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IN SILICO CHARACTERIZATION OF PUTATIVE DRUG TARGETS IN *PASTEURELLA MULTOCIDA* CAUSING FOWL CHOLERA

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ABSTRACT

The present investigation deals with the in silico characterization of putative drug targets in *Pasteurella multocida*. The predictions of target for the drug discovery were made by using Bioinformatics tools like kyto encyclopedia in genes and genomes data base. The results showed that, 73 pathways of *Pasteurella multocida* retrieved from web server were found to be non homologous to the host *Gallus gallus* (chicken) out of which 59 pathways were found to be in cytoplasm, 3 in periplasm,1 in outer membrane and 10 are unknown. The knowledge of the present study could make the drug discovery easier which have high affinity to the target site of the causative organism. Current vaccines against fowl cholera include bacterins, which provide only limited protection against homologous serotypes and live attenuated strains, which have been observed to revert to virulence. Therefore, the present work is carried out there is a need for more effective vaccines to control diseases caused by *P. multocida*.

KEYWORDS: fowl cholera, *Pasteurella multocida,* Phylogenetic tree, KEGG Metabolic pathway, Drug.

1. INTRODUCTION

Pasteurella multocida is a gram-negative bacteria pathogen that is the causative agent of fowl cholera in wild and domestic birds. It is also responsible for a range of diseases in mammals, including bovine hemorrhagic septicemia, swine atrophic rhinitis[1], bovine respiratory

disease (BRD), fowl cholera, atrophic rhinitis in swine, and pneumonia in sheep,[2, 3-5], *P. multocida* is one of the species targeted by sub-MIC antibiotics in production of animal feeds. Human disease caused by *P. multocida* usually results from bite and scratch wounds from cats or dogs, although human infection can also occur through non bite animal exposure.[6, 7, 8] Historically, *P. multocida* is significant as the first live-attenuated vaccine.[9, 10] In 2001, the *P. multocida* genome was sequenced.[11] In October, 1975 the disease was characterized by rapid onset, a short course, and signs of dullness and ruffled feathers in Utter Pradesh, India. There are many strains of *P. multocida* that infect different species of birds and mammals and cause varying degrees of disease.[2] Avian cholera is the most significant infectious disease of wild waterfowl in North America. Single outbreaks can kill thousands of birds, and outbreaks occur almost annually in some parts of the continent. Avian cholera infections have been reported in over 190 species of birds. Domestic poultry and other captive species are also susceptible to avian cholera. The various strains of *P. multocida* are more infectious in some species than others.. Avian strains of *P. multocida* typically do not infect humans, though mammalian strains can infect humans via animal bites, scratches, or wound contamination.[12] Avian cholera was first reported in wild waterfowl in North America in the winter of 1943-1944 in Texas and California.[3] In India avian cholera first reported in Uttar Pradesh *P. multocida* is released into the environment by dead and dying birds as well as asymptomatic carriers, and it can be transmitted to susceptible birds in a variety of ways. It can be transmitted during direct bird-to-bird contact, especially when birds are crowded together. The bacteria tend to collect on the surface of water and can become airborne when birds take flight and also when they land. Once in the air, the bacteria can be inhaled. Avian cholera can also be transmitted by way of ingestion of contaminated food or water.[13] *P. multocida* can also be introduced to new areas by humans by way of contaminated equipment, cages, and clothing. Avian cholera is transmitted easily between birds when they flock together in high densities. Birds are more susceptible to disease at times of stress, and avian cholera outbreaks often occur during the winter when birds are overcrowded and the weather is cold and damp. Other stressors influencing disease outbreaks include migration, harsh environmental conditions, and competition for limited food resources.[14] During outbreaks of avian cholera large numbers of birds are found dead, and very few sick birds are observed, indeed most wild birds infected with avian cholera are not observed when they are showing clinical signs. When sick birds are observed they exhibit lethargy, convulsions, and difficulty breathing. Recent research shows that some birds survive and become carriers of the bacteria. These birds are a likely source of infection for subsequent outbreaks.[15] *P. multocida* must be

isolated to reach a definitive diagnosis of avian cholera. Avian cholera shares clinical signs with several other diseases of wild birds, so it is important to conduct laboratory tests to confirm the cause of illness. Antibiotics may be used to effectively treat individual cases of avian cholera, but it is not feasible to treat wild birds when outbreaks occur.

2. MATERIALS AND METHODS

2.1 Microorganism

Pasteurella multocida sub sp. Multocida HNO6 was used in the present study. The sequence of the 16s r RNA gene is shown.[11]

2.2 Prediction of target for the drug discovery

Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used as a source of metabolic pathway information[20], all the metabolic pathways of the *Pasteurella multocida* were retrieved from the KEGG. Pathways which do not appear in the host but present in the pathogen have been considered as pathways unique to pathogen according to KEGG database annotation.[21] The corresponding amino acid sequences of the proteins involved in the pathway were retrieved from the KEGG database. They were subjected to a Basic Local Alignment Search Tool (BLAST) search against the non-redundant database with the e-value inclusion threshold set to 0.005.[30] The search was restricted to proteins from *Gallus gallus* through an option available in the BLAST program. This analysis was carried out for the detection of non homologues pathways in the host, which do not have hits below the e-value inclusion threshold of 0.005 and, were picked out as potential drug targets. All the non homologous pathway genes were believed to be essential genes of the pathogen. Essential genes are those indispensable for the survival of an organism, and their functions are therefore, considered a foundation of life.[22] These crucial genes were absent in the host.[23] All the essential pathways amino acid sequences were subjected to the PSORT (http://www.psort.org/psortb/index.html) analysis for the prediction the location of the pathway in the cell. This is required to find out the surface membrane proteins which could be probable drug targets. Further these surface membrane pathway protein sequences were characterized by using Peptide Statistic (PEPSTAT) program which is available online via European Molecular Biology Open Software Suite (EMBOSS) web server.[24]

ATGAAACCAGACCTATCTTCTCTTTGGCAAGAATGCCTTTTACAGCTTCAAGACCAGATT
TCACTAACGGATTTTAGCACCTGGTTACGTCCTTTACAGGCCGATTTTAGCGTACAAAAT
ACGATTGTTTTGTATGCCTCTAATGTGTTTATTAAACAAAAAGTGGACGAAAGTTATTTG
GCACAATTGACTAAAGTTGCACAAGAGCTCAGTGGCAATGCTGAGTTAGTGGTACAGGT
CAAAGTTGGGGTGAAACCTGAACCGAAGCCCGCGCAACCTTCTGCTTTGCCAACACATC
ACAATAAAGAAGAAAATAAACCGCAAACGGTTATTCGTTCTTATTTAAATCCCAAGCAT
GTGTTTGAAAATTTTGTGGAAGGTAAATCAAATCAACTTGCTCGTGCTGTTGCACAAAAA
GTGGCAGATAATCCTGGTGAACCGAGTTCCAATCCGCTTTTTCTGTATGGTGGTACAGGT
TTAGGGAAAACCCATTTATTGCATGCCATTGGCAATGGTATTCTATCTCGTAATACAAAT
GCGAGAGTCCTGTATATTCATGCTAATAATTTTATGCAACAAATGGTGAATGCGGTACGT
GATAACAAAATGGATGAATTTAAAAAATTCTACCGCTCTTTAGATGCGCTGTTAGTGGAT
GATATTCAATTCTTTGCAGAAAAAGAAAAAACCCAAGAAGAGT

Fig. 1 Sequence of 16s r-RNA of *Pasteurella multocida*

3. RESULTS

3.1 Bacteria

The bacteria was used in the present study *Pasteurella multocida sub* species *Multocida HN06.*

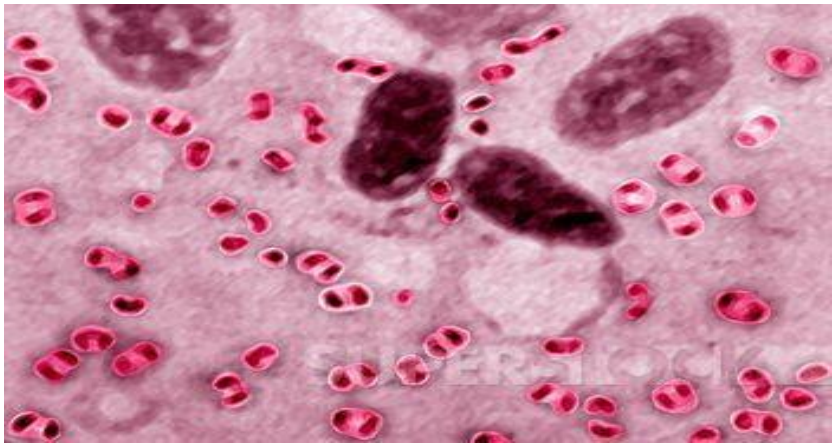

Fig. 2 *Pasterella multocida* (Medical microbiology with Baldwin at University of Missouri- Columbia)

3.2 Potential drug targets Identification

A total of 90 pathways of *Pasteurella multocida* were retrieved from KEGG database (http://www.kegg.jp/keggbin/ search_pathway_text) out of which 73 pathways were non homology to the host *Gallus gallus (*Table 1) as Fowl cholera causative agents are emerging multi drug resistant, the discovery of an alternative treatment for this disease has profound scope and significance. Drug discovery is time consuming and multistep process which includes test on many models and clinical trials.

3.3 Prediction of targets distribution in the cellular components

The KEGG Pathway of *P. multocida* HNO6 showed 73 pathway non homologous to host Gallus gallus (Table .1) All the 73 pathways amino acid sequence were Subjected to PSORT analysis (http://www.psort.org/psortb/index.html) (18). For prediction of location of pathway in cell, out of 73 pathways, 1 pathway were located in cell membrane (1%), 59 pathways in cytoplasm (81%), 3 pathways (4%) in periplasm ,10 pathways (14%) are unknown . Similar studies have been carried out by (21, 27). This is required to find out the cell wall proteins which could be probable drug targets. Glutamate dehydrogenase pathway was found to be present in the cell wall of the bacteria, cell wall Proteins were characterized by a PEPSTAT program (28). The abundance of each amino acid in the pathways and their molecular percent with respect to dayhoffstat and properties of residues is given in Table 2.

3.4 Glutamate dehydrogenase biosynthesis pathway

GLDH is an enzyme, present in most microbes and the mitochondria of eukaryotes, as are some of the other enzymes required for urea synthesis, that converts glutamate to α-ketoglutarate, and vice versa. In animals, the produced ammonia is usually used as a substrate in the urea cycle. Typically, the α- ketoglutarate to glutamate reaction does not occur in mammals, as glutamate dehydrogenase equilibrium favours the production of ammonia and α-ketoglutarate. Glutamate dehydrogenase also has a very low affinity for ammonia (high Michaelis constant of about 1 mM), and therefore toxic levels of ammonia would have to be present in the body for the reverse reaction to proceed (that is, α- ketoglutarate and ammonia to glutamate and NAD (P+). The enzyme represents a key link between catabolic and metabolic pathways, and is, therefore, ubiquitous in eukaryotes. GLDH can be measured in a medical laboratory to evaluate the liver function. Elevated blood serum GLDH levels indicate liver damage and GLDH plays an important role in the differential diagnosis of liver disease, especially in combination with amino transferases. GLDH is localized in mitochondria; therefore practically none is liberated in generalized inflammatory diseases of the liver such as viral hepatitides. Liver diseases in which necrosis of hepatocytes is the predominant event, such as toxic liver damage or hypoxic liver disease, are characterized by high serum GLDH levels.. In clinical trials, GLDH can serve as a measurement for the safety of a drug. Ammonia incorporation in animals and microbes occurs through the actions of glutamate dehydrogenase and glutamine synthatase. Glutamate plays the central role in mammalian and microbe nitrogen flow, serving as both a nitrogen donor and a nitrogen acceptor. In humans, the activity of glutamate dehydrogenase is controlled through ADP- ribosylation, a covalent

modification carried out by the gene sirt4. This regulation is relaxed in response to caloric restriction and low blood glucose. Under these circumstances, glutamate dehydrogenase activity is raised in order to increase the amount of α-ketoglutarate produced, which can be used to provide energy by being used in the citric acid cycle to ultimately produce ATP. In microbes, the activity is controlled by the concentration of ammonium and or the like-sized rubidium ion, which binds to an allosteric site on GDH and changes the Km (Michaelis constant) of the enzyme. The control of GDH through ADP-ribosylation is particularly important in insulin-producing β cells. Beta cells secrete insulin in response to an increase in the ATP: ADP ratio, and, as amino acids are broken down by GDH into α-ketoglutarate, this ratio rises and more insulin is secreted. sirt is necessary to regulate the metabolism of amino acids controlling insulin secretion and regulate blood glucose levels, alter the allosteric binding site of GTP cause permanent activation of glutamate dehydrogenase lead to disorder known as hyperinsulinism-hyperammonemia.[19]

Supplementary material

Table 1: Information about Non- homologues pathways and their locations in *p. multocida sub sp. Multocida HN06* searched against *Gallus gallus.*

Sl.No.	Gene id	Pathway	Location	No. of Amino acids
1	pmv1009	Adinylate kinase	Cytoplasm	214
2	pmv0037	Argonosuccinate lyase	Cytoplasm	382
3	pmv0296	Aspertate kinase III	Unknown	450
4	pmv0427	Formate dehydrogenase	Periplasm	809
5	pmv0580	Malate dehydrogenase	Unknown	311
6	pmv0972	Dihydrolipoamide acetyl transferase	Cytoplasm	632
7	pmv0413	Methylglyoxal synthase	Cytoplasm	152
8	pmv1837	Aspertate ammonia ligase	Cytoplasm	330
9	pmv1016	Citrate synthase I	Cytoplasm	428
10	pmv1621	Xylulose kinase	Cytoplasm	489
11	pmv0901	N-acetyl glutamate synthase	Cytoplasm	440
12	pmv0295	Adenylosuccinate synthetase	Cytoplasm	432
13	pmv0042	Glutamyl-t RNAsynthetase	Cytoplasm	480
14	pmv0303	Fructose-1,6-bisphosphatase	Unknown	333
15	pmv1912	Gluconolactonase	Cytoplasm	295
16	pmv1682	Diaminopimelate decarboxylase	Cytoplasm	416
17	pmv0651	Aminotransferase	Cytoplasm	396
18	pmv1688	Shikimate 5-dehycdrogenase	Cytoplasm	270
19	pmv0666	Acetyl –coA carboxylase	Cytoplasm	304
20	pmv1099	Fumerate reductase	Cytoplasm	616
21	pmv0738	Phosphotidyl cerophosphotase	Cytoplasm	245
22	pmv0946	Acetolactate synthase	Cytoplasm	163
23	pmv0195	Galactose mutarotase	Periplasm	343

24	pmv1523	Sucrose-6-phosphate hydrolase	Cytoplasm	480
25	pmv0132	Napthoate synthase	Cytoplasm	285
26	pmv0763	Acetate kinase	Cytoplasm	401
27	pmv1158	D-alamineligase	Cytoplasm	309
28	pmv0200	Amino peptidase	Cytoplasm	434
29	pmv0572	Pullulanase	Cytoplasm	672
30	pmv0911	phosphoserine aminotransferase	Cytoplasm	360
31	pmv1267	Glutamate dehydrogenase	Cell wall	449
32	pmv0785	Tetrathonate reductase	Cytoplasm	245
33	pmv1099	Fumerate reductase	Cytoplasm	616
34	pmv1238	6-phospho fructokinase	Cytoplasm	360
35	pmv0280	Uridine kinase	Cytoplasm	216
36	pmv0971	Dihydrolipomidedehydrogenase	Cytoplasm	688
37	pmv2265	Histidinoldehydrogenase	Unknown	432
38	pmv0651	Aminotrasferase	Cytoplasm	396
39	pmv1278	Catalase	Periplasm	484
40	pmv0317	Glutamate racemase	Cytoplasm	269
41	pmv1508	Diacylglycerol kinase	Cytoplasm	118
42	pmv1978	Triosephosphate isomerase	Cytoplasm	261
43	pmv0338	Cystine desulfurase	Cytoplasm	404
44	pmv0680	Ribofavin synthase	Cytoplasm	204
45	pmv1490	Nucleotidase	Cytoplasm	223
46	pmv0009	Phosphopentothenate synthase	Cytoplasm	400
47	pmv1728	Para-aminobenzoate synthase	Cytoplasm	188
48	pmv0666	Acetyl –co acarboxylase	Cytoplasm	304
49	pmv1949	Reductoisomerase	Cytoplasm	491
50	pmv1014	2-Oxogluterate dehydrogenase	Cytoplasm	404
51	pmv1559	Porphobilinogen deaminase	Cytoplasm	308
52	pmv0163	DNA polymerase I	Cytoplasm	930
53	pmv1326	Disaccharide synthase	Cytoplasm	392
54	pmv1166	Peptidoglycon synthetase	Cytoplasm	601
55	pmv1685	Phospholipase A	Unknown	290
56	pmv0951	Formyl tetrahydrofolatedeformylase	Cytoplasm	278
57	pmv1456	Malonyl transferase	Unknown	242
58	pmv2004	t-RNA methyl transferase	Cytoplasm	329
59	pmv1101	Fumerate reductase	Cytoplasm	132
60	pmv1623	Aspertate transaminase	Cytoplasm	396
61	pmv0600	Acyl co-A thoesterase II	Cytoplasm	292
62	pmv2219	Triose phosphate isomerase	Cytoplasm	349
63	pmv1360	2-isopropyl synthase	Cytoplasm	518
64	pmv0167	Mannitol –specific transforter subunit IIc	Cytoplasm	624
65	pmv1959	30s ribosomal protein s16	Cytoplasm	82
66	pmv1501	Phosphopyruvate hydratase	Cytoplasm	433
67	pmv2035	DNA-directed RNA polymerase	Cytoplasm	1417
68	pmv1196	Ribonuclease	Unknown	154
69	pmv1786	Cell division protein	Unknown	459
70	pmv1652	Preprotein translocase	Cytoplasm	441
71	pmv1986	Adenine glycosylase	Cytoplasm	378
72	pmv1372	Single strand DNA binding protein	Cytoplasm	166
73	pmv2284	Primosomal replication protein	Unknown	108

Table 2: Characterization of Glutamate dehydrogenase

PEPSTATS of EMBOSS_001 from 1 to 449

Molecular weight = 48312.99 Residues = 449

Average Residue Weight = 107.601 Charge = -1.0

Isoelectric Point = 6.1827

A280 Molar Extinction Coefficients = 45840 (reduced) 46090 (cystine bridges)

A280 Extinction Coefficients 1mg/ml = 0.949 (reduced) 0.954 (cystine bridges)

Improbability of expression in inclusion bodies = 0.685

Residue	Number	Mole%	DayhoffStat
A = Ala	53	11.804	1.373
B = Asx	0	0.000	0.000
C = Cys	5	1.114	0.384
D = Asp	19	4.232	0.769
E = Glu	28	6.236	1.039
F = Phe	22	4.900	1.361
G = Gly	49	10.913	1.299
H = His	4	0.891	0.445
I = Ile	15	3.341	0.742
J = ---	0	0.000	0.000
K = Lys	26	5.791	0.877
L = Leu	38	8.463	1.144
M = Met	12	2.673	1.572
N = Asn	17	3.786	0.881
O = ---	0	0.000	0.000
P = Pro	14	3.118	0.600
Q = Gln	24	5.345	1.371
R = Arg	18	4.009	0.818
S = Ser	24	5.345	0.764
T = Thr	23	5.122	0.840
U = ---	0	0.000	0.000
V = Val	38	8.463	1.282
W = Trp	4	0.891	0.685
X = Xaa	0	0.000	0.000
Y = Tyr	16	3.563	1.048
Z = Glx	0	0.000	0.000

Property	Residues	Number	Mole%
Tiny	(A+C+G+S+T)	154	34.298
Small	(A+B+C+D+G+N+P+S+T+V)	242	53.898
Aliphatic	(A+I+L+V)	144	32.071
Aromatic	(F+H+W+Y)	46	10.245
Non-polar	(A+C+F+G+I+L+M+P+V+W+Y)	266	59.243
Polar	(D+E+H+K+N+Q+R+S+T+Z)	183	40.757
Charged	(B+D+E+H+K+R+Z)	95	21.158
Basic	(H+K+R)	48	10.690
Acidic	(B+D+E+Z)	47	10.468

4. CONCLUSION

In the present study attempts were made to find out the putative targets of *Pasteurella multocida HN06* for the drug discovery with all the sources available in databases by computational methods. *Pasteurella multocida HN06* was used in the present study (11). A total 73 non homologues pathways amino acid sequence were screened for their location in bacterial cell out of which 59 pathway were found to be in cytoplasm 1 in the cell membrane, 3 in the periplasm and 10 are unknown. As pathways of cell wall were believed to be the most putative targets for the drug discovery the proteins involved in the cell wall pathway were further characterized for the determination of physicochemical properties of the protein. Perhaps the knowledge of the present study made the drug discovery easier which have high affinity to the target site. Possible drug discovery to manage fowl cholera with a help of bioinformatics tool is more significant, specific, reduce the time and complications involved in the clinical trials.

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