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Research Article

Comparison of Two *Stenotrophomonas maltophilia* Genomes Suggests CPBW01 is an Endosymbiont in *Leptinotarsa decemlineata*

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Abstract

Our previous results reveal that *Leptinotarsa decemlineata* feeding on field potato plants harbors *Stenotrophomonas maltophilia* strain CPBW01. Is CPBW01 an opportunist or an endosymbiont? In the present paper, the calculated ANI values revealed highest similarity of CPBW01 isolate to *S. maltophilia* ISMMS2; the phylogenetic trees of 16s rDNA and MLSA showed closest relationship of CPBW01 with ISMMS2. Moreover, the genomes of CPBW01 and ISMMS2 displayed a highly conserved synteny and gene order, and shared similar sizes and gene numbers. Among more than 4000 genes, CPBW01 had 103 strain-specific genes while ISMMS2 possessed 97 ones. CPBW01 genome included more mobile genetic elements that may allow it to sharpen its genome to adapt to the symbiont environment in insect cavity. CPBW01 owned more sophisticated chemotaxis signaling systems and stronger motility apparatus. Moreover, function forecasting of the CPBW01-specific genes indicated the isolate may work collaboratively with *L. decemlineata* to biosynthesize and break down several amino acids. In addition, strain-specific genes acting in nuclear acid metabolism, intercellular and intracellular signaling, secretion systems and extracellular enzymes, and lipid and polysaccharide metabolism disclosed an adaptation of CPBW01 and ISMMS2 to different environments. These pieces of evidence offer a solid support that *S. maltophilia* CPBW01 is an endosymbiont in *L. decemlineata*.

Keywords: *Leptinotarsa decemlineata*; *Stenotrophomonas maltophilia*; Genome; Synteny Analysis; Endosymbiont

Introduction

Many insects harbor symbiotic bacteria in their gut, body cavity, and/or other tissues [1-3]. These bacteria have formed a symbiotic interplay with their host insects over a long period of coevolution [4]. Some bacterial symbionts are indispensable for the growth, survival, and reproduction of their insect hosts through regulating larval development [5], synthesizing essential nutrients [6,7], assisting food digestion and energy utilization [8-12], providing food sources [13], or producing essential vitamins [14]. Other bacterial symbionts are not essential, but nonetheless influence a variety of host biological traits, such as modulation of immune responses [15], defense against natural enemies [14,16,17], tolerance to environmental stresses [18,19], resistance to noxious chemicals [20-22], adaptation to specific food plants [23,24], or regulation of sex ratios and related reproductive traits [25,26].

We focused on the *Stenotrophomonas* species in the present paper. The *Stenotrophomonas* genus currently has 17 species [27, 28], and occurs ubiquitously in water, soil, plants and animals [29]. These bacteria have been documented in several insect species such as coleopteran the Colorado Potato Beetle (CPB) *Leptinotarsa decemlineata* [30-32], *Henosepilachna vigintioctopunctata* [3] and *Rhynchophorus ferrugineus* [33], and *isopteran Psammotermes hypostoma* [10], identified by culture-independent PCR amplification of 16S rRNA [34].

It is known that CPBs exploit *Stenotrophomonas* in their oral secretions to suppress anti-herbivore defenses in tomato (*Solanum lycopersicum*) [30,31]. Moreover, we have identified *S. maltophilia* strain CPBW01 in CPB [32]. Up to now, in only another insect species, *P. hypostoma* [10], the bacterial species in the *Stenotrophomonas* genus has been identified, based on biochemical tests and sequencing of 16s rRNA gene. It is *S. maltophilia*. It acts as a cellulolytic symbiotic organism in the termite [10].

Considering the *Stenotrophomonas* spp. have a worldwide distribution, including plants [29], are the *Stenotrophomonas* spp. in CPB opportunists or symbionts? In the present paper, we calculated ANI values with the Ortho ANI program, and performed the phylogenetic analyses of 16s rDNA and Multilocus Sequence Analysis (MLSA) by concatenation of 6 housekeeping genes (with or without 16S RNA), and found CPBW01 was closer to an emerging human pathogen strain ISMMS2. We finally conducted a synteny analysis to compare the differences between CPBW01 and ISMMS2. Our results suggested that *S. maltophilia* CPBW01 was an endosymbiotic strain in CPB.

Materials and Methods

Bacteria culture

The *S. maltophilia* strain CPBW01 was routinely cultured in the minimal nutrient medium (Bushnell and Haas medium), with

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solanine as the unique carbon source [32].

ANI calculation

The Average Nucleotide Identity (ANI) was calculated using the algorithm described previously [35], with the web service EzBioCloud in the default setting. We used reference genomes from eight *S. maltophilia* strains, and eleven represent species within *Stenotrophomonas genus*.

16S rRNA Phylogenetic Analysis

The publicly available 16S rRNA sequences of type strains of *Stenotrophomonas* spp. (including CPBW01) were retrieved from the National Center for Biotechnology Information (NCBI) nucleotide database [36-40]. In particular, two *S. maltophilia* strains (i.e., ISMMS2 and U5) were included in order to confirm the phylogenetic status of CPBW01. At first, multiple sequence alignment of the 16S whole rRNA gene sequences of all strains was first performed by MEGA (v7). Specifically, Clustal W was used for multiple sequence alignment. Evolution history was reconstructed using the built-in maximum-likelihood method with 1,000 bootstraps.

Multi-locus sequence typing using multiple housekeeping genes

To further validate these clade assignments, Multilocus Sequence Analysis (MLSA) was performed by concatenation of housekeeping genes: *atpD, guaA, mutM, nuoD, ppsA, and recA* [41]. Multiple sequence alignment of these housekeeping genes in 11 *Stenotrophomonas* genomes [42-44] was performed using MEGA in order to infer their phylogeny. Additional MLSA using the six housekeeping genes plus 16S rRNA is also performed using MEGA (v7) to confirm the phylogenetic position of CPBW01.

Synteny analysis

The sequences from the genome of *S. maltophilia* CPBW01 were compared to those of ISMMS2 using bwa v0.7.17 (https://github.com/lh3/bwa) and bedtools v2.25.0 (https://bedtools.readthedocs.io). Structural variants were called from both genomic data by Delly v0.8.1 (https://github.com/dellytools/delly), the variants were classified as deletion, inversion, duplication, insertion, and translocation. Genomic synteny was analyzed using MUMmer v3.23 (http://mummer.sourceforge.net/) under default parameters [45], with the genome of *S. maltophilia* ISMMS2 as a reference. The comparison results were drawn using circus v5.16 (http://circos.ca) tool.

Meanwhile, MEGA (v7) software was used to compare genome of *S. maltophilia* CPBW01 and that of ISMMS2. The deleted and inserted gene sequence was identified as ISMMS2 -specific and CPBW01-specific ones, respectively. The gene sequences of each group were substantiated by comparison to the National Center for Biotechnology Information (NCBI) nucleotide database. The filtered gene sequences were named according to the annotated genomes of *S. maltophilia* CPBW01 and ISMMS2.

Function forecasting of genes

To understand gene functions from *S. maltophilia* CPBW01 and ISMMS, we used KAAS (https://www.genome.jp/tools/kaas/) to annotate strain-specific genes according to the functional genes from *Stenotrophomonas* (*S. maltophilia* K279a, *S. maltophilia* R551-3), *Xanthomonas* (*X. campestris* pv. campestris ATCC 33913, *X. campestris* pv. campestris 8004, *X. campestris* pv. campestris B100, *X. campestris* pv. vesicatoria, *X. citri* pv. citri 306, *X. oryzae* pv. oryzae MAFF 311018, *X. oryzae* pv. oryzae KACC 10331, *X. oryzae* pv. oryzae PXO99A, *X. albilineans*, *X. phaseoli*), and *Pseudoxanthomonas* (*P. suwonensis* 11-1).

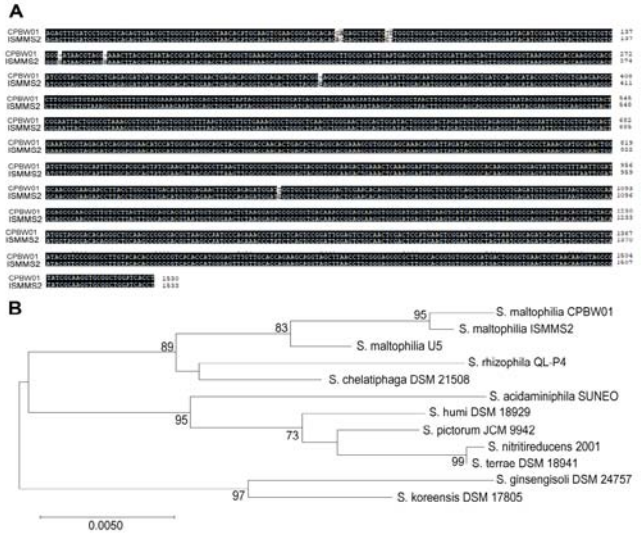

Figure 1: Alignment and phylogenetic analysis of 16S rRNA derived from *S. maltophilia* CPBW01 and ISMMS2 and related strains of *Stenotrophomonas* species. (A) Sequence alignment. Increasing background intensity (from light to dark) indicates an increase in sequence similarity. Gaps have been introduced to permit alignment. (B) The tree is constructed using the maximum-likelihood method based on the full-length sequence alignments. Bootstrap analyses of 1000 replications are carried out and bootstrap values > 50% are shown on the tree.

Results and Discussion

Similar genomes between *S. maltophilia* CPBW01 and ISMMS2

Genomic-wide relatedness comparison was calculated with the OrthoANI program using publicly available genomes from different *S. maltophilia* strains and different *Stenotrophomonas* species (Table 1). ANI results indicated that the genome of strain CPBW01 was closer to those of strain *S. maltophilia* ISMMS2, W18, OUC_Est10 and K279a (Table 1). *S. maltophilia* ISMMS2 and K279a are cultured from patient samples [36,38], while W18 and OUC_Est10 are isolated from slaughterhouse and oil-polluted soil respectively [37,39]. In contrast, the strain X28 and AB550 with lower ANI values are obtained from sludge and water respectively [40]. These data indicate that isolate CPBW01 should not be derived from host plants; it should be an endosymbiotic bacterium of *L. decemlineata*.

Comparison of the 16S rRNA genes from CPBW01 and ISMMS2 revealed that the sequences shared 99.5% similarity (8/1530 bp differences) (Figure A1). Sequence analysis of 16s rDNA of 10 *Stenotrophomonas* species and 3 bacterial strains within *S. maltophilia* obtained from Gen Bank database were represented in Figure 1B. The CPBW01 isolate joined together with *S. maltophilia* ISMMS2 to form a subclade, with bootstrap value of 95% supporting (Figure 1B).

To further validate these clade assignments, Multilocus Sequence Analysis (MLSA) was performed by concatenation of 6 housekeeping

Table 1: ANI values of strain CPBW01 to those from *Stenotrophomonas spp*.

Species/strain	Genome length (bp)	ANI value (%)	Average aligned length (bp)
S. maltophilia ISMMS2	4,509,724	98.08	2,882,922
S. maltophilia W18	4,737,900	93.04	2,815,900
S. maltophilia OUC_Est10	4,668,540	92.57	2,819,437
S. maltophilia K279a	4,851,120	92.44	2,796,610
S. maltophilia FDAARGOS_507	4,576,470	92.35	2,867,633
S. maltophilia X28	4,553,280	92.25	2,718,645
S. maltophilia AB550	4,942,920	91.4	2,716,433
S. maltophilia U5	4,451,020	87.03	2,617,543
S. pavanii LMG 25348	4,422,720	91.1	2,637,642
S. sp. PAMC25021	4,727,700	91.37	2,752,873
S. chelatiphaga DSM 21508	3,888,240	82.21	1,800,972
S. rhizophila QL-P4	4,198,320	81.67	1,826,838
S. acidaminiphila SUNEO	3,660,780	80.44	1,487,228
S. pictorum JCM 9942	2,890,680	79.78	1,195,667
S. terrae DSM 18941	4,327,860	79.03	1,489,264
S. nitritireducens 2001	4,541,040	79.02	1,530,551
S. humi DSM 18929	4.071,840	78.85	1,513,817
S. ginsengisoli DSM 24757	3,323,160	77.13	1,141,776
S. koreensis DSM 17805	3,000,840	76.98	1,059,334

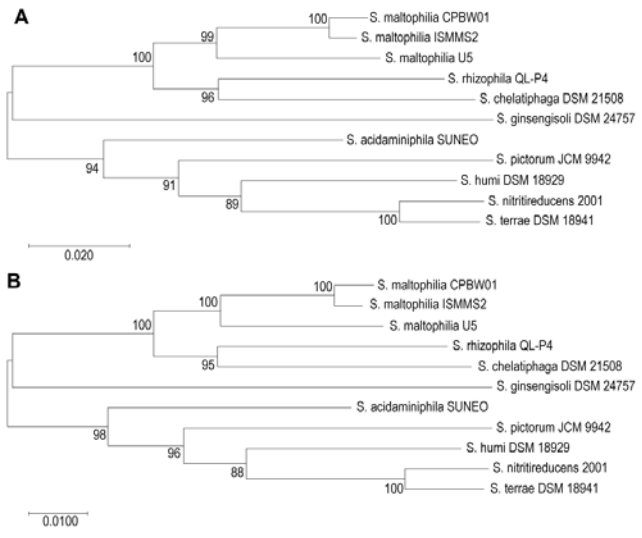

Figure 2: Phylogenetic analysis of Multilocus Sequence Analysis (MLSA) in *S. maltophilia* CPBW01 and ISMMS2 and related strains of *Stenotrophomonas* species. The phylogenetic analysis of MLSA by concatenation of 6 housekeeping genes (*atpD, guaA, mutM, nuoD, ppsA, and recA*), with or without 16S RNA (B and C) are performed. The trees are constructed using the maximum-likelihood method based on the full-length protein sequence alignments. Bootstrap analyses of 1000 replications are carried out and bootstrap values > 50% are shown on the tree.

genes, with or without 16S RNA (Figure 2). Both trees revealed high phylogenetic similarities with bootstrap values of 100% among CPBW01 and *S. maltophilia* ISMMS2.

All these results reveal that the genome sequence of *S. maltophilia* CPBW01 is similar to that of ISMMS2.

Table 2: General characteristics of *S. maltophilia* CPBW01 and ISMMS2.

Features	CPBW01 (this study)	ISMMS2 (Pak et al, 2015)
Genome size (bp)	4,444,327	4,509,724
Gene	4,050	4,081
rRNA	13	13
tRNA genes	75	74
Locality	*L. decemlineata*, Urumqi, Xinjiang, China	Patient blood, New York, USA

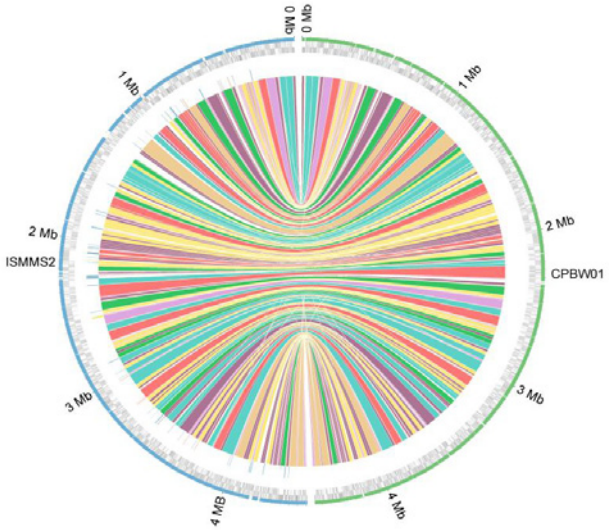

Figure 3: Synteny analysis of *S. maltophilia* CPBW01 and ISMMS2. Circular synteny figure was drawn using circus v5.16 (http://circos.ca) tool. From the outmost circles, circle (1) illustrates genome size from ISMMS2 (blue) and CPBW01 (green). The common sequences are linked with continuous lines. Circle (2) and (3) denote encoding genes on the backward and forward strands. The types of SV structure are as follows: deletion, blue; inversion, orange; duplication, yellow; insertion, red; translocation, green.

Comparison of genome sequence

Comparison of high-quality *Stenotrophomonas* genomes has been instrumental to advancing our understanding of how natural selection drives genomic change during the origin of novel traits. Genome sequence of our isolate *S. maltophilia* CPBW01 was compared with that of endophytic ISMMS2 strain [36] (Table 2, Figure 3). The genomes showed a highly conserved synteny and gene order (Figure 3), and shared similar sizes and gene numbers (Table 2).

Divergence in the gene of the two organisms might reflect adaptation to specific niches. We highlighted the differences between two genomes of CPBW01 and ISMMS2 and discovered that the inversions, duplications, insertions, and translocations scattered throughout (Figure 3, Table S1), implying the possible genetic evolution during strain development. Out of more than 4000 genes, the *S. maltophilia* CPBW01 genome contained 103 genes that were absent from *S. maltophilia* ISMMS2 (Table 3, Table S2). By contrast, the genome of *S. maltophilia* ISMMS2 comprised 97 genes that were lack in *S. maltophilia* CPBW01 (Table 4, Table S3).

Table 3: A summary of CPBW01-specific genes.

Functional protein	Number	Description
Transposase	4	Transposases contribute substantially to variation in genome. A high proportion of transposases may provide a basis for rapid divergence and survival of CPBW01 in insect hemolymph environments
Conjugal transfer proteins	1	May be involved in conjugal transfer of plasmids
AAA family ATPase	1	Remodeling and degrading DNA, using ATP as energy
HNH endonuclease	1	Degradation of nuclear acids
Nucleotidyltransferase	1	May metabolizes nuclear acids
Methyltransferase	2	Chemotaxis signaling system
Tetratricopeptide repeat domain protein	2	Assembly of periplasmic flagella, morphology and motility
Diguanylate cyclase	1	Mediation of quorum sensing
LysR	1	Regulation of gene expression
XRE	1	Involved in oxidant tolerance and virulence
Helix-turn-helix transcriptional regulator	1	Forming the regulation network to conducts intercellular and intracellular signals
Fimbria/pilus protein	9	Three fimbrial proteins, three pilus assembly proteins, a type IV pilus modification protein, a fimbria/pilus periplasmic chaperone and a fimbria/pilus outer membrane usher protein
Prepilin protein	3	Prepilin N cleavage/methylation domain-containing protein
RelE/ParE toxin	1	Type II toxin-antitoxin system RelE/ParE family toxin
RHS repeat protein	2	Inhibition of growth of neighboring cells
T4SS RHS protein	1	Inhibition of growth of neighboring cells
PIG-L family deacetylase	1	Glycosylphosphatidylinositol synthesis
YaiO family outer membrane beta-barrel protein	1	Integral membrane proteins
MFS transporter	1	Transmembrane transport of materials
ABC transporter	1	Transmembrane transport
ATP-binding cassette domain-containing protein	1	Transmembrane transport
TonB-dependent receptor	1	Uptake of specific substrates
Autotransporter outer membrane beta-barrel domain-containing proteins	1	Membrane protein
ADP-ribosylglycohydrolase family protein	1	The removal of ADP-ribose from ADP-ribosylated proteins
Polyphosphate kinase (PPK),	1	Biosynthesis of protein polyP
Antibiotic acetyltransferase	1	Antibiotic biosynthesis and signaling
Toxin	1	Intercellular competition and immunity
SLATT domain-containing protein	1	Involved in biological conflicts, immunity and signaling
Carbonic anhydrase	1	Formation of HCO3-
GNAT family N-acetyltransferase	2	Arginine biosynthesis
Acetyltransferase	1	Arginine biosynthesis
2OG-Fe(II) oxygenase	1	Metabolism of arginine and proline
Redoxin domain-containing protein	1	Lipid metabolism
Glycosyltransferase	3	Sugar metabolism
N-acetylglucosaminyltransferase	1	Metabolism of amino sugar and nucleotide sugar
Polysaccharide biosynthesis protein	1	Polysaccharide production
EamA family transporter	1	Drug/metabolite transporter
DUF family protein	4	A DUF2274, a DUF4433, a DUF4339 and a DUF4224 domain-containing proteins
Hypothetical protein	43	Unknown function

Table 4: A list of ISMMS2-specific genes.

Functional protein	Number	Description
Phage tail protein	4	Structural protein of phage
Phage tail tape measure protein	1	Structural protein of phage
Phage tail assembly protein,	1	Structural protein of phage
Phage major tail tube protein	1	Structural protein of phage
Phage baseplate protein	1	Structural protein of phage
Phage baseplate assembly protein	1	Structural protein of phage
Tail protein	1	Structural protein of phage
Tail fiber protein	1	Structural protein of phage
Baseplate assembly protein	1	Structural protein of phage
Site-specific integrase	2	Recombination of 2 DNAs
Integrase	1	Recombination of 2 DNAs
Recombinase family protein	1	Recombination of 2 DNAs
GIY-YIG nuclease family protein	1	Involved in DNA repair and recombination
Chromosome partitioning protein ParB	2	Involved in DNA repair and recombination
Nucleotide-binding protein	1	Nucleoid-associated protein
H-NS histone family protein	1	Nucleoid-associated protein
DEAD/DEAH box helicase	1	RNA metabolism
HAMP domain-containing protein	1	Chemotaxis signaling system
Response regulator transcription factor	1	Chemotaxis signaling system
His-Xaa-Ser system radical SAM maturase	2	Chemotaxis signaling system
YbjQ family protein	1	Mediation of QS signaling system
EAL domain-containing protein	1	Mediation of QS signaling system
Helix-turn-helix transcriptional regulator	2	Conducting intercellular and intracellular signals
Helix-turn-helix domain-containing protein	1	Conducting intercellular and intracellular signals
GntR family transcriptional regulator	1	Conducting intercellular and intracellular signals
Pilin	1	Type II secretion system
Wall-associated protein	1	Type II secretion system
HlyD family efflux transporter periplasmic adaptor subunit	1	Membrane protein
TlpA disulfide reductase	1	Biogenesis of cytochrome aa3 and development of symbiosis
Peptidase domain-containing ABC transporter	1	Transmembrane transport
ATP-binding protein	1	Binding of ATP
Nuclear transport factor 2 family protein	1	Facilitating protein transport into the nucleus
Aromatic alcohol reductase	1	Degradation of phenylalanine and tyrosine
Acyltransferase	1	Lipid metabolism
Glycosyltransferase	1	Sugar metabolism
Glycoside hydrolase family 104 protein	1	Sugar metabolism
Toprim domain-containing protein	1	A metal-assisted phosphodiester bond cleavage or formation
Insulinase family protein	1	Destroying or inactivating insulin
Tautomerase family protein	1	Unknown function
BLUF domain containing protein	1	Unknown function
DUF family protein	4	A DUF3667, a DUF5076, a DUF1016, and a DUF380 domain-containing proteins
Hypothetical protein	46	Unknown function

Mobile genetic elements and metabolism of nuclear acids

A high proportion of transposases and phages are associated with mobile genetic elements [46]. The *S. maltophilia* CPBW01 genome carries 4 Insertion Sequence (IS) elements that are absent in ISMMS2 (Table 3). Moreover, a CPBW01-specific conjugal transfer protein may be involved in conjugal transfer of the mobile genetic elements (Table 3).

S. maltophilia harbors giant phage [47]. Some ISMMS2-specific genes including four phage tail proteins, a phage tail tape measure protein, a phage tail assembly protein, a phage major tail tube protein, a phage baseplate protein, a phage baseplate assembly protein, a tail protein, a tail fiber protein, and a baseplate assembly proteins were involved in the survival of macrophages (Table 4). However, CPBW01 lacked these genes (Table 3).

An AAA family ATPase was specifically present in CPBW01 (Table 3), which may exert a common DNA remodeling or degradation function where the energy of ATP hydrolysis is coupled to translocation along polymeric substrate [48]. Moreover, a CPBW01-derived His-Asn-His (HNH) endonuclease and a nucleotidyltransferase domain-containing protein may be responsible for metabolism of nuclear acids (Table 3). Similarly, in ISMMS2 two site-specific integrases, an integrase and a recombinase family protein may catalyze recombination of 2 DNAs; a GIY-YIG nuclease family protein, two ParB chromosome partitioning proteins may be involved in DNA repair and recombination. A nucleotide-binding protein and an H-NS histone family protein are nucleoid-associated proteins in ISMMS2 (Table 4).

An ISMMS2-derived DEAD/DEAH box helicase was involved in all facets of RNA metabolism, including pre-mRNA splicing, micro RNA processing, RNA export, RNA editing, storage and decay, ribosome biogenesis, transcription, and translation [49,50].

In addition, the accuracy of protein biosynthesis is ensured by two processes involving tRNA: One is the correct attachment of the amino acid to the 3' terminus of tRNA, which is catalyzed by 20 aminoacyl tRNA synthetases, and the other is codon recognition by the tRNA anticodon [51]. CPBW01 has 75 tRNA genes whereas ISMMS2 genome codes for 74 tRNAs (Table 2).

Chemotaxis and flagella

Motile bacteria often use sophisticated chemotaxis signaling systems to direct their movements, enabling them to adapt to the natural habitats *via* moving toward favorable conditions and away from hostile surroundings. In general, bacterial chemotactic signal transduction pathways have three basic elements: (1) signal reception by bacterial chemoreceptors located on the membrane; (2) signal transduction to relay the signals from membrane receptors to the motor; and (3) signal adaptation to desensitize the initial signal input [52].

An ISMMS2-derived HAMP domain-containing protein, a response regulator transcription factor and two His-Xaa-Ser system radical SAM maturases (Table 4) belong to bacterial chemotactic signal transduction pathways [52]. Some genes encode proteins for chemotaxis of CPBW01. The resultant proteins included a methyltransferase domain-containing protein and a class I SAM-dependent methyltransferase (Table 3). These proteins may be the second and third basic elements [52].

Ultimately, the environmental signals perceived by the chemoreceptors are relayed to the flagellar motor to enable the bacteria to perform displacements corresponding to the type of stimulus perceived [52]. The flagellum is a bacterial motility apparatus that can be observed on the cell surface as long filamentous cellular appendices. The flagella motor is encoded by more than 50 genes [53]. Two tetratricopeptide repeat domain proteins in CPBW01 were absent in ISMMS2 (Table 3,4). These proteins have profound effects on assembly of periplasmic flagella, morphology and motility [54].

The discrepancy of chemotaxis and bacteria motility apparatus indicates evolutionary adaptation of two strains to different chemical environment between insect body and human blood.

Intercellular and intracellular signaling

In S. maltophilia, Quorum Sensing (QS) conducts intercellular and intracellular signal through a diffusible signal molecule, DSF [55-57]. DSF perception is linked to altered levels of the second messenger cyclic di-GMP [58]. A diguanylate cyclase in CPBW01 mediate quorum sensing (Table 3). In contrast, an YbjQ family protein present in ISMMS2 may act as a cyclic di-GMP effector to control bacterial virulence; an EAL domain-containing protein may mediate QS signaling system (Table 4).

In the prokaryotic kingdom, LysR, Helix-Turn-Helix (HTH), winged HTH, GntR, MarR and XRE family transcriptional regulators form the regulation network. They act as either activators or repressors, and regulate the expression of a diverse set of genes, including those involved in virulence, metabolism, quorum sensing, motility, morphogenesis, pathogenesis, stress tolerance and hypersensitive response [59-62]. A LysR, a XRE, and an HTH transcriptional regulator were CPBW01-specifically identified (Table 3). In contrast, other two HTH transcriptional regulators, an HTH domain-containing protein and a GntR family transcriptional regulator were found in ISMMS2 (Table 4).

These strain-specific intercellular and intracellular signals indicate evolutionary fitness of two strains to insect body v.s. human blood through various transcriptome.

Secretion systems and extracellular enzymes

Based on genome sequencing (Figure 3), *S. maltophilia* CPBW01 has type I, II, IV, V and VI secretion systems (T1SS, T2SS, T4SS, T5SS and T6SS), like some *S. maltophilia* isolates [38].

For T2SS, nine genes involving in fimbria/pilus construction, three prepilin-type N-terminal cleavage/methylation domain-containing proteins and a type II toxin-antitoxin system RelE/ParE family toxin were specifically present in CPBW01 (Table 3). In contrast, some genes coding for structure proteins such as a pilin and a wall-associated protein in ISMMS2 were absent in CPBW01 (Table 3,4).

It is known that T6SS and T4SS export Rearrangement Hotspot (Rhs) proteins which inhibit the growth of neighboring cells to mediate intercellular competition [63]. Three proteins in CPBW01, two RHS repeat protein and a T4SS RHS, were absent in ISMMS2 (Table 3,4).

Function variation of proteins

The differences in cellular surface proteins reveal environment fitness of bacteria. A Phosphatidylinositol Glycan Class L (PIG-L) family deacetylase gene was recognized in CPBW01 and a HlyD family efflux transporter periplasmic adaptor subunit was in ISMMS2 (Table 3,4). The former is involved in the biosynthesis of glycosylphosphatidylinositol [64], an anchor on the cell membrane for surface proteins to bind.

The outer membranes of gram-negative bacteria contain integral membrane proteins [65]. A YaiO family outer membrane β-barrel protein was present in CPBW01 but not in ISMMS2 (Table 3). A bacterial gene tlpA encoding a membrane protein TlpA family disulfide reductase was uncovered in ISMMS2 (Table 4).

Some transporters were different in two strains. A Major Facilitator Superfamily (MFS) transporter in CPBW01 were absent in ISMMS2 (Table 3,4). Moreover, an ABC transporter and an ATP-binding cassette domain-containing protein were present in CPBW01 (Table 3), and a peptidase domain-containing ABC transporter and an ATP-binding protein were present in ISMMS2 (Table 4).

Interacting with outer membrane receptor proteins, TonB system carries out high-affinity binding and energy dependent uptake of specific substrates into the periplasmic space. A TonB-dependent receptor was present in CPBW01 but not in ISMMS2 (Table 3,4).

Moreover, an autotransporter outer membrane beta-barrel domain-containing protein was present in CPBW01 but not in ISMMS2 (Table 3, 4). An ADP-ribosylglycohydrolase family protein in CPBW01 may catalyze the removal of ADP-ribose from ADP-ribosylated proteins, and a polyphosphate kinase helps in the biosynthesis of protein polyP (Table 3). An ISMMS2-derived nuclear transport factor 2 family protein facilitates protein transport into the nucleus (Table 4).

In addition, an antibiotic acetyltransferase, a toxin and an SLATT domain containing protein were present in CPBW01 but not in ISMMS2 (Table 3,4). These proteins should be associated with antibiotic biosynthesis, intercellular competition and immunity.

Amino acid metabolism

A CPBW01-specific carbonic anhydrase catalyzes the formation of HCO3-, which is essential for nitrogen metabolism. An acetyltransferase and two GNAT family N-acetyltransferase may be associated with arginine biosynthesis; a 2OG-Fe(II) oxygenase with the metabolism of arginine and proline (Table 3). In contrast, an aromatic alcohol reductase present in ISMMS2 may degrade phenylalanine and tyrosine (Table 4).

Herbivores tend to obtain free amino acids from plants. In the potato foliage, threonine, serine and proline are among the ten most abundant amino acids [66]. Given that free amino acids cannot be stored by insects, degrading excess amino acids that are not needed for protein synthesis is necessary for maintenance of a balanced amino acid composition in *L. decemlineata* hemolymph. The presence of a 2OG-Fe(II) oxygenase in CPBW01 (Table 3) indicates that the *S. maltophilia* isolate may work collaboratively with *L. decemlineata* to break down threonine, serine and proline. The indication suggests that *S. maltophilia* CPBW01 should be a symbiont of *L. decemlineata*.

Lipids

Lipid metabolism differed in the two strains. A CPBW01-specific redoxin domain-containing protein may catalyze lipid degradation (Table 3). In contrast, an acyltransferase was found in ISMMS2 (Table 4).

Polysaccharides and peptidoglycans

Sugars are materials for polysaccharide biosynthesis. Three CPBW01-specific glycosyltransferase and an N-acetylglucosaminyltransferase were identified. An ISMMS2-specific glycosyltransferase and a glycoside hydrolase family 104 protein genes were found (Table 3,4). Their products may be responsible for sugar metabolism.

Since galactose is absent and trehalose is the major blood sugar in *L. decemlineata* [67], the absence of enzymes for galactose metabolism and trehalose biosynthesis in CPBW01 is reasonable. Difference in enzymes for metabolism of polysaccharides and peptidoglycans suggests evolutionary fitness of two strains to insect body v.s. human blood.

Conclusion

In summary, our results suggested that CPBW01 was an endosymbiotic *S. maltophilia* strain, by following lines of evidence. (1) The genomes of CPBW01 and ISMMS2 had conserved genomic structures, and shared similar sizes and gene numbers. (2) The phylogenetic trees of 16s rDNA and MLSA showed closest relationship with *S. maltophilia*. (3) The calculated ANI values revealed highest similarity of CPBW01 to ISMMS2. (4) The synteny analysis indicated an evolutionary adaptation of CPBW01 and ISMMS2 to different chemical environment between insect body and human blood.

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