Biotechnology Reports 17 (2018) 6–9

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Short Communication

Complete genome sequence of N_2-fixing model strain *Klebsiella* sp. nov. M5al, which produces plant cell wall-degrading enzymes and siderophores

Zhili Yu[a,1], Shuying Li[a], Yuanyuan Li[a], Zhuang Jiang[a], Jinru Zhou[b], Qianli An[a,*]

[a] Ministry of Agriculture Key Lab of Molecular Biology of Crop Pathogens and Insects, Institute of Biotechnology, Zhejiang University, Hangzhou 310058, China
[b] YK Pao School, Shanghai 201620, China

ARTICLE INFO

Keywords:
Klebsiella oxytoca
Klebsiella michiganensis
Nitrogen fixation
Carbohydrate-active enzyme
Type II secretion system

ABSTRACT

The bacterial strain M5al is a model strain for studying the molecular genetics of N_2-fixation and molecular engineering of microbial production of platform chemicals 1,3-propanediol and 2,3-butanediol. Here, we present the complete genome sequence of the strain M5al, which belongs to a novel species closely related to *Klebsiella michiganensis*. M5al secretes plant cell wall-degrading enzymes and colonizes rice roots but does not cause soft rot disease. M5al also produces siderophores and contains the gene clusters for synthesis and transport of yersiniabactin which is a critical virulence factor for *Klebsiella* pathogens in causing human disease. We propose that the model strain M5al can be genetically modified to study bacterial N_2-fixation in association with non-legume plants and production of 1,3-propanediol and 2,3-butanediol through degradation of plant cell wall biomass.

The bacterial strain M5al (=ATCC BAA–1236 = DSM 3539 = NCIB 12204) was first designated as *Aerobacter aerogenes* [1,2], then *Klebsiella pneumoniae* [3], and later *K. oxytoca* [4]. M5al was isolated from soil and is able to convert atmospheric nitrogen to ammonia [2], degrade aromatic compounds [5,6], and produce industrial platform chemicals 1,3-propanediol and 2,3-butanediol [7]. M5al is free of capsular polysaccharides and thus was thought to be non-pathogenic and used safely [7]. M5al is easy to be genetically manipulated and has been extensively studied as a model strain in molecular genetics of N_2-fixation [8,9] and molecular engineering of microbial production of 1,3-propanediol and 2,3-butanediol [7,10].

The whole genome sequence of the strain M5al has attracted considerable attention. The genome of an M5al mutant VJSK009 was sequenced in 2004 (http://genome.wustl.edu/genomes/view/klebsiella_oxytoca_m5al). The genome of the strain M5al was first sequenced by Solexa technology and assembled to a 114-contig draft genome (GenBank accession no. AMPJ00000000.1) [11] and later sequenced by the PacBio Single Molecule, Real-Time (SMRT) technology [12] and assembled to a nearly complete 6-contig genome (GenBank accession no. LWKU00000000.1). Here, we present the complete genome sequence and discuss M5al's potential to colonize and provide nitrogen to plants.

The genome DNA of the strain M5al was inserted into a 15–20 kb insert library and sequenced by the PacBio SMRT technology at the Duke University Genome Sequencing & Analysis Core Resource. The sequencing resulted in 181,416 high-quality filtered reads with an average length of 13,226 bp. High-quality reads were assembled by the RS_HGAP_Assembly.3 in the SMRT analysis v2.3.0. The final assembly produced a complete genome of 307-fold coverage. The genome was annotated by the NCBI Prokaryotic Genome Annotation Pipeline [13]. The complete genome consists of a circular chromosome containing 5,800,138 bp with 55.9 mol% GC content, 5593 genes including 25 rRNA genes, 85 tRNA genes, and 7 non-coding RNA genes. The complete genome contains what appears to be a movable element (about 35 kb from locus_tag BWI76_10080 encoding a tRNA-Ser to BWI76_10240 encoding an integrase) and a frame-shifted transposase gene (BWI76_15780) inserted into a gene (split into BWI76_15775 and BWI76_15785) encoding a replication protein, which are missing from the previous 6-contig genome.

The strain M5al was submitted as *K. oxytoca* when the two previously sequenced genomes were deposited in GenBank and was changed to *K. michiganensis* in July 2016 based on overall genome relatedness. *K. michiganensis* is closely related to *K. oxytoca* [14]. However, the genome average nucleotide identities (ANIs) between M5al and the *K. michiganensis* type strain DSM 25444^T and between M5al and the *K. oxytoca* type strain NBRC 105695^T are 93.31% and 91.04%, respectively, lower than the threshold value of 95%–96% for species delimitation [15]. Therefore, M5al belongs to a novel species closely related to *K. michiganensis*.

Carbohydrate-active enzymes (CAZymes) catalyze the synthesis,

* Corresponding author.
E-mail address: an@zju.edu.cn (Q. An).
[1] Present address: Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.

https://doi.org/10.1016/j.btre.2017.11.006
Received 17 September 2017; Received in revised form 17 November 2017; Accepted 21 November 2017
Available online 22 November 2017

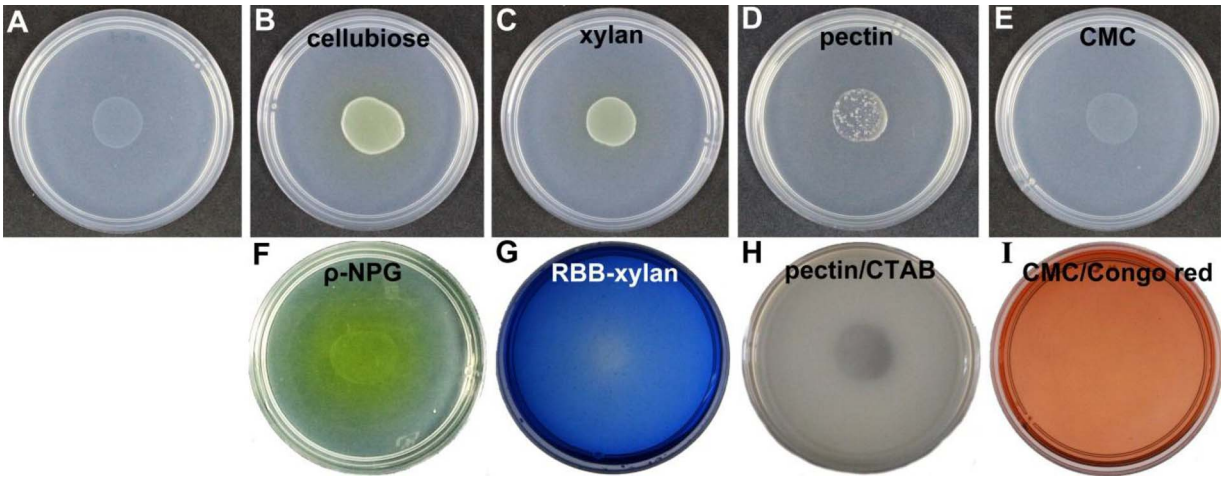

Fig. 1. Agar plate assays showing degradation of plant cell wall polysaccharides by activities of β-glucosidase, xylanase, and pectinase from *Klebsiella* sp. strain M5al. (**A–E**) M5al (1×10^7 cells) grown on an agar medium [0.2% (w/v) $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% KCl, and 1.5% purified agar] without addition of carbon sources (**A**), or supplemented with 0.2% cellubiose (**B**), 0.2% xylan (**C**), 0.2% pectin (**D**), or 0.2% carboxymethyl cellulose (CMC) (**E**) at 30 °C for 2 d. (**F–I**) Haloes, indicating activities of enzymes from M5al, formed on the agar medium containing ρ-nitrophenyl β-D-glucopyranoside (ρ-NPG, a chromogenic substrate of β-glucosidase; 0.15%) (**F**), 4-*O*-Methyl-D-glucurono-D-xylan dyed with Remazol brilliant blue R (RBB-xylan; 0.2%) (**G**), or pectin precipitated by hexadecyl trimethyl ammonium bromide (CTAB) (**H**). M5al did not use CMC (**E**) or degrade CMC stained by Congo red (**I**). RBB may inhibit M5al growth; degradation of RBB-xylan (**G**) was detected by putting a xylan agar plug (15 mm in diameter) grown with M5al for 2 d (**C**) on the center of the RBB-xylan plate.

degradation, and modification of complex carbohydrates [16]. CAZymes are important for the biotech industry, as the degradation of complex polysaccharides (cellulose, hemicellulose, pectin, and lignin) in the plant cell wall by microbes produces biofuels [16]. We identified 210 CAZymes in the M5al genome using the dbCAN web server (http://csbl.bmb.uga.edu/dbCAN/annotate.php). The CAZymes of M5al consist of 93 glycoside hydrolases (GH), 48 glycosyltransferases (GT), 8 polysaccharide lyases (PL), 29 carbohydrate esterases (CE), 10 auxiliary activities (AA), and 22 carbohydrate-binding modules (CBM). Notably, the CAZymes of M5al contain seven cellulases (two endoglucanases and five β-glucosidases), six xylanases, and seven pectinases (Table S1).

Most plant cell wall-degrading enzymes involved in enterobacterial phytopathogenesis are secreted through the type II secretion (T2S) system [17]. The plant cell wall-degrading enzymes to be secreted are first translocated from the cytoplasm into the periplasm through the Sec general secretory pathway or the twin-arginine translocation (Tat) system [18]. M5al contains genes encoding all the components of the T2S, Sec, and Tat systems (Table S2).

M5al grew on agar media containing cellubiose, xylan, or pectin as the sole carbon source and likely secreted β-glucosidases, xylanases, and pectinases into agar media (Fig. 1), indicating that the T2S, Sec, and Tat systems are functional. However, M5al did not show a clear endoglucanase activity for degradation of carboxymethyl cellulose (Fig. 1). We propose that M5al may produce 1,3-propanediol and 2,3-butanediol through degradation of plant cell wall biomass after activation of its endoglucanases and introduction of foreign exoglucanases.

Bacteria can use plant cell wall-degrading enzymes to weaken the plant cell walls and infect plant tissues. We introduced the plasmid pPROBE-pTet^r-TT [19] carrying the *gfp* reporter into M5al, inoculated rice seedlings grown in a semi-solid medium with the *gfp*-labeled M5al cells (1×10^7cells per seedling), and visualized bacterial infection of rice roots using laser scanning confocal microscopy. The *gfp*-labeled M5al cells preferentially colonized the root border cells surrounding root caps, root hair zones, and the junctions between primary roots and lateral roots (Fig. 2), showing typical colonization patterns of rhizobacteria [19,20]. The *gfp*-labeled M5al cells also colonized the cracks of emerging lateral roots and disrupted root epidermal cells but did not massively degrade the plant cell walls leading to root rotting symptoms (Fig. 2). Therefore, M5al controls the plant cell wall-degrading activities and is not pathogenic to the rice seedlings. Yoo et al. noted that M5al showed a low level of N_2 fixation in association with rice plants [21]. Likely, M5al can be used as a model strain to study the mechanisms of bacterial N_2 fixation contributing to non-legume plants.

Siderophores are low molecular weight iron chelators produced by microbes to sequester iron and perturb host iron homeostasis [22]. Bacteria can effectively uptake the nitrogenase metal cofactors Fe and Mo via siderophores to support N_2 fixation [23]. Moreover, siderophores act as both toxins and immunomodulators to the host to induce inflammatory cytokines and bacterial dissemination [22,24]. Siderophores, especially yersiniabactin, aerobactin, salmochelin, and colibactin, are critical virulence factors of *K. pneumoniae* causing invasive disease in immunologically competent human hosts [25]. The M5al genome contains the gene clusters for synthesis and transport of enterobactin (*Ent-Fep-Pfe* cluster) and yersiniabactin (*ybt* cluster) and the genes encoding the aerobactin receptor (*iutA*) and transporter (*FhuABCD*) (Table 1). Moreover, M5al is able to produce and secrete siderophores into agar media (Fig. S1). Enterobactin is the most common siderophore produced by the enterobacteria and has one of the highest ferric iron affinities [22]. To oppose iron acquisition by enterobactin, host epithelial cells and neutrophils secrete the protein siderocalin to bind and sequester enterobactin. However, yersiniabactin can evade siderocalin [22]. Therefore, further study is needed regarding the virulence of the yersiniabactin from M5al, as well as the function of the siderophores in relation to N_2 fixation and the production of 1,3-propanediol and 2,3-butanediol by M5al. The virulent yersiniabactin system should be knocked out for the safe use of the model strain M5al.

Nucleotide sequence accession number

The complete genome sequence of the strain has been deposited in GenBank under the accession number CP020657.

Author contributions

QA designed the study. ZY, SL, YL assembled and analyzed the genome. ZY, ZJ, JZ did the experiments. ZY, QA wrote the manuscript.

Conflict of interest statement

The authors declare no conflict of interest.