-17 | 10 | 11 | 4 | 8 | 8 | 8 | 2 | 141 |
| *E. coli* BM | 13 | 52 | 10 | 14 | 17 | 25 | 17 | 10 |

out of 24 strains with the O2:H6 serotype were identified with sequence type 141; they are characterized as nonpathogenic and are not related to the clinical manifestations of diseases.

The strain *E. coli* BM had the serotype O21:H4 and was assigned to sequence type 10 (ST-10). At the moment of accession (April 28, 2018), four strains with serotype O21:H4 were deposited to the EnteroBase database, two of them belonging to sequence type 10. Importantly, one of these strains, which is of human origin and is characterized as enteroaggregative, is associated with the diarrheal syndrome. Moreover, the heteropathogenic *E. coli* strains known to act as entero- and uropathogens belong to the same ST-complex (ST complex 10) (Kartsev et al., 2018).

Thus, it was shown in the course of the work that *E. coli* M-17 does not contain the antibiotic resistance and virulence determinants or incorporated plasmids; in terms of the pooled data of analysis of the whole-genome sequence and molecular typing, the strain is not pathogenic for humans and can be used for the manufacture of probiotics and biologically active food supplements.

In turn, the strain *E. coli* BM possesses a complex of antibiotic resistance and virulence genes, carries the mobile element genes, incorporated plasmids, and is found to be pathogenic for humans. The results of molecular typing enabled us to establish its sequence type—ST-10, and confirmed its identity with the epidemically significant clone. The results obtained rule out the use of this strain as a probiotic according to the present-day regulatory documents.

Our findings prove that the use of whole-genome sequencing that allows researchers to detect and analyze all determinants of interest and to perform molecular typing of the strain is a priority in the work with industrially promising strains.

## COMPLIANCE WITH ETHICAL STANDARDS

*Statement of the welfare of animals*. This work did not involve any experiments in animal objects.

*Conflict of interest*. The authors declare that there exists no conflict of interests.

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*Translated by E. Babchenko*