Spirulina: A Green Factory

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Spirulina (Arthrospira) :

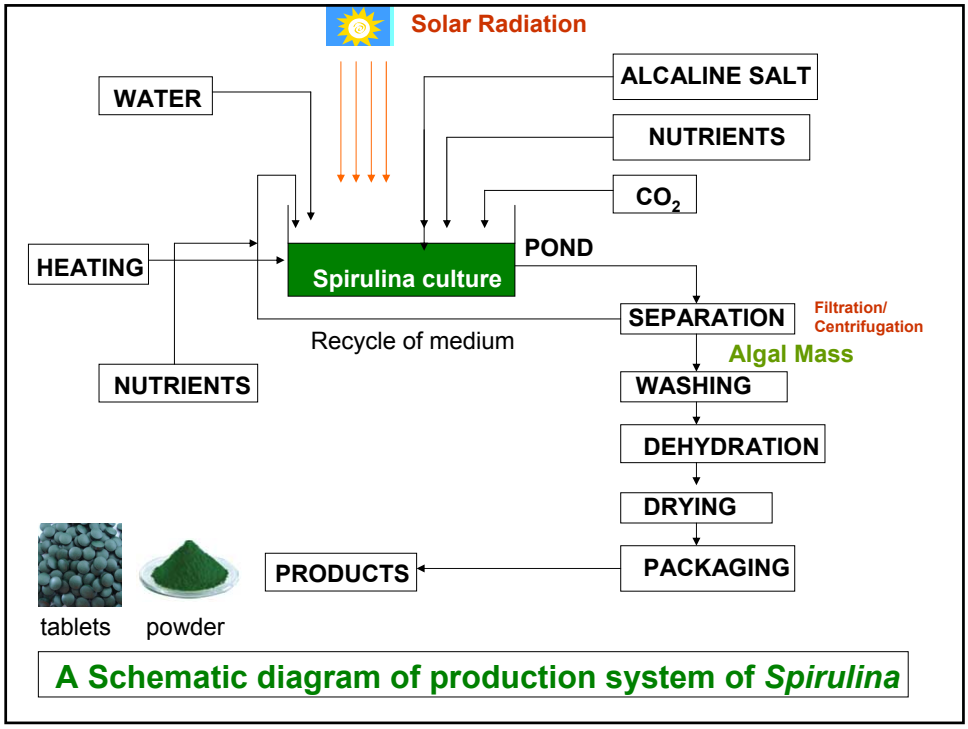
Is a photosynthetic, filamentous, spiral- shaped, multicellular, blue-green microalga

It belongs to Cyanobacteria, Oscillatoriaceae (family), Arthrospira (genus)

Most commercial production systems are based on large shallow raceways in which spirulina cultures are mixed by a paddle wheel under controlled conditions

It has a long history of use as a safe health food and as an important source of protein.

Arthrospira sp. **cultured in our Lab**



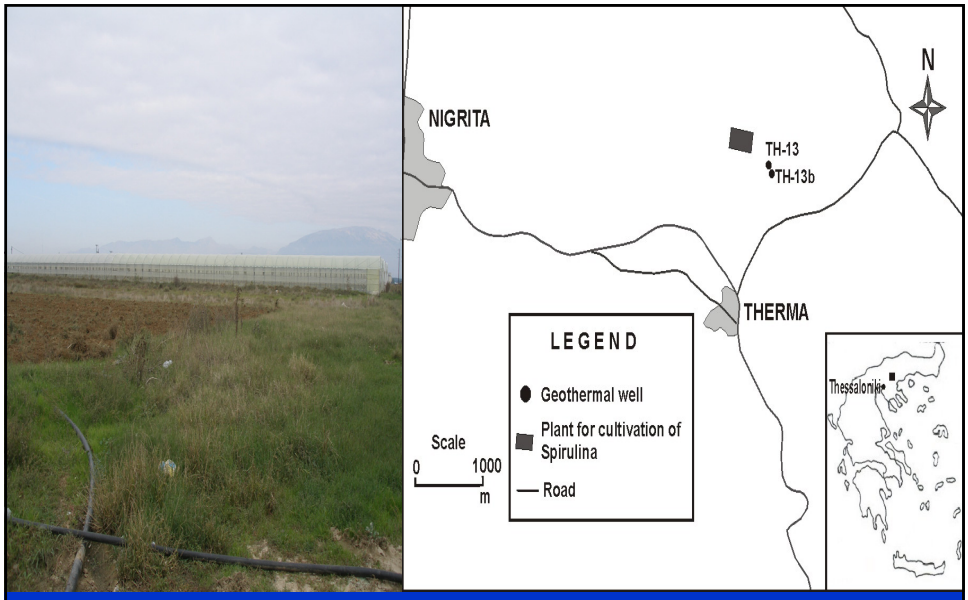
A Schematic diagram of production system of *Spirulina*

Geothermal Fluids used for Spirulina Production in Greece

- They represent a cheap and renuable energy form that is sequestered in underground aquifers
- They come from high depths
- They have high temperatures
- They usually contain dissolved CO_2

✓ The geothermal energy used in this way in greenhouse cultivation ponds contribute to increasing the daily yield by 20-30%

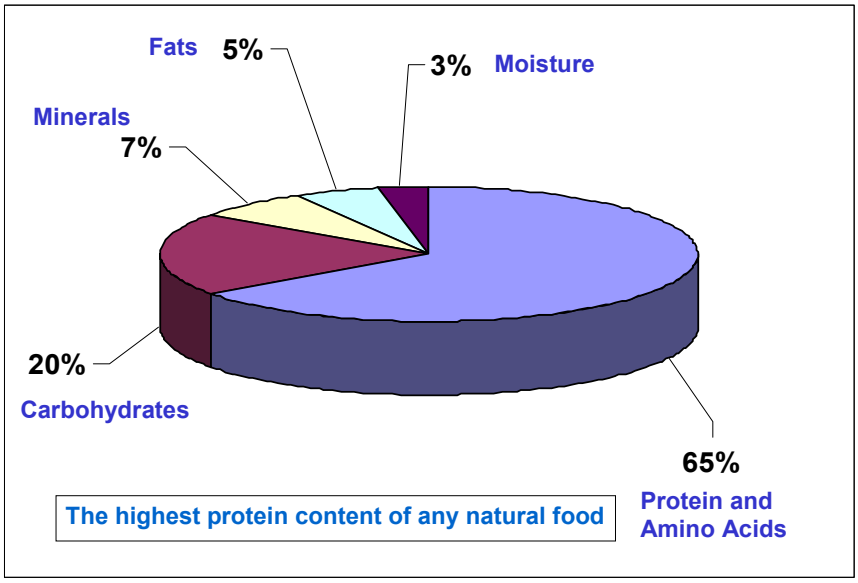
✓ The use of freely released geothermal CO2 reduces the production costs of *Spirulina* biomass by over 25%.



Location map of the Nigrita area with the sites of the geothermal production well (Th-13b) and the plant for cultivation of Spirulina

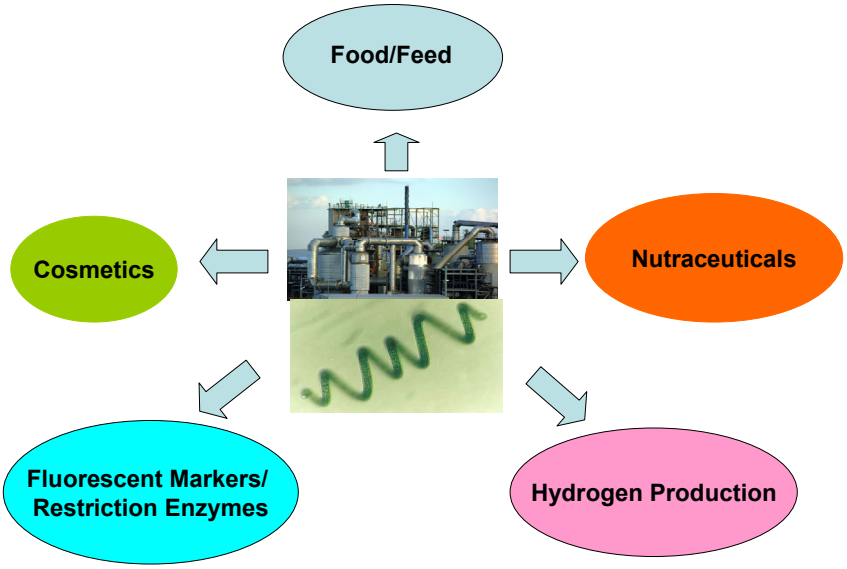
ALGAE A.C. Spirulina production pond

Composition of Spirulina



The highest protein content of any natural food

Spirulina: The Green Factory

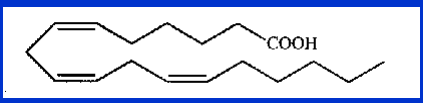


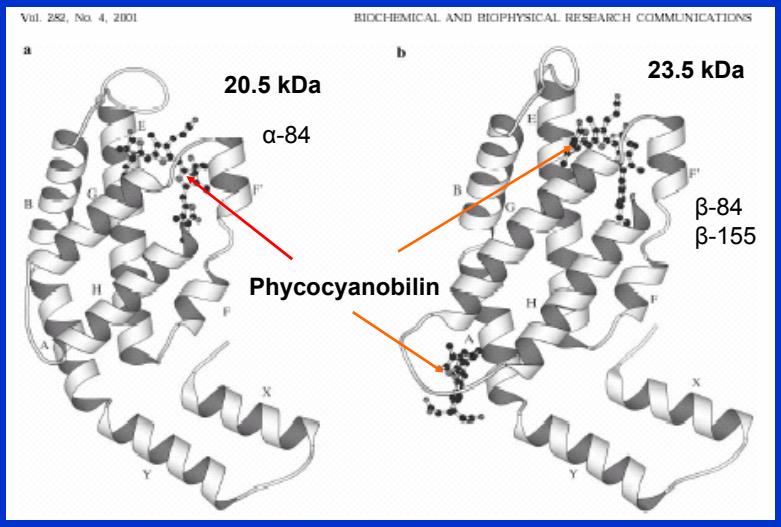
Potential Applications of *Spirulina* as Nutritional and Therapeutic *Supplement in Health Management*

- Antioxidant
- Anticancer effects
- Immunomodulation effects
- Anti-viral effects

Health benefits from Spirulina are mainly due to some major bioactive components:

- *Phycocyanin (Phycocyanin C/Allophycocyanin proteins)*
- *Spirulan (sulphated polysaccharide : MW~220,000 ; rhamnose (52%), uronic acids (16%))/ Immulina (High MW polysaccharide(~10^7) ; rhamnose (35.4%)*
- *Gamma Linolenic acid (GLA) (ω-6 PUFA)*

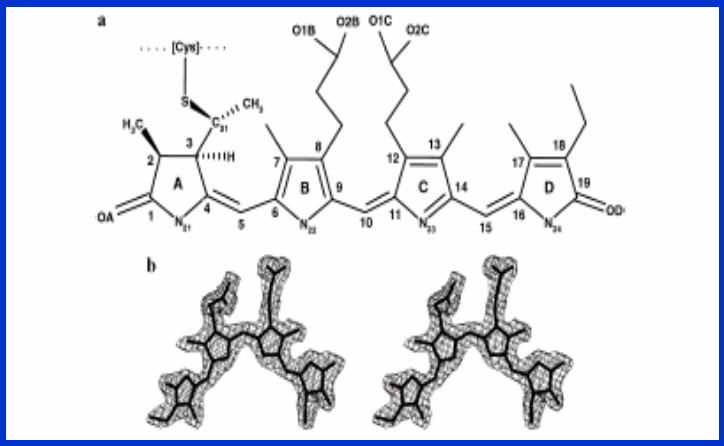




Ribbon representation of *spirulina platensis* Phycocyanin C subunits

(Phycocyanin C: Trimer of α/β heterodimers)

Phycocyanobilin (PCB) Chromophore



PCB is covalently attached to cysteines of the apoprotein via a thioether bond to C-31 on ring A

The final step in phycobiliprotein biosynthesis is the covalent chromophore attachment to the apoprotein by heterodimeric lyases

Commercial products containing Spirulina bioactive components

Chocolate confectionary

Old and new
Blue smarties (Nestlé)
with phycocyanin (2008)
(artificial coloring was removed)

Commercial GLA preparations contain plant-based oil GLA

Phycocyanin extract

A Spirulina high Mr Polysaccharide Nutraceutical

Present Research activities

Studies of *Spirulina* bioactive components in order to evaluate their potential use in biomedicine and cosmetics

- Lipid components
- Sulphated polysaccharides
- Phycocyanin and phycocyanobilin (PCB)
- Enzyme activities present in dried microalgal biomass

Lipid Components

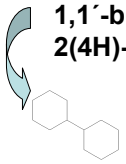
What is the chemical composition and Bioactivity of Volatile Lipid Components in Commercial Spirulina Dry Biomass ?

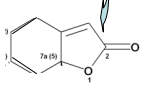
Major Volatile Lipid Components of *Spirulina* Dry Biomass Identified by GC-MS

heptadecane (77.88%)
6,10,14-trimethyl-2-pentadecanone (6.09%)
} Volatiles by Hydrodistillation

L-linalool (10.5%)
9(Z)-octadecenoic acid (10.19%)
hexadecanoic acid (5.0%)
cis-linalool oxide (4.08%)
} Pentane Extract

hexadecenoic acid
1,1´-bicyclohexyl
2(4H)-benzofuranone
} Fractions of Si gel Chromatography of Dichloromethane extract





Antimicrobial Action of Spirulina extracts and Si-Gel Chromatography Fractions

Antimicrobial activity was assayed against six human pathogenic bacteria and three fungi through both the disc diffusion (inhibition zones (mm)) as well as the dilution methods.

Tested samples	*S. aureus*	*S. epidermidis*	*P. aeruginosa*	*E. cloacae*	*K. pneumoniae*	*E. coli*	*C. albicans*	*C. tropicalis*	*C. glabrata*
Cyclohexane extract	8	10	NA	NA	NA	10	NA	8	NA
Dichloromethane extr.	11	11	NA	NA	NA	9	NA	9	8
Methanol extract	15	14	10	11	10	10	9	10	9
[1]Fraction 70-74	12	10	9	10	10	9	8	9	9
[1]Fraction 50-56	11	12	10	10	9	9	8	9	9
[1]Fraction 148-151	12	12	10	10	9	10	8	10	10
[1]Fraction 169-170	11	12	9	10	9	11	7	10	11
[1]Fraction 223-224	12	11	10	11	10	11	7	10	10
[1]Fraction 268	12	13	10	11	11	10	8	10	10
[1]Bicyclohexane	12	13	10	10	11	10	8	9	10
Netilmicin	17(0.004	19(0.004)	16(0.088)	17(0.008	18(0.008)	16(0.010	NT	NT	NT
Itraconazole	NT	NT	NT	NT	NT	NT	20	22	23
5-flucytocine	NT	NT	NT	NT	NT	NT	20(0.01)	21(0.001)	22(0.0001)

NA, Not Tested; NA, Not Active; Chromatography [1]Fractions of Dichloromethane extract

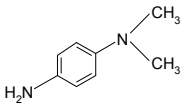
Anionic Polysaccharides

Spirulina anionic polysaccharides express Immunomodulating and Antiviral activities

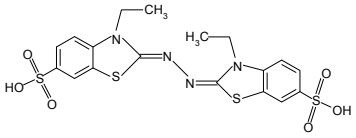
Free radical Scavenging Activity ?

Scavenging of stable free radicals by Spirulan

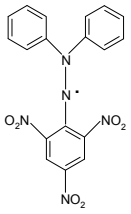
(spectrophotometric determination)



DMPD



ABTS



DPPH

EC_{50}= 0.2 mg/ml

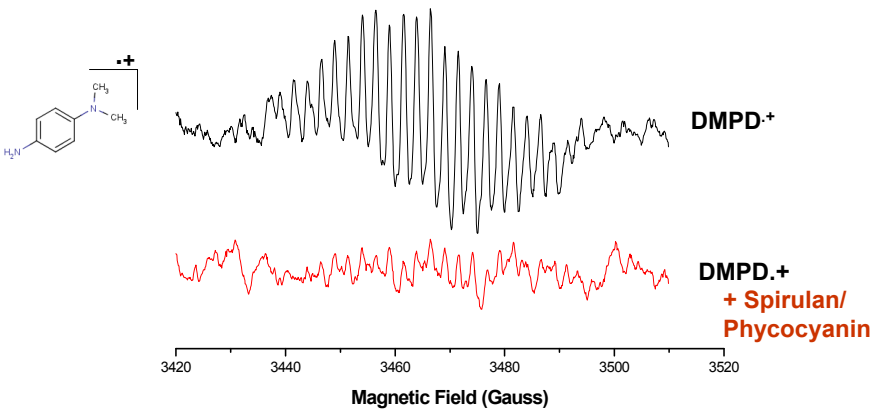
EC_{50}= 0.3 mg/ml

No

Polysaccharide Specificity

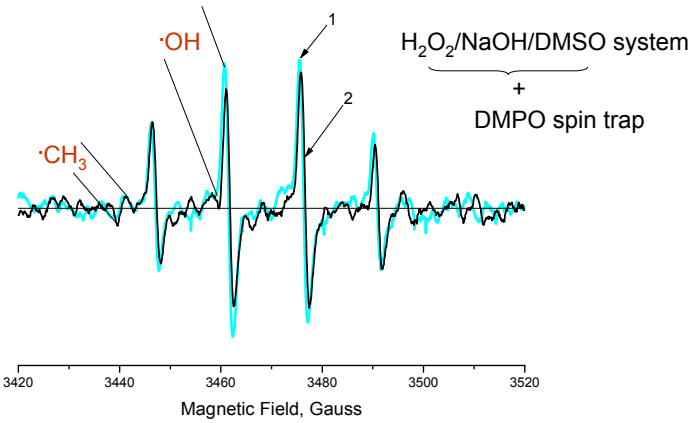
Chondroitin sulfate, Hyaluronic acid and Heparin express very low scavenging activities

Scavenging of DMPD.+ by Spirulan or Phycocyanin followed by EPR Spectroscopy



Tsapra et al. submitted

Spin-Trapping of Free Radicals in absence (1) and presence (2) of a *Spirulina platensis* Polysaccharide Fraction



Analysis of the EPR spectrum indicates that the polysaccharide scavenges hydroxyl radicals

Phycocyanobilin (PCB)

Phycocyanin : ~ 14% of Spirulina dry biomass

PCB: ~ 0.66% of Spirulina dry biomass
(15 g of Spirulina (a heaping tablespoon) contains about 100 mg PCB)

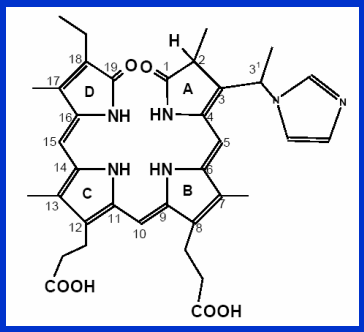
Phycocyanin exhibits a variety of pharmacological properties

Phycocyanobilin ?

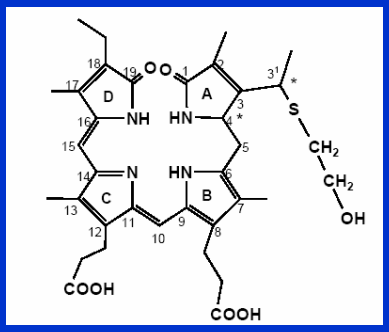
PCB Preparation

Spirulina → Phycocyanin —(Methanolysis)→ **PCB → Chromatography**

Since a Spontaneous formation of PCB adducts has been observed
(Tu et al (2009) JACS, 131, 5399)



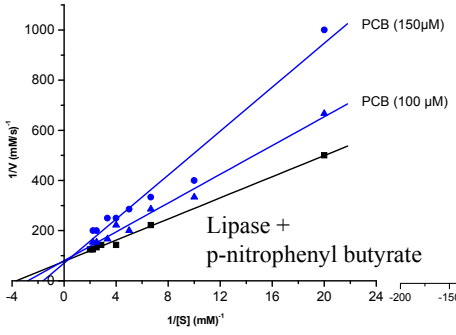
PCB-imidazol adduct

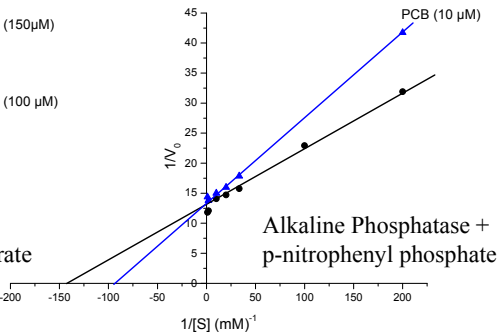


PCB-mercaptoethanol adduct

Therefore: Our aim was to examine the interaction of PCB with various biomolecules

Inhibition of Lipase and Alkaline Phosphatase by Phycocyanobilin (PCB)





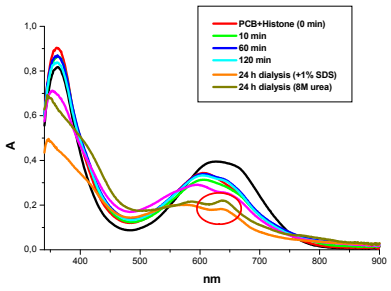
Ki=320 μM

K_i=18.9 μM

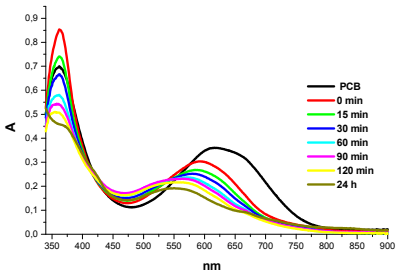
PCB irreversibly modified:
BSA, Histone H2B, Phosvitin, certain anionic polysaccharides and DNA

PCB interacts with:
the anions phosphates and borates as well as with the amine Tris

PCB interacts with surfactants:
SDS, Triton X100, CTAB



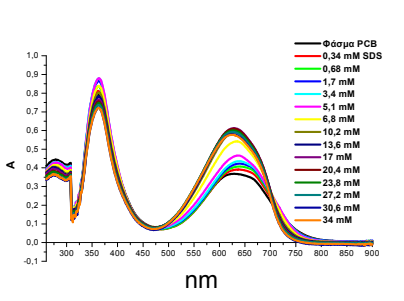
PCB+ Histone

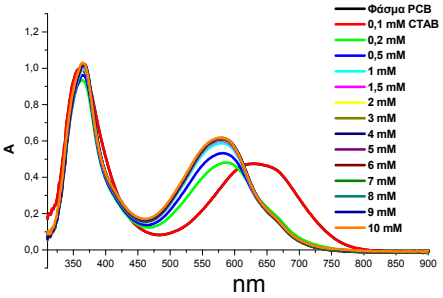


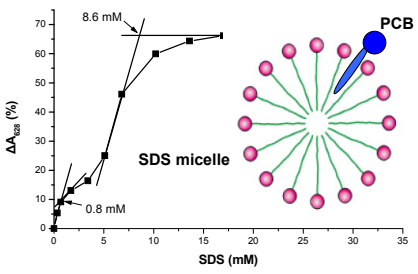
PCB + Pi (pH 9.0)

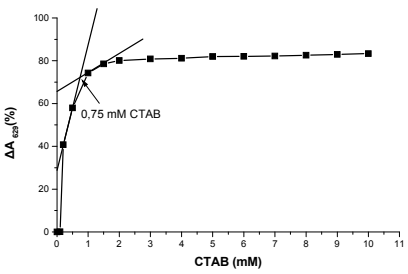
All the above interactions may cause autocatalytic PCB covalent modifications

Titration of PCB by SDS and CTAB









Peroxidase of *Spirulina* dry Biomass

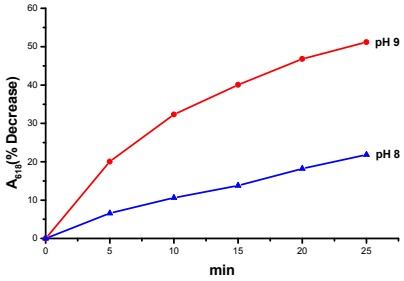
ROS-scavenging enzymes are increasingly used in skin health care products

Is Peroxidase Activity present in Dry Commercial Spirulina Biomass ?

Peroxidase from Spirulina Dried Biomass

- ✓Partial purification by ammonium sulfate fractionation and gel filtration
- ✓Molecular mass: 43.500 Da
- ✓Two pH optima: pH 4.0 and pH 8.0
- ✓Km for ABTS: 46 µM (best substrate)
- ✓Km for H_2O_2 : 21.2 mM

Spirulina Peroxidase (PO)-induced Spectral Change of Phycocyanin (PC) in presence of H_2O_2 and ABTS



Bleaching of PC was observed only in presence of ABTS

$$PO + H_2O_2 + ABTS \longrightarrow ABTS^{*} \xrightarrow{PC} PC_{mod}$$

(mediator)

Since Peroxidase activity survives the manufacturing conditions for *Spirulina* powder production, it may play significant role in cosmetic products in blocking hydrogen peroxide toxicity

Future Research activities

Metabolic Engineering, Metabolic Flux Analysis and determination of key metabolites using HPLC-MS/NMR techniques will be used for designing and modifying *Spirulina* pathways for the overproduction of important constituents of this microorganism

Synthesis of hydrophobic derivatives of PCB as membrane- targetted photosensitizers for photodynamic therapy

New cosmetic products containing Spirulina bioactive components

NHRF
Dr A. Xenakis
Dr V. Papadimitriou
Dr. E. Tzika

G. T. Sotiroudis, Msc
V. Tsapra, Msc
P. Koukouraki, Msc
I.Pontikos, Dipl. Th.
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University of Athens
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University of Malaga
Prof. C. Jimenez
Prof. R. Abdala

TEI, Athens
Prof. E. Nerantzis

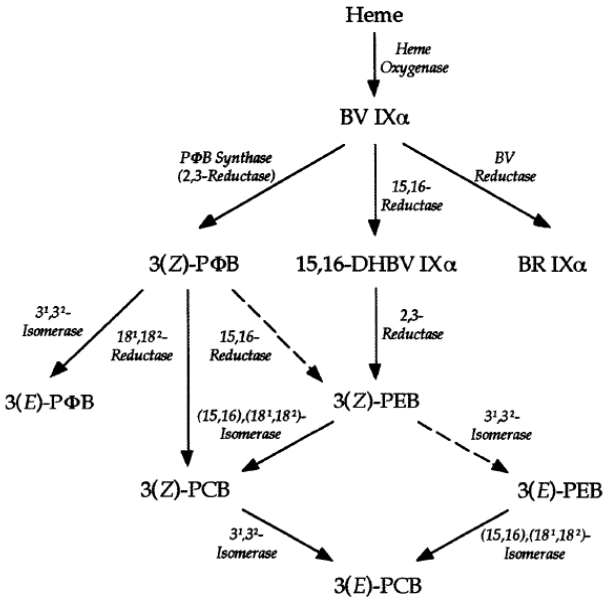
ALGAE AC, Nigrita,Serres

Bilateral Greece-Spain GSRT 006-γ Programme

01 PRAXE 11 GSRT Programme

Thank you for your attention

BIOSYNTHESIS OF BILINS



Arthrospira platensis str. Paraca, whole genome shotgun sequencing project

Genome Info:	Features:	BLAST homologs:	Links:	Review Info:
Refseq: NZ_ACSK00000000	Genes: **0**	COG	Genome Project	Publications: **None**
GenBank: ACSK00000000	Protein coding: **None**	TaxMap	Refseq FTP	Refseq Status: **WGS**
Length: 4,997,563 nt	Structural RNAs: **None**	TaxPlot	GenBank FTP	Seq.Status: **Draft**
GC Content: 44%	Pseudo genes: **None**	GenePlot	BLAST	Sequencing center: **University of Applied Sciences of Western Switzerland**
% Coding: **0%**	Others: **None**	gMap	TraceAssembly	Completed: **2009/07/20**
Topology: **other**	Contigs: 1820		CDD	Organism Group
Molecule: **DNA**			Other genomes for species:	

http://www.ncbi.nlm.nih.gov/sites

Arthrospira maxima CS-328, whole genome shotgun sequencing project

Genome Info:	Features:	BLAST homologs:	Links:	Review Info:
Refseq: NZ_ABYK00000000	**Genes: 5728**	COG	Genome Project	Publications: **None**
GenBank: ABYK00000000	Protein coding: 5690	TaxMap	Refseq FTP	Refseq Status: **WGS**
Length: 6,003,314 nt	**Structural RNAs: 38**	TaxPlot	GenBank FTP	Seq.Status: **Draft**
GC Content: 44%	Pseudo genes: **None**	GenePlot	BLAST	Sequencing center: **US DOE Joint Genome Institute (JGI-PGF)**
% Coding: 82%	Others: **None**	gMap	TraceAssembly	Completed: **2008/10/15**
Topology: **other**	Contigs: 129		CDD	Organism Group
Molecule: **DNA**			Other genomes for species:	

http://www.ncbi.nlm.nih.gov/sites/