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Analisis Bioinformatika Protein NRA Bakteri Streptococcus pyogenes Sebagai Dasar Pengembangan Reagen Diagnostik

Bioinformatics Analysis of NRA Proteins of the Bacteria Streptococcus pyogenes as a basic for the Development of Diagnostic Reagen

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Abstrak

Demam berdarah adalah penyakit yang disebabkan oleh bakteri Streptococcus pyogenes. Demam berdarah memiliki gejala sakit kepala yang menyakitkan, muntah, menggigil dan ruam merah. Peran laboratorium inspeksi dalam menegakkan diagnosis demam berdarah. Metode kultur merupakan gold standard yang mempunyai keterbatasan dalam efektifitas waktu pemeriksaan sehingga menyebabkan keterlambatan pengobatan. Peneliti menggugat menemukan molekul protein yang mampu memberikan dampak besar dalam pengembangan reagen diagnostik yang spesifik dan sensitif. Salah satu faktor virulensi bakteri S. pyogenes adalah protein Nra. Protein Nra memiliki 62% identitas urutan asam amino yang menyusunnya bagian dari pili bakteri. Protein Nra diharapkan dapat digunakan sebagai protein potensial untuk pengembangan biomarker secara mendalam di laboratorium. Sampel penelitian berupa sekuens protein Nra bakteri S. pyogenes yang diambil dari database NCBI dengan nomor tambahan ABJ15818.1. Analisis sifat fisik protein Nra menggunakan alat bioinformatika ProtParam disajikan secara deskriptif. Bakteri protein Nra S. pyogenes memiliki berat molekul 59988.00, periode isoelektrik 9.39, total asam amino 511 AA, nomor atom 8582 Da, indeks kestabilan 41.16, indeks alifatik 104.91, indeks GRAVY -0.071. Kesimpulannya protein Nra bakteri S. pyogenes bersifat basa, hidrofilik, tidak stabil, sulit berinteraksi dengan protein lain. Sifat fisika-kimia protein Nra bakteri S. pyogenes dapat menjadi referensi penemuan potensi molekul diagnostik untuk pengembangan reagen diagnostik demam berdarah yang spesifik dan sensitif.

Kata Kunci:
Streptococcus pyogenes
Protein Nra
Physico-chemical properties

Keywords:
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Abstract

Scarlet fever is disease caused Streptococcus bacteria pyogenes. Scarlet fever has symptom painful headache, vomiting, chills and arousal rash red. Inspection laboratory role in establishing the diagnosis of scarlet fever. The culture method is the gold standard own limitations in effectiveness time inspection so that cause lateness treatment. Researcher sued find capable protein molecules give impact big in development reagent specific and sensitive diagnostics. One factor virulence bacteria S. pyogenes proteins Nra . Protein Nra has 62% identity order the amino acids that make up it part of the bacterial pili . Protein Nra expected can used as a potential protein for deep biomarkers development laboratory. Research sample form Nra protein sequence bacteria Streptococcus pyogenes which was taken from NCBI database with accession number ABJ15818.1 . Analysis physical properties of Nra protein use device bioinformatics ProtParam served in a way descriptive. Protein Nra bacteria Streptococcus pyogenes own heavy molecule 59988.00, period isoelectric 9.39, total amino acid 511 AA, atomic number 8582 Da, index stability 41.16, index aliphatic 104.91, GRAVY index -0.071. In conclusion that the Nra protein bacteria Streptococcus pyogenes nature basic , hydrophilic , no stable , difficult interact with other proteins. Physico-chemical properties of Nra protein bacteria Streptococcus pyogenes can become reference invention potency molecule diagnostic For development reagent diagnostic specific and sensitive scarlet fever.

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INTRODUCTION

Scarlet fever (scarlet faver) or pharyngitis streptococcus is disease caused by the bacteria *Streptococcus β hemolyticus* Group A gave rise fever high. Bacteria *Streptococcus β hemolyticus* Group A or *Streptococcus pyogenes* is one bacteria pathogen prototype that causes mediated infection exotoxin. It

produces a number big exotoxins, superantigens, and related proteins wall producing cells diverse manifestation clinical, start from infection pyogenic classic, up to syndrome shock toxic (Wong & Yue n, 2012). The most common manifestation from infection *Streptococcus pyogenes* (*S. pyogenes*) is pharyngitis or Sick throat. Generally happens to children ages 5 to 15 years and n can cause invasive diseases such as infection bones, *necrotizing fasciitis*, inflammation muscles, meningitis and endocarditis (Danchine, *et al.*, 2007; Anggaraini et al, 2020; Ulfa & Putra, 2020).

Infection more scarlet fever often happen during season cold and spring in the area temperate medium. Plague can happened at home stairs, schools, facilities military, and other places where it occurs contact near man to human. Transmission happen via droplets and contaminated food bacteria *S. pyogenes*. *S. pyogenes* can cause infection superficial or systemic based on toxin and response immune mediation _ mechanism emergence disease (Wessels, 2020).

In the 19th century scarlet fever with high mortality _ occurs in Europe and America north, but in the 21st century it was reported scarlet fever especially occurred in China and Hong Kong as well as Asian and European countries (Yang, *et al.*, 2012). Usually For making a diagnosis inspection laboratory, such as inspection Microscopy, culture and identification biochemistry, antigen detection as well antibodies (yunita, 2013).

However, Usage microscope field dark own constraint in implementation technically (Tong *et al.*, 2017). Additionally, method diagnostic conventional such as culture and identification bokimia own limitations separately, that is in identification microorganisms reason need time around around 2-3 days, so matter This will cause lateness in start treatment and and required facility laboratory For carry out this culture (Aalbers, et al, 2011).

Rapid antigen detection tests (RADT) offer long turnaround time (TAT). more short and can done on the spot with source limited power _ or in place care. However , in comparison with throat culture method , RADT has less sensitivity _ adequate, between 70 and 90% (Stewart *et al.*, 2014; Elf *et al.* , 2018) . *The Infectious Diseases Society of America* (IDSA) and *the American Academy of Pediatrics* (AAP), issued recommendation latest for diagnosis and management GAS pharyngitis in 2012 due to low sensitivity of clinical diagnosis , doctor recommended For test children over 3 years old for GAS with use test rapid antigen detection test (RADT) or swab culture throat children Because more sensitivity _ superior (Stewart *et al.* , 2014; Thompson, 2020) . Accurate diagnosis and fast TAT can help in the correct diagnosis of *S. pyogenes* time, so increase maintenance patients and decisions treatment.

Therefore That need development method diagnostic demand invention molecule capable potential give impact big in development reagent specific and sensitive diagnostics _ For disease scarlet fever. Remember many factor virulence from bacteria *S. pyogenes* including capsules, *lipoteichoic acid*, M protein, M-Like protein, F protein, exotoxin pyrogen, Streptolysin S, Streptolysin O, streptokinase, dinase, C5a peptidase and Nra. So that important to identify _ characteristic physico-chemistry of Nra proteins bacteria *S. p y ogenes* as base development diagnostic use device Bioinformatics ProtParam.

Associated gene expression with *Group A* pilus *streptococcus* (GAS) has proven mediated by transcriptional regulators RALP family , incl RofA and Nra (Calfee *et al.* , 2018; Id, Davies and Mcmillan, 2020) . Nra is regulator pilus gene positive and also highlights importance level Nra intracellular For thermoregulation pilus expression (Nakata *et al.* , 2020) . Protein Nra has 62% identity order amino acids. Protein Nra arrange pilus expression is different in specific way for strains and/ or serotype (Calfee *et al.*,

2018) . Protein Nra Act as a positive regulator in some M3 strains and M49 strains and are functional as an autoregulator as well pilus gene expression in strain FCT- 3 positive or negative regulated by Nra with specific way (Nakata, Kreikemeyer and Foster, 2021) .

Bioinformatics is discipline moderate knowledge develop, and with with progress, will There is more Lots opportunity for scientists in study applied and pure (Ali *et al.*, 2021) . Bioinformatics platform designed For protein identification, quantification, and analysis downstream in bottom - up proteomics often requires effort interdisciplinary from expert math, expert statistics, and computer scientists (Chen, Hou and Tanner, 2020) . Bioinformatics covers analysis sequencing , genomics comparative , studies evolution molecular and phylogenetic , prediction protein and RNA structure , gene expression and regulation analysis , and analysis network biological , as well genetics disease humans , esp in particular , cancer , and analysis image medical (Auslander, Gussow and Koonin, 2021) . Physico-chemical parameters from all virulence proteins studied using the ProtParam server Expasy (https://web.expasy.org/protparam/) where order amino acid inserted in FASTA format to in box query on the ProtParam server ExPASy and delivered For identify characteristic physical and chemical from protein sequence. Server page count amount amino acids , wt molecules , quantity residue charged negative , index instability , pI theoretical , and average hydropathy (Rajapaksha, Gunasekara and Alwis, 2022; Achudhan, Kannan and Saleena, 2023) .

METHOD

Type of research This is study descriptive supported exploration _ studies literature. Samples in the form of Nra protein sequence bacteria *S. pyogenes* taken from NCBI database with accession number ABJ15818.1, shown in Figure 1.

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>ABJ15818.1 Nra [Streptococcus pyogenes]
MPYVKKKKDSFLVETYLEQSIRDKSELVLLLFKSPTIIFSHVAKQTGLTAVQLKYYCKELDDFFGNNLDI
TIKKGKIICCFVKPVKEFYLHQLYDTSTILKLLVFFIKNGTSSQPLIKFSKKYFLSSSSAYRLRESLIKL
LREFGLRVSKNTIVGEEYRIRYLIAMLYSKFGIVIYPLDHLDNQIIYRFLSQSATNLRTSPWLEEPFSFY
NMLLALSWKRHQFAVSIPQTRIFRQLKKLFIYDCLTRSSQQVIENAFSLTFSQGDLDYLFLIYITTNNSF
ASLQWTPQHIETCCHIFEKNDTFRLLLEPILKRLPQLNHSKQDLIKALMYFSKSFLFNLQHFVIEIPSFS
LPTYTGNSNLYKALKNIVNQWLAQLPGKRHLNEKHLQLFCSHIEQILKNKQPALTVVLISSNFINAKLLT
DTIPRYFSDKGIHFYSFYLLRDDIYQIPSLKPDLVITHSRLIPFVKNDLVKGVTVAEFSFDNPDYSIASI
QNLIYQLKDKKYQDFLNEQLQ
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Figure 1. Order Nra protein amino acids bacteria *S. pyogenes* (NCBI: ABJ15818.1)

Research methods Characterization of Physico-chemical Properties of Nra Protein from Streptococcus pyogenes bacteria bioinformatics can done with steps as following:

1. Identification Nra Protein Sequence: Sequence amino acids from the Nra protein identified from protein sequence databases using tool database searches such as BLAST (Basic Local Alignment Search Tool).
2. Analysis Nra Protein Sequence: Analysis Nra protein sequence can done with use tool bioinformatics like Expasy ProtParam. In analysis this, various characteristic physico-chemistry of the Nra protein like heavy molecules, isoelectric pH, content amino acids, and so on will calculated and known.
3. Prediction Nra Protein Structure: Prediction Nra protein structure done with use tool bioinformatics like Uniprot. In this process, the 3D structure of the Nra protein will predicted based on sequence amino acids.

The result of study This can help understand role of Nra protein in physiology bacteria and provide outlook about development therapy antibacterial new.

RESULT DAN DISSCUSSION

a. Identification Nra Protein Sequence

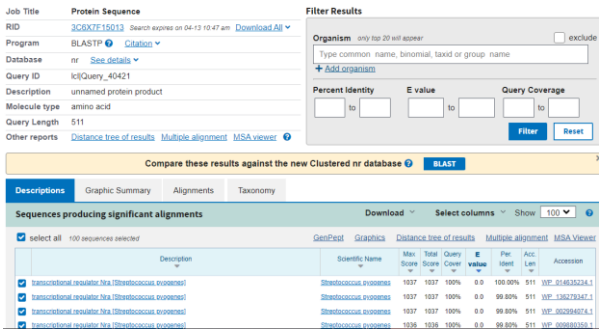

Figure 2. Identification Nra Protein Sequence with BLASP

b. Analysis Nra Protein Sequence

Parameter	Value Results	Interpretation	Information
Molecular Weight	59988.00		-
Number of Amino Acids	511		-
Point Value Isoelectric	9.39	Alkaline	pH, acid <7< alkaline
Atomic Composition	C:2792 H:4334 N:696 O:749 S:11		Primary structure
Formulas	C2792H4334N696O749S11		Primary structure
Number of Atoms	8582		-
Estimate Part Time	30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).		-
Index Stability	41.16	Less stable	<40 stable
Index Aliphatic	104.91		66.5-84.33
GRAVY Index	-0.071	Hydrophilic	(-) Hydrophilic + graph hydrophobic
Total amount of residueu negative (Asp+Glu)	43		**+/- (>/<)** J (-) > :: protein properties easy binds to other proteins J (+) > :: difficult binds to other proteins .
Total amount of residue positive (Arg+Lys)	63	hard binds to other proteins	**+/- (>/<)** J (-) > :: protein properties easy binds to other proteins J (+) > :: difficult binds to other proteins .

ProtParam is a Program from the Expasy website (http://us.expasy.org/tools/protparam.html) that is used For analyze Primary structure of the Nra protei. Where is this program will analyze heavy molecule, atomic composition, formula, atomic number, index instability, index aliphatic, and GRAVY. The physicochemical properties of proteins are very important For sustainability, efficiency, and stability in system biological.

The research results obtained show that the Nra protein with Accession number ABJ15818.1 is composed of 511 amino acids (511 aa) and has heavy molecule as much as 59988.00 Daltons.

Point isoelectric (pI) is the pH at which the protein does not have difference payload , no move in Medan electricity and every protein has isoelectric different (Ginting, 2019) . PI value _ can used For show characteristic base or sour from molecule zwitterionic , and compounds with pI > 7 can considered bases , and compounds with pI < 7 is possible considered sour (Naga *et al.* , 2010; Pergande and Cologna, 2017) . Analysis results Protparam show point isoelectric (pI) Nra protein own value > 7, namely 9.39, this show that protein _ nature base. For molecule complex like protein, period isoelectric useful in description characteristic sour or alkalinity and stability load molecule (Serban, Moldoveanu and Victor, 2017; Runthala *et al.* , 2023) .

atomic composition of the Nra protein is Carbon (C) = 2792, Hydrogen (H) = 4334, Nitrogen (N) = 696, Oxygen (O) = 749, Sulfur (S) = 11. Number of atoms of the Nra protein as many as 8582 with formula chemical C2792H4334N696O749S11.

Index results stability protein is considered stable with mark index <40 and protein >40 are called No stable (Kaur *et al.* , 2020; Runthala *et al.* , 2023) . Index This predict based protein stability composition sour the amino (Osorio, Rondón-villarreal and Torres, 2012; Panda and Chandra, 2012) . The results obtained of the Nra protein own index stability as big as 41.16, here show that this protein predicted not enough stable.

Index aliphatic role in stability thermal and quantity content hydrophobic amino acids (Panda & Chandra, 2012). The results obtained of the Nra protein own index aliphatic amounting to 104.91 which shows results that this protein predicted can stable over range wide temperature range (thermostable). Proteins with with index aliphatic tall have characteristic stable in a way thermal. The more tall mark index aliphatic then the protein increases thermostable ((Panda and Chandra, 2012; Ali *et al.* , 2017) . High range namely 74.14 to 80.45 can be stable over range wide temperature. _ This result almost similar with index aliphatic protein antifreeze ranges _ between 57.89 to 125.23 between sequence from different varieties.Index _ defined aliphatic (AI). as the relative volume of protein occupied by the chain side aliphatic considered as factor positive For enhancement stability globular protein thermal (Gouripur, Kaliwal and Kaliwal, 2016) .

Grand Average Index of Hydropathicity (GRAVY) was used For reflect mark hydrophobicity peptides , which measure amount all mark hydropathy amino acids are shared with long sequence (Chang and Yang, 2013) . Very low GRAVY index can produce more interaction Good with water (Gouripur, Kaliwal and Kaliwal, 2016) . GRAVY index of protein proteins Nra own mark index -0.071 which means this protein nature hydrophilic. Characteristic proteins hydrophilic will easy interact with water molecules (Ali *et al.* , 2017) . Proteins with negative GRAVY score considered hydrophilic in nature with good solubility (Kaur *et al*., 2020).

Total amount of residue negative (Asp+Glu) of the Nra protein as many as 43 meanwhile total amount of residue positive (Arg+Lys) of the Nra protein as many as 63. Total residue positive more Lots compared to residue negativ. Amount residue lots of positives This show that hard binds to other proteins (Palmer, Watts and Arnold, 2010)

CONCLUSION

Physico-chemical properties of the Nra protein with *accession number* ABJ15818.1 from bacteria *Streptococcus pyogenes* reason scarlet fever has done analysis in a way bionformatics. The results obtained is the Nra protein arranged of 511 amino acids (511 aa) and has heavy molecule as much as 59988.00 Daltons. Point Isoelectric (pI) has value > 7, namely 9.39, this show that protein nature base. Most atomic composition owned by H atoms = 4334. The number of atoms of the Nra protein as many as 8582 with formula chemical C2792H4334N696O749S11. Protein Nra own index stability as big as 41.16 which is of a nature not enough stable. Index aliphatic protein Nra amounting to 104.91 which shows results that this protein predicted can stable. Gravy index of Nra protein this is -0.071, nature hydrophilic. Amount residue positive a lot. Information This can used For develop therapy new more effective For overcome infection bacteria this and being base development methods and reagents specific and sensitive diagnostics for disease scarlet fever.

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