



GENOME ANNOUNCEMENT

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Draft genome of *Ochrobactrum intermedium* strain M86 isolated from non-ulcer dyspeptic individual from India

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Abstract

Background: *Ochrobactrum intermedium* is an emerging opportunistic pathogen of humans that is closely related to members of the genus *Brucella*. Earlier, we reported the case of an Indian subject with non-ulcer dyspeptic symptoms whose urease positive gastric biopsy revealed the presence of *Helicobacter pylori* along with non-*Helicobacter* like bacteria, eventually cultured and identified as *O. intermedium* strain M86.

Results: Here, we describe the unclosed draft genome of the strain M86 with a length of 5,188,688 bp and mean G+C content of 57.9%. We have also identified many putative gene clusters that might be responsible for its persistence in the gastric mucosa. Comparative analysis of genomic features of *Ochrobactrum intermedium* strain M86 and *Ochrobactrum intermedium* LMG 3301^T was also done.

Conclusions: This paper attempts to gain whole-genome based insights into the putative gene determinants of *O. intermedium* for survival in the highly acidic stomach lumen environment. Identification of genes putatively involved in the various metabolic pathways may lead to a better understanding of the survival of *O. intermedium* in acidic condition.

Background

The *Alphaproteobacteria* represent a biologically diverse group of bacteria with members like *Brucella*, *Bartonella*, *Agrobacterium* and *Ochrobactrum* that are capable of interacting with eukaryotic cells. *Ochrobactrum intermedium* is a Gram-negative, capsulating, aerobic bacilli belonging to the *Alphaproteobacteria*. It is the closest genetic relative of genus *Brucella* as evidenced by protein profiling, western blot, immunoelectrophoresis, amplified fragment length polymorphism, 16S rRNA gene and *RecA* gene sequence based studies [1]. Previous reports have suggested *O. intermedium* as an emerging pathogen in liver abscess post-liver transplantation and in the bladder cancer patient causing presumptive bacteremia [2,3]. But, clinical manifestations and diseases caused by *O. intermedium* are poorly characterised.

Several bacteria other than *Helicobacter pylori* have been detected earlier in gastric biopsies. The presence of *O.*

intermedium along with *H. pylori* was reported earlier in a subject from North-India diagnosed with non-ulcer dyspepsia [4]. A unique observation was the presence of severe fibrosis in the lamina propria of the gastric mucosa revealed during histological examination of the gastric antral biopsy. Whether this fibrosis was caused either partially or totally by *O. intermedium* was not clear. Other species of *Ochrobactrum* have also been associated along with *H. pylori*, e.g., *O. anthropi* with mild gastritis in squirrel monkeys [5]. Similarly, *Gastrospirillum hominis* [6], enterococci [7] and staphylococci have been associated with gastric disorders [8]. In some cases, they have been isolated from antral biopsies from patients with or without *H. pylori* colonization [9].

Importantly, both *H. pylori* and *O. intermedium* produce urease, and thus the detection of *H. pylori* by urease test in the presence of *Ochrobactrum* may be confounded. The role of *Ochrobactrum* in gastric pathology remains uncertain and requires detailed pathologic, microbiological and genetic investigations in order to evaluate the link between *H. pylori* and *O. intermedium* in the gastric niche. This

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paper attempts to gain whole-genome based insights into the putative gene determinants of *O. intermedium* for survival in the highly acidic stomach lumen environment.

Methods

Genome sequencing

Genomic DNA was isolated by PureLink[®] Genomic DNA Kit. The draft genome sequence of strain M86 was determined by Ion Torrent Personal Genome Machine (PGM[™]) sequencer using a 316 chip with 200-bp single-end shotgun sequencing. A total of 2,602,696 reads were obtained. PGM sequencing resulted in about 67X genome coverage with 148 contigs.

Assembly and annotation

The *de novo* approach was applied to finalize the unclosed draft genome using MIRA 3.4.0 version using default parameters [10]. Prediction and annotation of genes were done using RAST [11] server with SEED database and ISGA pipeline [12]. The data were further validated using gene prediction tools such as Glimmer [13]. Functional annotation was also performed by PGAAP using public database of National Centre for Biotechnology Information (NCBI). Prophages and putative phage like elements in the genome were identified

using prophage-predicting PHAST [14] Web server. Regions identified algorithmically as “intact” by PHAST, as well as regions sharing a high degree of sequence similarity and conserved synteny with predicted “intact” prophages, were identified as prophages.

Submission of genome sequence

The *Ochrobactrum intermedium* strain M86 whole genome shotgun (WGS) project was submitted to the GenBank and has the project accession AOE00000000 and consists of sequences AOE01000001-AOE01000148

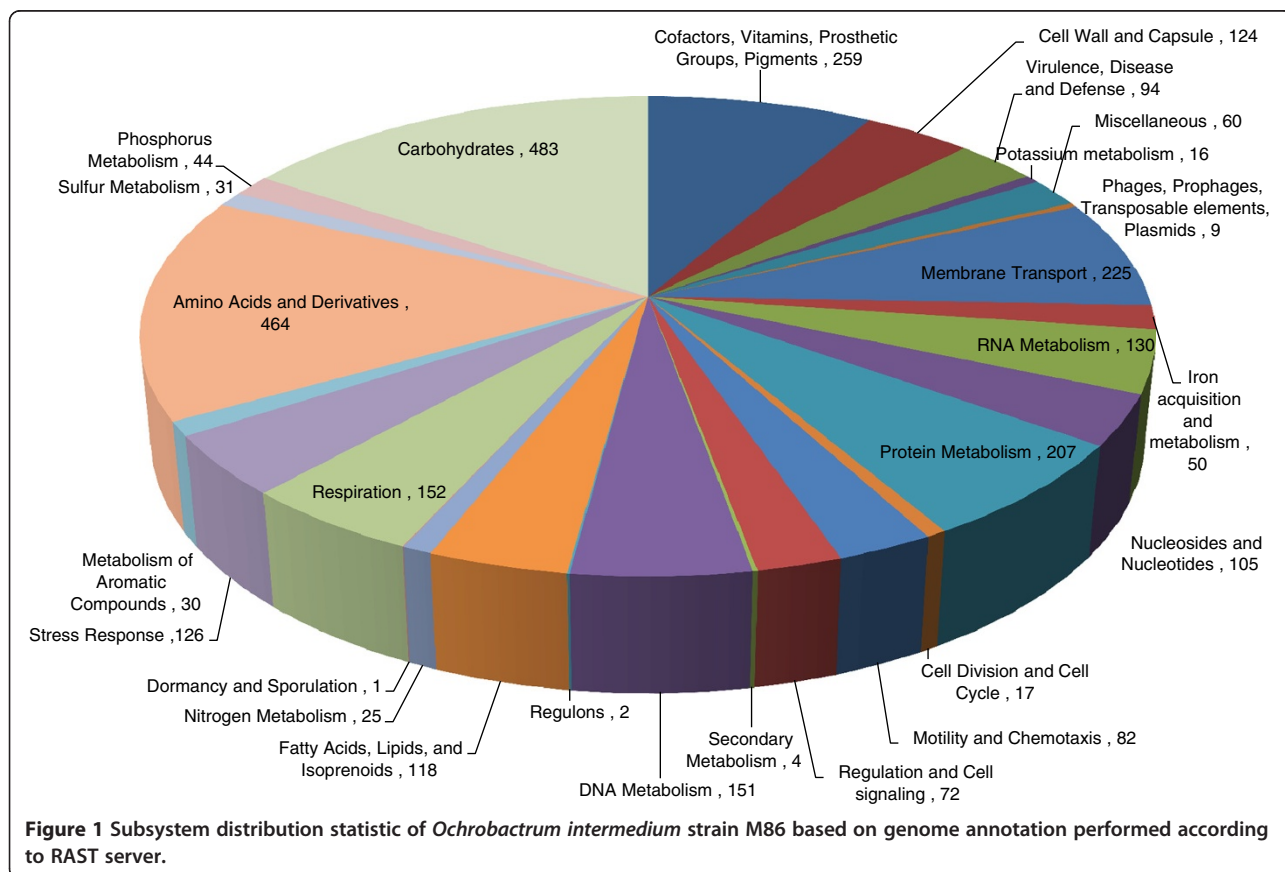
Quality assurance

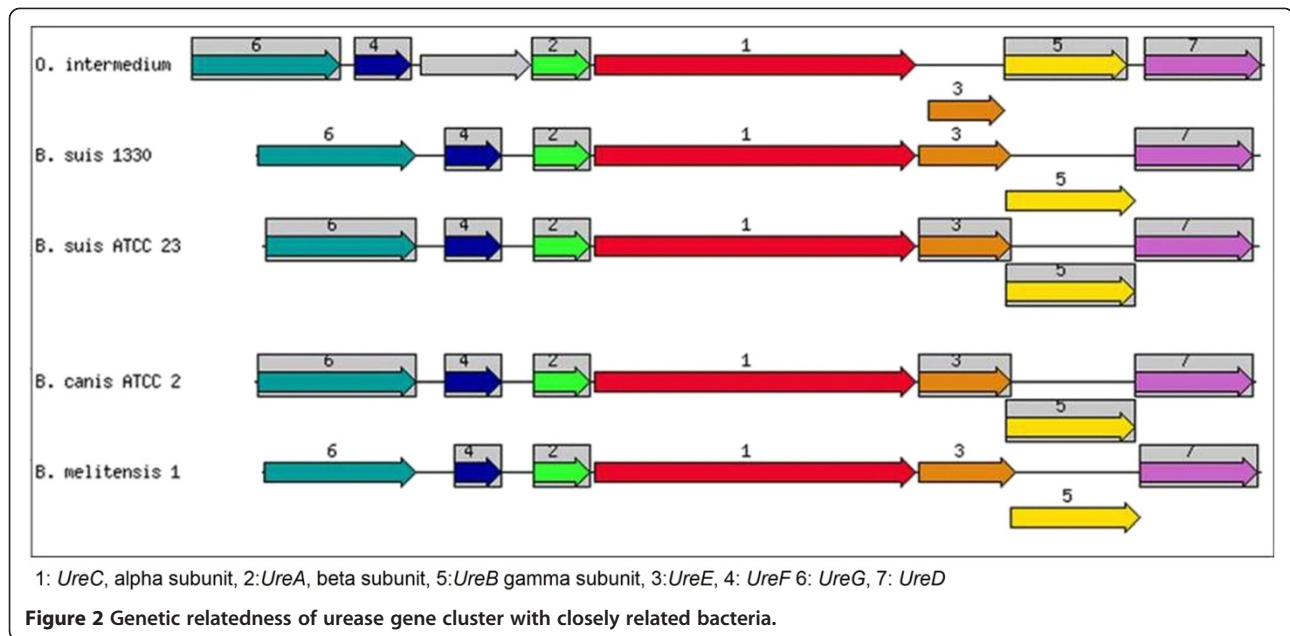
The genomic DNA was isolated from pure bacterial isolate and was further confirmed with 16S rRNA gene sequencing. Bioinformatic assessment of potential contamination of the genomic library by allochthonous microorganisms was done using PGAAP and RAST annotation systems.

Initial findings

Genome characteristics

Genome of *O. intermedium* strain M86 was sequenced on the IonTorrent Personal Genome Machine (PGM[™]) using 316 chip that resulted in 2,602,696 total reads with





a mean read length of 155 bp. *de-novo* assembly using the MIRA assembler v3.4.0 [10] with default parameters yielded ~67X coverage. A total of 148 contigs with >500 bp length were obtained. The unclosed draft genome sequence of strain M86 is of 5,188,688 bps and 5043 predicted coding DNA sequences (CDSs) and 66 RNA genes with mean G + C content of 57.9%. RAST server based annotation of the whole genome, showed the presence of 437 subsystems (related functional roles) [11]. Figure 1 describes the subsystem distribution of strain M86.

Putative gene clusters responsible for survival of *Ochrobactrum intermedium* strain M86 in the acidic environment of stomach lumen

H. pylori have several genes for biosynthesis of cytosolic urease for its survival in the acidic environment of stomach lumen [15]. Genome of strain M86 contains urease gene cluster (see Figure 2): out of which, *UreA*, beta subunit, *UreB* gamma subunit, *UreC*, alpha subunit, are part of core Urease enzyme, While four accessory proteins: *UreD*, *UreE*, *UreF* and *UreG* play important role in Ni²⁺ uptake and insertion into active site of apo-enzyme. Genetic relatedness of urease gene cluster with phylogenetically closely related bacteria is shown in Figure 2. A complete operon encoding the *VirB* gene involved in conjugative transfer is present in strain M86. Genes encoding osmotic stress, oxidative stress *HPIIb*, cold shock *GrpE*, heat shock *DnaK*, periplasmic stress *DegQ* and protection from reactive oxygen species, *sod* are found. Genes predicated to encode flagellar biosynthesis protein *FlhA* and *FlhB* has been identified in genome of strain M86 which are likely elementary to adaptation of new lifestyle. Enterobactin synthesis clusters of *entA*, *entB1*, *entB2*, *entC*, *entD*, *entE*,

Table 1 Comparisons of subsystem features between genome of *Ochrobactrum intermedium* strain M86 and *Ochrobactrum intermedium* LMG 3301^T

Subsystem features	Number of CDS present in <i>O. intermedium</i>	
	LMG 3301 ^T	M86
Amino acids and derivatives	448	464
Carbohydrates	460	483
Cofactors, vitamins, prosthetic groups, pigments	251	259
Protein metabolism	202	207
Membrane transport	188	225
Respiration	146	152
RNA Metabolism	131	130
DNA Metabolism	133	151
Cell wall and capsule	129	124
Stress response	121	126
Fatty acids, lipids, and isoprenoids	116	118
Nucleosides and nucleotides	105	105
Virulence, disease and defense	83	94
Motility and Chemotaxis	84	82
Regulation and cell signaling	66	72
Iron acquisition and metabolism	53	50
Miscellaneous	58	60
Phosphorus metabolism	46	44
Metabolism of aromatic compounds	29	30
Nitrogen metabolism	25	25
Cell division and cell cycle	17	17
Potassium metabolism	15	16
Sulfur metabolism	28	31

entF, *entG*, *entH* genes were also observed in the genome sequence of strain M86 suggesting its ability of iron acquisition by siderophore production. Presence of membrane transport machinery with dominance of Dipeptide-binding ABC transporter; periplasmic substrate-binding component was detected in the genome of strain M86. It has been found that all the clinical isolates and the type strains of *Ochrobactrum* were highly resistant to all forms of β -lactams except imipenem [16]. This resistance profile is consistent with the expression of the *AmpC* beta-lactamase characterized in *O. anthropi* [17,18]. The genome of strain M86 shows the presence of *AmpC* beta-lactamase gene which supports its resistance to β -lactams antibiotic observed by Dharne et al. [4]. Table 1 shows the comparison of genomic features of *Ochrobactrum intermedium* strain M86 and *Ochrobactrum intermedium* LMG 3301^T.

Prediction of phage islands

The presence of prophage sequences may also allow some bacteria to acquire antibiotic resistance, to exist in new environmental niches, to improve adhesion or to become pathogenic [14]. In addition, phages play a crucial role in genome plasticity and chromosome remodeling. The intact phage of 32 kb in length together with phage-like proteins and hypothetical proteins were identified in the genome of strain M86 by prophage-predicting PHAST [14] Web server.

Future directions

The genomic properties of *O. intermedium* are poorly characterized and, as a consequence, their role in human health and disease remains unclear. Elucidation of the physiological properties and identification of genes putatively involved in the various metabolic pathways may lead to a better understanding of the survival of *O. intermedium* in acidic condition. Further studies involving large scale genome sequencing and comparison of the *O. intermedium* strains isolated from several other non-ulcer dyspeptic individuals will help us apprehend the genomic features of its survival in the acidic condition of the stomach. Comparative genomic analyses of *O. intermedium* strain M86 and other environmental isolates of genus *Ochrobactrum* will permit us to understand its mechanisms for adaptation to new environments.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

GK and DD were involved in the genome assembly, annotation and manuscript preparation. SC and SS generated ion PGM data. MD and MP were involved in isolation of M86 strain. SM and VM provided the human stomach biopsy. GK and YS were involved in overall experimental design. All authors have read the manuscript and approved.

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