

**The phylogenetic variety and importance in the B-Lactamases of Elizabethkingia; anophilis, miracola and meningoseptica in pathogenicity.**

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With recent outbreaks of Elizabethkingia affecting the United States, it is important to further understand the method of action that this bacterium uses to infect patients. Given that Elizabethkingia is not regarded as a pathogen when found in nature, but has developed several advantages in antibiotic resistance, the sheer flexibility and variability of this organism to evade host immunity and pharmacological responses is cause for awe and alarm. Within this paper we will analyze the importance of Elizabethkingia as a pathogen, its proteophylogenetic importance as a carrier of  $\beta$  lactamases and the further development of a  $\beta$  lactamase library for the identification of these genes and proteins in future strains and other organisms.

Generally we find no cause of alarm when we speak of the Elizabethkingia Genus, as we see that it is a naturally ubiquitous gram-negative rod bacterium pertaining to the commensal bacteria of certain fish and amphibians. Elizabeth King for who the bacteria was named after was the first individual to isolate Elizabethkingia and originally dubbed it Flavobacterium meningosepticum in 1959. The reasoning behind this naming was due to the morphological characteristics of this bacterium and its ability to cause the ailments of sepsis and meningitis in infantile patients<sup>1</sup>. Yet with the dawn of the age of genetics and the ability to fully sequence the DNA of microorganisms, the world of phylogeny was

turned upside down and given new life through the process of 16s rRNA sequencing and categorization. Once this analysis was done to the Genus Chryseobacterium (which was the new house of Elizabethkingia since 1994), it was seen that the organisms for C. meningosepticum and C. miricola branched off together and housed their own Genus as opposed to the rest of the Chryseobacterium<sup>2</sup>.



**Fig 1. Elizabethkingia blood agar spread plates<sup>26</sup>**

According to Bergey's Manual of Systematics of Archaea and Bacteria, Elizabethkingia is characterized as

*"Straight, single rods with rounded ends, typically 0.5  $\mu\text{m}$  wide and variable in length, often forming filaments. Do not form endospores. Spherical degenerative forms do not appear in ageing liquid cultures. Nonmotile. Flagellar motility, gliding movement, and swarming growth have not been reported. Colonies are typically nonpigmented or weakly yellow*

*pigmented, circular, convex, smooth and shiny, with entire edges, and up to 2 mm in diameter. A strong aromatic odor is produced. Gram-stain-negative. Obligately aerobic, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Nitrate is not reduced. Chemoorganotrophic. Growth occurs at 22–37°C, but not at 5 or 42°C. Growth occurs readily on the usual commercial media. No growth factors are required. Growth on seawater media is possible. Growth on cefrimide and MacConkey agars is slow and weak, but is also strain-dependent. Catalase, oxidase, phosphatase, and  $\beta$ -galactosidase activities are present. Acid, but no gas, is produced from a number of carbohydrates in media with low peptone concentrations. Strong proteolytic activity occurs. Esculin, gelatin, and casein are hydrolyzed, but agar is not. Malonate is not utilized. Resistant to a wide range of antimicrobial agents. Menaquinone MK-6 is the predominant respiratory quinone. Predominant cellular fatty acids are C15:0 iso, C17:0 iso 3-OH, C17:1 iso  $\omega$ 9c, and summed feature 4 (comprising C15:0 iso 2-OH and/or C16:1  $\omega$ 7c/t). As they are ubiquitous in soil and freshwater, strains of *Elizabethkingia meningoseptica* occasionally occur in the hospital environment and clinical specimens; they are opportunistic pathogens of humans and various animals.”<sup>3</sup>*

### **Pathology & Virulence**

*Elizabethkingia* works as an opportunistic nosocomial pathogen mainly affecting premature births and infants. Given that these individuals are not fully immunocompetent, it has also been shown that immunodeficient individuals are at risk for infections of *Elizabethkingia*. With its optimal conditions of growth being right around room temperature and its ability to grow on freshwater show, medical equipment that is not properly sanitized or tap water can be prime sources of infection. Among the list of

nosocomial infections that Elizabethkingia is known to cause we have: meningitis in immunocompromised adults, pneumonia, post-operative bacteremia endocarditis. More recently and surprisingly, Elizabethkingia has been found in a dire case of necrotizing fasciitis in a diabetic patient and infecting soft tissue thus leading to sepsis within fully immunocompetent patients 48 cases of Elizabethkingia infection resulting in 17 fatalities were reported in Wisconsin over a 5-month period beginning in November 2015<sup>4,5,6</sup>. and once again in 2016 nine people were confirmed dead due to complications from an infection of Elizabethkingia.

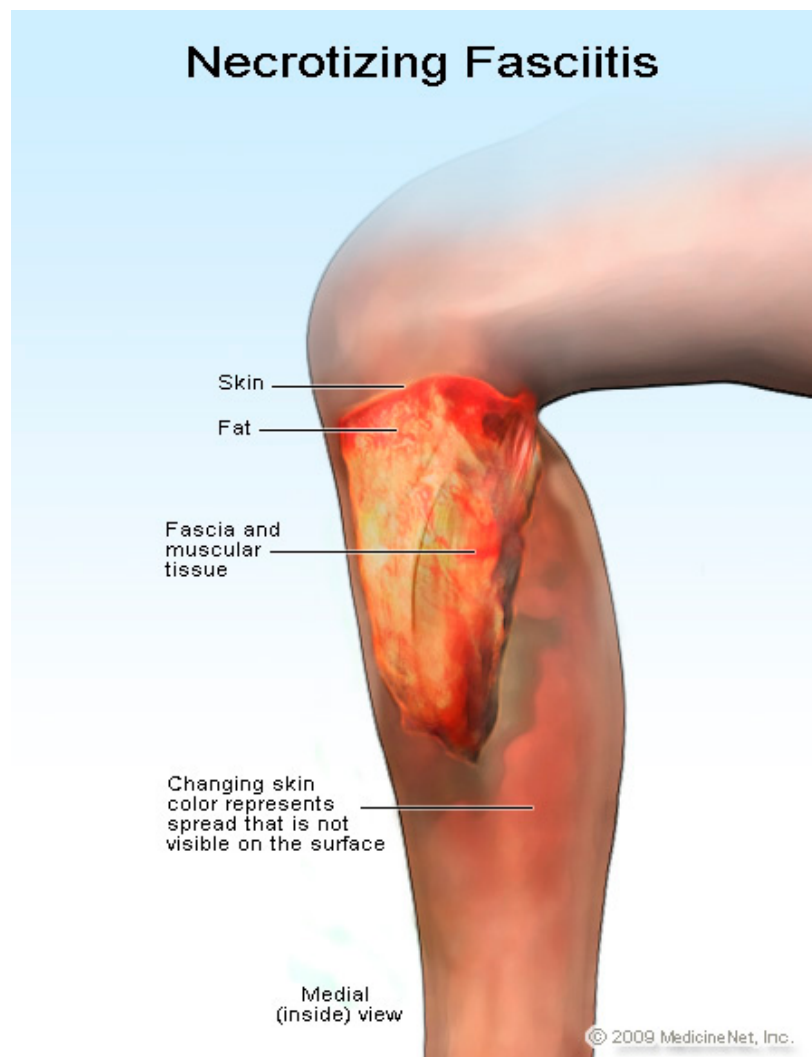


Fig 2. Demonstrates the effects of necrotizing fasciitis.<sup>4</sup>

As are most nosocomial opportunistic pathogens, Elizabethkingia demonstrates a high level of antibiotic resistance, and therefore serves as a perfect candidate for proteomic and enzymatic study. Elizabethkingia not only contains the genes necessary for the production of aminoglycosides, tetracycline, and chloramphenicol but its most interesting method of evading destruction is its application of a broad variety of B Lactamases.

### **The Categorization of B Lactamases**

There are two methods by which b-lactamases are classified. Molecular characteristics or functional properties offer us accurate descriptions of what the structure and therefore function the b-Lactamases offer. For the purposes of this paper we have chosen to bioinformatically analyze the molecular designations from classes A, B, C, and D. These individual classes serve to delineate the b-lactamases according to their primary structure and the domains that can be predicted due to their broad range conservation. In cases where clinical relevance is sought after, the functional classification method can be applied. This method divides b-lactamases into 3 groups (1,2,3) based on the substrates they are specific for and the treatment that they may require. Furthermore we can acquiesce subgroups, within the molecular classification method through functionality and merge the two methods this can be seen by the use of terms such as extended-spectrum b-lactamases, or ESBLs, and inhibitor-resistant TEM, or IRT, b-lactamases.(Bush)

### **Why B-Lactamases?**

B-lactam antibiotics are commonly utilized as broad spectrum antibiotics for the treatment of systemic and localized infections. Given the high reliance on these antibiotics

for a plethora of ailments, the natural selection of bacteria that are resistant to these agents have given a rise to pathogens that can destroy b-lactam antibiotics. These enzymes are ancient in nature, but given the more recent concentration of antibiotic use and ill patients in hospital settings, we see the genesis of “superbugs” able to resist several kinds of antibiotic attack as is our subject Elizabethkingia. B-Lactamases receive their name from their ability to degrade these previously mentioned b-lactam antibiotics [8]. Given the prehistoric nature of these enzymes, the naturally occurring isoforms exceed 1,300 [9]. The effects of the pathogens that possess these enzymatic complexes are extrapolated when placed in a hospital setting where b-lactams such as cephalosporins and carbapenems are heavily leaned on for the treatment of advanced infection.

## **Methods**

### **Clustal Omega**

Clustal Omega was utilized to create the phylogenetic tree Newicker sequences by using seeded guide trees and HMM profile-profile techniques to generate multiple sequence alignments between the known bla sequences and our candidate sequences.

### **Multiple Sequence Alignment**

A Multiple sequence alignment was done with our candidate b-lactamase sequences and known  $\beta$  lactamases of classes A,B,C and D. A multiple sequence alignment (MSA) is a sequence alignment of three or more biological sequences in the case of our analysis, these sequences were single letter protein sequences. Clustal produced results that allowed us to confirm sequence homology between our candidates of the Class A as they met the guidelines put together by the NIH for serine catalyzed b-lactamases. Clustal was run with numbers and the descriptions offered tell us the following: \*aa found in all sequences, : aa found in most sequences, .aa found in many sequences. Single amino acid or nucleotide changes appear as differing characters in a single alignment column, and insertion or deletion mutations appear as hyphens in one or more of the sequences in the alignment.

### **Treeview ETE**

Newick format produced by Clustal Omega was utilized to view a Python based phylogenetic tree through ETEtoolkit

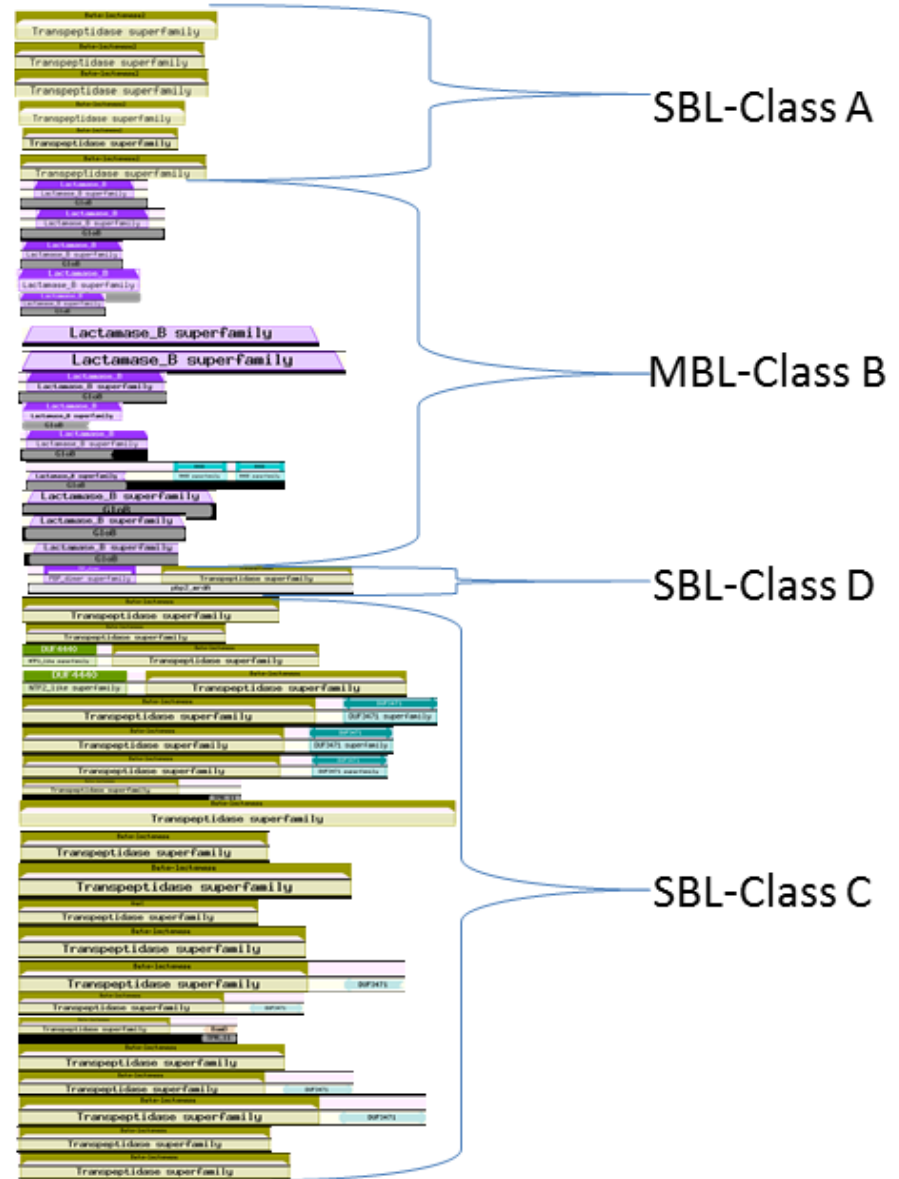
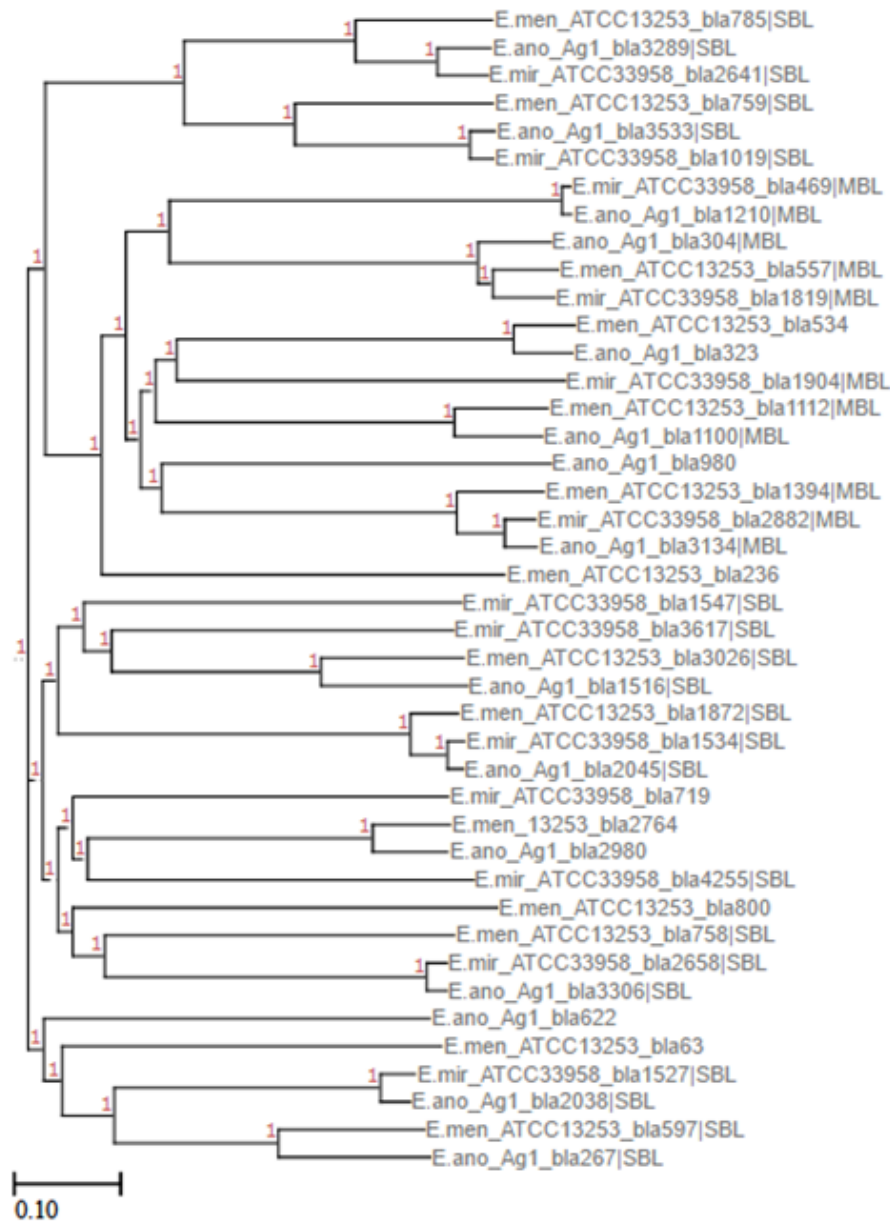
### **NCBI Conserved Domain Database**

The conserved domain database was utilized to identify the architectural characteristics of our candidate  $\beta$  lactamase proteins and furthermore associated with the phylogenetic tree to confirm architectural and evolutionary correlation.



# RESULTS

## Phylogenetic Tree of Classified B-Lactamases



## Multiple Sequence Alignment of Class A and C SBLs with motif localization

CLUSTAL O(1.2.1) multiple sequence alignment

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AAM74565.1|blaCARB-7|PSE      -----MKSLLVFALLMPSVVFASSSKFQSVQEIKGIESSLSARIGVA      43
AAD19217.1|blaCARB-6|PSE      -----MKFLLAFSLLIPSVVVFASSSKFQQVEQDVKAIEVSLSARIGVS      43
BAA02497.1|blaCARB-12|PSE     -----MKFLLVFSLLIPSVVVFASSSKFRQVEQDVKAIEVSLSARIGVS      43
AAK96394.1|blaCARB-1|PSE      -----MKFLLAFSLLIPSVVVFASSSKFQQVEQDVKAIEVSLSARIGVS      43
AAA25740.1|blaCARB-2|PSE      MLLYKMCDNQNYGVTY-MKFLLAFSLLIPSVVVFASSSKFQQVEQDVKAIEVSLSARIGVS      59
AAG23870.1|blaCARB-11|PSE     -----MKFLLAFSLLIPSVVVFASSSKFQQVEQDVKAIEVSLSARIGVS      43
E.men_ATCC13253_bla759|SBL     -----MT--FIKTYFTSLFLVGLIININAQTTNNELKKRIENIITGKKADVGS      47
E.ano_Ag1_bla3533|SBL         -----M--AKKAILGIMALMMSLPFFSQVHENTVLKKTLESIIISGKRATVGIS      46
E.mir_ATCC33958_bla1019|SBL    -----M--AKKAILGIIALLMSLSFFSQVHENTVLKKTLESIIISGKKATVGIS      46
AEI54993.1|blaPER-7|class     -----MNVIIKAVVTASTLLM-VSFSSFET-SAQSPLLKEQIESIVIGKKATVGVA      49
BAP39824.1|blaPER-8|class     -----MNVIIKAVVTASTLLM-VSFSSFET-SAQSPLLKEQIESIVIEKKATVGVA      49
BAU25894.1|blaPER-9|class     -----MNVIIKAVVTASTLLM-VSFSSFET-SAQSPLLKEQIESIVIGKKATVGVA      49
ACN22483.1|blaPER-5|class     -----MNVIIKAVVTASTLLM-VSFSSFET-SAQSPLLKQIESIVIGKKATVGVA      49
ACE77058.1|blaPER-4|class     -----MNVIIKAVVTASTLLM-VSFSSFET-SAQSPLLKEQIESIVIGKKATVGVA      49
AAU89132.1|blaPER-3|class     -----MNVIIKAVVTASTLLM-VSFSSFET-SAQSPLLKEQIESIVIGKKATVGVA      49
CAA79968.1|blaPER-1|class     -----MNVIIKAVVTASTLLM-VSFSSFET-SAQSPLLKEQIESIVIGKKATVGVA      49
ADD80743.1|blaPER-6|class     -----MNVIAKGVFTTTALLM-LSLSSWVW-SAQSPLLKEQIETIVTGKKATVGVA      49
CAA63714.1|blaPER-2|class     -----MNVITKCVFTASALLM-LGLSSFVW-SAQSPLLKEQIETIVTGKKATVGVA      49
AMJ32272.1|blaVEB-17|class    -----MKIV---KRILLVLL-SLFFTIAYSNAQTDNLTTLKIENVLKAKNARIGVA      46
AJH76942.1|blaVEB-10|class    -----MKIV---KRILLVLL-SLFFTIVYSNAQTDNLTTLKIENVLKAKNARIGVA      46
ALB25886.1|blaVEB-15|class    -----MKIV---KRILLVLL-SLFFTIVYSNAQTDNLTTLKIENVLKAKNARIGVA      46
AMJ32273.1|blaVEB-14|class    -----MKIV---KRILLVLL-SLFFTIVYSNAQTDNLTTLKIENVLKAKNARIGVA      46
ALB25884.1|blaVEB-13|class    -----MKIV---KRILLVLL-SLFFTIVYSNAQTDNLTTLKIENVLKAKNARIGVA      46
AKO63208.1|blaVEB-11|class    -----MKIV---KRILLVLL-SLFFTIVYSNAQTDNLTTLKIENVLKEKNARIGVA      46
ACA34904.1|blaVEB-6|class     -----MKIV---KRILLVLL-SLFFTIVYSNAQADNLTTLKIENVLKAKNARIGVA      46
ABM54868.1|blaVEB-4|class     -----MKIV---KRILLVLL-SLFFTIVYSNAQADNLTTLKIENVLKAKNARIGVA      46
AAS48620.1|blaVEB-3|class     -----MKIV---KRILLVLL-SLFFTIVYSNAQTDNLTTLKIENVLKAKNARIGVA      46
AAK29174.1|blaVEB-2|class     -----MKIV---KRILLVLL-SLFFTIVYSNAQADNLTTLKIENVLKAKNARIGVA      46
AAD01435.1|blaVEB-1|class     -----MKIV---KRILLVLL-SLFFTIVYSNAQTDNLTTLKIENVLKAKNARIGVA      46
AAK14293.1|blaVEB-9|class     -----MKIV---KRILLVLL-SLFFTIVYSNAQTDNLTTLKIENVLKAKNARIGVA      46
ACO56763.1|blaVEB-7|class     -----MKIV---KRILLVLL-SLFFTVEYSNAQTDNLTTLKIENVLKAKNARIGVA      46
ALB25887.1|blaVEB-16|class    -----MKIV---KRILLVLL-SLFFTVEYSNAQTDNLTTLKIENVLKAKNARIGVA      46
AKO63209.1|blaVEB-12|class    -----MKIV---KRILLVLL-SLFFTVEYSNAQTDNLTTLKIENVLKAKNARIGVA      46
ABN80430.1|blaVEB-5|class     -----MKIV---KRILLVLL-SLFFTVEYSNAQTDNLTTLKIENVLKAKNARIGVA      46
AGH33739.1|blaVEB-8|class     -----MKIV---KRILLVLL-SLFFTVEYSNAQTDNLTTLKIENVLKAKNARIGVA      46
E.men_ATCC13253_bla785|SBL    -----M---KKFTFLF--LLICQFSFAQQ--VSLLKEINNITKGKKATVAVS      40
E.ano_Ag1_bla3289|SBL        -----M---KRISIIIF--LFFSLFVFSQHSKPELLEKINTITKGKKATVAVS      42
E.mir_ATCC33958_bla2641|SBL   -----M---KKIILLF--LLTGQFVLAQH--TSILNDINAVTKDKKATVAVS      40
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|-----------------------------|--------------|----------------------|------|--|-----|
| AAM74565.1 blaCARB-7 PSE    | ILD          | TQNG-ESWDYNGDQRFPLT  | STFK | TIACAKLLYDAEHGKVNLNSTVEIKKADLV--TY         | 100 |
| AAD19217.1 blaCARB-6 PSE    | VLDT         | TQNG-EYWDYNGNQRFPPLT | STFK | TIACAKLLYDAEQGKVNPNSTVEIKKADLV--TY         | 100 |
| BAA02497.1 blaCARB-12 PSE   | VLDT         | TQNG-EYWDYNGNQRFPPLT | STFK | TIACAKLLYDAEQGKVNPNSTVEIKKADLV--TY         | 100 |
| AAK96394.1 blaCARB-1 PSE    | VLDT         | TQNG-EYWDYNGNQRFPPLT | STFK | TIACAKLLYDAEQGKVNPNSTVEIKKADLV--TY         | 100 |
| AAA25740.1 blaCARB-2 PSE    | VLDT         | TQNG-EYWDYNGNQRFPPLT | STFK | TIACAKLLYDAEQGKVNPNSTVEIKKADLV--TY         | 116 |
| AAG23870.1 blaCARB-11 PSE   | VLDT         | TQNG-EYWDYNGNQRFPPLT | STFK | TIACAKLLYDAEQGKVNPNSTVEIKKADLV--TY         | 100 |
| E.men_ATCC13253_bla759 SBL  | IIGGNKN-DKVQ | INGNQLYPML           | STFK | FPIALTVLHKVEKGELKMSQKIFIKKEELLDNTW         | 106 |
| E.ano_Ag1_bla3533 SBL       | IMGP         | DTK-EVTRINGDQMLPML   | STVK | FPLALTVLHEVEKGGKLSMDQKLFIKKEELEDTW         | 105 |
| E.mir_ATCC33958_bla1019 SBL | VIGP         | DTK-EVTGINGDQMLPML   | STVK | FPLALTVLHEVEKGGKLSMAQKLFIKKEELEDTW         | 105 |
| AEI54993.1 blaPER-7 class   | VWGP         | DDL-EPLLINPF         | EKFP | MQSVFKLHLAMLVLHQVDQGGKLDLNQTVIVNRAKVLQNTW  | 108 |
| BAP39824.1 blaPER-8 class   | VWGP         | DDL-EPLLINPF         | EKFP | MQSVFKLHLAMLVLHQVDQGGKLDLNQTVIVNRAKVLQNTW  | 108 |
| BAU25894.1 blaPER-9 class   | VWGP         | DDL-EPLLINPF         | EKFP | MQSVFKLHLAMLVLHQVDQGGKLDLNQTVIVNRAKVLQNTW  | 108 |
| ACN22483.1 blaPER-5 class   | VWGP         | DDL-EPLLINPF         | EKFP | MQSVFKLHLAMLVLHQVDQGGKLDLNQTVIVNRAKVLQNTW  | 108 |
| ACE77058.1 blaPER-4 class   | VWGP         | DDL-EPLLINPF         | EKFP | MQSVFKLHLAMLVLHQVDQGGKLDLNQTVIVNRAKVLQNTW  | 108 |
| AAU89132.1 blaPER-3 class   | VWGP         | DDL-EPLLINPF         | EKFP | MQSVFKLHLAMLVLHQVDQGGKLDLNQTVIVNRAKVLQNTW  | 108 |
| CAA79968.1 blaPER-1 class   | VWGP         | DDL-EPLLINPF         | EKFP | MQSVFKLHLAMLVLHQVDQGGKLDLNQTVIVNRAKVLQNTW  | 108 |
| ADD80743.1 blaPER-6 class   | VWGP         | DDL-EPLLINPF         | EKFP | MQSVFKMHLAMLVLHQVDQGGKLDLNQTVAVNRAAVLQNTW  | 108 |
| CAA63714.1 blaPER-2 class   | VWGP         | DDL-EPLLLNPF         | EKFP | MQSVFKLHLAMLVLHQVDQGGKLDLNQSVTVNRAAVLQNTW  | 108 |
| AMJ32272.1 blaVEB-17 class  | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKMW | 105 |
| AJH76942.1 blaVEB-10 class  | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKMW | 105 |
| ALB25886.1 blaVEB-15 class  | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKMW | 105 |
| AMJ32273.1 blaVEB-14 class  | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKTW | 105 |
| ALB25884.1 blaVEB-13 class  | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKTW | 105 |
| AKO63208.1 blaVEB-11 class  | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKTW | 105 |
| ACA34904.1 blaVEB-6 class   | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKMW | 105 |
| ABM54868.1 blaVEB-4 class   | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKMW | 105 |
| AAS48620.1 blaVEB-3 class   | IFNS         | NEK-DTFKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKTW | 105 |
| AAK29174.1 blaVEB-2 class   | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKTW | 105 |
| AAD01435.1 blaVEB-1 class   | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKTW | 105 |
| AAK14293.1 blaVEB-9 class   | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKTW | 105 |
| ACO56763.1 blaVEB-7 class   | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKTW | 105 |
| ALB25887.1 blaVEB-16 class  | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKTW | 105 |
| AKO63209.1 blaVEB-12 class  | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKTW | 105 |
| ABN80430.1 blaVEB-5 class   | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKMW | 105 |
| AGH33739.1 blaVEB-8 class   | IFNS         | NEK-DTLKIN           | NDFH | FPMQSVMKFPIALAVLSEIDKGNLSFEQKIEITPQDLLPKMW | 105 |
| E.men_ATCC13253_bla785 SBL  | VSGIEDNFQ    | LSNNGQLKMP           | MM   | SVFKFHIALAVLNQADKGNLTLQKILVKKSDLHENTW      | 100 |
| E.ano_Ag1_bla3289 SBL       | VLGIENDF     | QFSNANGNLKMP         | PML  | SVFKFHIALAVLNQVDKGNLTLQKILIKKSDLLENTW      | 102 |
| E.mir_ATCC33958_bla2641 SBL | VLGIENEF     | QFSNTNGNLKMP         | PML  | SVFKFHIALAVLNQVDKGNLTLQKILIKKSDLLENTW      | 100 |

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|                             |                                |                   |                 |       |     |   |   |      |
|-----------------------------|--------------------------------|-------------------|-----------------|-------|-----|---|---|------|
| AAM74565.1 blaCARB-7 PSE    | SPVLEKQVQGP--ITLSDACLATMTTSDN  | TAANIVINAVGDPKSI  | TDFLRQIGDKETRLD | 158   |     |   |   |      |
| AAD19217.1 blaCARB-6 PSE    | SPVIEKQVQQA--ITLDDACFATMTTSDN  | TAANIILSAVGGPKGVT | DFLRQIGDKETRLD  | 158   |     |   |   |      |
| BAA02497.1 blaCARB-12 PSE   | SPVIEKQVQQA--ITLDDACFATMTTSDN  | TAANIILSAVGGSKGVT | DFLRQIGDKETRLD  | 158   |     |   |   |      |
| AAK96394.1 blaCARB-1 PSE    | SPVIEKQVQQA--ITLDDACFATMTTSDN  | TAANIILSAVGGPKGVT | DFLRQIGDKETRLD  | 158   |     |   |   |      |
| AAA25740.1 blaCARB-2 PSE    | SPVIEKQVQQA--ITLDDACFATMTTSDN  | TAANIILSAVGGPKGVT | DFLRQIGDKETRLD  | 174   |     |   |   |      |
| AAG23870.1 blaCARB-11 PSE   | SPVIEKQVQQA--ITLDDACFATMTTSDN  | TAANIILSAVGGPKGVT | DFLRQIGDKETRLD  | 158   |     |   |   |      |
| E.men_ATCC13253_bla759 SBL  | SPFLEKYPQGNISITLEDALMWMCMCYSDN | NLTDILIRLIGGTD    | TVQKFIGT---KDFI | 163   |     |   |   |      |
| E.ano_Agl_bla3533 SBL       | SPFKEKYPQGNITITILEEALKWTVSYSDN | NLTDILRLIGGPET    | VQGFIDS---SSF   | 162   |     |   |   |      |
| E.mir_ATCC33958_bla1019 SBL | SPFKEKYPQGNITITILEEALKWTVSYSDN | NLTDILRLIGGPET    | VQGFIDS---SSF   | 162   |     |   |   |      |
| AEI54993.1 blaPER-7 class   | APIMKAYQGDQFSVPVQQLLQYSVSHSDN  | VACDLLFELVGGPAAL  | HDIQSMGIKETAVV  | 168   |     |   |   |      |
| BAP39824.1 blaPER-8 class   | APIMKAYQGDQFSVPVQQLLQYSVSHSDN  | VACDLLFELVGGPAAL  | HDIQSMGIKETAVV  | 168   |     |   |   |      |
| BAU25894.1 blaPER-9 class   | APIMKAYQGDQFSVPVQQLLQYSVSHSDN  | VACDLLFELVGGPAAL  | HDIQSMGIKETAVV  | 168   |     |   |   |      |
| ACN22483.1 blaPER-5 class   | APIMKAYQGDQFSVPVQQLLQYSVSHSDN  | VACDLLFELVGGPAAL  | HDIQSMGIKETAVV  | 168   |     |   |   |      |
| ACE77058.1 blaPER-4 class   | APIMKAYQGDQFSVPVQQLLQYSVSHSDN  | VACDLLFELVGGPAAL  | HDIQSMGIKETAVV  | 168   |     |   |   |      |
| AAU89132.1 blaPER-3 class   | APIMKAYQGDQFSVPVQQLLQYSVSHSDN  | VACDLLFELVGGPAAL  | HDIQSMGIKETAVV  | 168   |     |   |   |      |
| CAA79968.1 blaPER-1 class   | APIMKAYQGDQFSVPVQQLLQYSVSHSDN  | VACDLLFELVGGPAAL  | HDIQSMGIKETAVV  | 168   |     |   |   |      |
| ADD80743.1 blaPER-6 class   | SPMMKDHQGDQFSTVAVQQLLQYSVSHSDN | VACDLLFELVGGPAAL  | HAYIQSLGIKETE   | 168   |     |   |   |      |
| CAA63714.1 blaPER-2 class   | SPMMKDHQGDQFSTVAVQQLLQYSVSHSDN | VACDLLFELVGGPAAL  | HAYIQSLGVKEAA   | 168   |     |   |   |      |
| AMJ32272.1 blaVEB-17 class  | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| AJH76942.1 blaVEB-10 class  | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| ALB25886.1 blaVEB-15 class  | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| AMJ32273.1 blaVEB-14 class  | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| ALB25884.1 blaVEB-13 class  | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| AKO63208.1 blaVEB-11 class  | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| ACA34904.1 blaVEB-6 class   | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| ABM54868.1 blaVEB-4 class   | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| AAS48620.1 blaVEB-3 class   | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| AAK29174.1 blaVEB-2 class   | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| AAD01435.1 blaVEB-1 class   | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| AAK14293.1 blaVEB-9 class   | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| ACO56763.1 blaVEB-7 class   | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| ALB25887.1 blaVEB-16 class  | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| AKO63209.1 blaVEB-12 class  | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| ABN80430.1 blaVEB-5 class   | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| AGH33739.1 blaVEB-8 class   | SPIKEEFPNGT-TLTIEQILNYTVSESDN  | IGCDILLKLI        | GGTDSVQKFLNANH  | 164   |     |   |   |      |
| E.men_ATCC13253_bla785 SBL  | SPIREKYPQGNVNLPLSEIIYYTVAQSDN  | NGCDILRLRL        | GGTQAVQKFN      | 160   |     |   |   |      |
| E.ano_Agl_bla3289 SBL       | SPLREKYPDGNVELPLSEIIYYTVAQSDN  | NGCDILRLRL        | GGTQAVQKFN      | 162   |     |   |   |      |
| E.mir_ATCC33958_bla2641 SBL | SPLREKYPDGNVELPLSEIIYYTVAQSDN  | NGCDILRLRL        | GGTQAVQKFN      | 160   |     |   |   |      |
|                             | :*. :                          | : :.:             | : :**           | : :.: | : * | : | : | .. : |

|                             |  |             |
|-----------------------------|--|-------------|
| AAM74565.1 blaCARB-7 PSE    | RV <b>EP</b> ELNEG-KLGDLRDTTTPKAIATSTLNQLL--FGSTLSEASQKKLESWMVNNQVTGNL | 215         |
| AAD19217.1 blaCARB-6 PSE    | RI <b>EP</b> DLNEG-KLGDLRDTTTPKAIASSTLNQLL--FGSTLSEASQKKLESWMVNNQVTGNL | 215         |
| BAA02497.1 blaCARB-12 PSE   | RI <b>EP</b> DLNEG-KLGDLRDTTTPKAIASSTLNKFL--FGSALSEMNNKKLESWMVNNQVTGNL | 215         |
| AAK96394.1 blaCARB-1 PSE    | RI <b>EP</b> DLNEG-KLGDLRDTTTPKAIASSTLNKFL--FGSALSEMNNKKLESWMVNNQVTGNL | 215         |
| AAA25740.1 blaCARB-2 PSE    | RI <b>EP</b> DLNEG-KLGDLRDTTTPKAIASSTLNKFL--FGSALSEMNNKKLESWMVNNQVTGNL | 231         |
| AAG23870.1 blaCARB-11 PSE   | RI <b>EP</b> DLNEG-KLGDLRDTTTPKAIASSTLNKFL--FGSALSEMNNKKLESWMVNNQVTGNL | 215         |
| E.men_ATCC13253_bla759 SBL  | ND <b>EE</b> DMHKA-WDSQFINKITPNRATQLLDKFY--QGKILNRVNTKWLYEAMLNNKTGLKR  | 220         |
| E.ano_Ag1_bla3533 SBL       | ND <b>EE</b> GMHKD-WDSQFINKITPNSAALLLQEF--NGKILNKAHTQWLYNAMLNNASGKKR   | 219         |
| E.mir_ATCC33958_bla1019 SBL | ND <b>EE</b> GMHKD-WDSQFINKITPNYATLLLQEF--NGKILNKAHTQWLYNAMLNNASGKKR   | 219         |
| AEI54993.1 blaPER-7 class   | AN <b>EA</b> QMHAD-DQVQYQNWTSMKGAAEILKKFE--QKTQLSETSQALLWKWVETTTGPER   | 225         |
| BAP39824.1 blaPER-8 class   | AN <b>EA</b> QMHAD-DQVQYQNWTSMKGAAEILKKFE--QKTQLSETSQALLWKWVETTTGPER   | 225         |
| BAU25894.1 blaPER-9 class   | AN <b>EA</b> QMHAD-DQVQYQNWTSMKGAAEILKKFE--QKTQLSETSQALLWKWVETTTGPER   | 225         |
| ACN22483.1 blaPER-5 class   | AN <b>EA</b> QMHAD-DQVQYQNWTSMKGAAEILKKFE--QKTQLSETSQALLWKWVETTTGPER   | 225         |
| ACE77058.1 blaPER-4 class   | AN <b>EA</b> QMHAD-DQVQYQNWTSMKGAAEILKKFE--QKTQLSETSQALLWKWVETTTGPER   | 225         |
| AAU89132.1 blaPER-3 class   | AN <b>EA</b> QMHAD-DQVQYQNWTSMKGAAEILKKFE--QKTQLSETSQALLWKWVETTTGPER   | 225         |
| CAA79968.1 blaPER-1 class   | AN <b>EA</b> QMHAD-DQVQYQNWTSMKGAAEILKKFE--QKTQLSETSQALLWKWVETTTGPER   | 225         |
| ADD80743.1 blaPER-6 class   | AN <b>EA</b> QMHAD-DQVQYQNWTSMKAAQLLRKFE--QKQLSETSQALLWKWVETTTGPQR     | 225         |
| CAA63714.1 blaPER-2 class   | AN <b>EA</b> QMHAD-DQVQYQNWTSMKAAQVLQKFE--QKQLSETSQALLWKWVETTTGPQR     | 225         |
| AMJ32272.1 blaVEB-17 class  | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTTGSNR  | 223         |
| AJH76942.1 blaVEB-10 class  | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTTGSNR  | 223         |
| ALB25886.1 blaVEB-15 class  | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTTGSNR  | 223         |
| AMJ32273.1 blaVEB-14 class  | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRET-TGSNR  | 222         |
| ALB25884.1 blaVEB-13 class  | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTAGSNR  | 223         |
| AKO63208.1 blaVEB-11 class  | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTTGSNR  | 223         |
| ACA34904.1 blaVEB-6 class   | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTTGSNR  | 223         |
| ABM54868.1 blaVEB-4 class   | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTTGSNR  | 223         |
| AAS48620.1 blaVEB-3 class   | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTTGSNR  | 223         |
| AAK29174.1 blaVEB-2 class   | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTTGSNR  | 223         |
| AAD01435.1 blaVEB-1 class   | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTTGSNR  | 223         |
| AAK14293.1 blaVEB-9 class   | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTTGSNR  | 223         |
| ACO56763.1 blaVEB-7 class   | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTTGSNR  | 223         |
| ALB25887.1 blaVEB-16 class  | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTTGSNR  | 223         |
| AKO63209.1 blaVEB-12 class  | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTAGSNR  | 223         |
| ABN80430.1 blaVEB-5 class   | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTTGSNR  | 223         |
| AGH33739.1 blaVEB-8 class   | AN <b>EE</b> QM HKD-WNTQYQNWATPTAMNKLLIDTYNNKNQLLSKKSDFIWKIMRETTAGSNR  | 223         |
| E.men_ATCC13253_bla785 SBL  | HN <b>EE</b> EMHTKGVQVLYENYTTTSSMVKTLKSFY--KNMMISKKSTDFLMDIMLKTDTGMAK  | 218         |
| E.ano_Ag1_bla3289 SBL       | YN <b>EE</b> EMHKNDVKLTLYANYTTTASMVKTLKAFY--KGMFLSKRSTIFLMDIMTKTNTGMSK | 220         |
| E.mir_ATCC33958_bla2641 SBL | YN <b>EE</b> EMHKNDVKALYANYTTTASMVKTLKAFY--KGMFLSKRSTIFLMDIMTRTNTGMAK  | 218         |
|                             | * :. : : *   | :. : . * .. |

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|-----------------------------|---|-----|
| AAM74565.1 blaCARB-7 PSE    | LRSVLPVKWSIADRSAGGFF-----GARSITAIWVSEEKPIIVSIYLAQTEASMAERN      | 269 |
| AAD19217.1 blaCARB-6 PSE    | LRSVLPVKWSIADRSAGGFF-----GARSITAIWVSEEKTTIIVSIYLAQTEASMAERN     | 269 |
| BAA02497.1 blaCARB-12 PSE   | LRSVLPAGWNIADRSAGGFF-----GARSITAVVWSEHQAPIIVSIYLAQTQASMAERN     | 269 |
| AAK96394.1 blaCARB-1 PSE    | LRSVLPAGWNIADRSAGGFF-----GARSITAVVWSEHQAPIIVSIYLAQTQASMEERN     | 269 |
| AAA25740.1 blaCARB-2 PSE    | LRSVLPAGWNIADRSAGGFF-----GARSITAVVWSEHQAPIIVSIYLAQTQASMAERN     | 285 |
| AAG23870.1 blaCARB-11 PSE   | LRSVLPAGWNIADRSAGGFF-----GARSITAVVWSEHQAPIIVSIYLAQTQASMAERN     | 269 |
| E.men_ATCC13253_bla759 SBL  | LKGKLAADIKVAHRSGTSFTNDAGMTGAINSYGIIELPKNKKTCNYGFCSTRYL-----     | 273 |
| E.ano_Ag1_bla3533 SBL       | LKGNLPAGVKIAHRTGTSFTNKEGMTGAINDYGIIELPGKKKIYIAVVFVHNTYESFDESE   | 279 |
| E.mir_ATCC33958_bla1019 SBL | LKGNLPAGVKIAHRTGTSFTNKEGMTGAINDYGIIELPGKKKIYIAVVFVHNTYESFEASE   | 279 |
| AEI54993.1 blaPER-7 class   | LKGLLPAGTVVAHKTGTSG-VRAGKTAATNDLGIILLPDGRPLLVAVFVKDSAESSRTNE    | 284 |
| BAP39824.1 blaPER-8 class   | LKGLLPAGTVVAHKTGTSG-VRAGKTAATNDLGIILLPDGRPLLVAVFVKDSAESSRTNE    | 284 |
| BAU25894.1 blaPER-9 class   | LKGLLPAGTVVAHKTGTSG-VRAGKTAATNDLGIILLPDGRPLLVAVFVKDSAESSRTNE    | 284 |
| ACN22483.1 blaPER-5 class   | LKGLLPAGTVVAHKTGTSG-VRAGKTAATNDLGIILLPDGRPLLVAVFVKDSAESSRTNE    | 284 |
| ACE77058.1 blaPER-4 class   | LKGLLPAGTVVAHKTGTSG-VRAGKTAATNDLGIILLPDGRPLLVAVFVKDSAESSRTNE    | 284 |
| AAU89132.1 blaPER-3 class   | LKGLLPAGTVVAHKTGTSG-VRAGKTAATNDLGIILLPDGRPLLVAVFVKDSAESSRTNE    | 284 |
| CAA79968.1 blaPER-1 class   | LKGLLPAGTVVAHKTGTSG-VRAGKTAATNDLGIILLPDGRPLLVAVFVKDSAESSRTNE    | 284 |
| ADD80743.1 blaPER-6 class   | LKGLLPAGTVVAHKTGTSG-VRAGKTAATNDLGIILLPDGRPLLVAVFVKDSAESSRTNE    | 284 |
| CAA63714.1 blaPER-2 class   | LKGLLPAGTVVAHKTGTSG-VRAGKTAATNDLGIILLPDGRPLLVAVFVKDSAESSRTNE    | 284 |
| AMJ32272.1 blaVEB-17 class  | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| AJH76942.1 blaVEB-10 class  | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| ALB25886.1 blaVEB-15 class  | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| AMJ32273.1 blaVEB-14 class  | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 281 |
| ALB25884.1 blaVEB-13 class  | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| AKO63208.1 blaVEB-11 class  | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| ACA34904.1 blaVEB-6 class   | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| ABM54868.1 blaVEB-4 class   | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| AAS48620.1 blaVEB-3 class   | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| AAK29174.1 blaVEB-2 class   | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| AAD01435.1 blaVEB-1 class   | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| AAK14293.1 blaVEB-9 class   | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| ACO56763.1 blaVEB-7 class   | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| ALB25887.1 blaVEB-16 class  | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| AKO63209.1 blaVEB-12 class  | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| ABN80430.1 blaVEB-5 class   | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| AGH33739.1 blaVEB-8 class   | LKGQLPKNTIVAHKTGTSG-INNGIAAATNDVGVITLPGQLIFISVFVAESKETSEINE     | 282 |
| E.men_ATCC13253_bla785 SBL  | LPGLLPNVKMARKTGS SSGK-LPSGLTVAENDSGIVITLDNGKHFIAIAVFKNSTESEIINY | 277 |
| E.ano_Ag1_bla3289 SBL       | LPGLLPKVRMARKTGS SSGK-MKNGLTI AENDSGIVITLANGKHYAIAVFKDSMESEEVNC | 279 |
| E.mir_ATCC33958_bla2641 SBL | LPGLLPKVKMARKTGS SSGK-MKNGLTI AENDSGIITLANGKHYAIAVFKDSMESEEVNC  | 277 |

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|---------------------------|------------------------------|
| AAM74565.1 blaCARB-7 PSE  | DAIVKIGRSIFEVYTSQSR----- 288 |
| AAD19217.1 blaCARB-6 PSE  | DAIVKIGRSIFEVYTSQSR----- 288 |
| BAA02497.1 blaCARB-12 PSE | DAIVKIGRSIFDVYTSQSR----- 288 |
| AAK96394.1 blaCARB-1 PSE  | DAIVKIGHSIFDVYTSQSR----- 288 |

|                             |                            |     |
|-----------------------------|----------------------------|-----|
| AAA25740.1 blaCARB-2 PSE    | DAIVKIGHSFDFVYTSQSR-----   | 304 |
| AAG23870.1 blaCARB-11 PSE   | DAIVKIGHSFDFVYTSQSR-----   | 288 |
| E.men_ATCC13253_bla759 SBL  | -----                      | 273 |
| E.ano_Ag1_bla3533 SBL       | KIISDIGRAAYDYTYTKK-----    | 296 |
| E.mir_ATCC33958_bla1019 SBL | KIISDIGRATYDYTYTKK-----    | 296 |
| AEI54993.1 blaPER-7 class   | AIIAQVAQAAYQFELKKLSALSPN   | 308 |
| BAP39824.1 blaPER-8 class   | AIIAQVAQAAYQFELKKLSALSPN   | 308 |
| BAU25894.1 blaPER-9 class   | AIIAQVAQTAYQFELKKLSALSPN   | 308 |
| ACN22483.1 blaPER-5 class   | AIIAQVAQTAYQFELKKLSALSPN   | 308 |
| ACE77058.1 blaPER-4 class   | AIIAQVAQTAYQFELKKLSALSPN   | 308 |
| AAU89132.1 blaPER-3 class   | AIIAQVAQTAYQFELKKLSALSPN   | 308 |
| CAA79968.1 blaPER-1 class   | AIIAQVAQTAYQFELKKLSALSPN   | 308 |
| ADD80743.1 blaPER-6 class   | AIIAQVAQAAYQFELKKLSAVSPD   | 308 |
| CAA63714.1 blaPER-2 class   | AIIAQVAQAAYQFELKKLSAVSPD   | 308 |
| AMJ32272.1 blaVEB-17 class  | KIISDIAKITWDYLYLNK-----    | 299 |
| AJH76942.1 blaVEB-10 class  | KIISDIAKITWDYLYLNK-----    | 299 |
| ALB25886.1 blaVEB-15 class  | KIISDIAKITWDYLYLNK-----    | 299 |
| AMJ32273.1 blaVEB-14 class  | KIISDIAKITWNYLYLNK-----    | 298 |
| ALB25884.1 blaVEB-13 class  | KIISDIAKITWNYLYLNK-----    | 299 |
| AKO63208.1 blaVEB-11 class  | KIISDIAKITWNYLYLNK-----    | 299 |
| ACA34904.1 blaVEB-6 class   | KIISDIAKITWNYLYLNK-----    | 299 |
| ABM54868.1 blaVEB-4 class   | KIISDIAKITWNYLYLNK-----    | 299 |
| AAS48620.1 blaVEB-3 class   | KIISDIAKITWNYLYLNK-----    | 299 |
| AAK29174.1 blaVEB-2 class   | KIISDIAKITWNYLYLNK-----    | 299 |
| AAD01435.1 blaVEB-1 class   | KIISDIAKITWNYLYLNK-----    | 299 |
| AAK14293.1 blaVEB-9 class   | KIISDIAKITWNYLYLNK-----    | 299 |
| ACO56763.1 blaVEB-7 class   | KIISDIAKITWNYLYLNK-----    | 299 |
| ALB25887.1 blaVEB-16 class  | KIISDIAKITWNYLYLNK-----    | 299 |
| AKO63209.1 blaVEB-12 class  | KIISDIAKITWNYLYLNK-----    | 299 |
| ABN80430.1 blaVEB-5 class   | KIISDIAKITWNYLYLNK-----    | 299 |
| AGH33739.1 blaVEB-8 class   | KIISDIAKITWNYLYLNK-----    | 299 |
| E.men_ATCC13253_bla785 SBL  | SIIAQVSKAVFDYLNNTK-----    | 295 |
| E.ano_Ag1_bla3289 SBL       | GMIAQVSKIVWDALNKKKNKP----  | 299 |
| E.mir_ATCC33958_bla2641 SBL | GMIAQISKIVWDTLNNKKKNKP---- | 297 |

### **C Group 3**

CLUSTAL O(1.2.1) multiple sequence alignment

|  |  |                    |
|--|--|--------------------|
| CAK95242.1 blaADC-19 class                     | MRFKKISCLLLS----PLFIFNTSIYAGNTSKEQEI-----KKLVDQNFKPLLEKY     | 47                 |
| ACN62075.1 blaADC-18 class                     | MRFKKISCLLLP----PLFIFSTSIYAGNTPKEQEV-----KKLVDQNFKPLLDKY     | 47                 |
| CAK95238.1 blaADC-23 class                     | MRFKKISCLLLP----PLFIFSTSIYAGNTPKEQEV-----KKLVDQNFKPLLDKY     | 47                 |
| AIT76084.1 blaACC-4 cephalosporin-hydrolyzing  | -MRKKMQNTLKLLSVITCLAATVQGALAANIDESKI-----KDTVDDLIQPLMQKN     | 50                 |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | -MRKKMQNTLKLLSVITCLAATAQGALAANIDESKI-----KDTVDDLIQPLMQKN     | 50                 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | -MRKKMQNTLKLLSVITCLAATAQGALAANIDESKI-----KDTVDDLIQPLMQKN     | 50                 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | -MRKKMQNTLKLLSVITCLAATAQGALAANIDESKI-----KDTVDDLIQPLMQKN     | 50                 |
| BAP68758.1 blaACT-35 cephalosporin-hydrolyzing | MMKKSICC-----ALLLGISCSALATPVSEKQL-----AEVVANTVTPLMKAQ        | 43                 |
| AFU25650.1 blaACT-12 cephalosporin-hydrolyzing | MMKKSICC-----ALLLSTSCAALAAPLSETQL-----AKVVERTVTPLMKAQ        | 43                 |
| AHM76774.1 blaACT-22 cephalosporin-hydrolyzing | MMKKSICC-----ALLLSTSCAALAAPMSETQL-----AKVVARTVTPLMKAQ        | 43                 |
| E.mir_ATCC33958_bla719                         | -----MKYS  | 4                  |
| E.mir_ATCC33958_bla4255 SBL                    | ---MKTSSKFL-----AALLFISSFSFGQNISK--IDSII-----SDNYKKN         | 38                 |
| E.men_13253_bla2764                            | -MIKKLAFLEFL-----FALLFCKTEQOKTIDRNVIVDSTITAFQKKLLSQQVDSVFVKY | 54                 |
| E.ano_Ag1_bla2980                              | -MIKKLGLEFL-----FALLFCKTEQOKTIDRNVVVDSTITAFQKLTQQQIDTVFKY    | 54                 |
| .  |  |                    |
| CAK95242.1 blaADC-19 class                     | DVPGMAVGVIQNNKKYE-MYYGLQSVQDKKAVNSSTIFELGSVSKLF              | TATAGGYAKNKGK 106  |
| ACN62075.1 blaADC-18 class                     | DVPGMAVGVIQNNKKYE-IYYGLQSVQDKKAVNSSTIFELGSVSKLF              | TATAGGYAKAKGK 106  |
| CAK95238.1 blaADC-23 class                     | DVPGMAVGVIQNNKKYE-IYYGLQSVQDKKAVNSSTIFELGSVSKLF              | TATAGGYAKAKGK 106  |
| AIT76084.1 blaACC-4 cephalosporin-hydrolyzing  | NIPGMSVAVTVNGKNYI-YNYGLAAKQPQQPVNTENTLFEVGSLSKTF             | AATLASAQAQVSGK 109 |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | NIPGMSVAVTVNGKNYI-YNYGLAAKQPQQSVTENTLFEVGSLSKTF              | AATLASAQAQVSGK 109 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | NIPGMSVAVTVNGKNYI-YNYGLAAKQPQQPVNTENTLFEVGSLSKTF             | AATLASAQAQVSGK 109 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | NIPGMSVAVTVNGKNYI-YNYGLAAKQPQQPVNTENTLFEVGSLSKTF             | AATLASAQAQVSGK 109 |
| BAP68758.1 blaACT-35 cephalosporin-hydrolyzing | SVPGMAVAVIYQGKPHY-YTFGKADIAANKPVTPQTLFELGSISKTF              | TGVLGGDAIARGE 102  |
| AFU25650.1 blaACT-12 cephalosporin-hydrolyzing | SIPGMAVAVIYQGQPHY-FTFGKADVAANTPVTAQTLFELGSISKTF              | TGVLGGDAIARGE 102  |



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|--|---|----------------------|----------------|-----|
| AHM76774.1 blaACT-22 cephalosporin-hydrolyzing | SIPGMAVAVIYQGQPHY-FTFGKADVAANTPVTAQTLFELGSISKTF     | TGVLGGDAIARGE        | 102            |     |
| E.mir_ATCC33958_bla719                         | RIPGMQVAIIKNGKIIKLAAYGTANIPFNIPVTVNTLFPINSMTKCF     | TGTALVKLIENGQ        | 64             |     |
| E.mir_ATCC33958_bla4255 SBL                    | PEVGISVGFNNNEEHY-ISYGNLNAESQVQINKNSLFEIASITKILTS    | NLIAQAVLDHK          | 97             |     |
| E.men_13253_bla2764                            | HFNGS-IAIFKDSTLLYRKDNFGSNFRNKTKIDSNTIFAI            | GSVSKQF              | TAAVLVLLQQEQGK | 113 |
| E.ano_Ag1_bla2980                              | LFNGS-IAVFKDSTLLYRKENGYSNFKSKTKIDNNTIFAI            | GSVSKQF              | TAVLILLQKEQGK  | 113 |
|  | * :.. :   | * : : :..* : ** : .. | :              |     |
| CAK95242.1 blaADC-19 class                     | ISFDDTPGKYWKKLKNT---PIDQVNLQLATYTSGNLAL-QFPDEVQ     | -----TDQ             | 153            |     |
| ACN62075.1 blaADC-18 class                     | ISFDDTPGKYWKELKNT---PIDQVNLQLATYTSGNLAL-QFPDEVQ     | -----TDQ             | 153            |     |
| CAK95238.1 blaADC-23 class                     | ISFDDTPGKYWKELKNT---PIDQVNLQLATYTSGNLAL-QFPDEVQ     | -----TDQ             | 153            |     |
| AIT76084.1 blaACC-4 cephalosporin-hydrolyzing  | LSLDQSVSHYVPELRGS---SFDHVSVLNVGTHT-SGLQL-FMPEDIK    | -----NTT             | 155            |     |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | LSLDQSVSHYVPELRGS---SFDHVSVLNVGTHT-SGLQL-FMPEDIK    | -----NTT             | 155            |     |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | LSLDQSVSHYVPELRGS---SFDHVSVLNVGTHT-SGLQL-FMPEDIK    | -----NTT             | 155            |     |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | LSLDQSVSHYVPELRGS---SFDHVSVLNVGTHT-SGLQL-FMPEDIK    | -----NTT             | 155            |     |
| BAP68758.1 blaACT-35 cephalosporin-hydrolyzing | ISLDDPVTRYWPQLTGK---QWQGIRMLDLATYTAGGLPL-QVPDEVT    | -----DNA             | 149            |     |
| AFU25650.1 blaACT-12 cephalosporin-hydrolyzing | ISLGDVPTKYWPELTGK---QWQGVRLDLATYTAGGLPL-QVPDEVT     | -----DNA             | 149            |     |
| AHM76774.1 blaACT-22 cephalosporin-hydrolyzing | ISLSDPVTKYWPELTGK---QWQGVRLDLATYTAGGLPL-QVPDEVT     | -----DNA             | 149            |     |
| E.mir_ATCC33958_bla719                         | IKLDDYASVYLSNLP----EPWHKITIKQLLTHTS-GLPDIVDGDTGKM   | ----IVPGEDT          | 115            |     |
| E.mir_ATCC33958_bla4255 SBL                    | IKLDDYIDGFLPKEYILHQNLRNKIKISDLASHQS-GLPIDIDFAKIEQDP | QQPVNNVTA            | 156            |     |
| E.men_13253_bla2764                            | LKLTDKVSEYLPEFRIKE---YVNISISQLLNHTS-GLNI            | -----                | 149            |     |
| E.ano_Ag1_bla2980                              | LKLEDKVSQFLPDFRIKE---YENITINQLLNHTS-GLNI            | -----                | 149            |     |
|  | .. : : .  | : : : : : *          |                |     |

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| CAK95242.1 blaADC-19 class                     | QVLTFKDWKPKNSIGEYRQY <b>SN</b> PSIGLFGKVVALSMNKPFDQVLEKTIFFPDLGLKHSY-  | 212 |
| ACN62075.1 blaADC-18 class                     | QVLTFKDWKTKNAIGEYRQY <b>SN</b> PSIGLFGKVVALSMNKPFDQVLEKTIFFPPLHLKNSY-  | 212 |
| CAK95238.1 blaADC-23 class                     | QVLTFKDWKTKNAIGEYRQY <b>SN</b> PSIGLFGKIVALSMNKPFDQVLEKTIFFPPLHLKNSY-  | 212 |
| AIT76084.1 blaACC-4 cephalosporin-hydrolyzing  | QLMAYLKAWKPADAAGTHR <b>VY</b> SNIGTGLLGMIAAKSLGVSIEDAIEKTLPLQGMHHSY-   | 214 |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | QLMAYLKAWKPADAAGTHR <b>VY</b> SNIGTGLLGMIAAKSLGVSIEDAIEKTLPLQGMHHSY-   | 214 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | QLMAYLKAWKPADAAGTHR <b>VY</b> SNIGTGLLGMIAAKSLGMSYEDAIEKTLPLQGMHHSY-   | 214 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | QLMAYLKAWKPADAAGTHR <b>VY</b> SNIGTGLLGMIAAKSLGVSIEDAIEKTLPLQGMHHSY-   | 214 |
| BAP68758.1 blaACT-35 cephalosporin-hydrolyzing | SLLRFYQNWQPQWPGT <b>TRLY</b> ANASIGLFGALAVKPSGMPYEQAMTTRVLKPLKLDHTW-   | 208 |
| AFU25650.1 blaACT-12 cephalosporin-hydrolyzing | SLLRFYQSWQPQWAPGT <b>TRLY</b> ANASIGLFGALAVKPSGMRFEQAMTERVLKPLNLNHTW-  | 208 |
| AHM76774.1 blaACT-22 cephalosporin-hydrolyzing | SLLRFYQSWQPQWAPGT <b>TRLY</b> ANASIGLFGALAVKPSGMRFEQAMTERVLKPLNLNHTW-  | 208 |
| E.mir_ATCC33958_bla719                         | I <b>AL</b> EKVKTLP <b>IR</b> FAPGEK <b>SEY</b> NQ <b>T</b> NYVLLGQ <b>II</b> EKLSK <b>KP</b> FTSFVTEQ <b>EF</b> VPANMQ <b>Q</b> TNF                                   | 175 |
| E.mir_ATCC33958_bla4255 SBL                    | QTL <b>ATI</b> INN <b>CS</b> ELKDYGKYR <b>YST</b> IGY <b>T</b> LLGQ <b>IV</b> EKVY <b>GK</b> TYDE <b>II</b> RAK <b>II</b> KPLQ <b>MT</b> N-TL                          | 215 |
| E.men_13253_bla2764                            | -----MGNRLMF <b>K</b> SGTDFY <b>YS</b> NDGF <b>N</b> ALGK <b>IV</b> EKVSGK <b>SY</b> DQ <b>NI</b> TEL <b>F</b> TR <b>L</b> GMQ <b>HS</b> -ST                           | 202 |
| E.ano_Ag1_bla2980                              | -----MGNRLM <b>F</b> RS <b>G</b> T <b>G</b> FF <b>YS</b> NDGF <b>N</b> ALG <b>Q</b> IV <b>E</b> KVSGK <b>SY</b> D <b>EN</b> TEL <b>F</b> SK <b>I</b> GL <b>Q</b> HS-ST | 202 |

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|--|---|-----|
| CAK95242.1 blaADC-19 class                     | ---VNV <b>P</b> K <b>T</b> Q <b>M</b> Q <b>N</b> Y <b>A</b> F <b>G</b> Y <b>N</b> Q <b>E</b> N <b>Q</b> P <b>I</b> R-----V <b>N</b> P <b>G</b> P <b>L</b> D <b>A</b> P <b>A</b> Y <b>G</b> V <b>K</b> S <b>T</b> L <b>P</b> D <b>M</b> L <b>K</b> F <b>I</b> N          | 260 |
| ACN62075.1 blaADC-18 class                     | ---VNV <b>P</b> K <b>T</b> Q <b>M</b> Q <b>N</b> Y <b>A</b> Y <b>G</b> Y <b>N</b> Q <b>E</b> N <b>Q</b> P <b>I</b> R-----V <b>N</b> P <b>G</b> P <b>L</b> D <b>A</b> P <b>A</b> Y <b>G</b> V <b>K</b> S <b>T</b> L <b>P</b> D <b>M</b> L <b>T</b> F <b>I</b> N          | 260 |
| CAK95238.1 blaADC-23 class                     | ---VNV <b>P</b> K <b>T</b> Q <b>M</b> Q <b>N</b> Y <b>A</b> Y <b>G</b> Y <b>N</b> Q <b>E</b> N <b>Q</b> P <b>I</b> R-----V <b>N</b> P <b>G</b> P <b>L</b> D <b>A</b> P <b>A</b> Y <b>G</b> V <b>K</b> S <b>T</b> L <b>P</b> D <b>M</b> L <b>T</b> F <b>I</b> N          | 260 |
| AIT76084.1 blaACC-4 cephalosporin-hydrolyzing  | ---L <b>K</b> V <b>P</b> A <b>D</b> Q <b>M</b> E <b>N</b> Y <b>A</b> W <b>G</b> Y <b>N</b> K <b>K</b> D <b>E</b> P <b>V</b> H-----G <b>N</b> M <b>E</b> I <b>L</b> G <b>N</b> E <b>A</b> Y <b>G</b> I <b>K</b> T <b>T</b> S <b>S</b> D <b>L</b> L <b>R</b> Y <b>V</b> Q | 262 |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | ---L <b>K</b> V <b>P</b> A <b>D</b> Q <b>M</b> E <b>N</b> Y <b>A</b> W <b>G</b> Y <b>N</b> K <b>K</b> D <b>E</b> P <b>V</b> H-----V <b>N</b> M <b>E</b> I <b>L</b> G <b>N</b> E <b>A</b> Y <b>G</b> I <b>K</b> T <b>T</b> S <b>S</b> D <b>L</b> L <b>R</b> Y <b>V</b> Q | 262 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | ---L <b>K</b> V <b>P</b> A <b>D</b> Q <b>M</b> E <b>N</b> Y <b>A</b> W <b>G</b> Y <b>N</b> K <b>K</b> D <b>E</b> P <b>V</b> H-----V <b>N</b> M <b>E</b> I <b>L</b> G <b>N</b> E <b>A</b> Y <b>G</b> I <b>K</b> T <b>T</b> S <b>S</b> D <b>L</b> L <b>R</b> Y <b>V</b> Q | 262 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | ---L <b>K</b> V <b>P</b> A <b>D</b> Q <b>M</b> E <b>N</b> Y <b>A</b> W <b>G</b> Y <b>N</b> K <b>K</b> D <b>E</b> P <b>V</b> H-----V <b>N</b> M <b>E</b> I <b>L</b> G <b>N</b> E <b>A</b> Y <b>G</b> I <b>K</b> T <b>T</b> S <b>S</b> D <b>L</b> L <b>R</b> Y <b>V</b> Q | 262 |
| BAP68758.1 blaACT-35 cephalosporin-hydrolyzing | ---I <b>N</b> V <b>P</b> K <b>A</b> E <b>E</b> A <b>H</b> Y <b>A</b> W <b>G</b> Y <b>R</b> D <b>G</b> - <b>K</b> A <b>V</b> R-----V <b>S</b> P <b>G</b> M <b>L</b> D <b>A</b> Q <b>A</b> Y <b>G</b> V <b>K</b> T <b>N</b> V <b>Q</b> D <b>M</b> A <b>N</b> W <b>V</b> M | 255 |
| AFU25650.1 blaACT-12 cephalosporin-hydrolyzing | ---I <b>N</b> V <b>P</b> K <b>A</b> E <b>E</b> Q <b>H</b> Y <b>A</b> W <b>G</b> Y <b>R</b> D <b>G</b> - <b>K</b> A <b>V</b> H-----V <b>S</b> P <b>G</b> M <b>L</b> D <b>A</b> E <b>A</b> Y <b>G</b> V <b>K</b> T <b>N</b> V <b>K</b> D <b>M</b> A <b>S</b> W <b>V</b> V | 255 |

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| AHM76774.1 blaACT-22 cephalosporin-hydrolyzing | ---INVPKAEEQHYAWGYRDG-KAVH-----VSPGMLDAAEYGVKTNVKDMASWVV     | 255 |
| E.mir_ATCC33958_bla719                         | GDSFDVIKNMTESYSYKFNRN-GLWKRSSQLTRVFEEFSILTRPAAGINSTAKELADWLI | 234 |
| E.mir_ATCC33958_bla4255 SBL                    | TKDFNVK-NRRT----AHNPDGGIQE-----FFKW-NITAPAGLVKSTASDMVRFLK    | 261 |
| E.men_13253_bla2764                            | ALTYKSG-NFAS----AYLGNDKVQELVQNMPERLAEK-EIGTPAGGILSTIDDLHLWNQ | 256 |
| E.ano_Ag1_bla2980                              | ASTFKTG-NFAS----AYLGNDKVQEVVHNMPKRLAGK-EIGTPAGGILSTIDDLHLWNQ | 256 |

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| CAK95242.1 blaADC-19 class                     | ANLNPQKYP--K--DIQRAINETHQGFYQVGTMYQALGWEEFSYPALLQTLTLLDSNSEQIV | 316 |
| ACN62075.1 blaADC-18 class                     | ANLNPQKYP--K--DIQRAISETHQGFYQVGTMYQALGWEEFSYPAPLQTLTLLDSNSEQIV | 316 |
| CAK95238.1 blaADC-23 class                     | ANLNPQKYP--K--DIQRAINETHQGFYQVGTMYQALGWEEFSYPASLQTLTLLDSNSEQIV | 316 |
| AIT76084.1 blaACC-4 cephalosporin-hydrolyzing  | ANMGQLKLDANA--KMQQALTATHTGYFKSGEITQDLMWEQLPYPVSLPNLLTGNDMAM-   | 319 |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | ANMGQLKLDANA--KMQQALTATHTGYFKSGEITQDLMWEQLPYPVSLPNLLTGNDMAM-   | 319 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | ANMGQLKLDANA--KMQQALTATHTGYFKSGEITQDLMWEQLPYPVSLPNLLTGNDMAM-   | 319 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | ANMGQLKLDANA--KMQQALTATHTGYFKSGEITQDLMWEQLPYPVSLPNLLTGNDMAM-   | 319 |
| BAP68758.1 blaACT-35 cephalosporin-hydrolyzing | ANMAPENVA-DA--SLKQGIALAQSRYWRTGSMYQGLGWEMLNWPVEANTVVEGSDSKVA   | 312 |
| AFU25650.1 blaACT-12 cephalosporin-hydrolyzing | ANMAPDGVQ-DA--SLKQGMVLAQSRYWRTGSMYQGLGWEMLNWPVEAKTVVEGSDNKVA   | 312 |
| AHM76774.1 blaACT-22 cephalosporin-hydrolyzing | ANMAPDGVQ-DA--SLKQGMALAQSRYWRTGSMYQGLGWEMLNWPVEAKTVVEGSDNKVA   | 312 |
| E.mir_ATCC33958_bla719                         | HLTEGKFIDQKSLKIMWTPSLHNN-----GTTAPRGLGWSVS-----                | 271 |
| E.mir_ATCC33958_bla4255 SBL                    | AVLNKQ----TT---IGKAAIIMERVVYKDEKREMGGLNI-----                  | 295 |
| E.men_13253_bla2764                            | KLYSGKVLQPETLKKFTEKSAIRHHAVF----GKMGYACGIM-----                | 294 |
| E.ano_Ag1_bla2980                              | KLYGGKILKPETLKEFTKSAERQHAVF----GKMGYAYGIM-----                 | 294 |

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| CAK95242.1 blaADC-19 class | MKPNKVTAISKE---PSVKMFHKTGSTNGFGTYVVFIPKENIGLVMLTNKRIPNEERIKA | 373 |
| ACN62075.1 blaADC-18 class | MKPNKVTAISKE---PSVKMFHKTGSTNGFGSYVVFIPKENIGLVMLTNKRIPNEERIKA | 373 |

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|--|---|-----|
| CAK95238.1 blaADC-23 class                     | MKPNKVT AISKE---PSVKMFHKTGSTNGFGTYVVFIPKENIGLVMLTNKRIPNEERIKA | 373 |
| AIT76084.1 blaACC-4 cephalosporin-hydrolyzing  | -TKSVATPIVPLPPQENVWINKTGSTNGFGAYIAFVPAKMGIVMLANKNYSIDQRVTV    | 378 |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | -TKSVATPIVPLPPQENVWINKTGSTNGFGAYIAFVPAKMGIVMLANKNYSIDQRVTV    | 378 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | -TKSVATPIVPLPPQENVWINKTGSTNGFGAYIAFVPAKMGIVMLANKNYSIDQRVTV    | 378 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | -TKSVATPIVPLPPQENVWINKTGSTNGFGAYIAFVPAKMGIVMLANKNYSIDQRVTV    | 378 |
| BAP68758.1 blaACT-35 cephalosporin-hydrolyzing | LAPLPVVEVNPPAPPVKASWVHKTGSTGGFGSYVAFIPEKQIGIVMLANTSYPNPARVEA  | 372 |
| AFU25650.1 blaACT-12 cephalosporin-hydrolyzing | LAPLPVAEVNPPAPPVKASWVHKTGSTGGFGSYVAFIPEKELGIVMLANKSYPNPARVEA  | 372 |
| AHM76774.1 blaACT-22 cephalosporin-hydrolyzing | LAPLPVAEVNPPAPPVKASWVHKTGSTGGFGSYVAFIPEKKLGIVMLANKSYPNPARVEA  | 372 |
| E.mir_ATCC33958_bla719                         | -----LRKQHPFVYGS---GGMR SAMYYY PEDKIGFIILTNLRGANPEKLIE        | 315 |
| E.mir_ATCC33958_bla4255 SBL                    | -----VTDDKNTIYMKSGDSMGQSSIICYNRDKKWGIIILLDQRNSKMRQD-L         | 342 |
| E.men_13253_bla2764                            | -----LNTGKPEAYFHSGYVKGSPSLNIYYPGTKTSVIIILSNIADENKGKNAV        | 342 |
| E.ano_Ag1_bla2980                              | -----LNTGKPEAYFHSGYVKGSPSLNIYYPETKTSVIIILSNIADENKGKNAV        | 342 |

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| CAK95242.1 blaADC-19 class                     | AYAV-LN-----AIKK-----  | 383 |
| ACN62075.1 blaADC-18 class                     | AYAV-LN-----AIKK-----  | 383 |
| CAK95238.1 blaADC-23 class                     | AYAV-LN-----AIKK-----  | 383 |
| AIT76084.1 blaACC-4 cephalosporin-hydrolyzing  | AYKI-LS-----SLEGNK-----                                      | 390 |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | AYKI-LS-----SLEGNK-----                                      | 390 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | AYKI-LS-----SLEGNK-----                                      | 390 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | AYKI-LS-----SLEGNK-----                                      | 390 |
| BAP68758.1 blaACT-35 cephalosporin-hydrolyzing | AYYI-LE-----ALQ-----   | 381 |
| AFU25650.1 blaACT-12 cephalosporin-hydrolyzing | AYRI-LS-----ALQ-----   | 381 |
| AHM76774.1 blaACT-22 cephalosporin-hydrolyzing | AYRI-LS-----ALQ-----   | 381 |
| E.mir_ATCC33958_bla719                         | QLAGFYIPELHP-YTGTGLPPALGLLHTELLKTKYTKSKEIYKKLQLKDSQFNLSEASLN | 374 |

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|-----------------------------|---|-----|
| E.mir_ATCC33958_bla4255 SBL | -----LN-----K---IYDTVLK-----                | 352 |
| E.men_13253_bla2764         | -----FNPHEIKSITDNLQNVVTDLQDCLKPVEKKNE-----  | 376 |
| E.ano_Ag1_bla2980           | -----FNPHEVKNITDNLQNVIVDLQKDLLKPVEHKNE----- | 376 |

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|--|---|-----|
| CAK95242.1 blaADC-19 class                     | -----   | 383 |
| ACN62075.1 blaADC-18 class                     | -----   | 383 |
| CAK95238.1 blaADC-23 class                     | -----   | 383 |
| AIT76084.1 blaACC-4 cephalosporin-hydrolyzing  | -----   | 390 |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | -----   | 390 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | -----   | 390 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | -----   | 390 |
| BAP68758.1 blaACT-35 cephalosporin-hydrolyzing | -----   | 381 |
| AFU25650.1 blaACT-12 cephalosporin-hydrolyzing | -----   | 381 |
| AHM76774.1 blaACT-22 cephalosporin-hydrolyzing | -----   | 381 |
| E.mir_ATCC33958_bla719                         | DWGYTLHLHIGYMKQAIEIFSLNTQLYPSSGNTYDSLAEAYESSGNKLMALKNYRRSLEYS | 434 |
| E.mir_ATCC33958_bla4255 SBL                    | -----   | 352 |
| E.men_13253_bla2764                            | -----   | 376 |
| E.ano_Ag1_bla2980                              | -----   | 376 |

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|---|-------|-----|
| CAK95242.1 blaADC-19 class                    | ----- | 383 |
| ACN62075.1 blaADC-18 class                    | ----- | 383 |
| CAK95238.1 blaADC-23 class                    | ----- | 383 |
| AIT76084.1 blaACC-4 cephalosporin-hydrolyzing | ----- | 390 |

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|--|----------------------|-----|
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | -----                | 390 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | -----                | 390 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | -----                | 390 |
| BAP68758.1 blaACT-35 cephalosporin-hydrolyzing | -----                | 381 |
| AFU25650.1 blaACT-12 cephalosporin-hydrolyzing | -----                | 381 |
| AHM76774.1 blaACT-22 cephalosporin-hydrolyzing | -----                | 381 |
| E.mir_ATCC33958_bla719                         | PENTNAKEQIKLLDKQIKHH | 454 |
| E.mir_ATCC33958_bla4255 SBL                    | -----                | 352 |
| E.men_13253_bla2764                            | -----                | 376 |
| E.ano_Ag1_bla2980                              | -----                | 376 |

### **Class C Group 4**

CLUSTAL O(1.2.1) multiple sequence alignment

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|--|--|----|
| AFM80040.1 blaADC-63 class                     | -----MRFKKISCLLLSPLFIFSTSIYAGNTPKDQEIKKLVQNFKPLLEKYDVP       | 50 |
| CAK95241.1 blaADC-20 class                     | -----MRFKKISCLLLSPLFIFSTSIYADNTPKDQEIKKLVQNFKPLLDKYDVP       | 50 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | MRKKMQNTLKL-LSVITCLAATA-----QGALAANIDESKIKDTVDDLIQPLMQKNNIP  | 53 |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | MRKKMQNTLKL-LSVITCLAATA-----QGALAANIDESKIKDTVDDLIQPLMQKNNIP  | 53 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | MRKKMQNTLKL-LSVITCLAATA-----QGALAANIDESKIKDTVDDLIQPLMQKNNIP  | 53 |
| ACS44783.1 blaMOX-5 CMY-1/MOX                  | ----MQQRQSILWGALATLMWAG-----LAHAGDKAATDPLRPVVDASIRPLLKEHRIP  | 50 |
| ACS44784.1 blaMOX-6 CMY-1/MOX                  | ----MQQRQSILWGVLPTLMWAG-----LAHAGDRAATDPLRPVVDASIRPLLKEHRIP  | 50 |
| KGY70007.1 blaMOX-12 CMY-1/MOX                 | ----MQQRQSILWGALATLMWAG-----LAHAGDKAATDPLRPVVDASIRPLLKEHRIP  | 50 |
| ACS44785.1 blaMOX-7 CMY-1/MOX                  | ----MQQRQSILWGALATLMWAG-----LVHAGDKAATDPLRPVVDASIRPLLKEHRIP  | 50 |
| E.men_ATCC13253_bla800                         | -----MIKKLLSFSVLSSTGLVLFQAQQNIEM-K-----ADSIMKAYHIP---        | 37 |
| E.men_ATCC13253_bla758 SBL                     | ----MK--FKLGLRLLAFIFVA-VLS-GFRLSAQDNIHTDK-----IDRYLENIEKNNLD | 47 |
| E.mir_ATCC33958_bla2658 SBL                    | -----MYKTLFTSVLLS-VSSVLLSQSFNKEK-----LDTYFSTLEKNNKF          | 40 |
| E.ano_Ag1_bla3306 SBL                          | -----MYKTLFTSALLS-VSSVLLSQSFNKEK-----LDTYFSTLEKNNKF          | 40 |

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AFM80040.1|blaADC-63|class GMAVGVIQNNKKYEM-YYGLQSVQ---DKKAVNSSTIFELG**SVSKL**FTGTAGGYAKNKGK 106  
CAK95241.1|blaADC-20|class GMAVGVIQNNKKYEM-YYGLQSVQ---DKKTVNSSTIFELG**SVSKL**FTATAGGYAKNKGK 106  
AAF86694.1|blaACC-1b|cephalosporin-hydrolyzing GMSVAVTVNGKNYIY-NYGLAAKQ---PQQPVTENTLFEV**GSLSK**TFATLASAQAQVSGK 109  
AAF86700.1|blaACC-1d|cephalosporin-hydrolyzing GMSVAVTVNGKNYIY-NYGLAAKQ---PQQSVTENTLFEV**GSLSK**TFATLASAQAQVSGK 109  
AAF86699.1|blaACC-1a|cephalosporin-hydrolyzing GMSVAVTVNGKNYIY-NYGLAAKQ---PQQPVTENTLFEV**GSLSK**TFATLASAQAQVSGK 109  
ACS44783.1|blaMOX-5|CMY-1/MOX GMAVAVLKDGGKAHYF-NYGVADRE---RAVGVSEQTLFEI**GSVSK**TLTATLGAYAVVQGG 106  
ACS44784.1|blaMOX-6|CMY-1/MOX GMAVAVLKDGGKAHYF-NYGVADRE---RAVGVSEQTLFEI**GSVSK**TLTATLGAYAVVQGS 106  
KGY70007.1|blaMOX-12|CMY-1/MOX GMAVAVLKDGGKAHYF-NYGVADRE---RAVGVSEQTLFEI**GSVSK**TLTATLGAYAVVQGG 106  
ACS44785.1|blaMOX-7|CMY-1/MOX GMAVAVLKDGGKAHYF-NYGVADRE---RAVGVSEQTLFEI**GSVSK**TLTATLGAYAVVQGS 106  
E.men\_ATCC13253\_bla800 EMAYAVVTPDKIVVQHHTTGHRIIEINDKPNADIHDFH**LSNTKA**ITGFIAGYLTEQNK 97  
E.men\_ATCC13253\_bla758|SBL VGSISVYKEGKEVYRNFQKIPG-----QH-FNKNSVYQ**VSITK**LFTSVLIFKLIENS 101  
E.mir\_ATCC33958\_bla2658|SBL SGSAITQDNKLIYTRSVGYSIDIE---NKILNSDKTKYR**IGSISK**TFAVLILKSFEEGK 97  
E.ano\_Agl\_bla3306|SBL SGSAITQDNKLIYTRSVGYSIDIE---NKILNSDKTKYR**IGSISK**TFAVLILKSFEEGK 97

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AFM80040.1|blaADC-63|class ISFDDKPGKYWKELKNTNTP---IDQVNLQLATYTSGNLA-----LQFPDEVQTDQ 153  
CAK95241.1|blaADC-20|class ISFDDTSGKYWKELKNTNTP---IDQVNLQLATYTSGNLA-----LQFPDEVQTDQ 153  
AAF86694.1|blaACC-1b|cephalosporin-hydrolyzing LSLDQSVSHYVPELRGSS---FDHVSVLNVGTH-TSGLQ-----LFMPEDIKNTT 155  
AAF86700.1|blaACC-1d|cephalosporin-hydrolyzing LSLDQSVSHYVPELRGSS---FDHVSVLNVGTH-TSGLQ-----LFMPEDIKNTT 155  
AAF86699.1|blaACC-1a|cephalosporin-hydrolyzing LSLDQSVSHYVPELRGSS---FDHVSVLNVGTH-TSGLQ-----LFMPEDIKNTT 155  
ACS44783.1|blaMOX-5|CMY-1/MOX FELDDKASLFAPWLKGS---FDNITMGELATYSAGGLP-----LQFPPEEVSLE 153  
ACS44784.1|blaMOX-6|CMY-1/MOX FELDDKASLFAPWLKGSV---FDNITMGELATYSAGGLP-----LQFPPEEVSLE 153  
KGY70007.1|blaMOX-12|CMY-1/MOX FELDDKASLFAPWLKGS---FDNITMGELATYSAGGLP-----LQFPPEEVSLE 153  
ACS44785.1|blaMOX-7|CMY-1/MOX FELDDKASLFAPWLKGSV---FDNITMGELATYSAGGLP-----LQFPPEEVSLE 153  
E.men\_ATCC13253\_bla800 IKWDQKFDFLPELKDKNPKYINITLAQLLQHAGIQPFTSGAEYQ**KLPS**FKGSKAEKR 157  
E.men\_ATCC13253\_bla758|SBL LSPDTRLSEFFPTIQYSD-----KITIRNLEHSSGLNNYV**KKSG**KTIVLKEPRTDA--- 153  
E.mir\_ATCC33958\_bla2658|SBL LKPDDKLSIFFPQIKNAD-----QITISQLLQHRSGIHNI**TDDNS**YMDYYQEPQSEA--- 149  
E.ano\_Agl\_bla3306|SBL LKPDDKLSLFFPHIKNAD-----QITISQLLQHRSGIHNI**TDDNS**YMDYYQEPQSEA--- 149

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AFM80040.1|blaADC-63|class QVLTFFKDWKPKNPIGEYRQ**YSNPS**IGLFGKVVVALSMNKPFQVLEKTI**FP**PALGLKHSYV 213  
CAK95241.1|blaADC-20|class QVLTFFKDWKPKNPIGEYRQ**YSNPS**IGLFGKVVVALSMNKPFQVLEKTI**FP**PGLGLKHSYV 213  
AAF86694.1|blaACC-1b|cephalosporin-hydrolyzing QLMAYLKAWKPADAAGTHR**VYSNI**GTGLLGMIAAKSLGMSYEDAI**EKT**LLPQLGMHHSYL 215  
AAF86700.1|blaACC-1d|cephalosporin-hydrolyzing QLMAYLKAWKPADAAGTHR**VYSNI**GTGLLGMIAAKSLGVSYEDAI**EKT**LLPQLGMHHSYL 215  
AAF86699.1|blaACC-1a|cephalosporin-hydrolyzing QLMAYLKAWKPADAAGTHR**VYSNI**GTGLLGMIAAKSLGVSYEDAI**EKT**LLPQLGMHHSYL 215  
ACS44783.1|blaMOX-5|CMY-1/MOX KMQAYYRQWTPAYS**SRGSHRQYANPS**IGLFGYLAASSMKQ**PF**DRLEMQ**TMLP**PGLGLYHTYL 213  
ACS44784.1|blaMOX-6|CMY-1/MOX KMQAYYRQWTPAYS**SPGSHRQYANPS**IGLFGYLAASSMKQ**PF**DRLEMQ**TILP**PGLGLYHTYL 213  
KGY70007.1|blaMOX-12|CMY-1/MOX KMQAYYRQWTPAYS**SPGSHRQYANPS**IGLFGYLAASSMKQ**PF**DRLEMQ**TILP**PGLGLYHTYL 213  
ACS44785.1|blaMOX-7|CMY-1/MOX KMQAYYRQWTPAYS**SPGSHRQYANPS**IGLFGYLAASSMKQ**PF**DRLEMQ**TMLP**PGLGLYHTYL 213  
E.men\_ATCC13253\_bla800 QAFAYKLLTLPPVENNK**PYNYSNA**GYIAALMMEK**VSRKT**WEQ**LVQ**DV**LKDKL**KLQYNL- 216  
E.men\_ATCC13253\_bla758|SBL ELFAELKSQKPLFNP**GD**SV**YSNS**GYLLGK**IEH**KYK**DN**YGN**VL**R**KLIT**PK**NK**LL**FTNS** 213  
E.mir\_ATCC33958\_bla2658|SBL KLVDIITKAGSDFQ**PD**SK**YSYSNS**GYILLTY**ILEK**V**NK**SY**AEL**L**KEK**IT**KPL**GL**NST**YV 209  
E.ano\_Agl\_bla3306|SBL KLVDIITKAGSDFQ**PD**SK**YSYSNS**GYILLTY**ILEK**V**NK**K**PYAEL**L**KEK**IM**KPL**GL**NST**YV 209

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| AFM80040.1 blaADC-63 class                     | NVPKTMQNYAFGYNQENQPI-----RVNPGPLDAPAYGVKSTLPDMLSFIHANLNPQKY    | 268 |
| CAK95241.1 blaADC-20 class                     | NVPKTMQNYAFGYNQENQPI-----RVNPGPLDAPAYGVKSTLPDMLKFINANLNPQKY    | 268 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | KVPADQMENYAWGYNKKDEPV-----HVNMEILGNEAYGIKTTSSDLLRYVQANMGQLKL   | 270 |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | KVPADQMENYAWGYNKKDEPV-----HVNMEILGNEAYGIKTTSSDLLRYVQANMGQLKL   | 270 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | KVPADQMENYAWGYNKKDEPV-----HVNMEILGNEAYGIKTTSSDLLRYVQANMGQLKL   | 270 |
| ACS44783.1 blaMOX-5 CMY-1/MOX                  | NVPEQPMGHYAYGYWKEDKPF-----RVTPAMLAEEEPYGIKTTSSADLLRFVKANISGV-- | 266 |
| ACS44784.1 blaMOX-6 CMY-1/MOX                  | NVPEQAMGHYAYGYWKEDKPI-----RVTPGMLADEAYGIKTTSSADLLRFVKANISGV--  | 266 |
| KGY70007.1 blaMOX-12 CMY-1/MOX                 | NVPEQAMGHYAYGYWKEDKPI-----RVTPGMLADEAYGIKTTSSADLLRFVKANISGV--  | 266 |
| ACS44785.1 blaMOX-7 CMY-1/MOX                  | TVPEQAMGHYAYGYWKEDKPI-----RVTPGMLADEAYGIKTTSSADLLRFVKANIGGV--  | 266 |
| E.men_ATCC13253_bla800                         | GWPNRTNMNQPWGHWNKPNQLESVAPTTAYDLSLAEPAGDISMNIVDYSKFIQLNLQGLAG  | 276 |
| E.men_ATCC13253_bla758 SBL                     | ALQNPQNVSKSYKFSE-----QGWVKNPDFYFKNIIGVDISSNTSDLNKFINLLFEGRIL   | 269 |
| E.mir_ATCC33958_bla2658 SBL                    | GKKINSQNEAYSYSF-----GNKKS AETDMSIPIGAGAVSNPTDIVKFSNALFNGKLL    | 264 |
| E.ano_Agl_bla3306 SBL                          | GKKINSQNEAYSYSF-----GNKKS AETDMSIPIGAGAVSNPSDIVKFSNALFNGKLL    | 264 |

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| AFM80040.1 blaADC-63 class                     | --SADIQRAINETHQGRYQINTMYQALGWEEFSYPATLQTLTLLDSNSEQIVMKPNKVT AIS | 326 |
| CAK95241.1 blaADC-20 class                     | --PKDIQRAINETHQGFYQVGTMYQALGWEEFSYPAPLQTLTLLDSNSEQIVMKPNKVT AIS | 326 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | DANAKMQQALTATHTGYFKSGEITQDLMWEQLPYPVSLPNLLTGNDMAMTKSVATPIVPP    | 330 |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | DANAKMQQALTATHTGYFKSGEITQDLMWEQLPYPVSLPNLLTGNDMAMTKSVATPIVPP    | 330 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | DANAKMQQALTATHTGYFKSGEITQDLMWEQLPYPVSLPNLLTGNDMAMTKSVATPIVPP    | 330 |
| ACS44783.1 blaMOX-5 CMY-1/MOX                  | -DNAAMQQAIDLTHQGQYAVGEMTQGLGWERYYPVSEQTLLAGNSPAMIYNANPAAPAP     | 325 |
| ACS44784.1 blaMOX-6 CMY-1/MOX                  | -DNAAMQQAIDLTHQGQYAVGEMTQGLGWERYAYPVSEQTLLAGNSAAMIYNANPAAPAP    | 325 |
| KGY70007.1 blaMOX-12 CMY-1/MOX                 | -DNAAMQQAIDLTHQGQYAVGEMTQGLGWERYAYPVSEQTLLAGNSPAMIYNTNPAAPAP    | 325 |
| ACS44785.1 blaMOX-7 CMY-1/MOX                  | -DNAAMQQAIDLTHQGQYAVGEMTQGLGWERYAYPVSEQTLLAGNSPAMIYNAIPAVPAP    | 325 |
| E.men_ATCC13253_bla800                         | KNNILKAKT-----YQYLFNSADHYSIGWANAV-----                          | 304 |
| E.men_ATCC13253_bla758 SBL                     | EEKNLEVM-----KPVIGKETYGRGLMNFN-----                             | 294 |
| E.mir_ATCC33958_bla2658 SBL                    | NKESLEKM-----IT I--RDGYGYGLFTAQ-----                            | 287 |
| E.ano_Agl_bla3306 SBL                          | NKESLEKM-----ITV--RDGYGYGLFTTQ-----                             | 287 |

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| AFM80040.1 blaADC-63 class                     | K-EPSVKMYHKTGSTNGFG----TYVVFIPKENIGLVMLTNKRI PNEERIKAAAYVVLNAI | 381 |
| CAK95241.1 blaADC-20 class                     | K-EPSVKMFHKTGSTNGFG----TYVVFIPKENIGLVMLTNKRI PNEERIKAAAYAVLNAI | 381 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | LPPQENVWINKTGSTNGFG----AYIAFVPAKMGIVMLANKNYSIDQRVTVAYKILSSL    | 386 |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | LPPQENVWINKTGSTNGFG----AYIAFVPAKMGIVMLANKNYSIDQRVTVAYKILSSL    | 386 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | LPPQENVWINKTGSTNGFG----AYIAFVPAKMGIVMLANKNYSIDQRVTVAYKILSSL    | 386 |
| ACS44783.1 blaMOX-5 CMY-1/MOX                  | AAAGHPVLFKKTGSTNGFG----AYVAFVPAKGI VMLANRNPNEGTLKAGHAILTQL     | 381 |
| ACS44784.1 blaMOX-6 CMY-1/MOX                  | AARGHPVLFNKKTGSTNGFG----AYVAFVPAKGI VMLANRNSPIEGTLKAGHAILTQL   | 381 |
| KGY70007.1 blaMOX-12 CMY-1/MOX                 | AAAGHPVLFNKKTGSTNGFG----AYVAFVPAKGI VMLANRNPNEARIKAAHAILTKL    | 381 |
| ACS44785.1 blaMOX-7 CMY-1/MOX                  | AAAGHPVLFNKKTGSTNGFG----AYVAFVPAKGI VMLANRNSPIEARIKAAHAILTQL   | 381 |
| E.men_ATCC13253_bla800                         | --VNNKKYSDHL-GTDGTFLAYTQINQSEPKAY--I I LVNN-GSPEAQDGLFKFL---KI | 355 |
| E.men_ATCC13253_bla758 SBL                     | --FHGINFYGNTGGTYGTN----TILVYEPKSKISISLIIN-GEQYERDLFIKDV-VDII   | 346 |
| E.mir_ATCC33958_bla2658 SBL                    | --FNDLKGFGHAGGIDDFS----SLFAHYNVGNVSFALDSNVSDGYGNNLIKAL-LSAV    | 340 |
| E.ano_Agl_bla3306 SBL                          | --FNDLKGFGHSGGIDDFS----SLFVYVNVGNVSFALDSNVSEGYGNNLIKAL-LSAV    | 340 |

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| AFM80040.1 blaADC-63 class                     | KK-----  | 383 |
| CAK95241.1 blaADC-20 class                     | KK-----  | 383 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | EGNK-----  | 390 |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | EGNK-----  | 390 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | EGNK-----  | 390 |
| ACS44783.1 blaMOX-5 CMY-1/MOX                  | AR-----  | 383 |
| ACS44784.1 blaMOX-6 CMY-1/MOX                  | AR-----  | 383 |
| KGY70007.1 blaMOX-12 CMY-1/MOX                 | AR-----  | 383 |
| ACS44785.1 blaMOX-7 CMY-1/MOX                  | AR-----  | 383 |
| E.men_ATCC13253_bla800                         | LKKQYP-----  | 361 |
| E.men_ATCC13253_bla758 SBL                     | FNNHLSIHKS-----  | 356 |
| E.mir_ATCC33958_bla2658 SBL                    | YNKPYDIPEFKTYQADVNDFAKYIGTYASPSFPLKITITTDNTSLKAQATGQSEFTLTPT | 400 |
| E.ano_Ag1_bla3306 SBL                          | YNKPYDIPEFKTYQADVNDFAKYIGTYASPTFPLKIAITTDNTSLKAQATGQSEFTLTPT | 400 |

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|--|--------------------------------------|-----|
| AFM80040.1 blaADC-63 class                     | -----                                | 383 |
| CAK95241.1 blaADC-20 class                     | -----                                | 383 |
| AAF86694.1 blaACC-1b cephalosporin-hydrolyzing | -----                                | 390 |
| AAF86700.1 blaACC-1d cephalosporin-hydrolyzing | -----                                | 390 |
| AAF86699.1 blaACC-1a cephalosporin-hydrolyzing | -----                                | 390 |
| ACS44783.1 blaMOX-5 CMY-1/MOX                  | -----                                | 383 |
| ACS44784.1 blaMOX-6 CMY-1/MOX                  | -----                                | 383 |
| KGY70007.1 blaMOX-12 CMY-1/MOX                 | -----                                | 383 |
| ACS44785.1 blaMOX-7 CMY-1/MOX                  | -----                                | 383 |
| E.men_ATCC13253_bla800                         | -----                                | 361 |
| E.men_ATCC13253_bla758 SBL                     | -----                                | 356 |
| E.mir_ATCC33958_bla2658 SBL                    | DKNKFESQAGIKMEFYPDKKQFRLL-----       | 426 |
| E.ano_Ag1_bla3306 SBL                          | DKNKFESQAGIKMEFYPDKKQFRLLQNGLDILFTKE | 437 |

## **Class C Group 5**

CLUSTAL O(1.2.1) multiple sequence alignment

|                             |                                 |     |    |
|-----------------------------|---------------------------------|-----|----|
| ACN62070.1 blaADC-41 class  | MRFKKISCLLLSPLFIFSTSI-YAGN----- | TPK | 28 |
| ALA14812.1 blaADC-77 class  | MRFKKISCLLLSPLFIFSTSI-YAGN----- | TPK | 28 |
| ALA14816.1 blaADC-81 class  | MRFKKISCLLLSPLFIFSTSI-YAGN----- | TPK | 28 |
| AIT76094.1 blaDHA-18 class  | -MKKLSATLISALLAFSA-----PG-----  | FSA | 23 |
| AIT76102.1 blaDHA-19 class  | -MKKLSATLISALLAFSA-----PG-----  | FSA | 23 |
| AIT76108.1 blaDHA-13 class  | -MKKLSATLISALLAFSA-----PG-----  | FSA | 23 |
| CCK86742.1 blaCMY-89 class  | MMKKSICALLLTASFSTF-----AA-----  | AKT | 24 |
| AFK73452.1 blaCMY-81 class  | MMKKSICALLLTASFSTF-----AA-----  | AKT | 24 |
| AIT76099.1 blaCMY-114 class | MMKKSICALLLTASFSTF-----AA-----  | AKT | 24 |
| AKO62865.1 blaCMY-128 class | MMKKSICALLLTASFSTF-----AA-----  | AKT | 24 |

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|--|---|-----|
| AFK73449.1 blaCMY-80 class                     | MMKKSLLCCALLLTASFSTF-----AA-----AKT                             | 24  |
| AFZ85212.1 blaCMY-96 class                     | MMKKSLLCCALLLTASLSTF-----AA-----AKT                             | 24  |
| AEI70575.1 blaACT-9 cephalosporin-hydrolyzing  | MKTKSLCCALLLSTSCSVL-----AA-----PMS                              | 24  |
| ACJ05689.1 blaACT-5 cephalosporin-hydrolyzing  | MMKKSLLCCALLLGISCSAL-----AA-----PVS                             | 24  |
| AHM76779.1 blaACT-19 cephalosporin-hydrolyzing | MMKKSFCCALLLAIISGSAL-----AA-----PVS                             | 24  |
| E.ano_Ag1_bla622                               | -MKSI-ILIFLISLNVFSQNRESNIQ-----SVKQTKADKTIK                     | 36  |
| E.men_ATCC13253_bla63                          | -MKKH-SALFLLSLTLTFTACKKEGGKAQGNDEKEYESKLPNYGNVDLKNVFSPEDESKLAD  | 58  |
| E.mir_ATCC33958_bla1527 SBL                    | -MKKV-IIL-----FIAVSSI--V  | 15  |
| E.ano_Ag1_bla2038 SBL                          | -MKKV-IFL-----FIAVSSF--V  | 15  |
| E.men_ATCC13253_bla597 SBL                     | -----   | 0   |
| E.ano_Ag1_bla267 SBL                           | -----   | 0   |
|  |   |     |
| ACN62070.1 blaADC-41 class                     | DQEIKKLVLDQNFKPLLDKYDVPGMVAVGVIQNNKKYETYYGLQS-VQDKKAVSSSTIFELG  | 87  |
| ALA14812.1 blaADC-77 class                     | DQEIKKLVLDQNFKPLLEKYDVPGMVAVGVIQNNKKYEMYYYGLQS-VQDKKAVNSSTIFELG | 87  |
| ALA14816.1 blaADC-81 class                     | DQEIKKLVLDQNFKPLLEKYDVPGMVAVGVIQNNKKYEMYYYGLQS-VQDKKAVNRSTIFELG | 87  |
| AIT76094.1 blaDHA-18 class                     | ADNVAAVVDSTIKPLMAQQDIPGMVAVSVKGGKPYFNYGFAD-VQAKQPVTENTLFLG      | 82  |
| AIT76102.1 blaDHA-19 class                     | ADNVAAVVDSTIKPLMAQQDIPGMVAVSVKGGKPYFNYGFAD-VQAKQPVTENTLFLG      | 82  |
| AIT76108.1 blaDHA-13 class                     | ADNVAAVVDSTIKPLMAQQDIPGMVAVSVKGGKPYFNYGFAD-VQAKQPVTENTLFLG      | 82  |
| CCK86742.1 blaCMY-89 class                     | EQQIADIVNRTITPLMQEQAIIPGMVAVAIIYEGKPYFNYGFAD-IANKHPVTQOTLFLG    | 83  |
| AFK73452.1 blaCMY-81 class                     | EQQIADIVNRTITPLMQEQAIIPGMVAVAIIYEGKPYFNYGFAD-IVNNHPVTQOTLFLG    | 83  |
| AIT76099.1 blaCMY-114 class                    | EQQIADIVNRTITPLMQEQAIIPGMVAVAIIYEGKPYFNYGFAD-IANNHPVTQOTLFLG    | 83  |
| AKO62865.1 blaCMY-128 class                    | EQQIADIVNRTITPLMQEQAIIPGMVAVAIIYEGKPYFNYGFAD-IANNHPVTQOTLFLG    | 83  |
| AFK73449.1 blaCMY-80 class                     | EPQIADIVNRTITPLMQEQAIIPGMVAVIYQGKSYFNYGFAD-ITNNHPVTQOTLFLG      | 83  |
| AFZ85212.1 blaCMY-96 class                     | EPQIADIVNRTITPLMQEQAIIPGMVAVIYQGKPYFNYGFAD-ITNNHPVTQOTLFLG      | 83  |
| AEI70575.1 blaACT-9 cephalosporin-hydrolyzing  | EKQLSDVVERTVTPMLKAQAIIPGMVAVIYQGQPHYFTFGKAD-VTANKPVTTPQTLFLG    | 83  |
| ACJ05689.1 blaACT-5 cephalosporin-hydrolyzing  | EKQLAEVVANTVTPMLKAQAIIPGMVAVIYQGKPHYFTFGKAD-IAASKPVTTPQTLFLG    | 83  |
| AHM76779.1 blaACT-19 cephalosporin-hydrolyzing | EKQLAEVVANTVTPMLKTAQAIIPGMVAVIYQGKPHYFTFGKAD-IAASKPVTTPQTLFLG   | 83  |
| E.ano_Ag1_bla622                               | EPEYINQINELMTKSYERGLFNGNVLVAKKGVIVYQKSFSGFTD-ETRKTPPLTKNAIFNFG  | 95  |
| E.men_ATCC13253_bla63                          | KASVVRTIDNYYQHVEKGDLSGGVLAQKDDILYKRYGFR-ENNTVPIQDVMAMHVA        | 117 |
| E.mir_ATCC33958_bla1527 SBL                    | YGQKEKLDLFTSLYAAKEFNGNVLIAEKGVIVYKSFGLAN-EKTKQKLDKNTVFELA       | 74  |
| E.ano_Ag1_bla2038 SBL                          | YGQKEKLDLFTSLYAAKEFNGNVLIAEKGVIVYKSFGLAN-EKTKQKLDKNTVFELA       | 74  |
| E.men_ATCC13253_bla597 SBL                     | ----MKNTEILDYIINKTKFNGNIMIADGRKVLYTGSFGGETFNPGEKQELNRNSVFELA    | 56  |
| E.ano_Ag1_bla267 SBL                           | ----MKNVFDILENYIKKIKFNGNIMIERRKVLYAGSFGNNYHPPEEKRELSRSSVFELA    | 56  |
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|--|-------|--|-----|
| ACN62070.1 blaADC-41 class                     | SVSKL | FTATAGGYAKNKGKISFDDTPGKYWKELKNTPI-DQVNLLQLATYTSGNLALQFP  | 146 |
| ALA14812.1 blaADC-77 class                     | SVSKL | FTATAGGYAKNKGKISFDDTPGKYWKELKNTPI-DQVNLLQLATYTSGNLALQFP  | 146 |
| ALA14816.1 blaADC-81 class                     | SVSKL | FTATAGGYAKNKGKISFDDTPGKYWKELKNTPI-DQVNLLQLATYTSGNLALQFP  | 146 |
| AIT76094.1 blaDHA-18 class                     | SVSKT | FTGVLGAVSVAKKEMALNDPAAKYQPELALPQW-KGITLLDLATYTAGGLPLQVP  | 141 |
| AIT76102.1 blaDHA-19 class                     | SVSKT | FTGVLGAVSVAKKEMALNDPAAKYQPELALPQW-KGITLLDLATYTAGGLPLQVP  | 141 |
| AIT76108.1 blaDHA-13 class                     | SVSKT | FTGVLGAVSVAKKEMALNDPAAKYQPELALPQW-KGITLLDLATYTAGGLPLQVP  | 141 |
| CCK86742.1 blaCMY-89 class                     | SVSKT | FNGVLGGDAIARGEIKLSDPVTKYWPELTGKQW-RGISLLHLATYTAGGLPLQIP  | 142 |
| AFK73452.1 blaCMY-81 class                     | SVSKT | FNGVLGGDAIARGEIKLSDPVTKYWPELTGKQW-RGISLLHLATYTAGGLPLQIP  | 142 |
| AIT76099.1 blaCMY-114 class                    | SVSKT | FNGVLGGDAIARGEIKLSDPVTKYWPELTGKQW-RGISLLHLATYTAGGLPLQIP  | 142 |
| AKO62865.1 blaCMY-128 class                    | SVSKT | FNGVLGGDAIARGEIKLSDPVTKYWPELTGKQW-RGISLLHLATYTAGGLPLQIP  | 142 |
| AFK73449.1 blaCMY-80 class                     | SVSKT | FNGVLGGDAIARGEIKLSDPVTKYWPELTGKQW-QGISLLHLATYTAGGLPLQIP  | 142 |
| AFZ85212.1 blaCMY-96 class                     | SVSKT | FNGVLGGDAIARGEIKLSDPVTKYWPELTGKQW-QGISLLHLATYTAGGLPLQIP  | 142 |
| AEI70575.1 blaACT-9 cephalosporin-hydrolyzing  | SISKT | FTGVLGGDAIARGEISLGDVTKYWPELTGKQW-QGIRMLDLATYTAGGLPLQVP   | 142 |
| ACJ05689.1 blaACT-5 cephalosporin-hydrolyzing  | SISKT | FTGVLGGDAIARGEISLDDPVTRYWPQLTGKQW-QGIRMLDLATYTAGGLPLQVP  | 142 |
| AHM76779.1 blaACT-19 cephalosporin-hydrolyzing | SISKT | FTGVLGGDAIARGEISLDDPVTKFWPELTGKQW-QGIRMLDLATYTAGGLPLQVP  | 142 |
| E.ano_Ag1_bla622                               | SIVKQ | FNAVAIMMLVERGHLNLDLDPISKYNDLDP--KWSEKVTTRHLINYSAGPIRIENK | 153 |
| E.men_ATCC13253_bla63                          | SVSKP | ITAMAVMKLIEAGKLLNEPLTTLFPGFP---Y-PEITVEMLLKQRSGLPKYEHF   | 173 |
| E.mir_ATCC33958_bla1527 SBL                    | SVSKQ | FTAMGIVQLEKDGKLSYNDPLTKYFPELG--FY-KLITIDNLLHHTSGLPDYMEI  | 131 |
| E.ano_Ag1_bla2038 SBL                          | SVSKQ | FTAMGIVQLEKEGKLSYNDPLTKYFPELS--FY-KPITIDNLLHHTSGLPDYMSL  | 131 |
| E.men_ATCC13253_bla597 SBL                     | SVSKP | FTAFAMLKVLDVYHLSLHTDIRYFLPGFS---Y-KDITLFLHLLNHTSGLPDYMDL | 112 |
| E.ano_Ag1_bla267 SBL                           | SVSKP | FTAFAMLKVMDVYHLSLHTDVKYFLPDFP---Y-EGISVFQLLNHTSGLPDYMEI  | 112 |
|  | *     | : * : . . . . .  |     |

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| ACN62070.1 blaADC-41 class                     | DEV | -----QTDQQVLTFFKDWKPK--NPIGEYRQYNSPSIGLFGKVVVALSMNKPFDDQ   | 196 |
| ALA14812.1 blaADC-77 class                     | DEV | -----QTDQQVLTFFKDWKPK--NPIGEYRQYNSPSIGLFGKVVVALSMNKPFDDQ   | 196 |
| ALA14816.1 blaADC-81 class                     | DEV | -----QTDQQVLTFFKDWKPK--NPIGEYRQYNSPSIGLFGKVVVALSMNKPFDDQ   | 196 |
| AIT76094.1 blaDHA-18 class                     | DAV | -----KSRADLLNFYQQWQPS--WKPQDMRLYANSSIGLFGALTANAAGMPYEQ     | 191 |
| AIT76102.1 blaDHA-19 class                     | DAV | -----KSRADLLNFYQQWQPS--WKPQDMRLYANSSIGLFGALTANAAGMPYEQ     | 191 |
| AIT76108.1 blaDHA-13 class                     | DAV | -----KSRADLLNFYQQWQPS--WKPQDMRLYANSSIGLFGALTANAAGMPYEQ     | 191 |
| CCK86742.1 blaCMY-89 class                     | DDV | -----TDKAELLRFYQNWQPQ--WTPGAKRLYANSSIGLFGALVVKPSGMSYEE     | 192 |
| AFK73452.1 blaCMY-81 class                     | DYV | -----TDKAELLRFYQNWQPQ--WTPGAKRLYANSSIGLFGALVVKPSGMSYEE     | 192 |
| AIT76099.1 blaCMY-114 class                    | DYV | -----TDKAELLRFYQNWQPQ--WTPGAKRLYANSSIGLFGALVVKPSGMSYEE     | 192 |
| AKO62865.1 blaCMY-128 class                    | DDV | -----TDKTELLRFYQNWQPQ--WTPGAKRLYANSSIGLFGALVVKPSGMSYEE     | 192 |
| AFK73449.1 blaCMY-80 class                     | DDV | -----TDKAALLRFYQNWQPQ--WAPGAKRLYANSSIGLFGALAVKPSGMSYEE     | 192 |
| AFZ85212.1 blaCMY-96 class                     | DDV | -----TDKAALLRFYQNWQPQ--WAPGAKRLYANSSIGLFGALAVKPSGMSYEE     | 192 |
| AEI70575.1 blaACT-9 cephalosporin-hydrolyzing  | DEV | -----TDNTSLLRFYQHWQPQ--WKPQTTRLYANASIGLFGALAVKPSGMNFEQ     | 192 |
| ACJ05689.1 blaACT-5 cephalosporin-hydrolyzing  | DEV | -----TDNAALLRFYQNWQPQ--WKPQTTRLYANASIGLFGALAVKPSGMPFEQ     | 192 |
| AHM76779.1 blaACT-19 cephalosporin-hydrolyzing | EEV | -----TDNASLLRFYQHWQPQ--WKPQTTRLYANASIGLFGALAVKPSGMRVEQ     | 192 |
| E.ano_Ag1_bla622                               | MIV | -----PKNDEAWKILRKTDLTLFEPGKGYRYDNGNVFLQRRRIIEKVTGMTFQD     | 205 |
| E.men_ATCC13253_bla63                          | LEE | EAKVPKEKFI SNQFILDIVKKNPELARKPDTGFMYCNTNYALLALVVEKTTQMAFPE | 233 |
| E.mir_ATCC33958_bla1527 SBL                    | FDK | NWDKKKFATNKDIVEMLAKYKPELLFAPGDKYYSNTGYALLGLIIEKVSQSYGD     | 190 |
| E.ano_Ag1_bla2038 SBL                          | FDK | NWDKKKFATNKDIDMLAKYKPELLFVPGDKYYSNTGYALLGLIIEKVSQSYGD      | 190 |
| E.men_ATCC13253_bla597 SBL                     | FEK | HWDKTKIADNQDVLAMLSDYHPKIYFQPGQWDYNSNTGYVLLAVILEKITGFSFPE   | 171 |
| E.ano_Ag1_bla267 SBL                           | FEE | FWDKTIADNSDVLGLLIALRPKYVFNPGERWDYCNNTGYVILAVILEKITGFSFPE   | 171 |
|  |     | * * : : : *  |     |

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|--|---|-----|
| ACN62070.1 blaADC-41 class                     | VLEKTIFFPGLGLKHSYVNVPK---NQMQNYAFGYNQENQPIR-VNPGPLDAPA-----   | 245 |
| ALA14812.1 blaADC-77 class                     | VLEKTIFFPALGLKHSYVNVPK---TQMQNYAFGYNQENQPIR-VNPGPLDAPA-----   | 245 |
| ALA14816.1 blaADC-81 class                     | VLEKTIFFPALGLKHSYVNVPK---TQMQNYAFGYNQENQPIR-VNPGPLDAPAYG---A- | 248 |
| AIT76094.1 blaDHA-18 class                     | LLTARILAPLGLSHTFTITVPE---SVQSRAYAYGYKN-KKPVR-VSPGQLDAES-----  | 239 |
| AIT76102.1 blaDHA-19 class                     | LLTARILAPLGLSHTFTITVPE---SAQSQYAYGYKN-KKPVR-VSPGQLDAES-----   | 239 |
| AIT76108.1 blaDHA-13 class                     | LLTARILAPLGLSHTFTITVPE---SAQSQYAYGYKN-KKPVR-VSPGQLDAES-----   | 239 |
| CCK86742.1 blaCMY-89 class                     | AMTRRVLQPLKLAHTWITVPQ---SEQKNYAWGYRE-GKPVH-VSPGQLDAEA-----    | 240 |
| AFK73452.1 blaCMY-81 class                     | AMTRRVLQPLKLAHTWITVPQ---SEQKNYAWGYRE-GKPVH-VSPGQLDAEA-----    | 240 |
| AIT76099.1 blaCMY-114 class                    | AMTRRVLQPLKLAHTWITVPQ---SEQKNYAWGYRE-GKPVH-VSPGQLDAEA-----    | 240 |
| AKO62865.1 blaCMY-128 class                    | AMTRRVLQPLKLAHTWITVPQ---SEQKNYAWGYRE-GKPVH-VSPGQLDAEA-----    | 240 |
| AFK73449.1 blaCMY-80 class                     | AMTRRVLQPLKLAHTWIKVPQ---SEQKDYAWGYRE-GKPVH-VSPGQLDAEA-----    | 240 |
| AFZ85212.1 blaCMY-96 class                     | AMTRRVLQPLKLAHTWITVPQ---NEQKDYAWGYRE-GKAVH-VSPGQLDAEA-----    | 240 |
| AEI70575.1 blaACT-9 cephalosporin-hydrolyzing  | AMTKRVFKPLKLDHTWINVVK---EEEAHYAWGYRD-GKAIH-VSPGMLDAEA-----    | 240 |
| ACJ05689.1 blaACT-5 cephalosporin-hydrolyzing  | AMTTRVLKPLKLDHTWINVVK---AEEAHYAWGYRD-GKAVR-VSPGMLDAQA-----    | 240 |
| AHM76779.1 blaACT-19 cephalosporin-hydrolyzing | AMTKRVFKPLRLNHTWINVVK---AEEAHYAWGYRD-GKAVH-ISPGLDAEA-----     | 240 |
| E.ano_Ag1_bla622                               | FVTKNI IKPLKMTNSVFDKASGYKNRSTCYDMDNVRCPE-----M                | 245 |
| E.men_ATCC13253_bla63                          | AMQEIVFKPLKMKHSFIFQEKDISTAARS----FYN----NG-----RRMFPMDYLDL    | 278 |
| E.mir_ATCC33958_bla1527 SBL                    | YLSKKIFKPLGMANTRVYRSRYKPEISNYALGYVVDLSLGNK-KLLDDLKGKEYTYLDG   | 249 |
| E.ano_Ag1_bla2038 SBL                          | YLNKKIFKPLGMANTRVYRSRYKPEKISNYALGYVVDLSLGNK-KLLDDLKGKEYTYLDG  | 249 |
| E.men_ATCC13253_bla597 SBL                     | VLQKYIFRPLGMENTQVYNRRKHPHHISDYAFGVQKNPENKFMPLPDDIEGEEYVYLDG   | 231 |
| E.ano_Ag1_bla267 SBL                           | VLKKYIFRPLDMKNTMVYNRRKKPQLIPDYAFGVSNPETGKLLPKIKGEEYVYLDG      | 231 |

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|--|---|-----|
| ACN62070.1 blaADC-41 class                     | ----YGVKSTLPDMLKFINANLNPQKYP-ADIQRAINETHQGIFYQVIMYQALGWEEFSY  | 300 |
| ALA14812.1 blaADC-77 class                     | ----YGVKSTLPDMLSFINANLNPQKYP-TDIQRAINETHQGRYQVNTMYQALGWEEFSY  | 300 |
| ALA14816.1 blaADC-81 class                     | --PAYGVKSTLPDMLSFIHANLNPQKYP-ADIQRAINETHQGIFYQVNTMYQALGWEEFSY | 305 |
| AIT76094.1 blaDHA-18 class                     | ----YGVKSASKDMLRWAEMNMEPSRAGNADLEMAMYLAQTRYKTAAINQGLGWEMYDW   | 295 |
| AIT76102.1 blaDHA-19 class                     | ----YGVKSASKDMLRWAEMNMEPSRAGNADLEMAMYLAQTRYKTAAINQGLGWEMYDW   | 295 |
| AIT76108.1 blaDHA-13 class                     | ----YGVKSASKDMLRWAEMNMEPSRAGNADLEMAMYLAQTRYKTAAINQGLGWEMYDW   | 295 |
| CCK86742.1 blaCMY-89 class                     | ----YGVKSSVIDMARWVQANMDASHVQEKTLLQGGIELAQSRWYRIGDMYQGLGWEMLNW | 296 |
| AFK73452.1 blaCMY-81 class                     | ----YGVKSSVIDMARWVQANMDASHVQEKTLLQGGIELAQSRWYRIGDMYQGLGWEMLNW | 296 |
| AIT76099.1 blaCMY-114 class                    | ----YGVKSSVIDMARWVQANMDASHVQEKTLLQGGIELAQSRWYRIGDMYQGLGWEMLNW | 296 |
| AKO62865.1 blaCMY-128 class                    | ----YGVKSSVIDMARWVQANMDASLVQEKTLLQGGIELAQSRWYRIGDMYQGLGWEMLNW | 296 |
| AFK73449.1 blaCMY-80 class                     | ----YGVKSSVIDMARWVQVNMDSRVQEKTLLQGGIALAQSRWYRIGDMYQGLGWEMLNW  | 296 |
| AFZ85212.1 blaCMY-96 class                     | ----YGVKSSVIDMARWVQVNMDSRVQEKTLLQGGIALAQSRWYRIGDMYQGLGWEMLNW  | 296 |
| AEI70575.1 blaACT-9 cephalosporin-hydrolyzing  | ----YGVKTNIQDMASWLKANMNPDALSDSTLKGQIALAQSRWYRIGDMYQGLGWEMLNW  | 296 |
| ACJ05689.1 blaACT-5 cephalosporin-hydrolyzing  | ----YGMKTNVQDMANWVMANMAPENIADASLKGQIALAQSRWYRIGSMYQGLGWEMLNW  | 296 |
| AHM76779.1 blaACT-19 cephalosporin-hydrolyzing | ----YGVKTNVQDMANWVMANMAPENIADASLKGQIALAQSRWYRIGSMYQGLGWEMLNW  | 296 |
| E.ano_Ag1_bla622                               | EFISGWLWLDINDMYKWEAMNYNRLISRKSFETLLNPN-YAKEEGGSLG-----SY      | 296 |
| E.men_ATCC13253_bla63                          | IYGDKNVYTTPRDLYNFSKALFSENFLKPELKN-LIFQP-YSNEKAGINNYGIGFRMKIF  | 336 |
| E.mir_ATCC33958_bla1527 SBL                    | IVGDGMVNSTTGDLLKWDRALYGDKLVNQKDKD-LIFSS-IVTKDNKDRYGYGWVDTK    | 307 |
| E.ano_Ag1_bla2038 SBL                          | IVGDGMVNSTTGDLLKWDRALYGDKLVNQKDKD-LIFSS-IVTKDNKDRYGYGWVDTK    | 307 |
| E.men_ATCC13253_bla597 SBL                     | IQQDGTVNSTLDDLLIWNNAILEQYHIHEKHLD-IMFEP-TLLSRDRKFPYGLGWEIDEQ  | 289 |
| E.ano_Ag1_bla267 SBL                           | IQQDGTVNSTLDDLLIWNNAILEQYLVDEKYLD-IMFEP-TVNNEGQVFPYGLGWELDEK  | 289 |

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ACN62070.1|blaADC-41|class      PAPLQTLTLLDSNSEQIVMKPNKVTAIISK---EPSVKMFHKTGSTNGFGTYVVVFIPEKENIGL      357
ALA14812.1|blaADC-77|class      PATLQTLTLLDSNSEQIVMKPNKVTAIISK---EPSVKMYHKTGSTTGFQTYVVVFIPEKENIGL      357
ALA14816.1|blaADC-81|class      PATLQTLTLLDSNSEQIVMKPNKVTAIISK---EPSVKMYHKTGSTNGFGTYVVVFIPEKENIGL      362
AIT76094.1|blaDHA-18|class      PQQKDMIINGVTNEVALQPHPVTD-NQVQPYNRASVWHKTGATTGFGAYVAFIPEKQVAI      354
AIT76102.1|blaDHA-19|class      PQQKDMIINGVTNEVALQPHPVTD-NQVQPYNRASVWHKTGATTGFGAYVAFIPEKQVAI      354
AIT76108.1|blaDHA-13|class      PQQKDMIINGVTNEVALQPHPVTD-NQVQPYNRASVWHKTGATTGFGAYVAFIPEKQVAI      354
CCK86742.1|blaCMY-89|class      PLKADSIINGSDSKVALAALPAVEVNPVPAVKASVWHKTGSTGGFGSYVAFVPEKNLGI      356
AFK73452.1|blaCMY-81|class      PLKADSIINGSDSKVALAALPAVEVNPVPAVKASVWHKTGSTGGFGSYVAFVPEKNLGI      356
AIT76099.1|blaCMY-114|class      PLKADSIINGSDSKVALAALPAVEVNPVPAVKASVWHKTGSTGGFGSYVAFVPEKNLGI      356
AKO62865.1|blaCMY-128|class      PLKADSIINGSDSKVALAALPAVEVNPVPAVKASVWHKTGSTGGFGSYVAFVPEKNLGI      356
AFK73449.1|blaCMY-80|class      PLKADSIINGSDSKVALAALPAVEVNPVPAVKASVWHKTGSTGGFGSYVAFVPEKNLGI      356
AFZ85212.1|blaCMY-96|class      PLKADSIINGSDSKVALAALPAVEVNPVPAVKASVWHKTGSTGGFGSYVAFVPEKNLGI      356
AEI70575.1|blaACT-9|cephalosporin-hydrolyzing      PVEAKTVVEGSDNKVALAPLLVAEVNPPAPPVKASVWHKTGSTGGFGSYVAFIPEKELGI      356
ACJ05689.1|blaACT-5|cephalosporin-hydrolyzing      PVEANTVIEGSDSKVALAPLVAEVNPPAPPVKASVWHKTGSTGGFGSYVAFIPEKQIGI      356
AHM76779.1|blaACT-19|cephalosporin-hydrolyzing      PVEAKMVEGSDNKVALAPLVAEVNPPAPPVKASVWHKTGSTGGFGSYVAFIPEKQIGI      356
E.ano_Agl_bla622      -FE-----NEKLQRHNGISHKFESILLNDMKNDIIV      326
E.men_ATCC13253_bla63      DNG-----EKLTYHNGWWHGSNAVFGHLLKSKVTI      366
E.mir_ATCC33958_bla1527|SBL      -DP-----VGKIANHSGGWAGYITYIERDLHDHDKTI      337
E.ano_Agl_bla2038|SBL      -YP-----FGKIANHSGGWAGYITFIERDLDYDKTI      337
E.men_ATCC13253_bla597|SBL      RNN-----VGKVYHTGSPWGYFTCNSVYLEKGISI      320
E.ano_Agl_bla267|SBL      KNG-----EKQVYHTGGWPGYFTYNSLYLNSGISV      319
      .      :.*      :      .      :

ACN62070.1|blaADC-41|class      VMLTNKRIPNEERIKAAYAVLNAIKK-----      383
ALA14812.1|blaADC-77|class      VMLTNKRIPNEERIKAAYAVLNAIKK-----      383
ALA14816.1|blaADC-81|class      VMLTNKRIPNEERIKAAYAVLDAIKK-----      388
AIT76094.1|blaDHA-18|class      VILANKNYPNTERVKAAQAILSALE-----      379
AIT76102.1|blaDHA-19|class      VILANKNYPNTERVKAAQAILSALE-----      379
AIT76108.1|blaDHA-13|class      VILANKNYPNTERVKAAQAILSALE-----      379
CCK86742.1|blaCMY-89|class      VMLANKSYPNPVRVEAAWRILEKLQ-----      381
AFK73452.1|blaCMY-81|class      VMLANKSYPNPARVEAAWRILEKLQ-----      381
AIT76099.1|blaCMY-114|class      VMLANKSYPNPARVEAAWRILEKLQ-----      381
AKO62865.1|blaCMY-128|class      VMLANKSYPNPARVEAAWRILEKLQ-----      381
AFK73449.1|blaCMY-80|class      VMLANKSYPNPVRVEAAWRILEKLQ-----      381
AFZ85212.1|blaCMY-96|class      VMLANKSYPNLVRVEAAWRILEKLQ-----      381
AEI70575.1|blaACT-9|cephalosporin-hydrolyzing      VMLANKSYPNPARVEAAYRILSAL-----      380
ACJ05689.1|blaACT-5|cephalosporin-hydrolyzing      VMLANKSYPNPARVEAAYPILDALQ-----      381
AHM76779.1|blaACT-19|cephalosporin-hydrolyzing      VMLANKSYPNPARVEAAYHILDALQ-----      381
E.ano_Agl_bla622      ILASNNLNKVYSLG-----YIIRDIMLGKAYEIPKKSVMYRAIRKESFTDINKAKDITY      379
E.men_ATCC13253_bla63      IAIGNKYSSRVYST---LALSGLFEDFPLEKERLS-----KELTRLPVDEKPSDAGE--      415
E.mir_ATCC33958_bla1527|SBL      IMLQNNNSEATSSP---VR---QLRNILYDIRSVK-----VDIATLQKYAGK-Y      379
E.ano_Agl_bla2038|SBL      IILQNNNSEAASSP---VK---QLRNILYDIKPIK-----VDLATLQKYAGK-Y      379
E.men_ATCC13253_bla597|SBL      ILLSNKPDSEEDTE---EKILQTLLEDVVFNKEITI-----PLHY-----      356

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|--|---|-----|
| E.ano_Ag1_bla267 SBL                           | ILLCNKPDSED-VE---NEMLQKLEDAAFGKKSPR-----TLYS-----           | 354 |
|  | : *:  | :   |
| ACN62070.1 blaADC-41 class                     | -----   | 383 |
| ALA14812.1 blaADC-77 class                     | -----   | 383 |
| ALA14816.1 blaADC-81 class                     | -----   | 388 |
| AIT76094.1 blaDHA-18 class                     | -----   | 379 |
| AIT76102.1 blaDHA-19 class                     | -----   | 379 |
| AIT76108.1 blaDHA-13 class                     | -----   | 379 |
| CCK86742.1 blaCMY-89 class                     | -----   | 381 |
| AFK73452.1 blaCMY-81 class                     | -----   | 381 |
| AIT76099.1 blaCMY-114 class                    | -----   | 381 |
| AKO62865.1 blaCMY-128 class                    | -----   | 381 |
| AFK73449.1 blaCMY-80 class                     | -----   | 381 |
| AFZ85212.1 blaCMY-96 class                     | -----   | 381 |
| AEI70575.1 blaACT-9 cephalosporin-hydrolyzing  | -----   | 380 |
| ACJ05689.1 blaACT-5 cephalosporin-hydrolyzing  | -----   | 381 |
| AHM76779.1 blaACT-19 cephalosporin-hydrolyzing | -----   | 381 |
| E.ano_Ag1_bla622                               | LLKKTSENEYNFENPSELNTLGYELLRAGRI-KESIEIFKLAISEFPKNANLFDLGEAY | 438 |
| E.men_ATCC13253_bla63                          | -----   | 415 |
| E.mir_ATCC33958_bla1527 SBL                    | IKKNSKTFEVFFENNKLYVPLNP----QVRLELEAISTNKFKVRDFSPD-----VF    | 426 |
| E.ano_Ag1_bla2038 SBL                          | TKKNSKTFEVFFENNKLYVPLNP----QVKLELEAISTNKFKVRDFSPD-----VF    | 426 |
| E.men_ATCC13253_bla597 SBL                     | -----   | 356 |
| E.ano_Ag1_bla267 SBL                           | -----   | 354 |

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|--|---------------------------------------|-----|
| ACN62070.1 blaADC-41 class                     | -----                                 | 383 |
| ALA14812.1 blaADC-77 class                     | -----                                 | 383 |
| ALA14816.1 blaADC-81 class                     | -----                                 | 388 |
| AIT76094.1 blaDHA-18 class                     | -----                                 | 379 |
| AIT76102.1 blaDHA-19 class                     | -----                                 | 379 |
| AIT76108.1 blaDHA-13 class                     | -----                                 | 379 |
| CCK86742.1 blaCMY-89 class                     | -----                                 | 381 |
| AFK73452.1 blaCMY-81 class                     | -----                                 | 381 |
| AIT76099.1 blaCMY-114 class                    | -----                                 | 381 |
| AKO62865.1 blaCMY-128 class                    | -----                                 | 381 |
| AFK73449.1 blaCMY-80 class                     | -----                                 | 381 |
| AFZ85212.1 blaCMY-96 class                     | -----                                 | 381 |
| AEI70575.1 blaACT-9 cephalosporin-hydrolyzing  | -----                                 | 380 |
| ACJ05689.1 blaACT-5 cephalosporin-hydrolyzing  | -----                                 | 381 |
| AHM76779.1 blaACT-19 cephalosporin-hydrolyzing | -----                                 | 381 |
| E.ano_Ag1_bla622                               | FTNKQYDLALDSYKKAISLGGSNNGNAEKMIDKINLL | 475 |
| E.men_ATCC13253_bla63                          | -----                                 | 415 |
| E.mir_ATCC33958_bla1527 SBL                    | YEFKIMDD--GSIKCNMSQPAHNM-NEEGIKKI---- | 456 |
| E.ano_Ag1_bla2038 SBL                          | YEFKILDD--GSIKCNMSQPAHNM-NEEGIKKI---- | 456 |

E.men\_ATCC13253\_bla597|SBL  
E.ano\_Ag1\_bla267|SBL

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----- 354

## **Discussion**

### **Functional classifications**

A branch of functional studies is underway for full confirmation and classification of our proposed  $\beta$ -lactamases, but is not the primary focus of this study, given that results are only preliminary they have not been included within the confines of this paper.

### **Molecular classification**

As previously mentioned our approach towards confirmation of the extended variety of Elizabethkingia  $\beta$ -lactamases will focus on the molecular classifications of this enzyme family. In order to complete this analysis, an investigation of what makes an A,B,C or D  $\beta$  lactamase was necessary. The first  $\beta$  lactamases classified were the penicillinases from Staphylococcus aureus PC1 [10], Bacillus cereus 569/H [11], Bacillus licheniformis 749/C [12], and Escherichia coli/R6K, R-TEM [13,14,7]. B. cereus identified itself over the rest of the groups of  $\beta$  lactamases by showing a catalytic zinc giving rise to the metallo- $\beta$ -lactamase class, class B [14]. Shortly after the discovery of class B the sequencing of the AmpC gene from E.coli K-12 showed significant differences in sequence and motif structure than the classes previously described. AmpC is specific for the breakdown of cephalosporin as opposed to those of the A and B class [15]. Among the classes of the Serine  $\beta$ -lactamases, (A and C) there seemed to be one final outlier that was discovered by Huovinen et al. [16]. OXA-10 was the first class D  $\beta$ -Lactamase and serves as a reference for our study.



**Table 1** Distinguishing characteristics of the three major molecular classes of serine  $\beta$ -lactamases

| Molecular class | Molecular size (kDa) | Characteristic amino acid motifs   |                          |                                   |                            |                                   | References   |
|-----------------|----------------------|------------------------------------|--------------------------|-----------------------------------|----------------------------|-----------------------------------|--------------|
| A               | $\leq 31$            | <b>S<sup>70</sup>TSK</b><br>(SXXX) | - <sup>a</sup>           | <b>S<sup>130</sup>DN</b><br>(SXN) | <b>E<sup>166</sup>XXLN</b> | <b>K<sup>234</sup>TG</b>          | [20, 23, 24] |
| C               | $> 35$               | <b>S<sup>64</sup>TSK</b><br>(SXXX) | -                        | <b>Y<sup>150</sup>AN</b><br>(YXN) | -                          | <b>K<sup>315</sup>TG</b><br>(KSG) | [18, 25]     |
| D <sup>b</sup>  | $\leq 31$            | <b>S<sup>70</sup>TFK</b>           | <b>S<sup>118</sup>XV</b> | <b>Y<sup>144</sup>GN</b><br>(FGN) | -                          | <b>K<sup>216</sup>TG</b><br>(KSG) | [19, 21, 22] |

The most common motifs are shown in bold; alternative sequences are shown in parentheses, with X representing sequences that can accommodate multiple substitutions

<sup>a</sup> No corresponding motif

<sup>b</sup> Numbering according to Ref. [22]

**Table 1. Provided by Karen Bush et al. served as the guidelines utilized in order to be able to confirm Class A SBLs**

As we classified our largest family of  $\beta$ -lactamases, the Class Cs, there was so much diversity and noise within the sequences, that several subgroups were generated in order to be able to further analyze them based on their phylogenetic grouping. Class C  $\beta$ -lactams as previously mentioned house the cephalosporinases. Class C  $\beta$ -lactams tend to be approximately 360 amino acids in their coding sequences, compared to generally less than 310 amino acids for class A and class D enzymes.

In order for a  $\beta$ -lactamase to be classified as a Serine  $\beta$ -lactamase it is necessary to locate the catalytic serine in a motif with the SerX-X-Lys sequence [15-21]. In addition we located two other conserved sequences across all three classes of  $\beta$ -lactamases as shown in Table 1: (Ser/Tyr)-X-Asn motif and the Lys-(Thr/Ser)-Gly motif. When classifying the Class A SBLs a glutamic acid at or near position 166 is maintained across samples. We only compared one class D enzyme that loosely related to the NCBI database and searched for the conserved sequence of Ser-X-Val, beginning with serine at position 118 [18].

Conservation of motifs across bla sequences give us insight on the dependent factors for the proper function of these units [21, 22]. As we look to further classify these novel blas, a simple way to differentiate Class B MBLs is to sequester the metallic ions and look for inhibition. Luckily through this bioinformatics analysis of these sequences we have narrowed down the list of possible B class MBLs and further hones in on the classifications of the A, C and D classes. Now within the class of MBLs there is further subdivisions (eight in total) due to the variance of catalysis around and in conjugation with the zinc ion [23,24]. An interesting note within the structural analysis of the MBL subclass B1 and B3 is the fact that they need two  $Zn^{2+}$  ions to complete hydrolysis of b-lactams. In contrast, the members of subclass B2 more efficiently distribute the force  $Zn^{2+}$  provides by using a singular  $Zn^{2+}$  25.

**Table 2** Zinc ligands in the four subclasses of class B metallo- $\beta$ -lactamases

| B subclass | Typical enzymes                           | Substrate preference                    | Number of active site Zn <sup>2+</sup> atoms | Consensus amino acids at active site |                            | References   |
|------------|---|---|--|--------------------------------------|----------------------------|--------------|
|            |   |   |  | Ligands to Zn1                       | Ligands to Zn2             |              |
| B1a        | BCII, IMP family, VIM family, SPM-1, CcrA | All $\beta$ -lactams except monobactams | 2  | His116<br>His118<br>His196           | Asp120<br>Cys221<br>His263 | [27, 28]     |
| B1b        | NDM family <sup>a</sup>                   | All $\beta$ -lactams except monobactams | 2  | His116<br>His118<br>His196           | Asp120<br>Cys221<br>His263 | [29]         |
| B2         | CphA, Sfh-1                               | Carbapenems                             | 1  | NA <sup>b</sup>                      | Asp120<br>Cys221<br>His263 | [27, 28, 31] |
| B3         | L1, FEZ-1, CAU-1                          | All $\beta$ -lactams except monobactams | 2  | His/Gln116<br>His118<br>His196       | Asp120<br>His121<br>His263 | [27, 28]     |

<sup>a</sup> Defined to be in a new subclass of B1 because of low sequence homology between NDM-1 and other B1 MBLs [10]

<sup>b</sup> Not applicable; binding of Zn in this site results in enzyme inhibition of the CphA MBL [11]

## **Table 2. Classifies the subclasses of Class B MBLs**

**Table 3** Alignment of  $\beta$ -lactamase classification schemes, based on Refs. [12, 13], using arbitrary hydrolysis and inhibition parameters as activity assessments

| Molecular class | Functional group | Penicillinase activity <sup>a</sup> | Cephalosporinase activity <sup>b</sup> | ESBL activity <sup>c</sup> | Carbapenemase activity | Monobactamase activity | Clavulanic acid sensitive <sup>d</sup> | Representative enzymes    | References        |
|-----------------|------------------|-------------------------------------|--|----------------------------|------------------------|------------------------|--|---------------------------|-------------------|
| A               | 2a               | Y                                   | N                                      | N                          | N                      | N                      | Y                                      | PC1                       | [12, 37]          |
|                 | 2b               | Y                                   | Y                                      | N                          | N                      | N                      | Y                                      | TEM-1, SHV-1              | [12, 38]          |
|                 | 2be              | Y                                   | Y                                      | Y                          | N                      | Y                      | Y                                      | CTX-M-14, -15             | [38]              |
|                 | 2br              | Y                                   | Y                                      | N                          | N                      | N                      | N                                      | TEM-30, SHV-10            | [39, 40]          |
|                 | 2ber             | Y                                   | Y                                      | Y                          | N                      | <u>Y</u>               | <u>Y</u>                               | TEM-50, TEM-121           | [41, 42]          |
|                 | 2c               | Y                                   | N                                      | N                          | N                      | N                      | Y                                      | PSE-4, CARB-3             | [12, 43]          |
|                 | 2cc              | Y                                   | N                                      | <u>N</u>                   | N                      | N                      | Y                                      | RTG-4                     | [44]              |
|                 | 2e               | Y                                   | Y                                      | Y                          | N                      | V                      | Y                                      | SFO-1, FEC-1, L2          | [45, 46]          |
|                 | 2f               | Y                                   | Y <sup>f</sup>                         | Y                          | Y                      | Y                      | Y                                      | KPC-2, SME-1 <sup>f</sup> | [47]              |
|                 | B                | 3a <sup>e</sup>                     | Y                                      | Y                          | Y                      | Y                      | N                                      | N                         | IMP, VIM, NDM, L1 |
| 3b              |                  | <u>Y</u>                            | N                                      | N                          | Y                      | N                      | N                                      | CphA                      | [48]              |
| C               | 1                | N                                   | Y                                      | N                          | N                      | N                      | N                                      | AmpC, ACT-1               | [38, 49]          |
|                 | 1e               | N                                   | Y                                      | Y                          | N                      | N                      | N                                      | GC1, CMY-37               | [50, 51]          |
| D               | 2d               | Y                                   | N                                      | N                          | N                      | N                      | V                                      | OXA-1, OXA-10             | [12, 52]          |
|                 | 2de              | Y                                   | Y                                      | <u>Y</u>                   | N                      | N                      | V                                      | OXA-11, OXA-15            | [53]              |
|                 | 2df              | Y                                   | N                                      | Y                          | <u>N</u> <sup>h</sup>  | N                      | <u>N</u>                               | OXA-23, OXA-48            | [54, 55]          |

Activity data that are inconsistent with the published distinctive substrate profiles are shown in bold and underlined

<sup>a</sup> Y =  $k_{cat} > 5 \text{ s}^{-1}$ ; N =  $k_{cat} < 5 \text{ s}^{-1}$ ; V = variable within the functional group

<sup>b</sup> Hydrolysis of cephaloridine or cephalothin

<sup>c</sup> Based on hydrolysis of cefotaxime, ceftazidime, or cefepime

<sup>d</sup> Y =  $IC_{50} < 2 \mu\text{M}$ ; N  $\geq 2 \mu\text{M}$ ; V = variable among the functional group

<sup>e</sup> In spite of  $k_{cat}$  values generally  $\leq 1 \text{ s}^{-1}$ , resistance to cefepime and ceftiprome is seen in producing organisms

<sup>f</sup> SME enzymes compared to KPC carbapenemases have lower hydrolysis rates and lower catalytic efficiencies for expanded-spectrum cephalosporins, resulting in clinical susceptibility to those cephalosporins in SME-producing *S. marcescens* [47, 56]

<sup>g</sup> Includes subclasses B1 and B3

<sup>h</sup> In spite of  $k_{cat}$  values generally  $\leq 1 \text{ s}^{-1}$ , these enzymes confer resistance to carbapenem

**Table 3. Shows key substrates that will be implemented to measure functionality including penicillins (benzylpenicillin, carbenicillin, and cloxacillin), early cephalosporins (cephaloridine or cephalothin) in contrast to expanded-spectrum cephalosporins (cefotaxime or ceftazidime), the monobactam aztreonam, and carbapenems (usually imipenem)**<sup>7</sup>.

## **Conclusions & Future Directions**

As can be observed by the conserved motifs and branchings of our phylogenetic studies, Elizabethkingia has developed itself as a genus to be respected and extensively researched. Not only does it putatively house an extensive variety of antibiotic resistance factors, it is a goldmine for  $\beta$ -lactamases. Reiterating what was stated at the beginning of this paper, it is extremely important to monitor organisms like Elizabethkingia as we are actively observing them react to the extreme evolutionary pressure exerted upon them by humanity and our antibiotic dependence.

Through the completion of this study, we have classified several candidate  $\beta$ -lactamases through comparative molecular classifications and have left the door open for future functional classification to be more narrowed and efficient. We have separated a total of 6 Class A Serine B-Lactamases, 14 Class B Metallo-B-Lactamases, 20 Class C Serine-B-lactamases and 1 putative Class D Serine B-lactamase. Extensive motif annotation and identification for our Class C and A groups proved to further cement our claims. Our goal is to be able to further correlate phenotype with genotype in order to be able to have a full confirmation of our findings and submit this data to the National Institutes of Health. This research will help clinicians and researchers alike in the fight against novel super bugs and the development of treatments for those affected by them.

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