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Research Article

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Quorum sensing proteins in selected microorganisms (*Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Yersinia pestis):* A study in Bioinformatics

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ABSTRACT

Quorum sensing is the regulation of gene expression in response to fluctuations in cell-population density. Quorum sensing bacteria produce and release chemical signal molecules called auto-inducers that increase in concentration as a function of cell density. The detection of a minimal threshold stimulatory concentration of an auto-inducer leads to an alteration in gene expression. Gram-positive and Gram-negative bacteria use quorum sensing communication circuits to regulate a diverse array of physiological activities. These processes include symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation, and biofilm formation .It is well supported by the finding that the pathogenic and non -pathogenic organisms the proteins are stable in these organisms, which could be inferred by the instability index. Moreover, the conversation was found since it is stable. This work represents a series of interrelated modules designed to introduce you to modern biological techniques in the area of bioinformatics.

Keywords: Bacteria, Bioinformatics, communication, Quorum sensing, signals

INTRODUCTION

Quorum sensing is the ability of bacteria to communicate and coordinate behavior through signaling molecules. The reason for these systems seems to have evolved is to coordinate behavior or actions between individual bacterial cells depending on there number e. g. opportunistic bacteria can grow within a host without harming it, until they reach a certain concentration. When their numbers are sufficient to overcome the host's immune system, they can change their behavior and cause disease.

Bacteria which use quorum sensing produce and secrete signaling compounds called auto-inducers. These bacteria also have a receptor which can specifically detect the inducer. When the inducer binds to it's receptor, it activates transcription of certain genes, including those for inducer synthesis. When only a few other bacteria of the same kind are in the vicinity, diffusion reduces the concentration of the inducer on the surrounding medium, so the bacteria produce little inducer. With high concentrations of bacteria, the concentration of the inducer passes a threshold, so more inducer is synthesized. This form a positive feedback loop and the receptor becomes fully activated.

Two chemically different auto-inducers are involved in regulation, auto-inducer-1 and auto-inducer-2.Both Gram positive and Gram negative bacteria use quorum sensing to regulate a diverse array of physiological activities. These processes include symbiosis, virulence, competence, conjugation, antibiotic production, motility and spore and biofilm formation(*Miller M.B, Bassler BL, Quorum sensing in Bacteria. Ann Rev Microbiol,2001 55: 165-199*).

To form biofilm bacteria need to start a complicated genetic program to switch from a free- living planktonic lifestyle to a sessile existence. This stats with determination of their cell density by means of quorum sensing. From

the structure of quorum sensing auto-inducers, several derivatives have been developed which prevent biofilm formation in many Gram-positive bacteria, including *Staphylococcus aureus*, a particular problem in Hospitals. Some of these compounds are already in clinical studies.(Abraham WR, Controlling biofilm of Gram positive pathogenic bacteria. Curr Med Chem.2006 13: 1509-24).

Microorganisms communicate and cooperate to perform a wide range of multicellular behaviors such as nutrient acquisition, biofilm formation through quorum sensing. These complex multicellular complex behaviors are interesting from the perspective of social evolution-Why do microorganisms engage in these behaviors given that cooperative individuals can be exploited by selfish cheaters who gain the benefit of cooperation without saying there share of the cost? A new paper just published describes an overview of the different mechanisms through which cooperative behaviors can be stabilized, the novel problems that microorganisms pose and the new insights that can be gained from applying evolutionary theory to microorganisms (West SA, et al. Social evolution theory for microorganisms. Nat Rev Microbiol. 2006 4: 597-607).

Staphylococcus aureus are Gram-positive bacteria and cause diverse serious diseases in human and animals through the reproduction of toxins. The production of toxins is regulated by quorum sensing mechanisms, where proteins such as RNA III activating proteins (RAP) are secreted by the bacteria and induce virulence.

Bacillus subtilis are Gram- positive bacteria and the auto-inducer-2 production protein LuxS, is involved in an novel quorum sensing system and is thought to catalyze the degradation of S ribosyl homocysteine to homocysteine and the auto -inducer molecule 4,5 dihydroxy-2,3 -pentadione. The crystal structure of *Bacillus subtilis* Lux S has been determined at 1.2 A resolution, together with the binary complexes of Lux S with S- ribosyl homocysteine and homocysteine to 2.2 and 2.3 A resolution respectively.

In *E.coli*, the locus of enterocyte effacement (LEE) is a chromosomal pathogenicity island that encodes the proteins involved in the formation of the attaching and effacing lesions by the enterohemorrhagic *E.coli* (EHEC) and enteropahtogenic *E.coli*(EPEC). The LEE comprises of 41 open reading frames organized in five major operons,LEE1,LEE2,LEE3,Tir(LEE5), and LEE4 which encode a type III secretion system, the intimin adhesin, the translocated intimin receptor (Tir) and the other effector proteins. The first gene of LEE1 encodes the Ler regulator,, which activates all the other genes were activated by quorum sensing through Ler (V. Sperandio, J.L.Mellies, W. Nguyen, S.Shin, and J. B. Kaper, Proc. Natl. Acad. Sci.USA 96:15196-15201, 1999). In this study we report that a putative regulator in the *E.coli* genome is itself activated by quorum sensing .This regulator is encoded by open reading frame b3243; belongs to the Lys R family of regulators; is present in EHEC,EPEC and *E.coli* K-12; and shares homology with Aph B and Pttx R regulators of *Vibrio cholerae* and *Pseudomonas aeruginosa*, respectively . We confirmed the activation of b3243 by quorum sensing by using transcriptional fusions and renamed this regulator quorum sensing *E.coli* regulator (QseA). We observed that QseA activated trasncriptional of ler and therefore, of the other LEE genes.An EHEC qseA mutant had a striking reduction of type III secretion activity, which was complemented when qseA was provided in trans .Similar results were also observed with a qseA mutant of EPEC. The QseA regulator is part of the regulatory cascade that regulates EHEC and EPEC virulence genes by quorum sensing.

In *Yersinia pestis* the acyl - homoserine lactone molecular species (AHLs)
Produced by the *Yersinis pestis* AHL synthase Yspl were identified by biochemical and physical/chemical techniques. Bio assays of extracts from culture supernatants of the recombinant Yspl and wild type *Yersinia pestis* showed similar profiles of AHLs. Here its is not yet known how quorum sensing may be involved in the organism's lifestyle but the hierarchia regulation within the quorum sensing system of *Pseudomonas aeruginosa* and *Yersinia pseudotuberculosis* suggests that similar structure may exist within the ysp and ype quorum sensing systems of *Y.pestis.*

Identification of the principal AHLs employed by the ysp quorum sensing system may be valuable in the effort to characterize gene regulation and virulence in *Y. pestis.*
The aim is to Analyze proteins involved in quorum sensing bacteria pertaining to evolutionary relationship and structural and functional relationship. This work may give new insight to analyze bacterial communication.

Bioinformatics is the application of computer technology to the management of biological information. The need for Bioinformatics has arisen from the recent explosion of publicly available genomic information, such as that resulting from the Human Genome Project.

EXPERIMENTAL SECTION

Implication of Tools

To show the ways in which the NCBI online database classifies and organizes information on DNA sequences, evolutionary relationships and scientific publications.

To identify unknown nucleotide sequences from an insect endosymbiont by using the NCB search tool BLAST.

To identify 2 unknown nucleotide sequences of microbial origin by using the NCBI search tool BLAST. Once a protein has been determined through protemics techniques, Bioinformatics can be used to predict certain types of topology. Topology is the sequence of secondary structure elements within proteins.The most basic secondary structure elements within proteins are the alpha helix, the beta sheet and the random coil.However, some algorithms will predict topological features that are closely related to *in vivo* localization, such as signal sequences and trans membrane helices.

ProtParam is a tool which allows the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot or TrEMBL or for a user entered sequence. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half life, instability index, and grand average of hydropathicity(GRAVY).

SWISS MODEL is a fully automated protein structure homology modeling server, accessible via the ExPASy web server or from the program Deep view (swiss Pdb-Viewer). The purpose of this server is to make protein modeling accessible to all Biochemists and Molecular Biologists worldwide.

Clustal W is a general purpose multiple sequence alignment program for DNA or proteins . It produces biologically meaningful multiple sequence alignments of divergent sequences.It calculates the best match for the selected sequences and lines them up so that he identities, similarities and differences can be seen. Evolutionary relationships can be seen via viewing Cladograms or Phylograms.

RESULTS AND DISCUSSION

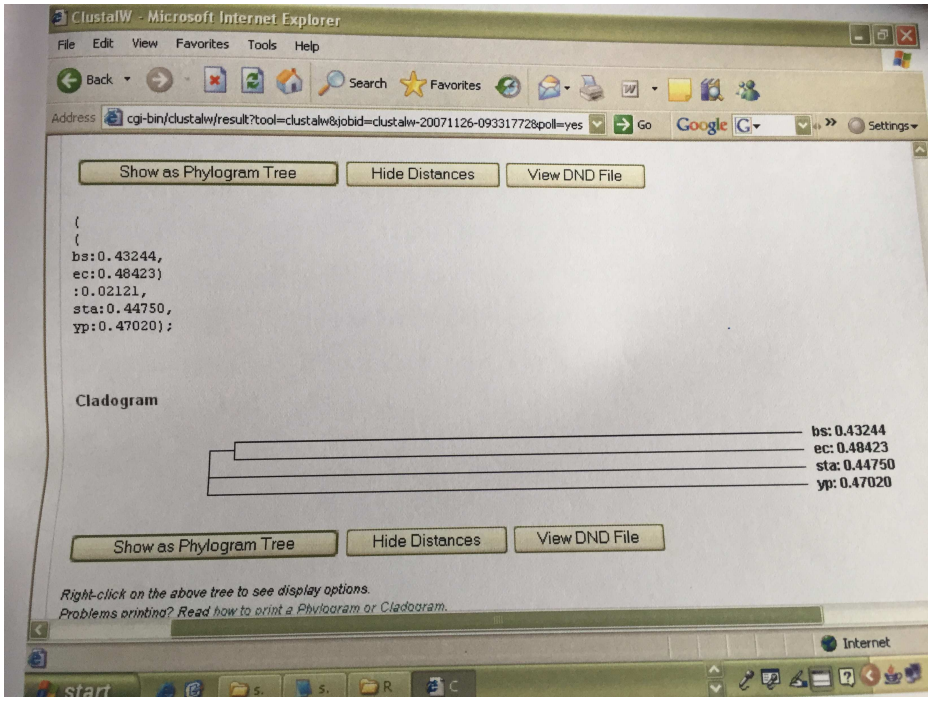

Figure 1

Table 1

Organism	Protein	Aminoacid	Percentage (%)
Staphylococcus aureus	RNAIII	Leucine	9.9
Bacillus subtilis	Lux S	Alanine	12.5
Escherichia coli	Regulator A	Alanine	12.8
Yersinia pestis	YspI	Leucine	12.5

Table 2

Organism	No of Amino acid	Molecular Weight	Theoretical pI	Instability Index	Aliphatic Index	Gravity
Staphylococcus aureus	243	27921.8	6.67	36.38	83.87	-0.493
Bacillus subtilis	120	13487.4	9.48	39.08	87.17	-0.342
Escherichia coli	196	21799.1	5.69	38.12	92.24	-0.037
Yersinia pestis	216	25496.3	6.60	40.70	99.26	-0.238

Table 3. Clustal W results

Number of Sequences	4
Sequence format	Pearson
Sequence type	aa
Clustal W version	1.83
Jal View	-
Output File	Clustal W-20071126-09331772.output
Alignment file	Clustal W-20071126-09331772.aln
Guide tree file	Clustal W-20071126-09331772.dnd
Your input file	Clustal W-20071126-09331772.input

Table 4. Scores

Seq A	Name	Len(aa)	Seq B	Name	Len(aa)	Score
1	*Bacillus subtilis*	120	2	ec	196	8
1	*Bacillus subtilis*	120	3	sta	243	10
1	*Bacillus subtilis*	120	4	yp	288	7
2	*Escherichia coli*	196	3	sta	243	4
2	*Escherichia coli*	196	4	yp	288	2
3	*Stphylococcus aureus*	243	4	yp	288	8

Discussion: The Cladogram shows **(Figure 1)** that there are both the pathogenic and non -pathogenic organisms. Though they diverge in their taxonomy they do show some convergence. The pathogenic and the non-pathogenic form a definite group. The organisms fall under definite pathogenic and non - pathogenic groups. The other two show divergence and even though they are from the same origin.

It is well supported by the finding that the pathogenic and non- pathogenic organisms the protein are stable in these organisms, which could be inferred by the instability index. Moreover, the conservation was found since it is stable.
The Highest percentage of Aminoacid was to be in *Escherichia coli.* The least was found to be in *Staphylococcus aureus. Bacillus subtilis and Yersinia pestis* was found to have similar percentage of amino acid sequences (**Table 1**).

In Table 2, the organism tend to posses an aliphatic index ranging from 83.78% -99.26%.The aliphatic index of a protein is the relative volume occupied by the aliphatic side chains(Alanine, Valine, Isoleucine, and Leucine). It may be regarded as a positive factor for the increase of thermostability of globular proteins. Since the organism have a high range of aliphatic index they are thermostable. Therefore, due to the presence of hydrocarbons they possess the necessary interaction in water which is the basic principle of quorum sensing. Cystine is responsible for the disulphide bridge formation. They might form a sub unit for other proteins. The highest Molecular weight was found to be in *Staphylococcus aureus*. The least molecular weight was *Bacillus subtilis* but exhibited highest pI.

The maximum instability index was found to be in *Yersinia pestis* which compared with *Staphylococcus aureus* which showed the minimum instability. *Bacillus subtilis* and *Escherichia coli* showed close difference in the index of instability.

The Gravity was least in *Escherichia coli* and the greatest was in *Staphylococcus aureus*. *Bacillus subtilius* and *Yersinia pestis* were in close proximity to each other.

Table 3 shows the Clustal W results which are a general purpose global multiple sequence alignment program for DNA or proteins. It produces biologically meaningful multiple sequence alignments of divergent sequences. It

calculates the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen. Evolutionary relationships can be seen via viewing Cladograms or Phylograms. As Shown in Figure 1 it shows the evolutionary relationship between each bacteria.

Table 4 shows the scores of the bacteria. The highest was found to be in *Staphylococcus aureus* in Sequence A and in Sequence B *Yersenia pestis* showed maximum value of 288.
The ratio sheets/helices despite their differences have conservation of secondary structure elements. These proteins tend to follow under a rare group of quorum sensing proteins.

Quorum sensing is the process by which many bacteria coordinate their gene expression according to the local density of their population by producing signaling molecules.

It coordinates certain behavior or actions between bacteria, based on the local density of the bacterial population. It can occur within a single bacterial species as well as between diverse species, and can regulate a host of different processes essentially serving as as simple communication network.

Bacteria that use Quorum sensing produce and secrete certain signaling compounds (called auto inducers or pheromones), eg: N-acyl homoserine lactones (AHL).These bacteria also have a receptor that can specifically detect the AHL (inducer). When the inducer binds the receptor, it activates transcription of certain genes, including those for inducer synthesis.There is a low likelihood of a bacterium detecting its own secreted AHL.

In *E.coli*,AI-2 is produced and processed bu the lsr operon. Part of it encodes an ABC transporter which imports AI-2 into cells during the early stationary(latent) phase of growth.AI-2 is then phosphorylated by the LsrK kinase, and the newly produced phospho-Ai-2 can either be internalized or used to suppress LsrK, a repressor of the lsr operon (thereby activating the operon).

Transcription of the lsr operon is also thought to be inhibited by dihydroxyacetone phosphate (DHAP) through its competitive binding to LsrR.

Glyceraldehyde 3-phosphate has also been shown to inhibit the lsr operon through cAMP- CAPK-mediated inhibition. This explains why when grown with glucose *E.coli*, will lose the ability to internalize AI-2(because of the catabolite repression).When grown normally Ai-2 presence is transient.

Quorum sensing via the accessory gene regulator (agr) system has been assigned a central role in the the pathogenesis of *Staphylococci* particularly *Staphylococcus aureus*. While the control of the virulence gene expression *in vitro* by agr has been relatively straightforward to describe, regulation of both the quorum sensing response itself and virulence genes *in vivo* is considerably more complex. The quorum response is highly dependent upon the environment in which the organism is grown and is strongly influenced by additional regulators that respond to signals other than cell density.

There is increasing evidence that the agr phenotype any influence the behavior to the chronic nature of some biofilm-associated infections.

Quorum sensing is important as it provides some very high insights to the mechanisms of parasitism and it provides a novel means to control infections of plants and animals.

As pheromones lie fully embedded within the protein.To activate the pheromones, several amino acid residues critical to RNA polymerase activation, or gene copying, .make contact with the “butterfly” of TraR. (Argone’s Rong-guang Zhang and SBC and collaborators, June 2002).

CONCLUSION

From the results obtained we can sum up that the inducers are nothing but proteins which in turn are amino acids sequences which play a vital role in quorum sensing in bacteria, thereby helping in the behavior of the bacteria and also the pathogenicity. Therefore, by knowing the sequences we can manipulate this communication to thwart harmful bacteria or aid helpful bacteria.

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