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Complete nucleotide sequence of the RA RagB/SusD gene

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Abstract. Riemerella anatipestifer (RA) is a Gram-negative rod-shaped bacterium associated with epizootic infections in poultry. RA infection is probably the most economically important infectious disease of farm ducks worldwide. The complete nucleotide sequence (1593bp) of RA RagB/SusD gene has been identified and sequenced in our laboratory. It is generally recognised that bioinformatics analysis plays an important role in the study of genes and proteins. Here in this article we intend to report some bioinformation about the nucleotide sequence of this gene for further research.

Introduction

Riemerella anatipestifer, the causative agent of septicemia anserum exsudativa (also called new duckling disease), belongs to the family Flavobacteriaceae of gram-negative bacteria. RA was first reported by Riemer in 1904 as a pathogen of geese. Since then it has been isolated from many avian species, including ducks, turkeys, chickens, and wild waterfowl, and can cause septicemic and exudative diseases in susceptible hosts [1]. To date, more than 21serotypes have been reported for RA, but there is no cross-immunoprotection among them [2]. Despite the devastating losses it causes in poultry production, not much is known about the pathogenesis and few data on virulence factors are available [3, 4, 5].

In the Riemerella anatipestifer genome, RagB/SusD gene, identified and sequenced in our laboratory[6], is conservative to the RagB/SusD gene of other bacteroidetes[7-20]. The complete nucleotide sequence of the RA ATCC 11845 strain RagB/SusD gene has been submitted in the GenBank Database and the accession number is NC_017045.1. The complete sequence of RA ATCC 11845 strain RagB/SusD gene is 1593bp with G/C content of 35.72%. Currently, there is little information on the molecular characteristics of RagB/SusD gene from RA. Here, we report the results of bioinformatics analysis about RagB/SusD gene.

Bacterium material and methods

The RA ATCC 11845 strain was identified by Cheng et al [6]. We got the RagB/SusD gene sequence form the NCBI submitted by our laboratory, and the accession number is NC_017045.1 in GenBank. And the commensurate amino acid sequence is deduced from the RA RagB/SusD gene by using the primer 5.0.

Sequence properties

RA RagB/SusD gene and the deduced amino acid sequence

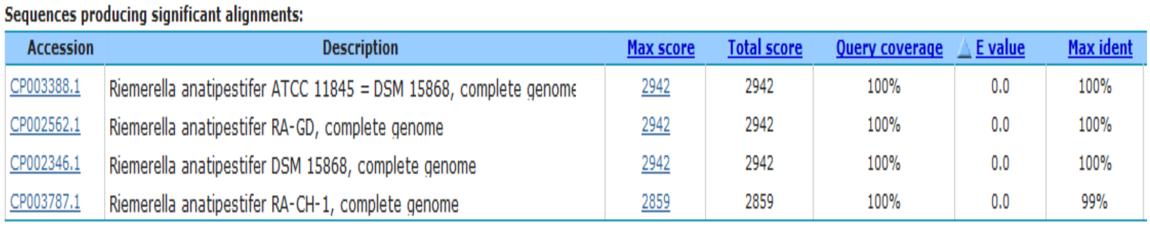
The RA RagB/SusD gene was found to be a 1593 bp segment. This RA RagB/SusD gene was expected to encode a protein comprising 530 amino acids with a putative molecular mass of 58.97243 kDa and an theoretical isoelectric point (pI) of 5.716. Including 60 basic amino acids(K,R), 65 acidic amino acid(D,E), 189 hydrophobic amino acids(A,I,L,F,W,V) and 139 polar amino acids(N,C,Q,S,T,Y) (Using DNAstar7.1).Using BLASTN2.2.26+ on NCBI ,we find the nucleotide sequence of RA ATCC 11845 RagB/SusD have 100% similarities with other three strains of RA RagB/SusD gene sequences with sequence alignment(Fig.1).

Compare RA RagB/SusD sequence with 19 referenced RagB/SusD protein sequences and construct the phylogenetic tree

Comparison of the amino acid sequence of RA RagB/SusD with those of Elizabethkingia anophelis Ag1、Ornithobacterium rhinotracheale DSM 15997、Chryseobacterium gleum ATCC 35910、Imtechella halotolerans K1、Flavobacteriaceae bacterium 3519-10、Fibrella aestuarina BUZ 2、Gramella forsetii KT0803、Mucilaginibacter paludis DSM 18603、Zobellia galactanivorans、Flavobacterium frigoris PS1、Joostella marina DSM 19592、Marivirga tractuosa DSM 4126、Solitalea canadensis DSM 3403、Flavobacteriaceae bacterium S85、Capnocytophaga ochracea DSM 7271、Capnocytophaga sp. oral taxon 329 str. F0087、Bacteroides coprosuis DSM 18011、Bacteroides faecis MAJ27、Prevotella denticola CRIS 18C-A revealed similarities of 70%、66%、59%、56%、55%、55%、53%、52%、50%、49%、50%、49%、53%、47%、48%、47%、44%、43%、44%, respectively(Using BLASTP2.2.26 on NCBI) (Fig.1). We gain the information of the 19 referenced RagB/SusD amino acid sequences of other bacteroidetes from NCBI. Phylogenetic tree is constructed by using the DNAstar7.1 based on the complete RA RagB/SusD protein sequence and the RagB/SusD protein sequences of 19 reference bacteroidetes(Fig.2). The results revealed that the RagB/SusD protein of RA has a close evolutionary relationship with Ornithobacterium rhinotracheale DSM 15997 and Elizabethkingia anophelis Ag1.

Bioinformatics presume the functions of RA RagB/SusD

Amino acid sequence analysis revealed that RA RagB/SusD was a outer membrane protein (Fig. 3). The RA RagB/SusD protein contained potential N-linked glycosylation sites, N-myristoylation sites, casein protein kinase II phosphorylation site and protein kinase C phosphorylation sites (https://www.predictprotein.org/submit) (Fig.4). We use an online sever (http://www.cbs.dtu.dk/services/NetPhos/) to predict that the RA RagB/SusD protein's serine sites, threonine sites and tyrosine sties, respectively (Fig.5). We also find that the predicted N-glycosylation sites at aa residues 16 and 450. And the RagB/SusD sequence may contain a signal peptide(As the RA RagB/SusD protein has been predicted that it doesn't transmembrane domain on line). Proteins with signal peptides are likely to be exposed to the N-glycosylation machinery and thus may be glycosylated (in vivo) even though they contain potential motifs[21].Collectively, those results indicate that the RagB/SusD protein may act an important role in the function of immue system, it may greatly influence the antibodies and genetic engineering vaccine, or in the diagnosis of RA.



Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
CP003388.1	Riemerella anatipestifer ATCC 11845 = DSM 15868, complete genome	2942	2942	100%	0.0	100%
CP002562.1	Riemerella anatipestifer RA-GD, complete genome	2942	2942	100%	0.0	100%
CP002346.1	Riemerella anatipestifer DSM 15868, complete genome	2942	2942	100%	0.0	100%
CP003787.1	Riemerella anatipestifer RA-CH-1, complete genome	2859	2859	100%	0.0	99%

Fig.1 The comparison results of RA RagB/SusD (BLASTN2.2.26+ on NCBI).

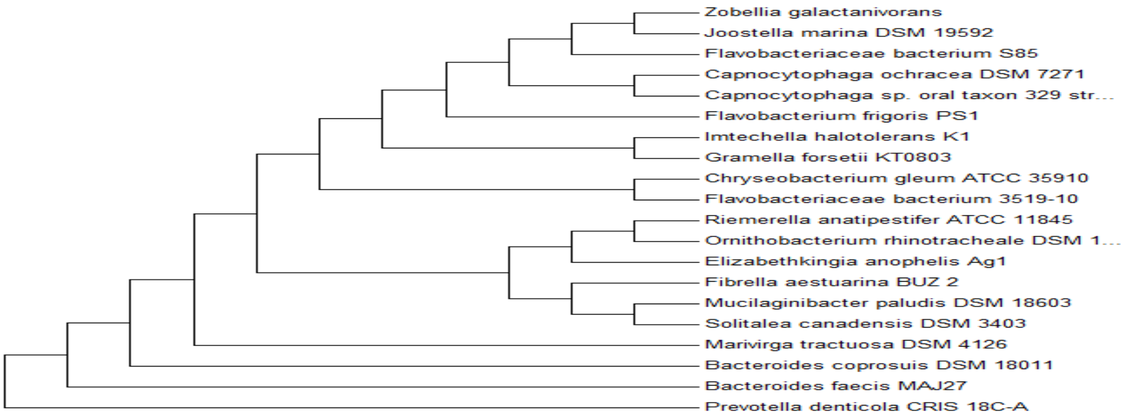
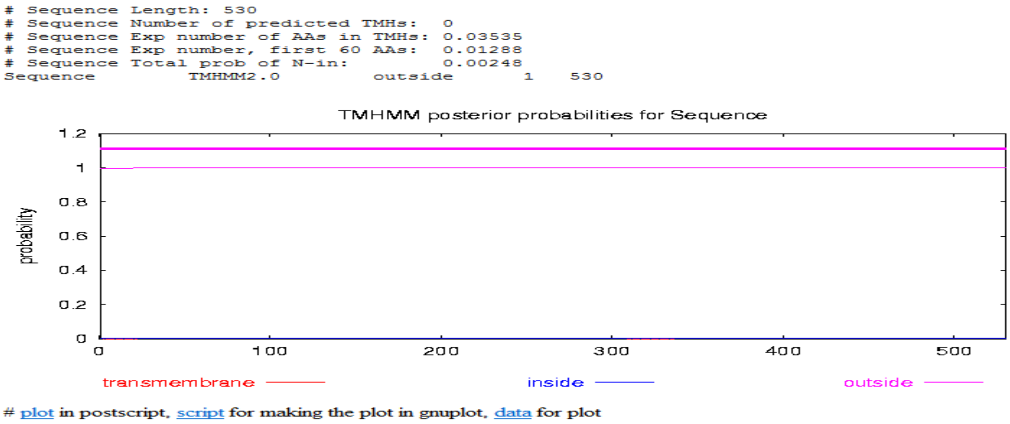


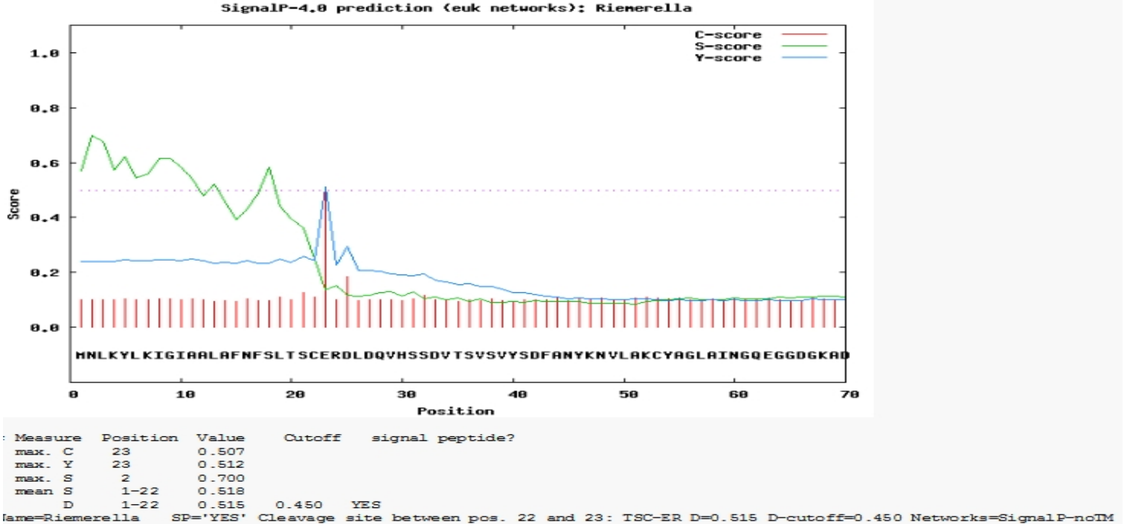
Fig.2 Phylogenetic tree of RagB/SusD based on 19 referenced RagB/SusD protein sequences from different species by using the Maximum parsimony method of MEGA (version 4.0). The protein database numbers of 19 reference bacteroidetes are shown



```
# Sequence Length: 530
# Sequence Number of predicted TMHs:  0
# Sequence Exp number of AAs in TMHs: 0.03535
# Sequence Exp number, first 60 AAs:  0.01288
# Sequence Total prob of N-in:        0.00248
Sequence        TMHMM2.0        outside      1   530
```

plot in postscript, script for making the plot in gnuplot, data for plot

Fig.3 The prediction for tranmembrane region of the RA RagB/SusD



```
 Measure  Position  Value    Cutoff   signal peptide?
  max. C    23       0.507
  max. Y    23       0.512
  max. S     2       0.700
  mean S     1-22    0.518
       D     1-22    0.515   0.450   YES
ame=Riemerella   SP='YES' Cleavage site between pos. 22 and 23: TSC-ER D=0.515 D-cutoff=0.450 Networks=SignalP-noTM
```

Fig.4 The result of signal peptide prediction of the RA ragB/susD protein

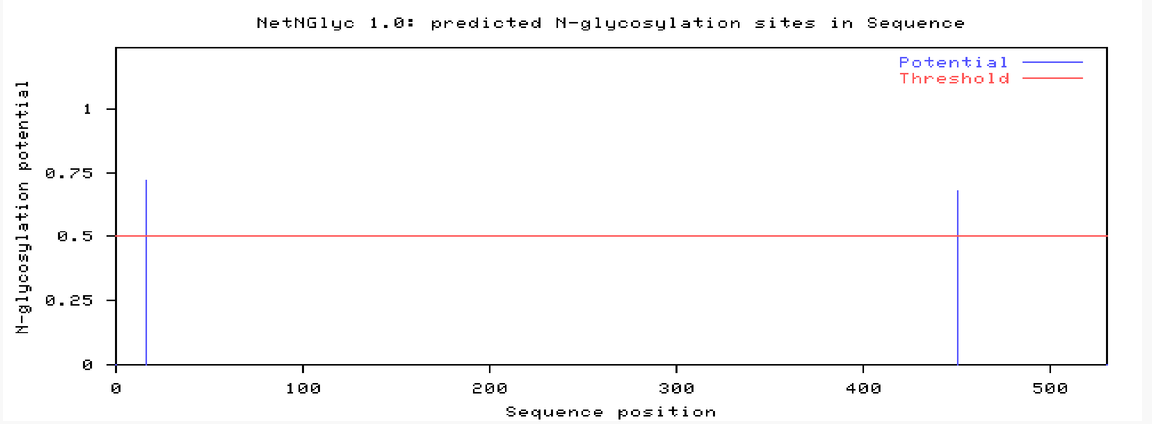


Fig.5 Prediction N-glycosylation sites in putative protein of RA RagB/SusD gene sequence.

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