The Macrotheme Review
A multidisciplinary journal of global macro trends

METAGENOMIC STUDY FROM THREE SPIRULINA STRAINS: GEOGRAPHICAL DIVERSITY

BENAHMED DJILALI Adiba*, BENAMARA Salem**, ZEGGAOUA Nassira***, CHAIBI Akila***, OUELHADJ Akli****, BOUKSAIM Mohamed***** and BENZARA Abdelmadjeid******.

**Biological and Agronomic Faculty of Science University Mouloud Mammeri of Tizi-Ouzou 15000, Algeria. Research Laboratory of Food Technology University M'hmed Bougara of Boumerdes 35000, Algeria.*
***Laboratory of Soft Technology and Biodiversity University M'hmed Bougara, Boumerdes 35000, Algeria.*
****Biologicaland Agro-veterinary Faculty ofScienceUniversity Saâd Dahlab Blida,Algeria.*
*****Biological and Agronomic Faculty of Science University Mouloud Mammeri of Tizi-Ouzou 15000, Algeria.*
******National Institute of Agronomic Research INRA Folds Back Morocco.*
*******National Institute of Agronomy (ENSA) El Harrach 16000, Algeria.*

Abstract

The aim of this study was the valorization of natural resources to produce Spirulina. For this work, the genomic description of the contents of three Spirulina strains were carried out with two strains of comestible Spirulina which produced in synthetic media in Burkina-Faso and Morocco and the new strains was isolated from Algerian natural medium using different natural resources. The IR analysis showed that the new strain and reference Spirulina from Burkina-Faso contain the same functional groupings. Based on structural and functional similarity, the new strain has changed its morphological structure and genetic composition. The similarity analysis of genetics parameters of these strains showed dissimilarity between a new strain and the comestible Spirulina from Burkina-Faso and Morocco. The analysis of the phylogenetic trees showed that the new strain is related specifically to the Oscillatoriophycideae family Scenedesmus obliquus type, which is known as a green alga of fresh water. While, the two strains from Spirulina from Burkina-Faso and Morocco which are identical were classified among the blue algae related to the Arthrospira and have the same molecular weight (22.67 KD) that is equal twice to the molecular weight of the stock (A) (10.21 kD).

Keywords: Spirulina, natural media, culture, metagenomic study

1. Introduction

Arthrospira which is *Spirulina platensis* green blue cyanobacteria is largely cultivated in the world. This species can be developed artificially in laboratory developed media and/or naturally under lake conditions.

Generally, it adapts to two many biotopes like, sands, fresh water, sea water (Tredeci and al. 1986; Wu and al. 1993; Halland 2006). Particularly, *Sp.platensis* can grow under alkaline

conditions pH higher or equal to 11 (Ciferri and Tiboni 1985). This pH caninhibit the proliferationof other species of cyanobacteria andpathogenic bacteria.

Moreover, it needs great quantities of salt NaCl (2 with water 270g/l) of carbonate and the high level of bicarbonate brought back by Materassi and al. (1984) and Fox(1999).

Several studies showed the beneficial effects of these cyanobacteria. They are classified in first rank of the food complements (Hudson and Karis 1974). It is significant to recall that the therapeutic potential of the Spirulina was already reported (Decree n° 2006/352 in Charpy2008). These last years, several substances with pharmacological interests resulting from *Sp.platensis* are available as therapeutic food (Degbey and al.2006), natural food dyes rich in phycocyanine (Jaouen and al. 1999; Spolaore and al. 2008), tablet containing powder of dates and Spirulina (Benahmed Djilali and al. 2011; Benahmed Djilali and al.2013), food of aquacultures(James and al. 2006; Kim and al. 2006) with 41570 Tones/an (Statistical FAO 2006), biodiesel microalgae (Chisti 2007) and others caused a detailed attention.

The world production of this microfood increased since 1995 by more than 4000Tones/an (Statistical CUBIA, 2000). Moreover, Algeria developed Spirulina in the synthetic medium in the south region of the country, precisely the area of Tamanrasset, but though the Spirulina produced locally does not meet the needs for the consumers.

Indeed, the production cost of Spirulina using synthetic media is often very high which results in a selling price out of by the mal nutrition phenomenon.

One of the aims of effective valorization of ecological resource is to produce Spirulina.

Nowadays, few producers manage specifically to perform the Spirulina culture without manure in Africa.

This work is articulated around the valorization of agricultural waste in order to put on the market a Bio Spirulina. Obtaining a Bio Spirulina requires a control and a perfect knowledge of the culture conditions and production of this microalga on a laboratory scale using natural media with minimum chemicals content, materials and of energy. Because of a lack of structural data on the cyanobacteria and more particularly Spirulina by developing agricultural waste, we proposed a step leading to their characterization.

The objectives of this study are:

- Contribution to the development of the Splirulina using natural resources;

- Knowledge of the culture of Spirulina on a laboratory scale on natural media complemented with a minimum chemicals quantity, materials and of energy;

- Marketing of as ubstance or product with high added value (feed ration rich in proteins and bioactive substances).

2. Material and methods

2.1. Biological material

Two strains of comestible Spirulina were used in this study. The first one was bought from France, though originally produced in a synthetic medium in Burkina-Faso (reference Spirulina). The second is originally from Morocco, produced in synthetic medium too.

2.2. Culture of Spirulina in the various natural media

2.2.1. Natural media

The culture was put out in the natural medium M_2 . This medium was optimized using different natural resources like sea water, tap water, powders of sand, poultry and ashes of wood (palm and fig) by implanting a mixture plan with $M_2 = 0{,}8S_4 + 0{,}1S_2 + 0{,}05S_3 + 0{,}05S_1$.

$S_1 = (9/1)(V/V)$ See water / Tap water;$S_2 = (1/1)(V/V)M_0/M_1$; M_0 =powder of sound (1%);M_1 = powder of poultry bone (1%); S_3= ashes of palm wood (10%); S_4 =ashes of fig wood (10%) with the knowledge that this medium is characterized by a salinity (9,5±0,1 g/l) and pH around (10,418±0,004).

2.2.2. Spirulina Batch culture

Batch culture allows analysis of the effect of the variation of natural resource on yield of growth and accumulation of specific pigments like phycocianin and several mineral compounds.

In the first time, the adaptation of the Spirulina from Burkina-Faso to the medium $M_2$was carriedout in erlenmeyer's of capacity 500ml while working under culture optimal conditions as recommended by several farmers (Fox 1999; Jourdan 2007). Respected temperature (30±02°C);daily illumination cycles with a maximal incident irradiance value of 4000 Luxes (Kosaric and al.1974), agitation and ventilation were ensured by an aquarium immersed pump (standard aquarium BOYU) under an air flow of 2 L/mn. The initial density ratio /culture medium respected is$(1\colon 100)(V/V)$. The initial medium density is 0,120.

And finally, a test of confirmation of culture in the same medium M_2 already optimized using the same Moroccan natural sources in laboratory of quality control on December 2011, Rabat – Morocco.It is noticed that 1% of Moroccan argan ashes wood was used as medium supplement. The objective of this study is to show the effect of the variation of the geographic sites on the yield of growth of Spirulina

2.3. Evaluation of the culture in the natural medium

Taking away was daily carried out in order to follow the biomass kinetics production. The biomass was evaluated by measuring the content of phycocyanin at 618 nm using a spectrophotometer (JASCO V-530). The phycocyanin component was extracted by using phosphate buffer0,05 M (pH=6,8) according to the methods reported brought back by Oliveira and al. (2008).

Yield of growth: in this research step, the green biomass was separated after 21 days from the medium M_2 by centrifugation (6000 tours/mn), then washed with sterile physiological water such as recommended by Cifferi (1983). Then the biomass was dried with 65°C during a few minutes at 65°Cusing a drying oven (MEMMERT) to have final moisture from 3 to 7 % according to Fox (1999).

The morphological structures of the powders of Spirulina from Burkina-Faso and new strain isolated from the medium M_2 were performed using the electronic microscope MEB (XL 20).

At ends of comparison, the powders of Spirulina from Burkina-Faso and new strain isolated from the medium M_2were analyzed using IR spectrophotometer with Fourier transform (SCHIMATZU–8900). The goals of this analysis are to show the absorption of the various functional groupings and the bioactive substances presence.

On another hand, the sensitivity of the three species *Pseudomonas aeruginosa, Aspergillus niger* and *Staphylococcus aureus* versus the phycocyanin extracts (C-PC) of the spirulina from Burkina-Faso and the new species isolated from the medium $M2$(stock A). To perform the sensitivity test, 10 µL of each extract were tested by the Mueller-Hinton agar diffusion method as reported by Rahal (2005). The concentration of each species was 10^7 UFC/ml, and the test was carried out in duplicate.

2.4. Identification of the new stock obtained usingthe medium$M2$

2.4.1. DNA exraction

The DNA was extracted from various Spirulina stocks using the Gen Elute Bacterial Genomic kit (Aldrich Sigma, Germany) and was quantified spectrophotometrically. The DNA was eluted with molecular-grade DNase-RNase water and stored at – 20 ^oC until further analysis.

2.4.2. PCR reaction

The analysis of 16S rRNA gene was performed with specific primers, forward primer 5'-GCTCAAGATTGAACGCTGGCG and reverse primer 5'-CGGTTACCTTGTTCGACTTCACC. PCR was carried out in a Thermo Cycler (Qtocycle HDV, Life Sciences) using Applied Biosystems reagent kit. A reaction with a total volume of 70 µL was set up by adding 4 µL DNA to 35 µL reagent and appropriate primers at 500 nM concentrations and topping up with molecular-grade water. Molecular-grade water was used us a reaction negative control. All samples and blanks were run according to the same procedures, 2 min at 94^oC for DNA denaturation, followed by 40 cycles at 94 $^oC$for 5 s, annealing temperature for 60 s, and 72 for 10 s for DNA elongation. At the end, 10 µL of PCR products were analysed in 1% DNA agarose gel and purified using the Exosap enzyme rests.

2.4.3. Sequencing analysis

Nucleotide sequence was determined by the dideoxy chain termination method using the BigDye Terminator v3.1 Cycler Sequencing Kit (Applied Biosystems), analysed using the Sotware Sequencing Analysis v5.3.1 (Applied Biosystems), and compared with the DNA using the BLAST server at NCBI (Altschul et al. 1990).

2.5. Metagenomic study

The goal of this approach is to have not only one genomic description of the contents of the sample but also an outline of the functional potential related to an environment. Individual metagenomic study of the three strains Spirulina from Burkina-Faso, Spirulina from Morocco and the new strain isolated from the medium $M2$was carried out thanks to the software of genomic annotations and the international data banks (SMS, INTERPROSSCAN, BLAST, EBI, MABL and NCBI) making it possible to have three dendrogrammes (Phylogenetic trees). The determination of the protein structure of the three stocks was made thanks to software RASMOL.

2.6. Statistical study

The Spirulina yields of growth obtained using medium $M2$ for the Moroccan and Algerian cultures were statistically treated with de Mann-Whitney test (Siegel, 1956) which allows the comparison of means of two independent samples. This non-parametric test is applied in the case of small numbers (<9) according to Siegel (1956). In order to compare the different parameters that characterize the three sequences of Spirulina from Burkina-Faso, Spirulina from Morocco and the new strain isolated from the medium $M2$, the similarity matrix was used. Because, similar

data are reported if the similarity is strictly greater than 95%. Statistical analysis was performed by XLSTAT Version 7.5.2 software.

3. Results and discussion

3.1. Evaluation of the Spirulina culture from the various natural media

The present study showed that the medium $M2$ was evaluated as an optimal medium with a good dried biomass yield (1,768±0,009g dry weight/100 ml/21 days, n=3) which was slightly higher when compared to the biomass yield obtained using the same medium$M2$ based of the Moroccan'scomponents (1,208±0,03g dry weight/100 ml/21days, *n*=3;with pH10,40±0,0013).

It is noticed that, the addition of the ashes of the argan wood to medium $M2$ produces a slight decrease on yields when Algerian wood ashes (1,208 ± 0,013 g dry weight/100 ml/21 days, $n = 3$) and Moroccan wood ashes (1,108 ± 0,03gdry weight/100 ml/21 days, $n = 3$) were used, but are not significant at a 95% probability ($Z = 0{,}775$, $\rho = 0{,}781$, $\alpha = 0{,}05$).But, this result is significant when compared to that reported by Materassi and al. (1984). These authors declare that the mean annual biomass yield obtained using sea water mixed with urea was (7,35 g dry weight/m^2/day) which was slightly lower value than that observed on the standard sodium bicarbonate medium and sea water (8,14 g dry weight /m^2/day) under controlled pH ranged from 8 to 8,3.

On another hand, reference Spirulina changes its morphology after 21 days of culture in the Algerian medium $M2$(helicoids structure of the trichome) (Fig 1(a)).It is also observed a correlation between the photosynthesis, the salinity and the morphology of Spirulina. Spirulina adapted to higher salinity showed laminated structure (Fig 1(b)).

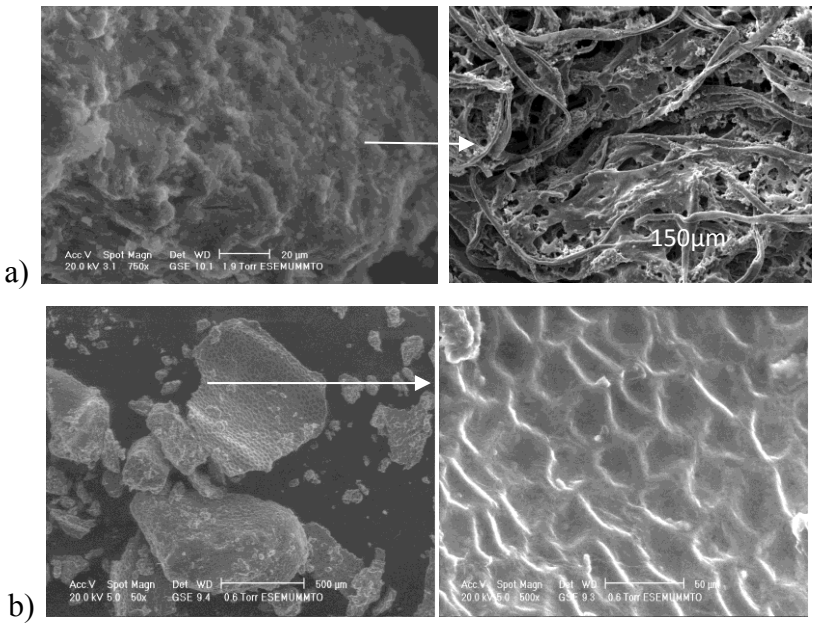


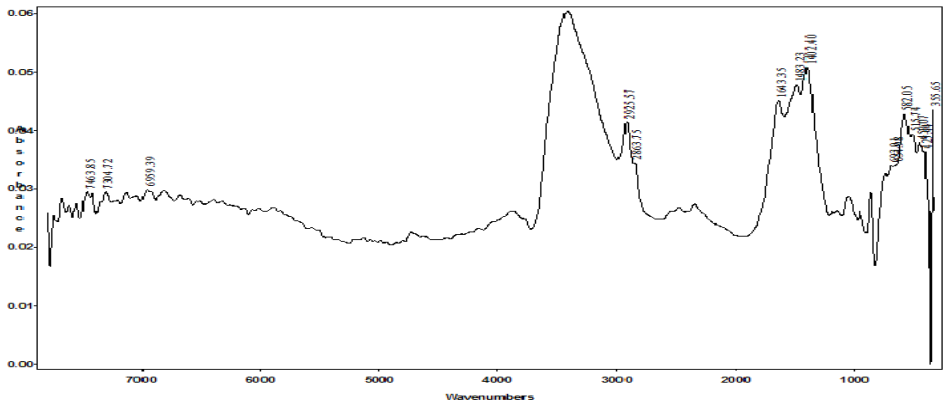
Fig 1 macroscopic structure of Spirulina isolated from Algerian natural medium $M2$

Our results are similar to those found by Lewin (1980); Jeeji Bai (1985) and Ben Dhiab and al. (2007), which showed that NaCl plays a significant role on the modification of the Spirulina morphology. The higher concentration inhibits the development of the helicoid structure and supports the laminated structure. Moreover, Bourreley (1979) showed that an insufficiency of sodium under alkaline conditions generate the phenomenon of the biomass photo-inhibition. Also, Kolchugina and Markarova (2005) suggested that sodium is a critical element and considered as limiting factor for the Spirulina growth. Particularly, several authors showed its role in photosynthesis as unique cation and can't be remplaced by other monovalent elements (k^+, Cs^+..).

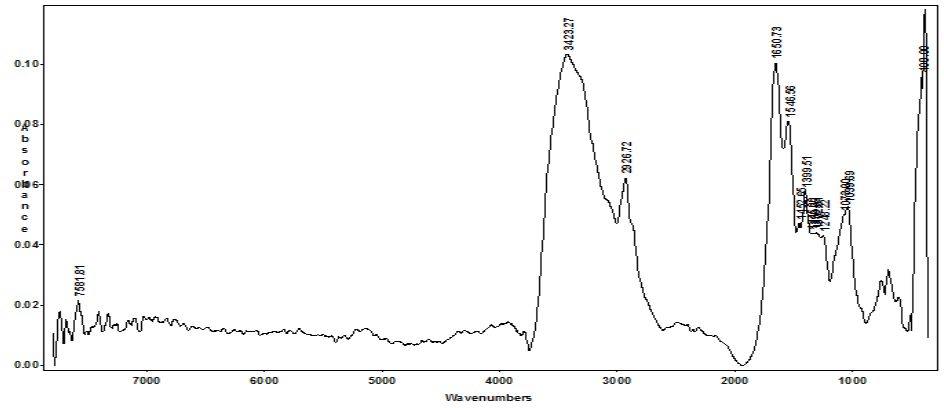
3.2. Antibacterial activity

The phycocyanin extract of the new strain isolated from the Algerian medium$M2$ showed weak average inhibition zones (9, 10 and 10,66 mm)when compared with reference Spirulina (14, 10 and 10,66mm) screw with-screw *P. aeruginosa, A. niger and S. aureus* respectly. This difference can be explained by the composition and the content of the Spirulina bioactive substances which can also be directly allotted to the culture conditions ($M2$ proves to be low in nitrogen) and the genetic properties related to the two stains.

Moreover, the phenomenon for accumulation of metals was negatively influenced by the substantial accumulation such as lipophylic and phenolic compounds which explain a difference in the position and the intensity of the peaks of functional groupings of these strains (Fig 2).



(a)



(b)

Fig 2 Absorption IR spectrum

(a) New strain isolated from the Algerian natural medium $M2$, (b) Spirulina from Burkina-Faso

These observations come to confirm those obtained with several studied algae species (Saitoh and al. 2001; Nakbanpate and al. 2002; Loukidou and al. 2004; Doshi and al. 2006).

However, the increase of antibacterial activity of reference Spirulina is allotted to the presence of the significant contents of active substances such as the essential fatty acids (C14: O (46,02%), C18: 2 (13,11) C18:3 (9,53%), C18:2 (13,11%) and with others volatile compounds such as polyphenols (5,1325% EAG, n=3) (Benahmed- Djilali and Benamara 2013).

3.3. Sequencing results

The results obtained are presented by the three following trees:

The obtained results of three strains of cyanobacteria from the different environments (Fig 3) show that the new strain isolated from the Algerian medium $M2$ is *Scenedesmus obliquus* species, a microscopic green alga of fresh water which known has a varied cellular structure. It is related specifically to the family of Oleaceae (Oscillatoriophycideae) (see reference KF290490 Gen Bank code) and its nearest orthologist which is "Phillyrealatifolia" insert clearly into the level of node 3. Phillyrealatifolia is known as a species rather close to the European olive-tree (Zarembinski and al. 1998).

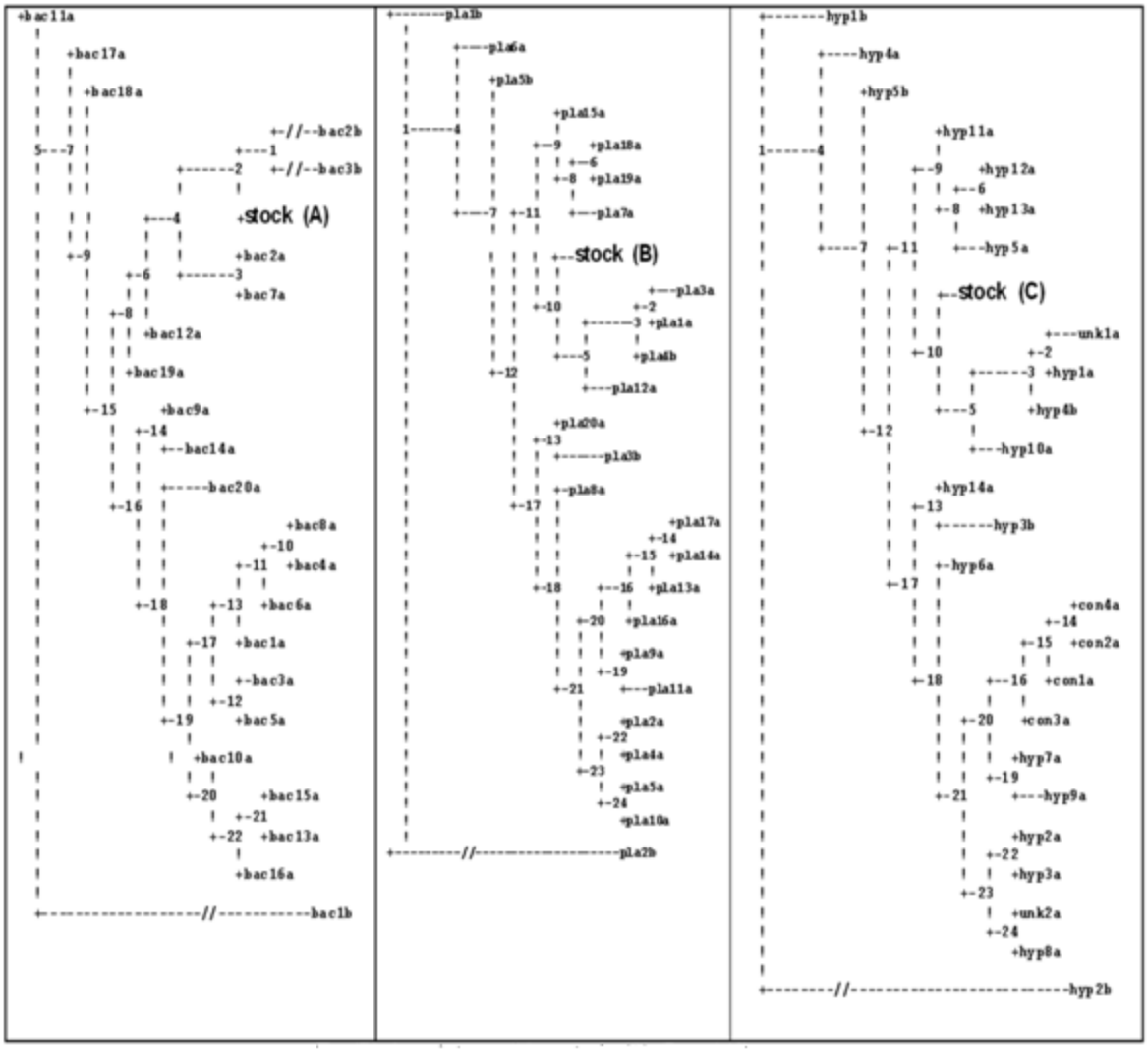


Fig 3 phylogenic trees of three strains (A), (B) and (C)

(A): New strain isolated from Algerian mediumM2 , (B): Spirulina from Burkina-Faso, (C): Spirulina from Morocco.

Noting that, the green algae have chlorophyll of the type *a* and *b* well as carotenoids. They are not mobiles and they measure approximately 5 to 30µm length and 8 to 10 µm of diameter (Ma and al.2003). It has a protein putatifa with unknown or hypothetical functions. For this strain, ORF n°1 selected was observed in the indirect direction at the time of the 2nd reading. This ORF codes for 9ÄA, it does not start with a MET and it does not contain a codon stop.

In order to find the orthologists of this strain, two groups were selected during this study. First, consists of firmicutes, eudicots monocots d-proteo bacteria having scores higher than 65 and the second forms an external group made up of apicomplexans, ascomycetes, a-proteo bacteria having scores lower than 35. However, two identical Spirulina from Burkina-Faso- and Morocco were classified among the blue algae of the Arthrospira kind are multicellular cyanobacteria. This algae type had *β*-carotene concentrations evaluated from ten to fifteen higher than the carrot one (Halidou and al. 2008). Those strains have the same orthologist that of the Heliobacteriummodesticaldum family it is an anaerobic bacterium, positive Gram,

photoheterotroph (Hai and al. 2012). Their sequences have also an identical molecular weight about (22,67 KD), which equals almost twice the molecular weight of the new strain isolated from Algerian medium M_2 (10,21 kD) and this is confirmed by the proteinic structure as shownin Fig 4.

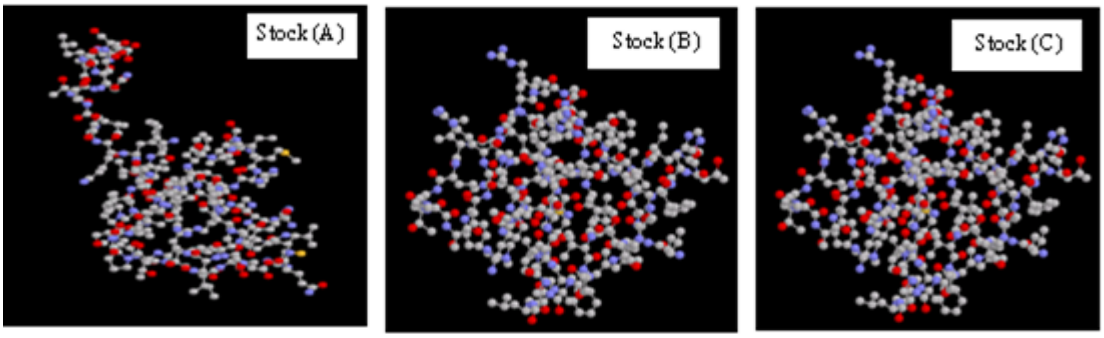


Fig 4 Structure 3D of proteins of the Strains (A), (B) and (C)

On another hand the new strain adapted to the Algerian medium $M_2$was modified genetically and this is confirmed with the change of conformation of its protein structure. It also results from it, a change of its function per contribution with two strains from Burkina-Faso and Morocco isolated from synthetic media.

Indeed, the stock (A) contains a secondary protein structure not assigned; it consists of 57groupes, 443 atoms and 454 bands. This protein consists of 68 MET and 67SER. While, Spirulina strains are completely similar and they have the same secondary protein structure not assigned, made up of 65groupes, 497atomes and 515 bands. Two structures having 53 CYS and 70 SER.

Then, the similarity analysis of genetic parameters (molecular weight, number of ORF, alignment, number of groups, number of atoms and number of amino acids (MET, CYS, SER) showed strains dissimilarity between a new strain while Spirulina strains are observed similar (Table 1).

Table 1 similarity test between the three strains

	Strain (A)	Strain (B)	Strain (C)
Strain (A)	**1,000**	0,000	0,000
Strain (B)	0,000	**1,000**	0,900
Strain (C)	0,000	0,900	**1,000**

(A) New strain cultivated in the Algerian medium M_2, (B) Spirulina of Burkina-Faso and (C) Moroccan Spirulina

Thus, this structural difference affirms the effects associated to the nature and the composition variation of the culture medium on the density of the Spirulina cells. This result is shown in concordance with those reported before by Wyman and Fay (1987); Walsby and Fay (1987); Giovannoni and al. (1988).

According to the comparison between the sequence of the new strain and that of the two Spirulina strains from Burkina-Faso and from Morocco, the modification in the succession of the

nucleic bases are probably due to genetic change. Such observation was also found and confirmed by Romano and al. (2000); Waterbury (2006). And the Fig 5 shows the profile of the genomes of these three strains.

4. Conclusion

In this study, the adaptation of Spirulina of Burkina-Faso was carried out in the natural medium $M2$. Based on structural and functional similarity, the new strain has changed its morphological structure, genetic composition. Our phylogénitic and genitic analysis confirmed that this strain is a species of the type *Scenedesmus obliquus,* green alga microscopic of fresh water.

Acknowledgments

This work was supported by grants from:

Responsible of Federation of the culture of Spirulina in France

Chemistry Laboratory University of Mouloud Mammeri of Tizi-Ouzou

Unit of Research and Development of the Intendance Beautiful Place Algeria

INRA, CNREST Rabat Morocco

Bibliographical references

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ,(1990) Basic local alignment

search tool. J Mol Biol 215, 3:403-10.

Benahmed Djilali A, Saidi N, Mekssoud A, Benamara S, (2013) Pharmacological and biological proprieties of a mixture of date powders (*Mech-Degla*) and Spirulina. The Macro Rev A multid J glob Macro Trends 2, 1:310-320.

Benahmed Djilali A, Saidi N, Mekssoud A, Benamara S,(2001) Preliminary characterization of food tablets from date (*Phoenix dactylifera L.*) and spirulina (*Spirulina sp*.) powders. J Powder Tech 208:725-730.

BenahmedDjilali A, and Benamara S, (2013) Culture of *Spirulina platensis* in media containing ashes. Ed Uni Euro.ISBN-10: 6131517800, ISBN-13: 978-6131517808 pp4.

Ben DhiabR, Ben Ouada H, Boussetta H, Franck F, Elabed A, Brouers MR, (2007) Growth, flurescence, photosynthetic O_2production and content pigment of salt adapted cultures of *Arthrospira (Spirulina platensis).*J Appl Phyco:19, 293-301.

Bourreley P, (1979) Initiatement with the systematic one. Tome 1: Green algas, Ed Nboue and Co pp 51.

Ciferri O, and Tiboni O, (1985) The biochemistry and industrial potential of *Spirulina.* Ann Rev Microbiol: 39,503-526.

Chisti Y, (2007) Biodiesel from microalgae. Biotech Adv:25,294 –306.

Decree n° 2006/352 of 20 March (2006) Official. J in Charpy and *al*. (2008). Spirulina can it be an asset for health and the development in Africa.

Degbey H, Hamadou B, Oumarou H, (2008) Evaluation of the effectiveness of the supplementation in Spirulina of the usual mode of the children reached of severe malnutrition. International Symposium one Cyanobacteria for Health, Sci and Devlop.104-108.

DoshiH, Ray A, Kothari IL, Gami B, (2006) Spectroscopic and scanning electron microscopy studies of bio-accumulation of pollutants by algae. Current Micro53: 148-157.

FAO, (2006) Fisheries Department, Fishery Information, Data and Statics Unit, Fishery Statistical time series, Aquaculture Production Quantities1950-2004. www.fao.org/fi/statist/FISOFT/FISHPLUS.asp.

Fox RD,(1999)Spirulina: Technique, Practice and Promise EDISUD, Aix in Provencepp246.

Giovannoni SJ, Turner SG, Olsen J, Barns S, Lane DJ, Pace NR, (1988)Evolutionary relationships among cyanobacteria and green chloroplasts. JBac170:3584–3592.

Halland (2006) Energy-Green-algae for carbon captures and Biodiesel ISIS.

Halidou-Doudou M, Degbey H, Daoud H, Leveque A, Donnen P, Hennart P, Dramaix-Wilmet M, (2008) Spirulina supplementation in the context of nutritional rehabilitation. J Epidemio and Pub Hea56:425-431.

Hai Y, Yisheng K, Hao Z, Xinliu G, Robert E, Blanken S, (2012) Expression and characterization of the diheme cytochrome c subunit of the cytochrome bc complex in Heliobacteriummodesticaldum.Arch Bioch and Bioph517:131-137.

Hudson BJF, and karis IG, (1974) The lipids of the alga Spirulina. J Sci and Food. Agr 25: 759-763.

James R., Sampath K, Thangarathinam R, Vasudevan I, (2006) Effect of dietary *Spirulina* level one growth, fertility, colouring and *leucocyte* count in red swordtail *Xiphophorushelleri.* Israeli Newspaper of Aquaculture Bamidegeh 58: 502-503.

Jaouen P, Lepine B, Nightingale NR, (1999) Clarification and concentration with membrane technology of phycocyanin solution extracted from *Spirulina platensis.* Tech Biotec13:877-881.

Jeeji-Bi NR, (1985) Comparative exclusion gold morphological transformation with box stady with *Spirulina fusiformis.*Arch Hydrobiol Suppl Algal Studay71, 191:38-39.

Jourdan JP, (2007) Report of the Mini Conference of Mialet on the production of the artisanal Spirulina. pp1-7.

Kim CJ, Yoon SK, Kim HI, Park YH, Oh HM, (2006) Effect of *Spirulina platensis* and probiotics have feed additive one growth of shrimp fenneropenaeus chinensis. J Microbiol Biotec16:1248-1254.

Kol chugina IB, and Markarova EN, (2005) Role of sodium ions and to their uptake by cells of cultured blue-Green algae *Spirulina platensis* and *Spirulina maxim.* Micro Flight74, 6: 646-649.

Lewin RA, (1980) Uncoiled variable of *Spirulina platensis* (cyanophyceae: Oscillatoriaceae). Algal Stud Arch Hydrobiol Suppl60, 26:48-52.

Loukidou MX, Zouboulis AI, Karapantsios TD, Matis KA,(2004) Equilibrium and kinetic modeling of chromium (VI) biosorption by *Aeromonas caviae* Colloids and surface A. Physico Chem Eng Asp242:93 –104.

Ma M, Zhu WZ, Wang ZJ, Witkamp GJ, (2003) Accumulation, assimilation and growth inhibition of copper on freshwater algae (*Scenedesmus subspicatus* 86.81 SAG) in the presence of EDTA and fulvic acid.Aquatic Toxico63:221-228.

Materassi R, Tredici M, Balloni W, (1984) Spirulina culture in sea water. Applied MicrobiolAnd Biotech 19:384-386.

Nakbanpate W, Thiravetyan P, Kalambaheti C, (2002) Comparison of gold adsorption by *Chlorella vulgaris* rice husk and activated carbon. To undermineEng15:549-552.

Oliveira EG, Rosa GS, Moraes MY, and Pinto LAA, (2008) Content Phycocyanin of *Spirulina platensis* dried in spouted bed and thin to bush-hammer. J Food Eeng and Process 31:34 -50.

Rahal K, (2005) Standardization of the antibiogramme in Human Medicine on a National Scale according to recommendations' of WHO, 4th Ed, Ed Ministry for Health, the Population and the Hospital Reform.

Romano I, Bellitti MR, Nicolaus B, Lama L, Manca MC, Pagnotta E, and Gambacorta A, (2000) Lipid profile: useful chemotaxonomic marker for classification of a new cyanobacterium in Spirulina genus. J Phytoch 54:94-2892.

SaitohT, Nakagaki NR, Uchida Y, Hiraide M, (2001) Spectrophotometric determination of some functional group on *Chlorella* for three evaluation to their contribution to metal uptake. Anal Sci17:793-795.

Siegel S, (1956) Nonparametric Statistics for the Behavioral Sciences. Nzw York: McGraw-Hill.

Spolaore P, Joannis-Cassan C, Duran E, Isambert A, (2008) Commercial Applications of Microalgae. News Paper Biosci Bioeng101: 87-96.

TredeciMR PapuzzoT, Tomaselli L, (1968) Outdoor mass culture of *Spirulina maxima* in sea-water.ApplMicrobiol Biotech24:47-50.

Walsby AE, and Fay C, (1987) Mechanisms of buoyancy regulation by planktonic cyanobacteria with gas vesicles. The cyanobacteria,Amsterdam: Elsevierpp377–392.

Waterbury JB, (2006) The Cyanobacteria : Isolation, Purification and Identification. Prokar. J 4:1053–1073.

Wu B, Tseng CK, Xiang W, (1993) Broad-scale cultivation of Spirulina in seawater based culture medium. Club-footed Mar 36:99-102.

Wyman M, and Fay P, (1987) Acclimation to the natural light climate. The cyanobacteria, Amsterdam Elsevier pp 347–376.

Zarembinski TI, Hung LW, Muller DHJ, Kim KK, Yokota H, Kim R, and Kim SH, (1998) Assignment of the biochemical function of a systematic hypothetical protein: a case of test of genomic structural. Acta Acad Sci 26,93: 15189.

Webcam:

[1]: (http://www.ncbi.gov/Blast.cgi).

[2] KF290490 750 bp DNA linear. Adiba BD, Nabil S, Mohamed B, Mostapha EF, Akli O, and Salem B,(2013) *Arthrospira platensis str*. Algerian 16S ribosomal RNA gene, partial sequence. BCT. http://www.ncbi.nlm.nih.gov/nuccore/KF290490