International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 9 | Special Issue 2 | 2019 | 284-293
| Research Article | Biological Sciences |Open Access |MCI Approved|
|UGC Approved Journal|

Comparative and Gene Expression Analysis of *Legionellosis*

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Received: 30 Jan 2019 / Accepted: 20 Feb 2019 / Published online: 01 Apr 2019
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Abstract

Legionellosis is the major problem in the world but the treatment of the disease is very difficult. The disease caused gene is *Legionella and the bacterium is Legionella pneumophila.* Kegg is used for the *Legionellosis* pathway identification. From the MGDB database list of genes present in the *Legionella pneumophila* are identified. The target gene for *Legionellosis* had been identified in NCBI. The expression of the target HtpB is viewed through Jcat. Comparative analysis of the target HtpB were done through Biocyc tool. Structural characterization of HtpB nucleotide and protein were carried out through tools like Protein colour,Yaspin,Ronn….etc

Keywords:

Keywords

Legionellosis, Legionella pneumophila, HTPB, Comparative analysis, Structural characterization.

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INTRODUCTION

Legionellosis is a potentially fatal infectious disease caused by Gram negative, aerobic bacteria belonging to the genus Legionella. Over 90% of legionellosis cases are caused by Legionella pneumophila, a ubiquitous aquatic organism that thrives in temperatures between 25 and 45 °C (77 and 113 °F), with an optimum around 35 °C (95 °F).legionellosis takes two distinct forms,

- Legionnaires' disease, also known as "Legion Fever", is the more severe form of the infection and produces high fever and pneumonia.
- Pontiac fever is caused by the same bacteria but produces a milder respiratory illness without pneumonia that resembles acute influenza. Pontiac fever also has a spontaneous resolution.

Legionnaires' disease acquired its name in July 1976 when an outbreak of pneumonia occurred among people attending a convention of the American Legion at the Bellevue-Stratford Hotel in Philadelphia. On January 18, 1977 the causative agent was identified as a previously unknown strain of bacteria, subsequently named Legionella. Some people can be infected with the Legionella bacteria and have only mild symptoms or no illness at all.

Outbreaks of *Legionnaires'* disease receive significant media attention. However, this disease usually occurs as single, isolated cases not associated with any recognized outbreak. When outbreaks do occur, they are usually in the summer and early autumn, though cases may occur at any time of year. The fatality rate of *Legionnaires'* disease has ranged from 5% to 30% during various outbreaks. "The death rate for patients who develop *Legionnaire's* disease while in the hospital is close to 50%, especially when antibiotics are started late," according to the NIH and U.S. National Library of Medicine service's MedlinePlus. Most infections occur in those who are middle-age or older.

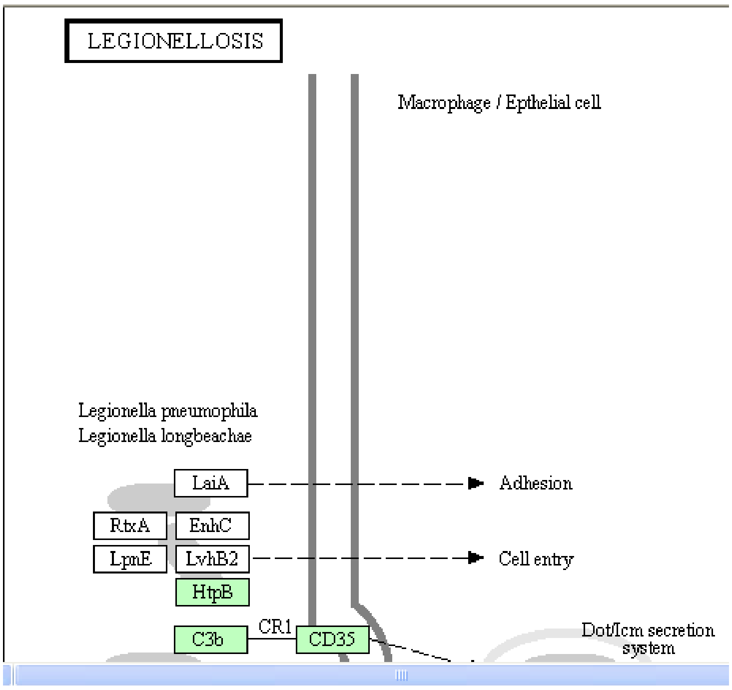
RESEARCH METHODOLOGY

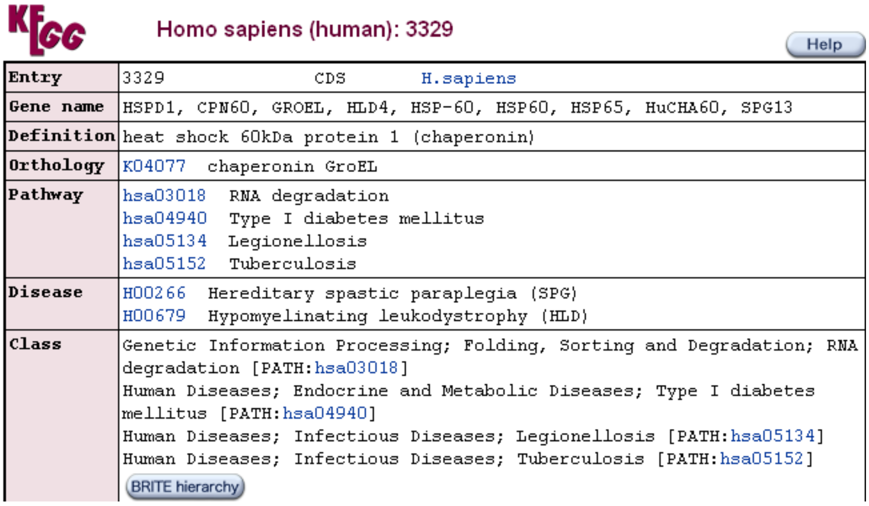
The metabolic pathways of *legionellosis* are obtained from kegg . The target gene for *Legionellosis* had been identified in NCBI. Gene identification in the species is obtained by using mbgd.The expression of the target HtpB is viewed through Jcat. Domain region of HtpB identified using domain linker prediction – short vector machine. Structural characterization of HtpB nucleotide and protein were carried out through tools like Protein colour,Yaspin,Ronn….etc. The sequence submitted to PFP for protein function studies. Biophysical Characterization of HtpB Protein were done using Dipole moment server.

RESULTS AND DISCUSSION

1. PATHWAY IDENTIFICATION

KEGG





Homo sapiens (human): 3329

Entry	3329 CDS H.sapiens
Gene name	HSPD1, CPN60, GROEL, HLD4, HSP-60, HSP60, HSP65, HuCHA60, SPG13
Definition	heat shock 60kDa protein 1 (chaperonin)
Orthology	K04077 chaperonin GroEL
Pathway	hsa03018 RNA degradation hsa04940 Type I diabetes mellitus hsa05134 Legionellosis hsa05152 Tuberculosis
Disease	H00266 Hereditary spastic paraplegia (SPG) H00679 Hypomyelinating leukodystrophy (HLD)
Class	Genetic Information Processing; Folding, Sorting and Degradation; RNA degradation [PATH:hsa03018] Human Diseases; Endocrine and Metabolic Diseases; Type I diabetes mellitus [PATH:hsa04940] Human Diseases; Infectious Diseases; Legionellosis [PATH:hsa05134] Human Diseases; Infectious Diseases; Tuberculosis [PATH:hsa05152]

The above result shows the metabolic pathways of *legionellosis Disease.*

2. SEQUENCE RETRIEVAL

NCBI

1. HtpB – Protein

>gi|149690|gb|AAA25298.1| 58-kDa common antigen [Legionella pneumophila]
MAKELRFGDDARLQMLAGVNALADAVQVTMGPRGRNVVLEKSYGAPTVTKDGVSVAKEIEFEHRFMNMGA
QMVKEVASKTSDTAGDGTTTATVLARSILVEGHKAVAAGMNPMDLKRGIDKAVLAVTKKLQAMSKPCKDS
KAIAQVGTISANSDEAIGAIIAEAMEKVGKEGVITVEDGNGLENELSVVEGMQLIAVHSPYFINNQQNMS
CELEHPFILLVDKKVSSIREMLSVLEGVAKSGRPLLIIAEDVEGEALATLVVNNMRGIVKVCAVKAPGFG
DRRKAMLQDIAILTKGQVISEEIGKSLEGATLEDLGSAKRIVVTKENTTIIDGEGKATEINARITQIRAQ
MEETTSDYDREKLQERVAKLAGGVAVIKVGAATEVEMKEKKARVEDALHATRAAVEEGIVAGGGVALIRA
QKALDSLKGDNDDQNMGINILRRAIESPMRQIVTNAGYEASVVVNKVAEHKDNYGFNAATGEYGDMVEMG
ILDPTKVTRMALQNAASVASLMLTTECMVADLPKKEEGVGAGDMGGMGGMGGMGGMM

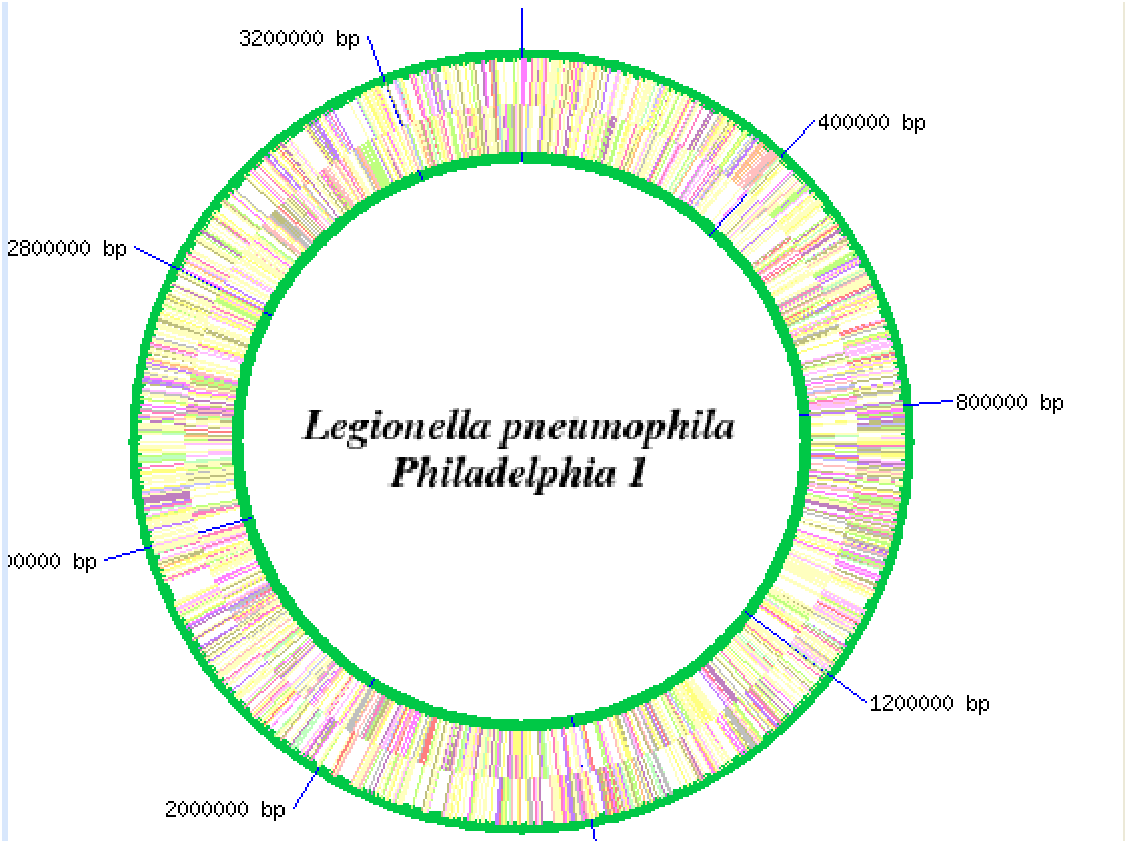
2.HtpB – Nucleotide

>gb|M91673.1|LPNHTPB:211-1854 Legionella pneumophila 58-kDa common antigen (htpB) gene, complete cds
ATGGCTAAAGAATTACGTTTTGGTGATGACGCTCGCCTACAAATGCTTGCTGGTGTTAATGCATTAGCAG
ATGCGGTTCAAGTTACTATGGGTCCACGTGGTCGTAATGTTGTATTGGAAAAATCTTATGGCGCTCCTAC
TGTAACTAAAGACGGTGTGTCTGTTGCCAAAGAAATTGAGTTTGAGCATCGTTTCATGAACATGGGCGCT
CAAATGGTTAAAGAAGTGGCTTCTAAAACTTCTGATACTGCTGGTGATGGTACTACTACTGCAACAGTAT
TGGCTCGTTCTATTCTTGTTGAAGGTCACAAAGCAGTTGCTGCTGGTATGAATCCAATGGATCTCAAACG
CGGTATTGATAAAGCAGTATTAGCAGTTACCAAAAAATTACAAGCTATGTCTAAGCCATGCAAAGACAGC
AAAGCTATTGCTCAAGTTGGAACTATTTCTGCTAATTCCGATGAAGCGATTGGTGCTATCATTGCTGAAG
CAATGGAAAAAGTTGGTAAAGAGGGTGTTATTACCGTTGAAGATGGTAATGGATTGGAAAATGAGCTTTC
TGTTGTTGAAGGTATGCAATTGATCGCGGTACATTCTCCATACTTTATCAACAACCAGCAAAACATGAGC
TGTGAACTTGAGCATCCATTCATTTTATTGGTTGACAAAAAAGTTTCCAGTATTCGTGAAATGTTGTCCG
TATTGGAAGGTGTTGCCAAATCTGGTCGTCCTTTATTGATCATTGCAGAAGATGTTGAAGGCGAAGCTTT
AGCTACTCTGGTAGTCAACAACATGCGCGGTATTGTAAAAGTATGTGCTGTCAAAGCGCCTGGTTTTGGT
GATCGCCGCAAAGCGATGTTGCAAGACATTGCTATTTTGACTAAGGGTCAAGTTATTTCTGAAGAAATTG
GCAAGAGCTTGGAAGGTGCTACTCTGGAAGATCTTGGTAGTGCTAAGCGAATCGTTGTTACCAAAGAAAA
CACTACTATCATTGATGGTGAAGGAAAGGCAACTGAAATTAATGCTCGTATTACTCAAATTCGTGCACAA
ATGGAAGAAACCACTTCTGATTACGATAGAGAAAAATTACAAGAGCGCGTTGCTAAACTAGCTGGTGGTG
TTGCTGTTATCAAAGTTGGCGCTGCTACAGAAGTTGAAATGAAAGAGAAGAAAGCACGTGTTGAAGATGC
TCTTCATGCTACTCGCGCTGCAGTAGAAGAAGGTATCGTTGCCGGTGGTGGTGTTGCCTTGATTCGTGCT
CAGAAAGCTCTTGATTCATTGAAAGGCGATAATGACGATCAAAATATGGGTATCAATATTTTACGTCGCG
CTATTGAATCTCCAATGCGTCAAATTGTTACTAACGCAGGATATGAAGCTTCTGTTGTAGTAAACAAGGT
AGCTGAGCACAAAGACAACTACGGTTTCAACGCTGCAACTGGTGAATACGGTGATATGGTTGAGATGGGT
ATTCTTGATCCAACCAAAGTAACCCGTATGGCTCTGCAAAATGCAGCTTCTGTAGCCAGTTTGATGTTGA
CTACTGAATGTATGGTTGCTGATCTGCCTAAGAAAGAAGAAGGTGTTGGTGCCGGCGATATGGGCGGCAT
GGGCGGAATGGGTGGCATGGGCGGAATGATGTAA

The above results show the Fasta format sequence of HtpB target.

3. GENE IDENTIFICATION

MGDB



Species	*Legionella pneumophila*
Strain	Philadelphia 1
Taxonomy ID	272624
Num. ORF	2943
Institution	Columbia Genome Center
Reference	Science 305 (5692), 1966-1968 (2004)
Sequences	chromosome-1 NC_002942.5 3,397,754
Function Category	MBGD ▾

List of Genes: *Legionella pneumophila* Philadelphia 1

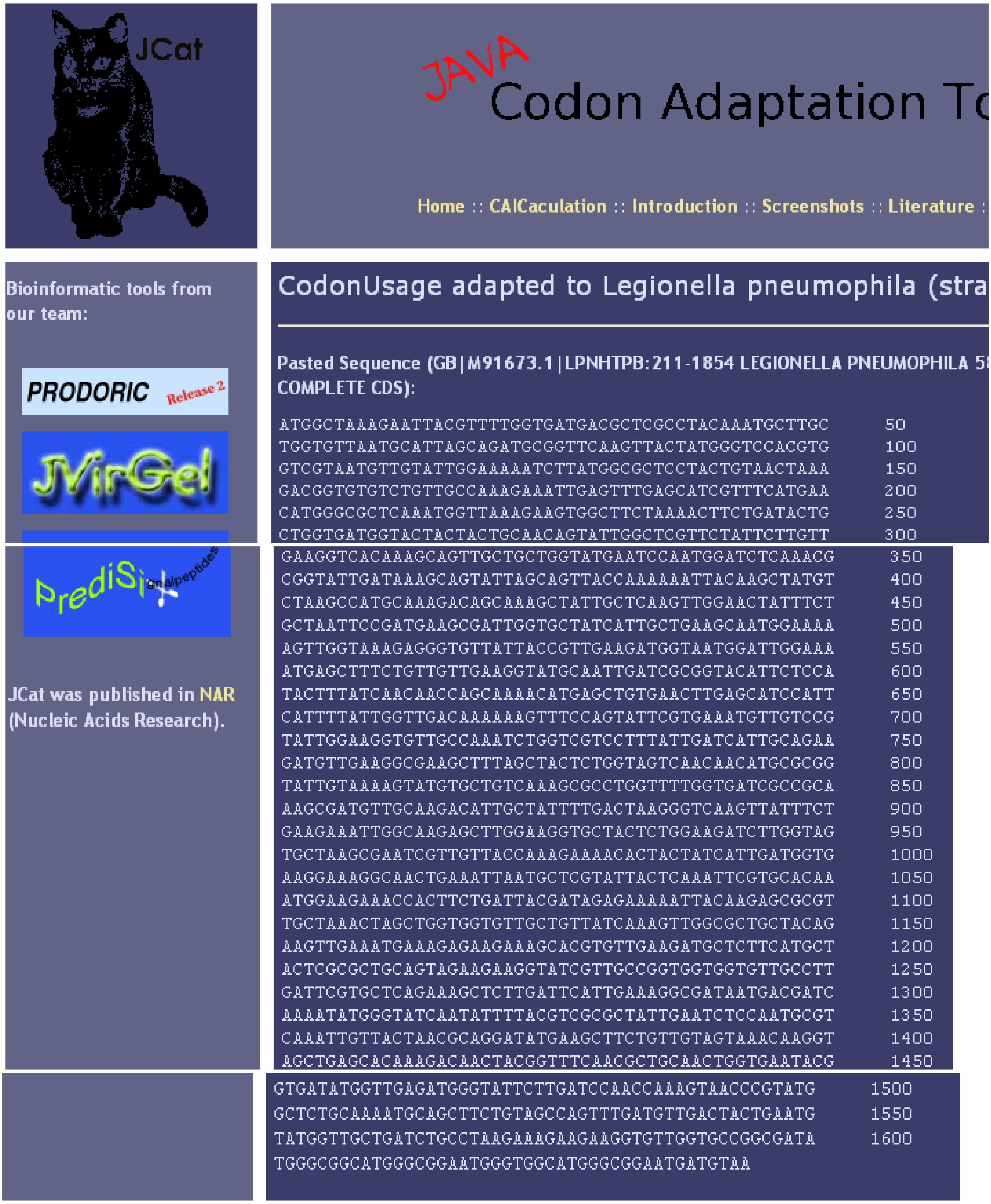
Id	name	from	to	dir	seq_acc	Descry
LPG0001	dnaA	654	2012	+	NC_002942.5	chromosomal replication initiation protein
LPG0002		2026	3129	+	NC_002942.5	DNA polymerase III beta chain
LPG0003		3126	4187	+	NC_002942.5	DNA recombination and repair protein ATPase RecF
LPG0004	gyrB	4482	6902	+	NC_002942.5	DNA gyrase subunit B
LPG0005		7271	8317	-	NC_002942.5	peptidylarginine deiminase
LPG0006	speA	8314	10206	-	NC_002942.5	arginine decarboxylase

LPG0007		10226	11083	-	NC_002942.5	Hydrolase
LPG0008		11612	12871	-	NC_002942.5	hypothetical protein

The above result shows the chromosomal structure and the list of genes in Legionella pneumophila species.

4. GENE EXPRESSION ANALYSIS

JCAT



Bioinformatic tools from our team:

JCat was published in NAR (Nucleic Acids Research).

CodonUsage adapted to Legionella pneumophila (stra

Pasted Sequence (GB|M91673.1|LPNHTPB:211-1854 LEGIONELLA PNEUMOPHILA 5
COMPLETE CDS):

```
ATGGCTAAAGAATTACGTTTTGGTGATGACGCTCGCCTACAAATGCTTGC      50
TGGTGTTAATGCATTAGCAGATGCGGTTCAAGTTACTATGGGTCCACGTG      100
GTCGTAATGTTGTATTGGAAAAATCTTATGGCGCTCCTACTGTAACTAAA      150
GACGGTGTGTCTGTTGCCAAAGAAATTGAGTTTGAGCATCGTTTCATGAA      200
CATGGGCGCTCAAATGGTTAAAGAAGTGGCTTCTAAAACTTCTGATACTG      250
CTGGTGATGGTACTACTACTGCAACAGTATTGGCTCGTTCTATTCTTGTT      300
GAAGGTCACAAAGCAGTTGCTGCTGGTATGAATCCAATGGATCTCAAACG      350
CGGTATTGATAAAGCAGTATTAGCAGTTACCAAAAAATTACAAGCTATGT      400
CTAAGCCATGCAAAGACAGCAAAGCTATTGCTCAAGTTGGAACTATTTCT      450
GCTAATTCCGATGAAGCGATTGGTGCTATCATTGCTGAAGCAATGGAAAA      500
AGTTGGTAAAGAGGGTGTTATTACCGTTGAAGATGGTAATGGATTGGAAA      550
ATGAGCTTTCTGTTGTTGAAGGTATGCAATTGATCGCGGTACATTCTCCA      600
TACTTTATCAACAACCAGCAAAACATGAGCTGTGAACTTGAGCATCCATT      650
CATTTTATTGGTTGACAAAAAAGTTTCCAGTATTCGTGAAATGTTGTCCG      700
TATTGGAAGGTGTTGCCAAATCTGGTCGTCCTTTATTGATCATTGCAGAA      750
GATGTTGAAGGCGAAGCTTTAGCTACTCTGGTAGTCAACAACATGCGCGG      800
TATTGTAAAAGTATGTGCTGTCAAAGCGCCTGGTTTTGGTGATCGCCGCA      850
AAGCGATGTTGCAAGACATTGCTATTTTGACTAAGGGTCAAGTTATTTCT      900
GAAGAAATTGGCAAGAGCTTGGAAGGTGCTACTCTGGAAGATCTTGGTAG      950
TGCTAAGCGAATCGTTGTTACCAAAGAAAACACTACTATCATTGATGGTG      1000
AAGGAAAGGCAACTGAAATTAATGCTCGTATTACTCAAATTCGTGCACAA      1050
ATGGAAGAAACCACTTCTGATTACGATAGAGAAAAATTACAAGAGCGCGT      1100
TGCTAAACTAGCTGGTGGTGTTGCTGTTATCAAAGTTGGCGCTGCTACAG      1150
AAGTTGAAATGAAAGAGAAGAAAGCACGTGTTGAAGATGCTCTTCATGCT      1200
ACTCGCGCTGCAGTAGAAGAAGGTATCGTTGCCGGTGGTGGTGTTGCCTT      1250
GATTCGTGCTCAGAAAGCTCTTGATTCATTGAAAGGCGATAATGACGATC      1300
AAAATATGGGTATCAATATTTTACGTCGCGCTATTGAATCTCCAATGCGT      1350
CAAATTGTTACTAACGCAGGATATGAAGCTTCTGTTGTAGTAAACAAGGT      1400
AGCTGAGCACAAAGACAACTACGGTTTCAACGCTGCAACTGGTGAATACG      1450
GTGATATGGTTGAGATGGGTATTCTTGATCCAACCAAAGTAACCCGTATG      1500
GCTCTGCAAAATGCAGCTTCTGTAGCCAGTTTGATGTTGACTACTGAATG      1550
TATGGTTGCTGATCTGCCTAAGAAAGAAGAAGGTGTTGGTGCCGGCGATA      1600
TGGGCGGCATGGGCGGAATGGGTGGCATGGGCGGAATGATGTAA
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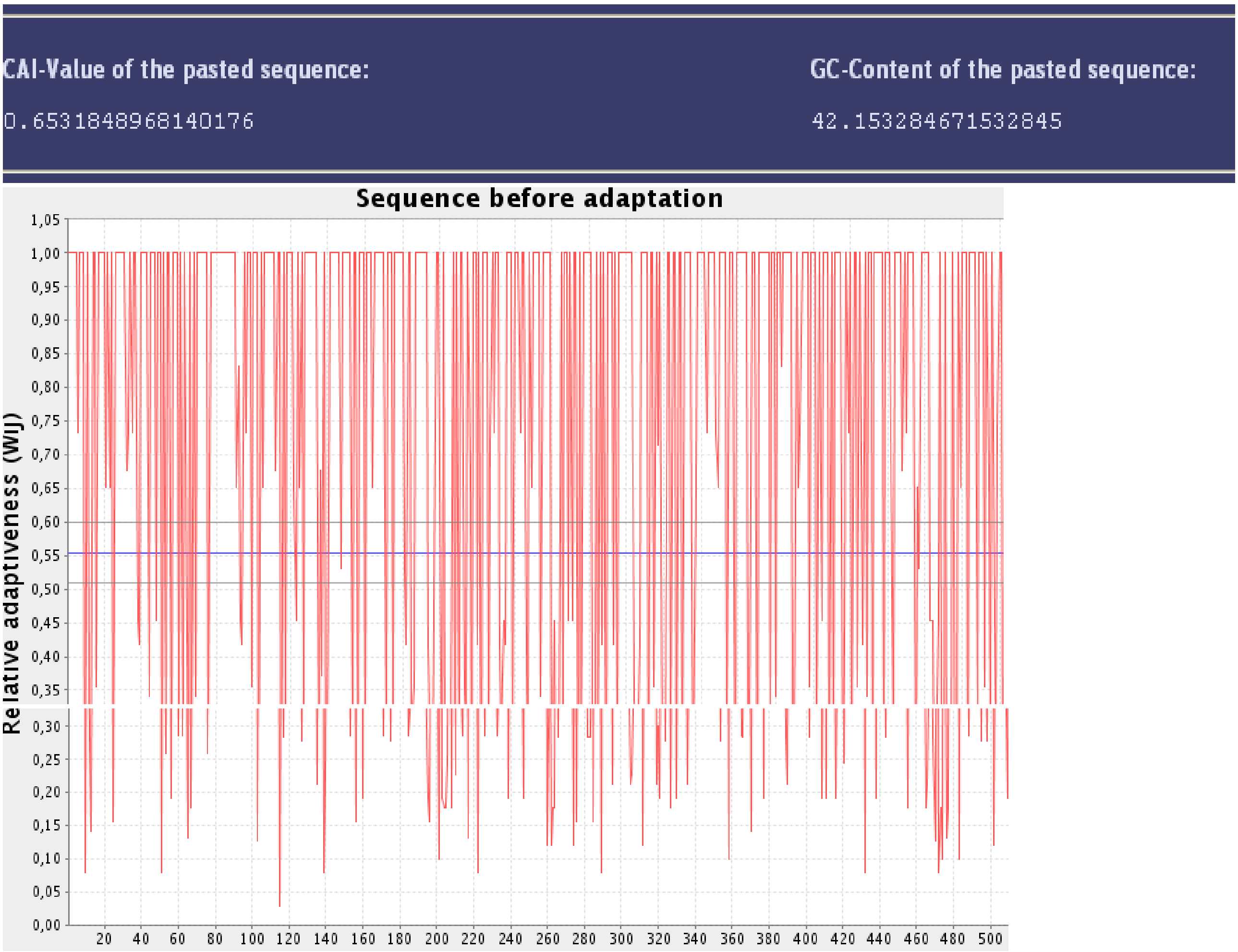


CAI-Value of the pasted sequence:
0.6531848968140176

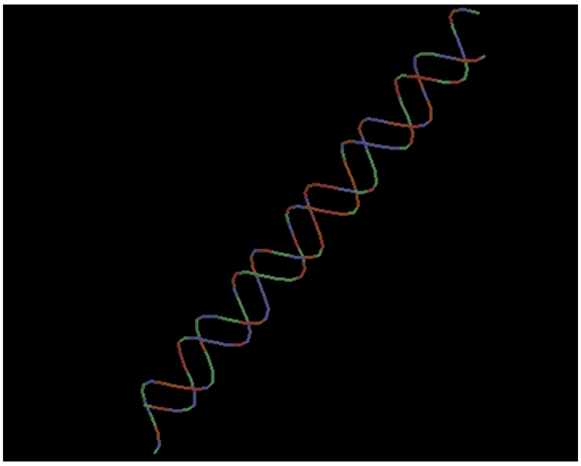
GC-Content of the pasted sequence:
42.153284671532845

The above result shows the CAI-value of the improved sequence is 1.0 and GC- content is 32. 360097323600975.Sequence before adaptation, after adaptation and their relative adaptiveness is 1.05-500.The codons of the GC-content of *legionella pneumophila* (strain paris):38.3731082861924 and the translation is 50-500.

5. STRUCTURE ANALYSIS

A) NUCLEOTIDE STRUCTURE ANALYSIS

i) DNA SEQUENCE TO STRUCTURE



This result shows the structure of dna sequence and indicated the nucleotides in the different colours.

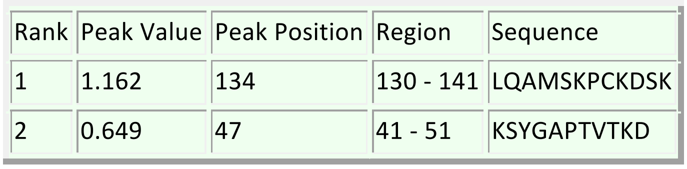
B) PROTEIN STRUCTURE ANALYSIS

a) DOMAIN ANALYSIS

i) DLP - SVM

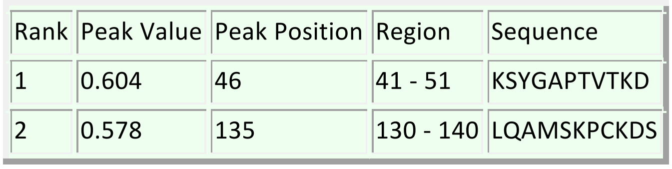
Candidate Region:

SVM-All



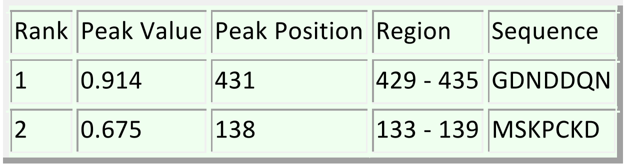
Rank	Peak Value	Peak Position	Region	Sequence
1	1.162	134	130 - 141	LQAMSKPCKDSK
2	0.649	47	41 - 51	KSYGAPTVTKD

SVM-Long



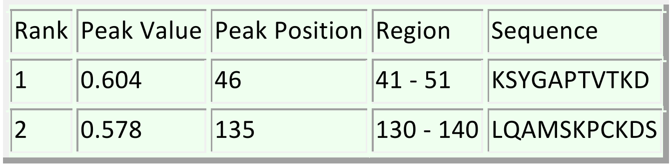
Rank	Peak Value	Peak Position	Region	Sequence
1	0.604	46	41 - 51	KSYGAPTVTKD
2	0.578	135	130 - 140	LQAMSKPCKDS

SVM-Short



Rank	Peak Value	Peak Position	Region	Sequence
1	0.914	431	429 - 435	GDNDDQN
2	0.675	138	133 - 139	MSKPCKD

SVM-Joint

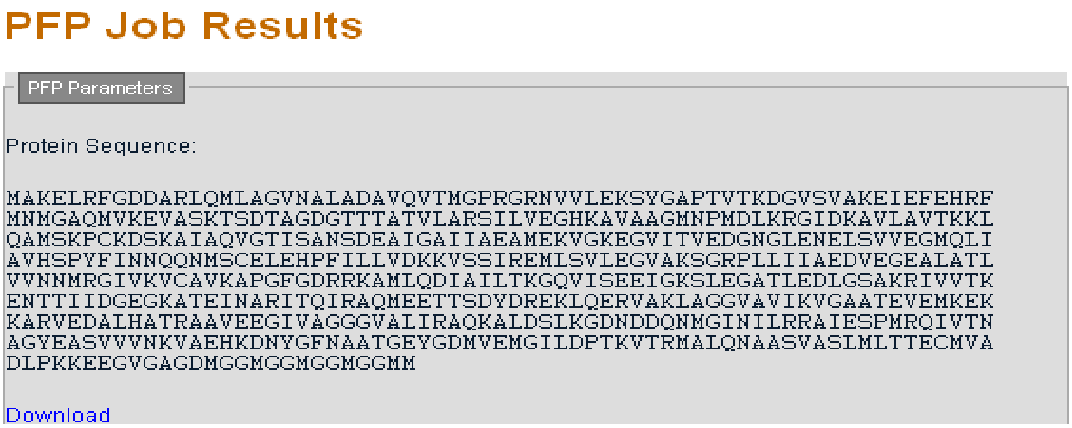


Rank	Peak Value	Peak Position	Region	Sequence
1	0.604	46	41 - 51	KSYGAPTVTKD
2	0.578	135	130 - 140	LQAMSKPCKDS

The above result shows the domain region of the sequence (independent character of the protein). This information is useful for protein annotation. For example, from 41 to 51 is the domain region in the sequence

b) PROTEIN FUNCTION PREDICTION

i) PFP: AUTOMATED PROTEIN FUNCTION PREDICTION SERVER



PFP Job Results

PFP Parameters

Protein Sequence:

```
MAKELRFGDDARLQMLAGVNALADAVQVTMGPRGRNVVLEKSYGAPTVTKDGVSVAKEIEFEHRF
MNMGAQMVKEVASKTSDTAGDGTTTATVLARSILVEGHKAVAAGMNPMDLKRGIDKAVLAVTKKL
QAMSKPCKDSKAIAQVGTISANSDEAIGAIIAEAMEKVGKEGVITVEDGNGLENELSVVEGMQLI
AVHSPYFINNQQNMSCELEHPFILLVDKKVSSIREMLSVLEGVAKSGRPLLIIAEDVEGEALATL
VVNNMRGIVKVCAVKAPGFGDRRKAMLQDIAILTKGQVISEEIGKSLEGATLEDLGSAKRIVVTK
ENTTIIDGEGKATEINARITQIRAQMEETTSDYDREKLQERVAKLAGGVAVIKVGAATEVEMKEK
KARVEDALHATRAAVEEGIVAGGGVALIRAQKALDSLKGDNDDQNMGINILRRAIESPMRQIVTN
AGYEASVVVNKVAEHKDNYGFNAATGEYGDMVEMGILDPTKVTRMALQNAASVASLMLTTECMVA
DLPKKEEGVGAGDMGGMGGMGGMGGMM
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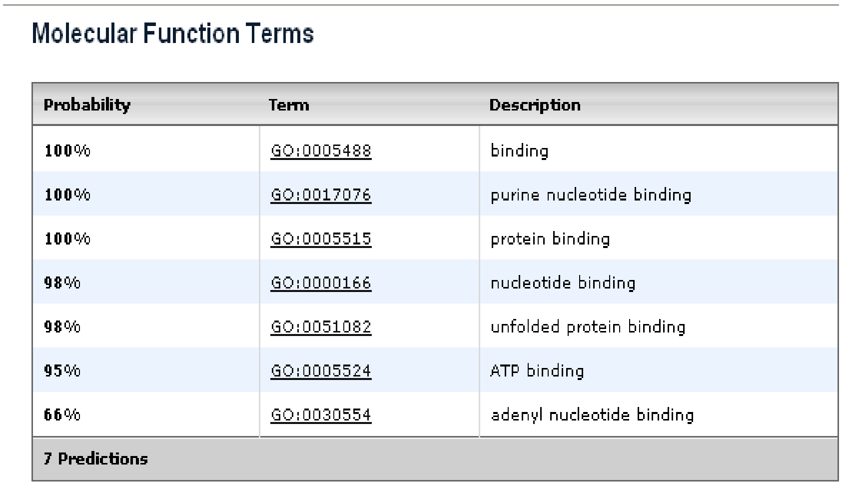
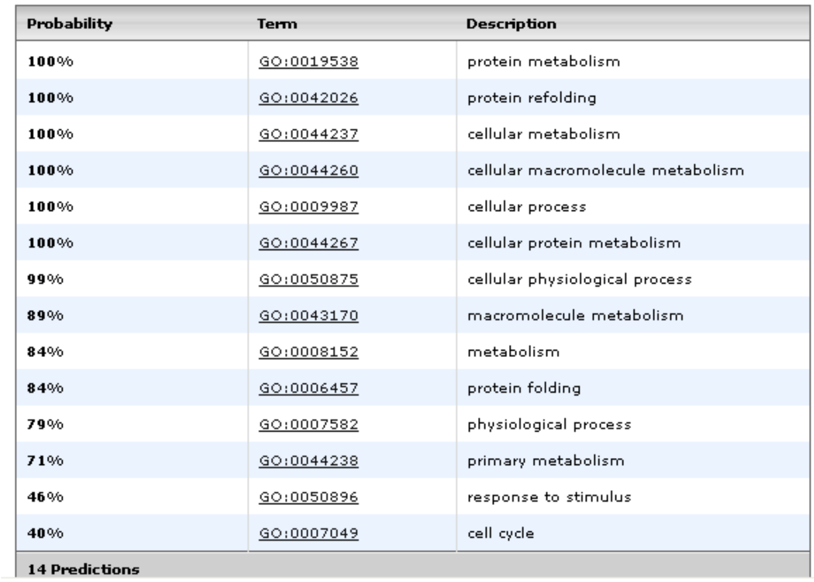


Molecular Function Terms

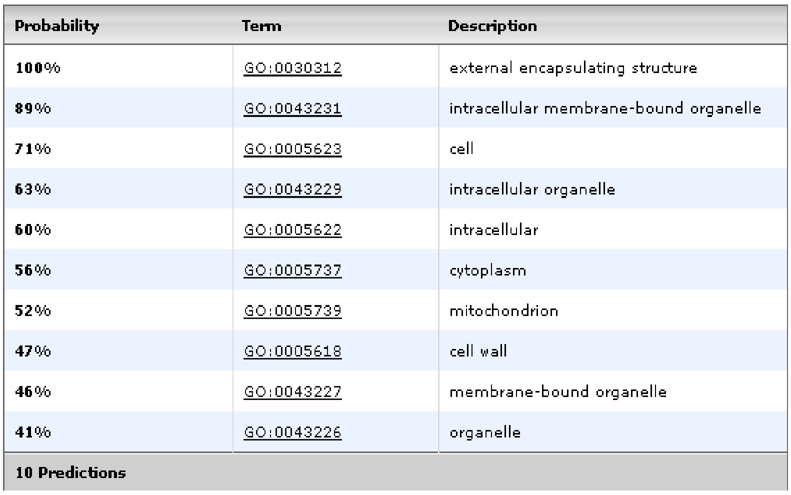
Probability	Term	Description
100%	GO:0005488	binding
100%	GO:0017076	purine nucleotide binding
100%	GO:0005515	protein binding
98%	GO:0000166	nucleotide binding
98%	GO:0051082	unfolded protein binding
95%	GO:0005524	ATP binding
66%	GO:0030554	adenyl nucleotide binding
7 Predictions		

Biological Process Terms



Probability	Term	Description
100%	GO:0019538	protein metabolism
100%	GO:0042026	protein refolding
100%	GO:0044237	cellular metabolism
100%	GO:0044260	cellular macromolecule metabolism
100%	GO:0009987	cellular process
100%	GO:0044267	cellular protein metabolism
99%	GO:0050875	cellular physiological process
89%	GO:0043170	macromolecule metabolism
84%	GO:0008152	metabolism
84%	GO:0006457	protein folding
79%	GO:0007582	physiological process
71%	GO:0044238	primary metabolism
46%	GO:0050896	response to stimulus
40%	GO:0007049	cell cycle
14 Predictions		

Cellular Component Terms



Probability	Term	Description
100%	GO:0030312	external encapsulating structure
89%	GO:0043231	intracellular membrane-bound organelle
71%	GO:0005623	cell
63%	GO:0043229	intracellular organelle
60%	GO:0005622	intracellular
56%	GO:0005737	cytoplasm
52%	GO:0005739	mitochondrion
47%	GO:0005618	cell wall
46%	GO:0043227	membrane-bound organelle
41%	GO:0043226	organelle
10 Predictions		

The above result shows the functional similarities between the target protein sequences. The target HtpB protein shows the binding value is 100 %.

CONCLUSION

Legionella pneumophila is a thin, ærobic, pleomorphic, flagellated, non-spore forming, Gram-negative bacterium of the genus Legionella. L.pneumophila is the primary human pathogenic bacterium in this group and is the causative agent of legionellosis or Legionnaires' disease. Legionellosis disease is serious and can be life-threatening. However, most people recover with antibiotic treatment. The target sequence collected using NCBI database. Highly expressed gene identification was done using Jcat tool. Domain region of HtpB identified using domain linker prediction – short vector machine and hydrophobicity region of HtpB identification done using protein colourer. The sequence submitted to PFP for protein function studies. In future the testing of wet lab protocol will lead in designing novel therapeutics.

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