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Proteomic Analysis and Identification of the Structural and Regulatory Proteins of the *Rhodobacter capsulatus* Gene Transfer Agent

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Abstract

The gene transfer agent of *Rhodobacter capsulatus* (GTA) is a unique phage-like particle that exchanges genetic information between members of this same species of bacterium. Besides being an excellent tool for genetic mapping, the GTA has a number of advantages for biotechnological and nanoengineering purposes. To facilitate the GTA purification and identify the proteins involved in GTA expression, assembly and regulation, in the present work we construct and transform into *R. capsulatus* Y262 a gene coding for a C-terminally His-tagged capsid protein. The constructed protein was expressed in the cells, assembled into chimeric GTA particles inside the cells and excreted from the cells into surrounding medium. Transmission electron micrographs of phosphotungstate-stained, NiNTA-purified chimeric GTA confirm that its structure is similar to normal GTA particles, with many particles composed both of a head and a tail. The mass spectrometric proteomic analysis of polypeptides present in the GTA recovered outside the cells shows that GTA is composed of at least 9 proteins represented in the GTA gene cluster including proteins coded for by Orf's 3, 5, 6–9, 11, 13, and 15.

Keywords

Gene transfer agent; *Rhodobacter capsulatus*; proteome

Introduction

The gene transfer agent (GTA) of *Rhodobacter capsulatus* carries out an unusual form of gene transfer in this α -proteo-bacterium. Unlike most bacteriophages, the GTA does not encapsidate DNA corresponding to the proteins that compose the particle itself. Instead, most of this GTA-specific information resides on the host bacterial chromosome in the GTA gene cluster.¹ The GTA can transfer small pieces (<5 kb) of randomly packaged host DNA from the donor *R. capsulatus* cell to the recipient cell.² The production and release of GTA particles apparently does not result in plaque formation (i.e., cell lysis).³ The mechanism of particle release is not known.

Other possible GTA-like particles have been identified by biochemical isolation and comparative genomic approaches.^{4–6} It is not known how frequently this mode of gene transfer is seen in the natural environment. Most strains of *R. capsulatus* are capable of producing and receiving the GTA.⁷ A GTA-like particle that infects the anaerobic spirochete *Brachyspira hyodysenteriae* has also been reported,^{8,9} suggesting that this mode of genetic transmission occurs more widely than previously anticipated.¹⁰

Since the original isolation of the particle,^{2,11,12} no further information has been gathered about the composition of the GTA that has not been indirectly gleaned from the nucleic acid sequence of that portion of the genomic DNA of *R. capsulatus* that contains the so-called GTA structural gene cluster.^{1,5,13,14} The GTA resembles a tailed bacteriophage, possessing icosahedral symmetry and a diameter of 30 nm.² While these earlier efforts resulted in very significant advances in our understanding of the basic repertoire of the gene products likely involved in the maturation and structure of the GTA, it does not reveal the composition of the isolated particle. Since the focus of some of our current work involves the use of GTA for nanotechnological applications,¹⁵ a thorough characterization of the polypeptide composition of this particle would allow us to gain understanding of, and control over, its cellular assembly.

In the present work, we identify the proteins that comprise the GTA particle, using a system by which we conjugated a His-tagged version of the 42 kDa major capsid protein Orf 5 into the *R. capsulatus* GTA overexpressing strain Y262, isolated chimeric GTA particles containing the processed Orf5 capsid protein, and identified at least 8 other members of the structural gene cluster.

Experimental Procedures

Strains, Growth Media and Reagents

The *R. capsulatus* Y262 overexpressing strain used in this study was provided by Carl Bauer (University of Indiana). The use of PY and RCV media to culture *R. capsulatus* Y262 cells and to produce GTA particles has been described previously.²

Construction of pPORF5 with a C-Terminal 6x His Tag (pPORF5CTH)

The conjugation plasmid, pPORF5CTH, contains the promoter region (beginning upstream of and including GTA orf1) and was designed to drive expression of a C-terminal His-tagged version of GTA orf5. The promoter for the GTA gene cluster in *R. capsulatus* was amplified from genomic DNA of the GTA overexpressing strain Y262² using the primer pair, 5'-ATAGGATCCCCCTCGAGCTGCGGCACCG-3' and 5'-ATACATATGCGCTTCCGGCTCCGACACC-3'. Orf5, the gene which codes for the major coat protein of GTA, was amplified with an in-frame His-Tag using the primer pair, 5'-AAACATATGAAGACCGAGACCAAGG-3' and 5'-TATAAGCTTTTCAGTGGTGGTGGTGGTGGTGGTGGCAGGCCGCAAACCTTCAAC-3'. The amplification conditions for both promoter-Orf 1 and His-Tagged orf5 fragments are the

following: initial 98 °C denaturation, 10 s; 59 °C annealing, 30 s; 72 °C extension, 50 s; 30 cycles. Amplification was performed using Phusion DNA polymerase essentially under conditions specified by the manufacturer. After purification with Qiagen PCR Purification Kit, the promoter fragment was digested with BamHI and NdeI and the His-Tagged orf5 was digested with NdeI and HindIII, yielding bands of ~1200 bp and ~1300 bp, respectively. These fragments were ligated into pRK442 vector digested with BamHI and HindIII to yield pPORF5-CTH.

R. capsulatus Y262 strain was grown in Sistrom's media for 2 days in the dark. *Escherichia coli* strain S17 containing pPORF5CTH was grown in LB media in 30 µg/mL of tetracycline overnight. The saturated S17 was then diluted 10-fold with LB and grown to OD^{600nm} of 0.3–0.4. One milliliter of the Y262 culture was mixed with 1.0 mL of S17 culture. The mixed cells were collected at room temperature at 9000g, washed with cold Sistrom's media, spun again, and resuspended in 100 µL of Sistrom's media. The cells were spotted on dry LB plates and grown anaerobically at 32 °C in the dark. After 6 h, the cells were restreaked onto RCV plates with streptomycin and tetracycline at 25 and 5 µg/mL, respectively, and grown for another 2 days in the same condition.

Growth Condition of Y262 Wild-Type and Y262 pPORF5CTH for GTA Production

The growth conditions used for *R. capsulatus* GTA overexpressing Y262 has been described previously,² and were used here for *R. capsulatus* Y262 harboring pPORF5CTH. Briefly, *R. capsulatus* pPORF5CTH was grown in RCV with streptomycin and tetracycline at 25 and 5 µg/mL, respectively. Strains were grown semianaerobically at 32 °C in the dark. After 2–3 days of growth in the RCV when the cells reached early stationary phase, OD^{600nm} ~ 0.8, they were mixed with fresh PY media at 3:1 ratio without additional antibiotics. The culture was grown anaerobically at 35 °C in the light for 2–3 days more to induce GTA production.

Purification of His-Tagged GTA Particles. Purification of His-Tagged GTA Particles Passed into the Media Outside the Cells

The general procedure for purification of GTA particles from *R. capsulatus* Y262 has been described.^{2,16} Y262 cells bearing pPORF5CTH, and producing the His-Tagged GTA particles, were spun at 8000g for 10 min. The supernatant was filtered through a Millipore 0.45 µm HV Durapore membrane. Stock 1.0 M Tris, pH 8.0, was added to the filtrate to bring the final concentration to 10 mM Tris, pH 8.0. The filtrate was then applied to a 2–3 mL NiNTA-agarose, and the column was subsequently washed with 10 mM Tris, pH 8.0, extensively. Finally, His-Tagged GTA was eluted with 40 mM imidazole in 10 mM Tris, pH 8.0. The eluted proteins were separated by SDS-PAGE, and either silver-stained,¹⁷ or electroblotted onto PVDF and incubated with anti-6× His antibodies (Roche). Prior to TEM imaging, samples were concentrated using a Centricon 10 membrane.

Mass Spectrometry of Chimeric His-Tagged GTA Particles

In-gel digestion of the silver stained protein bands was performed by the method of Shevchenko.¹⁸ An aliquot (33%–100%, depending on gel band stain intensity) of each digest was analyzed by a combination of nano-HPLC/µESI ionization on an LTQ linear ion trap mass spectrometer (Thermo Scientific Corporation, San Jose, CA) as previously described (Martin et al., 2000).¹⁹ The HPLC gradient (A = 100 mM acetic acid in water, B = 70% acetonitrile/100 mM acetic acid in water) was 0–60% B in 30 min, 60–100% B in 10 min, 100% B for 2 min, 100–0% B in 3 min and 0% B for 20 min. Collision activated dissociation (CAD) spectra were acquired in a data-dependent manner, with the first 10 most abundant *m/z* from each full MS being selected for MS/MS analysis. Acquired MS/MS spectra were analyzed using BioWorks 3.3.1 SP1 (Thermo Scientific Corporation, San Jose, CA). Protein information for *R. capsulatus* was obtained from www.integratedgenomics.com/ and a decoy database was

generated by reversing each protein sequence in the *R. capsulatus* database (in house software). DTA files corresponding to each MS analysis were searched against the *R. capsulatus* database and the decoy database using Sequest28 (rev13) from BioWorks 3.3.1 SP1 (Thermo Scientific Corporation, San Jose, CA). Search parameters allowed for a variable modification of oxidation at methionine residues and specified trypsin cleavage at lysine and arginine (except before a proline group), with up to two missed cleavages being allowed. Searches were performed with a precursor mass tolerance of 1.5 amu and a fragment mass tolerance of 0.5 amu. Peptide identifications with a minimum Xcorr of 2.0 from the real and decoy database were combined for each gel band analysis and sorted by the Sequest final score (Sf). For each gel band analysis, an Sf cutoff value was chosen which would give zero false positives in the final list of peptides. The peptide hits determined by this decoy database comparison were manually validated to confirm that the assignment was correct. For a given peptide MS/MS spectrum to meet the minimum manual validation criteria, fragment ions covering 80% of the peptide backbone were required. All high abundance fragment ions (>10% relative abundance) were required to be assigned to theoretical fragments. When these criteria were used, 1 out of 108 initial assignments were rejected in the manual validation process. At least two confirmed peptides hits in a single gel band analysis were required for a protein identification to be reported. The protein probability and final score values were determined for each protein using the validated peptides. The protein probability value is equal to that calculated for the single best-matched peptide from the protein, whereas the Sf value is an overall score calculated from the peptide Xcorr, ΔC_n , Sp and RSp values along with the peptide masses, charge states and number of matched peptides.

To confirm that predicted ORF assignments were consistent with the proteins observed, the contiguous DNA sequence for the GTA particle was translated into amino acid sequences. Each of the six potential open reading frames were translated using ORF finder from NCBI <http://www.ncbi.nlm.nih.gov/gorf/gorf.html>. Manual deletion of stop codons was performed in order to produce a FASTA file with a single contiguous amino acid sequence for each open reading frame. DTA files were searched against this database as described previously. No valid proteins from alternate reading frames were identified in this search.

SDS Polyacrylamide Gel Electrophoresis

Boiled protein extract were loaded in the wells of a 5% (stacking)/12% (resolving) polyacrylamide gel and subjected to SDS-PAGE. The His-tagged Orf5 protein was detected by Western blotting after reacting with anti-6 $\times$ His-tag primary antibody (Roche), HRP-conjugated secondary antibody, and developing with chemiluminescence reagents (PicoWest, Pierce Chemicals).

Transmission Electron Microscopy (TEM)

Negative staining of GTA particles was performed by absorbing 10 μ L of each sample to a plasma-etched Formvar/carbon-coated copper grid for 2 min, and staining with 10 μ L of 2% (w/v) phosphotungstic acid (pH 5.0) for 1 min at room temperature. Grids so prepared were examined without further processing at 80 kV in a JEOL 1230 transmission electron microscope and images were captured using a SIA-L3C digital camera system (Scientific Instruments and Applications, Inc., Atlanta, GA).

Bioinformatics

Homology searches were performed using BLASTp (<http://www.ncbi.nlm.nih.gov/BLAST/>).²⁰ Sequence alignments were performed using Clustal W (<http://www.ebi.ac.uk/clustalw/>)²¹

Results and Discussion

The GTA represents an unusual system for the exchange of genetic information between members of the same species. We have begun to characterize the components of the system in order to refine our understanding of the general structure of the particle, and to elucidate the precise way in which this phage-like particle transfers small pieces of its host DNA to other GTA-bearing *R. capsulatus* cells within the same population. As a first step in this process, we have identified the protein components of the GTA particle.

Our approach was to place a 6× His-tagged version of the naturally occurring Orf5 major capsid protein into the *R. capsulatus* GTA overexpressing Y262 genetic background, and to subsequently isolate the particle that is formed. To express the His-tagged Orf5 *in vivo*, we used a variation of the strategy developed by Lang and Beatty for the identification of the GTA cluster promoter element, replacing the β-galactosidase reporter used previously with the His-tagged Orf5 gene in the present work.¹ We expected that the His-tagged Orf 5 should be expressed under this promoter and up-regulated with increasing cell density.¹ We further expect that the GTA particles we purify would be chimeric in nature, that is, they are composed of both His-tagged subunits and endogenous Orf5 protein product in their capsid, as there is no reason to expect that endogenous copies of Orf5 would be mechanistically excluded from insertion. We were able to apply a onestep procedure using NiNTA chromatography to isolate a GTA particle consisting of 8 proteins, besides the His-tagged Orf5 capsid protein, encoded by the GTA cluster that are part of the virus-like agent.

In an effort to characterize the chimeric GTA particle shed to the outside of cells at stationary phase, we cleared the solution of cells, and harvested the GTA-containing growth media. We then applied the growth medium to a NiNTA-agarose column. If the 42 kDa His-tag precursor was incorporated into GTA, we would expect to observe binding of those GTA phage that were composed at least in part of capsid subunits bearing the 6× histidine tag at the C-terminus of the Orf5 protein in the eluted fractions from this column. The size of the Orf5 gene product should be 31 kDa, consistent with its processing by Orf4 prohead protease.^{1,2} We were able to identify the processed 31 kDa form of the Orf5 by Western blotting in the fractions eluted from NiNTA with 40 mM imidazole. Upon silver staining, we observed at least 5 other proteins in the same fraction besides the expected 31 kDa product. To identify whether these proteins were related to GTA (i.e., derived from or identical to gene products associated with the GTA structural gene cluster), we subjected each silver staining band to trypsin digestions and to subsequent mass spectrometric analysis. Figure 1 and Table 1 below summarize our observations.

The gene cluster coding for the structural proteins of the gene transfer agent of *R. capsulatus* is presented in Figure 2. We found peptides originating from no less than 9 predicted orfs from the GTA gene cluster. We have modified and updated the map presented earlier^{1,13} based on information obtained from the *R. capsulatus* genome web site and the mass spectrometric data presented elsewhere in this paper. Solid arrows represent orfs that code for predicted proteins that we did not detect in the purified GTA particle. Crosshatched arrows represent orfs that code for predicted proteins whose tryptic peptides we have detected in the purified GTA particle. The putative promoter region that drives expression of the cluster¹ is designated as “Pro”. An orf overlapping with an adjacent orf is shown above the solid black line. Insofar as it was possible, the designations were made consistent with earlier work.^{1,13} The RRC designations correspond to the same genes obtained from the *R. capsulatus* genomic database and are provided for comparison. Orfs not identified earlier, that fall between previously assigned Orfs, bear a half-unit notation, that is, Orf 3.5 falls between Orf 3 and Orf 4. Orfs with discrepancies in predicted start sites that are not currently resolved (Orfs 1–4) are represented by solid black boxes spanning the nucleotide positions suggested for starting.

Peptides were obtained from 9 out of a possible 17 predicted gene products in this region of the *R. capsulatus* chromosome. The orf designated 10.5 above, obtained from the *R. capsulatus* genome database, is in the same reading frame as “Orf 10.1” proposed earlier,¹³ and essentially equivalent to it but shorter by 10 amino acids. Orfs 13 has been assigned based on our peptide identifications. Orf 14, a possible cell wall peptidase/hydrolase, has been assigned based on the *R. capsulatus* genomic database. Orf 15 codes for one polypeptide of 138 kDa (RRC03501).

In addition to the proteins identified which are derived from the GTA cluster, we also detected four proteins that copurified with the His-tagged GTA particle. These four proteins originate from places on the chromosome outside of the GTA gene cluster and are also identified in Table 1.

The collection of polypeptides in the fraction we isolated from the NiNTA metal chelate affinity column suggest that we might have a near-complete or complete gene transfer agent particle, as many of the structural components are present. To determine if this was the case, we subjected the affinity purified GTA material to transmission electron microscopic imaging. Negative staining of the GTA particles using phosphotungstic acid revealed phage-like structure expected for GTA (Figure 3).² While many of the particles appeared to be capsids only, there were numerous particles with tails visible. The absence of tail fibers on many of the particles was expected given the relatively low abundance of these proteins on SDS-PAGE. The size of the particles was calculated to be 34.2 ± 4.3 nm (mean $\pm$ SD, $n = 20$) for particles with tail structures visible and 37.1 ± 3.6 nm (mean $\pm$ SD, $n = 90$) for particles without tail structures. The sizes of the particles were consistent with or larger than that found previously for GTA.^{2,15} It is also possible that the particles we isolated by NiNTA chromatography are a mixture of phage head intermediates from the prohead II stage to the mature capsid.²²

The fact that GTA particles can be found in the solution outside the cells that have assembled around a C-terminally modified capsid protein provides a solid basis for the idea that we can use such particles as platforms for specific modifications in a manner similar to that developed for other systems.^{23–30} Although it remains unclear whether the exit of the GTA from the cells requires cell lysis, purification can be accomplished directly from the culture medium. We were not able to isolate the relatively large amounts (mg level) of GTA from either the parent Y262, or our plasmid-bearing pPORF5CTH Y262, that was suggested to be possible.² Currently, we do not understand the cause of the relatively low yield. It has been noted in the early literature that GTA yields were sensitive to subtle changes in the culturing conditions.³¹

Outside the cells, we can detect the accumulation of the 31 kDa polypeptide, consistent with the rapid processing of this protein by the product of the Orf 4, a capsid-protein specific protease that is expected to be present and functional in the prohead stages of maturation, but not be an integral component of the mature particle. This is comparable to the maturation events of the coliphage HK97, whose gp5 major capsid protein bears considerable homology to Orf5 and is processed similarly.^{32,33}

Since we may not have captured all of the proteins associated with this particle under our conditions, it should be assumed that the proteins identified in the table above represent a “minimum GTA particle”. This particle is clearly a mature form of the GTA, as it consists of proteolytically processed capsid proteins, tail fibers and a portal protein. These proteins are only expected to be found in an assembled intact particle. It should be noted that the amounts of tail proteins found are relatively low, consistent with the observations of Yen² who found that proteins of this molecular weight class were variable in their preparations of purified GTA particles.

Four proteins, the products of Orfs 6–8 and 13, known to be a part of the GTA gene cluster, are components of the purified particle. Orf 15, earlier thought to produce four independent gene products, was reassigned using *R. capsulatus* genome project data to code for a single 138 kDa protein.¹³ This is now confirmed by our mass spectrometric data, where the coverage of this protein includes peptides found throughout the protein found at a molecular weight position on the gel consistent with the prediction for a single large protein Orf15. In this reading frame, the 138 kDa still retains its rhamnosyl transferase homology¹ and therefore its presumptive function, that of assisting the GTA to recognize its host.

We did not normally detect the appearance of an unprocessed form of the 42 kDa capsid protein (the precursor form of Orf5) in the medium outside the cells. In some experiments, upon elongated exposure times during Western blotting, we could detect a faint 42 kDa band outside the cells.

In our GTA preparations, we found four other proteins that do not originate from the GTA cluster, and whose role in GTA assembly or function, if any, is uncertain. RRC01836, identified only as “hypothetical exported proteins” in the *R. capsulatus* SB1003 genomic database, is one member of a group of highly related proteins including RRC01922 and RRC00032. When searched against the database using BLASTp,¹⁹ these proteins do not convincingly match existing entries, and show only fleeting homology to other bacteriophage proteins.

The mechanism of the release of the GTA particle from *R. capsulatus* cells remains unclear. The GTA may come from a small percentage of the population which lyses. Alternatively, GTA may be “shed” from stationary-phase *Rhodobacter* cells. One of the hallmarks of GTA is that infection of this particle does not result in plaque formation or cell lysis,^{3,34,35} and does not have a lytic cycle. GTA is deposited into the solution around cells in two waves, the greatest being at stationary phase.¹² At present, there is no evidence for an active GTA-specific holin/endolysin system in this bacterium. We speculate that the export protein RRC01836 may be involved in GTA export. Proof of direct involvement of the four non-GTA cluster derived proteins we have identified above in the assembly, structure or activity of the GTA particle awaits further detailed biochemical and genetic analysis of each individual polypeptide.

A reasonable model of the GTA structural gene cluster was originally presented by Lang and Beatty^{1,13} given the information available at that time. We can extend and modify the original description based on information available in public *R. capsulatus* database and from our mass spectrometric data.

We have confirmed that Orf 15 encodes a protein of 138 kDa,¹³ based on the composition of tryptic peptides we found associated with Band 1 on our SDS-polyacrylamide gel (Figure 1). The presence and amount of this protein band appears variable in our preparations, and may be subject to proteolysis (i.e., we detect a small fragment of it in band 6 (see Table 1)), or may only be loosely bound.

Peptides corresponding to Orfs 6–9, 11 and 13 have been found in the preparation of the purified GTA particle. Orfs 9 and 11 have been proposed to be tail-related proteins. The function of Orfs 6–8 and 13 are not known. We could confirm the reading frame of Orfs 13 as corresponding to the *R. capsulatus* genomic database assignments because we found peptides in Orf 13 that match this prediction.

We have included two predicted Orf 3.5 and Orf 10.5 to update the description of the GTA cluster obtainable from the *R. capsulatus* genomic database. We have tentatively assigned Orf 14 the predicted reading frame, but this awaits experimental verification. Orf 14 contains a domain which matches phage cell wall hydrolase/peptidase, so the assignment provided is probably correct.

We cannot improve on the tentative assignments of starting position of Orfs 1, 2, 3 or 4, as the peptides we obtained did not include peptides from these predicted gene products (Orfs 1, 2, 4) or we did not observe peptides from the regions of the protein that could allow us to further refine the map (Orf 3). We expect that the organizational map of the gene cluster we present in this work will require additions and refinements as we identify and define the function of the proteins that compose the GTA particle itself or are involved in its maturation.

In summary, we have developed a system by which we can produce and purify chimeric GTA particles from solution around *R. capsulatus* cells. We have characterized these particles as to protein composition, identifying the members of the GTA structural gene cluster present in the particles. We have been able to provide experimental data that clarifies the organization of the cluster itself and have presented this information as a framework for future refinements.

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Figure 1.

Polypeptides Isolated from GTA-containing Growth Media. The figure demonstrates the polypeptide profile obtained after SDS-PAGE of material harvested from the culture medium around *R. capsulatus* Y262 cells applied to NiNTA agarose and eluted with 10 mM Tris, pH 8.0, containing 40 mM imidazole. Silver staining of the gel reveals six protein bands (labeled 1–6, Figure 1). Each band was excised, trypsin digested, and subjected to mass spectrometric analysis.

Figure 2.

GTA gene organization. The organization of the GTA gene cluster found on the chromosome of *R. capsulatus* is represented in the illustration. Gene products derived from ORFs originating in the GTA cluster, and present as components of the purified particles isolated from media outside *R. capsulatus* cells, were identified by MS/MS analysis, and are crosshatched. RRC numbers and their corresponding ORFs can be found in Table 1.

Figure 3.

Transmission electron micrograph of chimeric GTA particles. Cells of *R. capsulatus* strain Y262 bearing the pORF5CTH plasmid were grown in RCV medium and subsequently transferred to PY medium for GTA production. Whole cells were removed by centrifugation, and the supernatant solution above the cells was recovered and filtered through a Durapore 0.45 μm membrane. The GTA-containing supernatant was chromatographed on NiNTA-agarose. GTA particles could be found in fractions eluted with 10 mM Tris, pH 8.0, containing 40 mM imidazole, these fractions were concentrated, and an aliquot was stained on a grid with phosphotungstic acid at pH 5.0. Bar represents 100 nm.

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Predicted size kDa	Comments	Peptide Coverage	Sequest protein scores /probability	Sequest peptide scores
32.9	Exported protein; function unknown	<p>MIALGLGLAANGPALRRYAVNGVAPVAVLDFERHFLSHPLALTRATSATYADALRAV QTAPADTPRYDYSTGKRALLLEASATNLLPNSAQFEASWGKTRASVLANAALAPNGTMT ADKLVEDTSNNSHEFVARTGTQIAAGTSVTASIFVKAERRWFALVTADSANAFRTTYFDL QTGTLGVVSSQGAAGHVAQIVAAAGNGWYRCSVTQTQASGNFNFYPSVASANGATSPGDBG ASGLYLWGAQLEAGAAVSSVIPTTEAAAVTRAADLASVAAGSYDLRRVDAAGTAVTKGV AHPGGALTIGAGSLYLLSLFPAGAL</p>	<p>Sf = 6.36 P(pro) = 3.3×10^{-16}</p>	<p>No. of peptides = 9 Sf cut-off = 0.86</p>
19.1	Function unknown	<p>MMLENVAVNGTALPVAFERHILRLDTGFADLGAEDALISYLRRAAMRGRTAKALIS RGFRALATAWRWGDMDOTLPAPVATYALRLVDAAAGVETPVAAGWRUVPDMARPREALG AMLPMPTGGRRVEDFTAGFASWSALPVDLAQAQVLLAAQVYELRHIDGAAEGGAMPPGV MALIERWRTVRVLGRRP</p>	<p>Sf = 8.5 P(pro) = 1×10^{-30}</p>	<p>No. of peptides = 6 Sf cut-off = 0.87</p>
42.1/31.4	Fragments of Oribacterium protein	<p>MKTETKARAGTGMPEGADPVAEVKLTALAGFLKEVKGFDQDDVKTRLQQQEERVTMLQTKTYA GRHALAAAATEEAPHOKAFAYLRTGDDDDGLRGLSLEGKALNSAVAEGGYLVDPQSETIR GVLRSTASLRQIASVNVVEATSFVDLVKTDMDGSGWASETAAALSETATPQIDRITIP LHELAAAMPKASQRLDDSAFDIETWLANRIADKFAAREAAAFISGDGVDPKPTGFLTKTKV ANGAWAWGSLGYVATGAAGDFAAVNADSDAVVDLVYALGAEYRANASFMNSKTAGAVRK MKDADGRFLWADSLAAGEPARLMGYPVLIAEDMPDIAANAYAJAFGDFGNGYTTIAERPDLRLV RDPFSAKPHVLFYASKRVGGDVSDFAAIKLLKFAAS</p>	<p>Sf = 1.83 P(pro) = 3.2×10^{-11}</p>	<p>No. of peptides = 2 Sf cut-off = 0.87</p>
9.09	Function unknown	<p>MDVFAKHAVSLESPAVRHYEITPSDSTDLARRPRALRVQTTGTLVLRDETGITVTVTVFA GEILPVRPVRVLTATGTTATAVCWE</p>	<p>Sf = 5.2 P(pro) = 4.1×10^{-14}</p>	<p>No. of peptides = 5 Sf cut-off = 0.70</p>
42.1/31.4	Fragments of Oribacterium protein	<p>MKTETKARAGTGMPEGADPVAEVKLTALAGFLKEVKGFDQDDVKTRLQQQEERVTMLQTKTYA GRHALAAAATEEAPHOKAFAYLRTGDDDDGLRGLSLEGKALNSAVAEGGYLVDPQSETIR GVLRSTASLRQIASVNVVEATSFVDLVKTDMDGSGWASETAAALSETATPQIDRITIP LHELAAAMPKASQRLDDSAFDIETWLANRIADKFAAREAAAFISGDGVDPKPTGFLTKTKV ANGAWAWGSLGYVATGAAGDFAAVNADSDAVVDLVYALGAEYRANASFMNSKTAGAVRK MKDADGRFLWADSLAAGEPARLMGYPVLIAEDMPDIAANAYAJAFGDFGNGYTTIAERPDLRLV RDPFSAKPHVLFYASKRVGGDVSDFAAIKLLKFAAS</p>	<p>Sf = 2.8 P(pro) = 3.4×10^{-12}</p>	<p>No. of peptides = 3 Sf cut-off = 0.70</p>
13.8	Function unknown	<p>MSYAVAGALQAAVYQQLRADAVLAALVGTAVYDAVPPGPPLAGTYVSLGPEDVADASDKTGA GAVHDFVISVITDAAAGFATAKAAAAVSDALVGADLVLSRGRVLVGLWFLRAKARRVEKADM RRIDLVRARVEG</p>	<p>Sf = 3.28 P(pro) = 1.3×10^{-15}</p>	<p>No. of peptides = 4 Sf cut-off = 0.70</p>
14.6	Function unknown	<p>MQVTADRIKKAAMAAISSREEDLIKEQILAHPTWKVGYITYVTGRRSEIEKGFVKSVIA CALNGGVIIHNRPGA VHGVIHAGLECLQGV'SATVVAESNLKLVALVSDGHWVAVAAHGES AFHPTTNHERIGFGVMIH</p>	<p>Sf = 1.85 P(pro) = 4.5×10^{-8}</p>	<p>No. of peptides = 2 Sf cut-off = 0.70</p>

April 7.