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META ANALYSIS OF LACTONASES FOR IDENTIFICATION OF CONSERVED SEQUENCES USING BIOINFORMATICS TOOL

Y.Aparna
Department of Microbiology
Bhavan's Vivekananda College
Sainikpuri,Secunderabad – 500094,
Telangana,India

J.Sarada
Department of Microbiology
Bhavan's Vivekananda College
Sainikpuri,Secunderabad – 500094,
Telangana,India

Abstract: Meta analysis is gaining importance as it is used to combine and study the data from various sources employing standard statistical methods. With the advances in sequencing analysis the data pertaining to conserved sequences in a protein, enzyme or DNA has become enormous which can be identified by using bioinformatics tool with multiple sequence analysis. This approach was used in present study to find the conserved sequences and similarity matching of an enzyme Lactonase from different strains of Enterobacter cloaceae.

Lactonase is highly specific enzyme active against AHLs which are signal molecules produced by quorum sensing mechanism and known to control the pathogenicity of infectious bacteria. This enzyme hydrolyses the lactone ring to produce corresponding acyl homoserines and reduces its activity . Lactonase is proved to be potent enzyme in degrading AHL's and thus find applications in controlling infections caused to humans, plants & animals.

Lactonase were commonly found in many gram positive Bacillus species like Bacillus mycoides, Bacillus thuringenesis and Bacillus cereus. However, AHL lactonases were also found in gram negative bacteria like Enterobacter cloaceae. MSA (multiple sequence alignment analysis) with smart Blast was carried out to find significant conserved sequences of Lactonase among different bacterial populations. Smart blast analysis was done for 13 different bacterial samples and lactonase enzyme was found to exhibit a minimum of 98% similarity in sequences which explains that the conserved regions are stable in almost all the members of Enterobacter cloaceae sps. The results indicate the existence of a mechanism which blocks the signalling circuit in bacterial species by exhibiting different modes of action.

Keywords: Lactonases, Meta analysis ,Smart blast analysis, Conserved sequences, Quorumquenching

I. INTRODUCTION

Meta analysis is a statistical procedure which is used to combine the data from different studies. This approach combines, summarises and review the qualitative and quantitative data from various selected studies which can be summarized and analyzed to draw conclusions. Meta analyses are systematic reviews which attempts to gather empirical evidence to answer specific research questions (1).

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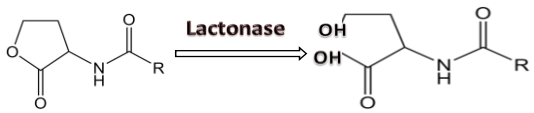
Quorum quenching N-acyl homserine lactonase also known as AHL Lactonase, belongs to family of hydrolases with EC 3.1.1.81. This enzyme catalyzes the hydrolysis of lactone rings and open HSL rings of AHL(Figure 1). Such reaction mechanisms could block quorum sensing an important signalling mechanism in expression of virulence characters in pathogens. These enzymes belong to MBL fold metallohydrolase superfamily which comprises of group of hydrolytic enzymes which are known to carry a wide variety of biological functions.



The above figure illustrates the degradation mechanism of Lactonase enzyme

Figure 1:Mode of action of AHL Lactonase enzyme

In the present study an attempt was made to know the conserved AHL Lactonase sequences from Enterobacter sps.AHL Lactonases are enzymes that interfere with quorum sensing circuits in bacteria (2).This phenomenn of interference is known as quorum quenching. A meta analysis on AHL lactonanses conserved in Enterobacter cloaceae sps was performed by bioinformatics tool using BLASTP.

II. MATERIALS AND METHODS:

QSI activity of Enterobacter cloaceae:
Organism exhibiting QSI activity was isolated by enrichment culture technique, characterized by 16s rRNA analysis and quorum quenching activity was confirmed by LCMS analysis.(3)

Research papers published on AHL Lactonases sequencing are identified by computerized searching electronic databases. This includes Pubmed,Science direct, Scirus, ISI web of Knowledge and google scholar(4,5,6,7,8)

Protein clusters: Reference protein sequences for AHL Lactonases fromEnterobacter cloaceae were identified using NCBI Entrez protein cluster database(9).

III. SMART BLAST Analysis: Simple Modular Architecture Research Tool is used for identification of similar and conserved domains in various organisms (10).

Accession numbers of Enterobacter cloaceae AHL Lactonase used in study: Total 13 sequences available inprotein cluster database used in the study were WP_063156506.1, WP_059306672.1,WP_050736909.1, WP_020882395.1, SAE94573.1, CZU82041.1, AHE72938.1, AKM87870.1, WP_047955721.1, WP_025203943.1,WP_014170421.1 AEW73993.1,EPR39490.1(4)

IV. Results and Discussion:

Smart Blast analysis was done for 13 different Enterobacter cloaceae sps producing AHL Lactonases. Multiple sequence alignment was carried out using blastp and looked for % of similarity. (Figure 2)

Sequences producing significant alignments:

Select: All None Selected:12

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

Description	Max score	Total score	Query cover	E value	Ident	Accession
N-acyl homoserine lactonase AttM [Enterobacter cloacae]	550	550	100%	0.0	100%	CZU82041.1
MBL fold metallo-hydrolase [Enterobacter cloacae]	547	547	100%	0.0	99%	WP_025203943.1
beta-lactamase [Enterobacter cloacae P101]	547	547	100%	0.0	99%	AHE72938.1
N-acyl homoserine lactonase AttM [Enterobacter cloacae EcWSU1]	544	544	100%	0.0	98%	AEW73993.1
MBL fold metallo-hydrolase [Enterobacter cloacae]	544	544	100%	0.0	98%	WP_014170421.1
MULTISPECIES: N-acyl homoserine lactonase family protein [Enterobacter cloacae complex]	544	544	100%	0.0	98%	WP_059306672.1
beta-lactamase domain protein [Enterobacter cloacae str. Hanford]	543	543	100%	0.0	98%	EPR39490.1
MULTISPECIES: N-acyl homoserine lactonase family protein [Enterobacter cloacae complex]	543	543	100%	0.0	98%	WP_020882395.1
N-acyl homoserine lactonase AttM [Enterobacter cloacae]	543	543	100%	0.0	99%	SAE94573.1
MULTISPECIES: N-acyl homoserine lactonase family protein [Enterobacter cloacae complex]	543	543	100%	0.0	99%	WP_050736909.1
MBL fold hydrolase [Enterobacter cloacae]	540	540	100%	0.0	98%	WP_047955721.1
beta-lactamase [Enterobacter cloacae]	540	540	100%	0.0	98%	AKM87870.1

Figure 2: Multiple sequence alignment of Enterobacter cloaceae sps

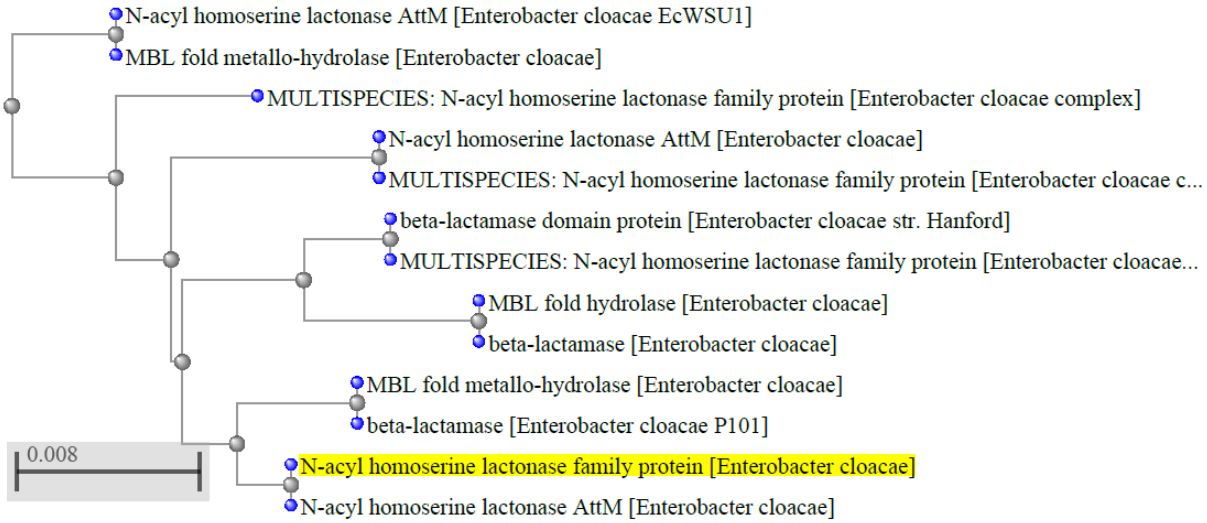


Figure 3:Phylogenetic analysis ofHLLactonase in Enterobacter sp

Claudogram depicts that AHL lactonases exhibit a minimum of 98% similarity in sequences which explains that the conserved regions/ domains are more common in all the members of Enterobacter cloaceae sps.

Smart blast analysis was done among various bacteria with Enterobacter cloacae N-acyl homoserine lactonase family protein with accession number WP_063156506.1 as quiery sequence.

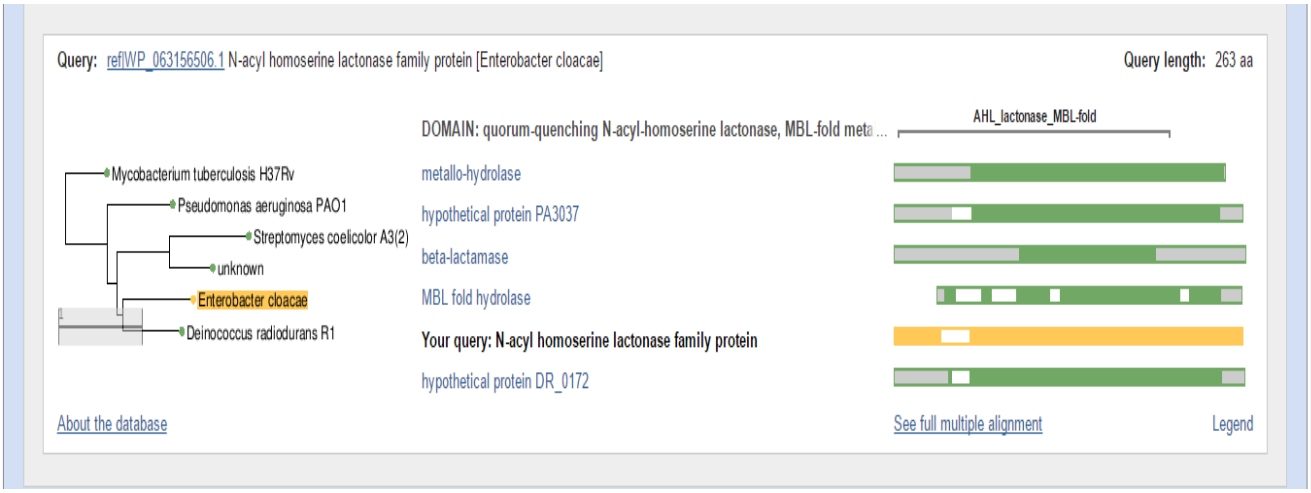


Figure 4:Smart blast analysis ofenterobacter AHLLactonase sequences

Smart blast analysis showed that N-AHL lactonase enzyme of Enterobacter cloaceae showed high sequence conservation with five different enzymes namely matallohydrolase isolated from Mycobacterium tuberculosis H37RV, beta lactamase from Streptomyces coelicolor A3(2), A hypothetical protein PA3037 from Pseudomonas aeruginosa PAO1 and MBL fold hydrolase from Methanothermobacter thermoautotrophicus. These regions however exhibited a typical MBL fold and belong to MBL superfamily. These results indicate the existence of a mechanism which blocks the signalling circuit in bacterial species by exhibiting different modes of action (Figure 4)

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VI. REFERENCES

1. A.B.Haidich, "Meta analysis in medical research", Hippokratia,2010:14;29-37.
2. Oh, K.B., H. Miyazawa, T. Naito, and H. Matsuoka, "Purification and characterization of an autoregulatory substance capable of regulating the morphological transition in Candida albicans. Proc. Natl. Acad. Sci. USA, 2001; 98: 4664-4668.
3. Y.Aparna and J.Sarada, "Anti Quorum Sensing Activity of Enterobacter spp. Isolated from Soil", Indian Journal of Science and Technology, Vol 10(8),Feb 2017
4. www.ncbi.nlm.nih.gov/entrez
5. www.sciencedirect.com
6. www.scirus.com/srsapp
7. www.isiwebofknowledhe.com
8. http://scholar.google.com
9. Kathleen O Neil,William KlimkeAnd Tatusova, "Protein clusters:A collection of proteins grouped by sequence similarity and function". Protein clusters help manual,NCBI,2010.
10. Jorg Schultz,RichardR.Copley,Tobias Doerks,Chris P.Ponting and Peer Bork, "SMART:a web based tool for the study of genetically mobile domains.Nucleic Acids". Res ,2000;28(1):231-234