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Evolutionary and Secondary Structure of Multi Drug Resistance Class 1 Integron from Gram Negative Bacteria

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Abstract: The study evaluated the evolutionary relationship and secondary structure of class 1 integron coded protein in order to infer evolutionary ancestry and understand their secondary structure possibly as a potential drug target. The prediction of the secondary structure of the class 1 integron coded protein was carried out using SOPMA tool. Results of the NCBI queries revealed significant identity with class 1 integron of the studied Gram negative bacteria. The nucleotide sequence alignment depicted several conserved regions with varying degree of transitions, transversions, insertions and deletions while the amino acid sequences of the nucleotides shows 64 conserved sites among all the sequences. The isolates contained comparatively higher random coil, alpha helix and extended strands in that order of sequence than the beta turns which were present in less percentages ranging from 8.56-11.48%. In conclusion, this study confirmed significant evolutionary relationship among the studied class 1 integron while higher representation of the random coil was observed than any other protein structures.

Key words: Evolutionary · Protein structure · Class 1 integrons · Gram negative Bacteria

INTRODUCTION

Integrons are genetic elements that contain the component of a site-specific recombination system that recognizes and captures mobile gene cassettes [1]. The basic structure of an integron possesses a gene for an integrase (intI), a recombination site (attI) and a promoter (P_C) that permits the expression of the gene cassettes incorporated in the variable region [2]. These bacterial genetic elements allows the shuffling of smaller mobile elements called gene cassettes and so they are called genetic construction kit for bacteria [3]. They usually harbor antibiotic resistance genes and hence play a vital role in the emergence of new multidrug resistant bacteria [4]. This is because they equip bacteria with the ability to scavenge foreign genes, especially antibiotic resistance genes. According to Nield *et al.*[5], subjection of antibiotic resistance gene cassettes to episodic selection lead to removal of unnecessary genes through excision events catalyzed by integrase which subsequently results in reduction of genetic burden.

Integrons by themselves are not mobile [6, 7], but they may be part of mobile elements like transposons and plasmids [8, 9] which further enhance the spread of antibiotic resistance genes. Large conjugative plasmids harbouring both class 1 and class 2 integrons have been reported from *Salmonella* [10]. There are at least eight classes of integrons [11, 12], but those found in clinical isolates belong to four main classes according to their integrases and associated cassettes [13, 14]. Further classification attempts, as well as some of the evolutionary history of integrons, have recently been reviewed [14]. Class 1 integrons have been described as the main mobilizers of antibiotic-resistance genes amongst enteric bacteria.

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Gram-negative enteric bacteria (GNEB) are common causes of nosocomial infection, infecting nearly every organ and body cavity while wide spread use of antibiotics to treat GNEB infections has led to the emergence of multi-drug resistant GNEB and subsequently increase cost of treatment [15, 16]. Drug resistance on its own, is a large and growing problem in infection control due to the increase in the proportion of death as well as the cost of effective antimicrobial agents [15, 16].

In GNEB, different classes of integrons have been identified in several clinical isolates, where they contribute significantly to the prevalence and dissemination of antibiotic resistance genes [11, 12]. This study examined the evolutionary relationship and the secondary structure of class 1 integron coded protein from different clinical enteric Gram negative bacteria.

MATERIALS AND METHODS

In Silico: The sequences JN108891, AB161449, AY069972, NF915770, AB186122 and DQ402098 were obtained using nucleotide BLAST (http:// blast.ncbi.nlm.nih.gov/ Blast.cgi) [17] and subjected to evolutionary analysis using the MEGA explorer [18]. Pairwise distances were calculated using Kimura 2 parameter. In MEGA explorer, translate option was used for converting the gene sequence into amino acid sequence. Sequence similarity search with BLASTP and best homologous protein was found using the multiple sequence alignment. Secondary structure of the protein sequences were predicted using SOPMA tool [19].

RESULTS

The phylogenetic analysis of the class one integron of GNEB retrieved from NCBI gene bank revealed significant evolutionary relationships despite being from different enteric organisms. As shown in Fig.1 below, all the retrieved class 1 nucleotide sequences shared several conserved regions except in few organisms with lesser levels of conserved regions as observed in a strain of *Citrobacter freudii* (DQ402098) and *Serratia marcescens* (AY069972) , both of which form a simplicifolious clade as an indication that the distribution of their nucleotide sequence are substantially different from the distribution in the remaining chunks. In terms of relatedness of their class 1 integrons, organisms in chunk 3 are more closer than those in chunk 1 and 2 . Although, organisms on chunk 3 shared higher level of homology in their nucleotide sequences, their level of resemblance was different according to the different clades, as organisms on clade 1 are closer than those on clade 2. The secondary structure of the class 1 integron coded protein from the different isolates also depicted significant representation of the random coil, alpha helix and the extended strands in that order of sequence than the beta turns that ranged between 8.56-11.48%.

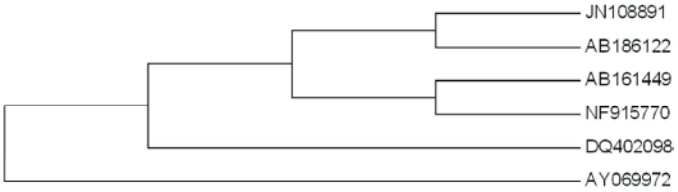
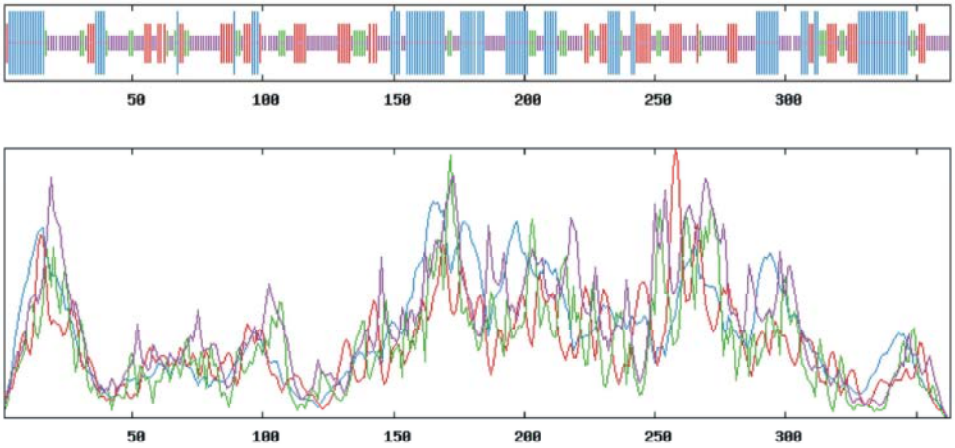


Fig. 1: Phylogenetic relationship among the studied class 1 integron

10 20 30 40 50 60 70
| | | | | | |
DVCYGAATMLRSRAVALKQSPLRKNCETITNGSYIEEWSYREWPYSMECQRTAPVSYYLPMAVGWTQDFI
heehhhhhhhhhhhhhhhtccccccccccccttceeehhhhtcccccccttcccceeeccceeetccthee
NGSITQPKVCGRNTFKFYIQERIDLSINRCFNQPKENNGSCHCFRWWGDIQKPDRSSRYTTYIYNRHRAG
ttccccccccccceeeeehttceeehhheccccctttcccceeeeeccccccccccccceeeeectttttt
RCLLSNPQQFASFYPRLRLHKLLPNLAKGLTSGSNGFANLSRLLYQKPRQVCDPLCQALRLHENRTLRSE
ceeeccccchhhhcchhhhhhhhhhhhhhhhcttcchhhhhhhhhhcccccccchhhhhhhhhtttccchh
CTMDRKNEMPKPKVWEGNSCLAIERHERQLPAFFLYLLECNPRAGPCITVKNQSRALGSNSSNSSRLPLR
hhhctttccccceettcceeehhhhhtccchheeeeeeccttccceeeeeccccccetccccccccccee
GVCSWCKPKVSELQDRVRAPMGCSEKVECTFYANLGWFLLDSRAVFVSMHWFTKILVAFVHKCLNKFRQG
eeccccccchhhhhhhhhcccccccchhheehhtttteeeecttceeeehhhhhhhhhhhhhhhhhhhctt
TLLTSRPCKRMHA

ceeeccccccccc
Sequence length: 363
SOPMA:
Alpha helix (Hh): 106 is 29.20%
3_{10} helix (Gg): 0 is 0.00%
Pi helix (Ii): 0 is 0.00%
Beta bridge (Bb): 0 is 0.00%
Extended strand (Ee): 69 is 19.01%
Beta turn (Tt): 41 is 11.29%
Bend region (Ss): 0 is 0.00%
Random coil (Cc): 147 is 40.50%
Ambiguous states (?): 0 is 0.00%
Other states: 0 is 0.00%

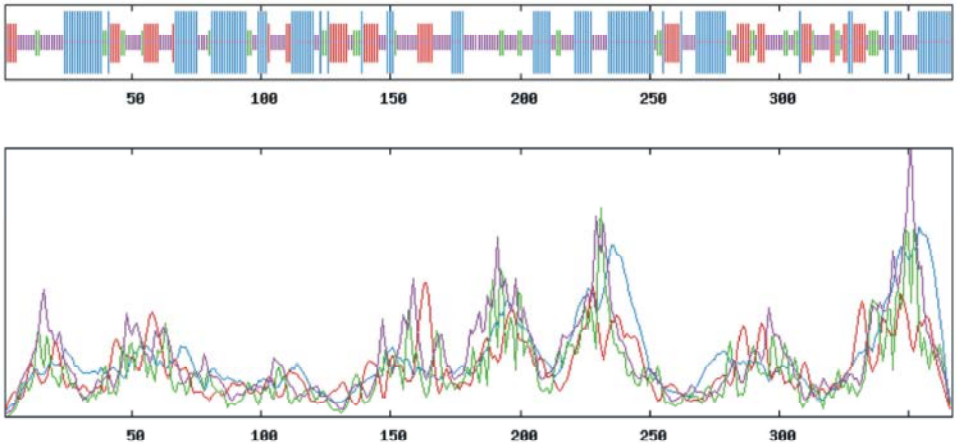


Parameters:
Window width: 17
Similarity threshold: 8
Number of states: 4

Fig. 2: Chromatographical representation of the secondary structure of JN108891

10 20 30 40 50 60 70
| | | | | | |
RCLMLWSSNDVTQQGSRPKTKLTSEEELNYHWLYRRMELSGMALIFHGVPKVNSSCLKLLPITNGCWLDA
heeeeeccccccctttccccccccchhhhhhhhhhhhhhhhttheeeettcccccteeeeeeccccchhh
RLLNQWEHYPTESMRSHVQVLHLTMRTYSFHQLKMLPTRKRIMSLFQVVGRYTKASIKIHYIYLQTSSRK
hhhhhhccccthhhhhhhhhhhhhhttcchhhhecccccceehhhhhhhhhcchttheeeeeeecttttth
VMFTFLKSPAILGQFLPKTSPLTIIVTKSGKRVNKWQQRIRKPVTPFVPKAAPGLRSAVPGVKATKSYIT
eeeeeeccchhhtcccccccceeeeeeccccccchhhhhcccccccccccctttccccttcccchhhhh
KRMHHGEENAQTQSVGRKLLPGNRAAATVARIFSVILARMSIESRTMHYCMKMKSIKSSWIEQQLFQIAL
hhccttccccchhhhhhhccccchhhhhhhhhhhhhhhhhhhcttheeeeeehccccchhhhhhhhhhhh
AGVGLFLVQTQSVRVARPSTCTNGMQKGMYLLCQSWMVPAFEIGCIRMKYALVLNQNTGGVCSQMPQVAR
ttcceeeeettceeeccccccctttcctttheeeetcccccceettceehheeeeectttttcchhcchhhcc
DAPDVAPLLKALDALST
cccchhhhhhhhhhhh
Sequence length: 367
SOPMA:
Alpha helix (Hh): 131 is 35.69%

3_{10} helix (Gg): 0 is 0.00%
Pi helix (Ii): 0 is 0.00%
Beta bridge (Bb): 0 is 0.00%
Extended strand (Ee): 65 is 17.71%
Beta turn (Tt): 39 is 10.63%
Bend region (Ss): 0 is 0.00%
Random coil (Cc): 132 is 35.97%
Ambiguous states (?): 0 is 0.00%
Other states: 0 is 0.00%

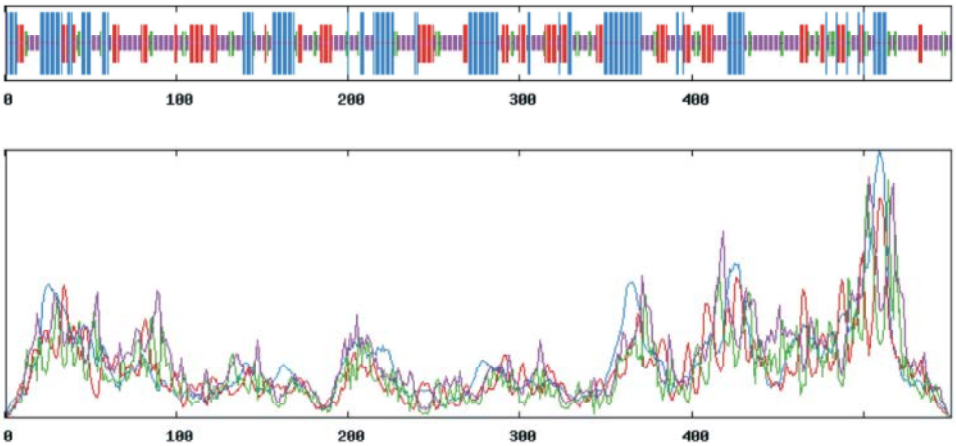


Parameters:
Window width: 17
Similarity threshold: 8
Number of states: 4

Fig. 3: Chromatographical representation of the secondary structure of AB186122

10 20 30 40 50 60 70
| | | | | | |
FGCEQLCACIRFQGRDVRSVPCLNLLRHLTNATSILVNQCILHTNTAYLESSRNHPRLAKVHSTFSLHPI
cchhhhhheeeettccccccchhhhhhhhhhhhheeehhheectthhhhhhcccccthhhhcceeeeccc
GARTRSCNSDTLGLHQGTNLPPQGQSGRSCCYSDPRALDFHFHTVMHGPALYGLHSSKYHRKNAGNCRSC
ccccccccceeeecttccccccccccceeccctcceeeeeeeeccccceeeeccccccctttcccccch
RSIARQEFPSHTLGLGISFFLSMVHSLRNVRFSCSLNAWHSGSQTWRGFWYKRRDRFANPLLPLVNPFAR
hhhhhtccccccetcchhhhhhhhhhhhhhcteeeccccccttcceeeeeeeccccccccthccccchhh
FGNYNLCRRSLGKLANCWGFQESKHHLPARCLLIYVVYLLDRSGFCISPHHLKQHDPLFSLGWLKHLLME
ccccchhhhhhhhhhhhttccccccccchheeeeeeeeecttttccccccccccccceeehhhhhhhhhh
RSIRSHCQMNLNVLRPHTFGWVMLPLIQKSCVQPTAIGYRLTGAVHLWHSMEYQGHSRLHSSILPLVIVS
hhhhhhhhcceeeecttccceeeechhccccccteeeeeeecheeeehhhcctttccccttccceeeeh
QFFLRVLNCFRATALLRNIVAAPHQTSTHGVTRLLLGCPRHRLYKKTVITSHENRHCAVTTAAFGQDSGP
hhhhhhhhhhhhhhhhhhhhhhcccccccctteeeeeeccccchhccheeeeccttteeeeeeeeccccccc
VAAHTLLALQLTNRTGLCPLGSCLHPFPRCASPGNLGQQRSRGISVLAGERAQGFGLHATSGIGGLAVLL
chhhhhhhhhhccttccccccccccccccccctttccccccccceeeeetcccttceeehcttttheeeee
RQGAVHGSALASGDRKTSAVAALAGGADPGSGSHPRFSGRRASFVRPASVWNGHADQGFAT
httcccchheehttcchhhhhhhhhttccttcccccccccccceeeccccccccccctttccc
Sequence length: 551
SOPMA:
Alpha helix (Hh): 141 is 25.59%

3_{10} helix (Gg): 0 is 0.00%
Pi helix (Ii): 0 is 0.00%
Beta bridge (Bb): 0 is 0.00%
Extended strand (Ee): 112 is 20.33%
Beta turn (Tt): 57 is 10.34%
Bend region (Ss): 0 is 0.00%
Random coil (Cc): 241 is 43.74%
Ambiguous states (?): 0 is 0.00%
Other states: 0 is 0.00%

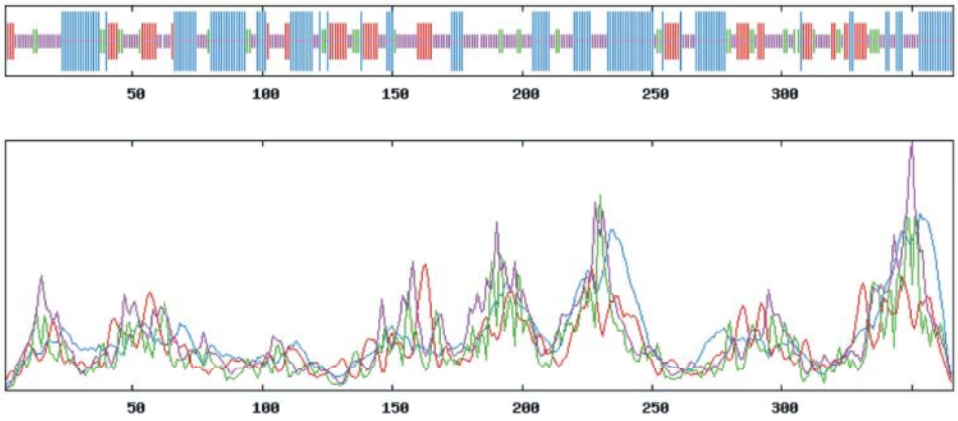


Parameters:
Window width: 17
Similarity threshold: 8
Number of states: 4

Fig. 4: Chromatographical representation of the secondary structure of AB161449

10 20 30 40 50 60 70
| | | | | | |
CLMLWSSNDVTQQGSRPKTKLTSEEELNYHWLYRRMELSGMALIFHGVPKVNSSCLKLLPITNGCWLDAR
eeeeeccccccccttccccccccchhhhhhhhhhhhhhhhttheeeettcccccteeeeeeccccccehhhh
LLNQWEHYPTESMRSHVQVLHLTMRTYSFHQLKMLPTRKRIMSLFQVVGRYTKASIKIHYIYLQTSSRKV
hhhhhccccthhhhhhhhhhhhhhttcchhhheccccccceehhhhhhhhhhcchttheeeeeeecctttthe
MFTFLKSPAILGQFLPKTSPLTIIVTKSGKRVNKWQQRIRKPVTPFVPKAAPGLRSAVPGVKATKSYITK
eeeeeccchhhtcccccccceeeeeeccccccchhhhhcccccccccccccttcccccttcccchhhhhh
RMHHGEENAQTQSVGRKLLPGNRAAATVARIFSVILARMSIESRTMHYCMKMKSIKSSWIEQQLFQIALA
hccttcccchhhhhhhcccccchhhhhhhhhhhhhhhhhhhcttheeeeeehccccchhhhhhhhhhht
GVGLFLVQTQSVRVARPSTCTNGMQKGMYLLCQSWMVPAFEIGCIRMKYALVLNQNTGGVCSQMPQVARD
tcceeeeettceeeccccccttccttheeeetccccccceettceehheeeeectttтcchhcchhhccc
APDVAPLLKALDALST
ccchhhhhhhhhhhh
Sequence length: 366
SOPMA:
Alpha helix (Hh): 130 is 35.52%
3_{10} helix (Gg): 0 is 0.00%
Pi helix (Ii): 0 is 0.00%
Beta bridge (Bb): 0 is 0.00%
Extended strand (Ee): 65 is 17.76%

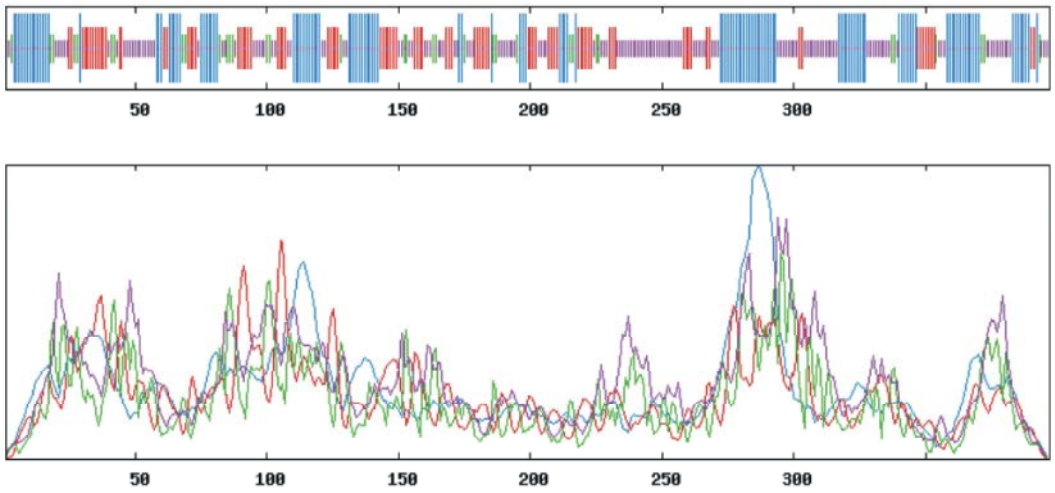
Beta turn (Tt): 39 is 10.66%
Bend region (Ss): 0 is 0.00%
Random coil (Cc): 132 is 36.07%
Ambiguous states (?): 0 is 0.00%
Other states: 0 is 0.00%



Parameters:
Window width: 17
Similarity threshold: 8
Number of states: 4

Fig. 5: Chromatographical representation of the secondary structure of NF915770

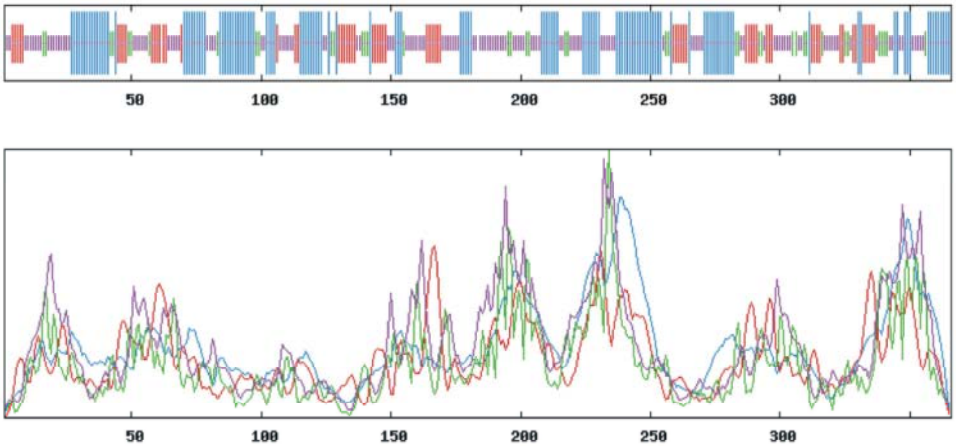
10 20 30 40 50 60 70
| | | | | | |
ALRRGSMFDVMEQQRCYAAGQSPNKVNLGRIVKLSLMVAISKNGVIGNGPDIPWSAKGEQLLFKAITYNQ
hccthhhhhhhhhhhhhhttccccceettheeeeeeeeeecttceeccccccccccccchhheehhhhhtt
WLLVGRKTFESMGALPNRKYAVVTRSSFTSDNENVLIFPSIKDALTNLKKITDHVIVSGGGEIYKSLIDQ
eeeechhhhhhhtcctttceeeeeeccccctttceeeeecchhhhhhhhhhhhcceeeeetcchhhhhhhhh
VDTLHISTIDIEPEGDVYFPEIPSNFRPVFTQDFASNINYSYQIWQKGQVAATDSQTCHAFCTKSRARFA
hhheeeeeeeccttceeeeeccttccceeecchhtccceeeeeehttccccccthhheeeeccccceee
IRCARRGYMKIVHYEANAPWIGRMKCPNPKCGKETPAWQSSGMSDSCPHFFCDTCSNVIHREQDHALLYE
ehhhhttheeeeeecttccceeeccccccccccccccccccccccccccceeeeccccceeccchhhhhhhh
NEINQELLDRIAATLPDCPCGGRFVPGANPKCPSCKTEYVHQWDAVKRLNVPFMPILDGSCLIRDRLYSY
hhhhhhhhhhhhhhhcccccccceeccccccccccccchhhhhhhhhhhhcccccccccttchhhhhhheee
EVCIGSKPKYWWRLFTNALTSLGKGRSRRAPAKSVRCTKHIIAHSQT
eeeetcchhhhhhhhhhhhhttcccccccccchhhhhhheehtccc
Sequence length: 397
SOPMA:
Alpha helix (Hh): 126 is 31.74%
3_{10} helix (Gg): 0 is 0.00%
Pi helix (Ii): 0 is 0.00%
Beta bridge (Bb): 0 is 0.00%
Extended strand (Ee): 89 is 22.42%
Beta turn (Tt): 34 is 8.56%
Bend region (Ss): 0 is 0.00%
Random coil (Cc): 148 is 37.28%
Ambiguous states (?): 0 is 0.00%
Other states: 0 is 0.00%



Parameters:
Window width: 17
Similarity threshold: 8
Number of states: 4

Fig. 6: Chromatographical representation of the secondary structure of DQ402098

10 20 30 40 50 60 70
| | | | | | |
RGARGLMLWSSNDVTQQGSRPKTKLTSEEELNYHWLYRRMELSGMALIFHGVPKVNSSCLKLLPITNGCW
ttcceeeeeccccccctttccccccccchhhhhhhhhhhhhhhhttheeeettcccccteeeeeeccccce
LDARLLNQWEHYPTESMRSHVQVLHLTMRTYSFHQLKMLPTRKRIMSLFQVVGRYTKASIKIHYIYLQTS
hhhhhhhhhcccctthhhhhhhhhhhhhhhttcchhhhecccccceehhhhhhhhhhcchtttheeeeeeecct
SRKVMFTFLKSPAILGQFLPKTSPLTIIVTKSGKRVNKWQQRIRKPVTPFVPKAAPGLRSAVPGVKATKS
ttheeeeeeccchhhtccccccccceeeeeeccccccchhhhhcccccccccccccttcccccttcccchh
YITKRMHHGEENAQTQSVGRKLLPGNRAAATVARIFSVILARMSIESRTMHYCMKMKSIKSSWIEQQLFQ
hhhhhccttcccchhhhhhhcccccchhhhhhhhhhhhhhhhhhhcttheeeeeehccccchhhhhhhhh
IALAGVGLFLVQTQSVRVARPSTCTNGMQKGMYLLCQSWMVPAFEIGCIRMKYALVLNQNTGGVCSQMPQ
hhhttcceeeeettceeeccccccccttcctttheeeetcccccceettceehheeeeectttttcchhcchh
VARDAPDVAPLLKAMH
hccccchhhhhhhhh
Sequence length: 366
SOPMA:
Alpha helix (Hh): 126 is 34.43%
3_{10} helix (Gg): 0 is 0.00%
Pi helix (Ii): 0 is 0.00%
Beta bridge (Bb): 0 is 0.00%
Extended strand (Ee): 65 is 17.76%
Beta turn (Tt): 42 is 11.48%
Bend region (Ss): 0 is 0.00%
Random coil (Cc): 133 is 36.34%
Ambiguous states (?): 0 is 0.00%
Other states: 0 is 0.00%



Parameters:
Window width: 17
Similarity threshold: 8
Number of states: 4

Fig. 7: Chromatographical representation of the secondary structure of AY066972

DISCUSSION AND CONCLUSION

The use of protein structure and nucleotide sequences to understand evolution of cell or the function of genes cannot be overemphasized. According to Zuckerkandl and Pauling [20], evolutionary relationships between organisms can be studied by comparing their DNA sequences. In this study, a very high level of similarities was observed in the DNA sequences of the studied class 1 integron from different enteric bacteria. This is an indication that class I integron may have a common ancestors with most of them probably being transferred as an inserts in plasmid and/or transposons [20]. The fact that two organisms namely *Citrobacter freundii* and *Serratia marscencens* connoted themselves differently on a simplicifolious clade is an indication that the distribution of their nucleotide sequence are substantially different from that of the other organisms in the remaining chunks. However, such observation may not be unconnected to the fact that such acquired class I integron has rearranged due to different levels of mutation resulting from substitution in form of transition, tranversion, deletion and even insertions. In determining the secondary structure of the class 1 integrons, the sequences obtained were analyzed using bioinformatic tools and queried against the nucleotide database using the BLAST algorithm [17]. The BLAST analysis revealed homology with the available class 1 integrons of Gram negative enteric bacteria gene sequence in the Genbank. The prediction of secondary structure of the Gram negative enteric bacteria of class 1 integron coding gene was carried out using SOPMA. The isolates contain comparatively higher random coil, alpha helix and extended strands than beta turns which are present in less percentages of 8.56-11.48%. This knowledge is important as it helps us design low-molecular-weight synthetic agents that reproduce their essential features [21] by using synthetic agents to mimic the helices. This also in particular, has immense interest in drug discovery because of the central role that the a-helical regions play in many biological processes. Again, information about the amino acid residues in the class 1 integron coded protein is needed to study the binding of target drugs in these regions. This is because changes in these residues results in the modification of drug binding sites. In conclusion, this study confirmed common evolutionary ancestors for the class 1 integrons and the high representation of random coil, alpha helix and extended strands in that of sequence for the class 1 integron coded proteins.

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