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Research Article

TWO NEW STRAINS OF MICROALGAE Scenedesmus sp. RECENTLY ISOLATED AND IDENTIFIED BY 18S SEQUENCING FROM THE CAN GIO MANGROVE BIOSPHERE RESERVE

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ABSTRACT

The microalgae Scenedesmus, belonging to the Chlorophyta phylum, is a widely distributed freshwater algae species. Many species from this genus are found in the Can Gio mangrove biosphere reserve, where they play a significant role in the aquatic environment. In this study, two microalgae strains, Scenedesmus CG01 and CG03, were newly isolated from two different sampling sites within the Can Gio mangrove biosphere reserve. These two strains exhibit similar morphological features typical of the genus Scenedesmus. PCR amplification, sequencing, and identification based on the 18S DNA sequence confirmed that both strains, CG01 and CG03, belong to Scenedesmus sp. The 18S DNA sequences of these strains differ from those of Scenedesmus sp. CCAP 217/7 and CCAP217/8 in GenBank. The phylogenetic analysis of the 18S sequences revealsthat CG01 and CG03 are in a distinct cluster, separate from the remaining samples. This research on the isolation and accurate identification of species provides an important foundation for future studies on the application of microalgae.

Keywords: 18S; Can Gio; Scenedesmus

1. Introduction

Scenedesmus is a diverse and widely found genus of microalgae, commonly present in freshwater ponds, lakes, rivers, and streams, but seldom in brackish water environments. These microalgae can live in various environmental conditions, but prefer water bodies with mild acidity and low salinity (Canter-Lund & Lund, 1995). In addition to aquatic environments, some *Scenedesmus* species can also grow in moist soil. Similar to many other algal species, *Scenedesmus* microalgae is a group of productive organisms and an important

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food source for other species in the water body (Janse van Vuuren, 2006). Some microalgae species in this group have been utilized as food or raw material for biodiesel production (Assunção et al., 2023; Colla et al., 2020). Studies on microalgae biodiversity in the Can Gio mangrove biosphere reserve have also shown that the microalgae *Scenedesmus* have been found in freshwater environments. However, studies have mainly focused on sampling, formalin fixation, and morphological identification of the microalgae (Pham, 2017).

Morphologically, *Scenedesmus* exists as unicells or cells, and can be elliptical, oval, spherical, or rhombic. They are also commonly found in coenobia with multicellularity inside a parental mother wall with the ends of round or pointed cells aligned or staggered, sticking together, with spikes or without spines. The spikes are formed in the head of the terminal cell in the coenobia, their function is to help them defend against predation. The coenobia may consist of 2, 4, or 8 cells, and may even reach 16 to 32 cells (Janse van Vuuren, 2006). This genus exhibits a wide range of diversity, encompassing numerous species, which renders morphological identification potentially inaccurate. The inaccuracy of identification based on morphological traits stems from the diminutive size and simple structure of these microalgae. Furthermore, the rapid emergence of new species contributes to the issue, as many microalgae possess similar external morphologies (Gross, 2004). The development of identification techniques based on DNA barcoding has provided an additional basis for identifying microalgae species. In addition, DNA sequence data also provides evidence about the evolutionary origin of green microalgae, thereby contributing to correcting incorrect classifications in the old classification system (Baudelet et al., 2017).

In this study, monoclonal microalgal strains of the genus *Scenedesmus* were isolated from various locations within Can Gio mangrove biosphere reserve. These strains were meticulously identified through a dual approach, analyzing morphological characteristics and 18S ribosomal DNA sequences. Accurate taxonomic identification of microalgae species is an important basis for future research on microalgae applications.

2. Materials and methods

2.1. Collecting microalgae samples

Two algae-containing water samples obtained at two different coordinates in the Can Gio mangrove biosphere reserve are denoted as sample 1 (10.487603, 106.873403) and sample 2 (10.651512, 106.783311). The water samples were kept cold and transported to the laboratory for analysis within 48 hours. In the laboratory, the water samples were examined under an Olympus BX-10 microscope with a $100 \times$ objective to identify the presence of green algae.

2.2. Isolation of monoclonal microalgae

The goal of isolating and purifying algae is to obtain monoclonal algal strains without any contamination by other microorganisms (Fernandez-Valenzuela et al., 2021). The collected samples were observed under a Leica ATC 2000 microscope using S-eye camera software. Single microalgae cells were isolated from the natural water samples using a micropipette and transferred into the BG11-H medium to promote proliferation. Green microalgae species can grow on the agar surface. The isolation of monoclonal algal strains was continued by culturing on agarose (Gross, 2004). The solid medium was prepared by adding 10 g agarose/L of BG11-H medium, then boiled at 95°C. When the medium was cooled to around 60°C, it was poured into Petri dishes, cooled, and stored at 4°C. The cultures were cultured at a temperature of 24–26°C, under white LED light with an intensity of 40 μ mol photon/m²/s, a light/dark cycle of 14/10 h, for a period of 2 to 4 weeks until the appearance of algal colonies. Monoclonal algal colonies were then transferred into the BG11-H medium and further cultured for proliferation (Andersen, 2005). The culture medium was supplemented every two weeks and subcultured to maintain monoclonal strains of microalgae.

2.3. Methods for determining morphological characteristics

Monoclonal algal cells were observed under an optical microscope to examine their morphological characteristics and to determine the genus or family of the algal samples. Key morphological features, including cell size, shape, chloroplast structure, and modes of reproduction, was recorded, analyzed, and compared with reference microalgae in algal collections (Culture Collection of Algae and Protozoa, Central Collection of Algal Cultures) as well as with descriptions in the literature on the genus *Scenedesmus*.

2.4. DNA extraction and PCR reactions

The total DNA of monoclonal algal cells was extracted using the ABT kit (Biological Solutions Co., Ltd, Vietnam) and served as the template for the PCR reaction. The 18S rRNA gene sequence was selected for amplification using specific primer pairs. Each PCR reaction contained the following components: 12,5 μ L 2X Mytaq Mix, 0,5 μ L primer 18S-FA2 (5'-ACCTGGTTGATCCTGCCAGTA-3'), 0,5 μ L primer 18S-RB2 (5'-GATCCTTGCAGGTTCACCTACG-3') (Maltsev & Konovalenko, 2017), 0,5 μ L DNA template, and 11,5 μ L H₂O. The annealing temperature for the primer pairs was optimized by testing within the range of 50–53 °C. The products of the DNA extraction and PCR reactions were analyzed by electrophoresis on a 1% agarose gel.

2.5. Microalgae identification method based on DNA sequence comparison

The 18S rRNA PCR products were sent to 1st Base, Malaysia, for bidirectional sequencing using the primer pairs 18S-FA2 and 18S-RB2. The resulting DNA sequences were viewed and edited using ChromasPro Version 2.6.6 and SeaView 5.0.5 software. These

programs were used to align and merge the sequences to create consensus sequences. These consensus sequences were then compared with sequences available in GenBank using the BLAST tool (https://blast.ncbi.nlm.nih.gov/) for identification of monoclonal algal strains.Based on the BLAST results, species identification was conducted. If the identity between the query sequence (the 18S sequence of the isolated microalgal strain) and the sequences available in GenBank exceeds 99.0%, the strain was considered to belong to the same species as GenBank sample. If the identity is between 98.0% and 99.0%, this suggested that the microalgal strain in this study and the published strains may not be definitively the same species. In such cases, the symbol "cf." (an abbreviation for the Latin term "confer," meaning "compare to") was used, as in *Chlorella* cf. *vulgaris*, to indicate some uncertainty in the identification. If the sequence identity was less than 98.0%, the symbol "sp." is used, as in *Chlorella* sp. (Fawley & Fawley, 2020). In addition, the morphology of the isolated strain was also compared with the species in the BLAST results to provide the most accurate taxonomic data possible.

2.6. Phylogenetic tree construction

The sequences used for phylogenetic tree construction include 18S DNA sequences obtained from this study and sequences published in GenBank. The sequences selected from GenBank must have reliable species names from previously published research and are phylogenetically closely related to the isolated microalgal strains. These sequences were aligned using the MUSCLE algorithm in MEGA software. Neighbour-joining (NJ) trees, based on Kimura 2-Parameter (K2P) distances, were generated using MEGA to illustrate the molecular phylogeny. Some sequences from GenBank were also selected as outgroups during the phylogenetic analysis of microalgae strains isolated from the Can Gio mangrove biosphere reserve. The phylogenetic trees were constructed with 1,000 iterations to determine the bootstrap value.

3. Results and discussion

3.1. Results of isolation of a single microalgae strain

Green microalgae strains proliferated well on the surface of agarose containing BG11-H medium and algal colonies appeared after 1–2 weeks and are visible by eye. Following isolation and proliferation, two strains of green microalgae, initially identified as belonging to the genus *Scenedesmus* based on morphological characteristics, were successfully isolated. These two microalgae strains were denoted CG01 (isolated from sample 1) and CG03 (isolated from sample 2), respectively.

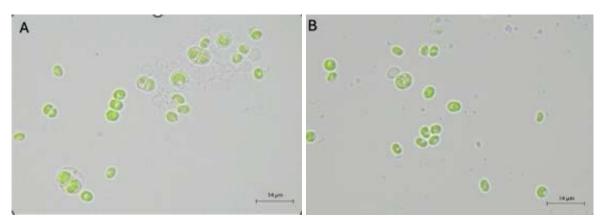


Figure 1. Morphology of two strains of green microalgae CG01 (A), CG03 (B) isolated from the Can Gio reserve

Two microalgae strains of *Scenedesmus* CG01 and CG03 exhibit similar morphology. Most of the cells are unicellular, green, ovoid, or oval, and vary in size, about 5–7 μ m long and about 2–4 μ m wide (Figure 1). Most cells of the two microalgae strains *Scenedesmus* sp. CG01 and CG03 have cup-shaped chloroplasts containing a pyrenoid (Figure 2A). The presence of a nucleus and chloroplasts containing a pyrenoid inside is one of the basic characteristics for identifying this group (Janse van Vuuren, 2006; *Scenedesmus*, n.d.).

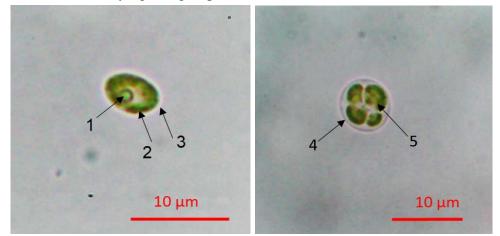


Figure 2. Cell structure Scenedesmus CG01 and CG03 (A) and asexual reproduction by spores (B) (\times 100) (1: pyrenoids; 2: chloroplasts; 3: cell walls; 4: a common wall of 4 spores, 5: spores)

The *Scenedesmus* microalgal strains CG01 and CG03 undergo asexual reproduction through sporulation, during such process, the mother cell produces 2-4 endospores. Subsequently, the mother cell forms a thick, transparent outer wall without spines, resulting in an enlarged size compared to its normal state (Figure 2B). Once the endospores attain a certain size, the sporangial cell wall ruptures, releasing the daughter spores. Previous studies have demonstrated that this asexual mode of sporulation is a common reproductive mechanism in this microalgal genus. While sexual reproduction has been documented in

some *Scenedesmus* species, it is an extremely rare occurrence (Janse van Vuuren, 2006; *Scenedesmus*, n.d.).

3.2. Results of DNA extraction, PCR, and 18S sequencing

Total DNA electrophoresis results showed high DNA extraction efficiency; DNA quality guaranteed for PCR reaction (Figure 3A).

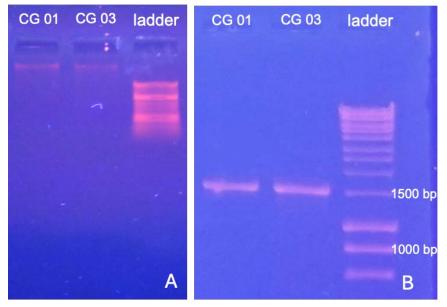


Figure 3. Electrophoresis results of total DNA (A) and PCR products of the 18S region of two isolated strains of green microalgae (B)

PCR reactions were performed using template DNA extracted from two *Scenedesmus* strains, CG01 and CG03. After adjusting the primer annealing temperature to 51°C, the PCR reactions were successfully performed on both strains (Figure 3B). The PCR product size was approximately 1,500 bp. The sequencing results and consensus 18S sequences of the two *Scenedesmus* strains are summarized in Table 1.

	- J	0	
Strain	18S-FA2 primer (bp)	18S-RB2 primer (bp)	Consensus sequence (bp)
CG01	1003	985	1512
CH03	991	989	1524

Table 1. 18S sequencing results after correction and consensus sequences

 of two isolated microalgae strains

3.3. The identification of algae strains is based on the 18S sequencing BLAST results

Comparisons of the 18S nucleotide sequences of strain CG01 and CG03 with those in GenBank showed the highest identity (99.85%) to *Scenedesmus* sp. CCAP 217/7 and CCAP217/8 (accession numbers FN298924.1 and FN298925.1) (Figure 4). However, the BLAST results showed that the query cover between the available sequences in GenBank and the 18S sequence of CG01 was only 66%. Alignment showed that the 18S sequence of CG01 had a segment that did not match the sequences of microalgae species available on

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GenBank. This segment is 478 bp long, from position 378 to 855 of the CG01 sequence (Figure 5). The BLAST result of the CG03 microalgae strain also yielded similar results. This can be explained by the fact that the CG01 and CG03 microalgae strains carry a mutation that inserts a 478 bp sequence segment into the 18S region of the genome.

~	select all 100 sequences selected	<u>GenBank</u>		Graphics		Distance tree of results		MSA Viewer	
	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
~	Scenedesmus sp. CCAP 217/8 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene	Scenedesmus s	1256	1882	66%	0.0	99.85%	2944	FN298925.1
~	Scenedesmus sp. CCAP 217/7 18S rRNA gene (partial), ITS1, 5.8S rRNA gene. ITS2 and 28S rRNA gene	Scenedesmus s	1256	1882	66%	0.0	99.85%	2944	FN298924.1
~	Coelastrum sphaericum isolate CCAP 217/2 small subunit ribosomal RNA gene, partial sequence	Coelastrum sph	1251	1865	66%	0.0	99.71%	1095	MG022737.1
~	Coelastrum sphaericum strain CCAP 217/3 small subunit ribosomal RNA gene. partial sequence	Coelastrum sph	1251	1865	66%	0.0	99.71%	1096	OR178768.1
~	Pectinodesmus regularis strain CCAP_276-56_consensus_sequence small subunit ribosomal RNA gene_p.	Pectinodesmus	1251	1876	66%	0.0	99.71%	2557	OR168647.1
~	Coelastrum pseudomicroporum strain KLL-G006 clone c 18S ribosomal RNA gene, partial sequence: inter	Coelastrum pse	1251	1859	66%	0.0	99.71%	2426	KP726229.1
~	Coelastrum pseudomicroporum strain KLL-G006 clone a 18S ribosomal RNA gene, partial sequence; inter	Coelastrum pse	1251	1865	66%	0.0	99.71%	2426	KP726228.1
~	Pectinodesmus sp. GB1d genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete se	Pectinodesmus	1251	1882	66%	0.0	99.71%	2420	AB917099,1
~	Coelastrum proboscideum strain SAG 217-2 18S ribosomal RNA gene, complete sequence	Coelastrum pro	1251	1865	66%	0.0	99.71%	1755	KF673364.1
~	Scenedesmus regularis genomic DNA containing 18S rRNA gene. ITS1, 5.8S rRNA gene. ITS2, 28S rRNA	Pectinodesmus	1251	1878	66%	0.0	99.71%	2760	FR865732.1
~	Scenedesmus sp. HP1-22-8 genes for 18S rRNA, internal transcribed spacer 1, partial sequence	Scenedesmus s	1251	1887	66%	0.0	99.71%	2506	AB255365.1
~	Coelastrum astroideum var. rugosum strain UTEX2442 18S ribosomal RNA gene. partial sequence: interna	Coelastrum astr	1251	1865	66%	0.0	99.71%	2510	GQ375093.1
~	Coelastrum astroideum var. rugosum strain Tsarenko 18S ribosomal RNA gene, partial sequence	Coelastrum astr	1251	1865	66%	0.0	99.71%	1778	AF388377.1
~	Coelastrum sphaericum strain SAG 32.81 18S ribosomal RNA gene, partial sequence	Coelastrum sph	1251	1865	66%	0.0	99.71%	1761	AF388376.1

Figure 4. BLAST results of DNA sequencing 18S of green microalgae strains CG01 and CG03

The difference in the homologous zones of strain CG01 compared to the other strain's sequences was a single nucleotide polymorphism (SNP). Examination of the bi-directional sequencing results for CG01 and CG03 confirmed that the sequencing peaks at the SNP site were clear and reliable. Both strains of *Scenedesmus* sp. CCAP 217/7 and CCAP217/8 have image data available on the Culture Collection of Algae and Protozoa (CCAP: https://www.ccap.ac.uk/catalogue/strain-217). Morphological comparisons of the strain CG01/CG03 with those of *the Scenedesmus* sp. CCAP 217/7 and CCAP217/8 showed a high degree of morphological similarity (*CCAP*, n.d.).

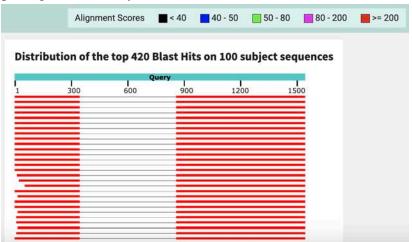


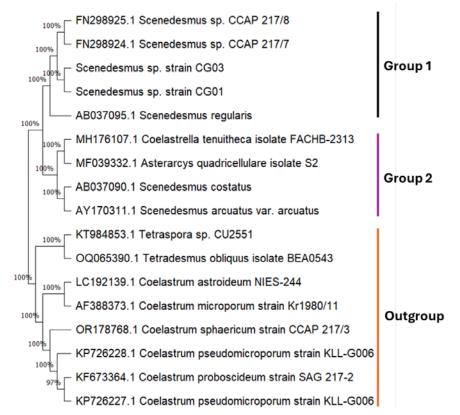
Figure 5. BLAST result of the 18S sequence of CG01 strain shows two match ranges: range 1 (site 1 to 344 of query sequence) and range 2 (site 856 to 1512 of query sequence)

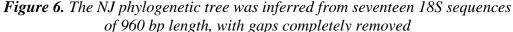
The difference in the homologous region of strain CG01 compared to the sequences of *Scenedesmus* sp. CCAP 217/7 and CCAP217/8 was one single nucleotide polymorphism (SNP). Examination of the bidirectional sequencing results of CG01 and CG03 showed that the sequencing peaks at the SNP were clear and reliable. These two strains of *Scenedesmus* sp. CCAP 217 both have image data in the Culture Collection of Algae and Protozoa (CCAP: https://www.ccap.ac.uk/catalogue/strain-217). The results of comparing the morphology of strain CG01 with those of the strains *Scenedesmus* sp. CCAP 217/7 and CCAP217/8 showed a high similarity in morphology (CCAP, n.d.).

Additionally, the BLAST results revealed that the 18S sequence of CG01 and CG03 shared a high similarity (99.71%) with various other species. For instance, they exhibit similarity with *Coelastrum sphaericum* isolate CCAP (accession number MG022737.1) and *Pectinodesmus regularis* (Synonyms: *Scenedesmus regularis*) strain CCAP_276-56 (accession number OR168647.1). However, despite these similarities, morphological comparative analyses identified distinct disparities between the strains. Therefore, based on the BLAST results, the green microalgal strains CG01 and CG03 are categorized as *Scenedesmus* sp. The taxonomic characteristics of strains CG01 and CG03 are as follows: Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; CS clade; Sphaeropleales; Scenedesmaceae; *Scenedesmus* (Nucleotide BLAST, n.d.). The difference in 18S sequences also shows that the two microalgal strains isolated in Can Gio mangrove biosphere reserve were *Scenedesmus* strains with many new characteristics.

3.4. A phylogenetic tree based on the 18S sequences

The data used to construct the phylogenetic tree included 15 selected sequences from GenBank and 2 sequences from algal strains CG01 and CG03. Alignment was performed using SeaView software with the MUSCLE option. The 478 bp insertion in the 18S of CG01 and CG03 strains was removed from the analysis data because this sequence region had no similarity to sequences available in the gene bank. Manual gap removal resulted in a 960 bp analysis region.





The results of the phylogenetic tree construction showed that all eight samples used as outgroups were concentrated in one group with an absolute bootstrap value (100) (Figure 6). The phylogenetic tree constructed by the NJ method showed that the microalgal samples were divided into three groups. The samples CG01, CG03, CCAP 217/7, and CCAP217/8 were in group 1 with absolute bootstrap values. However, the two strains CG01 and CG03 were separate from the two strains *Scenedesmus* CCAP 217/7 and CCAP217/8. Group 2 with microalgae species was closely related phylogenetically to the algal strains CG01 and CG03. The grouping results once again confirmed that the two identified microalgal strains belonging to the genus *Scenedesmus* were correct.

4. Conclusions

Microalgae strains CG01 and CG03 were successfully isolated and cultivated from two distinct sampling locations in Can Gio. These two microalgal strains exhibited morphological characteristics typical of the genus *Scenedesmus*. Comparative analysis of the 18S rDNA sequences of these two newly isolated strains with those available in GenBank revealed that these two newly isolated strains were *Scenedesmus* sp.. Studies on the growth and bioactive compounds synthesis of these two newly isolated *Scenedesmus* strains will provide information on future applications.

- Conflict of Interest: Authors have no conflict of interest to declare.
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HAI CHỦNG VI TẢO Scenedesmus sp. MỚI ĐƯỢC PHÂN LẬP TỪ KHU DỰ TRỮ SINH QUYỄN RỪNG NGẬP MẶN CẦN GIỜ VÀ ĐỊNH DANH DỰA TRÊN TRÌNH TỰ 18S

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TÓM TẮT

Vi tảo Scenedesmus thuộc ngành Chlorophyta là một trong những loài tảo nước ngọt được phân bố rộng khắp trên thế giới. Nhiều loài vi tảo thuộc chi Scenedesmus xuất hiện và đóng vai trò quan trọng trong môi trường nước ở khu dự trữ sinh quyển rừng ngập mặn Cần Giờ. Trong nghiên cứu này, hai chủng vi tảo được làm thuần, Scenedesmus CG01 và CG03, đã được phân phân lập mới từ hai vị trí thu mẫu khác nhau ở khu dự trữ sinh quyển rừng ngập mặn Cần Giờ. Hai chủng vi tảo này có đặc điểm hình thái giống nhau và điển hình của chi Scenedesmus. Kết quả PCR, giải trình tự và định danh dựa trên trình tự DNA 18S giúp xác định 2 chủng CG01 và CG03 này là Scenedesmus sp. Trình tự DNA của 2 chủng này có một số điểm khác biệt so với trình tự DNA tương ứng của Scenedesmus sp. CCAP 217/7 và CCAP217/8 trong ngân hàng gene. Cây phát sinh loài dựa trên trình tự 18S cho thấy hai chủng CG01 và CG03 ở một nhóm tách biệt với các mẫu còn lại. Các nghiên cứu phân lập và định danh chính xác tên loài cung cấp cơ sở quan trọng trong các nghiên cứu ứng dụng vi tảo tiếp theo sau.

Từ khoá: 18S; Cần Giờ; Scenedesmus

APPENDIX

ALIGNMENT OF THE 18S SEQUENCE OF THE MICROALGAL STRAIN CG01 WITH THAT OF THE STRAIN SCENEDESMUS SP. CCAP 217/8

<u>Lownload</u> ✓ <u>GenBank Graphics</u> sort by: E value ✓ <u>Next</u> ▲ <u>Previous</u> ≪<u>Descriptions</u>
 Scenedesmus sp. CCAP 217/8 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), strain CCAP 217/8
 Sequence ID: <u>FN298925.1</u> Length: 2944 Number of Matches: 2

	: 376 to		<u>Graphics</u>		Vext Match	
icore 256 bi	ts(680)	Expect 0.0	Identities 682/683(99%)	Gaps 0/683(0%)	Strand Plus/Plus	
uery	856	GAGAAACGGCTAC	CACATCCAAGGAAGGCAG	AGGCGCGCAAATTAC	CCAATCCTGATAC	915
bjct	376	GAGAAACGGCTAC	CACATCCAAGGAAGGCAGC	AGGCGCGCGCAAATTAC	ccaatcctgatac	435
uery	916	GGGGAGGTAGTGA	CAATAAATAACAATACCGG	GCATTTTATGTCTG	TAATTGGAATGAG	975
bjct	436	GGGGAGGTAGTGA	CAATAAATAACAATACCGC	dc4++++4+d+6+6+d	TAATTGGAATGAG	495
uery	976	тасаатстааатс	CCTTAACGAGGATCCATTO	GAGGGCAAGTCTGGT	GCCAGCAGCCGCG	1035
bjct	496	tacaatctaaatc	CCTTAACGAGGATCCATTC	GAGGGCAAGTCTGGT	GCCAGCAGCCGCG	555
uery	1036	GTAATTCCAGCTC	CAATAGCGTATATTTAAGT	TGTTGCAGTTAAAAA	GCTCGTAGTTGGA	1095
bjct	556	GTAATTCCAGCTC	CAATAGCGTATATTTAAGT	tgttgcagttaaaa	GCTCGTAGTTGGA	615
uery	1096	TTTCGGGTGGGTT	CTAGCGGTCCGCCTATGGT	GAGCACTGCTATGGG	сттсстттстотс	1155
bjct	616	+++cgggtgggt+	CTAGCGGTCCGCCTATGG	GAGTACTGCTATGGC		675
uery	1156	GGGGACGGGCTTC	TGGGCTTCACTGTCCGGGA	CTCGGAGTCGACGTC	GTTACTTTGAGTA	1215
bjct	676	GGGGACGGGCTTC	tgggcttcactgtccggg	CTCGGAGTCGACGTC	GTTACTTTGAGTA	735
uery	1216	AATTAGAGTGTTC	AAAGCAGGCTTACGCCCTG	AATACTTTAGCATGO	GAATAACACGATAG	1275
bjct	736	AATTAGAGTGTTC	AAAGCAGGCTTACGCCCTG	AATACTTTAGCATGO	GAATAACACGATAG	795
uery	1276	GACTCTGGCCTAT	CTTGTTGGTCTGTAGGACC	GGAGTAATGATTAAG	AGGGACAGTCGGG	1335
bjct	796	GACTCTGGCCTAT	cttgttggtctgtaggaco	GGAGTAATGATTAAG	AGGGACAGTCGGG	855
uery	1336	GGCATTCGTATTT	CATTGTCAGAGGTGAAATT	CTTGGATTTATGAAA	GACGAACTACTGC	1395
bjct	856	GGCATTCGTATT	CATTGTCAGAGGTGAAAT	CTTGGATTTATGAA	GACGAACTACTGC	915
uery	1396	GAAAGCATTTGCC	AAGGATGTTTTCATTAATC	AAGAACGAAAGTTGO	GGGGCTCGAAGACG	1455

Range 2: 56 to 400 GenBank Graphics

Vext Match A Previous Match & First Match

Score 614 bits(332)	Expect 3e-170	Identities 341/345(99%)	Gaps 1/345(0%)	Strand Plus/Plus
Query 1		AACTGC-TATACTGTGAAA		
Sbjct 56	GCATGTCTAAGTATA	AACTGCTTATACTGTGAAA	LTGCGAATGGCTCAT	TAAATCAGTTA
Query 60	TAGTTTATTTGGTGG	ТАССТТАСТАСТСССАТАА	CCGTAGTAATTCTAG	AGCTAATACGT
Sbjct 116	tagtttatttggtgg	taccttactactcggataa	ccgtagtaattctad	AGCTAATACGT
Query 120	GCGTAAATCCCGACT	TCTGGAAGGGACGTATATA	TTAGATAAAAGGCCG	ACCGGGCTTTG
Sbjct 176	dcdtaaatcccdact	tctggaagggacgtatata	ttagataaaaggeed	accgagctttg
Query 180	CCCGACCCGCGGTGA	ΑΤCATGATATCTTCACGAA	GCGCATGGCCTTGTG	CCGGCGCTGTT
Sbjct 236	ĊŦĊĠĂĊĊĊĠĊĠĠŦĠĂ	AtcAtgAtAtcttcAcgAA	6666746666644666	66666664644
Query 240		CCCTATCAACTTTCGATGG	TAGGATAGAGGCCTA	CCATGGTGGTA
Sbjct 296	ccattcaaatttctg	ċċċtatċaaċtttċċatċċ	taggatagaggççta	ccateeteeta
Query 300	ACGGGTGACGGAGGA	TTAGGGTTCGATTCCGGAG/	AGGGAGCCTGA 34	4
Sbjct 356	ACGGGTGACGGAGGA	ttAĠĠĠttĊĠÁttĊĊĠĠĂĠ,	AGGGAGCCTGA 40	0