



Research Article

Molecular Insights into Beneficial Effects of Tea-Plant Growth and Selenium Enrichment by *Herbaspirillum camelliae*

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Abstract: *Herbaspirillum camelliae* WT00C, as a tea-plant endophytic bacterium, not only colonizes specifically in tea plants but also promotes tea-plant growth and selenium enrichment. Different from diazotrophic endophytes *H. seropedicae*, *H. frisingense* and *H. rubrisubalbicans*, *H. camelliae* WT00C does not display nitrogen-fixing activity. To understand the molecular mechanisms of promoting the growth of tea plant and Se-enrichment, we sequenced and annotated the genome of *H. camelliae* WT00C. The results showed that the genome was composed of 6,079,821 base pairs with a total of 5,537 genes. The genomic survey also revealed that *H. camelliae* WT00C was a multifunctional bacterium metabolizing a variety of carbon and nitrogen sources and defending against biotic and abiotic stress. Although this bacterium did not have intact nitrogen-fixing genes, its genome held the genes responsible for indole-3-acetic acid (IAA) biosynthesis, 1-aminocyclopropane-1-carboxylate (ACC) deamination, siderophore synthesis, ammonia formation, urea metabolism, glutathione and selenocompound metabolisms. Biosynthesis of IAA, siderophore, ammonia, urea and ACC deaminase could explain why two bacterial strains promote tea-plant growth and development. Selenocompound metabolism in this bacterium might also benefit tea-plant growth and Se-enrichment. In addition, the genome of *H. camelliae* also contained a multitude of protein secretion systems T1SS, T3SS, T4SS and T6SS, in which T4SS did not exhibit in other members of the genus *Herbaspirillum*.

Keywords: *Herbaspirillum*, endophytes, genome annotation, selenocompound metabolism, secretion system

1. Introduction

Herbaspirillum camelliae WT00C and WT00F were two gram-negative, endophytic bacterial strains isolated from the tea plant in Wuhan city, China [1]. Two strains were already identified as a novel species of the genus *Herbaspirillum* based on their physiochemical and genetic characteristics as well as 16S rRNA gene sequences [2]. Differences between the strains WT00C and WT00F were that (a) the strain WT00C displayed catalase activity but the strain WT00F did not, and (b) the strain WT00F decomposed lactose to produce acid but the strain WT00C did not [1]. Unlike *H. seropedicae*, *H. rubrisubalbicans*, *H. frisingense* and *H. lusitanum* [3-5], *H. camelliae* was unable to fix nitrogen. However, biochemical assays showed that these two strains produced ammonia, siderophores and indole-3-acetic acid (IAA) [1]. Production of ammonia, siderophores and IAA implied that *H. camelliae* might have the potential to promote the growth and development of tea plants. Further studies found that *H. camelliae* entered plants via plant vulnus, and only colonized in

tea plant. Inoculating the bacterial cells to tea cuttings by soaking for an hour markedly stimulated lateral root formation and the growth of newborn shoots. Sprinkling the bacterial cells to the upper incision of tea twigs in the field also enhanced the growth of newborn shoots. More importantly, both strains WT00C and WT00F did not cause plant disease symptoms in tea plants. Thus, *H. camelliae* was thought to be potential bioaccelerator for tea cultivation [6]. Two strains also had a strong capability of reducing selenate/selenite to form red elemental selenium (Se⁰) and improving the Se-enrichment of tea plants [7]. Nevertheless, the detailed molecular mechanism of promoting tea-plant growth and Se-enrichment by two bacterial strains has not been fully understood.

In an attempt to understand bacterial effects on promoting tea-plant growth and Se-enrichment, we sequenced the genome of *H. camelliae* WT00C and analyzed its metabolic profiles. In this paper, we reported those metabolic pathways involving probably in promoting tea-plant growth and Se-enrichment, and explained why *H. camelliae* could act as a bioaccelerator stimulating tea-plant growth and improving Se-enrichment in tea plants. Our study not only helps us to deeply understand the biological functions and properties of *H. camelliae* as a novel bioaccelerator at the molecular level, but also benefits us to further exploit new purposes of two strains in agriculture or other aspects.

2. Materials and methods

2.1 Bacterial strain

H. camelliae WT00C, deposited in CCTCC (AB 2018017^T) and KCTC (62527^T), was routinely grown in the nutrient broth (NB) containing 0.5% peptone, 0.3% yeast extract and 0.5% NaCl (pH 6.8) at 28 °C, 200 rpm. This strain was also able to grow at 37 °C, 200 rpm in LB medium (1% tryptone, 0.5% yeast extract, and 0.5% NaCl, pH 7.2). The detailed process was described by Wang et al. [1] and Liu et al. [2].

2.2 Preparation of genomic DNA

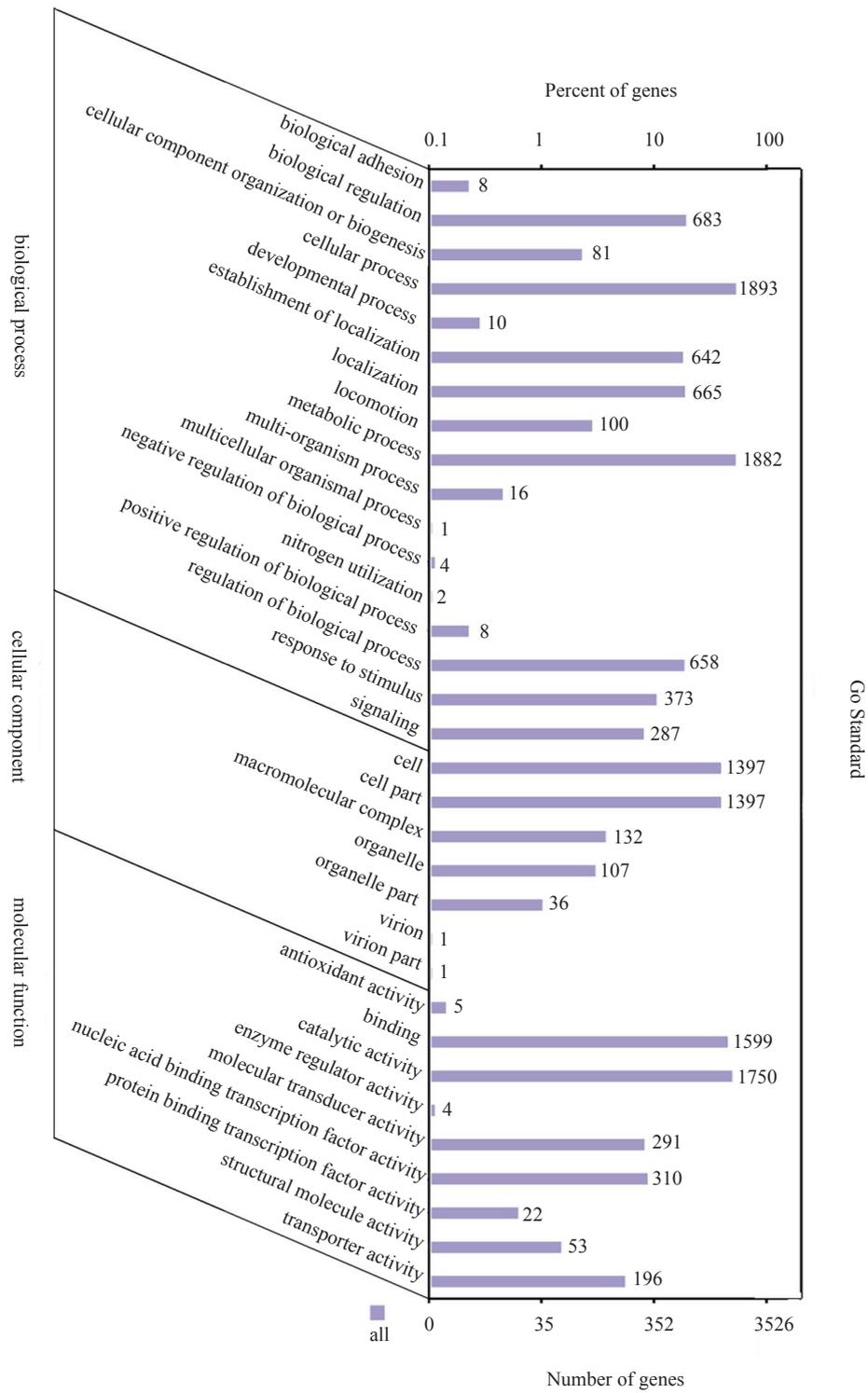
To prepare genomic DNA, *H. camelliae* WT00C was activated by inoculating into 5 ml NB broth and growing at 28 °C overnight. Then, 1 ml of the bacterial culture was put into 100 ml of fresh LB broth (10 g/L tryptone, 5 g/L yeast extract and 10 g/L NaCl) and incubated at 37 °C until OD₆₀₀ of 0.8 recorded on a spectrophotometer (Shimadzu UV-2550). The bacterial culture was collected by centrifugation at 8000 rpm at 4 °C. DNA was isolated and purified by using the phenol-chloroform method after bacterial cells were lysed with lysozyme and SDS. The purified DNA was sent to BGI (Wuhan, China) for sequencing.

2.3 Genome sequencing and annotation

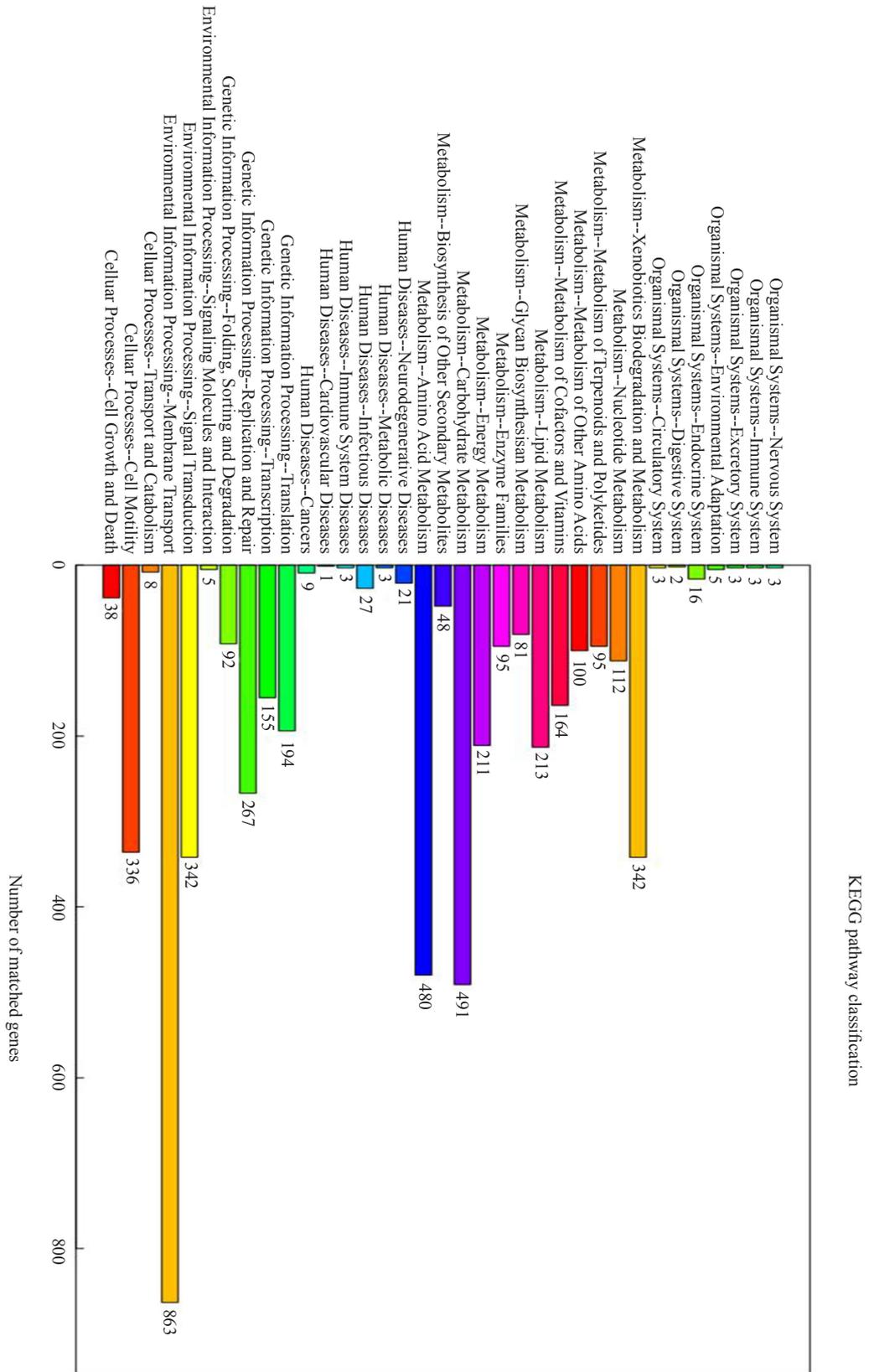
As stated by the manufacturer's instructions, the genomic DNA of *H. camelliae* WT00C was sequenced with an Illumina HiSeq 2000 instrument. The genomic DNA prepared from *H. camelliae* WT00C was used to construct two random sequencing libraries containing small fragments (500 bp) and large fragments (6 kb). In both 500 bp and 6,000 bp libraries, the mean read length was 90 bp. These reads were then filtered and assembled into contigs by using SOAPdenovo v1.05 (<http://soap.genomics.org.cn>). 4 scaffolds consisting of 11 contigs were finally constructed step by step using all the paired-end information of reads. Repeat sequences and gene islands were predicted via Tandem repeat finder (TRF) [8], IslandPath-DIOMB and SIGI-HMM software [9]. Open reading frames (ORFs) were identified by an integrated automatic annotation platform with Glimmer 3.0 [10] and Blast softwares [11]. The function of translation product for each ORF was inferred by using the Blast package to search the public databases NR (non-redundant) (NCBI), UniProtKB/Swiss-Port (EMBL-EBI), Inter Pro [12], Gene Ontology (GO) [13] and Kyoto Encyclopedia of Genes and Genomes (KEGG) [14]. The genomic data of the strain WT00C was finally deposited in the GenBank database (accession numbers: KV880769.1).

3. Results and discussion

3.1 General features for the genome of *H. camelliae* WT00C



(a)



(b)

Figure 1. Genome information of *H. camelliae* WT00C. (a) Gene ontology annotation; (b) KEGG pathway classification

The genome of *H. camelliae* WT00C, a tea-plant-specific endophyte [1, 6], was sequenced by BGI and annotated. The genome was composed of 6,079,821 base pairs with 62.36% G + C. Whole-genome held 5,537 ORFs, and the DNA regions encoding proteins covered 87.1% of the bacterial genome. 57 tRNA genes representing all 20 amino acids and 9 rRNA genes including 5 S, 16 S and 23 S were also identified. General features of the genome of *H. camelliae* WT00C were summarized in Table 1. The results obtained from the GO and KEGG annotations were also summarized in Figure 1.

Table 1. Genomic features of *H. camelliae* WT00C

Content	Number
Size (bp)	6,079,821
G + C%	62.36
Total number of gene	5,537
Average ORF size (bp)	955
Protein coding regions (%)	87.01
Number of tandem repeat	263
Total tandem repeat length (bp)	21,508
Tandem repeat length/genome (%)	0.3538
Number of minisatellite DNA	140
Number of microsatellite DNA	61
rRNA (5 S, 16 S, 23 S)	9
tRNAs	57

3.2 Auxin biosynthesis

IAA is one of the main auxin in plants. It controls many important physiological processes, such as root and root hair growth, tissue differentiation, and responses to light and gravity [15]. Bacteria promoting plant growth are probably able to produce and secrete phytohormones [16]. IAA assay showed that *H. camelliae* WT00C and WT00F produced IAA of 18.7 mg/L and 24.5 mg/L respectively in LB medium [1]. In the metabolic pathway of *H. camelliae* WT00C, IAA is synthesized via indole-3-acetaldehyde which is catalyzed by ALDH (aldehyde dehydrogenase [EC 1.2.1.3]), or indole-3-acetonitrile by nitrilase [EC 3.5.5.1], or indole-3-acetamide by amidase [EC 3.5.1.4]. In the last route, indole-3-acetamide is the product of the hydration of indole-3-acetonitrile catalyzed by nitrile hydratase. [EC 4.2.1.84]. Pedrosa et al. [17] proposed that the most probable route for IAA synthesis in *H. seropedicae* SmR1 was via indolepyruvate catalyzed by tryptophan aminotransferase [EC 2.6.1.27] and IOR (indolepyruvate ferredoxin oxidoreductase, [EC 1.2.7.8]). Nevertheless, this route was not found in the metabolic pathway of *H. camelliae* WT00C. In *H. camelliae* WT00C, IAA biosynthesis may perhaps initiate from anthranilate to indole-3-acetonitrile, tryptophan to indole-3-acetamide or indole-3-acetaldehyde by the synthesis pathway of IAA in rhizobacteria [18].

3.3 ACC deaminase and modulation of endogenous ethylene levels

Ethylene is a versatile and gaseous phytohormone. It plays a vital role in modulating plant growth and development. Ethylene is also a stress hormone helping plants to survive under various biotic and abiotic stresses. Ethylene affects all stages of plant development including the growth and development of a plant's roots, stems, leaves, flowers and fruits [19]. When the stress occurs, ethylene level in plants are typically increased to respond positively to the presence of metals, temperature extremes, organic and inorganic chemicals, drought, inundation, ultraviolet light, insect or nematode damage, mechanical wounding and phytopathogens [20]. The high level of ethylene in plants

obviously exacerbates the effects of the stress triggering ethylene responses. Thus, lowering the magnitude of stress and ethylene level simultaneously decreases plant damage caused by the stress [20].

In plants, ethylene is synthesized via the catalysis of ACC synthase and ACC oxidase. ACC synthase activated by IAA under biotic and abiotic stress conditions catalyses L-methionine to form S-adenosylmethionine (SAM) and then to form ACC (1-aminocyclopropane 1-carboxylate). ACC is further converted to ethylene by ACC oxidase [20, 21]. In the genome of *H. camelliae* WT00C, the gene encoding ACC deaminase [EC 3.5.99.7] was cloned. The cloned ACC deaminase gene was composed of 1082 bp, and its protein with a molecular mass of 38.5 kDa contained 355 amino acids. The assay of enzyme activity showed that the purified enzyme catalyzed ACC to form 2-oxobutanoate and ammonium. ACC deaminase was thought to modulate ethylene levels in plants, decrease the stress response caused by ethylene and allow plant growth under stress conditions [22]. Thus, ACC deaminase in bacterial cells has an obvious competitive effect against ACC oxidase in plants. As shown in Figure 2, decomposition of ACC by ACC deaminase in *H. camelliae* WT00C likely reduces the synthesis of ethylene in tea plant. IAA and ACC deaminase synthesized coordinatively in *H. camelliae* WT00C may play an important role in promoting plant growth and development, as shown in *Burkholderia phytofirmans* PsJN [23].

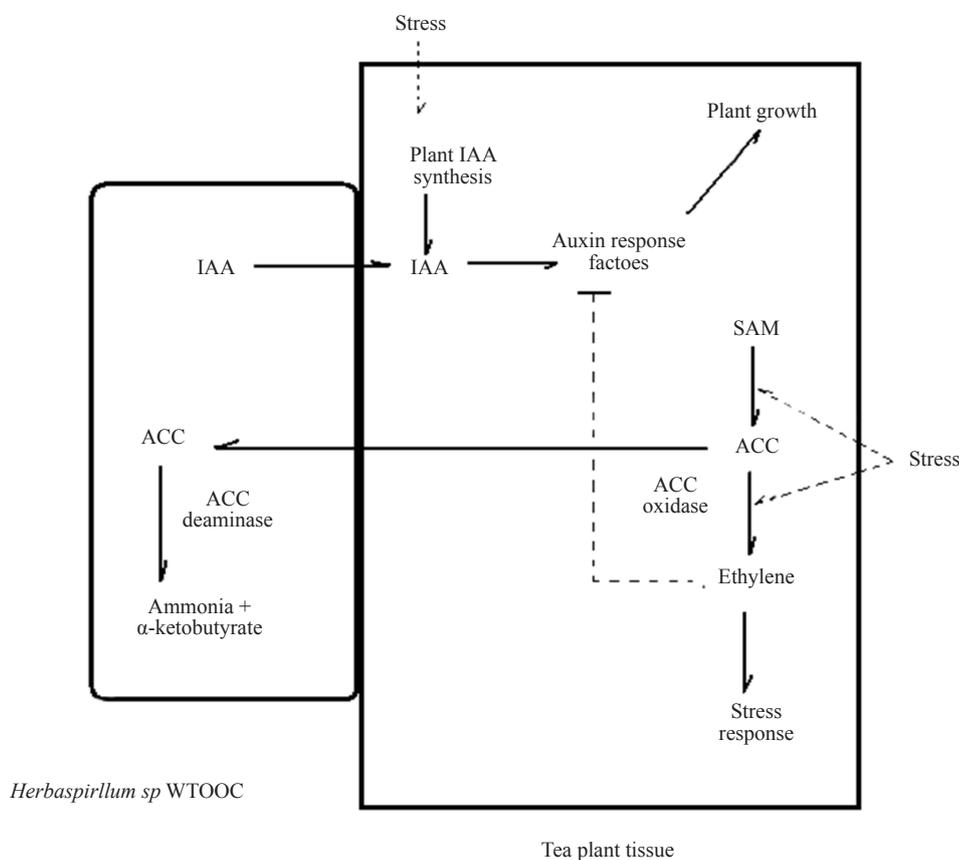


Figure 2. A schematic model for the growth promotion of ACC deaminase and IAA in *H. camelliae* WT00C. ACC: 1-aminocyclopropane-1-carboxylate; IAA: indole-3-acetic acid; SAM, S-adenosyl methionine

3.4 Siderophore production

Siderophores are small iron-chelating compounds. In bacteria, Fe^{2+} -dependent repressors can bind to the upstream of genes responsible for siderophore synthesis when intracellular iron concentrations are high. At low concentrations, Fe^{2+} dissociates from the repressor, and then the repressor, in turn, dissociates from the upstream of genes, which finally leads to transcription of those genes. The ferric uptake regulator (*Fur*) or diphtheria toxin repressor (*DtxR*) usually

regulates Fe²⁺ dissociation from the Fe²⁺-repressor complex. Meanwhile, the whole Fe-siderophore complex, in many cases, transports actively across cell membranes. In gram-negative bacteria, the Fe-siderophore complex is transported into the periplasm via TonB-dependent receptors. Thus, ferric ion in Fe-siderophore complex may be reduced to Fe²⁺ outside the cells. Siderophores are also able to chelate other metals, such as copper, zinc, lead, aluminium, cadmium, vanadium, plutonium, manganese, indium, uranium and gallium chromium [24-27].

Universal chemical assay [28] demonstrated that both *H. camelliae* WT00C and WT00F strains were capable of producing siderophore [1]. KEGG annotation revealed that *H. camelliae* WT00C might perhaps synthesize the siderophore enterochelin because three subunits VibB, EntB and DhbB of isochorismate lyase/aryl carrier protein [EC 3.3.2.1] and EntF (the component F of enterobactin synthetase [EC 2.7.7.-]) were present. In addition, the genome survey also found a very large DNA fragment (> 27 kb, similar to the Hsero_2343 of *H. seropedicae*). This large DNA fragment was located in downstream of TonB-dependent siderophore receptor *CirA* and the sigma factor PrfI encoding for two non-ribosomal peptide synthases and an amino acid adenylation protein. These enzymes might be involved in the non-ribosomal synthesis of siderophores in *H. camelliae* WT00C. The genome of *H. camelliae* WT00C also held 20 TonB-dependent siderophore receptors and a ABC-type hydroxamate-type ferric siderophore uptake system. Pedrosa et al. [17] suggested that iron uptake occurred through active transport mechanisms by means of an ABC-type system and TonB/ExbB/ExbD. In the rice-endophyte *Azoarcus*, a plethora of TonB-dependent siderophore receptors were also found [29, 30]. Those iron receptors may endow *H. camelliae* WT00C with strong competitiveness in iron-limited environments so that this bacterium can effectively compete with other bacteria. Furthermore, siderophores were also thought to improve plant growth through a direct effect on the plant or the control of noxious organisms or some other routes [31].

3.5 Nitrogen metabolism and ammonia formation

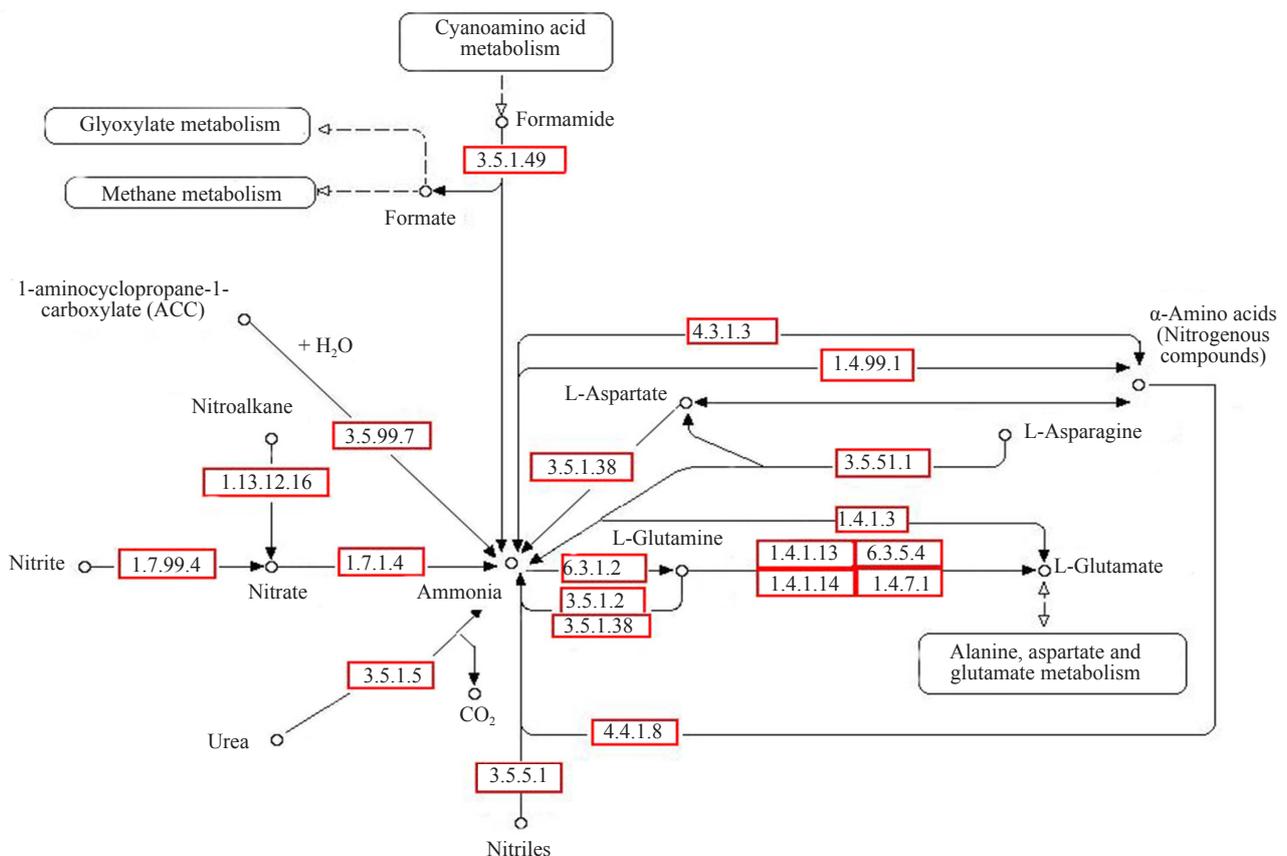


Figure 3. A schematic diagram showing nitrogen metabolic pathways of *H. camelliae* WT00C. The number in each square box represents EC number assigned by European community to each enzyme

As an important source of nitrogen for many plant species, ammonia is the dominant nutrient content for plant growth and development and is easily absorbed by plants. Interestingly, two strains of *H. camelliae* held the capability of producing ammonia under the culture conditions [1]. In *H. seropedicae*, ammonia mainly comes from atmospheric nitrogen via nitrogen fixation by nitrogenases, because it holds nitrogen-fixing genes (*nif*) such as *nifA*, *nifB*, *nifD*, *nifE*, *nifH*, *nifK*, *nifN*, *nifQ*, *nifS*, *nifU*, *nifV*, *nifW*, *nifX*, *nifZ*, and *nifZ1* [17]. In contrast, *H. camelliae* did not show any nitrogen-fixing activity. Amplification of *nifH* and *nifD* genes by polymerase chain reaction (PCR) was also failed when the specific primer pairs were used [1]. In the genome of *H. camelliae* WT00C, only two analogues of *nifA* and *nifU* genes relevant to nitrogen fixation are found. As a consequence, *H. camelliae* does not have a nitrogen-fixing capability.

In accordance with the metabolic pathways of *H. camelliae* WT00C, ammonia mainly comes from amino acid metabolism. For instance, ammonia formation occurs via L-aspartate catalyzed by glutaminase-asparaginase [EC 3.5.1.38], L-glutamin by glutaminase [EC 3.5.1.2], L-asparagine by asparaginase [EC 3.5.1.1] and L-glutamate deamination by glutamate dehydrogenase [EC 1.4.1.3] (see Figure 3). In addition, ammonia also comes from formamide catalyzed by formamidase [EC 3.5.1.49], 1-aminocyclopropane-1-carboxylate (ACC) by ACC deaminase [EC 3.5.99.7], nitrite by nitrite reductase [EC 1.7.1.4], and urea or nitriles by urease [EC 3.5.1.5] or nitrelase [EC 3.5.5.1]. As shown in Figure 3, nitrite is the production of nitrate and nitroalkane catalyzed by nitrate reductase [EC 1.7.99.4] and nitronate monooxygenase (NMO EC 1.13.12.16).

Figure 3 also showed that ammonia was used to synthesize L-glutamate by glutamate dehydrogenase (GDH EC 1.4.1.3) or L-glutamine by glutamine synthetase [EC 6.3.1.2]. L-glutamine is then catalyzed by NAD(P)H-glutamate synthase [EC 1.4.1.13/14], ferredoxin-glutamate synthase [EC 1.4.7.1] or asparagine synthase [EC 6.3.5.4] to form L-glutamate. Finally, L-glutamate enters the metabolic pathway of amino acids. Thus, ammonia is excreted from the interior to the exterior by the bacterium only when it becomes overabundant.

3.6 Urea metabolism

Urea, as a nitrogen-release fertilizer, is synthesized in the body of many organisms as part of the urea cycle. Like *H. seropedicae*, *H. camelliae* was capable of synthesizing and degrading urea. In our previous study, both strains displayed urease activities [1]. In the metabolic pathways of *H. camelliae* WT00C, there is a complete urea cycle for arginine biosynthesis. Four key enzymes ornithine carbamoyltransferase [EC 2.1.3.3], argininosuccinate synthase [EC 6.3.4.5], argininosuccinate lyase [EC 4.3.2.1] and arginase [EC 3.5.3.1] in the urea cycle are present in the bacterial genome. In the last step of the urea cycle, arginase converts L-arginine into L-ornithine and urea. Then urea is degraded into carbon dioxide and ammonia by urease [EC 3.5.1.5] or by urea carboxylase [EC 6.3.4.6] and allophanate hydrolase [EC 3.5.1.54].

Like the *H. seropedicae*, *Janthinobacterium* and *Corynebacterium glutamicum* [32], the urease operon in the genome of *H. camelliae* WT00C contains *ureA*, *ureB* and *ureC* genes and 6 accessory genes *ureD*, *ureE*, *ureF*, *ureG*, *ureH*, and *ureJ*. The operon constitution is similar to that of *H. seropedicae* SmR1 [17], except that *ureH* does not occur in the latter operon. In the genome of *H. camelliae* WT00C, ABC-type urea transport operon (*urt*ABCDE) was also found in the upstream of the *ure* gene cluster. In view of the fact that a σ^{54} -dependent promoter is located in the upstream of *urtA* and both *urt* and *ure* genes are expressed under nitrogen deprivation, the *urt* and *ure* genes are probably controlled by the nitrogen regulatory (Ntr) system [33].

3.7 Glutathione and selenocompound metabolisms

In plants, animals, fungi and some bacteria, glutathione (GSH) is an important antioxidant, which prevents damage to cellular components caused by reactive oxygen species including free radicals, heavy metals, peroxides and lipid peroxides [34]. Glutathione is also crucial for biotic and abiotic stress management in plants. In the glutathione-ascorbate cycle, glutathione is a pivotal component for the precursor of phytochelatin and glutathione oligomers chelating heavy metals as well as reducing poisonous hydrogen peroxide [35, 36]. Glutathione is required to defend efficiently against plant pathogens [37]. 5'-Adenylylsulfate (APS) reductase in the sulfur assimilation pathway uses glutathione as electron donor, and glutaredoxin also uses glutathione as substrate. These oxidoreductases participate in flower development and plant defense signaling [38].

The genome of *H. camelliae* WT00C held a complete pathway for glutathione biosynthesis. As shown in Figure

also important for the function of thyroid gland. An increase of dietary selenium intakes reduces the effects of mercury toxicity [17]. Selenium deficiency occurs in a number of severe or chronic diseases (e.g. cancer, diabetes, HIV/AIDS and tuberculosis) [40-44]. Selenium-rich tea was thought to be a good choice for selenium-deficient patients as dietary supply. The conversion of selenate into non-toxic elemental selenium or incorporation of selenium into bacterial proteins to form selenoproteins in *H. camelliae* WT00C facilitated selenium accumulation in tea when the bacterium colonized in tea plant [7]. Red elemental selenium (Se^0) purified from *H. camelliae* WT00C cells improved immunoactivities of crucian carp and zebrafish against spring viraemia of carp virus [45].

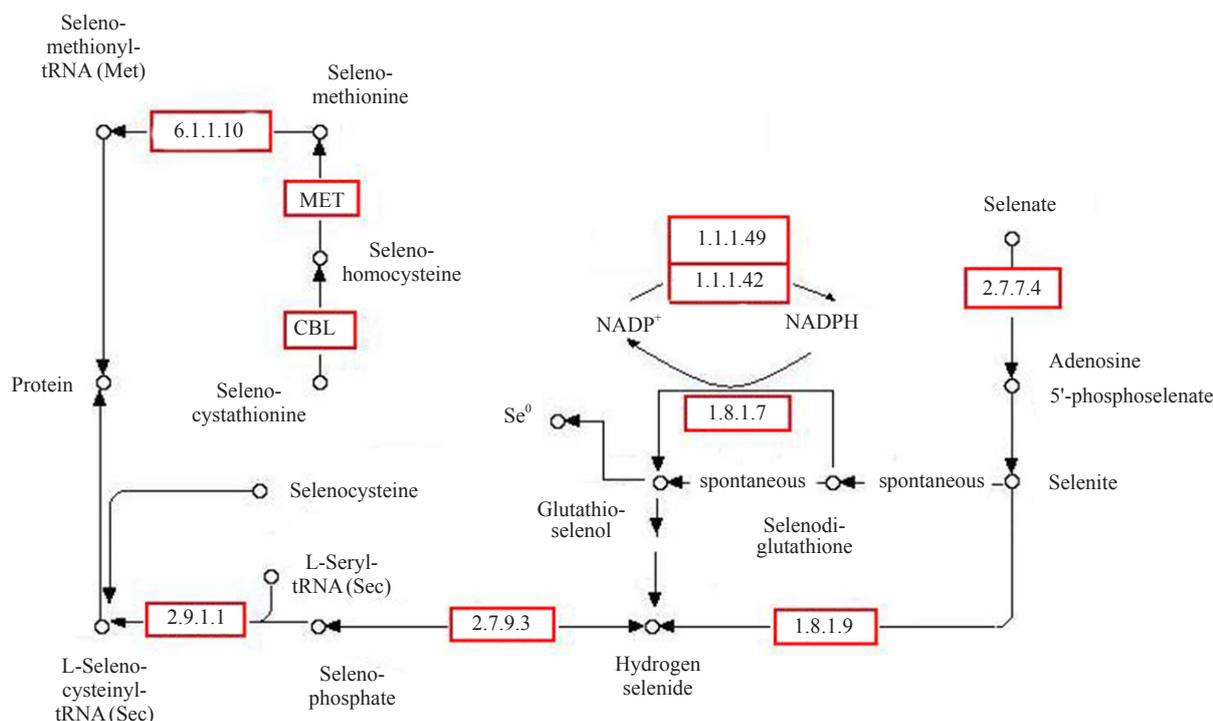


Figure 5. A diagram showing selenocompound metabolism in *H. camelliae* WT00C. The number in each square box represents EC number. MET: methionine synthase; CBL: cystathionine beta lyase

3.8 Bacterial secretion systems and protein secretion

Eight types of secretion systems (T1SS, T2SS, T3SS, T4SS, T5SS, T6SS, T7SS and T8SS) have been reported in prokaryotic bacteria, in which only T7SS occurs in Gram-positive bacteria [46-49]. T2SS, T5SS, T7SS and T8SS use a two-step process to perform secretion, which includes a stopover in the periplasm with the help of Sec or Tat export machinery. In contrast, T1SS, T3SS, T4SS and T6SS use a one-step mechanism to deliver exoproteins. T1SS, T2SS and T5SS deliver proteins into the extracellular medium, and T3SS, T4SS and T6SS deliver proteins into host cells [47, 50]. In pathogenic bacteria, T3SS, T4SS and T6SS are implicated in the delivery of toxic effector proteins into the cytoplasm of eukaryotic cells [17]. The genomic survey revealed that *H. camelliae* WT00C held T1SS (ABC transporters), T3SS, T4SS, T4SS pili and T6SS. The detailed gene information for each secretion system was shown in Figure 6. In addition, Sec-related genes *ftsY*, *gidC*, *ffh*, *secA*, *secB*, *secY*, *secE*, *yajC*, *secD*, *secF* and *secG* were also found in the bacterial genome except for *secM* (secretion monitor). Different from the Sec system, only *tatA*, *tatB* and *tatC* genes for Tat translocase systems were found in the genome of *H. camelliae* WT00C. The absence of *tatE* gene encoding a component of Tat export machinery implied that the Tat system might not function properly. Although partial T2SS gene analogues including *gspD*, *gspF*, *gspG*, *gspE*, *gspA* and *gspH* were found, these genes dispersed over different locations and did not occur as a gene cluster.

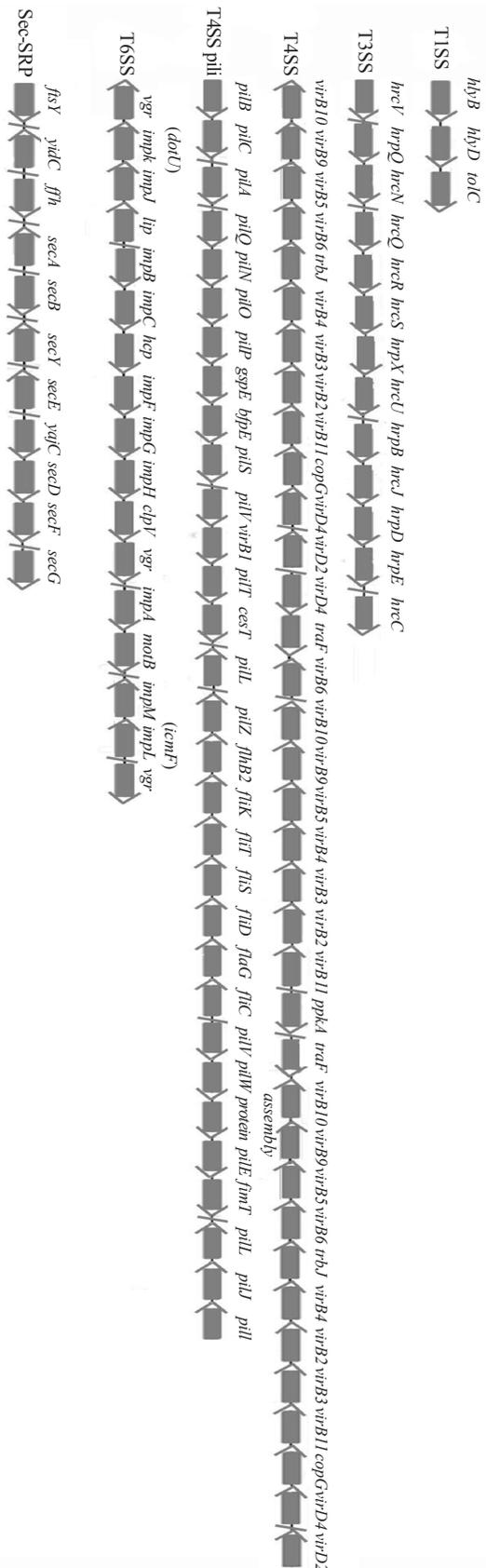


Figure 6. The gene clusters of the secretion systems in *H. camelliae* WT00C

Effector proteins secreted via T3SS were thought to control host metabolism and circumvent plant defense mechanisms [17]. T3SS might also optimize beneficial interactions between host and bacterial cells because effector proteins secreted by T3SS responded to flavonoids exuded by plant roots. The biologic effects of those effectors, either enhancing or diminishing nodulation, depended on the host legume [51-53]. Interestingly, *H. camelliae* WT00C held an intact T4SS and T4SS pili genes in its genome, whereas *H. seropedicae* only held T4SS pili without T4SS. T4SS was initially found in *Agrobacterium tumefaciens*. *A. tumefaciens* used T4SS to deliver T-DNA (a portion of Ti plasmid) into the host leguminous plant. This secretion system mediated intracellular transfer of macromolecules including DNA and proteins via a mechanism that was ancestrally related to one of bacterial conjugation machineries [54, 55]. Two effector proteins AnkA and ATS-1 were also secreted via T4SS [56]. Moreover, VirE2 (a type IV secretion substrate) was found to interact with the VirD4 (T4SS-coupling proteins) and had six functions in *A. tumefaciens* [57, 58]. T6SS was initially identified in *Pseudomonas aeruginosa* and *Vibrio cholerae* [59, 60], and then found in 1/4 proteobacterial genomes including plant, animal and human pathogens as well as environmental, soil or marine bacteria [61, 62]. Although most studies of T6SS secretion in the early stage focused mainly on its pathogenesis role in higher organisms, more recent studies suggested that T6SS played a broader physiological role in defense against simple eukaryotic predators and interactions between bacteria [63, 64]. In the *H. camelliae* WT00C genome, the T6SS gene clusters contained 17 genes, in which Hcp and VgrG proteins were thought to be the substrates secreted universally by T6SS. Structural analysis revealed a striking similarity between those proteins and the tail spike of T4 phage, and the activity of this system in function was thought to resemble phage infection [65]. Thus, T3SS, T4SS and T6SS in non-pathogenic bacteria (e.g. *H. seropedicae* SmR1 and *H. camelliae* WT00C) are mainly involved in microbe-plant recognition and interaction.

4. Concluding remarks

The genomic survey has revealed that *H. camelliae* WT00C is a metabolically versatile bacterium able to produce plant-growth modulators auxin, siderophore enterochelin, ammonia and other metabolites involving in nutrient metabolism and improvement of stress tolerance in tea plants. Although *H. camelliae* is devoid of nitrogen-fixing genes, its plant-growth-promoting capacity may mainly derive from IAA biosynthesis, urea and ammonia metabolism, ACC deamination and its modulation of endogenous ethylene levels in tea plant as well as other metabolites involving in plant stress tolerance. The formation of ammonia mainly depends on the many genes for amino acid metabolism, NO_3^- and NO_2^- assimilation. Whereas ACC deaminase may play a crucial role in modulating the production of ethylene in plants so that tea plant can thrive under biotic or abiotic stress. In addition, glutathione, siderophore, glycogen, trehalose, spermine, peroxidases may also play a role in enhancing stress tolerance or promoting the growth and development of tea plants. Different from *H. seropedicae*, *H. rubrisubalbicans* and *H. frisingense* isolated from gramineous plants, *H. camelliae* colonizes only in tea plants of *Camellia* genus, but not in gramineous and brassicaceous plants [6]. *H. camelliae* is devoid of glycohydrolases (e.g. polymethyl galacturonase [EC 3.1.1.11], β -1,3-glucanase [EC 3.2.1.4]) involved in plant cell wall degradation. This may explain why this bacterium invades tea plant only through plant vulnus other than other manners (e.g. irrigation and sprinkling) [6]. *H. camelliae* also displays an impressive variety of protein secretion systems that may benefit bacterial invasion, colonization and its endophytic life. *H. camelliae* holds a typical T4SS, which is absent in other members in the genus *Herbaspirillum*. T4SS of gram-negative bacteria usually delivers DNA or protein substrates from bacterial cells to host cells through conjugation machinery [54]. Exchange of substance between the bacterium and its host plant via T4SS may perhaps help *H. camelliae* to establish its endophytic lifestyle in tea plants. In addition, this bacterium has an intact pathway of selenocompound metabolism as shown in Figure 5. Selenate reduction and selenoprotein synthesis will benefit not only red elemental selenium and selenoprotein synthesis but also selenium enrichment in tea plants.

Acknowledgements

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