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Short Communication

In Silico Sequence Analysis of Coagulase from *Staphylococcus aureus*

***Asidah N. M.[1,3], Ruzianisra M.[2] and Chong T. T.[3]**

[1]Department of Biotechnology, Faculty of Agriculture and Biotechnology, Universiti Sultan Zainal Abidin, Gong Badak Campus, 21300 Kuala Terengganu, Terengganu Darul Iman, MALAYSIA.
[2]Faculty of Pharmacy, Universiti Teknologi Mara (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, MALAYSIA.
[3]Institute of Biological Science, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, MALAYSIA.

***Corresponding author; E-mail: norasidah@unisza.edu.my**

ABSTRACT

Coagulase production is generally accepted as being characteristic of pathogenic and potentially pathogenic strains. Coagulase can cause clot formation in the immediate vicinity of the bacterium. The protein has been shown to contribute to bacterial virulence in wound infections owing to its ability to delay the healing processes. In this study, we conducted *in silico* sequence analysis using coagulase from the Gram-positive human pathogen *Staphylococcus aureus* with GenBank Accession no. CAC 84776.1. Various bioinformatics tools were used to predict the properties of the *S. aureus* coagulase. The N-terminal portion of coagulase was predicted to form transmembrane helices whereas the majority of the protein was hydrophilic in nature and was predicted to form several alpha-helices. Coagulase was also predicted to localize extracellularly and has a pI value of 8.41. The *in silico* analysis carried out offers an alternative way to obtain structural information and will assist in determining the structural prediction of the *S. aureus* coagulase.

Keywords: Coagulase, *Staphylococcus aureus*, sequence analysis, structure prediction

ABSTRAK

Penghasilan koagulase umumnya diterima sebagai ciri strain bakteria patogen dan yang berpotensi sebagai patogen. Koagulase boleh menyebabkan pembentukan gumpalan secara langsung di kawasan sekitar bakteria. Protein ini juga telah terbukti menyumbang kepada kevirulenan bakteria pada jangkitan luka disebabkan oleh kebolehan melambatkan proses penyembuhan. Dalam kajian ini, analisis jujukan *in silico* dengan menggunakan koagulase daripada *Staphylococcus aureus* iaitu patogen manusia Gram-positif dan mempunyai no. aksesi GenBank CAC 84776.1 telah dilakukan. Pelbagai peralatan bioinformatik telah digunakan untuk meramalkan sifat koagulase *S. aureus*. Di bahagian N-terminal pada koagulase diramalkan membentuk heliks trans-membran manakala sebahagian besar protein bersifat hidrofilik yang diramalkan membentuk beberapa heliks-alfa. Koagulase juga diramalkan berada dibahagian luar sel dengan nilai pI sebanyak 8.41. Analisis secara *in silico* ini menawarkan cara alternatif untuk memperolehi maklumat tentang stuktur dan dapat membantu dalam menentukan ramalan struktur koagulase *S. aureus*.

Kata kunci: Koagulase, *Staphylococcus aureus*, analisis jujukan, ramalan struktur

INTRODUCTION

Bacteria which are members of the genus *Staphylococcus* are major human pathogens (Easmon & Adlam, 1983). They can be divided into two groups based on the production of coagulase, which is capable of coagulating blood plasma. The synthesis of this enzyme is restricted to some species in the genus, among of which is *Staphylococcus aureus* (Kloos & Bannerman, 1995; Koneman, 1997). *S. aureus* causes a range of acute and pyogenic infections, including abscesses, central nervous system infections, endocarditis, osteomyelitis, pneumonia, urinary tract infections, chronic lung infections associated with cystic fibrosis, and several syndromes caused by exotoxins and enterotoxins, including food poisoning, scalded skin and toxic shock syndromes (Lindsay *et al.*, 1998). *S. aureus* is also a main cause of hospital-acquired (nosocomial) infections of surgical wounds and infections related to indwelling medical devices (Archer, 1998).

Coagulase has been studied for more than 100 years (Loeb, 1903), and is secreted by virtually all *S. aureus* isolates (Field & Smith, 1945; Smith *et al.*, 1947). Studies have shown that the accessory gene regulator (*agr*) up-regulates the production of many exoproteins, including Toxic shock syndrome toxin–1 (TSST-1), enterotoxins B and C, and V8 protease (*sspA*); and down-regulates the synthesis of cell wall-associated proteins, including fibronectin-binding proteins, and

fibrinogen-binding proteins (coagulase) during post-exponential and stationary growth phase (Foster *et al.*, 1990; Lindberg *et al.*, 1990; Novick, 2000). *S. aureus* plays the role as a bacterial community at the center of lesions and are separated from infiltrating immune cells by an amorphous pseudocapsule (Cheng *et al.*, 2009). Abscesses grow in size and eventually rupture, providing for pathogen entry into the blood circulation and dissemination to uninfected tissues (Cheng *et al.*, 2009).

Evidence in support of a role for coagulase was suggested by N-terminal and central parts of the enzyme displaying sequence variation, which has been exploited for the classification of strains (Phonimdaeng *et al.*, 1990; Panizzi *et al.*, 2006). Hence, coagulase production has been used as a diagnostic test, differentiating *S. aureus* isolates from commensal staphylococci, for example *S. epidermidis* (Duthie & Lorenz, 1952). During host infection, coagulase conformationally activates the central coagulation zymogen, prothrombin, thereby triggering the cleavage of fibrinogen to fibrin (Panizzi *et al.*, 2006). The crystal structure of the active complex revealed binding of the D1 and D2 domains to prothrombin and insertion of the Ile1-Val2 N-terminus of coagulase into the Ile16 pocket of prothrombin, inducing a functional active site in the zymogen through conformational change (Friedrich *et al.*, 2003). The search for protective immunity against invasive *S. aureus* disease has been the aim since the discovery of this microbe (Rogers & Melly, 1965). However this pursuit has not yet been successful and a staphylococcal vaccine is currently not available (Projan *et al.*, 2006).

Realizing the importance of understanding coagulase function due to the pathogenicity of *S. aureus*, this report aims to conduct the sequence analysis of coagulase to gain insight of coagulase structure-function.

MATERIALS AND METHODS

Sequence Analysis

Sequence analysis were fully conducted *in silico* using coagulase with GenBank Accession no. CAC 84776.1 to obtain useful information of coagulase in *S. aureus*. There are a large number of coagulase sequences from *S. aureus* in the database but most of these are partial sequences. Thus the coagulase sequence with accession no. CAC 84776.1 was selected as the representative full coagulase sequence and covered residues 1–690 amino acids and expressed the function in platelet binding in *S. aureus*, according to Heilmann *et al.* (2002).

Prediction Methods

A few bioinformatics tools, home pages, programs and database were used to give some insight and prediction about the coagulase in *S. aureus*.

Molecular weight and isoelectric point

Compute pI/Mw tool (Bjellqvist *et al.*, 1993; Gasteiger *et al.*, 2005). The isoelectric point and molecular weight of coagulase was calculated using the Expasy program at http://au.expasy.ch/tools/pi_tool.html.

Transmembrane segments

TMpred (Hoftmann & Stoffel, 1993). The transmembrane segment and their orientation were predicted by the method of TMpred available at http://www.ch.embnet.org/software/TMPRED_form.html. For visual representation, the two dimensional (2D) model of coagulase, 'TransMembrane protein Re-Presentation in 2 Dimensions' tool was used (Spyropoulos *et al.*, 2004). Several important biological and physical sequence attributes were embedded in the graphical representation.

Solvent accessiblity and secondary structure prediction

Jpred (Cuff & Barton, 2000; Cole *et al.*, 2008) is a prediction service that enables the prediction of both the secondary structure and solvent accessibility of proteins at http://www.compbio.dundee.ac.uk/www-jpred/.

Subcellular localization

PSORTb (Gardy *et al.*, 2003; 2005; Yu *et al.*, 2010) Computational prediction of the subcellular localization of proteins is a valuable tool for genome analysis and annotation, since a protein's subcellular localization can provide clues regarding its function in an organism. The prediction of protein localization sites in the cells was carried out using the PSORT program at http://www.psort.org/psortb/

RESULTS AND DISCUSSION

Coagulase is an extracellular protein that forms a complex with human prothombin and activates it without the usual proteolytic cleveage. The resulting complex directly initiates blood clotting. Putative conserved domains were detected for the coagulase superfamily in the coagulase sequence of *S. aureus* (accession no. CAC 84776.1) which is 690 amino acids residue in length (Fig. 1A). The extracellular nature of coagulase was predicted by PSORTb which enables the prediction of prokaryotic localization sites.

The isoelectric point (pI or IEP) and theoretical molecular weight of coagulase was determined to be 8.41 and 77849.13 respectively. The pI is the pH

value at which the molecule carries no net electrical charge or the negative and positive charges are equal. The pI value can affect the solubility of a molecule at a given pH. Such molecules have minimum solubility in water or salt solutions at the pH which corresponds to their pI and often precipitate out of solution. At a pH below their pI, proteins carry a net positive charge (H^+); above their pI they carry a net negative charge (OH^-). Proteins can thus be separated according to their isoelectric point (overall charge) on a polyacrylamide gel using a technique called isoelectric focusing, which uses a pH gradient to separate proteins.

The hydrophobicity values were calculated by the program TMpred. The TMpred program makes a prediction of transmembrane segment and their region by looking at the hydrophobicity of blocks or "windows" of amino acids along the sequence. A string of about twenty hydrophobic amino acids in a row indicates potential transmembrane helices. The algorithm used in TMpred also takes into account information from an analysis of TMbase, a database of naturally occurring transmembrane proteins. A score of above 500 indicates possible transmembrane helices (Hoftmann & Stoffel, 1993). Only one region of coagulase, at the N-terminal domain, was predicted for possible transmembrane helices (Fig. 1A and 1B). Therefore, coagulase is likely a soluble protein due to its mainly hydrophilic nature.

In the case of solvent accessibility, the area of a protein surface that is exposed to the surrounding solvent was predicted using JPred (Fig. 3). When comparing the conservation of solvent accessibility of coagulase with similar structures, level of solvent accessibility of 5% to 25% showed more propensities for the residues to be buried compared than 0%. Residues that are buried in the interior of the protein play an important role in stabilizing the structure, yet they could not be a part of an active site of coagulase or an interaction site for a component of signal transduction, all of which require spatial accessibility of the residues to the solvent.

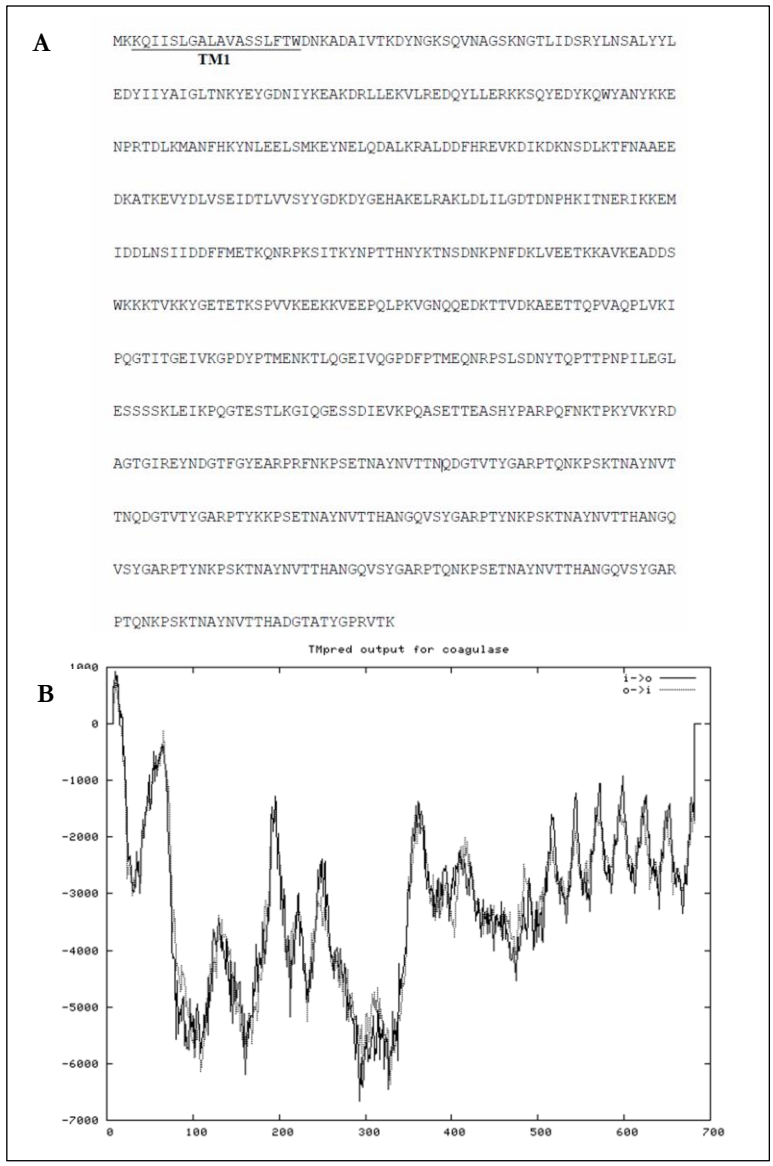


Fig. 1. (A) The amino acid sequence of coagulase from *S. aureus* (accession no. CAC 84776.1). (B) Hydrophobicity plot of coagulase as determined by TMpred. The amino acid residues that are predicted to form transmembrane helices are marked as TM1 in (A).

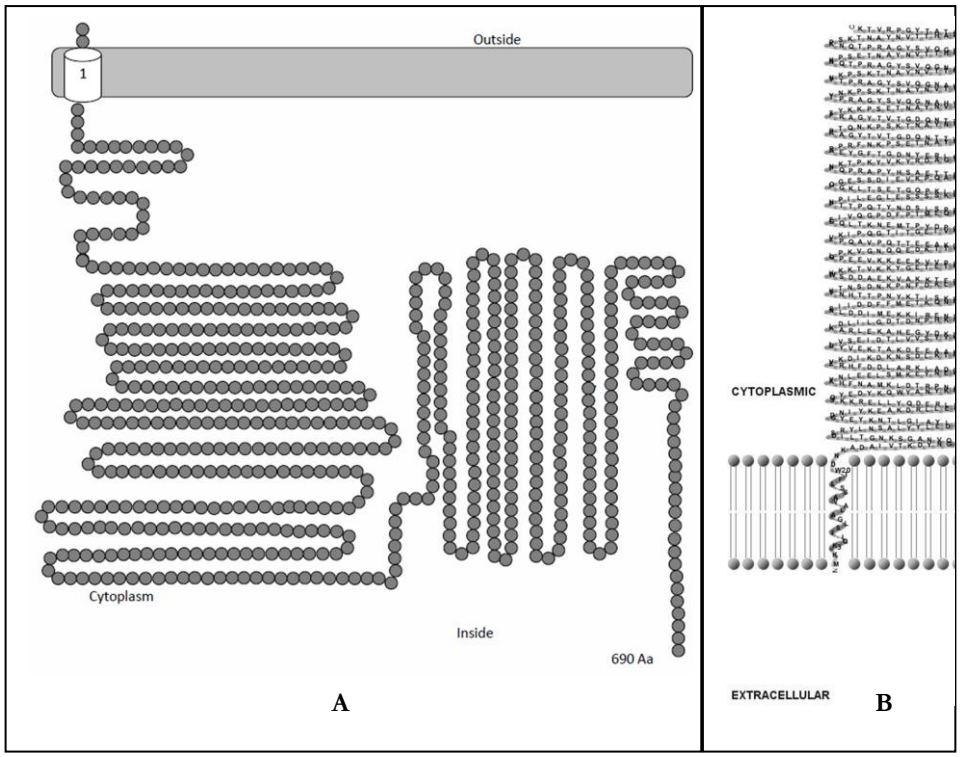
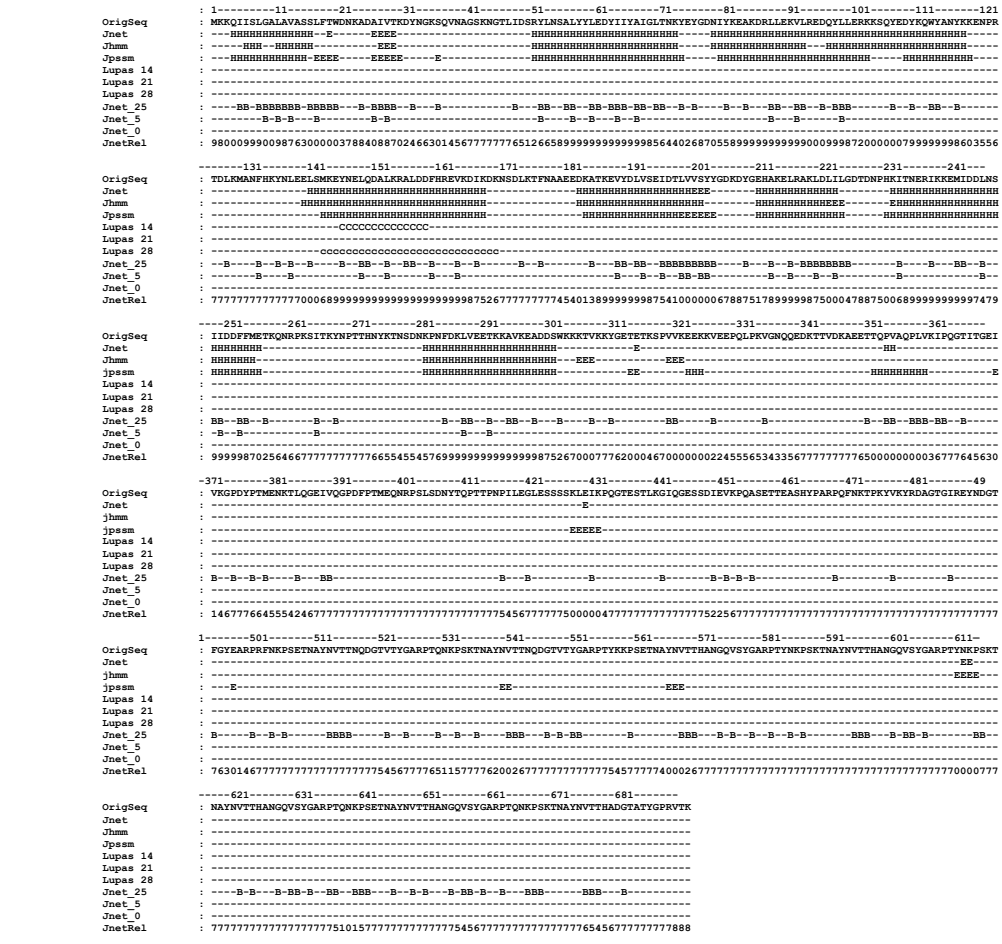


Fig. 2. (A) Diagrammatic representation of the predicted topology structure of *S. aureus* coagulase. The transmembrane helix is shown as a cylinder marked '1', and amino acid residues are shown as circles. (B) 2D model of *S. aureus* coagulase showing the transmembrane N-terminal alpha-helical segment using the 'TransMembrane protein representation in 2 Dimensions' tool.



Notes:

Jnet - Final secondary structure prediction for query
jhmm - Jnet hmm profile prediction
jpssm - Jnet PSIBLAST pssm profile prediction
Lupas - Lupas Coil prediction (window size of 14, 21 and 28)
Note on coiled coil predictions - = less than 50% probability
c = between 50% and 90% probability
C = greater than 90% probability
Jnet_25 - Jnet prediction of burial, less than 25% solvent accesibility
Jnet_5 - Jnet prediction of burial, less than 5% exposure
Jnet_0 - Jnet prediction of burial, 0% exposure
JnetRel - Jnet reliability of prediction accuracy, ranges from 0 to 9, bigger is better.

Fig. 3. Results of JPred prediction for *S. aureus* coagulase secondary structure and solvent accessibility. Predicted alpha-helices are denoted by 'H', beta-sheets by 'E', and random coils by '-' in the Jnet, jhmm and jpssm outputs. For predictions of relative solvent accessibility, a residue is predicted to be buried ('B') or exposed ('-') at 0% (Jnet_0), 5% (Jnet_5) and 25% (Jnet_25) cut-off points.

CONCLUSION

Coagulase from *S. aureus* plays a significant role in the pathogenesis of the bacteria via its procoagulant and fibrinogen-binding activity. Coagulase has been shown to be an extracellular protein that forms a complex with human prothrombin and activates it without the usual proteolytic cleavage. The resulting complex directly intiates blood clotting. In this *in silico* analysis, coagulase was shown to have a p*I* of 8.41 and only one region at its N-terminal was predicted to form transmembrane helices. A large portion of coagulase is likely soluble due to its hydrophilic nature. A number of alpha-helices were predicted and this correlated well with the crystal structure of part of the coagulase protein that has been resolved. The information from this *in silico* analysis will help to gain insights into the action of the coagulase protein in *S. aureus* and contribute to the understanding of the architecture of the structure of the full length coagulase protein.

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