Indian J Microbiol (Jan–Mar 2014) 54(1):120–121
DOI 10.1007/s12088-013-0433-9

SHORT COMMUNICATION

Draft Genome Sequence of the Methyl Parathion (Pesticide) Degrading Bacterium *Pseudomonas* spp. MR3

Manoj V. Parakhia · Rukam S. Tomar · Megha R. Vadukia · Bipin J. Malviya · Visha M. Rathod · Jalpa R. Thakkar · Kinjal J. Parmar · Rashmin M. Dhingani · B. A. Golakiya

Received: 20 July 2013 / Accepted: 15 October 2013 / Published online: 22 October 2013

Abstract *Pseudomonas* spp. MR3 was isolated from the surrounding soil of pesticide manufacturing industries of Ankleshwar, Gujarat. Under laboratory conditions these microbes were able to degrade up to 500 ppm of methyl parathion within 72 h. Genome sequencing of *Pseudomonas* spp. MR3 was carried out inIon Torrent (PGM), next generation sequencer. The data obtained revealed 1,268 contigs with genome size of 2.99 Mb and G + C content of 60.9 %. The draft genome sequence of strain MR3 will be helpful in studying the genetic pathways involved in the degradation of several pesticides.

Keywords Methyl parathion · Draft genome · *Pseudomonas* · Strain MR3

Introduction

Methyl parathion is a broad spectrum insecticide/miticide, formulated as a microencapsulate (20.9 % active ingredient) and as an emulsifiable concentrate (27.59–52.7 % active ingredient). The excessive use of natural resources and large scale synthesis and use of methyl parathion have generated a number of environmental problems such as contamination of air, water and terrestrial ecosystems [1]. Soil microflora is one of the basic agents for detoxification of pesticides. Some investigators found that soil contaminated with pesticides could be decontaminated by inoculation with specifically adapted microorganisms [2]. In the present study, soil samples were collected from the surrounding area of the pesticide manufacturing industries of Ankleshwar, Gujarat, India. Nineteen different bacteria isolated from the soil samples were distinguishable on the basis of their morphology and growth pattern on the selected media. All the isolates were checked for methyl parathion degradation under different concentrations through high performance liquid chromatography (HPLC). Among the nineteen isolates, *Pseudomonas* spp. MR3 strain showed highest degradation rate with degradation of 500 ppm of methyl parathion within 72 h and hence was selected for whole genome sequencing.

Whole-genome sequencing of *Pseudomonas* spp. MR3 was done using Ion Torrent (PGM), next generation sequencer (NGS) from Life Technologies at Department of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat. A 314 chip was loaded with enriched library of 260 bp to generate sequencing data. A total number of 564,810 reads of average length 145 nucleotides (nts), with a coverage of 15× of a 4.5-Mb genome (expected size) were produced by the machine. Assembly was done by using MIRA assembler v 3.4.1.0 [3], which revealed a total of 1,261 contigs. The final genome draft was used for genome annotation by Rapid Annotations using Subsystems Technology (RAST) system [4], RNAmmer 1.2 [5] and ARAGORN software [6]. The size of genome was found to be 2.99 Mb with G + C content of 60.9 %. A total of 79 RNAs genes were found, 3 copies of 5S rRNA were found, 2 copies of 16S rRNA and two for 23S rRNA synthesis. We have also reconfirmed the 16S rRNA gene sequence by Sanger's sequencing and it was found to be

M. V. Parakhia (✉) · R. S. Tomar · M. R. Vadukia · B. J. Malviya · V. M. Rathod · J. R. Thakkar · K. J. Parmar · B. A. Golakiya
Department of Biotechnology, Junagadh Agricultural University, Junagadh 362 001, Gujarat, India
e-mail: mvparakhia@gmail.com

R. M. Dhingani
College of Food Processing Technology and Bio-Energy, Anand Agricultural University, Anand, Gujarat, India

100 % identical with that obtained from whole genome sequence data analyzed by RNAmmer. The 16S rRNA sequence of *Pseudomonas* spp. MR3 available in GenBank database (Submission ID: 1626232) showed 99 % identity with the 16S rRNA gene sequence of *Pseudomonas putida* KT2440 (Accession number NR 074596.1). RAST annotation showed, *P. putida* KT2440 (score 510), *P. putida* F1 (score 504), and *P. putida* GB-1 (score 490) to be the closest neighbors of the strain MR3. This annotation also indicated the presence of genes involved in metabolisms of phosphorus, sulfur, aromatic compounds, nitrogen, protein, potassium and iron acquisition. Genes for Phosphono acetaldehyde hydrolase (EC 3.11.1.1), Salicylate hydroxylase (EC 1.14.13.1), Catechol 1,2-dioxygenase (EC 1.13.11.1), 1H-3-hydroxy-4-oxoquinaldine 2,4-dioxygenase and Monoamine oxidase (1.4.3.4) found to be involved in the catabolism of organophosphate compound were also identified. By using the tblastn, gene sequence of mpd gene at contig 310 with a length of 1,470 bp was identified which lead to the production of hydrolase enzyme responsible for the degradation of methyl parathion.

Nucleotide Sequence Accession Numbers

This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank with the accession number ARYY 00000000. The version described in this paper is the first version, NZ_ARYY00000000.1 GI:499136694. Bioproject registered under Accession number: PRJNA203097 ID: 203097.

Acknowledgments This research was carried out at Department of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat, India.

References

1. Cisar JL, Snyder GH (2000) Fate and management of turf grass chemicals. ACS Symp Ser 743:106–126
2. Cho TH, Wild JB, Donnelly KC (2000) Utility of organophosphorus hydrolase for the remediation of mutagenicity of methyl parathion. Environ Toxicol Chem 19:2022–2028
3. Chevreux B (2005) MIRA: an automated genome and EST assembler. German Cancer Research Center, Germany
4. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O (2008) The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75
5. Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW (2007) RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35:3100–3108
6. Laslett D, Canback B (2004) ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32:11–16