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THE IDENTIFICATION OF SOME DUNALIELLA SPECIES UTILIZING THE GENETIC RELATEDNESS OF PROTEIN, ISOZYME AND DNA BANDING PATTERNS.

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Abstract

Although the different species of *Dunaliella* are nearly identified but some species in culture collection are still misnamed. In this work the primary and tertiary semantides were analyzed for the identification of four species of *Dunaliella*. The results of tertiary semantides including protein profile and isozymes pattern revealed that there is no species relation between the four species specially between *D. salina* and *D. bardawil*. The results of PCR-RFLP-DNA banding pattern strengthened those obtained from the isozymes and protein profile pattern and proved that *D. bardawil* is neither a synonymous species of *D. salina* nor of *D. parva* but *D. bardawil* should be regarded as separate species. This basic molecular biology technique recommended to be used in ascertaining the genetic relatedness and diversity of taxa.

Introduction

Algae of the genus *Dunaliella* are grown as a food source in aquaculture and *D. salina* and *D. bardawil* are the richest algal sources of β- carotene and glycerol. Masjuk (1973) recognized 29 species of *Dunaliella* but despite of these excellent and comprehensive works, Borowitzka and Borowitzka (1988), recorded that some of *Dunaliella* species in culture collection are still misnamed. This confusion of names and species makes comparison of results by different authors extremely difficult. Masjuk and Radenko (1973) regarded *D. bardawil* as a nomen nuda and is actually a strain of *D. salina* Toed. , Ben-Amotz and Avron (1983) regarded this genus as a new species called *D. bardawil*. Borowitzka and Borowitzka (1988) reported that *D. bardawil* is a misnamed species of *D. salina*, and *D. salina* is a misnamed species of *D. parva*. On the contrary Cowan *et al.*(1992) regarded *D. salina* that grows at bardawil lake as a variety of *D. salina* (*D. salina* var. *bardawil*). Finally, Olmos, *et al.,* (2000), ascertained that *D. bardawl* should be catalogued as other species of the genus *Dunaliella* that produces large amounts of □– carotene. Each of these authors had his own arguments. It is clear from the above ideas that the proposed names of some species of *Dunaliella* still doughtly accepted by some algologists. Therefore, the need for more trustful parameters for taxonomy is now necessary. The use of primary-, secondary-, tertiary- and epi-semantides are recommended by many

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chemotaxonomists to be the most reliable parameters for chemotaxonomy (Jayarao, *et al.*, 1991 and Olmos, *et al.*, 2000).

Consequently, the urgent need for trustful chemotaxonomic parameters in *Dunaliella*, especially, *D. salina* and *D. bardawil* is quite evident. The incorporation of chemical data into numerical taxonomy may be another approach, but according to Ragan and Chapman (1978), there still remains the vexing question of what data, how much data and weighting.

For these reasons, the present work was conducted mainly to develop a series of methodologies that would allow "in an easy and precise way" the identification of the four species of the genus *Dunaliella* namely *D. salina*, *D. bardawil*, *D. tertiolecta* and *D. parva*. in order to shed light on the interrelationship of these four *Dunaliella* species specially *D. salina* and *D. bardawil* through the analyses of some semantic molecules as criteria for chem-classification. Of these are the techniques of protein profile, isozymes pattern and PCR-RFLP DNA profile.

The resulting bands of these techniques may then be used for distinguishing sub- species (Gow and Gadd, 1995). The last technique has been used in the identification of various genera of higher plants (Hu and Quiros, 1991; Williams, *et al.*, 1991; Yang and Quiros, 1993). RAPD –PCR was used also in the identification of some algae such as *Sargassum* (Chai-Ling Ho, *et al.*, 1995) and *Anabaena* and *Microcystes* (Neilan, 1995 and Ashraf, 1998).

Materials and Methods

The biological material used in this work were the axenic four species of the unicellular green alga, *Dunaliella*. Namely *D. salina* , *D. bardawil*, *D. tertiolecta* and *D. parva* were obtained from UTEX-the culture collection of algae, at the University of Texas at Austin.

The axenic cultures of the four studied *Dunaliella* species were grown in MH medium (Loeblich, 1982) and grown in50 ml MH medium in 250 ml Erlenmeyer pyrex-glass flasks under controlled laboratory conditions (temperature at 25˚C ± 3˚C and light at 4000 lux). The starting inoculum was 1 ml (containing 0.4 x 10^{6} cells/ml). Culture experiments were conducted under a regime of 16 hour light/ 8 hour dark.

The cells of 8-10 days old cultures were harvested by centrifugation at 10.000 r.p.m. for 20 min using angle rotor centrifuge. The algal pellets obtained after centrifugation kept in refrigerator for further analyses.

Protein profile and isozymal analyses

For protein analyses the frozen cell pellets was ground and extracted by using 0.5 M HCl buffer. After centrifugation the supernatant was dialyzed by using the activated dialyses tubing over a sucrose bed. The concentrated samples were transferred into small tubes and few drops of glycerol were added to increase viscosity and 2-3 drops of bromo phenol blue were added as an electrophoretic marker. The obtained samples were kept in the freezer for total protein profile

analyses and isozymes. Discontinuous disc electrophoresis technique was applied according to Scandalios (1969).

Gels containing the segregated soluble proteins were fixed in 12.5% trichloroacetic acid for 10 min. and then stained overnight in a 0.04% PAGE- blue G-90 dye in 3.0% perchloric acid. The gels were then fixed in 7% acetic acid.

For isozyme analyses the recovered gels were transferred directly to the appropriate staining reaction mixture as described in the original literatuer, Malate Dehydrogenase, MDH (Shaw and Koen, 1964) and α-Estrase, Glutamate Dehydrogenase, GDH and Succinate Dehydrogenase, SDH (Shaw and Prasad, 1970).

Extraction, purification and PCR amplification of DNA

DNA was extracted according to Neilan (1995). PCR reaction was performed in a total volume of 100µl, containing 50ng of chromosomal DNA dissolved in TE (Tris EDTA) buffer (pH8) (Sambrook, *et al.,* 1989) and 200 ng of each of the two primers (MA1 and MA2) were added. The reaction mixture was overlaid with a minute drops of light mineral oil (Sigma). The amplification was performed using a thermal cycler (Perkin Elmer PCR system 2400) programmed for 1 cycle at 95˚C for 5 min and 40 cycles of 40 sec at 94˚C, 1min at 37˚C and 2 min at 72˚C, and a final extension at 72˚C for 12 min.

Primer code	Sequences
5’----------3’	
MA1	GGGGATCCGTAGTCATATGCTTGTCTC
MA2	CGGAATTCCTTCTGCAGGTTCACC

Digestion of the PCR products

Once the size of the PCR products had been identified, 5µl of each reaction were digested with BstUI restriction enzymes under appropriate conditions. PCR products were digested for 3h in a 30- µl volume. Digestion was stopped using loading buffer solution (Sambrook, *et al.*, 1989) and kept at -20˚C.

RFLP analyses

Digestion of the DNA PCR products from the four species of *Dunaliella* were analyzed by electrophoresis in 1.5 % agarose gels with 0.5x TBE buffer (pH 8) and detected by staining with ethidium bromide solution (stock solution of 10 mg/ml in distilled water) for 30 min. and analyzed to determine the RFLP profile of the species. Gels were then photographed under UV light.

Results and Discussion

Protein profile of the four studied *Dunaliella* species

From the results obtained concerning total soluble protein profile (Table 1 and Figure 1) in approximaetely all gel the bands were distributed throughout the gel. Some bands were cathodic, others were anodic while few bands showed cathodic, anodic simmetry.

The data obtained from protein profile were statistically analyzed to evaluate the average similarity matrices, dendrograms and steriograms of the investigated *Dunaliella* species. Electrophoretic separation of total proteins was recommended by many authors for the identification and differentiation of micro-organisms (Yamad *et al.*, 1987; Ibrahim *et al.*, 1990 ; Ibrahim and Abu- seada, 1991 and Livna *et al.*1992).

Almost in all gels 18 different individual bands (as a total) with different Rf- values were obtained. Six bands were found to be common in all the four species of *Dunaliella*. On the other hand, some bands were found to be specific for each species. The average similarity matrix and dendrogram (Table 2) showed that the highest values were between *D. salina* and *D.parva* (88%) while the lowest values were between *D. salina* and *D.bardawil* (55%).

Table (1): Protein profile bands pattern of the four studied *Dunaliella* species.

Species / **Band**	***D. Salina***	***D. bardawil***	***D. tertiolecta***	***D. Parva***
1	-	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	-	+	+
6	+	+	+	+
7	+	-	+	+
8	+	+	+	+
9	+	+	+	+
10	+	-	+	+
11	-	+	-	-
12	+	-	-	+
13	-	+	-	-
14	-	-	-	+
15	-	-	+	-
16	+	-	+	+
17	-	+	-	+
18	-	+	-	-

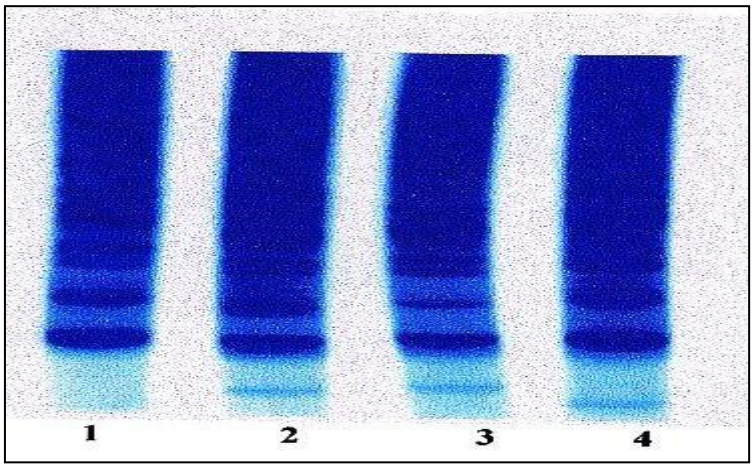

Figure (1) Protein profle bands pattern of *D. salina* (lane 1), *D. bardawil* (lane 2), *D. tertiolecta* (lane 3) and *D. parva* (lane 4).

This indicates that *D. salina* and *D. bardawil* are separate species. This fact coincides with idea of Ben-Amotz and Avron (1983) who regarded *D. bardawil* as a new species , and against the idea of Masjuk (1973) and Cowan *et al*., (1992). The first author regarded *D. bardawil* as a strain of *D. salina* and the second author regarded *D. bardawil* as a variety of *D. salina* and called it *D. salina* var. *bardawil*.

Table (2): The similarity matrix of the four studied *Dunaliella* species in accordance to their total soluble protein profile bands pattern.

Operational taxonomic units	***D. Salina***	***D. bardawil***	***D. tertiolecta***	***D. parva***
D. salina	---	55%	87%	88%
D. bardawil	---	---	61%	64%
D. tertiolecta	---	---	---	85%
D. parva	---	---	---	---

Isozymes pattern of the four studied Dunaliella species:

The isozymes pattern of the four *D.* species concerning α- estrase, malate dehydrogenase, glutamate dehydrogenase and succinate dehydrogenase were studied by gel electrophoresis. Zymograms (Fig. 2-5), represented the patterns of these enzymes. The results obtained from all the four studied *Dunaliella* species, concerning these four isozymes profile gave (7) bands for α- estrase, (5) bands for

both malate and succinate dehydrogenase , while glutamate dehydrogenase gave (6) bands.

The average similarity matrix and dendrogram of isozymes profile (Tables 3-6) revealed that:For α- esterase enzyme *D. salina* showed resemblance to the other four members of *Dunaliella* (80% resemblance). The least resemblance was between *D. bardawil* and *D. tertiolecta* (60%). For malate dehydrogenase (MDH) a complete similarity (100%) was found between *D. salina* and *D. tertiolecta* and between *D. bardawil* and *D. parva* was observed while, the least relationships (57% resemblance) were between *D. salina* and *D. bardawil , D. salina* and *D. parva, D. bardawil* and *D. tertiolecta , D. tertiolecta* and *D. parva*. This enzyme represents the most polymorphic one, hence it has the maximum number of bands (7 bands) of all the studied enzyme system. Many of literatures reported the same results of this enzyme (Thomas and Delcaripo, 1971; Mohammad, 1981; Shaalan and Chapman, 1983 a, b & c; Shaalan and Mohammad, 1985; Mohammad and Shaalan, 1985; Abd-El-Kareem, 1993 and Abd-El-Salam, 1997).

From the above mentioned discussion, concerning the results of malate dehydrogenase, it appeared that these results confederate those of Ben- Amotz and Avron (1983) and stand against those proposed by Masjuk (1973) and Cowan et al., (1992).

For succinate dehydrogenase enzyme the pattern of the four species of *Dunaliella* showed complete similarity between *D. salina* and *D. parva* and between *D. bardawil* and *D. tertiolecta.*

For glutamate, dehydrogenase *D. salina* and *D. parva* gave complete similarity (100% relationship). The resemblance between the other two species *D. bardawil* and *D. tertiolecta* were the least.

Pontikis *et al*.(1980) pointed out that the most commonly used markers in chemotaxonomy are the cytological traits. In this field, an increase in efficiency and resolving power could be achieved by using isozymes electrophoresis. The different molecular forms of an enzyme arising from any cause are called isozyme (Markerd and Moller, 1959).

These isozymes form a banding pattern (zymogram) that used in marker assisted characterization and selection within a single species many variants of an enzyme may often be recognized (Rothe, 1994). The appearance of bands on the gel reflex the genotype of isolates, since the loci (alleles) were identified according to the relative mobilities of their bands (Abd El-Salam, *et al*., 1981).

From the above discussion, it could be concluded that there is no species relation between the four *Dunaliella* species specially *D. salina* and *D. bardawil*.

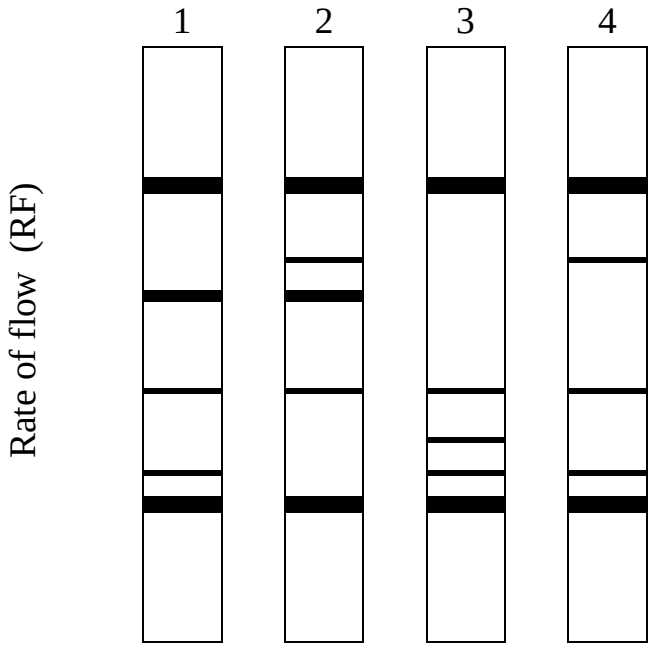

Figure (2): Line diagram of α- Esterase zymograms of the four studied *Dunaliella* species. *D.salina* (lane 1), *D.bardawil* (lane 2), *D. tertiolecta* (lane 3) and *D. parva* (lane 4).

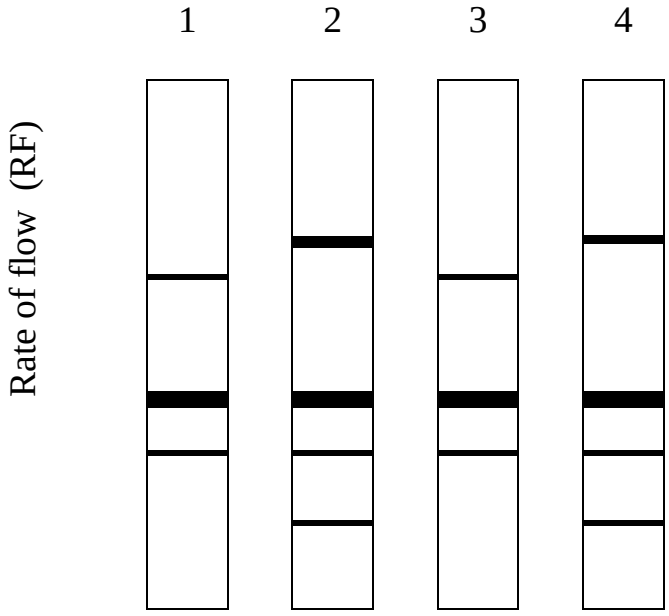

Figure (3): Line diagram of Malate dehydrogenase zymograms of the four studied *Dunaliella* species. *D.salina* (lane 1), *D.bardawil* (lane 2), *D. tertiolecta* (lane 3) and *D. parva* (lane 4).

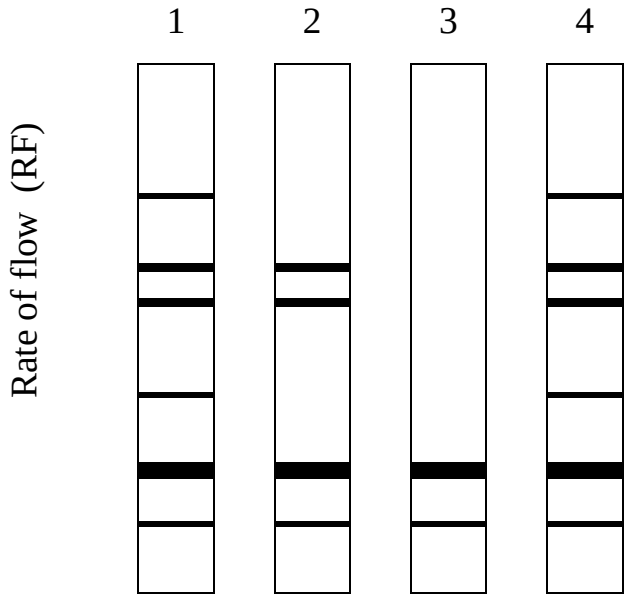

Figure (4): Line diagram of Glutamate dehydrogenase zymograms of the four studied *Dunaliella* species. *D.salina* (lane 1), *D.bardawil* (lane 2), *D. tertiolecta* (lane 3) and *D. parva* (lane 4).

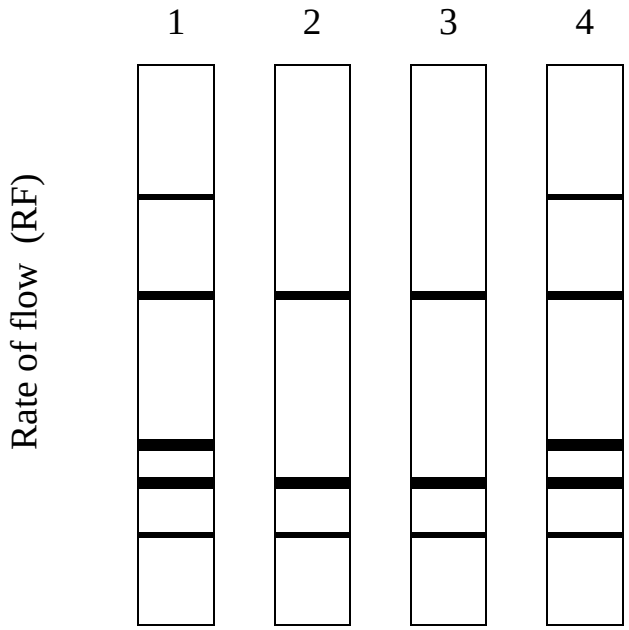

Figure (5): Line diagram of Succinate dehydrogenase zymograms of the four studied *Dunaliella* species. *D.salina* (lane 1), *D.bardawil* (lane 2), *D. tertiolecta* (lane 3) and *D. parva* (lane 4).

Table (3): The similarity matrix of the four studied *Dunaliella* species in accordance to their α-Esterase zymograms.

Operational taxonomic units	***D. Salina***	***D. bardawil***	***D. tertiolecta***	***D. parva***
D. salina	---	80%	80%	80%
D. bardawil	---	---	60%	80%
D. tertiolecta	---	---	---	80%
D. parva	---	---	---	---

Table (4): The similarity matrix of the four studied *Dunaliella* species in accordance to their Malate dehydrogenase zymogram (MDH).

Operational taxonomic units	***D. Salina***	***D. bardawil***	***D. tertiolecta***	***D. parva***
D. salina	---	57%	100%	57%
D. bardawil	---	---	57%	100%
D. tertiolecta	---	---	---	57%
D. parva	---	---	---	---

Table (5): The similarity matrix of the four studied *Dunaliella* species in accordance to their Glutamate dehydrogenase zymogram (GDH).

Operational taxonomic units	***D. Salina***	***D. bardawil***	***D. tertiolecta***	***D. parva***
D. salina	---	80%	50%	100%
D. bardawil	---	---	66%	80%
D. tertiolecta	---	---	---	50%
D. parva	---	---	---	---

Table (6): The similarity matrix of the four studied *Dunaliella* species in accordance to their Succinate dehydrogenase zymogram (SDH).

Operational taxonomic units	***D. Salina***	***D. bardawil***	***D. tertiolecta***	***D. parva***
D. salina	---	75%	75%	100%
D. bardawil	---	---	100%	75%
D. tertiolecta	---	---	---	75%
D. parva	---	---	---	---

PCR-RFLP - DNA technique:

The restriction fragment length polymorphism of DNA (RFLP) techniques in conjugation with polymerase chain reaction (PCR) was used since it is sensitive and rapid because the entire genome of an organism is used as the basis for generating the DNA profile. The results obtained from DNA banding patterns of the four *Dunaliella* species generated by RFLP- PCR showed a good reproducibility (Table 7 and Fig.6).

A total of 23 DNA bands for the four studied *Dunaliella* species were detected by performing a PCR reaction using two primers (MA1 and MA2) followed by a digestion with BstUI restriction enzyme. This procedure identified and differentiated the four different species of *Dunaliella*. In general the sizes of the bands of the fragments are ranged from lower than 1K bp up to 8K bp approximately. The profiles were generated in most cases ranged from 5 to 7 bands. The uninformative bands, which were common, of all tested isolates were the bands number 1,7 and 11. The informative bands, which were specific to only one isolate, were 5 bands. These bands were number 2 for the isolate (*D. salina*), number 3 for the isolate (*D.tertiolecta*), numbers 4 and 5 for the isolate (*D.bardawil*), and the band number 8 for the isolate (*D. parva*). While the band number 6 shared with the isolates (*D. salina* and *D. tertiolecta*) and the bands number 9 and 10 were shared with the isolates (*D. salina* and *D. parva*) (Table 7).

The average similarity matrix (Table 8) revealed that the highest values were between *D. salina* and *D. parva* (76%). While the least were between *D. salina* and *D. bardawil, D. bardawil* and *D. tertiolecta* (50%) and between *D bardawil* and *D. parva, D. tertiolecta* and *D. parva* (54%). This indicates the incomplete similarity between the four species specially between *D. salina* and *D. bardawil*. The results confirmed also that *D. salina*, *D. bardawil, D. tertiolecta* and *D. parva* are four different species of *Dunaliella*.

Table (7): PCR- RFLP DNA banding pattern of the four studied *Dunaliella* species.

Species / Band	*D. Salina*	*D. Bardawil*	*D. tertiolecta*	*D. Parva*
1	+	+	+	+
2	+	-	-	-
3	-	-	+	-
4	-	+	-	-
5	-	+	-	-
6	+	-	+	-
7	+	+	+	+
8	-	-	-	+
9	+	-	-	+
10	+	-	-	+
11	+	+	+	+

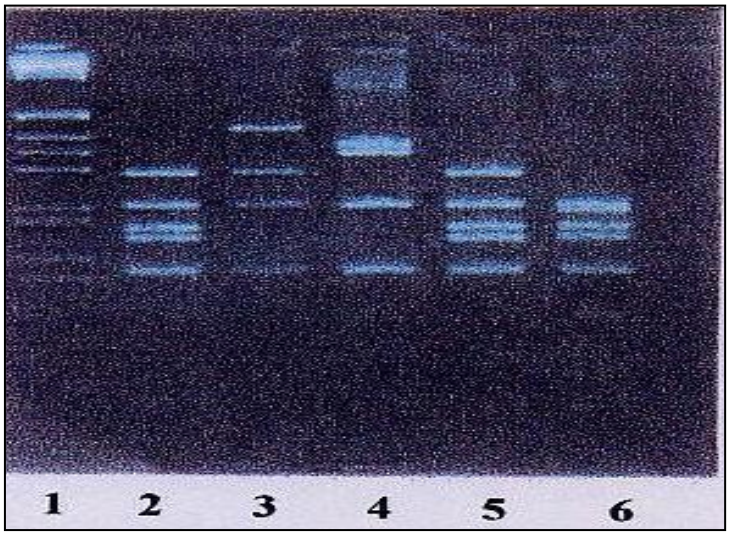

Figure (6): PCR-RFLP DNA Banding Pattern of the Four Studied Dunaliella Species marker (lane 1) *D.salina* (lane 2 and 5), *D. tertiolecta* (lane 3), *D.bardawil* (lane 4) and *D. parva* (lane 6).

These results support the idea recommended by many authors that the PCR-RFLP-DNA technique could be used in ascertaining the genetic relatedness and diversity of taxa (Olsen *et al*., 1986 ;Gobel *et al*., 1987; Hu and Quiros, 1991; Williams, *et al.*, 1991; Yang and Quiros, 1993; Gow and Gadd, 1995; Chai-Ling Ho, *et al.*, 1995; Neilan, 1995 and Ashraf, 1998).

The results of DNA banding may gave a relable conclusion that strengthen the obtained results of isozymes and protein patterns. These basic molecular biology techniques could be used in ascertaining the genetic relatedness and diversity of taxa. The latter ideas were ascertained by many authors.

Table (8): The similarity matrix of the four studied *Dunaliella* species in accordance to their PCR- RFLP DNA banding pattern.

Operational taxonomic units	***D. Salina***	***D. bardawil***	***D. tertiolecta***	***D. parva***
D. salina	---	50%	61%	76%
D. bardawil	---	---	50%	54%
D. tertiolecta	---	---	---	54%
D. parva	---	---	---	---

However, our results support the idea of Ben- Amotz and Avron (1983) and Olmos *et al*., (2000) that *D. bardawil* is not a misnamed species of *D. salina*, nor could *D. salina* be a misnamed species of *D. parva*, as was reported by Borowitzka and Borowitzka (1988). *D. bardawil* represents, and should be catalogued as, other species of the genus *Dunaliella* that produce large amounts of β- carotene. These results raise great possibilities about the application of these methodologies in the identification of natural samples.

References

Abd El-Salam, A.Z.; El-Adl, A.M.; Kosba, Z.A. and Adham, I.M. (1981). Isozyme polymorphism in Drosophila. Egypt. J. Genet. Cytol. 10:210- 215.

Abd-El-kareem,M.S.M. (1993). Some chemotaxonomic studies on members of Rhodophyceae growing along the mediterranean sea- shore of Alexandria. Ph.D. Thesis. Fac of Sci. Alex. Univ. Alex. Egypt.

Abd-El-Salam, E. M.F. (1997). Comparative taxonomic studies on Charophytes in relation to other green plants.Ph. D. Thesis, Fac.of Sci. Alex. Univ. Alexandria, Egypt.

Ashraf, B. A.H. (1998). Genetic studies on some microalgae. M.Sc. Thesis, Fac.of Agriculture, Ain Shams Univ. Cairo, Egypt.

Ben-Amotz, A. and Avron, M. (1983). Accumulation of metabolites by halotolerant algae and its industrial potential. Annual Review of Microbiology. 37: 95-119.

Borowitzka, M.A. and Borowitzka, L.J (1988). *Dunaliella* in, Borowitzka, M.A. and Borowitzka, L.J. (eds). Microalgal Biotechnology. Cambridge Univ. Press, 27-58.

Chai-Ling Ho; Morphang, S. and Pang, T. (1995). Application of polymerase chain reaction (PCR) testing randomly amplified polymorphic DNA (RAPD) primers in the molecular identification of selected *Sargassum* species. Eur. I. Phycol. 30: 273-280.

Cowan, A.K.; Rose, P.D. and Horne, L.G. (1992). *Dunaliella salina*: A model system for studying the response of plant cell to response. J. Experimental. Botany. 43: 1535-1547.

Gobel, U. B.; Geiser, A. and Stanbridge, E.J. (1987). Oligonucleotides probes complementary to variable regions of ribosomal RNA discriminate between *Mycoplasma* species. J.General Microbiology. 133: 1969-1974.

Gow, N.A.R. and Gadd, G.M. (1995). The growing fungus (eds. Chapman and Hall), London SEI 8HN, UK.

Hu, J. and Quiros, C.F. (1991). Identification of broccoli and cauliflower cultivars with RAPD markers. Plant cell Rep. 10: 505-511.

Ibrahim, S.A. and Abu Seada M.N. I. (1991). Phylogenetic relationship between various yeasts by means of isoelectric focusing of soluble protein in polysaccharide and agarose gels. Zagazig Agric. Res. 18(1): 69-82.

Ibrahim, S.A.; Laila, M.A. and Donhauser, S. (1990). Fingerprinting and alcohol dehydrogenase polymorphism in different yeasts. Egypt J. Genet. Cytol. 19: 131-142.

Jayarao, B. M.; Doré, J. E. ; Baumbach, G. A. ; Matthews, K. R. and Oliver, S. P. (1991). Differentiation of *Streptococcus uberis* from *Streptococcus parauberis* by polymerase chain reaction and restriction fragment length polymorphism analyses of 16S ribosomal DNA. Journal of Clinical Microbiology. 29: 2774-2778.

Livna, A.; Nelson, E.Y. and Sukenik, A. (1992). Immunological cross reactivity among photosynthetic proteins from various marine unicellular algal species. Botanica Marina. 35: 181-187.

Loeblich, L.A. (1982). Photosynthesis and pigments influenced by light intensity and salinity in the halophilic *Dunaliella salina* (Chlorophyta). J. Mar. Biol. Ass. U.K., 62: 493-508.

Markerd, C.L. and Moller, F. (1959). Multiple forms of enzymes tissue, onto genetic and specific patterns. Proceeding of National Academy of Science of the USA. 45: 753-63.

Masjuk, N.P. (1973). Morfologija, Sistemetika, Ekologija, Geograficeskoe Rasprostranenie roda *Dunaliella* Teod. Naukova Dumka, Kiev. 244

Masjuk, N.P. and Radenko, M. I. (1973). New taxons from the genus Dunaliella Teod. III. Ukr. Bot. Zh. 30: 468.

Mohammad, Y.A. (1981). Biochemical taxonomy of *Chlamydomonas* and related volvocalean genera. Ph. D. thesis. University of California. Los Angeles. U.S.A.

Mohammad, Y.A. and Shaalan, S.H. (1985). Electroohoretic studies of isozymes in some Rhodophycean marine algae from Alexandria. Bull. Fac. Sci. Alex. Univ. 25: 10-22.

Neilan, B.A. (1995). Identification and phylogenetic analysis of taxigenic cyanobacteria by multiplex randomly amplified polymorphic DNA PCR. Applied and Environment Microbiology. pp.2286-2291.

Olmos, J.; Paniagua, J. and Contreras, R. (2000). Molecular identification of *Dunaliella* sp. Utilizing the 18S rDNA gene. Letters in Applied Microbiology. 30: 80-84.

Olsen, G.J.; Lane, D.J.; Giovannoni, S.J. and Pace, N.R. (1986). Microbial ecology and evolution: a ribosomal RNA approach. Annals of Review of Microbiology. 40:337-365.

Pontikis, C.A.; Loukas, M. and Kousounis, G.(1980). The use of biochemical markers to distingish olive cultivars. Journal of Horticulture Sciene. 55 (4): 333-343.

Ragan, M.A. and Chapman, D.J. (1978). A biochemical phylogeny of the protists. Academic Press New York, San Francisco, London.

Rothe, G.M. (1994). Data evaluation population genetics and evolution in Rothe G.M. (ed.) Electrophoresis of enzymes. Springer Verlag Berlin Heidelberg. P 273.

Sambrook, K.T.; Frisch, E.F. and Maniatis, T. (1989). Molecular Cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Scandalios, J.C. (1969). Genetic control of multiple molecular forms of enzymes in plants: a review. Biochem. Gen. 3:37-79.

Shaalan, S.H.; and Chapman, D.J. (1983 a). Chemotaxonomy of symbiotic Zoochlorella. I.Biochemical heterogeneity of isolates from different strains of *Hydra viridis* (Florida strain). Bulletin of the faculty of science, Alexandria.23: 41-59.

Shaalan, S.H.; and Chapman, D.J. (1983 b). Chemotaxonomy of symbiotic Zoochlo rella. II. Effect of symbiotic process and culture conditions on the total soluble protein and isozyme profiles of some isolates from *Hydra viridis* (Florida strain). Bulletin of the faculty of science, Alexandria.

Shaalan, S.H.; and Chapman, D.J. (1983 c). Chemotaxonomy of symbiotic Zoochlorella. III.Biochemical heterogeneity of isolates from different strains of *Hydra viridis* and from different animal hosts. Bulletin of the faculty of science, Alexandria.23: 59.

Shaalan, S.H.; and Mohammad, Y.A. (1985). Chemotaxonomy of some marine algae belonging to family Ulvaceae from Alexandria sea shore. J. Fac. Mar. Sci. 4: 183-191.

Shaw, C.R. and Koen, A.L. (1964). Aspartate dehydrogenase activity of malate dehydrogenase. Biochim. Biophys. Acta. 92:397.

Shaw, C.R. and Prasad, R. (1970). Starch gel electrophoresis of enzymes-A compilation of recipes. Biochemical genetics. 4: 297-320.

Thomas, P. and Delcaripo, J.B. (1971). Electrophoretic analysis of enzymes from 32 isolates of protosiphon. Phycologia. 9: 285-292.

Williams, J.G.K.; KubeliK, A.R. Rafalski, J.A. and Tingey, S.V. (1991). DNA polymorphism amplified by arbitrary are useful as genetic markers. Nuclic Acids Res. 18: 6531-6535.

Yamad, Y.; Aizawa, K.; Matsuoto, A.; Nakagawa, Y. and Bono, I. (1987). Electrophoretic comparison of enzymes in strains of fission yeasts genera Schizosacchromyces, Octosporomyces and Hasengawea. J. Appl. Microbiol. 33: 363-369.

Yang, X. and Quiros, C.F. (1993). Identification and classification of celery cultivars with RAPD markers. Theor. Appl. Genet. 86: 205-212.

تعريف بعض انواع جنس دونـاليللا باستخدام طرز حزم البروتين والمتشابهات الإنزيمية والوحدات المنفصلة للحمض النووي DNA

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على الرغم من تعريف الكثير من انواع طحلب دونـاليللا الا انه مـا زال هنـاك شك في تعريف بعضها مما ادى الى صعوبة في مقارنـة نتـائج هذه الانـواع ولـذا فقد تناولت هذه الدراسـة اربعـة انـواع من جنس دونـاليللا باستخدام نتائج الدراسات الفسيولوجية والكيميائية التصنيفية للتفريق بينها. اثبتت نتـائج فصـل البـروتين و المتشـابهات الانزيميـة بـالهجرة الكهربائيـة انـه لا يوجـد هنـاك علاقـة ثابتـة بـين الأربعـة انـواع للدونايللا خصوصا بين دونـاليللا سـالينا ودونـاليللا بارداويل. كما سـاعدت نتـائج نظـام الوحدات المنفصلة للحمض النووي DNA باستخدام تقنية جهاز الدورات الحرارية المتكررة (PCR) واستخدام (RFLP) في تأكيد نتائج الفصل بالهجرة الكهربائية للبروتين والمتشابهات الانزيمية.

وتم التأكد أن دونـاليللا بارداويل ليست نوع مرادف لـدونـاليللا سـالينا أو دونـاليللا بارفـا لكنهـا (أي دونـاليللا بارداويل) تمثـل نـوع مستقل ويجـب اعتبارهـا نـوع آخـر للـدونـاليللا . وقد اعتبـر ان هـذه التقنيـات المرتبطة بالبيولوجيا الجزيئية يمكن استخدامها كأساس في اثبات التشابهات والاختلافات الجينية للكائنات.