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Original article

Identification of potential drug targets in *Yersinia pestis* using metabolic pathway analysis: MurE ligase as a case study

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ABSTRACT

Sporadic outbreaks of plague, lack of a vaccine, emergence of multidrug-resistant strains of *Yersinia pestis*, and its potential use in bioterrorism, call for an urgent need to develop new drugs for plague. We have used comparative metabolic pathway analysis to identify 245 drug-target candidate enzymes in *Y. pestis* CO92 which are non-homologous to host *Homo sapiens* and likely to be essential for the pathogen's survival. Further analysis revealed that 25 of these are potential choke point enzymes. As a case study, structure of a choke point enzyme, MurE ligase, was modeled and docking studies performed against a library of compounds leading to identification of a potential inhibitor. This approach enables rapid potential drug-target identification, thereby facilitating search for new antimicrobials.

1. Introduction

Plague is often dismissed as an ancient disease, with little relevance in the present day world. The historical prevalence of the disease has been well documented [1,2]. However, sporadic outbreaks in the last two decades and the potential use of the causative organism as an agent of bioterrorism make it a relevant threat even now [2]. Though the incidence of the disease has drastically reduced with improvement in health awareness and availability of antibiotics, it can by no means be considered as eradicated.

The causative organism of plague, *Yersinia pestis* is an obligate parasite. Based on metabolic differences (ability to convert nitrate to nitrite and glycerol fermentation), it has three biotypes *viz*., antiqua, mediaevalis and orientalis, with no difference in their virulence [1]. It infects mammals as their primary hosts, with fleas acting as agents of transmission. Transmission of the pathogen is also possible through air, making it an even bigger threat as a biological weapon. Plague continues to afflict thousands of people annually, in absence of any effective vaccine against it. Current treatment relies solely on antibiotics. In such a scenario, emergence of strains resistant to multiple drugs is a cause of great concern [3,4]. Also, several thousand new cases are reported each year, predominantly in Africa [5]. All the cases in recent history have been attributed to the orientalis biovar. In 2001, the complete genome of a clinical isolate belonging to the orientalis biovar, *Y. pestis* CO92 was sequenced [6]. Endemic plague foci persist in many countries in Africa; the former Soviet Union; the Americas, including the southwestern United States; and parts of Asia. From 1989 to 2003, 38,310 human plague cases and 2845 deaths have been reported to the World Health Organization by 25 countries [7]. All these factors suggest that plague still poses a grave threat to human health, and more potent alternatives to current treatment methods are urgently required. Hence, in the present study we attempt to find a set of potential drug targets in the *Y. pestis* CO92 strain of the orientalis biovar, responsible for most of the recent outbreaks of the disease.

Availability of complete genome sequences of human pathogens is a crucial asset in obtaining biological information about them. *In silico* analysis of these genomes and the information extracted from them is a useful strategy to develop tools to counter these pathogens. One aspect of this strategy is the analysis of the complete metabolic network of these pathogens to understand their physiology in depth. A deep understanding of the metabolic networks,

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intra-cellular processes, and the physiology of these pathogens can help us in determining the factors responsible for their pathogenicity, virulence, and survival. This in turn, can be used to find novel drugs and vaccines against them.

In this study, comparative metabolic pathway analysis was performed between the pathogen *Y. pestis* CO92 and its host *Homo sapiens* in order to detect the enzymes which are unique to pathogen. Enzymes which are specific to the pathogen, and show no significant homology to any protein in the host organism can serve as potential drug targets, as there is little risk of a potential drug adversely interacting with a host protein. Also, inactivation of proteins which are essential for survival could be lethal for the pathogen. Based on this a list of potential drug targets for plague have been compiled. To further refine the list, those enzymes which either uniquely consume a specific substrate or uniquely produce a specific product in a metabolic network (choke points) [8], were shortlisted through choke point analysis. The analysis revealed a set of 245 enzymes present in *Y. pestis* CO92 as potential drug targets for the treatment of plague, with 25 of them as choke points. Many of the enzymes identified as potential drug targets by our approach, have also been identified as drug targets or putative drug targets by The Center for Structural Genomics of Infectious Diseases (CSGID) Consortium (http://csgid.org/csgid/), which determines the three-dimensional structures of proteins from major human pathogens. Thus, this approach can be used for a fast and accurate assessment of the genomes of human pathogens for detecting potential drug targets. As a case study, the three-dimensional structure of one of the potential targets, MurE ligase enzyme was modeled in order to find a suitable inhibitor through docking studies.

2. Results and discussion

2.1. Metabolic pathways analysis

Comparative metabolic pathway analysis of *H. sapiens* and *Y. pestis* CO92 shows that there are 24 pathways unique to the pathogen and 72 pathways common to both, as listed in Tables 1 and 2, respectively.

Table 1
Metabolic pathways present in *Yersinia pestis* CO92 but not in the host *Homo sapiens*.

S. No.	Metabolic pathway	KEGG pathway ID
1.	Geraniol degradation	ype00281
2.	gamma-Hexachlorocyclohexane degradation	ype00361
3.	Benzoate degradation via hydroxylation	ype00362
4.	Fluorobenzoate degradation	ype00364
5.	Novobiocin biosynthesis	ype00401
6.	D-Alanine metabolism	ype00473
7.	Streptomycin biosynthesis	ype00521
8.	Polyketide sugar unit biosynthesis	ype00523
9.	Lipopolysaccharide biosynthesis	ype00540
10.	Peptidoglycan biosynthesis	ype00550
11.	1- and 2-Methylnaphthalene degradation	ype00624
12.	1,4-Dichlorobenzene degradation	ype00627
13.	Fluorene degradation	ype00628
14.	Benzoate degradation via CoA ligation	ype00632
15.	Trinitrotoluene degradation	ype00633
16.	3-Chloroacrylic acid degradation	ype00641
17.	C5-Branched dibasic acid metabolism	ype00660
18.	Caprolactam degradation	ype00930
19.	Biosynthesis of siderophore group nonribosomal peptides	ype01053
20.	Two-component system	ype02020
21.	Bacterial chemotaxis	ype02030
22.	Flagellar assembly	ype02040
23.	Phosphotransferase system (PTS)	ype02060
24.	Bacterial secretion system	ype03070

Table 2
Metabolic pathways present in both the pathogen *Yersinia pestis* CO92 and the host *Homo sapiens*.

S. No.	Metabolic pathway	KEGG pathway ID
1.	Glycolysis/gluconeogenesis	ype00010
2.	Citrate cycle (TCA cycle)	ype00020
3.	Pentose phosphate pathway	ype00030
4.	Pentose and glucuronate interconversions	ype00040
5.	Fructose and mannose metabolism	ype00051
6.	Galactose metabolism	ype00052
7.	Ascorbate and aldarate metabolism	ype00053
8.	Fatty acid biosynthesis	ype00061
9.	Fatty acid metabolism	ype00071
10.	Ubiquinone and other terpenoid-quinone biosynthesis	ype00130
11.	Oxidative phosphorylation	ype00190
12.	Purine metabolism	ype00230
13.	Pyrimidine metabolism	ype00240
14.	Alanine, aspartate and glutamate metabolism	ype00250
15.	Glycine, serine and threonine metabolism	ype00260
16.	Cysteine and methionine metabolism	ype00270
17.	Valine, leucine and isoleucine degradation	ype00280
18.	Valine, leucine and isoleucine biosynthesis	ype00290
19.	Lysine biosynthesis	ype00300
20.	Lysine degradation	ype00310
21.	Arginine and proline metabolism	ype00330
22.	Histidine metabolism	ype00340
23.	Tyrosine metabolism	ype00350
24.	Phenylalanine metabolism	ype00360
25.	Tryptophan metabolism	ype00380
26.	Phenylalanine, tyrosine and tryptophan biosynthesis	ype00400
27.	beta-Alanine metabolism	ype00410
28.	Taurine and hypotaurine metabolism	ype00430
29.	Selenoamino acid metabolism	ype00450
30.	Cyanoamino acid metabolism	ype00460
31.	D-Glutamine and D-glutamate metabolism	ype00471
32.	Glutathione metabolism	ype00480
33.	Starch and sucrose metabolism	ype00500
34.	Other glycan degradation	ype00511
35.	Amino sugar and nucleotide sugar metabolism	ype00520
36.	Glycerolipid metabolism	ype00561
37.	Inositol phosphate metabolism	ype00562
38.	Glycerophospholipid metabolism	ype00564
39.	Arachidonic acid metabolism	ype00590
40.	alpha-Linolenic acid metabolism	ype00592
41.	Sphingolipid metabolism	ype00600
42.	Pyruvate metabolism	ype00620
43.	Glyoxylate and dicarboxylate metabolism	ype00630
44.	Propanoate metabolism	ype00640
45.	Butanoate metabolism	ype00650
46.	One carbon pool by folate	ype00670
47.	Methane metabolism	ype00680
48.	Thiamine metabolism	ype00730
49.	Riboflavin metabolism	ype00740
50.	Vitamin B6 metabolism	ype00750
51.	Nicotinate and nicotinamide metabolism	ype00760
52.	Pantothenate and CoA biosynthesis	ype00770
53.	Biotin metabolism	ype00780
54.	Lipoic acid metabolism	ype00785
55.	Folate biosynthesis	ype00790
56.	Porphyrin and chlorophyll metabolism	ype00860
57.	Terpenoid backbone biosynthesis	ype00900
58.	Limonene and pinene degradation	ype00903
59.	Nitrogen metabolism	ype00910
60.	Sulfur metabolism	ype00920
61.	Aminoacyl-tRNA biosynthesis	ype00970
62.	Biosynthesis of unsaturated fatty acids	ype01040
63.	ABC transporters	ype02010
64.	Ribosome	ype03010
65.	RNA degradation	ype03018
66.	RNA polymerase	ype03020
67.	DNA replication	ype03030
68.	Protein export	ype03060
69.	Base excision repair	ype03410
70.	Nucleotide excision repair	ype03420
71.	Mismatch repair	ype03430
72.	Homologous recombination	ype03440