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Choke point analysis of the metabolic pathways of *Acinetobacter baylyi*: A genomics approach to assess potential drug targets

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Numerous species of the genus *Acinetobacter* have been known to cause various nosocomial infections. An insight into the pathogenesis of *Acinetobacter baylyi* reveals that it is a potent organism in causing nosocomial infections. In this study, choke point analysis of the entire metabolic network of *A. baylyi* is performed to assess potential drug targets. Potential drug targets are proposed based on the analysis of the top 8 choke points in the bacterial network. A comparative study between the reported top 8 bacterial choke points and the human metabolic network was performed. Further biological inferences were made on results obtained by performing a homology search against the human genome. The study was successful in listing out of the potential drug targets from these pathways which may be useful for the discovery of broad-spectrum drugs.

Key words: Choke points, *Acinetobacter baylyi*, human genome, drug discovery, metabolic pathway, opportunistic pathogen.

INTRODUCTION

Acinetobacter spp. plays a significant role in the colonization and infection of immunocompromised patients. They have been implicated in a variety of nosocomial infections, including bacteremia, urinary tract infection and secondary meningitis, but their predominant role is as agents of nosocomial pneumonia, particularly ventilator-associated pneumonia in patients confined to hospital intensive care units (Bergogne-Bérézin and Towner, 1996). Such infections are often difficult to be treated by the clinician as these strains have developed resistance to the classical antibiotics used. This calls for an urgent need for identification of novel drug targets to effectively combat nosocomial infections. Infact, these bacteria have a significant capacity for long term survival in the hospital environment, with corresponding enhanced opportunities for transmission between patients, either via human reservoirs or via inanimate materials. In this study, *Acinetobacter baylyi* (also called *Acinetobacter* sp*.* ADP1), an opportunistic pathogen (Chen et

al., 2008), is used as a model organism for the identification of novel drug targets for effective treatment of nosocomial infections.

A. baylyi is highly competent for natural transformation (Young et al., 2005). Due to this ability, ADP1 can effectively express a wide variety of foreign genes including antibiotic resistance cassettes, essential metabolic genes, negatively selectable catabolic genes and even intact operons from highly divergent bacteria (Metzgar et al., 2004). This makes it an opportunistic pathogen which is capable of causing secondary infections in association with other gram negative bacilli. Moreover, *Pseudomonas aeruginosa* is phylogenetically the closest organism to ADP1 (Jacobs et al., 2003) and shares 1655 orthologous genes with it (V de Berardinis et al., 2008). One thousand one hundred and seventy seven orthologous genes are shared between *E. coli* and ADP1 (V de Berardinis et al., 2008). Due to a considerable degree of homology between the genes of ADP1 and these pathogenic gram-negative bacilli, it is a potent causal organism of secondary infections in association with them. Also, its high competence for natural transformation (Barbe et al., 2004) may favour uptake of DNA from pathogenic strains in the hospital environment. An

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Table 1. The reaction and its EC number are given. The In Human column denotes whether or not the enzymatic activity has a similar enzyme in human as determined by BLAST alignment with an expectation of less than 0.0001.

Target enzyme	EC number	References	Chokepoint	In human
Lipoyl synthase	2.8.1.8	Kok et al., 1995	Yes	Yes
DNA directed RNA polymerase	2.7.7.6	V de Berardinis et al., 2008	Yes	Yes
UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase (murein peptide ligase)	6.3.2.-	Chakravorty et al., 2007	Yes	No
ssDNA exonuclease, 5'→3' specific, Mg Dependent	3.1.-.-	Kickstein et al., 2007	Yes	No
UDP-N-acetylmuramate-alanine ligase	6.3.2.8	V de Berardinis et al., 2008	Yes	No
Tryptophan synthase	4.2.1.20	Cohn and Crawford, 1976	Yes	No
Phosphopantetheinyl transferase	2.7.8.-	V de Berardinis et al., 2008	Yes	No
GTP cyclohydrolase I	3.5.4.16	El Yacoubi Basma, 2006	Yes	Yes

insight into its pathogenesis has revealed that it also cause nosocomial infections particularly bacteremia in immunocompromised patients (Chen et al., 2008). As certain genes are present in *A. baylyi*, which show resistance to traditional antibiotics (Gomez et al., 2006), there is an urgent need for the identification of novel drug targets.

Understanding the cellular mechanisms and interactions between cellular components is instrumental to the development of new effective drugs and vaccines. Metabolic pathway analysis is increasingly becoming important for assessing inherent properties in biochemical reaction networks. Choke point strategy enables to classify reactions that either uniquely consume a specific substrate or produce a specific product (Yeh et al., 2004). Such reactions, called choke point reactions, are believed to be essential to the pathogen's survival and are therefore potential drug targets. This study identifies such choke points in the metabolic network of *A. baylyi* as potential drug targets.

MATERIALS AND METHODS

Sequence Retrieval and pathway analysis

KEGG (Kyoto Encyclopedia of Genes and Genomes (Kanehisa et al., 2002) pathway database was used as a source of metabolic pathway information. KEGG provides with a typical metabolic network consisting of reactions, metabolites and enzymes. Metabolic pathway identification numbers of the host *Homo sapiens* and the pathogen *A. baylyi* were extracted from the KEGG database. A network based comparative study of the choke points was performed between *A. baylyi* and *Homo sapiens*. BLASTp (Altschul et al., 1997) package was used to infer homology between the sequences. Further, gene ontologies archived in different databases viz. UniProt, Microbial Genome Database and Genoscope were used to predict functions of the enzymes coded by these genes.

Choke point analyses

Here the choke point analyses method of Yeh et al. (2004) has been adopted.

Chokepoint analyses are particularly straightforward to perform with a computational representation of metabolism and there are

certain reactions that are excluded from the chokepoint analysis, namely proteolytic reactions (as the specificity of these reactions is not captured), reactions that do not have clearly defined substrates (e.g., protein disulfide isomerase), and reactions with important side effects (many ATPases). Although these enzymes could be good drug targets, the rationale of the method is not expected to apply in these cases.

If an enzyme catalyzes at least one chokepoint reaction, it is classified as a potential drug target. All the potential metabolic drug targets are listed in Table 1.

To assess the usefulness of identifying chokepoint enzymes for proposing drug targets, chokepoints and non-chokepoints against proposed drug targets from the literature is compared. A complete literature search for proposed *Acinetobacter* drug targets is attempted that were metabolic enzymes and met the criteria outlined above. Of the 12 proposed targets with biological evidence, 8 are chokepoints in Table 1. Due to the high percentage of enzymes identified as choke points, one additional criteria observed in addition to being a choke point enzyme for identifying potential metabolic drug targets is that an enzyme not having an isozymes would make it more likely to be a good drug target. This is because one enzyme would be easier to inhibit than a family of enzymes. NADH Dehydrogenase I in *Acinetobacter* has two isozymes and according to the criteria outlined above, it has not been included in the list of potential drug targets identified in Table 1.

There are further aspects on which the list of potential drug targets can be narrowed down. The drug should adversely affect the parasite but not the human host; therefore, if the drug target has a homologous enzyme in human, it should not be essential or have differential inhibition in human (perhaps due to different protein structure or different regulation). Potential drug targets should be expressed in the human stages of the pathogen.

RESULTS

Potential drug targets are proposed based on the analysis of the top 8 choke points in the bacterial network. A comparative study between the reported top 8 bacterial choke points and the human metabolic network was performed. Further biological inferences were made on results obtained by performing a homology search against the human genome. The study was successful in listing out the potential drug targets from these pathways, which may be useful for the discovery of broad-spectrum drugs.
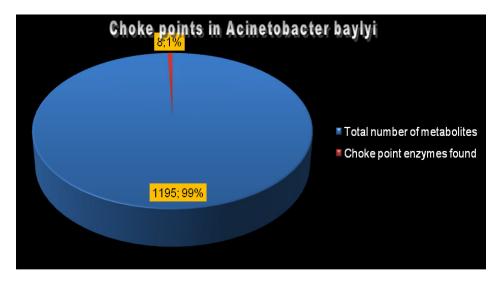
A total of 8 choke point enzymes have been identified

Figure 1. Choke point enzymes in the metabolome of *Acinetobacter baylyi.*

from the metabolome of *A. baylyi,* which comprise 1% of the metabolome of the pathogen (Figure 1). Strategy applied has been explained in Materials and Methods. Of the 8 choke points 3 choke points have homologs in human and may interfere in drug-pathogen enzyme interaction causing some side effects. Therefore 62.5% of the reported targets are choke point enzymes having no human homologs. The 8 choke points are given in Table 1.
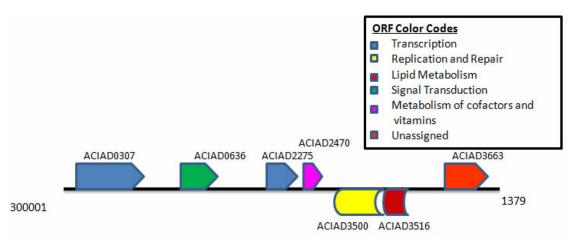
DISCUSSION

Drug target identification based on 'omics' networks (di Bernardo et al., 2005; Giaever et al., 2004; Yeh et al., 2004) is a very promising approach that has recently become possible. The concept of choke points (Dawson and Elliot, 1980) in a given network contributes effectively in the identification of drug targets.

Often drugs are identified by a unique pathogen specific metabolic activity approach, as in the case of reverse transcriptase in HIV (Imamichi, 2004). However, the screening of the entire pathogen network to find choke point based potential drug targets followed by a study with the human metabolic network provides additional results. Further, some enzymes for e.g. Lipoyl synthase, show a significant similarity at e-inclusion threshold value of 0.0001, thus have a human homologue. Such enzymes may still be used as drug targets because the e-inclusion value has been set so as to detect closely related homologues. No homologue in such a case may lead to an inference that it is not a drug target as the two protein sequences are divergent due to evolutionary lineage. In such cases the target may be effective without causing any side effects to the host. However, experimental validation of these drug targets may remove any ambiguity. The targets obtained in this

study are obtained by reliable computational approaches and have a sound grounding for future analysis. Annotations provided in *Acineto*Cyc (Genoscope), KEGG Metabolic Pathways and UniProt (Expasy proteomics server) are analyzed to investigate further on the predictability of the enzymes as potential drug targets. Figure 2 depicts the loci of the Open Reading Frames of the genes encoding the choke point enzymes. Such a representation highlights the pathways in which these enzymes are involved and their essentiality.

All the 8 choke point enzymes identified in the study are essential for the pathogen's survival. GTP cyclohydrolase-I is the first enzyme of the Tetrahydrofolate pathway (THF) and serve as a cofactor during the synthesis of purines, glycine, serine and also the initiator formyl methionyl t-RNA (El Yacoubi et al., 2006). The presence of homologous enzymes in both human and bacteria has precluded the development of GTP-cyclohydrolase-I as a viable target. Murein peptide ligase (Mpl) acts in response to DNA damage. Moreover an Mpl deficient mutant is susceptible to β-lactamases. Since *E. coli* mutants do not show susceptibility to β-lactamases so Mpl is a potential target for species-specific antibacterial compounds. Tryptophan synthase is responsible for the synthesis of L-tryptophan from indole and L-serine. It is thus essential for amino acid biosynthesis. DNA dependent RNA polymerase catalyzes the transcription of DNA into RNA using four ribonucleotides as substrates. UDP-N-acetylmuramate-alanine ligase is essential for cell wall formation and peptidoglycan biosynthesis. *recJ* encoded ssDNA exonuclease is essential for genetic transformation and it has been shown that its deletion is lethal (Kickstein et al., 2007) Lipoate synthase is essential for the biosynthesis of cofactors, prosthetic groups, and carriers. Phosphopantethenyl transferase is essential for siderophore synthesis. The iron acquisition systems of many pathogenic and saprophytic bacteria

Figure 2. Diagrammatical representation of the chokepoint enzymes coding genes on the genome map of *A. baylyi*. Open Reading Frame (ORF) colour codes depict the various metabolic pathways in which these chokepoint enzymes are involved.

rely on the production of small molecules called sidero-phores. Siderophores allow the pathogen to thrive in the human microenvironment by the uptake of free iron. By identifying choke point reactions, enzymes essential for the pathogen's survival have been identified. The identification of choke point enzymes has led to the enrichment of drug targets as compared to the non choke point enzymes.

Conclusion

The genome sequence has allowed to fill in much of the metabolism of *A. baylyi*, revealing that many pathways normally present in eukaryotes are absent from this organism. It is also possible that some 'missing' pathways are actually present, but they may not be identifiable. In addition, by identifying choke point reactions, it has been tried to identify enzymes that are essential to the parasite's survival. There is an enrichment of drug targets in chokepoints as compared with non-chokepoints. This leads to the conclusion that the classification of an enzyme as a chokepoint has some bearing on whether or not it would make a good drug target. The choke point analysis is limited, because the capabilities of the parasite to transport an accumulating metabolite out of the cell or a limiting metabolite into the cell have not been considered. One reason that chokepoints may not be essential could be that they create unique intermediates to an essential product that are not essential themselves because of another pathway to the essential product. Finally, there could be chokepoint reactions that are not essential due to other pathways that achieve the same metabolic goal within the organism, such that blocking the reaction has no deleterious effects on the parasite. The provisional targets which have been cited here needs to be examined further, both computationally and experimentally for these additional features.

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