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Conjugative IncX1 Plasmid Harboring Colistin Resistance Gene *mcr-5.1* in *Escherichia coli* Isolated from Chicken Rice Retailed in Singapore

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Colistin is regarded as one of the last-resort antimicrobials for treatment of Gram-negative bacterial infections (1). Several cases of plasmid-borne colistin resistance genes *mcr-1*, *mcr-3*-like, and *mcr-4.2* in clinical *Enterobacteriaceae* isolates, including *Escherichia coli*, have been reported in Singapore (2–4). However, the *mcr-5* gene has not been reported in clinical isolates in Singapore. Previously, we reported the antimicrobial resistance (AMR) genotype and phenotype of *E. coli* SGEHI2010ENV103 isolates from ready-to-eat food in Singapore and documented the first isolate carrying *mcr-5.1* in Singapore (5). In this study, we further analyzed this isolate. Here, we report the first complete nucleotide sequence of a transferable plasmid harboring *mcr-5.1* in *E. coli* isolated from ready-to-eat chicken rice in Singapore. (Chicken rice is a common dish in Singapore which is composed of cooked chicken and seasoned rice, served with sauce and cucumber garnishes.)

This *E. coli* isolate was obtained through the retail food surveillance program by the National Environmental Agency (NEA). It was isolated from retail chicken rice from a hawker center in Singapore in 2010. The MIC of colistin for this isolate was further determined to be 8 μg/ml using broth dilution assay as described by CLSI (6). Double-disk synergy testing was performed as previously described (5), and no extended-spectrum-β-lactamase (ESBL) phenotype was detected. Conjugation experiments were performed using the filter mating method (7). Sodium azide-resistant *E. coli* strain J53 was the recipient strain, and transconjugants were selected using 4 μg/ml colistin plus 200 μg/ml sodium azide. PCR confirmed the presence of *mcr-5* in transconjugants after 24 h of coculture (8), suggesting that the *mcr-5.1* genes were able to be transferred to recipient *E. coli* strain J53. The transfer frequency determined was 10^{-6}. This suggests that this *mcr-5.1*-carrying plasmid might have the potential to transfer to other strains. The stability test for the plasmid carrying *mcr-5.1* was performed as previously described (9). The plasmid was still stable after 20 successive days of subculture in LB broth (~200 generations) without colistin selection, demonstrating the stability of the plasmid. The drug MICs of the transconjugant and *E. coli* J53 were determined using MicroScan Neg MIC panel type 44 (Beckman Coulter, Inc., Brea, CA, USA), in accordance with the manufacturer's instructions (data not shown). The colistin MIC of transconjugants was further determined by broth dilution assay, and the results showed an 8-fold increase (from 1 to 8 μg/ml).

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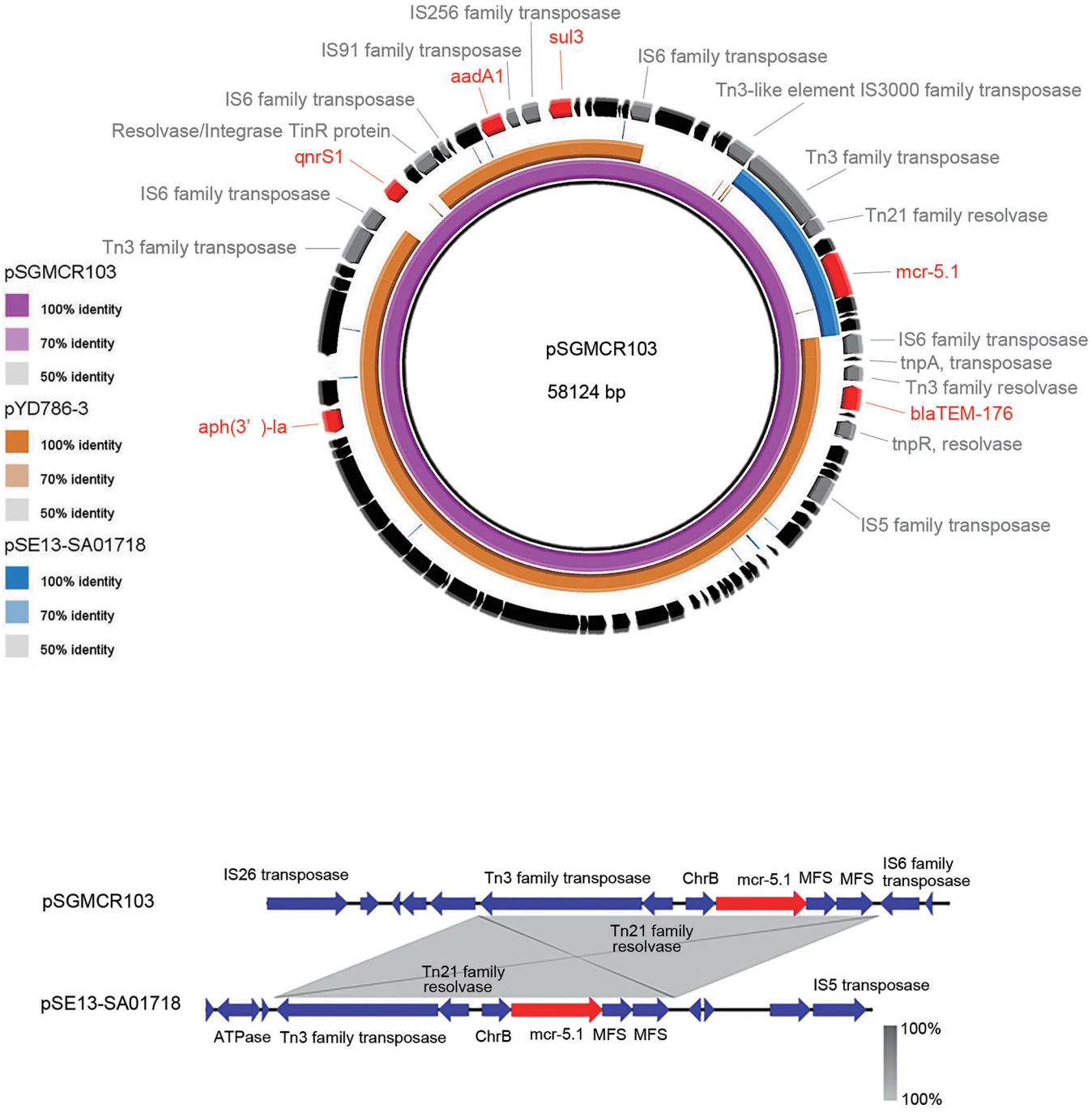

FIG 1 (Top panel) Sequence map of plasmids pSGMCR103, pYD786-3 (GenBank accession number KU254580.1), and pSE13-SA01718 (GenBank accession number KY807921.1). The outermost circle shows the predicted coding sequences of pSGMCR103. The red parts indicate antimicrobial resistance genes; the gray parts indicate the genes encoding mobile element protein. The figure was generated by the use of BRIG (http://brig.sourceforge.net/). (Bottom panel) Genetic environment of the *mcr-5.1* gene in comparison to pSE13-SA01718. ME, mobile element; MFS, gene encoding major facilitator superfamily (MFS)-type transporter; ChrB, gene encoding the protein involved in chromate resistance. The figure was drawn by the use of EasyFig 2.2.2 (http://mjsull.github.io/Easyfig/).

The total DNA of SGEHI2010ENV103 was extracted and purified using a QIAamp DNA minikit and sequenced using a Pacbio RS II system and a HiSeq 2500 system. The closed plasmid was assembled by Unicycler on the Galaxy platform (https://usegalaxy.org/) using both long-read and short-read data with default parameters (10). It was annotated by Rapid Annotation using Subsystem Technology (RAST) (11) and corrected by the use of BLASTn (12). AMR genes or related site mutations, plasmid type, serotype, and sequence type were determined using the online tools on the Center for Genomic

Epidemiology website (https://cge.cbs.dtu.dk/services/), and default settings were used during analysis.

The serotype and sequence type of SGEHI2010ENV103 are determined to be O99:H38 and ST8361, respectively. The sequence of pSGMCR103 revealed a circular plasmid of 58,124 bp in length with 47.7% G+C content. PlasmidFinder showed that it has 98.93% identity with the IncX1 replicon. Members of the IncX group of plasmids are commonly found in *Enterobacteriaceae* with a narrow host range. They are known to encode fimbriae which enable conjugative transfer (13). Among the five subtypes of the IncX group, only IncX4 was previously reported to be related to *mcr* genes in *E. coli*, *Salmonella* spp., *Klebsiella* spp., etc. (14, 15). This is the first time that the IncX1 group of plasmids carrying *mcr* gene has been reported.

The plasmid sequence closest to pSGMCR103 in NCBI is that of plasmid pYD786-3 (GenBank accession number KU254580.1) with 77% query coverage and 99% identity, which was carried by one *E. coli* isolate from a human urine specimen collected in the United States. A comparison of these two plasmids is shown in the top panel of Fig. 1. They share antimicrobial resistance genes *aph(3′)-Ia*, *aadA1* (aminoglycoside resistance), $bla_{\text{TEM-176}}$ (beta-lactam resistance), and *sul3* (sulfonamide resistance). In addition, pSGMCR103 also carries quinolone resistance gene *qnrS1* and colistin resistance gene *mcr-5.1*. Gene *mcr-5.1* was harbored on a Tn*3* transposon-like element, which is similar to pSE13-SA01718 (accession number KY807921.1) carried by a *Salmonella* isolate reported before. All three components of Tn*3* were found on this plasmid, including beta-lactamase (encoded by gene *bla*), Tn*3* transposase (encoded by gene *tnpA*), and Tn*3* resolvase (encoded by gene *tnpR*) (Fig. 1, top panel). Its genetic environment is shown in the bottom panel of Fig. 1. Also, other insertion elements such as the IS*5*, IS*6*, IS*91*, and IS*256* family (shown in the top panel of Fig. 1) were found on the plasmid, which may indicate the recombinational activity of the plasmid.

To our knowledge, this is the first report of a complete sequence analysis of a plasmid carrying *mcr-5* in *E. coli* in ready-to-eat food, and it is the first report of the association of *mcr-5* with the IncX1 plasmid. This has enlarged the range of plasmid types known to harbor *mcr-5*. The Tn*3*-like mobile element may enhance the transferability of the *mcr-5* gene and make it easier for that gene to coexist with the $bla_{\text{TEM-176}}$ beta-lactamase gene.

Data accessibility. The sequence of plasmid pSGMCR103 was deposited at GenBank with accession number MK731977. The raw reads of Pacbio sequencing for isolate SGEHI2010ENV103 are available under project identifier PRJNA531074 in the Sequence Read Archive (SRA).

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We declare that we have no conflicts of interest.

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