The Czs heavy metal efflux pump and its expression in *Elizabethkingia anophelis* when exposed to β-lactam drugs

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ABSTRACT

Cobalt, zinc, and cadmium are heavy metals which can often be found in clinical settings (on handrails, medical equipment, etc.). These metals have been found to have antimicrobial properties. *Elizabethkingia anophelis*, an emerging nosocomial infection in the Great Lakes region of North America, has proteins that may counteract these properties and make the organism more dangerous in a clinical setting. We used information from the RAST database and compared it to RNA sequencing data from *E. anophelis* to determine the effect of two different antibiotics on these proteins. We found that the frequency of transcription of these proteins responded to the introduction of β lactams (i.e. penicillins, to which *E. anophelis* is resistant) either as a stress response by the bacteria, or as a concerted effort to pump β lactams from the cell. Our research may impact how cobalt, zinc, and cadmium-containing compounds are used in clinical settings when combating *E. anophelis* and how general practices regarding the combating of drug-resistant microbes are carried out.

INTRODUCTION

Elizabethkingia anophelis is a gram-negative, rod-shaped non-motile aerobic bacillus that is resistant to most common antibiotics (excepting erythromycins and others of that family) (Etymologia 2016). Since 2015, the organism has caused several nosocomial infections in immunosuppressed individuals near the American Great Lakes. Due to their constant (but imperfect) exposure to antibiotics and antiseptics, so-called "Superbugs" such as MRSA (Multi-drug resistant Staphylococcus Aureus), MDR-TB (Multi-drug resistant Tuberculosis) and Clostridium difficile have seen a renaissance. *E. anophelis* is another "Superbug." Our research specifically deals with *E. anophelis*' resistance to cobalt, zinc, and cadmium via the Czc, a four-protein ion pump related to the Cus pump, which is responsible for resistance to germicidal copper and silver ions in similar organisms and which is found in all kingdoms. Both pumps are driven by the proton-motive force and do not require ATP (Nies 1995). Both of these pumps are a part of the RND permease superfamily identified by Tseng et al in 1999. We sought to determine what effect, if any, the exposure of *E anophelis* to two β-lactams, Imipenem and Cefotex, would have on the frequency of expression of our proteins.

MATERIALS AND METHODS

We searched RAST, a database of all potential proteins in *E. anophelis* for "cadmium, cobalt, and zinc." RAST contains a database of the genomic information isolated from *E. anophelis* and the genes' presumptive proteins, based on similar analyses of other similar organisms. We narrowed our search to twenty total genes whose presumptive proteins were related to cadmium, cobalt, and zinc efflux. We then compared the standard expression frequency of these genes in mRNA to the expression frequency when the organism was exposed to Imipenem (a carbapenem) or Cefotex (a cephalosporin), two β lactams. We determined that any gene that had an increase or decrease of expression of 50% or more was significant (i.e. fold change of 1.5 or greater), and any protein expressed fewer than three times without antibiotic exposure (fewer than six times total) was likely insignificant.

RESULTS

peg (RAST-specific)	Fold Change Cefotax/ control	Fold Change Imipenem/ control	Sum transcripts	Control mRNA	Cefotex mRNA counts	Imipenem mRNA counts	Significance
368	-1.1	1.7	544	149	138	257	Imipenem
369	-1.1	-1.3	1316	481	456	379	No
370	-1.1	-1.2	1672	610	547	515	No
575	1.5	-1	7	2	3	2	No
712	1	-1.1	250	84	86	80	No
996	-1.5	1.1	407	146	100	161	Cefotax
997	-2	1.2	64	24	12	28	Cefotax
999	-1.4	1.4	84	27	20	37	No
1471	-3.8	1.8	3389	1117	297	1975	Yes
1775	1.1	1.1	35	11	12	12	No
1820	1.4	-1	81	24	34	23	No
1885	-1	3	10	2	2	6	Imipenem
1886	-1.1	1.5	65	19	18	28	Imipenem
1887	-1.2	1.3	19	6	5	8	No
1963	-1	2	4	1	1	2	No
3302	2	-1	4	1	2	1	No
3434	1.3	-1.3	1259	418	525	316	No
3435	1.4	-1.6	245	80	114	51	Imipenem
3436	1.8	-1.7	1718	507	910	301	Yes
3438	1.4	-1.7	15	5	7	3	Imipenem

Fig.1: Table of genes, fold change, number of transcriptions, and significance (No=insignificant either in number of control transcripts, total transcripts, or because fold change<1.5).

Of the twenty genes we investigated, we found that nine were significant: the frequency of transcription responded to both drugs for genes 3436 and 1471; it responded only to Imipenem for genes 368, 1185, 1186, 3435, 3438; and it responded only to Cefotex for genes 996 and 998. Our analysis also shows that these genes are often grouped into triplets or quartets (genes 368, 369, and 370; genes 996, 997, and 999; genes 1885, 1886, and 1887; genes 3434, 3435, 3436, and 3438) indicating a three or four-protein grouping like that of CusA and other efflux pumps. Furthermore, genes outside these groupings were usually insignificant either in fold change or in number of original transcriptions.

DISCUSSION

Our analysis of the change in transcriptions between control *E. anophelis* and β-lactam-exposed *E. anophelis* confirms that for several Cd-Co-Zn efflux genes, the introduction of a β-lactam drug to the organism causes a significant change in expression frequency (fold change >1.5). This may be a result of the organism's generic stress response to invaders, or it may be a specific "anti-hospital" reaction, whereby the organism responds to the antiseptic environment of a hospital, e.g. response to antibiotics, antiseptics and disinfectants, and antimicrobial materials such as those impregnated with cadmium, cobalt, and zinc; or less likely, silver and copper.

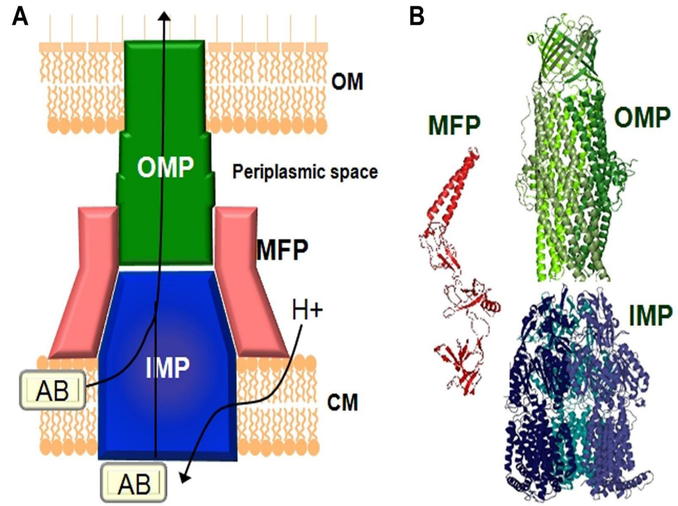

Fig. 2: Rendering of the CusA pump, a four-protein complex, similar to the Csz pump, which is responsible for silver and copper cation efflux. Csz is structurally and functionally similar.

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