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Exposed Proteomes of Brachyspira pilosicoli responsible for the overall decline in poultry and pig's meat *production*

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ABSTRACT

Brachyspira pilosicoli is a gram-negative spiral-shaped, obligate anaerobe bacterium belongs to the spirochete family, the pathogen causing diarrhea, reduced growth rate, reduced egg and meat production, and quality. In recent years, there has been a substantial rise in the rate of Brachyspira pilosicoli infections, as well as the emergence of the virulent and antibiotic-resistant Brachyspira pilosicoli strains. So there is an urgent need for the identification of the therapeutic potential target and development of new drugs for the treatment and prevention of Brachyspira Pilosicoli infections. In the current investigation, we used approach by reference protein (RefSeq. Protein) the individual 1378 protein sequence was entered in DELTA-BLAST against proteomes of pigs (taxid: 9821) pigs & lt; 2 & gt; (taxid: 9823), chickens (taxid: 9031) and result of 27 protein sequences no similarity was found, these 27 protein sequences were again compared against the database of essential single genes individually and 9 of them had no similarity and 18 protein sequences were taken out which had similarity and their protein name, biological process, molecular function and role in virulence have been mentioned and discussed.

Keywords— *Brachyspira, Zoonotic*

1. INTRODUCTION

The seven officially named and several unofficially named species of the anaerobic *spirochetes* are colonizing the large intestine of the mammals and birds. These seven official species belong to the genus *Brachyspira* [Stanton TB et al 2006]. Here *Brachyspira hyodesenteria* is the agent of the swine dysentery, *B. intermedia* is the pathogen mainly of the adult chickens and *B. Pilosicoli* is the one which is responsible for the cause of intestinal *spirochetosis*. This *B. Pilosicoli* has a wide range of infection when compared to the other two main pathogenic species which have been colonizing in the mammals and birds as well as in human beings [Trott DJ et al 1998]. The colonizing of the *B. Pilosicoli* species has been rapidly grown in developing countries due to unhygienic conditions. The infected pigs and chickens in the farm show the depressed rate of growth and production [Trott DJ et al 1997, Margawani KR, et al 2004, Munshi MA et al 2004, Nelson EJ et al 2009].

Rectal bleeding, chronic diarrhea with abdominal pain and focal colitis have been observed in the individuals who are suffering from the *B. Pilosicoli* infection. In an in vitro study, it showed that by using Caco-2 cells has shown that *B. Pilosicoli* strains initially targets the cell junctions where one cell end of the spirochete invaginates into the Caco-2 cell membranes [Naresh R, et al 2009]. When the mode of infection has been started here the whole cell surface will be colonized by the *B. Pilosicoli* species. Then a false brush border will be formed by the infections In this model colonized monolayers demonstrated accumulation of the actin at the cell junction, loss of tight junction integrity, fragmentation and condensation of the nuclear material consistent with apoptosis, here a spirochaetamia with *B. Pilosicoli* has been recorded in the immunocompromised human beings [Trott DJ et al 1997, Bait-Merabet L et al 2008] *Brachyspira Pilosicoli* is a food born and water-born disease i;e the water and food sources which are contaminated by the *B.Pilosicoli* [Margawani KR et al 2004, Oxberry SL et al 1998, Verlinden M, et al 2012]. Of these two species i.e; *B. Hydosynteria* and *B.Pilosicoli* here *B. Pilosicoli* has been shown that about 30% of the individuals have been colonized by this species including Oman [Barrett SP et al 1990] and Papua New Guinea [Trott DJ 1997], but recent investigations showed that it is uncommon in developed countries [Lee JI et al 1992, Tompkins DS et al 1986, Brooke CJ et al 2001] No epidemiological studies have been conducted on the B. Aalborg because this organism is the one which is fastidious and taking up to three weeks to grow under anaerobic incubation on the specialized media. Till today *B. Aalborg* have been found in the humans and here it has been isolated in the five studies i;e in three it was grown in the colorectal biopsies [Hovind-Hougen K et al 1981, Kraaz W, et al 2000] and in two it was grown from feces [Brooke CJ et al 2003, Calderaro A et al 2003].

Table 1: *Brachyspira* species, their host, and pathogenicity

Species	Host	Pathogenicity
B. aalborgi	Human, primates	Mild to moderate (Hovind-Hougen K et al 1981)
B. alvinipulli	Chicken, goose, Red-breasted, merganser, dog	Mild to severe (Stanton TB et al 2015)
B. hyodysenteriae	Chicken, goose, mallard, common rhea, pig, rat, mouse	Severe (Harris DL et al 1972, Taylor DJ et al 1971)
B. innocent	Chicken, pig, dog, horse	None (Kinyon JM et al 1979, Stanton TB. et al 1992)
B. intermedia	Chicken, Pig	Mild to moderate (Stanton TB et al 1997)
B. murdochii	Chicken, pig, rat	None (Stanton TB et al 1997)
B. pilosicoli	Chicken, pheasant, grey partridge, feral water birds, common rhea, pig, dog, human.	Mild to moderate (Trott DJ, et al 1996)

2. OVERVIEW OF THE DISEASE

2.1 Signs and symptoms

The infections of the *Brachyspira Pilosicoli* are observed commonly in the egg-laying chickens in the poultry farms. Many cases have been reported worldwide especially in Europe, the US and Australian countries where a huge number of chickens have been reared, so here the Gastro-Intestinal infections have been observed commonly here [Hampson DJ, et al 2013, Stephens CP et al 1999]. Infection of the *Brachyspira Pilosicoli* starts from milder to higher, which leads to higher death rate of chickens[Hampson DJ, et al 2013, Stephens CP, et al 1999, Erlandson K et al 2005] Before, at the initial stage of infection the diarrhea, feces with altered color and consistency will be observed [Taylor P et al 1993]. This infection starts resulting in the low growth rate of the chickens and layers will be delayed up to 7-9 weeks in the egg-laying processes and also the poor egg quality will be produced by the infected hens. [Taylor P et al 1993, Griffiths IB, et al 1987, Taylor P et al 2002].

The laid egg will be sometimes cracked and the outer shell will be contaminated by feces [Swayne DE et al 1992]. The chicks hatched by the infected mother will have the same similarity i.e.; decreased weight paler feces, delayed lay one set, despite the absence of the contamination. These results made raised questions of the potential epigenetic variations by infections to *Brachyspira Pilosicoli*. At the intestinal level, the cross-section of the infected chickens shows that the *Brachyspira pilosicoli* fixed to the cells of the intestinal wall. The attached part's tissues look often like bleeding, the intestinal wall shows the loss of microvilli. Here due to the loss of microvilli the increased amount of water will be seen in feces [Taylor P et al 1993, Beardsworth PM et al 2004].

2.2 Zoonotic potential

In humans it is mostly seen in the immune-compromised patients, this is confirmed by biopsy i;e it shows its symptoms through diarrhea and through abdominal pain [Trott DJ et al 1995, Calderaro A et al 2007]. As a result of septicemia in some of the rare cases, this infection causes death in human beings [Prim N, et al 2011]. These cases have been only observed in the elderly and immunocompromised patients or in the population having a dense area with poor hygienic conditions [Lee JI et al 1992, Trott DJ et al 1997, Margawani KR et al 2004]. It has been notified that the *B. Pilosicoli* is able to survive and be transmitted to the consumers via contaminated meat of chickens and pigs which has been infected [Verlinden M, et al 2012].

In 2012, Mappley et al. [Mappley LJ, et al 2012] carried out the genetic comparison of the three strains of the *B. Pilosicoli* which was isolated from the humans, pigs, and chickens, which showed the genotype of these three strains were very similar. But some difference was notified in the genome size and arrangement and in some putative coding regions for the carbohydrates, amino acids and nucleotide metabolism transport [Mappley LJ, et al 2012]. These data highlight some fundamental genetic differences between these species isolated from the chicken and humans in comparison to strains isolated from pigs. [Mappley LJ, et al 2012].

3. MATERIALS AND METHODS

EDGAR ("Efficient Database framework for comparative Genome Analyses using BLAST score Ratios") an online available search tool helped in taking the *B. pilosicoli* bacteria for the comparative analysis i;e to compute the core genome, the pan-genome, singleton genes, synteny plots, and Venn diagrams. Here the species of the *Brachyspira Pilosicoli* were selected in the parameter selection. A minimum of five species of *B. Pilosicoli* was selected in the parameter selection. Venn diagram was built in the EDGAR which built the core genome contained about 1378 protein sequences. The availability of these protein sequences was found in EDGAR bioinformatics and they were downloaded and converted to FASTA format. FASTA format protein sequences were then blasted using the NCBI BLAST tool which finds regions of similarity between biological sequences. The program compares protein sequences to sequence databases and calculates the statistical significance. Next, the studies say that the mortality rate and the number of infected organisms were the humans, hens, and pigs, in these species the bacteria colonized in the intestinal parts of it and showed its pathogenicity. The availability of the genome sequence was seen in the NCBI BLAST and it was confirmed that the availability was there.

Next, in the blast, the protein query was entered individually than in choosing the search set options the reference protein (Refseq_protein) was selected. While choosing the organisms here pigs (taxid: 9821), pigs < #2> (taxid: 9823) and chickens (taxid: 9031) were selected. These mentioned organisms were selected because of the pathogenesis of the bacteria in these species was very high. Then in the program selection here, DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST) Algorithm was selected. Next, the whole built options were BLAST then here. The Whole 1378 proteins were BLAST using the

Protein Blast here and the result obtained was further noted, it had 28 proteins sequences had no similarities out of 1378 protein sequences. Here we considered the no similarities found sequences as the drug targets which were then submitted to the DEG BLAST. The submitted protein sequences were then DEG BLAST here and the result obtained was here out of 28 query sequences 18 of them were found to be no similar against the Prokaryotes (no hit), single gene and multiple genes were also considered and here 18 query sequence were taken out, 9 of them were left out and CEG (Cluster Of Essential genes) was done. Results obtained showed us that further studies can be carried into consideration with the protein models which can be built using obtained protein sequences.

4. RESULT

Table 2: Protein query and their functions

Protein Query	Protein name	Biological Process	Role in Virulence
BP951000_0622	MerR family transcriptional regulator	GO:0006355 regulation of transcription, DNA-templated	Metal-binding domain (Brown, N. L., et al 2003, Changela, A., et al 2003, Matthew J. Bush et al 2017)
BP951000_0591	methionine-tRNA ligase	None predicted	Catalyzes the chemical reaction (Leung K-Y et al 2013, Van Haandel L et al et al 2012, Tian Q et al 2015& Kozo Tomita 2017)
BP951000_0785	methyl-accepting chemotaxis protein B	GO:0007165 signal transduction	Sensing environmental cues (Abu Iftiaf Md Salah Ud-Din1 et al 2017, Berleman JE et al 2005 & He K, Bauer CE (2014))
BP951000_1875	methyl-accepting chemotaxis sensory transducer	GO:0007165 signal transduction	Sensing environmental cues (Abu Iftiaf Md Salah Ud-Din1 et al 2017, Berleman JE et al 2005 & He K, Bauer CE (2014))
BP951000_1776	methyl-accepting chemotaxis protein B	GO:0007165 signal transduction	Sensing environmental cues (Abu Iftiaf Md Salah Ud-Din1 et al 2017, Berleman JE et al 2005 & He K, Bauer CE (2014))
BP951000_1844	methyl-accepting chemotaxis sensory transducer	GO:0007165 signal transduction	Sensing environmental cues (Abu Iftiaf Md Salah Ud-Din1 et al 2017, Berleman JE et al 2005 & He K, Bauer CE (2014))
BP951000_1845	methyl-accepting chemotaxis sensory transducer	GO:0006935 chemotaxis GO:0007165 signal transduction	Sensing environmental cues (Abu Iftiaf Md Salah Ud-Din1 et al 2017, Berleman JE et al 2005 & He K, Bauer CE (2014))
BP951000_0500	methyl-accepting chemotaxis protein B	GO:0007165 signal transduction	Sensing environmental cues (Abu Iftiaf Md Salah Ud-Din1 et al 2017, Berleman JE et al 2005 & He K, Bauer CE (2014))
BP951000_1276	methyl-accepting chemotaxis protein B	GO:0006935 chemotaxis GO:0007165 signal transduction	Sensing environmental cues (Abu Iftiaf Md Salah Ud-Din1 et al 2017, Berleman JE et al 2005 & He K, Bauer CE (2014))
BP951000_0882	methionyl-tRNA formyltransferase	GO:0009058 biosynthetic process GO:0071951 conversion of methionyl-tRNA to N-formyl-methionyl-tRNA	Involvement in the biosynthetic process (Xi Peng et al 2016 & Zhang, Y. M., J. K. Liu and T. Y. Wong (2003))
BP951000_2210	metal-dependent amidohydrolase	None predicted	Helps in enzyme Biodegradation (Alexandre A. de Castro 2016, Singh BK (2009), Theriot CM, Grunden AM (2011) & Bigley AN, Raushel FM (2013))
BP951000_1654	methyltransferase	None predicted.	Epigenetic modification (Siyi Chena, et al 2017, W. Reik, J. Walter 2001 & K.D. Robertson 2005)
BP951000_0520	membrane-bound lytic murein transglycosylase D	GO:0000270 peptidoglycans metabolic process	The essential catalyst of bacterial cell wall function (E. Scheurwater, 2008, Dusan Hesek, et al 2017)
BP951000_0389	metal-dependent phosphohydrolase	None predicted.	regulation of response against nutritional stress conditions (Shama Khan1 et al 2015, Gross M, Marianovsky I, Glaser G (2006))

BP951000_1097	methyltransferase GidB	GO:0006364 rRNA processing	Epigenetic modification (Siyi Chena et al 2017, W. Reik et al 2001 & K.D. Robertsn et al 2005)
BP951000_1849	metal-dependent amidase aminoacylase carboxypeptidase	GO:0008152 metabolic process	Involvement in Peptidolytic growth (Veronica M., Lin, L et al 2007)
BP951000_0058	metal-dependent amidase/aminoacylase/carboxypeptidase AbgB	GO:0008152 metabolic process	Involvement in Peptidolytic growth (Lin, L et al 2007)
BP951000_0378	methylenetetrahydrofolate reductase	GO:0006555 methionine metabolic process GO:0055114 oxidation-reduction process	Causes gene mutation in chromosome 1 (Mark W. Morningstar, et al 2017)

5. DISCUSSION

The above-mentioned proteins are the target proteins and they were obtained because of their high pathogenicity factor and involvement in the metabolic activities, mutation, hydrolase activity, signal transduction and many more.

In our subtractive genomics approach, the essential genome sequences were available in NCBI Protein BLAST, in which no similarities were found in only between 28 proteins sequences virulence caused ones were brought back from the NCBI BLAST and searched against Database of Essential Genes (DEG) tool respectively. When searched under DEG BLAST showed only 18 of them showed the no similarities (no hit) which were considered as the druggable targets. The Interpro an online tool helped to known the individual proteins' biological process and molecular function and the research studies on these proteins showed us that the involvement of them in the virulence factors and showed the matching of them with the other proteins. Hence concluded that the Bacteria *B. Pilosicoli* selected these above-mentioned proteins as its targets because of their involvement in the virulence factors, which make them differ from other bacteria.

6. CONCLUSION

With consideration to the above results, it can be concluded that the obtained proteomes have the highest rate of pathogenicity role in hens, pigs and also zoonotic potentials. There is a need for investigations regarding this proteomics which play a major role with respect to their pathogenicity. Further studies can be carried out by using these proteomes to feature investigate the protein models to be built and to find out the drug targets to the above-obtained results. The intention of ligand-protein docking is to expect the most important binding mode(s) of a ligand with a protein of recognized three-dimensional structure. Successful docking techniques seek high-dimensional spaces correctly and use a scoring feature that efficaciously ranks candidate dockings, they also can be used to carry out virtual screening on large libraries of compounds, rank the results, and recommend structural hypotheses of the way the ligands inhibit the target, that's useful in lead optimization.

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