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Environmental adaptability and stress tolerance of Laribacter hongkongensis: a genome-wide analysis

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Abstract

**Background:** Laribacter hongkongensis is associated with community-acquired gastroenteritis and traveler’s diarrhea and it can reside in human, fish, frogs and water. In this study, we performed an in-depth annotation of the genes in its genome related to adaptation to the various environmental niches.

**Results:** L. hongkongensis possessed genes for DNA repair and recombination, basal transcription, alternative σ-factors and 109 putative transcription factors, allowing DNA repair and global changes in gene expression in response to different environmental stresses. For acid stress, it possessed a urease gene cassette and two arc gene clusters. For alkaline stress, it possessed six CDSs for transporters of the monovalent cation/proton antiporter-2 and NhaC Na⁺:H⁺ antiporter families. For heavy metals acquisition and tolerance, it possessed CDSs for iron and nickel transport and efflux pumps for other metals. For temperature stress, it possessed genes related to chaperones and chaperonins, heat shock proteins and cold shock proteins. For osmotic stress, 25 CDSs were observed, mostly related to regulators for potassium ion, proline and glutamate transport. For oxidative and UV light stress, genes for oxidant-resistant dehydratase, superoxide scavenging, hydrogen peroxide scavenging, exclusion and export of redox-cycling antibiotics, redox balancing, DNA repair, reduction of disulfide bonds, limitation of iron availability and reduction of iron-sulfur clusters are present. For starvation, it possessed phosphorus and, despite being asaccharolytic, carbon starvation-related CDSs.

**Conclusions:** The L. hongkongensis genome possessed a high variety of genes for adaptation to acid, alkaline, temperature, osmotic, oxidative, UV light and starvation stresses and acquisition of and tolerance to heavy metals.

**Background**

In 2001, Laribacter hongkongensis, a novel genus and species that belongs to the Neisseriaceae family of β-subclass of the Proteobacteria, was discovered from the blood and empyema pus of a patient with underlying alcoholic cirrhosis [1]. Subsequently, it was observed that L. hongkongensis was associated with community-acquired gastroenteritis and traveler’s diarrhea in human [2-5]. L. hongkongensis was capable of living under a variety of ecological niches. In addition to humans, L. hongkongensis resides in the intestines of a variety of freshwater fish, most commonly those of the carp family, including grass carps (Ctenoharyngodon idellus), bighead carps (Aristichthys nobilis) and mud carps (Cirrhina molitorella), as well as those of frogs [4,6-9]. Moreover, it can also survive and replicate as a free living bacterium in water obtained from drinking water reservoirs [10]. To survive in these ecological niches, L. hongkongensis needs the capability of protecting DNA damages by endogenous and exogenous metabolites and regulating the expression of a variety of genes, which makes it able to adapt to different temperatures, pH and osmotic pressures, as well as oxidative and ultraviolet light stresses.

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In this article, we present an overview of the genes of the *L. hongkongensis* genome related to DNA repair and recombination and regulation of gene expression. In addition, the putative genes and mechanisms that enable *L. hongkongensis* to adapt to different temperatures, pH and osmotic pressures, as well as oxidative and ultraviolet light stresses were also presented. These genes of *L. hongkongensis* were compared to those of *Neisseria gonorrhoeae*, *Neisseria meningitidis* and *Chromobacterium violaceum*, the other three bacteria of the *Neisseriaceae* family of β-proteobacteria with complete genome sequences available [11-13]. Human is the only known reservoir and host for *N. gonorrhoeae* and *N. meningitidis*. *N. gonorrhoeae* is most commonly associated with sexually transmitted infections and *N. meningitidis* is most commonly associated with pneumococcal meningitis and bacteremia. *C. violaceum* is highly versatile and can be found abundantly in multiple ecosystems, including water and soil, in tropical and subtropical regions. It is associated with infrequent but potentially fatal infections in humans.

**Results and discussion**

**DNA repair**

Several pathways are involved in the repair of mutagenic and cytotoxic effects of DNA damage that can arise through endogenous and exogenous stress in bacteria. **Damage reversion (Direct repair)**

Reversion of the damaged base is the simplest DNA repair mechanism, which involves a single-step reaction by specific enzymes. Photoreactivation and alkylation repair are two of the most well-known damage reversion mechanisms. Photoreactivation is carried out by photolyase, which acts upon lesions induced by UV irradiation in a light-dependent reaction. A gene homologous to *phrB*, which encodes a photolyase, was found in the *L. hongkongensis* genome (Table 1). Alkylation repair is mediated by the enzymes alkyltransferases, encoded by *ogt* and *ada*, as well as iron-dependent dioxygenases, encoded by *alkA*, which remove added alkyl groups from duplex DNA. Genes encoding for all three enzymes could be found in the *L. hongkongensis* genome (Table 1). Since *L. hongkongensis* can survive in natural water environments and is therefore often exposed to sunlight, these enzymes may be important for protection against such DNA damage. This is in contrast to *N. meningitidis* which lacks *alk* and some meningococcal and gonococcal strains which lack photolyase activity, which may reflect the lack of light exposure in the neisserial habitat [14].

**Base excision repair**

*L. hongkongensis* is exposed to reactive oxygen species generated during normal cellular metabolism, as well as from oxidative bursts from its host. One of the most important protective defense mechanisms against such DNA damage is the base excision repair (BER) pathway, which recognizes a wide range of DNA lesions. This includes the most frequently encountered form of oxidative DNA damage: production of 7, 8-dihydro-8-oxo-2′-deoxyguanosine (8oxodG) which can lead to ambiguous base pairing (either A or C) during DNA replication. The BER pathway is carried out by two types of enzymes: glycosylases and AP-endonucleases. Glycosylases excise the damaged base from the sugar phosphate backbone, leaving abasic (AP) sites, and endonucleases incise the 5′ or 3′ phosphodiester from the AP site to generate a nucleotide gap. There are eight glycosylases and endonucleases in the *L. hongkongensis* genome. Among the glycosylases, the uracil DNA glycosylase (UNG) is the most well-characterized enzyme found in various bacteria and eukaryotes. It is responsible for the excision of uracil residues from DNA which can arise as a result of misincorporation of dUMP residues by DNA polymerase or due to cytosine deamination. Similar to *C. violaceum* [15], the most closely related bacterial species of the *Neisseriaceae* family with complete genome sequence available, the *L. hongkongensis* genome contains two copies of UNG (Table 1). The complete 8oxodG system (GO system) is also present, which involves MutM/FPG, MutT and MutY, which act together to protect the bacterium against the effects of 8oxodG in *E. coli* [16]. MutM or FPG is formamidopyrimidine DNA glycosylase that recognizes oxidized purines such as 8oxodG and imidazole ring-opened purines; while MutY is an atypical glycosylase which removes adenine from DNA when it is mispaired with 8oxodG, preventing GC to TA transversions [17]. In *N. meningitidis*, it has been shown that MutY has a prominent role in DNA repair, with *mutY* mutants exhibiting high spontaneous mutation rates [14].

**Nucleotide excision repair**

Nucleotide excision repair (NER) involves a group of highly conserved proteins and repairs bulky lesions caused by exogenous damage such as UV light that generate a large helical distortion [18,19]. NER is carried out by the UvrABC complex in *E. coli*, which excises a 24- to 32-bp DNA fragment that contains the damaged lesion [20]. A functional NER pathway has also been demonstrated in *N. gonorrhoeae* [21]. Similar to *N. gonorrhoeae*, *N. meningitidis* and *C. violaceum* [14,15,21], homologues of all enzymes in this pathway are present in the *L. hongkongensis* genome (Table 1).

**Mismatch repair**

The mismatch repair (MMR) system recognizes and removes single-base mismatches as well as small nucleotide insertions or deletions (forming small loops) that result from errors during replication. In *E. coli*, MMR is carried out by a number of enzymes working at a
Table 1 Single-strand breaks repair proteins in *L. hongkongensis* and their closest homologues

| Repair pathways/Types of enzymes | Gene | Protein | Function of protein encoded | CDS | Closest match organism | Amino acid Identity (%) | Best E-value |
|----------------------------------|------|---------|-----------------------------|-----|------------------------|------------------------|--------------|
| **Direct repair**                |      |         |                             |     |                        |                        |              |
|                                  | *phrB* | PhrB protein | Repairs UV radiation-induced DNA damage by catalyzing light-dependent monomerization of cyclobutyl pyrimidine dimers between adjacent bases | LHK_02646 | L. nitroferrum | 58.73 | 3.00E-131 |
|                                  | *ogt*  | Ogt     | Repairs alkylated guanine by transferring alkyl group at O-6 position to a cysteine residue in the enzyme | LHK_00364 | Dechloromonas aromatica | 46.67 | 4.00E-30  |
|                                  | *ada*  | Ada     | Repairs alkylated guanine in DNA by transferring alkyl group at the O-6 position to a cysteine residue in the enzyme | LHK_00147 | Colwellia psychrerythraea | 44.29 | 1.00E-60  |
| **Base excision repair**         |      |         |                             |     |                        |                        |              |
| DNA glycosylases                 | *alkA* | AlkA    | Excises damaged DNA polymer formed due to alkylation lesions by hydrolyzing deoxyribose N-glycosidic bond | LHK_01743 | Thiobacillus denitrificans | 61.95 | 2.00E-62  |
|                                  | *mutY* | MutY    | Adenine glycosylase active on G-A mispairs. Also corrects error-prone DNA synthesis due to oxidized guanine | LHK_02781 | L. nitroferrum | 63.29 | 1.00E-92  |
|                                  | *ung*  | UNG     | Excises uracil residues arising from misincorporation of dUMP residues by DNA polymerase or cytosine deamination | LHK_00013 | L. nitroferrum | 56.14 | 3.00E-58  |
|                                  |        |         |                             |     |                        |                        |              |
| Bifunctional glycosylases        | *mutM* | MutM    | Recognizes and removes damaged bases. Cleaves DNA backbone to generate single-strand break at site of base removal | LHK_00316 | Neisseria flavescens | 57.25 | 9.00E-90  |
|                                  | *nth*  | Endonuclease III | Apurinic and/or apyrimidinic endonuclease activity and DNA N-glycosylase activity | LHK_01218 | Methylococcus capsulatus | 72.04 | 1.00E-81  |
| AP endonucleases                 | *xthA* | Exodeoxyribo-nuclease III | Removes damaged DNA at cytosines and guanines | LHK_02447 | C. violaceum | 67.06 | 5.00E-94  |
|                                  | *exoA* | Exodeoxyribo-nuclease | Posseses 3’ to 5’ exonuclease, 3’ phosphatase activities and makes DNA single-strand breaks at apurinic sites | LHK_03213 | L. nitroferrum | 73.73 | 1.00E-108 |
| **Nucleotide excision repair**   |      |         |                             |     |                        |                        |              |
| Global genome repair factors     | *uvrA* | Protein UvrA | DNA-binding ATPase, forms recognition complex composed of 2 UvrA and 2 UvrB subunits and scans DNA for abnormalities | LHK_01605 | L. nitroferrum | 82.89 | 0         |
|                                  | *uvrB* | Protein UvrB | Causes local melting of the DNA helix, probes one DNA strand for the presence of a lesion | LHK_00960 | L. nitroferrum | 82.18 | 0         |
|                                  | *uvrC* | Protein UvrC | Incises 5’ and 3’ sides of lesion | LHK_02627 | L. nitroferrum | 71 | 0         |
| **Transcription coupled repair factors** |      |         |                             |     |                        |                        |              |
| DNA-directed RNA polymerase (RNAP) complex | *rhoB* | RNAP subunit beta | Subunit of DNA-dependent RNA polymerase | LHK_00246 | L. nitroferrum | 85.26 | 0         |
|                                  | *rhoC* | RNAP subunit beta | Subunit of DNA-dependent RNA polymerase | LHK_00247 | C. violaceum | 87.09 | 0         |
|                                  | *rhoA* | RNAP subunit alpha | Subunit of DNA-dependent RNA polymerase | LHK_00279 | L. nitroferrum | 90.83 | 1.00E-171 |
|                                  | *rhoE* | RNAP delta factor | Participates in initiation and recycling phases of transcription | LHK_01458 | L. nitroferrum | 63.82 | 7.00E-54  |
|                                  | *rhoZ* | RNAP omega subunit | Promotes RNA polymerase assembly | LHK_00457 | Methylobacillus flagellatus | 73.91 | 1.00E-21  |
Table 1 Single-strand breaks repair proteins in *L. hongkongensis* and their closest homologues (Continued)

| Transcription-repair coupling factor (TRCF) | mfd | TRCF | DNA mismatch repair proteins in *L. hongkongensis* and their closest homologues (Continued) |
|---------------------------------------------|-----|------|------------------------------------------------------------------------------------------|
| Mismatch excision repair                    |     |      | DNA mismatch repair protein MutS                                                         |
| Mismatch and loop recognition factors       |     |      | Mismatch recognition                                                                      |
| DNA exoribonucleases                        |     |      | Bidirectionally degrades single-stranded DNA                                             |
| DNA polymerase III holoenzyme               |     |      | Subunit of DNA polymerase, a 3’-5’ exonuclease posseses proofreading function              |
| Other MMR factors                           |     |      | Subunits of DNA polymerase, tau subunit serves as scaffold in dimerization of the core complex while gamma subunit interacts with delta subunit to transfer beta subunit on DNA |
| DNA adenine methylase                       |     |      | Methylates DNA sequence GATC and protects DNA from cleavage by restriction endonuclease  |
| Very short patch repair protein             |     |      | Endonuclease, nicks double-stranded DNA                                                  |

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sequential manner: MutS recognizes the mismatch; MutL is recruited and binds as a dimer; the bound MutS-MutL complex in turn recruits the MutH endonuclease; MutH nicks the nascent DNA strand, distinguishing it from the parental strand by its undermethylation of GATC sequences; MutU (also known as UvrD) and other exonucleases (such as RecJ or ExoI) mediate the removal of up to 1000 bases (upstream or downstream) of the strand that contain the lesion [22,23]. This strand is then repaired by the actions of DNA polymerase I. Similar to N. meningitidis and C. violaceum [14,15], the L. hongkongensis genome contains the most important enzymes of the MMR pathway except that mutH is absent, suggesting that this gene has been lost in related bacterial lineages (Table 1). In N. meningitidis, it has been shown that mutS mutants had a significantly increased frequency of phase variation and moderate increases in the rate of missense mutations [24]. However, other mechanisms are likely involved in determining meningoococcal mutability. Further studies are required to investigate if MutH function is not required or another protein carries out the MutH strand-specificity function in these bacteria of the Neisseriaceae family. In contrast to C. violaceum, N. meningitidis and N. gonorrhoeae which possess only one copy of the Dam protein, which is responsible for DNA methylation, the L. hongkongensis genome contains three copies of dam. These three Dam homologues are phylogenetically most closely related to the Dam of C. violaceum, with two of the three copies having identical nucleotide sequences encoded on two highly similar prophages (Figure 1). It has previously been reported that the Dam methylase from C. violaceum has high similarity to a bacteriophage Dam homologue, suggesting acquisition via a horizontal transfer event [15]. Although our analysis shows that the Dam proteins from L. hongkongensis and C. violaceum are only distantly related to homologues found in other bacteriophages, the phylogenetic clustering of enzymes from different classes of bacteria supports that this enzyme is frequently horizontally transferred between bacteria (Figure 1).

Recombinational repair

Recombinational repair is activated in response to double-strand breaks (DSBs) in DNA which can lead to broken chromosomes and cell death. Such damage is repaired by homologous recombination in a process known as double-strand break repair (DSBR); which involves initiation, strand pairing and exchange, branch migration and branch resolution. Similar to the pathogenic Neisseria species and C. violaceum [15,25,26], the L. hongkongensis genome possesses all the important genes in this pathway, including the recA gene universally found in bacteria (Table 2). RecA has an important role in pathogenic Neisseria species, being involved in repeat-associated events, including those associated with pilus antigenic variation and transformation in N. meningitidis [25]. The L. hongkongensis genome contains two pathways for repair initiation (RecBCD and RecFOR), and two pathways for branch migration and

![Figure 1 Phylogenetic tree showing the relationships of the three copies of Dam methylases from L. hongkongensis (LHK_01749, LHK_02602 and LHK_00398) to those from other bacteria. The unrooted tree was constructed by neighbor-joining method using Kimura's two-parameter correction, with bootstrap values calculated from 1000 trees. The scale bar indicates the estimated number of substitutions per 20 bases. Bacterial names and accession numbers are given as cited in the GenBank database. Phylum or class is indicated in parentheses. Genes identified in bacteriophages are highlighted in grey.](http://www.cellandbioscience.com/content/1/1/22)
| Repair pathways/Types of enzymes | Gene | Protein | Function of protein encoded | CDS | Closest match organism | Amino acid Identity (%) | Best E-value |
|---------------------------------|------|---------|----------------------------|-----|------------------------|------------------------|---------------|
| Initiation                      | recB | Exodeoxyribonuclease V beta chain | Catalyzes unwinding of double-stranded DNA and cleavage of single-stranded DNA, stimulates local genetic recombination | LHK_01202 | Pseudomonas entomophila | 45.33 | 0 |
| RecBCD pathway                  | recC | Exodeoxyribonuclease V gamma chain | ATP-dependent exonuclease and helicase, DNA-dependent ATPase and ATP-stimulated endonuclease | LHK_01203 | Pseudomonas aeruginosa | 48.11 | 0 |
| RecFOR pathway                  | recD | Exodeoxyribonuclease V alpha chain | ATP-dependent exonuclease, ATPase and ATP-stimulated endonuclease | LHK_01201 | Pseudomonas putida | 50.47 | 1.00E-126 |
| Branch migration and resolution | recF | DNA replication and repair protein RecF | DNA metabolism, DNA replication and normal SOS inducibility | LHK_01798 | Bordetella petrii | 43.67 | 4.00E-91 |
|                                 | recO | DNA repair protein RecO | Acts with RecF and RecR | LHK_01467 | L. nitroferrum | 50.41 | 6.00E-43 |
|                                 | recR | Recombination protein RecR | Acts with RecF and RecO | LHK_00965 | L. nitroferrum | 70.71 | 5.00E-79 |
|                                 | recQ | ATP-dependent DNA helicase RecQ | Helicase involved in the RecFOR recombination pathway | LHK_02771 | C. violaceum | 68.49 | 0 |
| Branch migration and resolution | recG | ATP-dependent DNA helicase RecG | Catalyzes branch migration in processing Holliday junction intermediates to mature products. Unwinds DNA with a 3’ to 5’ polarity | LHK_02776 | L. nitroferrum | 71.3 | 0 |
|                                 | ruvA | Holliday junction ATP-dependent DNA helicase RuvA | Forms complex with RuvB, RuvAB is a helicase that mediates Holliday junction migration by localized denaturation and reannealing | LHK_03111 | C. violaceum | 59.7 | 1.00E-54 |
|                                 | ruvB | Holliday junction ATP-dependent DNA helicase RuvB | Possesses weak ATPase activity, stimulated by the RuvA protein in the presence of DNA. Forms complex with RuvA | LHK_00086 | L. nitroferrum | 92.35 | 8.00E-165 |
|                                 | ruvC | Crossover junction endodeoxyribonuclease RuvC | Resolves Holliday junction intermediates in recombination, cleaves cruciform structure in supercoiled DNA | LHK_03190 | L. nitroferrum | 79.89 | 6.00E-58 |
| Other recombination repair related proteins | priA | Primosomal protein PriA | Replication restart protein, catalyzes reactivation of replication forks that have stalled at sites of DNA damage | LHK_02821 | L. nitroferrum | 58.37 | 0 |
|                                 | radA | DNA repair and recombination protein RadA | Binds and assembles on single-stranded DNA, promotes DNA strand exchange between homologous DNA molecules | LHK_02039 | L. nitroferrum | 79.42 | 0 |
|                                 | rusA | Crossover junction endodeoxyribonuclease RusA | Resolves Holliday junction intermediates made during homologous genetic recombination and DNA repair | LHK_01785 | Ralstonia eutropha | 62.04 | 3.00E-40 |
|                                 | rdgC | Recombination-associated protein RdgC | Inhibits RecA promoted DNA strand exchange, ATPase activity, and RecA-dependent LexA cleavage, a potential negative regulator of RecA | LHK_00720 | L. nitroferrum | 58.92 | 3.00E-92 |
|                                 | recX | Regulatory protein RecX | Inhibits RecA recombinase and coprotease activities | LHK_00794 | Burkholderia phymatum | 52.45 | 8E-25 |
|                                 | yqgF | Putative Holliday junction resolvase | Nuclease resolves Holliday junction intermediates | LHK_02882 | L. nitroferrum | 66.67 | 8E-46 |
|                                 | bet | Single-stranded DNA annealing protein | Mediates annealing of (partially) single-stranded regions of DNA containing regions of complementary sequence | LHK_01498 | Providencia rettgeri | 69 | 9E-74 |
|                                 | exo | Alkaline exonuclease | Single-stranded DNA exonuclease that digests double-stranded DNA ends with 5’- to 3’-polarity to generate long 3’-ssDNA ends | LHK_01497 | Klebsiella pneumoniae subsp. rhinoscleromatis | 70 | 7E-76 |
resolution (RuvABC and RecG). In addition to recombination repair, the RecBCD and RecN are also involved in recombination during transformation, and RecO, RecQ and RecJ in antigenic variation in N. gonorrhoeae [25,27]. However, it remains to be seen if these components possess similar function in related species including L. hongkongensis.

Interestingly, homologues of the Bet and Exo recombinational repair proteins from bacteriophage lambda are present within a probable 11kb defective prophage region on the L. hongkongensis chromosome. Bet is a single-stranded DNA annealing protein (SSAP, sometimes also referred to as a synaptae), and Exo is a single-stranded DNA alkaline exonuclease with 5’- to 3’- polarity [28]. The bet and exo genes are positioned immediately adjacent to one another along with an additional copy of a single-stranded DNA binding protein of phage origin (ssb2, LHK_01496), which is homologous to, but distinct from, the presumed major functioning ssb of neisserial origin (LHK_01479). Such arrangements of phage-related DNA recombination proteins are commonly found in bacteria [29], acquired presumably by phage integration followed by subsequent genetic rearrangement. If actively transcribed, functional pairs of Exo and Bet proteins will promote DNA recombination events analogous to those mediated by the RecA/RecBCD/RecFOR pathways, and would be expected to increase the rates of gene/genome rearrangements [28]. The Bet and Exo proteins may also function synergistically with RecA. The transcriptional status of the genes within this presumed defective prophage region remain to be established.

It has previously been noted that low-GC Gram positive species tend to possess RecT SSAPs rather than Bet-family proteins [29], although this relationship has not been re-examined more recently. The LHK_01498 gene is the only bet homologue present in the Neisseriaceae. However, there is a (functionally-equivalent) recT homologue present in Kingella oralis ATCC51147 (the only recT-family recombinase present in the Neisseriaceae) which does appear to have partnering exonuclease. Due to likely (partial) genetic reassortment in a phage host prior to incorporation into the L. hongkongensis genome, the bet, exo and ssb2 genes have apparently unrelated phylogenies (data not shown). The 162aa Ssb2 protein homologue is 69% identical to the presumed functional Ssb protein within the cell (175aa), but protein alignment reveals that it is lacking a stretch of ca. 25 amino acids near the C-terminus (data not shown). Interestingly, structural studies on the E. coli Ssb-DNA complex have shown that this unstructured region loops out from the Ssb tetramer [30]. This region is not involved in DNA binding, but is thought to be responsible for interacting with the DNA primase and clamp loader proteins [31]. This suggests that the Ssb and Ssb2 proteins are designed to work with quite different replication or DNA repair protein systems.

**SOS Response**

The SOS response is activated when replication is blocked by DNA damage. The pathway is responsible for activation of a variety of physiological responses, including cell cycle inhibition and various DNA repair pathways. In E. coli, the SOS response involves more than 40 genes which are induced when there is a large amount of DNA damage, allowing increased repair and restoration of replication [32]. The pathway is controlled by a dual-component system, with RecA being the activator and LexA the repressor. The RecA protein forms a complex with single-stranded DNA, which leads to cleavage of LexA repressor and expression of the SOS regulon. Although genes related to SOS response, including dinB, dinG, umu-D and dnaA, could be identified, the lexA is absent in L. hongkongensis genome, a phenomenon also observed in C. violaceum, N. meningitidis and N. gonorrhoeae [14,15,33] (Table 3). This suggests that the lexA gene is lost in the common ancestor of these bacteria during evolution. Moreover, the recA, uvrA and uvrB genes of N. gonorrhoeae are known to lack the characteristic lexA-binding site or SOS boxes, the general hallmarks of an active SOS response. In fact, it has been experimentally confirmed that a functional SOS response is absent in N. gonorrhoeae [34]. Similarly, SOS boxes cannot be identified in the homologues of SOS-inducible genes in N. meningitidis [14,35,12], suggesting that the SOS response may also be absent in related bacteria of the same family. Similar to the two Neisseria species and C. violaceum [15], SOS boxes are also absent in the SOS-related genes in L. hongkongensis. Further studies are required to determine if SOS response is constitutive or absent in this group of bacteria.

**DNA replication**

Bacterial DNA replication mechanisms are responsible for the accurate duplication of genetic material during cell division. The whole process involves the interplay of many different proteins with a variety of functions. A total of 36 coding sequences (CDSs) potentially involved in DNA replication are present in the L. hongkongensis genome, including 12 initiation factors, 11 elongation factors, 2 termination factors and 5 topoisomerases (Table 4). Since many of these proteins are essential to the bacterial cell and therefore preserved during bacterial evolution, they are often highly conserved among phylogenetically closely related bacteria.

**Replication initiation**

In L. hongkongensis, there is an oriC containing eight 9-bp repeat elements known as DnaA boxes, which are
| Repair pathways/Types of enzymes          | Gene  | Protein    | Function of protein encoded                                                                 | CDS       | Closest match organism            | Amino acid identity (%) | Best E-value |
|------------------------------------------|-------|------------|---------------------------------------------------------------------------------------------|-----------|-----------------------------------|-------------------------|--------------|
| TLS (translesion DNA synthesis factors)  |       |            | Poorly processive, error-prone DNA polymerase involves in translesional DNA synthesis       | LHK_01833 | *L. nitroferrum*                  | 69.32                   | 2.00E-128    |
|                                         |       |            | Essential for induced (or SOS) mutagenesis, modifies DNA replication machinery to allow bypass synthesis across a damaged template | LHK_01580 | *Legionella pneumophila* subsp. *pneumophila*        | 48.65                   | 9.00E-32    |
| Y-family DNA polymerases                 |       |            |                                              |           |                                   |                         |              |
|                                         | dinB  | DNA Polymerase IV | Poorly processive, error-prone DNA polymerase involves in translesional DNA synthesis       | LHK_01833 | *L. nitroferrum*                  | 69.32                   | 2.00E-128    |
|                                         | umuD  | Protein UmuD | Essential for induced (or SOS) mutagenesis, modifies DNA replication machinery to allow bypass synthesis across a damaged template | LHK_01580 | *Legionella pneumophila* subsp. *pneumophila*        | 48.65                   | 9.00E-32    |
|                                         |       |            |                                              |           |                                   |                         |              |
| Other SOS response factors               |       |            |                                              |           |                                   |                         |              |
|                                         | dinG  | Probable ATP-dependent helicase DinG         | Damage-inducible helicase, unwinds DNA duplex with a 5'-3'-polarity                       | LHK_02134 | *L. nitroferrum*                  | 64.79                   | 0            |
|                                         | dnaA  | Chromosomal replication initiator protein DnaA | Initiates and regulates chromosomal replication                                           | LHK_03240 | *L. nitroferrum*                  | 76.72                   | 0            |
| Modulation of nucleotide pools          | dut   | dUTPase   | Produces dUMP, immediate precursor of thymidine nucleotides and decreases intracellular concentration of dUTP | LHK_01910 | *L. nitroferrum*                  | 78.45                   | 1.00E-46    |
|                                         | rnaA  | Ribonucleoside-diphosphate reductase 1 subunit alpha | Catalyzes biosynthesis of deoxyribo-nucleotides from the corresponding ribonucleotides | LHK_01803 | *L. nitroferrum*                  | 71                      | 0            |
|                                         | rnaB  | Ribonucleoside-diphosphate reductase 1 subunit beta | Catalyzes biosynthesis of deoxyribo-nucleotides from the corresponding ribonucleotides | LHK_01801 | *C. violaceum*                    | 83.1                    | 5.00E-177    |
|                                         | rnaE  | Ribonucleoside-diphosphate reductase 2 subunit alpha | Catalyzes biosynthesis of deoxyribo-nucleotides from the corresponding ribonucleotides | LHK_01596 | *L. nitroferrum*                  | 79.73                   | 0            |
|                                         | mutT  | Mutator MutT protein | Removes oxidatively damaged guanine from DNA and the nucleotide pool, degrades 8-oxo-dGTP to monophosphate | LHK_02262 | *C. violaceum*                    | 60.12                   | 2.00E-56    |
| Other factors involved in DNA repair    |       |            |                                              |           |                                   |                         |              |
|                                         | ligA  | DNA ligase | Catalyzes phosphodiester linkages between 5'-phosphoryl and 3'-hydroxyl groups in double-stranded DNA, essential for DNA replication and repair | LHK_02877 | *Cupriavidus taiwanensis*          | 65.83                   | 0            |
|                                         | recJ  | Single-stranded-DNA-specific exonuclease RecJ | Single-stranded-DNA-specific exonuclease required for many recombinational events            | LHK_02397 | *L. nitroferrum*                  | 71.58                   | 0            |
|                                         | polA  | DNA polymerase I | DNA polymerase exhibits 3’ to 5’ and 5’ to 3’ exonuclease activity                      | LHK_02983 | *C. violaceum*                    | 68.03                   | 0            |
|                                         | ssb   | Single-stranded DNA binding protein, SSB    | Forms homotetramer and binds single-stranded DNA to protect susceptible ssDNA from nucleolytic digestion and prevents secondary-structure formation | LHK_01479 | *M. flagellatus*                  | 82.24                   | 4.00E-46    |
|                                         | ssb2  | Single-stranded DNA binding protein         | Forms a homotetramer and binds single-stranded DNA to protect susceptible ssDNA from nucleolytic digestion and prevents secondary-structure formation | LHK_01496 | *L. nitroferrum*                  | 72.86                   | 0            |
|                                         |       |            |                                              |           |                                   |                         |              |
|                                         | recA  | Protein RecA | Catalyzes ATP-dependent uptake of single-stranded DNA by duplex DNA, and hybridization of homologous single-stranded DNA | LHK_00793 | *L. nitroferrum*                  | 86.75                   | 4.00E-137    |
|                                         | recN  | DNA repair protein RecN | Coordinates alignment of broken segments with intact duplexes to facilitate recombination | LHK_01210 | *C. violaceum*                    | 82.43                   | 5.00E-159    |
|                                         | uvrD  | DNA helicase II | ATPase and helicase involves in post-incision events of nucleotide excision repair and methyl-directed mismatch repair | LHK_00065 | *C. violaceum*                    | 65.68                   | 0            |
|                                         | rep   | ATP-dependent DNA helicase Rep | Helicase and ATPase involves in DNA replication, binds to single-stranded DNA, initiates unwinding at a nick | LHK_00318 | *L. nitroferrum*                  | 72.86                   | 0            |
| Types of enzymes | Gene | Protein | Function of protein encoded | CDS | Closest match organism | Amino acid identity (%) | Best E-value |
|-----------------|------|---------|----------------------------|-----|------------------------|-------------------------|--------------|
| Initiation factors | hupB1 | DNA-binding protein hu-beta | Beta chain of heterodimeric histone-like DNA-binding protein, wraps DNA to stabilize and prevent denaturation under extreme environmental conditions | LHK_02545 | C. violaceum | 79.78 | 2.00E-33 |
| | hupB2 | DNA-binding protein hu-beta | Beta chain of heterodimeric histone-like DNA-binding protein, wraps DNA to stabilize and prevent denaturation under extreme environmental conditions | LHK_02180 | C. violaceum | 46.59 | 1.00E-14 |
| | iihA/ himA | Integration host factor subunit alpha | One of the two subunits of integration host factor, a specific DNA-binding protein | LHK_02751 | L. nitroferrum | 91.84 | 8.00E-46 |
| | iihB/ himD | Integration host factor subunit beta | One of the two subunits of integration host factor, a specific DNA-binding protein | LHK_00870 | L. nitroferrum | 82.35 | 2.00E-39 |
| | dnaA | Chromosomal replication initiator protein DnaA | Initiates and regulates chromosomal replication | LHK_03240 | L. nitroferrum | 76.72 | 0 |
| | dnaB | Replicative DNA helicase | Initiation and elongation, DNA-dependent ATPase | LHK_01738 | C. violaceum | 76.48 | 0 |
| | dnaG | DNA primase | Polymerase synthesizes small RNA primers for the Okazaki fragments on both template strands at replication forks | LHK_01504 | N. gonorrhoeae | 40.73 | 3.00E-76 |
| | ssb | Single-stranded DNA binding protein, Ssb | Forms homotetramer and binds single-stranded DNA to protect susceptible ssDNA from nucleolytic digestion and prevents secondary-structure formation | LHK_01479 | M. flagellatus | 82.24 | 4.00E-46 |
| | ssb2 | Single-stranded DNA binding protein | Forms a homotetramer and binds single-stranded DNA to protect susceptible ssDNA from nucleolytic digestion and prevents secondary-structure formation | LHK_01496 | L. hongkongensis | 68 | 3E-57 |
| | fis | DNA-binding protein Fis | Nucleoid-associated protein | LHK_03207 | C. violaceum | 73.68 | 1.00E-25 |
| | hvrA | H-NS like protein | Binds tightly to dsDNA, increases thermal stability and inhibits transcription | LHK_00853 | C. violaceum | 58.82 | 4.00E-28 |
| | icIA | Chromosome initiation inhibitor | In vitro inhibitor of chromosomal replication initiation | LHK_00797 | Acinetobacter baumannii | 43.24 | 1.00E-63 |
| Elongation factors | dnaE | DNA polymerase III subunit alpha | Subunit of DNA polymerase | LHK_01389 | L. nitroferrum | 74.13 | 0 |
| | dnaN | DNA polymerase III subunit beta | Subunit of DNA polymerase, initiates replication | LHK_03241 | L. nitroferrum | 72.5 | 3.00E-131 |
| | holC | DNA polymerase III subunit chi | Subunit of DNA polymerase | LHK_01415 | C. violaceum | 50 | 2.00E-27 |
| | holA | DNA polymerase III subunit delta | Subunit of DNA polymerase, interacts with gamma subunit to transfer beta subunit on DNA | LHK_00117 | C. violaceum | 67.28 | 7.00E-79 |
| | holB | DNA polymerase III subunit delta’ | Subunit of DNA polymerase | LHK_02696 | L. nitroferrum | 57.36 | 3.00E-75 |
| | dnaQ | DNA polymerase III subunit epsilon | Subunit of DNA polymerase, a 3'-5' exonuclease possesses proofreading function | LHK_00881 | C. violaceum | 71.74 | 6.00E-85 |
| | | | | LHK_01009 | C. violaceum | 62.7 | 4.00E-60 |
| | | | | LHK_02526 | C. violaceum | 51.52 | 3.00E-105 |
| Protein | Description | Homolog | Accession | Identity | E-value |
|---------|-------------|---------|-----------|----------|---------|
| dnaX    | DNA polymerase III subunits gamma and tau | Subunits of DNA polymerase, tau subunit serves as scaffold in dimerization of the core complex while gamma subunit interacts with delta subunit to transfer beta subunit on DNA | LHK_00963 | C. violaceum | 82.17 | 2.00E-154 |
| mphA    | Ribonuclease HI | Endonuclease degrades RNA of RNA-DNA hybrids, specifies the origin of replication by suppressing initiation at origins other than the oriC locus, removes RNA primers from the Okazaki fragments of ligation strands | LHK_00880 | L. nitroferrum | 77.3 | 2.00E-59 |
| mphB    | Ribonuclease HI | Endonuclease degrades RNA of RNA-DNA hybrids | LHK_00722 | L. nitroferrum | 71.88 | 1.00E-68 |
| polA    | DNA polymerase I | DNA polymerase exhibits 3' to 5' and 5' to 3' exonuclease activity | LHK_02983 | C. violaceum | 68.03 | 0 |
| ligA    | DNA ligase | Catalyzes phosphodiester linkages between 5'-phosphoryl and 3'-hydroxyl groups in double-stranded DNA, essential for DNA replication and repair | LHK_02877 | C. taiwanensis | 65.83 | 0 |
| dam     | DNA adenine methylase | Methyllates DNA within the sequence GATC and protects the DNA from cleavage by restriction endonuclease | LHK_01749 | C. violaceum | 83.92 | 8.00E-131 |
| hda     | DnaA-homolog protein hda | Mediates interactions of DnaA with beta subunit sliding clamp, controls initiation of DNA replication by inhibiting reinitiation of replication | LHK_00510 | C. violaceum | 66.82 | 1.00E-77 |
| gyrA    | DNA gyrase subunit A | Negatively supercoils closed circular double-stranded DNA, catalyzes interconversion of topological isomers of double-stranded DNA rings, including catenanes and knotted rings. Consists of subunit A and B. Responsible for DNA breakage and rejoining, forms A2B2 tetramer | LHK_01836 | L. nitroferrum | 82.09 | 0 |
| gyrB    | DNA gyrase subunit B | Negatively supercoils closed circular double-stranded DNA, catalyzes interconversion of topological isomers of double-stranded DNA rings, including catenanes and knotted rings. Consists of subunit A and B. Catalyzes ATP hydrolysis, forms A2B2 tetramer | LHK_03242 | L. nitroferrum | 83.06 | 0 |
| parC    | DNA topoisomerase 4 subunit A | Essential for chromosome segregation, relaxation of supercoiled DNA. Performs decatenation during replication of circular DNA molecule. Composed of subunits ParC and ParE | LHK_00093 | L. nitroferrum | 74.47 | 0 |
| parE    | DNA topoisomerase 4 subunit B | Essential for chromosome segregation, relaxation of supercoiled DNA. Performs decatenation during replication of circular DNA molecule. Composed of subunits ParC and ParE | LHK_00606 | L. nitroferrum | 82.01 | 0 |
| topoA   | DNA topoisomerase 1 | Conversion of one DNA topological isomer to another | LHK_03143 | C. violaceum | 80.13 | 0 |
potential binding sites for the initiator protein DnaA. The DnaA in *L. hongkongensis* is highly conserved when compared to those in closely related bacteria, with 76.7% amino acid identity with the homologue from *Lutielia nitroferrum*. The four domains of DnaA previously identified to possess distinct functions are also present [36]. As DnaA assembles with oriC to form a large nucleoprotein complex, the DNA melts to generate single DNA strands necessary for the binding of a helicase, DnaB, and the replisomal machinery [36]. Although bacteria do not possess histones, their genomes are arranged in tightly compacted arrangements known as nucleoids, which are important for maintaining an optimal DNA topology for replication initiation.

Six nucleoid-associated proteins, also referred to as histone-like factors, were identified in the *L. hongkongensis* genome, including two HU-beta proteins, one HN-S protein, two integration host factors (IHF) and one factor for inversion stimulation (Fis). HU-beta and HN-S proteins bind DNA non-specifically and contribute to the global condensation of bacterial chromosomes [37]. IHF and Fis recognize specific DNA sequences and assist in organizing supercoiled domains [36,38]. Earlier studies have shown that IHF stimulates DnaA-mediated unwinding of oriC, whereas Fis inhibits DUE melting [39,40].

**Replication regulation**

The *L. hongkongensis* genome contains three copies of *dam* and one copy of the *hda* gene which are likely involved in the regulation of the replication process. Dam is an adenine methyltransferase responsible for the methylation of GATC sites of the oriC in *E. coli* which is important for origin sequestration, thus preventing re-initiation. Hda, a homologue of DnaA, is involved in the regulatory inactivation of DnaA (RIDA), which directly stimulates ATP hydrolysis by DnaA after the initiator melts the DUE [36]. It has been shown that *hda*-deficient cells display an over-initiation phenotype in *E. coli* [41,42].

**Control of gene expression**

As for other bacteria, the principal mechanism for control of gene expression is through regulation of the amount of mRNA produced from the corresponding gene. This is primarily determined by the affinity of RNA polymerase for the promoter. In *L. hongkongensis*, this is exemplified by the difference in mRNA levels of argB-20 and argB-37 at different temperatures, resulting in different amounts of the two enzymes, N-acetyl-L-glutamate kinase (NAGK)-20 and NAGK-37 respectively [43]. Genes that encode proteins which control basal transcription, including the five-subunit RNA polymerase core enzyme (αββ′ω) and σ-factors for binding specifically to different classes of promoters and hence selective expression of different groups of genes, are present in the *L. hongkongensis* genome. The primary σ-factor, σ^70_, is responsible for recognizing the promoters for transcription of most of the housekeeping genes. Furthermore, the *L. hongkongensis* genome contains other alternative σ-factors, including σ^28_ (FliA), σ^32_ (RpoH), σ^30_ (RpoS), σ^24_ (RpoE) and σ^34_ (RpoN), which allow it to bring about global changes in gene expression in response to different environmental stresses (Table 5). The types of alternative σ-factors in *L. hongkongensis* are the same as those in *C. violaceum*, except that there are two copies of σ^28_ (the flagellar σ-factor) in the *C. violaceum* genome but only one copy of σ^28_ in the *L. hongkongensis* genome. In the genomes of *N. gonorrhoeae* and *N. meningitidis*, no σ^28_ and σ^38_ (the starvation/stationary phase σ-factor) are observed. In addition to RNA polymerase and the σ-factors, the *L. hongkongensis* genome also encodes transcriptional activators and repressors, which belong to a variety of families of transcription factors. These transcription factors bind to sites near the target promoter and stimulate or repress the activity of the corresponding σ-RNA polymerase holoenzyme. In the *L. hongkongensis* genome, 109 coding sequences (CDSs) that encode putative transcription factors were identified (Table 6). Among the 46 families of bacterial transcription factors, *L. hongkongensis* contains genes that encode putative transcription factors in 22 of them. The largest groups belong to the LysR families. In most of the families, the number of genes in the *L. hongkongensis* genome that encode putative transcription factors in that family is in between that of *C. violaceum* and the *Neisseria* species (Table 7). This is in line with the ability of *C. violaceum* to survive in a wide range of environments and the fastidious growth requirements and limited host range of *N. gonorrhoeae* and *N. meningitidis*. One of the exceptions is that *L. hongkongensis* possesses three CDSs that encode putative transcription factors of the cold shock family, more than those in the genomes of *C. violaceum*, *N. gonorrhoeae* and *N. meningitidis*. This may be related to the adaptability of *L. hongkongensis* to environments of low temperatures, such as those of freshwater fish and frogs.

**Tolerance to acid stress**

*L. hongkongensis* is able to grow at pH of as low as 2 (unpublished data), and its tolerance to acid stress is much higher than that of *N. gonorrhoeae*, *N. meningitidis* and *C. violaceum*. This is in line with the recovery of *L. hongkongensis* from stool samples of patients with gastroenteritis, as it has to pass through the highly acidic environment of the stomach before reaching the intestine. Therefore, it is not surprising that *L. hongkongensis* possesses abundant mechanisms for tolerating
acid stress compared to *N. gonorrhoeae*, *N. meningitidis* and *C. violaceum*. The genome of *L. hongkongensis* contains a complete urease gene cassette and two arc gene clusters. The urease cassette contains eight CDSs encoding three structural (UreA, UreB and UreC) and five accessory proteins (UreE, UreF, UreG, UreD and UreI), whereas each arc cluster consists of four CDSs encoding the three enzymes, arginine deiminase, ornithine carbamoyltransferase and carbamate kinase, of the arginine deiminase pathway, and a membrane bound arginine-ornithine antiporter. Urease hydrolyzes urea into carbon dioxide and ammonia, whereas the arginine deiminase pathway converts L-arginine to carbon dioxide, ATP, and ammonia. The ammonia generated from both pathways raises the pH and counteracts the acid stress. A similar urease gene cassette is not present in the genomes of *N. gonorrhoeae*, *N. meningitidis* and *C. violaceum*, whereas one arc gene cluster is present in the *C. violaceum* genome, but not in that of *N. gonorrhoeae* or *N. meningitidis*.

In addition to the urease cassette and arc clusters, the *L. hongkongensis* genome also contains three CDSs that encode putative chaperones of which their transcription can also potentially be induced by acid shock. These include dnaK, mopA1 and htpG. Furthermore, other gene products may help the bacterium to survive in acidic environment or their expression can be induced by acid stress (Table 8). The functions of some of these products are listed below:

### Table 5 CDSs related to transcription in *L. hongkongensis, N. meningitidis, N. gonorrhoeae* and *C. violaceum*

| Product | Gene | *L. hongkongensis* | *N. meningitidis* | *N. gonorrhoeae* | *C. violaceum* |
|---------|------|-------------------|------------------|----------------|---------------|
| ATP-dependent helicase | hrpA | + | + | + | + |
| ATP-dependent RNA helicase | hIE | + | + | + | + |
| DNA-directed RNA polymerase (alpha subunit) | rpoA | + | + | + | + |
| DNA-directed RNA polymerase (beta subunit) | rpoB | + | + | + | + |
| DNA-directed RNA polymerase (beta subunit) | rpoC | + | + | + | + |
| DNA-directed RNA polymerase (omega subunit) | rpoZ | + | + | + | + |
| RNA helicase | dbpA | - | - | - | + |
| Sigma factor 32 | rpoH | + | + | + | + |
| Sigma factor 38 | rpoS | + | + | + | + |
| Sigma factor A (sigma 70) | rpoD | + | + | + | + |
| Sigma factor E (sigma 24) | rpoE | + | + | + | + |
| Sigma factor for flagellar operon | fla | + | - | - | 4* |
| Sigma factor N (sigma 54) | rpoN | + | + | + | + |
| Transcription elongation factor GreA | greA | + | + | + | + |
| Transcription elongation factor GreB | greB | + | + | + | + |
| Transcription termination factor Rho | rho | + | + | + | + |
| N utilization substance protein A | nusA | + | + | + | + |
| N utilization substance protein B | nusB | + | + | + | + |
| Transcription anti-termination protein NusG | nusG | + | + | + | + |

*Two copies of the gene are present.*

### Table 6 Families of transcription factors (TFs) in *L. hongkongensis*

| Family | Number of TFs | Family | Number of TFs |
|--------|---------------|--------|---------------|
| LysR | 25 | PadR | 0 |
| AraC/XylS | 9 | RpiR | 0 |
| OmpR | 9 | ArgR | 0 |
| NtrC/Fis | 8 | DnrB | 0 |
| TetR | 8 | LexA | 0 |
| Cro/Ci/Xre | 8 | TrmB | 0 |
| LuxR | 7 | PenR/Blal/MecI | 0 |
| GntR | 6 | SfsA | 0 |
| ArsR | 4 | CopG/RepA | 0 |
| MarR | 4 | ModE | 0 |
| Cold shock domain | 3 | Pab | 0 |
| MerR | 3 | CtsR | 0 |
| AsnC | 2 | CodY | 0 |
| CRP-FNR | 2 | TrpR | 0 |
| DecR | 2 | MtrR | 0 |
| Fur | 2 | ROS/MUCR | 0 |
| BoA/YrbA | 2 | MetJ | 0 |
| IclR | 1 | GutM | 0 |
| Rtf2 | 1 | Crl | 0 |
| LytTR | 1 | ComK | 0 |
| HrcA | 1 | FliD | 0 |
| SinB | 1 | Rcr | 0 |
| Lac | 0 | NifT/Fxu | 0 |

The genome of *L. hongkongensis* contains a complete urease gene cassette and two arc gene clusters. The urease cassette contains eight CDSs encoding three structural (UreA, UreB and UreC) and five accessory proteins (UreE, UreF, UreG, UreD and UreI), whereas each arc cluster consists of four CDSs encoding the three enzymes, arginine deiminase, ornithine carbamoyltransferase and carbamate kinase, of the arginine deiminase pathway, and a membrane bound arginine-ornithine antiporter. Urease hydrolyzes urea into carbon dioxide and ammonia, whereas the arginine deiminase pathway converts L-arginine to carbon dioxide, ATP, and ammonia. The ammonia generated from both pathways raises the pH and counteracts the acid stress. A similar urease gene cassette is not present in the genomes of *N. gonorrhoeae*, *N. meningitidis* and *C. violaceum*, whereas one arc gene cluster is present in the *C. violaceum* genome, but not in that of *N. gonorrhoeae* or *N. meningitidis*.
gene products are unknown, but the survival of the respective bacteria at low pH had been shown to be affected if the corresponding gene was deleted [44-49].

Tolerance to alkaline stress

*L. hongkongensis* is able to grow at pH as high as 9.0 (unpublished data). While this may be related to its ability to survive the alkaline pH in the host intestine, growth at such alkalinity is still in line with many other non-extremophilic bacteria. Adaptive mechanisms to achieve cytoplasmic pH homeostasis in bacteria include transporters and enzymes that promote proton capture and retention, production of acidic metabolites and cell surface changes [50]. Among all these mechanisms, the most widely studied ones involve the transporters.

In the genome of *L. hongkongensis*, there are four CDSs coding for putative transporters which belong to the monovalent cation/proton antiporter-2 (CPA2) family. Two of the CDSs code for putative Na⁺/H⁺ exchangers (LHK_02296, LHK_00707) while the other two code for the putative genes *kef* (LHK_02848) and *kefB* (LHK_02018). No CDS encoding putative homologues of monovalent cation/proton antiporter-1 (CPA1) or monovalent cation/proton antiporter-3 (CPA3) has been identified. Transporters of the monovalent cation/proton antiporter (CPA) superfamily support key physiological functions of bacteria by catalyzing active efflux of Na⁺ and/or K⁺, with respective H⁺ influx, to maintain cytoplasmic pH homeostasis and tolerate fluctuations in osmolarity [51]. Since cytoplasmic bacterial parasites or symbionts are sheltered by the host cell, it has been postulated that their genomes encode few genes for Na⁺/H⁺ antiporters [51]. A comparison of *L. hongkongensis*, *C. violaceum*, intracellular pathogens *N. gonorrhoeae* and *N. meningitidis*, as well as the two model bacterial organisms, *Bacillus subtilis* and *Escherichia coli*, are shown in Table 9. It can be observed that *L. hongkongensis* and *C. violaceum* have more genes predicted to encode CPA2 superfamily transporters when compared to *N. meningitidis* and *N. gonorrhoeae*.

In addition to the four CDSs coding for the putative transporters of the CPA2 family, two CDSs putatively coding for transporters of the NhaC Na⁺/H⁺ antiporter (NhaC) family are also present (LHK_00646, LHK_02247) in the *L. hongkongensis* genome. Both are predicted to code for the gene *nhaC*. Nevertheless, gene sequences of the two CDSs are significantly different, indicating a possible difference in phylogenetic origin. The *nhaC* homologue in the alkaliphilic bacteria *Bacillus firmus* has been confirmed experimentally to produce NhaC, which has Na⁺/H⁺ antiporter activity [52]. Table 9 also compares the number of identified NhaA, NhaB, NhaC and NhaD

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**Table 7 Distribution of transcription factors families in *L.hongkongensis*, *N. meningitidis*, *N. gonorrhoeae* and *C. violaceum***.

| Transcription factor family | L. hongkongensis HLHK9 | C. violaceum ATCC 12472 | N. gonorrhoeae FA 1090 | N. meningitidis MC58 |
|----------------------------|-------------------------|--------------------------|------------------------|----------------------|
| AraC/XylS                  | 9                       | 25                       | 3                      | 3                    |
| ArsR                      | 4                       | 4                        | 4                      | 2                    |
| AsnC                      | 2                       | 6                        | 2                      | 2                    |
| Cold shock domain         | 3                       | 2                        | 1                      | 1                    |
| CRP-FNR                   | 2                       | 3                        | 1                      | 1                    |
| DecR                      | 2                       | 4                        | 1                      | 1                    |
| GntR                      | 6                       | 15                       | 2                      | 2                    |
| IclR                      | 1                       | 2                        | 1                      | 1                    |
| LacI                      | 0                       | 2                        | 0                      | 0                    |
| LuxR                      | 7                       | 12                       | 1                      | 1                    |
| LysR                      | 25                      | 67                       | 5                      | 6                    |
| MarR                      | 4                       | 18                       | 2                      | 3                    |
| MerR                      | 3                       | 8                        | 1                      | 1                    |
| NtrC/Fis                  | 8                       | 19                       | 4                      | 3                    |
| Ompr                      | 9                       | 11                       | 1                      | 1                    |
| TetR                      | 8                       | 17                       | 2                      | 2                    |
| CRO/CU/Xre                | 8                       | 9                        | 12                     | 9                    |
| Fur                       | 2                       | 1                        | 1                      | 1                    |
| HcrA                      | 1                       | 1                        | 0                      | 0                    |
| SirB                      | 1                       | 1                        | 1                      | 1                    |
| Rft2                      | 1                       | 2                        | 2                      | 2                    |
| BolA/YrbA                 | 2                       | 2                        | 2                      | 2                    |
| LytTR                     | 1                       | 4                        | 0                      | 0                    |
family transporters in the genomes of *L. hongkongensis* to those in *C. violaceum*, *N. meningitidis*, *N. gonorrhoeae*, *B. subtilis* and *E. coli*.

**Acquisition of and tolerance to heavy metals**

To adapt to natural freshwater, *L. hongkongensis* should be able to acquire essential heavy metal ions and expel them, or their toxic counterparts, when their levels reach toxicity. Many heavy metals belong to the transition elements. Their electronic configurations provide them with an exquisite ability to form complex compounds. Metal ions such as iron(II), cobalt(II), nickel(II) and copper(II) are essential to many physiological functions, yet are toxic at high concentrations. Certain species, such as silver(I), cadmium(II) and mercury(II), however, are relatively toxic to bacteria [53].

**Iron**

Iron is required by both prokaryotes and eukaryotes for the synthesis of important proteins such as cytochromes. Bacteria employ a variety of mechanisms to acquire iron, such as siderophore-mediated uptake, metal inorganic transport systems (MIT) and ATP-binding cassette (ABC) transport systems.

No gene for siderophore production was found in the *L. hongkongensis* genome. Since heme-bound iron and iron-containing proteins may not be readily available outside of a host [54], transporter-mediated transport of ionic iron would be the probable mechanism of iron acquisition during the environmental persistence of *L. hongkongensis*. A locus coding for the periplasmic ferric iron binding protein FbpA, permease FbpB and a putative iron-transport system ATP-binding protein is present (LHK_02634-02636). Putative homologous loci, containing three similar CDSs, is present in *C. violaceum* (CV1908-1910), *N. gonorrhoeae* (NGO0215-0217) and *N. meningitidis* (NMB0632-0634). The gene coding for the putative iron-transport system ATP-binding protein in *L. hongkongensis* (LHK_02636) is probably homologous to the *fbpC* gene in *N. meningitidis*. The FbpABC system has been shown to be a specific ferric iron transport system with high affinity to Fe$^{3+}$ in *Haemophilus influenzae* [55].

In addition, two CDSs are the putative homologues of the genes coding for the high-affinity ABC transport system for ferrous iron in *E. coli* (*feoABC*) are present in the *L. hongkongensis* (LHK_03044-03045). The two CDSs code for the putative homologues of *feoA* and *feoB* respectively. The putative homologue of *feoB* is also present in *C. violaceum*. No putative homologues of *feoA* or *feoC* are found in *N. gonorrhoeae* and *N. meningitidis*.

**Nickel**

Nickel is an essential component of urease, which is implicated in the acid tolerance of *L. hongkongensis*. The CorA and HoxN systems have been proposed as an important nickel and cobalt transport system in bacteria [53]. No putative CDS coding for genes of the CorA system is present in the *L. hongkongensis* genome, yet a CDS coding for a histidine-rich glycoprotein with functional domain of the high-affinity nickel transport protein *NicO* was identified (LHK_02812). The *NicO* protein is related to the NixA of the HoxN family, which has been implicated in the urease-dependent pathogenesis of *Helicobacter pylori* [56]. A locus of four CDSs coding for *dppD*, *dppE*, *dppF* (LHK_00939-00942) was found. They belong to an ABC transporter subfamily and are predicted to transport dipeptides, oligopeptides and nickel. The *dppA* homologue (LHK_00667) is located distant from the *dppBCDF* locus. This is in contrast to *C. violaceum*, where the CDS coding for the putative *dppA* gene is contiguous to the *dppBCDF* locus. This separation of *dppA* from

| Product | Gene | *L. hongkongensis* | *N. meningitidis* | *N. gonorrhoeae* | *C. violaceum* |
|---------|------|---------------------|-------------------|-----------------|----------------|
| Acid shock RNA protein | asr | + | - | - | + |
| Acid-resistance protein, possible chaperone | hdaA | + | - | - | - |
| Sigma factor 38 | rpaS | + | - | - | - |
| Ferric uptake regulator protein | fur | + | + | + | + |
| DNA polymerase I | polA | + | + | + | + |
| β-ketoacyl-ACP synthases II | fabF | + | + | + | + |
| Lysine-cadaverine antiporter | cadB | + | - | - | + |
| Arginine decarboxylase | adiA | + | - | - | + |
| Ada transcriptional dual regulator | ada | - | - | - | + |
| Lysine decarboxylase | cadA | - | - | - | + |
| OmpR transcriptional dual regulator | ompR | - | + | + | + |

*Two copies of the gene are present

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Table 8 Other CDSs related to acid stress in *L. hongkongensis*, *N. meningitidis*, *N. gonorrhoeae* and *C. violaceum*.

| Product | *L. hongkongensis* | *N. meningitidis* | *N. gonorrhoeae* | *C. violaceum* |
|---------|--------------------|--------------------|-----------------|----------------|
| Acid shock RNA protein | asr | + | - | - | + |
| Acid-resistance protein, possible chaperone | hdaA | + | - | - | - |
| Sigma factor 38 | rpaS | + | - | - | - |
| Ferric uptake regulator protein | fur | + | + | + | + |
| DNA polymerase I | polA | + | + | + | + |
| β-ketoacyl-ACP synthases II | fabF | + | + | + | + |
| Lysine-cadaverine antiporter | cadB | + | - | - | + |
| Arginine decarboxylase | adiA | + | - | - | + |
| Ada transcriptional dual regulator | ada | - | - | - | + |
| Lysine decarboxylase | cadA | - | - | - | + |
| OmpR transcriptional dual regulator | ompR | - | + | + | + |

*Two copies of the gene are present

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and N. gonorrhoeae is very similar to the homologues were found in magnesium and cobalt efflux protein gene, putative N. meningitidis and cobalt transports system as found in. No putative homologue of the ABC-type magnesium and cobalt efflux protein (LHK_00289) were present. Apart from the HoxN and NiCoT described, three CDSs [65]. It may also contribute to Pb2+ efflux [66]. Gram-negative bacteria such as E. coli and paralogues of cadA-1 and CadA-like protein coding genes are present in C. violaceum as zntA and copA; in N. meningitidis and N. gonorrhoeae as putative transport ATPase genes.

Cobalt
Cobalt is found in coenzyme B12, which is responsible for methyl group transfer and rearrangement [61,62]. Apart from the HoxN and NiCoT described, three CDSs that encode a putative ABC-type cobalt transport system (LHK_01956-01958) and one that codes for a putative magnesium and cobalt efflux protein (LHK_00289) were also found. No putative homologue of the ABC-type cobalt transport system was found in C. violaceum, N. meningitidis and N. gonorrhoeae. For the putative magnesium and cobalt efflux protein gene, putative homologues were found in C. violaceum, N. meningitidis and N. gonorrhoeae as corC.

Cadmium
A CDS coding for a cadmium-translocating P-type ATPase (CadA-1, LHK_00449) was found in the genome. CadA and CadA-like proteins have been implicated in the transport of various heavy metals include, but not limiting to, cadmium, cobalt, mercury, lead and zinc [53]; CadA has been shown to be responsible for the Cd2+ efflux in both Gram-positive bacteria such as Staphylococcus aureus[63] and Bacillus spp. [64]; and Gram-negative bacteria such as Ralstonia metallidurans [65]. It may also contribute to Pb2+ efflux [66]. cadA-1 is very similar to the E. coli gene zntA, which has been shown to be responsible for the intrinsic resistance of E. coli to zinc and cadmium [67]. Probable homologues and paralogues of cadA-1 and CadA-like protein coding genes are present in C. violaceum as zntA and copA; in N. meningitidis and N. gonorrhoeae as putative transport ATPase genes.

Copper
A locus of two CDSs (LHK_03034-03035) coding for a putative copper translocating ATPase and a conserved heavy metal associated domain were also found in the genome. The putative copper translocating ATPase gene has a homologue, copA, in E. coli; copA in E. coli has been shown to be important in resistance to the toxic effects of copper, and is induced by silver and copper ions [68]. Putative homologues of this copper translocating ATPase gene (LHK_03035) are also present in C. violaceum (copA), N. meningitidis and N. gonorrhoeae.

Tolerance to temperature stress
L. hongkongensis inhabits the intestines of freshwater fish, frogs and human [4,6-9]. It is also able to survive freely in freshwater environment [10]. In contrast to human, the body temperatures of freshwater fish and frogs vary with the environmental temperature. The ability to survive in such a wide range of habitats is in line with its ability to survive from 15°C to 42°C, although its growth rate is higher at higher temperatures [8]. In an experiment that examined the differential gene expression of L. hongkongensis at 20°C to 37°C using proteomics study, we found that there were 12 differentially expressed protein spots involved in various functions [43]. Seven spots were more highly expressed at 20°C than at 37°C and five more highly expressed at 37°C than at 20°C. Among these were NAGK-37 that was up-regulated at 37°C and NAGK-20 that was up-regulated at 20°C. These two isoenzymes of NAGK catalyze the second step of the arginine biosynthesis pathway.

In addition to the differentially expressed genes detected by 2-dimensional gel electrophoresis, the L. hongkongensis genome also contains other genes that could be of importance for adaptation to different temperatures. These include genes related to chaperones and chaperonins, heat shock proteins and cold shock proteins. Overall, the number of CDSs in the L. hongkongensis genome encoding putative chaperones and heat shock proteins is lower than that in C. violaceum, but higher than those in the Neisseria species (Table 10). This phenomenon is similar to that observed in the number of distribution of transcription factors in L. hongkongensis genome encoding putative chaperones and heat shock proteins is lower than that in C. violaceum, but higher than those in the Neisseria species (Table 10). This phenomenon is similar to that observed in the number of distribution of transcription factors in L. hongkongensis genome encoding putative chaperones and heat shock proteins is lower than that in C. violaceum, but higher than those in the Neisseria species (Table 10). This phenomenon is similar to that observed in the number of distribution of transcription factors in L. hongkongensis genome encoding putative chaperones and heat shock proteins is lower than that in C. violaceum, but higher than those in the Neisseria species (Table 10). This phenomenon is similar to that observed in the number of distribution of transcription factors in L. hongkongensis genome encoding putative chaperones and heat shock proteins is lower than that in C. violaceum, but higher than those in the Neisseria species (Table 10).
Tolerance to osmotic stress

*L. hongkongensis* can survive in and adapt to a variety of ecological niches, including water and the intestines of freshwater fish, frogs and humans, with different osmotic stress. A total of 25 CDSs in the *L. hongkongensis* genome could be related to control of osmotic pressure (Table 11). Most of these CDSs encode proteins and their regulators for transport of potassium ion, proline and glutamate. Among the 25 CDSs, 11 of them are related to potassium ion transport (nine and two for potassium uptake and efflux respectively); whereas only nine CDSs present in the *C. violaceum* genome and three in the *N. gonorrhoeae* and *N. meningitidis* genomes are related to potassium ion transport (Table 11).

Table 9 Cation/proton antiporters identified in *L. hongkongensis*, *N. meningitidis*, *N. gonorrhoeae* and *C. violaceum*; and the model bacterial organisms, *B. subtilis* and *E. coli*

| Features | *L. hongkongensis* | *N. meningitidis* MC58 | *N. gonorrhoeae* FA 1090 | *C. violaceum* ATCC12472 | *B. subtilis* 168b | *E. coli* K12-MG1655b |
|----------|--------------------|------------------------|--------------------------|---------------------------|------------------|----------------------|
| Genome size (Mbp) | 3.17 | 2.27 | 2.15 | 4.75 | 4.22 | 4.64 |
| Total no. of transporter proteins | 442 | 103 | 96 | 564 | 298 | 354 |
| No. of identified transporters per Mb genome | 139 | 45.4 | 44.7 | 119 | 71.0 | 76.3 |
| No. of cation/proton antiporters | 6 | 4 | 4 | 3 | 6 | 7 |
| Monovalent cation/proton antiporter-1 (CPA1) family | 0 | 1 | 1 | 0 | 1 | 2 |
| Monovalent cation/proton antiporter-2 (CPA2) family | 4 | 1 | 1 | 3 | 2 | 3 |
| Monovalent cation (K⁺ or Na⁺): proton antiporter-3 (CPA3) family | 0 | 0 | 0 | 0 | 1 | 0 |
| NhaA Na⁺:H⁺ antiporter family | 0 | 0 | 0 | 0 | 0 | 1 |
| NhaB Na⁺:H⁺ antiporter family | 2 | 2 | 2 | 0 | 2 | 0 |
| NhaC Na⁺:H⁺ antiporter family | 2 | 2 | 2 | 0 | 2 | 0 |
| *aGenome size data obtained from www.ncbi.nlm.nih.gov/projects/genome/, calculations based on data from www.membranetransport.org and with updated number of transporters in our annotation*
| *bRetrieved from www.membranetransport.org*
| *cNot listed on www.membranetransport.org, see Krulwich et. al.*

Tolerance to osmotic stress

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Table 10 CDSs related to temperature stress in *L. hongkongensis*, *N. meningitidis*, *N. gonorrhoeae* and *C. violaceum*

| Product | *L. hongkongensis* HLHK9 | *N. meningitidis* MC58 | *N. gonorrhoeae* FA 1090 | *C. violaceum* ATCC 12472 |
|---------|--------------------------|------------------------|--------------------------|---------------------------|
| Chaperone Hsp40, co-chaperone with DnaK | dnaJ | + | + | + |
| Chaperone Hsp70, co-chaperone with DnaJ | dnaK | + | + | + |
| Co-chaperone GrpE | grpE | + | + | + |
| Chaperone subunit of chaperonin GroEL-GroES | groEL | + | + | + |
| Regulator subunit of chaperonin GroEL-GroES | groES | + | + | + |
| ATP-dependent protease specificity component and chaperone | cpaA | + | + | + |
| ClpB chaperone | clpB | + | + | + |
| ClpP serine protease | clpP | + | + | + |
| Hsc66 chaperone, member of Hsp70 protein family | hscA | + | + | + |
| Hsc20 co-chaperone of Hsc66 | hscB | + | + | + |
| Heat shock protein of Hsp90 family | hspG | + | - | - |
| Heat shock protein, integral membrane protein | hspX | + | + | + |
| Molecular chaperone Hsp33 | hslO | + | + | + |
| ATPase component of the HslVU protease | hslU | + | - | - |
| Peptidase component of the HslVU protease | hslV | + | - | - |
| Heat shock protein Hsp15 | hslR | + | + | + |
| Cold shock transcription factor | cjaA | + | + | + |

*Two copies of the gene are present*
Table 11 CDSs related to control of osmotic pressure in *L. hongkongensis, C. violaceum, N. meningitidis* and *N. gonorrhoeae*

| Product                                                                 | Gene       | Function                                              | L. hongkongensis | C. violaceum ATCC 12472 | N. meningitidis MC58 | N. gonorrhoeae FA 1090 |
|------------------------------------------------------------------------|------------|-------------------------------------------------------|-----------------|--------------------------|----------------------|------------------------|
| Sodium/hydrogen exchanger                                              |            | Sodium efflux, hydrogen influx                        | LHK_00707       | CV2903                   | -                    | -                      |
|                                                                         |            |                                                       | LHK_02296       | CV4147                   | -                    | -                      |
| Potassium uptake protein                                               | trkA       | Potassium uptake                                      | LHK_01490       | -                        | NMB1614              | NGO1154                |
| Potassium uptake protein                                               | trkH       | Potassium uptake                                      | LHK_01488       | -                        | NMB0661              | NGO0230                |
| Glutathione-regulated potassium-efflux system protein                 | ksbB       | Potassium efflux, hydrogen influx                     | LHK_02018       | CV3326                   | NMB0209              | -                      |
| Glutathione-regulated potassium-efflux system protein                 | ksf        | Potassium efflux, hydrogen influx                     | LHK_02848       | -                        | -                    | NGO1774                |
| Potassium-transporting ATPase, A subunit                               | kdpA       | Potassium uptake                                      | LHK_01572       | CV1599                   | -                    | -                      |
| Potassium-transporting ATPase, B subunit                               | kdpB       | Potassium uptake                                      | LHK_01573       | CV1598                   | -                    | -                      |
| Potassium-transporting ATPase, C subunit                               | kdpC       | Potassium uptake                                      | LHK_01574       | CV1597                   | -                    | -                      |
| Osmosensitive potassium channel signal transduction histidine kinase   | kdpD       | Protein kinase of two-component regulatory system     | LHK_01575       | CV1596                   | -                    | -                      |
| Two component transcriptional regulator                               | kdpE       | kdp operon transcription regulation                   | LHK_01576       | CV1595                   | -                    | -                      |
| Potassium-transporting ATPase                                          | kdpF       | Potassium uptake                                      | LHK_01577       | CV1596                   | -                    | -                      |
| ATP-sensitive inward rectifier potassium channel related transmembrane protein |            | -                                                     | CV1109          | CV1109                   | -                    | -                      |
| Low affinity potassium transport system protein                        | kup        | Potassium uptake                                      | LHK_01720       | CV2731                   | -                    | -                      |
|                                                                         |            |                                                       | LHK_00121       | CV0573                   | -                    | -                      |
| Glucose-methanol-choline oxidoreductase                                | betA       | Glycine betaine synthesis                             | -               | -                        | -                    | -                      |
| NAD-dependent betaine aldehyde dehydrogenase                          | betB       | Glycine betaine synthesis                             | -               | -                        | -                    | -                      |
| High-affinity choline transport protein                                | betT       | Choline uptake                                        | LHK_01689       | CV4302                   | NMB1277              | NGO0529                |
| Large conductance mechanosensitive channel                            | mscL       | Compatable solute efflux                              | LHK_02562       | CV1360                   | -                    | -                      |
| Small conductance mechanosensitive channel                            | mscS       | Compatable solute efflux                              | LHK_01942       | CV2330                   | NMB0213              | NGO2057                |
|                                                                         |            |                                                       | LHK_02394       | CV2385                   | -                    | -                      |
|                                                                         |            |                                                       | LHK_02965       | CV2962                   | -                    | -                      |
|                                                                         |            |                                                       |                 | CV4288                   | -                    | -                      |
| Osmotically inducible lipoprotein                                      | osmB       | -                                                     | LHK_01892       | CV3209                   | -                    | -                      |
| Osmotically inducible lipoprotein                                      | osmC       | -                                                     | LHK_01612       | -                        | -                    | -                      |
| Sodium glutamate symport carrier protein                              | gntS       | Sodium and glutamate uptake                           | -               | CV1105                   | NMB0085              | NGO1890                |
| CDS related to control of osmotic pressure in L. hongkongensis, C. violaceum, N. meningitidis and N. gonorrhoeae (Continued) |
|----------------------------------------------------------------------------------------------------------------------------------|
| **Proton glutamate symport protein** | Hydrogen and glutamate uptake | LHK_02672 | CV1198 | CV3409 |
| **Proline/betaine transporter** | proP | Proline, glycine betaine and ectoine uptake | LHK_02126 | CV1299 | CV2901 |
| **ABC-type proline/glycine betaine transport systems, ATPase** | proV | Proline and glycine betaine uptake | - | CV1197 | - |
| **Proline-specific permease** | proY | Proline uptake | - | CV1138 | - |
| **Osmoprotectant transport system substrate-binding protein** | - | Osmoprotectants uptake | - | CV1195 | CV4392 |
| **Osmoprotectant transport system permease protein** | - | Osmoprotectants uptake | - | CV1194 | CV1196 |
| **Osmoprotectant transport system ATP-binding protein** | - | Osmoprotectants uptake | - | CV4393 | CV4395 |
| **Outer membrane porin** | ompC | Hydrophilic molecules uptake by passive diffusion | - | CV0217 | - |
| **Outer membrane porin** | ompF | Hydrophilic molecules uptake by passive diffusion | - | - | - |
| **Osmolarity sensor protein** | envZ | Protein kinase of two-component regulatory system | - | - | - |
| **Transcriptional regulator** | ompR | ompC and ompF transcription regulation | - | CV0216 | - |
| **Aquaporin Z** | aqpZ | Water influx and efflux | - | CV2864 | - |
| **Glycerol uptake facilitator protein** | glpF | Glycerol and water uptake | - | CV0252 | - |
| **Glycerol kinase** | glpK | Protein kinase of regulatory system | LHK_03100 | CV0251 | - |
| **Glycerol-3-phosphate regulon repressor** | glpR | Repressor in glp operons transcription regulation | LHK_03101 | CV0112 | CV0136 |
In addition to the 11 CDSs related to potassium transport, five other CDSs encode mechanosensitive channel proteins. These channels allow a quick and transient increase in compensatory solute (e.g. proline and glutamate) flux out of bacterial cells in response to large turgor pressure generated by water influx due to osmotic downshock when the bacterial cells are transferred to environments of low osmolarity [69]. Interestingly, a \textit{betT} gene that encodes a transport protein for choline uptake is present in the \textit{L. hongkongensis} genome. However, the \textit{betA} and \textit{betB} genes, that encode enzymes for metabolizing choline to glycine betaine, the osmotically active compound, are absent [70]. Similarly, the \textit{glpR} and \textit{glpK} genes are present. However, the \textit{glpF} gene, another gene in the \textit{glpFK} operon that encodes the glycerol uptake facilitator protein, is absent [71]. Therefore, the contributions of \textit{betT}, \textit{glpR} and \textit{glpK} and their corresponding choline and glycerol transport systems to tolerance of osmotic stress in \textit{L. hongkongensis} are unknown. The expressions of two other CDSs, \textit{osmB} and \textit{osmC}, which encode two osmotically inducible lipoproteins, have been found to be affected by change in osmolarity in \textit{E. coli} [72,73]. Both \textit{osmB} and \textit{osmC} are membrane proteins of unknown function. In \textit{E. coli}, it was observed that deletion of \textit{osmC} will render the bacterium more sensitive to oxidative stress because of its peroxidase activity [74].

**Tolerance to oxidative stress and ultraviolet light stress**

Oxidative stress on aerobic bacteria is mainly mediated by partially reduced oxygen species, or reactive oxygen species, most notably superoxide and hydrogen peroxide, that are inevitable by-products of aerobic metabolism. These reactive oxygen species can cause damage to DNA, proteins and membranes. As a result, all aerobic bacteria possess various mechanisms to scavenge superoxide and hydrogen peroxide [75], as well as to protect the cells from damaged by these reactive oxygen species. In most bacteria, inducible responses to superoxide stress and hydrogen peroxide stress are mediated through the transcription factors SoxR(S) and OxyR respectively, which command the induction of a battery of defensive proteins, including superoxide dismutase and catalase respectively [76].

In the \textit{L. hongkongensis} genome, genes for oxidant-resistant dehydratase (\textit{fumC}, \textit{acnA}), superoxide scavenging (\textit{sodB}), hydrogen peroxide scavenging (\textit{ahpC}, \textit{cpx}), exclusion and export of redox-cycling antibiotics (\textit{acrA}, \textit{acrB}, \textit{tolC}), redox balancing (\textit{nfnB}), DNA repair (\textit{xthA}, \textit{nhr}, \textit{mutM}, \textit{mutY}, \textit{mutT}), reduction of disulfide bonds (\textit{trxA}, \textit{trxB}, \textit{gpxA}, \textit{gshA}, \textit{gshB}, \textit{grxA}, \textit{grxC}, \textit{gor}) [77], limitation of iron availability (\textit{bfr}, \textit{dps}, \textit{fur}) and reduction of iron-sulfur clusters (\textit{fpr}, \textit{yggX}) are present (Table 12). Transcriptions of most of the genes are regulated by SoxR(S) and/or OxyR transcription factors in other bacteria (Table 12) [78]. In addition, some genes may be regulated by other transcription factors, such as RpoS, FNR [79], Fur and Lrp [80,81]. Interestingly, SoxR(S) is not present in the genomes of \textit{N. gonorrhoeae}, \textit{N. meningitidis} and \textit{N. lactamica} and the role of SoxR(S) is presumably taken up by other transcription factors [82]. Notably, SoxR(S) was also not found in the \textit{L. hongkongensis} genome by BLASTp search.

In addition to oxidative stress, ultraviolet light is another environmental stress that damages the DNA of a bacterium. The genomes of \textit{L. hongkongensis}, \textit{C. violaceum}, \textit{N. gonorrhoeae} and \textit{N. meningitidis} all contain one copy of \textit{phrB} which encodes a photolysase for direct repair of DNA; and one copy each of \textit{uvrA}, \textit{uvrB}, \textit{uvrC} and \textit{uvrD} in the nucleotide excision repair system.

**Starvation related CDSs**

\textit{L. hongkongensis} is arguably fastidious: it is asaccharolytic, metabolizing none of the common sugars, requiring malate, adipate or caprate as its carbon source [1,4,43]; in the laboratory, its optimal growth requires brain-heart infusion (BHI) instead of commonplace lysogeny broth (LB) (unpublished data). Thus the pivotal study published in 2007, describing the isolation of \textit{L. hongkongensis} from six of the 10 surveyed drinking water reservoirs in Hong Kong, prompts inquiries into the mechanisms of survival and persistence of this bacterium in nutrient-poor environments [10]. In many natural waters, nutrients are scarce. An average of the reservoirs from which \textit{L. hongkongensis} were isolated demonstrates such: the permanganate value, a surrogate for organic carbon content, had a yearly mean of 1.25 mg O₂/L; ammoniacal nitrogen, 0.05 mg N/L; and total phosphorus, 0.015 mg P/L [83]. This is in stark contrast with even so-called “minimal medium”, in which the malate content measures 2000 mg/L; ammoniacal nitrogen 9.0 mg N/L and total phosphorus 17 mg P/L [84]. Clearly, \textit{L. hongkongensis} has exquisite adaptive abilities which enable its survival in environments such as the drinking water reservoirs.

**General starvation**

With limited nutrients, bacteria do not continue their exponential growth indefinitely. Instead, they move into the stationary phase; cells lose viability and enter the death phase; in prolonged periods of nutrient depletion, a resistant subpopulation survives and the extended stationary phase ensues [85]. To adapt to stress conditions as such, alternative sigma factors enable bacterial RNA polymerase to transcribe an alternative subset of its genes. In the stationary phase, the starvation/stationary phase sigma factor, σ\text{SI} encoded by \textit{rpoS}, is used to upregulate the expression of a number of genes. Some of these genes may be clustered with \textit{rpoS}: in
Table 12 CDSs related to tolerance of oxidative stress in *L. hongkongensis*, *C. violaceum*, *N. meningitidis* and *N. gonorrhoeae*.

| Role                                | Gene   | Protein          | Regulated by         | L. hongkongensis HLHK9 | C. violaceum ATCC12472 | N. meningitidis MC58 | N. gonorrhoeae FA1090 |
|-------------------------------------|--------|------------------|----------------------|------------------------|------------------------|----------------------|-----------------------|
| Transcriptional regulator          | soxR   | SoxR             | H₂O₂, O₂             | -                      | CV2793                 | -                    | -                     |
|                                     | soxS   | SoxS             | -                    | -                      | -                      | -                    | -                     |
|                                     | oxyR   | OxyR             | H₂O₂                 | LHK_02531              | CV3378                 | NMB0173              | NGO1813               |
|                                     | ohrR   | Organic hydroperoxide resistance transcriptional regulator | Organic peroxides | -                      | CV0210                 | -                    | -                     |
|                                     | fnr    | Fumarate/nitrate reductase regulator | O₂ | LHK_00352              | CV3647                 | NMB0380              | NGO1579               |
|                                     | perR   | PerR             | H₂O₂                 | -                      | -                      | NMB1266              | NGO0542               |
|                                     | lrp    | Leucine-responsive protein | Leucine | LHK_01860              | CV1913                 | NMB0573              | NGO1294               |
| Oxidant-resistant dehydratase isozymes |       |                  |                      |                        |                        |                      |                       |
|                                    | fumC   | Fumarase C       | SoxRS, SoxS, FNR, FNR | LHK_00495              | CV1120                 | NMB1458              | NGO1029               |
|                                    | acnA   | Aconitase A      | SoxRS, SoxS, FNR, Fur | LHK_02153              | CV1121                 | NMB0433              | -                     |
| Superoxide scavenging              | sodA   | Manganese superoxide dismutase | SoxRS, FNR | LHK_01716              | CV0867                 | NMB0884              | NGO0405               |
|                                    | sodB   | Iron superoxide dismutase | LHK_02309 | CV2504                 | -                      | NMB1398              | -                     |
|                                    | sodC   | Copper-zinc superoxide dismutase | H₂O₂, RpoS, FNR | - | - | NMB0216 | NGO1767 |
| Hydrogen peroxide scavenging       | ahpC   | Alky hydroperoxide reductase | OxyR, PerR | LHK_00938 | CV3799 | - | - |
|                                    | ahpF   | Alky hydroperoxide reductase | OxyR, PerR | - | - | NMB1716-NMB1715 | NGO1365-NGO1364 |
|                                    | cpx    | Cytochrome c peroxidase | FNR | LHK_02666 | CV0300 | - | NGO1769 |
| catalase/peroxidase                | catalase | Hydroperoxidase I | OxyR, RpoS | LHK_01300 | LHK_02436 | - | - |
| Exclusion and export of redox-cycling antibiotics | micF | Antisense RNA to porin OmpF | SoxRS, Lrp, OmpR | LHK_01426-LHK_01425-LHK_01424 | CV0435-CV0434-CV0433 | NMB1716-NMB1715-NMB1714 | NGO1365-NGO1364-NGO1363 |
|                                    | acrA   | Drug export system | SoxRS | LHK_01953 | CV2244 | NMB0804 | NGO0388 |
|                                    | acrB   | Drug export system | SoxRS | LHK_02129-LHK_02130-LHK_02131 | CV2240-CV2241-CV2242 | NMB1716-NMB1715-NMB1714 | NGO1365-NGO1364-NGO1363 |
|                                    | toIC   | Drug export system | SoxRS | LHK_02929-LHK_02930-LHK_02931 | CV2240-CV2241-CV2242 | NMB1716-NMB1715-NMB1714 | NGO1365-NGO1364-NGO1363 |
| Redox balancing                    | nfnB   | Nitroreductase    | SoxRS | LHK_01953 | CV2244 | NMB0804 | NGO0388 |
| DNA repair                         | xthA   | Exodeoxyribonuclease III | H₂O₂, RpoS | LHK_02447 | CV0877 | NMB0399 | NGO1561 |
|                                    | nth    | Endonuclease III  | LHK_01218 | CV3293 | NMB0533 | NGO0139 | - |
| Strain | CDS Related to Oxidative Stress |
|--------|---------------------------------|
| L. hongkongensis | **Endonuclease IV** |
|          | **FNR** |
|          | LHK_00316 CV4062 NMB1295 NGO0610 |
|          | LHK_002781 CV3703 NMB1396 NGO0710 |
|          | LHK_002262 CV1787 NMB1064 - |
|          | LHK_00322 CV0032 NMB0453 NGO1506 |
|          | LHK_00604 CV1112 |
|          | LHK_01693 CV1586 |
|          | LHK_01823 CV1767 CV3401 CV3611 |
| C. violaceum | **Formamidopyrimidine-DNA glycosylase** |
|          | **FNR** |
|          | LHK_00316 CV4062 NMB1295 NGO0610 |
|          | LHK_002781 CV3703 NMB1396 NGO0710 |
|          | LHK_002262 CV1787 NMB1064 - |
|          | LHK_00322 CV0032 NMB0453 NGO1506 |
|          | LHK_00604 CV1112 |
|          | LHK_01693 CV1586 |
|          | LHK_01823 CV1767 CV3401 CV3611 |
| N. meningitidis | **Adenine glycosylase** |
|          | **FNR** |
|          | LHK_00316 CV4062 NMB1295 NGO0610 |
|          | LHK_002781 CV3703 NMB1396 NGO0710 |
|          | LHK_002262 CV1787 NMB1064 - |
|          | LHK_00322 CV0032 NMB0453 NGO1506 |
|          | LHK_00604 CV1112 |
|          | LHK_01693 CV1586 |
|          | LHK_01823 CV1767 CV3401 CV3611 |
| N. gonorrhoeae | **7,8-dihydro-8-oxoguanine triphosphatase** |
|          | **FNR** |
|          | LHK_00316 CV4062 NMB1295 NGO0610 |
|          | LHK_002781 CV3703 NMB1396 NGO0710 |
|          | LHK_002262 CV1787 NMB1064 - |
|          | LHK_00322 CV0032 NMB0453 NGO1506 |
|          | LHK_00604 CV1112 |
|          | LHK_01693 CV1586 |
|          | LHK_01823 CV1767 CV3401 CV3611 |

**Protein repair**

| Strain | CDS Related to Protein Repair |
|--------|--------------------------------|
|          | **Protein-methionine-S-oxide reductase** |
|          | **msrAB** |
|          | LHK_01369 (msrB) CV2325 (msrB) |
|          | CV3212 (msrB) NMB0044 (msrAB) NGO2059 (msrAB) |

**Reduction of disulfide bonds**

| Strain | CDS Related to Disulfide Reduction |
|--------|-----------------------------------|
|          | **Thioredoxin 1** |
|          | **TrxA** |
|          | LHK_01690 CV1584 NMB1366 NGO0652 |
|          | LHK_00591 CV1325 NMB0006 NGO0057 |
|          | LHK_01462 CV4257 NMB1845 NGO1923 |
|          | LHK_01491 CV4279 NMB1958 NGO2124 |
|          | LHK_02476 |
|          | LHK_02092 |
|          | LHK_02481 CV3708 NMB0946 NGO0926 |
|          | LHK_00424 CV1107 NMB1621 - |
|          | LHK_00595 CV3555 NMB1346 |
|          | LHK_00615 CV3787 |
|          | LHK_00857 CV4276 NMB1037 NGO0608 |
|          | LHK_0093 CV4275 NMB1359 NGO1217 |
|          | LHK_00503 CV3620 NMB0773 NGO0351 |
|          | LHK_02837 CV1126 NMB1790 NGO0114 |
|          | LHK_01492 CV2037 NMB0947 NGO0925 |

**Reduction of iron-sulfur clusters**

| Strain | CDS Related to Iron-Sulfur Cluster Reduction |
|--------|---------------------------------------------|
|          | **NADPH-ferredoxin reductase** |
|          | **fpr** |
|          | LHK_02993 CV0086 NMB1044 NGO0687 |
|          | LHK_00654 CV3356 NMB2021 NGO2083 |
|          | LHK_00654 CV3356 NMB2021 NGO2083 |
|          | LHK_00654 CV3356 NMB2021 NGO2083 |
| Table 12 CDSs related to tolerance of oxidative stress in *L. hongkongensis*, *C. violaceum*, *N. meningitidis* and *N. gonorrhoeae*. (Continued) |
|---------------------------------------------------------------|
| **Organic hydroperoxide resistance**                          |
| ohrA              | Organic hydroperoxide resistance protein                      | OhrR | -       | CV0209 | - | - |
| ohrB              | Hydrogenperoxide resistance protein                           | Sigma B | -       | CV2493 | - | - |
| **Disulfide bond formation in periplasm**                     |
| dsbA              | Disulfide oxidoreductases                                     | Cpx two component system | LHK_02939 | CV3998 | NMB0278 | NMB0294 | NGO1548 |
| dsbB              | Hydroperoxide resistance protein                              | Sigma B | -       | CV2493 | - | - |
| dsbG              | Thioldisulfide interchange protein                            | OxyR | -       | CV2637 | - | - |
| **Increase cellular pools of reduced pyridine nucleotides for glutathione-dependent repair reactions** |
| zwf                | Glucose-6-phosphate 1-dehydrogenase                           | SoxRS | LHK_01919 | CV0145 | NMB1392 | NGO0715 |
| **Limit iron availability**                                  |
| bfr                | Bacterioferritin                                              | Fur | LHK_01239 | CV3399 | NMB1206 | NGO0794 |
| dsps               | DNA-binding protein                                           | OxyR, RpoS | LHK_01835 | CV4253 | - | - |
| fur                | Ferric uptake regulator                                       | PerR, OxyR, SoxRS | LHK_01431 | CV1797 | NMB205 | NGO1779 |
| **Protein binding**                                          |
| hslO               | Molecular chaperone Hsp33                                     | H$_2$O$_2$ & temperature | LHK_02184 | CV2000 | NMB2000 | NGO1189 |
| **Others**                                                   |
| rimK               | Ribosomal protein S6 modification protein                     | SoxRS | -       | - | - | - |
| ribA               | Cyclic GMP hydrolase                                          | SoxRS | LHK_02390 | CV2005 | NMB1254 | NGO1134 |
A CDS coding for the putative gene surA precursor is present in the *L. hongkongensis* genome (LHK_03194). This survival protein precursor was also found in *C. violaceum*, *N. meningitidis*, *N. gonorrhoeae* and *E. coli*. SurA, the periplasmic chaperone protein encoded by this gene, is responsible for the proper folding and insertion of a subset of outer membrane proteins in *E. coli* [89]. It is of interest, however, to note that the SurA precursor protein is only expressed at 37°C, but not the environmental temperature of 20°C, when *L. hongkongensis* is cultured in the rich medium of BHI [43]. It is unknown, therefore, whether temperature may have a more generalized effect on the starvation response of *L. hongkongensis*.

### Carbon starvation

In the *L. hongkongensis* genome, only one CDS coding for the putative carbon starvation gene cstA2 was found (LHK_00676). This is similar to *N. gonorrhoeae* and *N. meningitidis*, but different from the *C. violaceum* genome, which contains two CDSs coding for the putative genes cstA1 and cstA2. The *E. coli* homologue of the *L. hongkongensis* cstA2 gene is cstA. CstA is a starvation-induced peptide transporter in *E. coli*, and has been implicated in peptide utilization [90].

CDSs coding for putative genes *sspA* and *sspB* are present in the *L. hongkongensis* genome (LHK02886-02887). Putative homologues of *sspA* and *sspB* are also present in *C. violaceum*, *N. meningitidis*, *N. gonorrhoeae* and *E. coli*. In *E. coli*, *sspA* and *sspB* code for the stringent starvation proteins SspA and SspB. Whilst SspA is essential to expression of SspB, it has also been found to be upregulated in the starvation response to glucose, nitrogen, phosphate and amino acids [91]. SspA and SspB are probably not implicated in theugar starvation response of *L. hongkongensis*, if any, since the bacterium is asaccharolytic. It is uncertain, nevertheless, whether carbon starvation, i.e. of malate, caprate and adipate, may lead to upregulation of the putative *sspA* and *sspB* genes in *L. hongkongensis*.

### Phosphorus starvation

It has long been observed that phosphate is often the limiting nutrient of algal and bacterial growth in freshwater environments [92-94]. Bacteria have evolved various mechanisms to enhance the uptake of phosphate, even by cell envelope elongation to increase the surface area to volume ratio [95]; albeit a relationship is yet to be ascribed to the seagull or spiral rod shape of *L. hongkongensis*. From the freshwater reservoir data stated above, phosphate is probably the scarcest nutrient amongst carbon, nitrogen, phosphorus and iron with its concentration of 0.015 mg P/L (or 0.5 μM). On the other hand, however, it is worthwhile to note the more recent finding that phosphate depletion may enhance bacterial resistance to multiple antimicrobials [96,97].

Phosphate homeostasis in bacteria is mainly achieved by the PhoR/PhoB two-component regulatory system (TCRS). In *L. hongkongensis*, the putative genes coding for the PhoR/PhoB are adjacent to each other (LHK_00166-00165), as in *C. violaceum* (CV_0563-0562). The *N. gonorrhoeae* and *N. meningitidis* homologue of the phoR and phoB genes, however, could not be identified.

The PhoR/PhoB TCRS is closely related to the phosphate-specific transport (Pst) system. In *E. coli*, there is a pstSCAB-phoU operon in which the genes *pstS*, *pstC*, *pstA*, *pstB* and *phoU* are clustered. This is not the case in *L. hongkongensis*, *C. violaceum*, *N. gonorrhoeae* and *N. meningitidis*. In *L. hongkongensis*, the putative *pstSCAB* locus (LHK00524-00521) is well separated from the CDS coding for the putative *phoU* gene (LHK_00885). In *C. violaceum*, this separation is also seen (*pstSCAB*: CV_0938-0935; *phoU*: CV_1261); the *pstSCAB* locus is also clustered with the putative *pitA* gene, which codes for a low-affinity inorganic phosphate transporter (CV_0934). In contrast to such, the CDS that encodes the putative *pitA* gene in *L. hongkongensis* is separated from the putative *pstSCAB* locus (LHK_02538). It is believed that the *PstS*, *PstC*, *PstA* and *PstB* proteins, together with *PhoU*, are responsible for the formation of an ABC transporter in the capture of periplasmic inorganic phosphate. In an abundance of phosphate, the *Pst* system, together with the histidine kinase *PhoR*, repress the transcription regulatory protein *PhoB*. When the extracellular phosphate concentration is below a threshold value, for example 4 mM in *E. coli*, autophosphorylation on a *PhoR* histidine residue occurs; the phosphorylation is subsequently transferred form *phospho-PhoB*, which modulates *Pho* regulon activities [98,99].

### Conclusions

The *L. hongkongensis* genome possessed a high variety of genes for DNA repair and recombination and regulation of gene expression, as well as adaptation to acid, alkaline, temperature, osmotic, oxidative, UV light and starvation stresses as well as acquisition of and tolerance to heavy metals (Figure 2)
Methods

All CDSs in the *L. hongkongensis* genome were annotated as described in our previous publication and classified functionally according to the Clusters of Orthologous Groups system [43]. Annotated genes were mapped to pathways according to the Kyoto Encyclopedia of Genes and Genomes database to help identify stress-response pathways. The CDSs were members of COG L (replication, recombination and repair), COG K (transcription), COG F (nucleotide transport and metabolism) and COG O (post-translational modification, protein turnover, chaperones). Additional CDSs were examined by keyword search using the following words and their variants: stress response, regulation, adaptation, temperature, ultraviolet, acid, alkali, pressure, oxidative, homeostasis and resistance. Manual confirmation of the assigned function was performed by sequence similarity search using BLAST against the NCBI nr database, and assisted by conserved domain search (CD-search), identification of signature sequence motifs and sequence analysis using InterProScan. Cellular localization of putative proteins was predicted using PSORTb where appropriate [100]. Phylogenetic relationships were determined using Clustal x version 1.81. *oriC* was predicted by Ori-finder http://tubic.tju.edu.cn/doric/.

**Abbreviations**

Boxocl, 7, 8-dihydro-8-oxo-2′-deoxyguanosine; d24 (RpoE): RNA polymerase sigma-E factor; d28 (FilA): RNA polymerase sigma factor for flagellar operon; d32 (RpoH): RNA polymerase sigma-32 factor; d38 (RpoS): RNA polymerase sigma factor RpoS; d54 (RpoN): RNA polymerase sigma-54 factor; d70 (RpoD): RNA polymerase sigma factor; o-factor(s); o-RNA: Sigma-RNA; A: Adenine; ABC: ATP-binding cassette; AcnA: Aconitate hydratase; ArgB: N-acetyl-L-glutamate kinase gene; ATP: Adenosine triphosphate; BER: Base excision repair; BetA: Choline dehydrogenase; BetB: Betaine aldehyde dehydrogenase; BetC: High affinity choline transporter protein; BetD: Bacteroides; BHI: Brain-heart infusion medium; Bp: Base pair; Cytosine; CadA: Cadmium efflux ATPase CadA; CDS: Coding sequences(s); Cnr: Nickel and cobalt resistance protein Cnr; CopA: Copper-exporting P-type ATPase A; CorA: Magnesium transport protein CorA; CPA: Monovalent cation/proton antiporter; CPA1/2/3: Monovalent cation/proton antiporter-1/2/3; Cpx: Cytochrome peroxidase; CspA/D: Cold shock protein CspA/D; CstA/B: Carbon starvation-induced
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Authors’ contributions
PCYW, KYY and SKPL designed and supervised the study. RYTF, TCCH, GKMV, AKLT, JLLT, WC, RMW and SOTC annotated the genome. HT performed bioinformatics analysis. SKPL, RYTF, TCCH, RMW and PCYW drafted the manuscript. All authors corrected the manuscript. All authors read and approved the final manuscript.

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