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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* is 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 pyogenic 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 endonuceleases in the *L. hongkongensis* genome. Among the glycosylases, the uracil DNA glycocosylase (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 imidzole 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	Regulatory protein 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 arised from misincorporation of dUMP residues by DNA polymerase or cytosine deamination	LHK_00013	*L. nitroferrum*	56.14	3.00E-58
				LHK_00723	*Ralstonia pickettii*	59.26	2.00E-33
Bifunctional glycosylases	*mutM* (*fpg*)	Formamido-pyrimidine-DNA glycosylase	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 (*xthA2*)	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	*rpoB*	RNAP subunit beta	Subunit of DNA-dependent RNA polymerase	LHK_00246	*L. nitroferrum*	85.26	0
	rpoC	RNAP subunit beta	Subunit of DNA-dependent RNA polymerase	LHK_00247	*C. violaceum*	87.09	0
	rpoA	RNAP subunit alpha	Subunit of DNA-dependent RNA polymerase	LHK_00279	*L. nitroferrum*	90.83	1.00E-171
	rpoE	RNAP delta factor	Participates in initiation and recycling phases of transcription	LHK_01458	*L. nitroferrum*	63.82	7.00E-54
	rpoZ	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	Recognizes RNAP-DNA-RNA complex blocked at template strand lesion, replaces RNAP, releases truncated transcript and recruits UvrABC repair system	LHK_00629	*L. nitroferrum*	73.95	0
Mismatch excision repair							
Mismatch and loop recognition factors	*mutS*	DNA mismatch repair protein MutS	Mismatch recognition	LHK_00373	*C. violaceum*	67.73	0
Molecular matchmarker	*mutL*	DNA mismatch repair protein MutL	Promotes formation of a stable complex between two or more DNA-binding proteins	LHK_01012	*C. violaceum*	55.51	0
DNA exonucleases	*xseA*	Exodeoxyribo-nuclease 7 large subunit	Bidirectionally degrades single-stranded DNA	LHK_01101	*C. violaceum*	59.51	4.00E-125
	xseB	Exodeoxyribo-nuclease 7 small subunit	Bidirectionally degrades single-stranded DNA	LHK_02322	*C. violaceum*	65.28	6.00E-20
DNA polymerase III holoenzyme	*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 posseses 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
	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
Other MMR factors	*dam*	DNA adenine methylase	Methylates DNA sequence GATC and protects DNA from cleavage by restriction endonuclease	LHK_01749	*C. violaceum*	83.92	8.00E-131
				LHK_02602	*C. violaceum*	75	9.00E-113
				LHK_00398	*C. violaceum*	75	9.00E-113
	vsr	Very short patch repair protein	Endonuclease, nicks double-stranded DNA	LHK_03243	*Limnobacter* sp. MED105	61.38	5.00E-48

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 under-methylation 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 meningococcal 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 *Neisseriaceum* 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
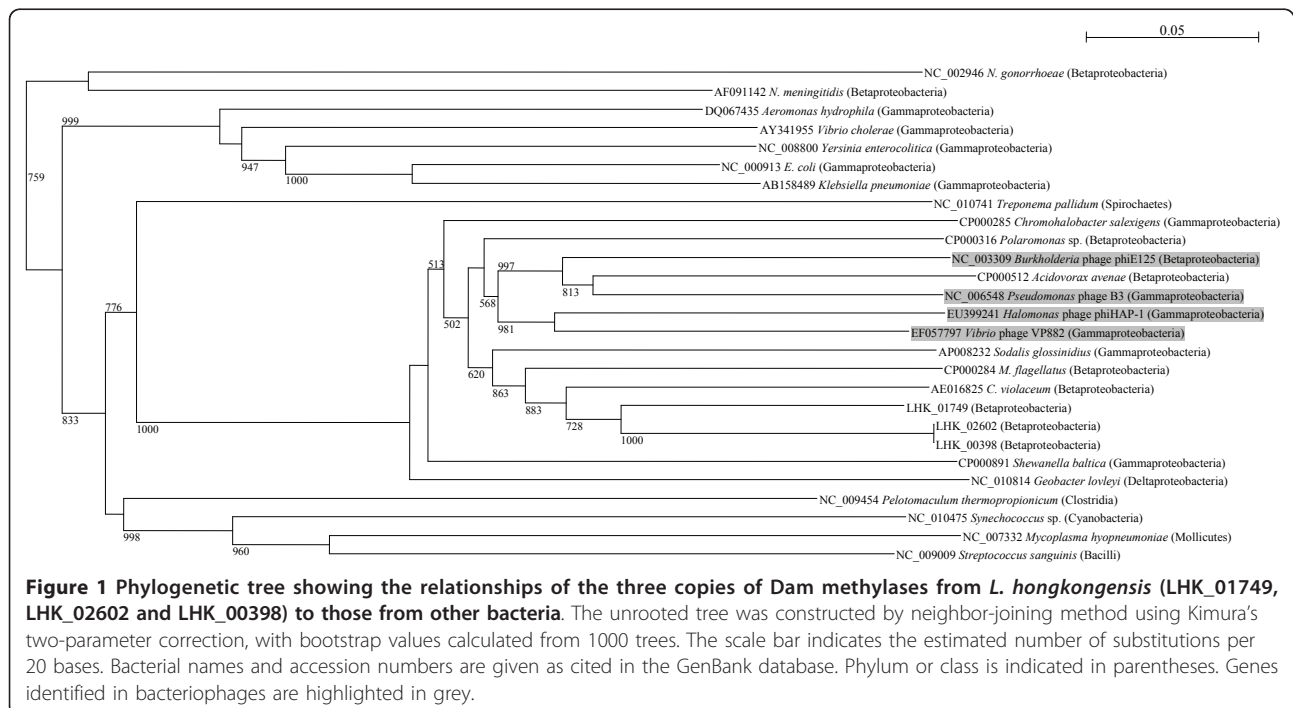

Figure 1 Phylogenetic tree showing the relationships of the three copies of Dam methylases from *L. hongkongensis* (LHK_01749, LHK_02602 and LHK_00398) to those from other bacteria. The unrooted tree was constructed by neighbor-joining method using Kimura's two-parameter correction, with bootstrap values 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.

Table 2 Homologous recombination 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
Initiation							
RecBCD pathway	*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
	recC	Exodeoxyribonuclease V gamma chain	ATP-dependent exonuclease and helicase, DNA-dependent ATPase and ATP-stimulated endonuclease	LHK_01203	*Pseudomonas aeruginosa*	48.11	0
	recD	Exodeoxyribonuclease V alpha chain	ATP-dependent exonuclease, ATPase and ATP-stimulated endonuclease	LHK_01201	*Pseudomonas putida*	50.47	1.00E-126
RecFOR pathway	*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	Holiday 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	Holiday 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 N′	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 synaptase), 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 is 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

Table 3 Other proteins involved in DNA repair

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							
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
	nrdA	Ribonucleoside-diphosphate reductase 1 subunit alpha	Catalyzes biosynthesis of deoxyribo-nucleotides from the corresponding ribonucleotides	LHK_01803	L. nitroferrum	71	0
	nrdB	Ribonucleoside-diphosphate reductase 1 subunit beta	Catalyzes biosynthesis of deoxyribo-nucleotides from the corresponding ribonucleotides	LHK_01801	L. nitroferrum	83.1	5.00E-177
	nrdE	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. hongkongensis HLHK9 (SSB protein)	68	3E-57
	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	62.43	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

Table 4 Replication proteins and their closest homologues

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_02345	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
	ihfA/ 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
	ihfB/ 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
				LHK_01506	N. gonorrhoeae	40.73	3.00E-76
	dnaG	DNA primase	Polymerase synthesizes small RNA primers for the Okazaki fragments on both template strands at replication forks	LHK_00463	C. violaceum	65.42	2.00E-155
	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 HLHK9 (Ssb protein)	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
				LHK_00959	C. violaceum	53.47	2.00E-18
	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 polymerse 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

Table 4 Replication proteins and their closest homologues (Continued)

	dnaX	DNA polymerse 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
	rnhA	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 lagging strands	LHK_00880	*L. nitroferrum*	77.3	2.00E-59
	rnhB	Ribonuclease HII	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
Termination factors	dam	DNA adenine methylase	Methylates DNA within the sequence GATC and protects the DNA from cleavage by restriction endonuclease	LHK_01749	*C. violaceum*	83.92	8.00E-131
				LHK_02602	*C. violaceum*	75	9.00E-113
				LHK_00398	*C. violaceum*	75	9.00E-113
	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
Topoisomerases	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 topoisomerse 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
	topA	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 *Lutiella 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 ($\alpha_2\beta\beta'\omega$) 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^{38} (RpoS), σ^{24} (RpoE) and σ^{54} (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

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

Product	Gene	*L. hongkongensis* HLHK 9	*N. meningitidis* MC58	*N. gonorrhoeae* FA1090	*C. violaceum* ATCC12472
ATP-dependent helicase	*hrpA*	+	+	+	+
ATP-dependent RNA helicase	*rhlE*	+	+	+	+
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	*fliA*	+	-	-	+[a]
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*	+	+	+	+

[a]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	DtxR	0
TetR	8	LexA	0
CRO/Cl/Xre	8	TrmB	0
LuxR	7	PenR/BlaI/MecI	0
GntR	6	SfsA	0
ArsR	4	CopG/RepA	0
MarR	4	ModE	0
Cold shock domain	3	PaiB	0
MerR	3	CtsR	0
AsnC	2	CodY	0
CRP-FNR	2	TrpR	0
DeoR	2	MtlR	0
Fur	2	ROS/MUCR	0
BolA/YrbA	2	MetJ	0
IclR	1	GutM	0
Rrf2	1	Crl	0
LytTR	1	ComK	0
HrcA	1	FlhD	0
SirB	1	RtcR	0
LacI	0	NifT/FixU	0

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

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
DeoR	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/CI/Xre	8	9	12	9
Fur	2	1	1	1
HrcA	1	1	0	0
SirB	1	1	1	1
Rrf2	1	2	2	2
BolA/YrbA	2	2	2	2
LytTR	1	4	0	0

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 homologue 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

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

Product	Gene	*L. hongkongensis* HLHK9	*N. meningitidis* MC58	*N. gonorrhoeae* FA1090	*C. violaceum* ATCC12472
Acid shock RNA protein	*asr*	+[a]	-	-	+
Acid-resistance protein, possible chaperone	*hdeA*	+[a]	-	-	-
Sigma factor 38	*rpoS*	+	-	-	+
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*	-	+	+	+

[a]Two copies of the gene are present

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; the toxic complexes formed by these ions preclude their physiological use by common 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 dppB, dppC, dppD and 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 the

dppBCDF locus, however, is not unique; it is also observed in *H. influenzae*[57]. The relatively well-characterized nickel/cobalt resistance system Cnr [58] and nickel/cobalt/cadmium resistance system Ncc [59] find no direct homologous systems in the *L. hongkongensis*; whilst the putative gene coding for a protein of similar function, in terms of sequence homology and functional domains, is traced to a CDS which encodes the putative NolG efflux pump of the resistance-nodulation-cell division superfamily (LHK_02819). In addition, two CDSs coding for an ABC transporter for cobalt (LHK_01077-01078) were found; it is uncertain whether this member of the nickel(II)-cobalt(II) uptake transporter (NiCoT) family may also transport nickel due to variations in the exact level of binding specificity [60]. Homologues of putative genes encoding dppA, dppBCDF (LHK_00667, LHK_00939-00942) and the histidine-rich glycoprotein (LHK_02812) were identified in the genome of *C. violaceum* but not published genomes of *N. gonorrhoeae* or *N. meningitidis*. No putative homologue of the locus containing genes coding for the ABC transporter for cobalt (LHK_01077-01078) was identified in *C. violaceum*, *N. gonorrhoeae* or *N. meningitidis*.

Cobalt

Cobalt is found in coenzyme B_{12}, 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 Cd^{2+} 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 Pb^{2+} 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*, *N. gonorrhoeae*, *N. meningitidis* and *C. violaceum* as described above. On the other hand, the *L. hongkongensis* genome possesses two copies of cspA which encodes cold shock transcription factor and one copy of cspD which encodes cold shock protein homologue (Table 10). There is only one copy of cspA in the genomes of *N. gonorrhoeae*, *N. meningitidis* and *C. violaceum*, whereas cspD is absent from the genomes of *N. gonorrhoeae* and *N. meningitidis*.

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* HLHK9	*N. meningitidis* MC58[b]	*N. gonorrhoeae* FA 1090[b]	*C. violaceum* ATCC12472	*B. subtilis* 168[b]	*E. coli* K12-MG1655[b]
Genome size (Mb)[a]	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[c]	0
NhaA Na^+:H^+ antiporter family	0	0	0	0	0	1
NhaB Na^+:H^+ antiporter family	0	0	0	0	0	1
NhaC Na^+:H^+ antiporter family	2	2	2	0	2	0
NhaD Na^+:H^+ antiporter family	0	0	0	0	0	0

[a]Genome 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
[b]Retrieved from www.membranetransport.org
[c]Not listed on www.membranetransport.org, see Krulwich *et. al.*

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

Product	Gene	*L. hongkongensis* HLHK 9	*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*	+[a]	+	+	+[a]
Regulator subunit of chaperonin GroEL-GroES	*groES*	+[a]	+	+	+[a]
ATP-dependent protease specificity component and chaperone	*clpA*	+	+	+	+
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	*htpG*	+	-	-	+
Heat shock protein, integral membrane protein	*htpX*	+	+	+	+[a]
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	*cspA*	+[a]	+	+	+
Cold shock protein homologue	*cspD*	+	-	-	+

[a]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* HLHK 9	*C. violaceum* ATCC 12472	*N. meningitidis* MC58	*N. gonorrhoeae* FA 1090
Sodium/hydrogen exchanger	-	Sodium efflux, hydrogen influx	LHK_00707 LHK_02296	CV2903 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	kefB	Potassium efflux, hydrogen influx	LHK_02018	CV3326	NMB0209	-
Glutathione-regulated potassium-efflux system protein	kef	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	-	-	-	-
ATP-sensitive inward rectifier potassium channel related transmembrane protein	-	Potassium uptake	-	CV1109	-	-
Low affinity potassium transport system protein	kup	Potassium uptake	LHK_01720 LHK_00121	CV2731 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	Compatible solute efflux	LHK_02562	CV1360	-	-
Small conductance mechanosensitive channel	mscS	Compatible solute efflux	LHK_01830 LHK_01942 LHK_02394 LHK_02965	CV0295 CV2330 CV2385 CV2962 CV4288	NMB0042 NMB0213	NGO1771 NGO2057
Osmotically inducible lipoprotein	osmB	-	LHK_01892	CV3209	-	-
Osmotically inducible lipoprotein	osmC	-	LHK_01612	-	-	-
Sodium glutamate symport carrier protein	gltS	Sodium and glutamate uptake	-	CV1105	NMB0085	NGO1890

Proton glutamate symport protein	-	Hydrogen and glutamate uptake	LHK_02672	CV1198 CV3409	-	-
Proline/betaine transproter	*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 CV4393 CV4395	-	-
Osmoprotectant transport system ATP-binding protein	-	Osmoprotectants uptake	-	CV4394	-	-
Outer membrane porin	*ompC*	Hydrophilic molecules uptake by passive diffusion	-	CV3424	-	-
Outer membrane porin	*ompF*	Hydrophilic molecules uptake by passive diffusion	-	-	-	-
Osmolarity sensor protein	*envZ*	Protein kinase of two-component regulatory system	-	CV0217	-	-
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 *betT* gene that encodes a transport protein for choline uptake is present in the *L. hongkongensis* genome. However, the *betA* and *betB* genes, that encode enzymes for metabolizing choline to glycine betaine, the osmotically active compound, are absent [70]. Similarly, the *glpR* and *glpK* genes are present. However, the *glpF* gene, another gene in the *glpFK* operon that encodes the glycerol uptake facilitator protein, is absent [71]. Therefore, the contributions of *betT*, *glpR* and *glpK* and their corresponding choline and glycerol transport systems to tolerance of osmotic stress in *L. hongkongensis* are unknown. The expressions of two other CDSs, *osmB* and *osmC*, which encode two osmotically inducible lipoproteins, have been found to be affected by change in osmolarity in *E. coli* [72,73]. Both *osmB* and *osmC* are membrane proteins of unknown function. In *E. coli*, it was observed that deletion of *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 *L. hongkongensis* genome, genes for oxidant-resistant dehydratase (*fumC*, *acnA*), superoxide scavenging (*sodB*), hydrogen peroxide scavenging (*ahpC*, *cpx*), exclusion and export of redox-cycling antibiotics (*acrA*, *acrB*, *tolC*), redox balancing (*nfnB*), DNA repair (*xthA*, *nth*, *mutM*, *mutY*, *mutT*), reduction of disulfide bonds (*trxA*, *trxB*, *gpxA*, *gshA*, *gshB*, *grxA*, *grxC*, *gor*) [77], limitation of iron availability (*bfr*, *dps*, *fur*) and reduction of iron-sulfur clusters (*fpr*, *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 *N. gonorrhoeae*, *N. meningitidis* and *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 *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 *L. hongkongensis*, *C. violaceum*, *N. gonorrhoeae* and *N. meningitidis* all contain one copy of *phrB* which encodes a photolyase for direct repair of DNA; and one copy each of *uvrA*, *uvrB*, *uvrC* and *uvrD* in the nucleotide excision repair system.

Starvation related CDSs

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 *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 *L. hongkongensis* were isolated demonstrates such: the permanganate value, a surrogate for organic carbon content, had a yearly mean of 1.25 mg O_2/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, *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^{38}, encoded by *rpoS*, is used to upregulate the expression of a number of genes. Some of these genes may be clustered with *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	H_2O_2, O_2	-	CV2793	-	-
	soxS	SoxS	SoxR	-	-	-	-
	oxyR	OxyR	H_2O_2	LHK_02531	CV3378	NMB0173	NGO1813
	ohrR	Organic hydroperoxide resistance transcriptional regulator	Organic peroxides	-	CV0210	-	-
	fnr	Fumarate/nitrate reductase regulator	O_2	LHK_00352	CV3647	NMB0380	NGO1579
	perR	PerR	H_2O_2	-	-	NMB1266	NGO0542
	lrp	Leucine-responsive protein	Leucine	LHK_01860	CV1913	NMB0573 NMB1650	NGO1294 NGO1407
Oxidant-resistant dehydratase isozymes							
	fumC	Fumarase C	SoxRS, RpoS, FNR	LHK_00495	CV1120	NMB1458	NGO1029
	acnA	Aconitase A	SoxRS, FNR, Fur, RpoS	LHK_02153 LHK_02309	CV1121 CV2054	NMB0433	-
Superoxide scavenging							
	sodA	Manganese superoxide dismutase	SoxRS, FNR	-	-	-	-
	sodB	Iron superoxide dismutase		LHK_01716	CV0867 CV2504	NMB0884	NGO0405
	sodC	Copper-zinc superoxide dismutase	H_2O_2, RpoS, FNR	-	-	NMB1398	-
Hydrogen peroxide scavenging							
	ahpC	Alky hydroperoxide reductase	OxyR, PerR	LHK_00938	CV3739	-	-
	ahpF	Alky hydroperoxide reductase	OxyR, PerR	-	-	-	-
	cpx	Cytochrome c peroxidase	FNR	LHK_02666	CV0300	-	NGO1769
	catalase/ peroxidase	Hydroperoxidase I	OxyR, RpoS	LHK_01300 LHK_02436	-	-	-
	catalase	Hydroperoxidase II	H_2O_2, RpoS	LHK_01264	CV3549	NMB0216	NGO1767
Exclusion and export of redox-cycling antibiotics							
	micF	Antisense RNA to porin OmpF	SoxRS, Lrp, OmpR	-	-	-	-
	acrA-acrB-tolC	Drug export system	SoxRS	LHK_01426-LHK_01425-LHK_01424	CV0435-CV0434-CV0433	NMB1716-NMB1715-NMB1714	NGO1365-NGO1364-NGO1363
				LHK_02129-LHK_02130-LHK_02131	CV2240-CV2241-CV2242		
				LHK_02929-LHK_02930-LHK_02931			
Redox balancing							
	nfnB	Nitroreductase	SoxRS	LHK_01953 LHK_03211	CV2244	NMB0804	NGO0388
DNA repair							
	xthA	Exodeoxyribonuclease III	H_2O_2, RpoS	LHK_02447	CV0877	NMB0399	NGO1561
	nth	Endonuclease III		LHK_01218	CV3293	NMB0533	NGO0139

Table 12 CDSs related to tolerance of oxidative stress in *L. hongkongensis*, *C. violaceum*, *N. meningitidis* and *N. gonorrhoeae*. *(Continued)*

nfo	Endonuclease IV	SoxRS	-	-	-	-
mutM	Formamidopyrimidine-DNA glycosylase	FNR	LHK_00316	CV4062	NMB1295	NGO0610
mutY	Adenine glycosylase		LHK_02781	CV3703	NMB1396	NGO0710
mutT	7,8-dihydro-8-oxoguanine triphosphatase		LHK_02262	CV1787	NMB1064	-
mutT homologues	MutT/NUDIX family protein		LHK_00322	CV0032	NMB0453	NGO1506
			LHK_00604	CV1112		
			LHK_01693	CV1586		
			LHK_01823	CV1767		
				CV3401		
				CV3611		
Protein repair						
msrAB	Protein-methionine-S-oxide reductase		LHK_01369 (*msrB*)	CV2325 (*msrA*) CV3212 (*msrB*)	NMB0044 (*msrAB*)	NGO2059 (*msrAB*)
Reduction of disulfide bonds						
trxA	Thioredoxin 1	ppGpp	LHK_01690	CV1584	NMB1366	NGO0652
-	Thioredoxin		LHK_00591	CV1325	NMB0006	NGO0057
			LHK_01462	CV4257	NMB1845	NGO1923
			LHK_01491	CV4279	NMB1958	NGO2124
			LHK_02476			
			LHK_02092			
trxC	Thioredoxin 2	OxyR	-	CV1106	-	-
trxB	Thioredoxin reductase		LHK_1482	CV1895 CV2813	NMB1324	NGO0580
-	Peroxiredoxin		LHK_02841	CV3708	NMB0946	NGO0926
gpxA	Glutathione peroxidase		LHK_00424	CV1107 CV3555 CV3787	NMB1621	-
gshA	Glutamate-cysteine ligase		LHK_03085	CV4276	NMB1037	NGO0608
gshB	Glutathione synthase		LHK_03093	CV4275	NMB1559	NGO1217
grxA	Glutaredoxin 1	OxyR	LHK_00503	CV3620	NMB0773	NGO0351
grxB	Glutaredoxin 2	RpoS, ppGpp	-	-	NMB1734	NGO1381
grxC	Glutaredoxin 3		LHK_02837	CV1126	NMB1790	NGO0114
gor	Glutathione reductase	OxyR, RpoS, ppGpp	LHK_01492	CV2037	NMB0947	NGO0925
Reduction of iron-sulfur clusters						
fpr	NADPH-ferredoxin reductase	SoxRS	LHK_02993	CV0086 CV4045	NMB1044 NMB1450	NGO0687 NGO0734
fldA	Flavodoxin	SoxRS	-	-	-	-
fldB	Flavodoxin	SoxRS	-	-	-	-
yggX	Iron trafficking protein	SoxRS	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 NMB0407	NGO1548 NGO1717
dsbB	Oxidoreductase		LHK_01744	CV3193	NMB1649	NGO1292
dsbG	Thiol:disulfide 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 CV3552	NMB1206 NMB1207	NGO0794 NGO0795
dps	DNA-binding protein	OxyR, RpoS	LHK_01835 LHK_03179	CV4253	-	-
fur	Ferric uptake regulator	PerR, OxyR, SoxRS	LHK_01431	CV1797	NMB0205	NGO1779
Protein binding						
hslO	Molecular chaperone Hsp33	H_2O_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

L. hongkongensis a *surE-pcm-nlpD-rpoS* cluster was observed (LHK_00356-00353). This is similar to *C. violaceum* ATCC12472 (CV_3679-3682), and was also observed in other pathogens such as *E. coli*, *Salmonella* Typhimurium [86] and *Yersinia pestis* [87]. In the *L. hongkongensis surE-pcm-nlpD-rpoS* cluster, there is an overlap between the *surE* and *pcm* genes; which was also observed in *C. violaceum*. Despite this overlapping, as shown in *E. coli*, the *pcm* gene can either be co-transcribed with the *surE* gene or transcribed on its own [88].

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 the sugar 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

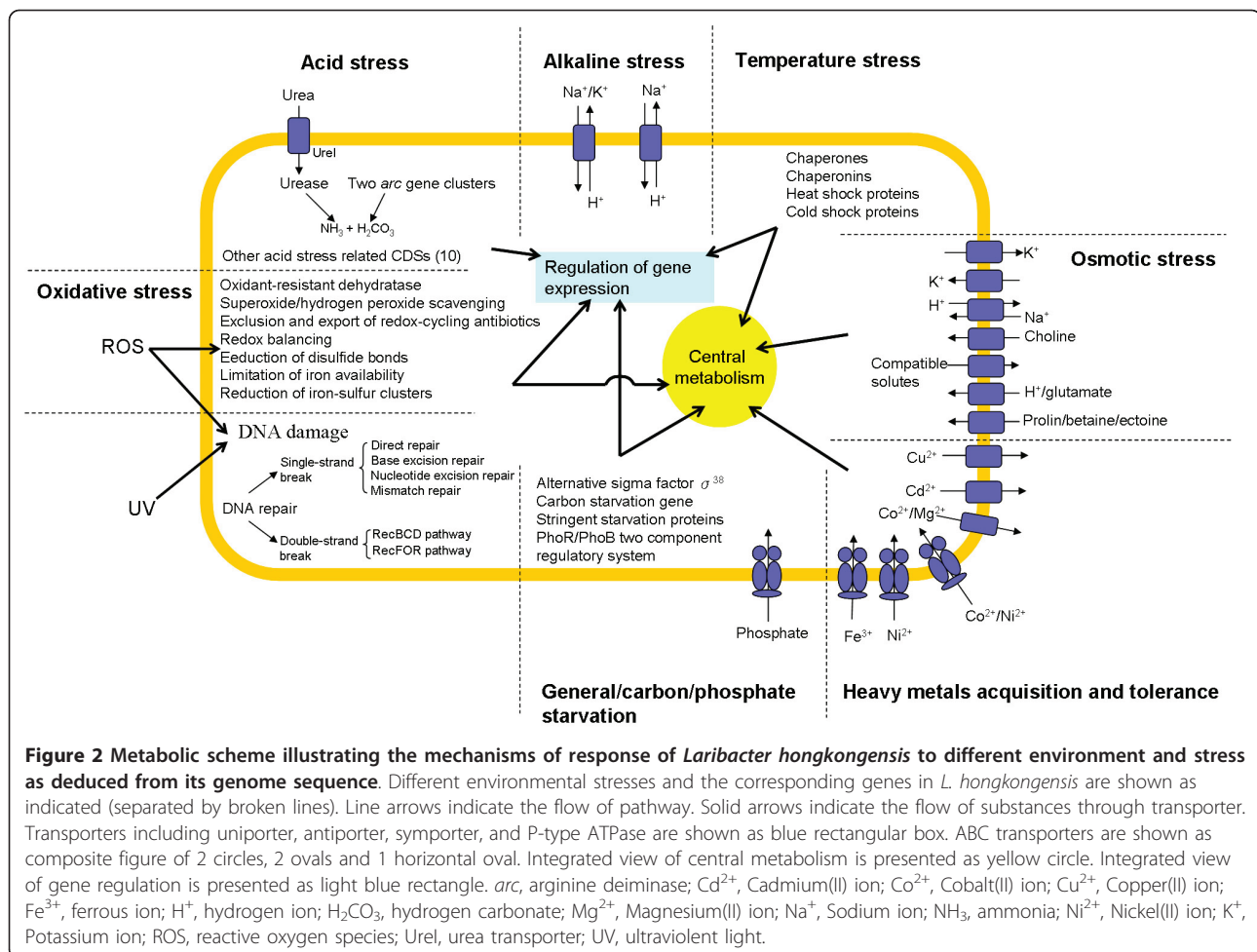It has long been observed that phosphate is often the limiting nutrient of algal and bacterial growth in freshwater environments [92-94]. Bacteria have evolved various mechanisms to enhance the uptake of phosphate, even by cell envelope elongation to increase the surface area to volume ratio [95]; albeit 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. vioalceum*, *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)

Figure 2 Metabolic scheme illustrating the mechanisms of response of *Laribacter hongkongensis* to different environment and stress as deduced from its genome sequence. Different environmental stresses and the corresponding genes in *L. hongkongensis* are shown as indicated (separated by broken lines). Line arrows indicate the flow of pathway. Solid arrows indicate the flow of substances through transporter. Transporters including uniporter, antiporter, symporter, and P-type ATPase are shown as blue rectangular box. ABC transporters are shown as composite figure of 2 circles, 2 ovals and 1 horizontal oval. Integrated view of central metabolism is presented as yellow circle. Integrated view of gene regulation is presented as light blue rectangle. *arc*, arginine deiminase; Cd^{2+}, Cadmium(II) ion; Co^{2+}, Cobalt(II) ion; Cu^{2+}, Copper(II) ion; Fe^{3+}, ferrous ion; H^+, hydrogen ion; H_2CO_3, hydrogen carbonate; Mg^{2+}, Magnesium(II) ion; Na^+, Sodium ion; NH_3, ammonia; Ni^{2+}, Nickel(II) ion; K^+, Potassium ion; ROS, reactive oxygen species; UreI, urea transporter; UV, ultraviolent light.

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 × version 1.81. *oriC* was predicted by Ori-finder http://tubic.tju.edu.cn/doric/.

protein CstA/B; DNA: Deoxyribonucleic acid;DnaK: Chaperone protein DnaK; DppB/C: Dipeptide transport system permease protein DppB/C; DppD/F: Dipeptide transport ATP-binding protein DppD/F; Dps: Deoxyribonucleic acid protection during starvation protein; DSB(s): Double-strand break(s); DSBR: Double-strand break repair; dsDNA: Double-stranded deoxyribonucleic acid; DUE: DNA-unwinding element; dUMP: Deoxyuridine 5'-monophosphate; dUTP: Deoxyuridine 5'-triphosphate; FbpA: Major ferric iron-binding protein FbpA; FbpB: Ferric transport system permease protein FbpB; FbpC: Ferric ions import ATP-binding protein FbpC; FeoA/B/C: Ferrous iron transport protein A/B/C; Fis: Factor for inversion stimulation; FNR: Fumarate and nitrate reduction regulatory proteins; Fpr: Ferredoxin-NADP reductase; FumC: Fumarase C; Fur: Furric uptake regulation protein Fur; G: Guanine; GlpF: Glycerol uptake facilitator protein; GlpK: Glycerol kinase GlpK; GlpR: Glycerol-3-phosphate regulon repressor protein GlpR; Gor: Glutathione reductase; GO system: 8oxodG system; GpxA: Glutathione peroxidase; GrxA/C: Glutaredoxin-1/3; GshA: Glutamate-cysteine ligase; GshB: Glutathione synthase; HoxN: High-affinity; nickel transport protein HoxN; HtpG: High temperature protein HtpG; IHF(s): Integration host factor(s); Kef: Glutathione-regulated potassium efflux protein Kef; KefB: Glutathione-regulated potassium efflux protein KefB; LB: Lysogeny broth; Lrp: Leucine-responsive regulatory protein; LysR: Transcriptional activator protein LysR; mg N/L: Milligrams of nitrogen per liter; mg O2/L: Milligrams of oxygen per liter; mg P/L: Milligrams of phosphorus per liter; MIT: Metal inorganic transport system; MMR: Mismatch repair; MopA: 60 kDa chaperonin; alternative name for GroEL or Cpn60; mRNA: Messenger ribonucleic acid; MutM: Formamidopyrimidine-DNA glycosylase; MutT: Mutator MutT protein; MutY: Adenine/thymine-specific adenine glycosylase; NAGK: N-acetyl-L-glutamate kinase; Ncc: Nickel-cobalt-cadmium resistance protein Ncc; NER: Nucleotide excision repair; NfnB: Oxygen-insensitive NAD(P)H nitroreductase; NhaA/B/C/D: Sodium/proton antiporter NhaA/B/C/D; NicO/A: High-affinity nickel transport protein NicO/A; NiCoT: Nickel(II)-cobalt(II) uptake transporter; NlpD: Lipoprotein NlpD; NolG: Nodulation protein NolG; Nth: Endonuclease III; O: Oxygen; oriC: Origin of replication; OsmB/C: Osmotically-inducible lipoprotein OsmB/C; OxyR: Hydrogen peroxide-inducible genes regulator OxyR; Pcm: Protein-L-isoaspartate O-methyltransferase; PhoB: Phosphate regulon transcriptional regulatory protein PhoB; PhoR: Phosphate regulon sensor protein PhoR; PhoU: Phosphate transport system protein PhoU; PhrB: Deoxyribodipyrimidine photo-lyase; PitA: Low-affinity inorganic phosphate transporter PitA; Pst: Phosphate-specific transport system; PstS/C/A/B: Phosphate-specific transport system protein S/C/A/B; RIDA: Regulatory inactivation of DnaA; RNA: Ribonucleic acid; RNAP: Deoxyribonucleic acid-directed ribonucleic acid polymerase; RpoS: RNA polymerase sigma factor RpoS; SodB: Superoxide dismutase SodB; SoxR: Redox-sensitive transcriptional activator SoxR; SSAP(s): Single-stranded deoxyribonucleic acid annealing protein(s); ssDNA: Single-stranded deoxyribonucleic acid; SspA/B: Stringent starvation protein SspA/B; SurA/E: Stationary-phase survival protein SurA/E; T: Thymine; TCRS: Two-component regulatory system; TLS: Translesion deoxyribonucleic acid synthesis; TolC: Outer membrane protein TolC; TRCF: Transcription-repair coupling factor; TrxA: Thioredoxin; TrxB: Thioredoxin reductase; UNG: Uracil deoxyribonucleic acid glycosylase; UreA/C/B: Urease subunit gamma/alpha/beta; UreE/F/G/D/I: Urease accessory protein UreE/F/G/D/I; UV: Ultraviolet; UvrA/B/C/D: UvrABC nucleotide excision repair system protein A/B/C/D; XthA Exodeoxyribonuclease III; YggX: Ferrous-trafficking protein; ZntA: Lead, cadmium, zinc and mercury-transporting ATPase ZntA

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