Data in Brief

Genome sequence of Enterobacter sp. ST3, a quorum sensing bacterium associated with marine dinoflagellate

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A B S T R A C T

Phycosphere environment is a typical marine niche, harbor diverse populations of microorganisms, which are thought to play a critical role in algae host and influence mutualistic and competitive interactions. Understanding quorum sensing-based acyl-homoserine lactone (AHL) language may shed light on the interaction between algal-associated microbial communities in the native environment. In this work, we isolated an epidermal bacterium (was tentatively named Enterobacter sp. ST3, and deposited in SOA China, the number is MCCC1K02277-ST3) from the marine dinoflagellate Scrippsia trochoidea, and found it has the ability to produce short-chain AHL signal. In order to better understand its communication information at molecular level, the genomic map was investigated. The genome size was determined to be 4.81 Mb with a G + C content of 55.59%, comprising 6 scaffolds of 75 contigs containing 4647 protein-coding genes. The functional proteins were predicted, and 3534 proteins were assigned to COG functional categories. An AHL-relating gene, LuxR, was found in upstream position at contig 1. This genome data may provide clues to increase understanding of the chemical characterization and ecological behavior of strain ST3 in the phycisphere microenvironment.

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1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/assembly/GCA_001469415.1/.

2. Introduction

As producers and decomposers, bacterioplankton are a key component of microbial food webs and play significant roles in biogeochemical cycles [1]. In “algae–bacteria” symbiosis, bacteria extensively participate in substance cycles, oxidation–reduction activities, and regulate the balance of phytoplankton ecosystems, which is the most basic and active link in the whole phycosphere habitat [2]. In recent years, chemical ecology has emerged as a new approach to evaluate the structural and functional diversity, as well as the dynamic equilibrium of the algae–bacteria symbiont [3]. Many key life processes, including food acquisition, movement behavior, defense, and signal communication are mediated by signal interactions at the individual, population, and community levels [4–5]. Understanding signal regulated ecological processes will help to define the crucial molecular interactions between algae and bacteria.

Infochemicals are frequent in the phycosphere, and various cross-talking behaviors of heterotrophic bacteria have been identified in algae–bacteria symbiosis relationships [6]. These processes are often controlled by cell density-dependent regulation of gene expression, which is mediated by diffusible signal molecules whose concentration correlates with the population density. This process is termed quorum sensing (QS), and one of the most well-known QS signals is N-acetylhomoserine lactone (AHL) [7]. AHLs can induce gene expression either directly by interacting with a transcriptional regulator, or indirectly by activating a signal cascade [8]. Bacteria use AHL to regulate a variety of phenotypes such as biofilm formation, exopolysaccharide production, virulence factor production, and motility, which are essential for the successful establishment of a symbiotic relationship with their eukaryotic hosts [9]. Previous studies have shown that alga-associated bacterial isolates (of epi- or endophytic origin) produce AHLs and exhibit various functions, including facilitating the settlement of zoospores associated with marine dinoflagellate.
[10], regulating the morphogenesis pattern [11], affecting individual reproduction [12], and promoting the liberation of carpospores [13]. Recently, our group isolated an *Enterobacter* sp. ST3 strain from the dinoflagellate *Scrippsiella trochoidea* (the sample environmental features list in Table 1). The electron micrograph images showed that ST3 strain is a motile and long rod-shaped bacterium (Fig. 1). It has broad environmental suitability and the optimal growth temperature and pH values were 30 °C and 8.0, respectively (Fig. 2). As a member of γ-proteobacteria, *Enterobacter* sp. play multi-roles in symbiosis environment, such as exopolysaccharide produce, iron metabolism, heavy metals biosorption, and pollutant biodegradation [14–17]. In algae–bacteria symbionts, *Enterobacter* sp. is often found as a main species and participant in the matter cycles of the phycosphere [18]. Of particular note, this isolate ST3 possesses cross-talking language activities, and can secrete short-chain (C6) AHL molecules (Fig. 3). Although the phenotype was previously observed, this type of functionality has not been elucidated at the gene level with genomic approaches. In addition, we speculated that its ecological function was regulated by quorum sensing, but there is a lack of direct evidence for confirmation on the genomic level. In order to better understand genetic underpinning of the bacterium roles, here, we performed whole-genome sequencing of this bacterium and searched for its AHL encoding gene(s).

### 3. Experimental design, materials and methods

The genomic DNA of *Enterobacter* sp. ST3 was extracted using the DNA extraction kit (Mo Bio, CA, USA) according to the manufacturer’s instructions. The whole-genome shotgun project of *Enterobacter* sp. ST3 was performed using pair-end sequencing in an Illumina Miseq sequencing platform (Illumina, CA, USA), which was performed by Shenzhen Hengchuan Gene-Tech. Co., Ltd. The reads were assembled with SOAPdenovo (V.2.04) [19], and the sequence was annotated using the RAST annotation server [20]. tRNA and rRNA genes were predicted by tRNAscan-SE [21] and RNAmmer [22], respectively. Genes

| Table 1 | Genome and environmental features. |
|---------|-----------------------------------|
| Item    | Description                       |
| MIGS data | Bacteria                          |
| Investigation type | NSFC (41476092)                     |
| Project name | Jin Zhou                          |
| Collected by | 05-23-2013                        |
| Collection date | 23°35′N, 114°17′E               |
| Country | China                             |
| Environment | Surface sea water                  |
| Biotic relationship | Attached bacterium with algae   |
| Trophic level | Aerobe                            |
| Relate to oxygen | LB medium                        |
| Sequencing method | Illumina MiSeq                   |
| Assembly | SPAdes v. 3.5.                     |
| Finishing strategy | Whole genome                    |
| Annot source | RAST/NCBI blastx                |
| Estimated size | 5–6 M bp                         |
| Geo_loc_name | Shenzhen, China                   |
| Sample material | Surface water from the Yantian Port |
| Temp | 27 °C                             |
| Salinity | 30.7 PSU                          |
| Motility | Yes                              |
| Genome assembly data | ST3                              |
| Assembly name | 260×                             |
| Genome coverage | Illumina HiSeq 2000          |
| Sequencing technology |                |

Fig. 1. Electron micrographs of cells of “Enterobacter sp. ST3”. Preparation and EM conditions were as described by Hahnke et al. [25]. The magnification times: a (8000×), b (25,000×), c & d (40,000×), and e & f (60,000×).
were predicted using Glimmer 3.02 [23] and annotated by searching against the NVBI-nr and KEGG databases.

The whole genome of Enterobacter cloacae ST3 comprised 4,815,521 nucleotides and the G + C content was 55.59% (Table 2). It contains 75 contigs with an N50 contig length of 127,978 bp. The whole genome encodes nine 5 s rRNA, two 16 s RNA, five 23 s RNA, and seventy-nine tRNA genes. The genome predicted a total of 4647 genes with 3534 protein-coding genes. Based on functional categories of COG (http://www.ncbi.nlm.nih.gov/COG/), a total of 426 genes were annotated to be involved in carbohydrate transport and metabolism, which enables the strain to adapt to and compete in algae–bacteria symbiosis (Fig. 4).

The other 523 genes were predicted to have general functions. In addition, 171 genes were predicted to encode signal transduction molecules.

In addition, we analyzed the candidate genes related to AHL production. We found an AHL-related gene (LuxR family transcriptional regulator) located in the upstream position (contig 1), which indicates that Enterobacter sp. ST3 may be able to mediate its cell density with AHL signals and exhibit its functions in a fluctuating phycosphere environment. This gene showed relatively high sequence identity to the LuxR gene of another Enterobacter species (GenBank: WP_017692678.1). Some scientists found some bacterial ecological function (for example algicidal activity) was regulated by quorum sensing from the genomic level [24]. The genome sequence of Enterobacter sp. ST3 should enable further research to gain deeper insights into the molecular mechanisms of algae–bacteria interactions, and may facilitate the development of new ideas for improving the understanding of algae bloom formation and carbon cycle processes.

4. Nucleotide sequence accession number

The genome sequences were deposited in GenBank with the accession number LMBL00000000. The version described in this paper is the first version.

Conflict of interest

The authors declare that there is no conflict of interests on work published in this paper.

Table 2
Genome features of Enterobacter sp. ST3.

| Attributes                  | Values         |
|-----------------------------|----------------|
| Genome size                 | 4,815,521 bp   |
| GC content %                | 55.59%         |
| Number of Contigs           | 75             |
| Total contig size           | 4,798,623 bp   |
| Largest contig              | 295,927 bp     |
| Scaffolds                   | 6              |
| Total scaffold size         | 4,815,521 bp   |
| Largest scaffold            | 4,805,842 bp   |
| Protein coning genes        | 4647           |
| tRNAs                       | 79             |
| rRNAs                       | 16             |
| Minisatellite DNA           | 44             |
| Microsatellite DNA          | 0              |
| Genes with a predicted function | 21          |
| Potential AHL encoding site | Contig 1       |
| Encoding-AHL gene length    | 158 bp         |
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References

[1] J.A. Steele, P.D. Countway, L. Xia, P.D. Vigil, J.M. Beman, D.Y. Kim, C.E. Chow, R. Sachdeva, A.C. Jones, M.S. Schwalbach, J.M. Rose, I. Hewson, A. Patel, F. Sun, D.A. Caron, J.A. Fuhrman, Marine bacterial, archaeal and protistan association networks reveal ecological linkages. ISME J. 5 (9) (2011) 1414–1425.

[2] J. Zhou, G.F. Chen, X.S. Zhu, L. Chen, Z.H. Cai, A review of the relationship between algae and bacteria in harmful algal blooms. Acta Ecol. Sin. 34 (2) (2014) 269–281 (in Chinese, abstract in English).

[3] K.L. Van Alstyne, T.A. Nelson, R.L. Ridgway, Environmental chemistry and chemical ecology of "green tide" seaweed blooms. Integr. Comp. Biol. 55 (3) (2015) 518–532.

[4] A. Ianora, M.G. Bentley, G.S. Caldwell, R. Casotti, A.D. Cembella, J. Engström-Öst, C. Halsband, E. Sonnenschein, C. Halsband, C.A. Llewellyn, A. Paldavičienė, R. Pilkaityte, G. Pohnert, A. Razinkovas, G. Romano, U. Tillmann, D. Vaiciute, The relevance of marine chemical ecology to plankton and ecosystem function: an emerging field. Mar. Drugs. 9 (9) (2011) 1625–1648.

[5] S.A. Arun, L.K. Himeno, H.M. van Tol, B.P. Durham, L.T. Carlson, K.R. Heal, R.L. Morales, C.T. Berthiaume, M.S. Parker, B. Djuanda, A.E. Ingalls, M.R. Parsek, M.A. Moran, E.V. Armbrust, Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. Nature 522 (7554) (2015) 98–101 (2015).

[6] S.A. Amin, M.S. Parker, E.V. Armbrust, Interactions between diatoms and bacteria. Microbiol. Mol. Biol. Rev. 76 (2) (2012) 667–684.

[7] P. Williams, K. Winzer, W. Chan, M. Câmara, Look who's talking: communication and quorum sensing in the bacterial world. Phil. Trans. R. Soc. B. 362 (2007) 1119–1134.

[8] C. Fuqua, M.R. Parsek, E.P. Greenberg, Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu. Rev. Genet. 35 (2001) 439–468.

[9] J.E. González, N.D. Keshavan, Messing with bacterial quorum sensing. Microbiol. Mol. Biol. Rev. 70 (4) (2006) 859–875.

[10] I. Joint, K. Tait, M.E. Callow, J.A. Callow, D. Milton, P. Williams, M. Câmara, Cell-to-cell communication across the prokaryote-eukaryote boundary. Science 298 (5596) (2002) 1297–1299.

[11] R.P. Singh, C.R.K. Reddy, Seaweed–microbial interactions: key functions of seaweed-associated bacteria. FEMS Microbiol. Ecol. 88 (2014) 213–230.

[12] K. Tait, I. Joint, M. Daykin, D. Milton, P. Williams, M. Câmara, Disruption of quorum sensing in seawater abolished attraction of zoospores of the green alga Ulva to bacterial biofilms. Environ. Microbiol. 7 (2005) 229–240.

[13] R.P. Singh, R.S. Baghel, C.R. Reddy, B. Jha, Effect of quorum sensing signals produced by seaweed-associated bacteria on carpospore liberation from Gracilariadura. Front. Plant. Sci. 6 (2015) 117.

[14] C.L. Li, Study iron metabolism related gene function of Enterobacter cloacae in Ningbo coastal of sewage Outfall. The master degree thesis of Ningbo University, 2013.

[15] A. Iyer, K. Mody, B. Jha, Biosorption of heavy metals by a marine bacterium. Mar. Pollut. Bull. 50 (1) (2005) 340–343.

[16] A. Iyer, K. Mody, B. Jha, Characterization of an exopolysaccharide produced by a marine Enterobacter cloacae. Indian J. Exp. Biol. 43 (5) (2005) 467–471.

[17] S.J. Tan, Algal Bloom and Evaluation of Ecological Remediation Effects by Artificial ReefThesis for Master Degree Tsinghua University, China, 2013.

[18] S.J. Tan, Algal Bloom and Evaluation of Ecological Remediation Effects by Artificial ReefThesis for Master Degree Tsinghua University, China, 2013.

[19] R. Luo, B. Liu, Y. Xie, Z. Li, W. Huang, J. Yuan, G. He, Y. Chen, Q. Pan, Y. Liu, J. Tang, G. Wu, H. Zhang, Y. Shi, Y. Liu, C. Yu, B. Wang, Y. Liu, C. Han, D.W. Cheung, S.M. Yiu, S. Peng, X.Q. Zhang, G. Liu, X. Liao, Y. Li, H. Yang, J. Wang, T.W. Lam, J. Wang, SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience 1 (1) (2012) 18.

[20] R.K. Azizi, D. Bartels, A.A. Best, M. Dejongh, T. Díaz, R.A. Edwards, K. Formisma, S. Gerdès, E.M. Glass, M. Kuhl, F. Meyer, G.J. Olsen, R. Olson, A.L. Osterman, R.A. Overbeek, L.K. McNeil, D. Paarmann, T. Paczian, B. Parello, G.D. Pusch, C. Reich, R.
Stevens, O. Vassieva, V. Vonstein, A. Wilke, O. Zagnitko, The RAST server: rapid annotations using subsystems technology. BMC Genomics 9 (2008) 75.

[21] T.M. Lowe, S.R. Eddy, TRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25 (1997) 955–964.

[22] K. Lagesen, P. Hallin, E.A. Rødland, H.H. Staerfeldt, T. Rognes, D.W. Ussery, RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35 (2007) 3100–3108.

[23] A.L. Delcher, K.A. Bratke, E.C. Powers, S.L. Salzberg, Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23 (6) (2007) 673–679.

[24] L. Zheng, Z. Cui, L. Xu, C. Sun, R.J. Powell, R.T. Hill, Draft genome sequence of Rhodobacteraceae strain PD-2, an algicidal bacterium with a quorum-sensing system, isolated from the marine microalga Prorocentrum donghaiense. Genome Announc. 3 (1) (2015), e01549–14.

[25] R.L. Hahnke, C.M. Bennke, B.M. Fuchs, A.J. Mann, E. Rhiel, H. Teeling, R. Amann, J. Harder, Dilution cultivation of marine heterotrophic bacteria an abundant after a spring phytoplankton bloom in the North Sea. Environ. Microbiol. 17 (10) (2015) 3515–3526.