Draft Genome Sequence of Enterobacter. Sp. E20, Isolated from Glyphosate Polluted Soil

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Abstract: *Enterobacter*. Sp. E20 was isolated from glyphosate-polluted soil in China. This bacterium presented the capacity for high glyphosate tolerance when growing in M9 minimal medium, which showed significant difference from that of the model *Escherichia coli* strain K12. We presented the draft genome sequence of the strain *Enterobacter*. Sp. E20. Genes involved in the glyphosate tolerance were identified. The genomics information will facilitate the study of glyphosate tolerant mechanism.

1. Introduction

The glyphosate resistance gene CP4, which is widely used in agricultural production for genetically modified crops, and the mutant *TIPS* of *E. coli*, are protected by foreign patents. In addition to the action sites closely related to glyphosate resistance, most of them are protected by patents, and these patents have restricted the development of GM crops in China. Based on this, in order to broaden the glyphosate resistance gene resources, it is necessary to develop a new glyphosate resistance gene, which lays a foundation for the cultivation of transgenic crops in China.

A number of soil microbes may emerge some new mechanisms to survive when they were under the continuous stress in extreme environment, such as heat, cold, high salinity, radiation, glyphosate and so on (Dutta and Paul, 2012; Sharma et al., 2013). Surprisingly,some pivotal genes in the specific pathways may appear or be changed in adaptation to the abominable environment (Dragosits et al., 2013). It is common to adapt to glyphosate stress for some microbes in glyphosate-polluted soil (Cao et al., 2013). Generally, microorganisms could detoxify glyphosate by overexpression of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) to resist the inhibition of shikimate pathway (Gaines et al., 2010; Salas et al., 2012), or by degradation of glyphosate (Dick and Quinn, 1995; Pipke and Amrhein, 1988).

2. Results

In the process of adapting extreme microorganisms to the harsh environment for a long time, extreme microbes may produce molecular regulation mechanisms adapted to external stress. Exploring the molecular mechanism of microbial growth under adverse conditions in response to stress, helping to mine key genes for stress tolerance, applying modern agricultural biotechnology, improving crops, and improving agricultural production.

Enterobacter Sp. E20 was isolated from glyphosate-polluted soil around an aged chemical company in Zhejiang Province, China. This bacterium could grow well in M9 minimal medium containing 3400 mM glyphosate and showed an eximious capacity for high glyphosate tolerance(Figure 1). The glyphosate tolerance of E20 is much greater than other strains on the M9 defective medium. Identification of categoric strains of E20 and excavation of their glyphosate tolerance-related genes are important for the cultivation of glyphosate-resistant crops. The amplification of *16srDNA* of the E20 showed that the strain possesses a high homology to

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Escherichia coli strain. The *aroA* gene of E20 (*aroA20*) was isolated by PCR method using the primers which designed against the conserved sequence of some *aroA* genes. Sequence analysis revealed that AroA20 EPSPS belongs to class I EPSPS, and might be a glyphosate sensitive enzyme. Incomprehensibly, the E20 strain possessed a high glyphosate resistance, which distinguished from other microbes containing class I EPSPSs. So, we can speculate that AroA20 must have been modified by site directed mutagenesis to get glyphosate resistance. The novel strain may possess a complex adaptation mechanism. Genomic analyses of E20 could provide insights into the mechanism of extreme environment adaptation. Therewith, the genome of E20 was sequenced and the pivotal genes involved in glyphosate resistance were identified. This work could provide useful information for the development of transgenic glyphosate-tolerant plants.

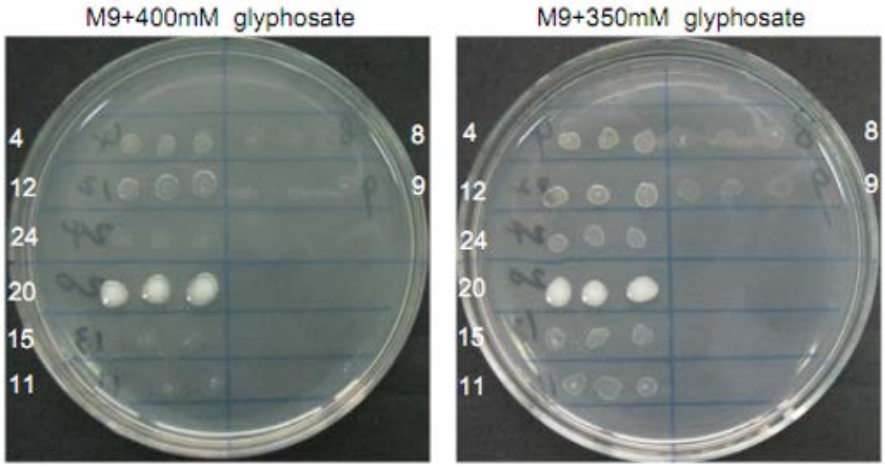

Figure. 1 Evaluation of the tolerance for different strains on M9 solid medium containing 350 mM and 400 mM glyphosate

Eight strains for *16srDNA* sequencing were selected, which could grow well in 300 mM glyphosate liquid and solid M9 medium. 2 μL of the same concentration of bacteria solution was selected on M9 containing 350 mM and 400 mM glyphosate. The medium was cultured at 37 degrees for 36 hours to identify its growth condition.

The *Enterobacter* Sp. E20 genome was sequenced by a whole-genome shotgun strategy using Illumina HiSeq 2000. Genomic libraries of 180 bp, 800 bp, and 3 kb were constructed and sequenced, providing total about 450-fold coverage of the genome. De novo assembly was performed using SOAPdenovo2 (Luo et al., 2012), resulting in 44 scaffolds more than 500bp length. The gaps inside the scaffolds were closed with GapFiller (Boetzer and Pirovano, 2012). Protein-encoding genes were predicted by combining the results of Glimmer 3.02 (Delcher et al., 2007). tRNA and rRNA genes were identified by tRNAscan-SE (Lowe and Eddy, 1997) and RNAmmer (Lagesen et al., 2007), respectively. Functional annotation was performed by searching against the NCBI nr, Swiss-Prot (Boeckmann et al., 2003), InterProScan (Quevillon et al., 2005), COG (Tatusov et al., 2001), and KEGG (Kanehisa and Goto, 2000) databases.

Table 1 Genome features of Enterobacter. Sp. E20

Features	Chromosome
Length (bp)	4,714,013
G+C content	55.8%
CDS	4,248
rRNA genes	3
tRNA genes	70

The draft genome sequence of *Enterobacter* Sp. E20 is composed of 4,714,013 bases, with a G+C content of 55.8%(Table 1). It contains 4,248 open reading frames (ORFs), 3 rRNAs, and 70 tRNA genes. There are 3,922 genes involving 22 COG function categories and 2,669 genes annotated into 2,394 KEGG orthologous groups by KAAS, which are involved in 193 metabolic

pathways. The availability of the draft genome sequence of Enterobacter Sp. E20 will strongly provide considerable information for exploring abiotic stress tolerance genes and accounting for the mechanism for the singular glyphosate tolerant strain that possess class I EPSPS gene in glyphosate stress adaptation.

In our further studies, it was found that *Enterobacter* 20 has high resistance in the early glyphosate resistance screening and can tolerate glyphosate stress up to 400 mM. The *aroA* encoding gene was further cloned, but its EPSPS was sensitive to glyphosate.

When AroA20 and related EPSPS constructed phylogenetic tree for cluster analysis, it was found that AroA20 has the closest evolution relationship with *E. coli* EPSPS, but *E. coli* is highly sensitive to glyphosate. The difference in glyphosate resistance between the strain and the *aroA* gene is a key point for further research.

Transcriptome analysis of *Enterobacter* 20 strain under glyphosate stress conditions may reveal its response to glyphosate stress environment under glyphosate stress conditions, and also facilitate the mining of new functional genes.

3. Nucleotide Sequence Accession Number

The complete genome sequence of *Enterobacter* Sp. E20 has been deposited in GenBank under the accession number JRUR00000000. The strain is available from the corresponding author upon request.

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