



Research Article

GENOME WIDE ANALYSIS OF INTERGENIC REGIONS IN *MYCOPLASMA* GENOMES FOR FUNCTIONAL CHARACTERIZATION: AN *IN SILICO* APPROACH

ANGAMUTHU KANDAVELMANI AND JEGADEESAN RAMALINGAM

Department of Plant Molecular Biology and Bioinformatics, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu, India

*Corresponding Author: Email - kandavelmani@gmail.com

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Abstract- Advances in sequencing technology have unraveled the whole genome sequence of many *Mycoplasma* species which has provided a major milestone in the study of minimal genomes. During the course of analyzing the complete genomes of 11 *Mycoplasma* species, it was observed that around 7–17 % of these genome sequence lie in the intergenic space. A genome-wide analysis using *in silico* methods was carried out to explore the functional elements concealed in these intergenic sequences. A total of 6840 intergenic sequences were extracted from these 11 genomes and were subjected to a series of systematic analysis based on bioinformatics approach. An extensive analysis of all the 6840 intergenic sequences, markedly affirmed the protein coding potentials of 195 intergenic sequences. Of these 195 sequences, functional domains were predicted for 37 sequences from 8 *Mycoplasma* genomes. The outcome of the present study would facilitate a better understanding of the evolution, pathogenicity, drug resistance, virulence mechanism and development of novel antibiotics to combat *Mycoplasma* infections.

Keywords- *in silico*, intergenic, minimal genome, concealed, coding potential

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Introduction

Bacterial intergenic regions have become one of the major thrust areas of research as the functional elements concealed in these regions still remain unraveled. Bacterial intergenic regions encompass various control elements and signals for the regulation of transcription [1]. Microbial intergenic regions have also been found to harbor functional elements like transposons [2]. Genome-wide analysis of the intergenic regions through computational and experimental methods in the genome of *Mesorhizobium huakuii* 7653R has resulted in the identification of putative novel small non-coding RNAs [3]. Intergenic regions have largely been thought to contain small non-coding RNAs [4]. However, studies suggest that intergenic regions may possess functional small-length coding regions that are not normally annotated in gene prediction programs [5-7]. A high degree of sequence conservation and genetic diversity have been discovered from the analysis of intergenic regions of *Trypanosoma cruzi* genome [8]. As intergenic spacer regions are highly conserved, these sequences are used as potential tools for strain typing of *Mycoplasma synoviae* species [9]. In general, intergenic regions of prokaryotes are relatively shorter than that of eukaryotes. In contrast to eukaryotes, evolutionary pressure has minimized the amount of non-functional DNA in prokaryotic genomes. Most of the bacterial and archeal genomes were found to have 6-14 % of non-coding DNA [10]. Prokaryotes make use of the operon system to reduce the number of regulatory elements that control transcription of genes [11].

Mycoplasmas are the smallest and simplest known free-living and self-replicating organisms with smallest genomes (0.58–2.20Mb). During the course of their reductive evolution from Gram-positive bacteria, *Mycoplasmas* have lost several genes involved in metabolism, cellular process and energy production, transcription regulation, cell division and heat shock response [12].

Mycoplasmas make a heavy use of operon system to potentially reduce the number of regulatory elements and to increase the gene density. As *Mycoplasmas* have limited biosynthetic capabilities, most *Mycoplasmas* exist as parasites with strict host and tissue specificities. *Mycoplasmas* are widespread in nature as obligate parasites of humans, mammals, reptiles, fish, arthropods, and plants [13]. *Mycoplasmas* cause chronic inflammatory disease of the respiratory system, urogenital tract and joints. As *Mycoplasmas* are conditional pathogens, they are found to be associated with various diseases, including pneumonia, arthritis, meningitis and chronic urogenital tract disease [14, 15]. *Mycoplasmas* are also found to be associated with various pathological conditions and intrauterine infections, including pyelonephritis, pelvic inflammatory disease, chorioamnionitis, endometritis, and postpartum fever, leading to important complications such as preterm birth, low birth weight, spontaneous abortion, stillbirth, premature birth, infertility, and perinatal mortality [16-22]. *Mycoplasmas* have also been found to be associated with bacterial vaginosis [23]. *Mycoplasmas* are difficult to culture in the laboratory because of their complex nutritional requirements and specialized requirement of growth conditions. Despite the use of metagenomic sequencing method for the identification of *Mycoplasmas* that are not detected by conventional methods [24, 25], study of *Mycoplasmas* by *in silico* methods remains more significant. *In silico* experiments act as precursor in steering researchers to design experiments with a higher competence and reduced cost. A complete understanding of the biology of an organism necessarily starts with knowledge of its genetic makeup. The advent of new rapid sequencing methods has made available the whole genome sequences of a number of *Mycoplasma* species thereby allowing for a computational insight towards understanding of their complete biology and evolutionary perspectives.

In the present study, a genome wide analysis of intergenic regions from 11 *Mycoplasma* genomes was carried out. Various *in silico* strategies have been employed to explore the functional elements concealed in the intergenic space.

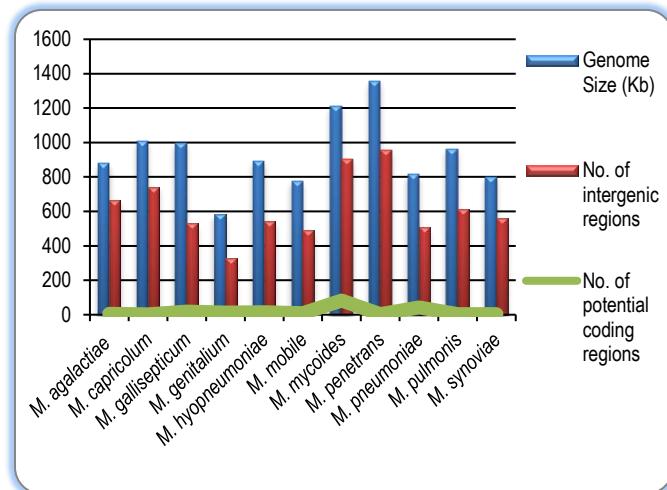


Fig-1 Genome size versus number of intergenic and potential coding regions identified Kb – Genome length in kilo base pairs

Table-1 Intergenic regions extracted from the whole genome sequences

SN	Species	Genome Size (bp)	No. of IG regions†	No. of overlapping gene pairs	Length of IG region (bp)*	% IG region§
1	<i>M. agalactiae</i>	8,77,438	667	112	96664	11.02
2	<i>M. capricolum</i>	10,10,023	742	102	106649	10.56
3	<i>M. gallisepticum</i>	9,96,422	532	218	108807	10.92
4	<i>M. genitalium</i>	5,80,076	326	179	44268	7.63
5	<i>M. hyopneumoniae</i>	8,92,758	544	172	79042	8.85
6	<i>M. mobile</i>	7,77,079	489	149	64170	8.26
7	<i>M. mycoides</i>	12,11,703	904	141	208475	17.21
8	<i>M. penetrans</i>	13,58,633	957	109	146429	10.78
9	<i>M. pneumoniae</i>	8,16,394	507	208	87645	10.74
10	<i>M. pulmonis</i>	9,63,879	614	192	83585	8.67
11	<i>M. synoviae</i>	7,99,476	558	145	73862	9.24

†Total number of intergenic regions obtained in each genome; *Total length of intergenic sequences extracted in each genome; §Percentage of the extracted intergenic sequence in the whole genome sequence; bp–base pair

Table-5 Summarized results of the potential coding regions identified in 11 complete *Mycoplasma* genomes

SN	Species	Genome Size (bp)	No. of potential coding regions	Length of potential coding regions (bp)	% potential coding region
1	<i>M. agalactiae</i>	8,77,438	5	1338	0.15
2	<i>M. capricolum</i>	10,10,023	2	1377	0.13
3	<i>M. gallisepticum</i>	9,96,422	18	9443	0.94
4	<i>M. genitalium</i>	5,80,076	13	6198	1.06
5	<i>M. hyopneumoniae</i>	8,92,758	14	3000	0.33
6	<i>M. mobile</i>	7,77,079	9	6639	0.85
7	<i>M. mycoides</i>	12,11,703	82	38916	3.21
8	<i>M. penetrans</i>	13,58,633	3	696	0.051
9	<i>M. pneumoniae</i>	8,16,394	42	13215	1.61
10	<i>M. pulmonis</i>	9,63,879	4	774	0.08
11	<i>M. synoviae</i>	7,99,476	3	741	0.09

bp–base pair

Materials and Methods

Sequence Retrieval

Whole genome sequences from 11 species namely, *Mycoplasma agalactiae* PG2 (NC_009497.1), *Mycoplasma capricolum* subsp. Capricolum ATCC 27343 (NC_007633.1), *Mycoplasma gallisepticum* str. R (low) (NC_004829.2), *Mycoplasma genitalium* G37(NC_000908.2), *Mycoplasma hyopneumoniae* J

(NC_007295.1), *Mycoplasma mobile* 163K (NC_006908.1), *Mycoplasma mycoides* subsp. *mycoides* SC str. PG1(NC_005364.2), *Mycoplasma penetrans* HF-2(NC_004432.1), *Mycoplasma pneumoniae* M129 (NC_000912.1), *Mycoplasma pulmonis* UAB CTIP (NC_002771.1) and *Mycoplasma synoviae* ATCC 25204 (NZ_CP011096.1) were retrieved from the Genome database of the National Centre for Biotechnology Information [www.ncbi.nlm.nih.gov/genome].

Extraction of intergenic sequences

Making use of the genome annotations available at NCBI's genome database, whole genome sequences of all the 11 *Mycoplasma* species were scanned to extract the gaps between the genes. Certain genes were found to be overlapping which is a common occurrence in prokaryotes to attain genome compaction. These overlapping genes were excluded and only intergenic nucleotide sequences were extracted. A total of 6,840 intergenic sequences were extracted from the 11 *Mycoplasma* genomes.

Sequence Comparison for identification of homology

The extracted intergenic sequences were subjected to sequence similarity search to identify homologs. The BLASTx program [26] was used to identify high-scoring homologous sequences. The BLASTx program first translated the input intergenic nucleotide sequences into protein sequence in all six possible reading frames. These protein sequences were then searched against the non-redundant protein sequence database to identify protein sequence matches. The statistical significance of the identified protein sequence matches was also calculated by the BLASTx program. Intergenic sequences showing protein coding potentials and their respective orthologs were filtered out for further analysis.

Identification of protein coding region and gene prediction

A sum of 286 intergenic sequences which showed the presence of ortholog evidence was analyzed using the programs, GeneMark [27] and GeneMark.hmm [28]. Identification of protein coding region and gene prediction were carried out using the prokaryotic versions of the programs GeneMark and GeneMark.hmm respectively. Both the programs incorporate a non – homogeneous Markov chain models for coding region prediction. In addition, GeneMark employs Bayesian algorithm whereas GeneMark.hmm uses a hidden Markov model (HMM). Both these programs predicted coding potential for 195 intergenic sequences.

Calculation of coding potential

The 195 intergenic sequences which were predicted to possess protein coding potentials were analyzed using Coding Potential Calculator [29], a Support Vector Machine based classifier. The program incorporates six biologically meaningful sequence features to assess the protein coding potential.

Function prediction by domain identification

Pfam is the consortium of protein families which are represented by multiple sequence alignment and hidden Markov models [30]. The Pfam program was used to predict the function of intergenic sequences based on domain search. The input nucleotide sequences were translated into protein sequence in six possible frames. A protein domain search was conducted to identify the functional domains present in these translated sequences.

Results and Discussion

The whole genome sequences of the 11 *Mycoplasma* species under study were scanned using the genome annotations available at the genome database of the National Centre for Biotechnology Information. Some of the genes in these *Mycoplasma* species were found to be overlapping. [Table-1] shows the total numbers of overlapping gene pairs identified in 11 *Mycoplasma* genomes under study. Overlapping genes are found to be a common occurrence of prokaryotic genomes and they can be thought of as the results of evolutionary pressure to minimize the genome size. It has been shown that overlapping genes are a consistent feature of microbial genomes [31]. However, it has been indicated that incidental elongation of the coding regions has led to the existence of many overlapping genes in the genomes of *M. genitalium* and *M. pneumoniae* [32].

Table-4 Functional domains identified in the translated intergenic sequences through Pfam search

Species	IG Start	IG Stop	IG Length	Pfam Domain	Score
<i>M.agalactiae</i>	227187	2,28,764	1578	DUF285	15.09
	498315	4,98,690	376	Ribosomal_L33	61.26
<i>M.gallisepticum</i>	255298	2,58,275	2978	FIVAR	40.21
	431265	4,31,927	663	Flavodoxin_1	25.58
	462768	4,64,303	1536	Myco_haema	287.3
	929511	931116	1606	Transposase_mut	40.89
<i>M.genitalium</i>	84045	8827	4183	Adhesin_P1	95.02
	167181	170006	2826	MgpC	315.4
	213550	216015	2466	MgpC	41.23
	229068	232008	2941	MgpC	1.85
	428113	430579	2467	MgpC	309.04
<i>M.hypopneumoniae</i>	90812	91550	739	Dnaj	104.96
	349246	350488	1243	Methylase_S	16.76
<i>M.mobile</i>	449935	451156	1222	Competence	73.44
	476091	476760	670	RecO_N	21.5
	564403	565470	1068	PseudoU_synth_2	60.15
<i>M.mycooides</i>	148917	151265	2349	Transposase_8	3.56
	165938	166865	928	Rve	33.6
	279951	280595	645	Transposase_8	4.06
	363554	366577	3024	Transposase_11	107.69
	430966	434875	3910	Transposase_11	126.19
	441059	441882	824	DUF28	241.19
	466225	471547	5323	Transposase_11	126.19
	638016	641974	3959	Transposase_11	109.45
	760709	762511	1803	Transposase_11	108.92
	888052	889735	1684	Transposase_8	3.56
	927749	929169	1421	Transposase_11	121.66
	968221	969186	966	Rve	33.6
	1051370	1051861	492	ABC_tran	14.86
	1053003	1055841	2839	Transposase_11	104.02
	1141349	1141849	501	DUF285	62.34
	1174931	1177813	2883	Transposase_11	125.83
	1198688	1201578	2891	Transposase_11	125.83
<i>M.penetrans</i>	2587	3001	415	S4	10.37
<i>M.pneumoniae</i>	116710	118311	1602	Adhesin_P1	115.62
	168082	169041	960	Adhesin_P1	19.06
	335269	336478	1210	Lipoprotein_10	53.65

Excluding these overlapping gene pairs, sequences of gaps between the genes were extracted from the genomes of *Mycoplasma* species which resulted in a total of 6840 intergenic sequences [Table-1]. These intergenic sequences were then subjected to similarity search using BLASTx. The program translated the input intergenic nucleotide sequences in all six possible reading frames and matched against the non-redundant protein sequence database. Homologs with E-values <10-6, which imply the presence of protein coding segments, were then extracted. Of the 6840 intergenic sequences analyzed, 286 sequences were found to have significant sequence similarities with protein sequences from *Mycoplasma* and other species. These 286 intergenic sequences which showed the presence of ortholog evidence were further analysed using the ab initio tools Genemark and Genemark.hmm respectively to identify the protein coding regions and to predict the genes harbored in them. Combined results of BLASTx, GeneMark and GeneMark.hmm shows the coding potential for a total of 195 intergenic sequences. The coding potential of these sequences were then calculated using Coding Potential Calculator, a Support Vector Machine based classifier [Table-2].

The orthologs of these 195 intergenic sequences obtained through BLASTx search are presented in [Table-3]. These 195 intergenic sequences were further examined using Pfam to identify domains of known function. Pfam search on the protein sequences of these candidate genes showed that 37 of them had a known domain [Table-4]. [Table-5] summarizes the potential coding regions identified in the 11 complete genomes of *Mycoplasma*.

Of the analyzed *Mycoplasma* genomes, *M. mycooides* was found to harbour the highest number of intergenic sequences with coding potential. *M. penetrans* which is having the largest genome among the 11 *Mycoplasma* genomes under study is found to possess only 3 intergenic regions with coding potential. Though, there was a strong correlation between the genome size and the percentage of intergenic region, no correlation was observed between the genome size and the identified number of intergenic regions with protein coding potential [Fig-1]. All the predicted genes satisfied the criteria of both ab initio and presence of ortholog evidence.

Most of the newly predicted coding regions were found to be homologous with known and hypothetical proteins from closely related bacterial species including their own genome which in turn makes the predictions more reliable. Functional domains have been identified for 37 candidate genes using Pfam search. *Mycoplasma* genomes were found to harbor multiple copies of gene sequences. Analysis of *M. genitalium* has revealed the occurrence of multiple copies of the gene MgPa, which is responsible for adhesion. *M. pneumoniae* genome has been reported to contain multiple copies of genes encoding cytadhesion proteins [13]. Adhesion of *Mycoplasma* to the host epithelium is an essential step for colonization and for infection. Loss of cytadherence due to mutation results in the loss of infectivity. Thus, adherence has been proved to be a major virulence factor for *Mycoplasma* pathogenesis [13, 33]. In the genomes of *M. genitalium* and *M. pneumoniae* cytadherence has been reported to be a complex multifactorial process which requires a group of adhesion proteins namely P1, P30, P116, MgPa and HMW1-3 [13, 34]. It has also been reported that only portions of the proximal regions of the genes encoding for adhesion related proteins occur in multiple copies in these genomes [35]. However, the present study shows that in the genomes of *M. genitalium* and *M. pneumoniae*, most of the functional domains predicted through Pfam search are related to adhesion proteins and that their corresponding DNA sequence show strong protein coding potential [Table-2]. The present study also shows that the genome of *M. genitalium* harbors MgPC domain (adhesion) with highest score [Table-4].

Most the functional domains identified in the translated intergenic sequences of *M. mycooides* through Pfam search are related to transposase [Table-4]. However, many transposase genes have been already reported in the original annotation of *M. mycooides* genome [36]. Transposase enzymes play a vital role in dictating genomic rearrangement events. Putative transposase genes have been identified in the intergenic transcribed spacer regions of *M. imitans* genome [37]. With further experimental analysis, the putative transposase genes predicted in the present study could also be included in the annotation.

Computational analysis followed by microarray based experimental studies has identified the existence of expression signals in the intergenic regions of *Mycobacterium tuberculosis* [38]. Furthermore, with the use of computational methods and experimental validations, 6 novel small RNA genes have been successfully identified in the intergenic regions of *Salmonella enterica Typhimurium* [39]. Hence it is reasonable to presume that there could be unidentified coding segments concealed in the intergenic regions of *Mycoplasma* genomes under study. However, *in silico* substantiations of the present study could urge Mycoplasmologists to further investigate these repeat elements to be used as genetic markers.

Conclusion

Genome-wide *in silico* analysis has identified the functional elements concealed in the intergenic regions of complete genomes of 11 species of *Mycoplasma*. This would help Mycoplasmologists to design novel experimental methods for the efficient identification of antibacterial targets and genetic markers in these genomes. The findings of the present study could also pave way for the elucidation of evolution and virulence mechanism of *Mycoplasma*.

This in turn would facilitate the drug design process to bring forward novel antibiotics against *Mycoplasmas* and to combat antibiotic resistance.

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***Principal Investigator: Dr A Kandavelmani**

University: Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu
Author Contributions

Author Contributions: Both author equally contributed

Author statement: Both authors read, reviewed, agree and approved the final manuscript

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Table-2 Intergenic sequences from 11 *Mycoplasma* genomes with protein coding potentials

SN	IG Start	IG End	IG Length	Gene Start	Gene End	Gene Length	Strand	CPC Score
<i>M.agalactiae</i>								
1	5993	6326	334	5994	6326	333	-	0.99997
2	622612	622881	270	622614	622850	237	+	0.67453
3	764420	765348	929	764772	764975	204	+	0.20957
4	801638	801946	309	801640	801945	306	-	0.1378
5	824718	825105	388	824720	824977	258	+	1.30342
<i>M.capricolum</i>								
6	227187	228764	1578	227451	228662	1212	+	2.13288
7	498315	498690	376	498466	498630	165	+	0.12997
<i>M.gallisepticum</i>								
8	6078	6368	291	6079	6165	87	+	0.4255
9	147012	149311	2300	147333	147887	555	-	1.4463
10	188644	189838	1195	188646	188924	279	-	2.6741
11	255298	258275	2978	255298	255366	69	+	5.66456
12	399521	401928	2408	399523	399684	162	+	3.12165
13	431265	431927	663	431265	431498	234	+	0.2263
14	462768	464303	1536	462770	463745	975	+	3.24752
15	522679	524140	1462	522758	522925	168	+	2.92266
16	576074	578622	2549	576283	577506	1224	-	7.01267
17	585392	586234	843	585717	585977	261	-	0.79256
18	595070	597948	2879	595402	596886	1485	-	7.00315
19	698461	699809	1349	698465	699073	609	-	0.80244
20	725306	726705	1400	725390	725980	591	-	3.21039
21	804557	807427	2871	804967	806574	1608	-	5.0006
22	929511	931116	1606	929673	929849	177	+	1.64162
23	945783	947567	1785	946262	946519	257	-	0.61213
24	963747	965068	1322	964065	964298	234	-	5.33426
25	968392	969453	1062	968513	968980	468	-	4.83178
<i>M.genitalium</i>								
26	84045	88227	4183	84082	84408	327	+	2.56225
				86025	86114	90	-	1.59723
27	167181	170006	2826	167182	167613	432	+	2.98776
28	174794	175804	1011	174977	175360	384	+	1.1701
29	213550	216015	2466	213552	214571	1020	+	2.74111
30	220439	220606	168	220440	220598	159	+	0.37385
31	229068	232008	2941	229168	229335	168	+	2.84887
32	273319	273789	471	273320	273745	426	+	0.47267
33	312809	315300	2492	312855	313250	396	+	2.83069
34	315378	315700	323	315467	315697	231	+	1.0705
35	327053	327432	380	327054	327431	378	-	3.24502
36	428113	430579	2467	428235	429662	1338	+	3.71496
37	455948	456754	807	456055	456264	210	+	1.95053
38	578582	579223	642	578584	579222	639	-	1.09749
<i>M.hopneumoniae</i>								
39	90812	91550	739	90897	90998	102	+	1.88916
40	119923	120295	373	119925	120053	129	-	0.0535
41	228251	228736	486	228638	228736	99	-	0.52602
42	285645	286428	784	286213	286338	126	+	1.88278
43	349246	350488	1243	349735	349860	126	+	1.32357
44	363448	364020	573	363449	363703	255	+	0.2464
45	385509	385719	211	385510	385719	210	+	0.00753
46	392581	393351	771	392718	392927	210	+	0.24054
47	479967	480221	255	479967	480221	255	+	0.87454
48	506138	506370	233	506138	506368	231	-	0.22366

49	555366	555803	438	555366	555692	327	-	0.95874
50	731046	731202	157	731046	731201	156	-	0.20662
51	775977	776482	506	775979	776482	504	+	4.85977
52	892062	892333	272	892063	892332	270	-	0.386835
<i>M.mobile</i>								
53	44446	44745	300	44447	44740	294	-	0.23588
54	201647	202393	747	201847	202392	546	-	0.31483
55	344911	345496	586	345075	345239	165	+	0.24087
56	449935	451156	1222	450060	451154	1095	+	2.94279
57	476091	476760	670	476091	476756	666	+	4.08823
58	482794	483248	455	482796	483248	453	-	0.0386257
59	564403	565470	1068	564722	565429	708	+	2.54534
60	739974	742109	2136	740432	741814	1383	+	6.58653
61	757981	759865	1885	758416	759744	1329	-	2.89462
<i>M.mycoides</i>								
62	56758	56989	232	56760	56987	228	-	1.08819
63	81703	82308	606	81794	82111	318	-	1.5832
64	116950	117661	712	117155	117439	285	-	1.20412
65	133924	134935	1012	133926	134750	825	-	1.82739
66	141980	144915	2936	143148	143300	153	-	3.67143
67	147747	148799	1053	147857	148039	183	-	1.2613
68	148917	151265	2349	149276	150049	774	+	2.12295
69	163385	165394	2010	163524	164501	978	-	1.61787
70	165938	166865	928	166133	166807	675	+	0.52509
71	186263	187583	1321	186264	186518	255	+	1.88113
72	244994	246808	1815	245059	245181	123	-	2.5083
				245981	246589	609	+	0.78422
73	262031	263021	991	262157	262834	678	-	1.59224
74	265192	265990	799	265212	265988	777	-	1.25495
75	267813	268197	385	267813	268082	270	+	0.51962
76	277825	278429	605	278134	278337	204	+	0.31386
77	279951	280595	645	279952	280002	51	+	3.6287
78	283455	284173	719	283457	284035	579	-	0.73807
79	325337	326509	1173	325337	326509	1173	+	4.9151
80	350917	351234	318	350919	351233	315	-	1.20105
81	363554	366577	3024	363671	364951	1281	+	2.4318
82	387059	391127	4069	387197	387517	321	+	1.07672
				387558	387710	153	-	3.31367
83	393713	393921	209	393713	393919	207	-	0.77984
84	418227	420248	2022	418405	419079	675	-	1.58934
				431821	433302	1482	+	1.24913
85	440015	440440	426	440015	440431	417	+	1.95001
86	430966	434875	3910	431066	431614	549	-	2.63008
				431821	433302	1482	+	1.24913
87	441059	441882	824	441061	441783	723	+	3.38243
88	457190	459981	2792	457190	457243	54	+	3.82352
89	466225	471547	5323	466331	466483	153	+	1.71818
				466524	467501	978	-	1.85246
90	484620	485129	510	484642	484773	132	-	0.36655
91	486201	486527	327	486289	486525	237	-	0.47261
92	486831	487466	636	486833	487069	237	-	0.03781
93	594624	595272	649	594753	595088	336	+	1.96412
94	598680	600218	1539	598730	599083	354	-	0.93598
95	638016	641974	3959	638075	638218	144	-	1.16264
				640146	641633	1488	+	2.94233
96	682160	682408	249	682170	682406	237	-	1.17383
97	684945	686291	1347	685180	685596	417	+	0.90474

98	690345	690846	502	690356	690844	489	-	2.3173
99	723281	723523	243	723357	723521	165	-	0.03987
100	724961	725478	518	724973	725476	504	-	3.04047
101	728190	730384	2195	728420	728572	153	-	3.43502
102	733525	733750	226	733525	733749	225	-	0.74707
103	742397	742931	535	742397	742933	537	+	2.06591
104	744504	745178	675	744919	745176	258	-	1.29105
105	755533	756521	989	755534	755803	270	+	3.6707
106	760709	762511	1803	760829	761641	813	+	3.69436
107	770634	771517	884	770726	771322	597	-	1.01951
108	826519	828588	2070	826755	827045	291	-	2.96052
109	830706	831159	454	830924	831148	225	-	0.78398
110	846303	846529	227	846349	846522	174	-	0.97782
111	853578	853774	197	853722	853772	51	-	0.26519
112	882388	883459	1072	882482	882712	231	-	1.57831
113	888052	889735	1684	888389	888826	438	+	1.15268
114	894131	894324	194	894131	894322	192	-	1.4093
115	903755	904928	1174	903799	904926	1128	-	3.21204
116	917312	919405	2094	917314	917403	90	+	1.0598
117	927749	929169	1421	927954	929168	1215	-	4.87856
118	940111	940806	696	940112	940222	111	+	0.24929
119	950737	951301	565	950783	951247	465	-	1.29382
120	957343	958258	916	957708	958004	297	+	1.78249
121	968221	969186	966	968223	968411	189	+	1.02816
122	1002154	1003570	1417	1002350	1002418	69	-	1.518
123	1006991	1008926	1936	1007031	1008008	978	-	1.69789
124	1037357	1037638	282	1037359	1037637	279	+	2.12408
125	1041484	1043475	1974	1041683	1041835	153	-	3.5681
126	1051370	1051861	492	1051372	1051860	489	+	2.03761
127	1053003	1055841	2839	1053005	1053217	213	+	2.72732
128	1078330	1078578	249	1078343	1078576	234	-	0.07797
129	1080022	1080406	385	1080024	1080329	306	+	0.61293
130	1081151	1081474	324	1081153	1081389	237	+	0.7245
131	1107107	1107495	389	1107236	1107493	258	-	0.86195
132	1114809	1115277	469	1115018	1115275	258	-	0.83845
133	1123929	1124312	384	1123941	1124312	372	-	1.46713
134	1137119	1138792	1674	1137258	1137596	339	+	0.976371
135	1139066	1139674	609	1139067	1139624	558	+	2.40066
136	1141349	1141849	501	1141444	1141848	405	+	0.68509
137	1154487	1155084	598	1154527	1154988	462	-	0.45648
138	1169131	1169581	451	1169131	1169580	450	-	2.04634
139	1174931	1177813	2883	1174932	1175117	186	+	2.06948
140	1178241	1178841	601	1178284	1178745	462	-	0.4542
141	1192888	1193338	451	1192888	1193337	450	-	2.04634
142	1198688	1201578	2891	1198975	1199439	465	+	2.06907
143	1202005	1203541	1537	1202048	1202722	675	-	0.85241
<i>M.pneumatis</i>								
144	2587	3001	415	2776	3000	225	+	0.17502
145	8996	9285	290	8998	9285	288	+	1.3476
146	946080	946264	185	946080	946262	183	-	0.438888
<i>M.pneumoniae</i>								
147	57298	57885	588	57601	57885	285	+	2.13356
148	114255	114571	317	114257	114568	312	+	0.29127
149	116710	118311	1602	116917	117174	258	+	2.67295
150	126650	126947	297	126650	126946	297	+	0.800816
151	131753	132567	815	131899	132513	615	+	2.46409
152	133387	134054	668	133389	133673	285	+	1.29464
153	141261	141939	679	141641	141922	282	+	0.50463
154	146290	147148	859	146291	146842	552	+	0.93534
155	147542	148089	548	147544	148089	546	+	0.72275

156	165027	166482	1456	165029	165259	231	+	0.71227
157	168082	169041	960	168082	168702	621	+	0.54591
158	170734	171048	315	170736	171047	312	+	1.20902
159	190048	190620	573	190050	190190	141	+	0.62459
160	245570	246048	479	245599	246048	450	-	0.1334
161	284463	285189	727	284542	285189	648	+	0.78332
162	322492	324196	1705	322493	322594	102	-	1.29995
163	334414	334767	354	334414	334767	354	+	1.25543
164	335269	336478	1210	335362	335637	276	+	1.20889
165	336827	337769	943	336827	337006	180	+	0.40999
166	343034	343763	730	343035	343277	243	+	0.1471
167	360397	360643	247	360536	360643	108	+	0.50177
168	404933	409870	378	409562	409870	309	+	0.90442
169	435581	435923	343	435618	435923	306	+	0.80642
170	438532	439219	688	438534	438827	294	+	1.63826
171	446742	447769	1028	446742	447362	621	-	2.46408
172	465177	465426	250	465177	465425	249	-	1.20812
173	530639	530855	217	530639	530854	216	-	0.48426
174	535777	536088	312	535779	536087	309	-	1.71772
175	566598	566890	293	566600	566890	291	+	0.81612
176	592221	592397	177	592222	592395	174	-	0.47217
177	603728	604154	427	603730	603870	141	-	0.81411
178	604587	604896	310	604620	604781	162	-	0.70882
179	610471	611024	554	610615	611019	405	+	2.62053
180	620015	620560	546	620017	620070	54	-	0.06484
181	621845	622873	1029	621845	622498	654	-	1.07569
182	624251	624861	611	624317	624466	150	-	0.22727
183	626140	626916	777	626140	626766	627	-	0.94984
184	705117	705533	437	705191	705553	363	-	0.59677
185	706232	706734	503	706405	706671	267	-	0.95577
186	738995	739423	429	738995	739351	357	-	0.87155
187	760949	761503	555	761420	761503	84	+	2.2871
188	775340	776336	997	776253	776336	84	+	0.34432
<i>M.pulmonis</i>								
189	468389	468564	176	468390	468563	174	-	1.0139
190	470287	470560	274	470326	470559	234	-	1.60585
191	475694	475985	292	475694	475759	66	+	1.28786
192	654868	656085	1218	654868	655167	300	+	0.09366
<i>M.synoviae</i>								
193	172859	173237	379	172860	173237	378	-	0.91765
194	242211	242418	208	242270	242365	96	-	0.12937
195	288995	289493	499	266630	266896	267	-	0.56908

IG Start, IG End-Genomic start and end positions of intergenic sequences respectively; IG Length-Length of intergenic sequences in base pairs; Gene start, Gene End – Genomic start and end positions of predicted genes within the intergenic region respectively; Gene Length- Length of the predicted gene in base pairs; Strand-Coding Strand; CPC Score- Coding potential score of the predicted gene calculated by CPC

Table-3 Intergenic sequences from 11 *Mycoplasma* genomes and their respective orthologs

S.No	IG Start	IG End	Frame	Orthologs
<i>M.agalactiae</i>				
1	5993	6326	-1	tr A5IXE5 A5IXE5_MYCAA
2	622612	622881	3	tr O85034 O85034_MYCHO
3	764420	765348	1	tr Q6MU49 Q6MU49_MYCMS
4	801638	801946	-2	tr Q4A6Z6 Q4A6Z6_MYCS5
5	824718	825105	3	tr Q9KHU4 Q9KHU4_MYCAA
<i>M.capricolum</i>				
6	227187	228764	1	gb AAF24202.1 AF165135_2
7	498315	498690	2	ref NP_326320.1
<i>M.gallisepticum</i>				
8	6078	6368	2	ref NP_696913.1
9	147012	149311	-3	gb AAG18473.1 AF143819_2
10	188644	189838	-1	ref NP_072679.1
11	255298	258275	1	gb AAF29524.1 AF210770_2
12	399521	401928	1	ref NP_622755.1
13	431265	431927	2	ref NP_240122.1
14	462768	464303	3	gb AAF91414.1 AF275312_3
15	522679	524140	3	ref NP_405485.1
16	576074	578622	-1	pir S48753
17	585392	586234	-3	gb AAF91414.1 AF275312_3
18	595070	597948	-1	gb AAF91413.1 AF275312_2
19	698461	699809	-2	ref NP_072739.1
20	725306	726705	-3	gb AAF67108.1 AF224059_1
21	804557	807427	-2	gb AAB50154.1
22	929511	931116	2	gb AAG18473.1 AF143819_2
23	945783	947567	1	ref NP_563226.1
24	963747	965068	-1	ref NP_077876.1
25	968392	969453	-3	ref NP_077884.1
<i>M.genitalium</i>				
26	84045	88227	2	ref NP_072855.1
27	167181	170006	2	ref NP_072855.1
28	174794	175804	1	ref NP_072855.1
29	213550	216015	1	gb AAA88888.1
30	220439	220606	1	ref NP_072853.1
31	229068	232008	3	gb AAA88901.1
32	273319	273789	2	ref NP_072854.1
33	312809	315300	1	ref NP_072855.1
34	315378	315700	3	ref NP_110065.1
35	327053	327432	-2	gb AAG34751.2 AE000044_8
36	428113	430579	3	ref NP_072855.1
37	455948	456754	2	ref NP_110223.1
38	578582	579223	-2	ref NP_110376.1
<i>M.hopneumoniae</i>				
39	90812	91550	2	gb AAB01923.1
40	119923	120295	-2	ref NP_326155.1
41	228251	228736	-3	pir T03806
42	285645	286428	2	ref NP_326246.1
43	349246	350488	3	ref NP_208262.1
44	363448	364020	2	ref NP_078120.1
45	385509	385719	2	ref NP_326311.1
46	392581	393351	3	ref NP_274409.1
47	479967	480221	1	ref NP_078313.1
48	506138	506370	-3	ref NP_326311.1
49	555366	555803	-1	ref NP_326604.1
50	731046	731202	-2	gb AAG00942.1 AF272977_2
51	775977	776482	3	ref NP_326057.1
52	892062	892333	-2	ref NP_325984.1
<i>M.mobile</i>				
53	44446	44745	-3	ref NP_282657.1
54	201647	202393	-2	ref NP_326011.1
55	344911	345496	2	ref NP_438386.1
56	449935	451156	3	ref NP_326292.1
57	476091	476760	1	ref NP_325990.1
58	482794	483248	-1	gb ZP_00036984.1
59	564403	565470	2	ref NP_326168.1
60	739974	742109	3	ref NP_222925.1
61	757981	759865	-2	ref NP_562175.1
<i>M.mycooides</i>				
62	56758	56989	-2	ref NP_325984.1
63	81703	82308	-3	gb AAD16446.1
64	116950	117661	-1	ref NP_604103.1
65	133924	134935	-3	gb ZP_00063462.1

66	141980	144915	-1	gb AAD16446.1
67	147747	148799	2	gb AAK64567.3
68	148917	151265	2	gb AAM29175.1
69	163385	165394	-3	gb AAD16446.1
70	165938	166865	1	gb AAM29175.1
71	186263	187583	3	emb CAD21855.1
72	244994	246808	-3	gb AAC44572.1
73	262031	263021	-2	ref NP_326558.1
74	265192	265990	-3	gb AAM29175.1
75	267813	268197	1	ref NP_602531.1
76	277825	278429	1	sp Q50239 YI6A_MYCMY
77	279951	280595	2	sp Q50239 YI6A_MYCMY
78	283455	284173	-1	ref NP_229864.1
79	325337	326509	1	ref NP_703923.1
80	350917	351234	-2	ref NP_326517.1
81	363554	366577	1	gb AAD16446.1
82	387059	391127	-3	gb AAD16446.1
83	393713	393921	-3	pir S48585
84	418227	420248	-3	gb AAM29175.1
85	430966	434875	1	gb AAD16446.1
86	440015	440440	1	ref NP_655377.1
87	441059	441882	3	ref NP_654473.1
88	457190	459981	1	gb AAD16449.1
89	466225	471547	2	gb AAD16446.1
90	484620	485129	-3	ref NP_561650.1
91	486201	486527	-3	ref NP_219422.1
92	486831	487466	-2	emb CAA83720.1
93	594624	595272	1	gb AAF24203.1 AF165135_3
94	598680	600218	-3	sp Q50239 YI6A_MYCMY
95	638016	641974	1	gb AAD16446.1
96	682160	682408	-3	ref NP_295234.1
97	684945	686291	1	gb AAK94953.1 AF396969_2
98	690345	690846	-3	sp P19679 RS2_SPICL
99	723281	723523	-3	ref NP_077875.1
100	724961	725478	-3	emb CAA88819.1
101	728190	730384	-3	gb AAD16446.1
102	733525	733750	-2	ref NP_077874.1
103	742397	742931	2	ref NP_561501.1
104	744504	745178	-1	gb ZP_00059656.1
105	755533	756521	3	gb AAF15254.1 AF162998_2
106	760709	762511	2	gb AAD16446.1
107	770634	771517	-1	gb AAM29175.1
108	826519	828588	-1	gb AAD16446.1
109	830706	831159	-3	pir S48592
110	846303	846529	-2	gb ZP_00045981.1
111	853578	853774	-3	ref NP_214304.1
112	882388	883459	-1	gb AAK64570.3
113	888052	889735	2	sp Q50239 YI6A_MYCMY
114	894131	894324	-3	emb CAA83689.1
115	903755	904928	-3	ref NP_326049.1
116	917312	919405	2	gb AAD16446.1
117	927749	929169	-2	gb AAK13041.1
118	940111	940806	1	ref NP_395977.1
119	950737	951301	-1	sp Q50239 YI6A_MYCMY
120	957343	958258	3	sp Q50239 YI6A_MYCMY
121	968221	969186	2	gb AAM29175.1
122	1002154	1003570	-3	pir S77744
123	1006991	1008926	-1	gb AAD16446.1
124	1037357	1037638	3	sp Q48960 GIDB_MYCCA
125	1041484	1043457	-1	gb AAD16446.1
126	1051370	1051861	3	ref NP_326027.1
127	1053003	1055841	1	gb AAD16446.1
128	1078330	1078578	-3	gb AAL37452.1 AF328916_2
129	1080022	1080406	3	gb AAL15430.1
130	1081151	1081474	3	ref NP_206851.1
131	1107107	1107495	-3	ref NP_072799.1
132	1114809	1115277	-3	ref NP_072799.1
133	1123929	1124312	-1	ref NP_561202.1
134	1137119	1138792	2	gb AAK85864.1
135	1139066	1139674	2	gb AAK13041.1
136	1141349	1141849	3	gb AAK13041.1
137	1154487	1155084	-1	gb AAM29175.1
138	1169131	1169581	-2	ref NP_472958.1

139	1174931	1177813	3	gb AAD16446.1
140	1178241	1178841	-1	gb AAM29175.1
141	1192888	1193338	-2	ref NP_472958.1
142	1198688	1201578	2	gb AAD16446.1
143	1202005	1203541	-2	sp Q50239 YI6A_MYCMY
<i>M.penetrans</i>				
144	2587	3001	1	ref NP_077910.1
145	8996	9285	3	gb AAL67403.1 AF447814_71
146	946080	946264	-3	ref NP_326424.1
<i>M.pneumoniae</i>				
147	57298	57885	1	ref NP_109737.1
148	114255	114571	3	ref NP_109830.1
149	116710	118311	2	gb AAK92038.1 AF290000_1
150	126650	126946	1	ref NP_109970.1
151	131753	132567	3	ref NP_109830.1
152	133387	134054	3	ref NP_109830.1
153	141261	141939	3	emb CAB37845.1
154	146290	147148	2	ref NP_662051.1
155	147542	148089	3	ref NP_326607.1
156	165027	166482	1	ref NP_109829.1
157	168082	169041	1	gb AAK92038.1 AF290000_1
158	170734	171048	3	ref NP_109890.1
159	190048	190620	1	gb AAK92038.1 AF290000_1
160	245570	246048	3	gb AAK92038.1 AF290000_1
161	284463	285189	2	ref NP_072758.1
162	322492	324196	-1	ref NP_110031.1
163	334414	334767	1	ref NP_109888.1
164	335269	336478	1	ref NP_110194.1
165	336827	337769	1	ref NP_109833.1
166	343034	343763	2	ref NP_110097.1
167	360397	360643	2	gb AAB93999.1
168	409493	409870	1	ref NP_110031.1
169	435581	435923	2	ref NP_110053.1
170	438532	439219	3	ref NP_109838.1
171	446742	447769	-3	ref NP_110064.1
172	465177	465426	-2	sp Q9EXD4 Y26A_MYCPN
173	530639	530855	-2	ref NP_110124.1
174	535777	536088	-2	ref NP_110124.1
175	566598	566890	3	ref NP_109830.1
176	592221	592397	-3	ref NP_073008.1
177	603728	604154	-1	sp P75290 YE97_MYCPN
178	604587	604896	-2	ref NP_326424.1
179	610471	611024	2	sp Q9EXD6 YE13_MYCPN
180	620015	620560	-3	ref NP_109737.1
181	621845	622873	-1	ref NP_109737.1
182	624251	624861	-2	ref NP_109737.1
183	626140	626916	-1	ref NP_072758.1
184	705117	705553	-1	ref NP_110275.1
185	706232	706734	-1	ref NP_110274.1
186	738995	739423	-1	ref NP_109977.1
187	760949	761503	1	ref NP_110322.1
188	773753	774638	-1	ref NP_110155.1
<i>M.pulmonis</i>				
189	468389	468564	-2	ref NP_326229.1
190	470287	470560	-2	ref NP_326229.1
191	475694	475985	2	ref NP_326220.1
192	654868	656085	1	ref NP_326352.1
<i>M.synoviae</i>				
193	172859	173237	-1	ref NP_078315.1
194	242211	242418	-3	gb AAB58471.1
195	288995	289493	-3	gb AAD42255.1 AF085698_1

IG Start, IG End-Genomic start and end positions of intergenic sequences respectively