Comparative genomic analysis revealed mechanisms involved in high production of avermectin in *S. avermitilis* 9-39

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Key words: Functional genomics; high production; avermectin;

Introduction. Avermectin and its analogs are major commercial antiparasitic agents widely used in the fields of animal health, agriculture, and human infections [1]. The genetic basis for high titer is poorly understood in industry strain selected by mutant-and-screen method [2,3]. In an effort to better examine the mechanism of avermectin high production, we sequenced the entire genome of strain 9-39 (high producer) and performed a whole-genome comparative analysis to profile genomic variations between the mutant strain 9-39 and the wild type strain MA-4680[4]. This analysis strongly characterized the genetic basis of the high-producing of avermectin and the difference between the high producer and the wild-type strain, revealed by comparative genomics analysis, which will trigger the further avermectin improvement.

Methods. The nucleotide sequence of *S. avermitilis* strain 9-39 was determined with the next generation sequencing technology. DNA libraries with 500 bp and 2 kb inserts were constructed and sequenced with the Illumina Genome Analyzer sequencing technique.

Results.

The complete genome of 9-39 contains 9,767,982 bases (average GC content, 70.7%) and encodes at least 8,148 potential open reading frames. Comparison with the genome of ATCC31267 revealed that the major core genome region (6.5-Mb around the *oriC* region) was highly conserved with all known essential genes.

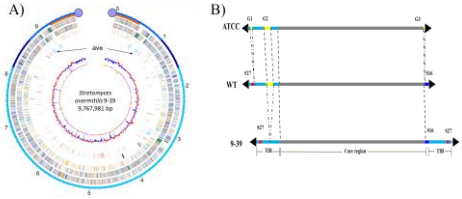

Fig.1 Schematic representation of A) the *S. avermitilis* 9-39 chromosome and gene clusters for secondary metabolism.; B) position of the lost/gain special large fragments in the genome of ATCC, WT and 939.

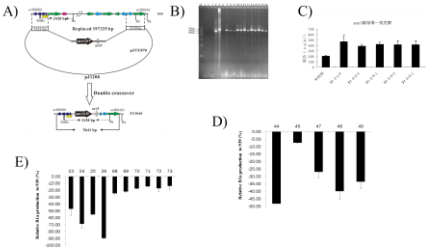

Fig.2 Large fragments deletion and fermentation analysis of conjugants. A) schematic representation of homologous recombination; B) Positive conjugants was confirmed by PCR amplification; Fermentation analysis of C) conjugants with large fragment of G2 region knockout; D) conjugants with large fragment of S16 region knockout; E) conjugants with large fragment of S27 region knockout.

Phosphoglucomutase (*pgmA*), which was involved in the pathway of starch and sucrose metabolism, was found to be located in the G2 region. While another copy of maltose transporter gene and maltose metabolism relative genes are found in strain 939 specific fragment S16, which are involved in the starch and maltose metabolic pathway.

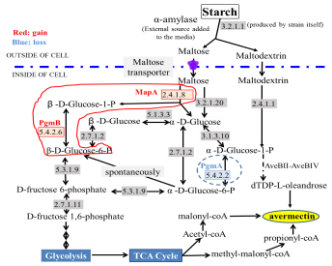

Fig.3 Variations of metabolic pathways for starch and maltose in avermectin high producing strain 939

Conclusions. 1.The plasticity of the terminal region in line chromosome might be associated to the horizontal transfer of genetic information.

2. Enhanced gene dosage is one of the factors for the overproduction of AVM, and demonstrates that large-scale genome rearrangements can be a result of classical strain improvement by mutagenesis.

3.G2 region deletion in the chromosome of 9-39 is helpful for the avermectin production; and the additional β-phosphoglucomutase and maltose transporters might affect carbon metabolism and accelerate the precursor supplying of avermectin production.

Acknowledgements. This work was supported in part by grants from National Natural Science Foundation of China (31100075, 31170095, 31000004). The author gra tefu11y acknowledges the support of K. C. Wong Education Foundation, Hong Kong.

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