







# Microalgae: biological activites for cosmetics

Silvia Buono

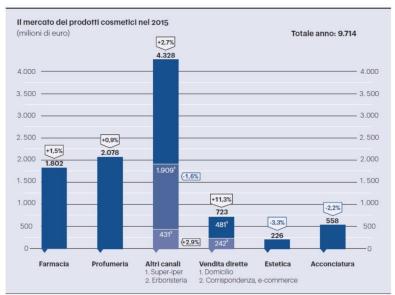


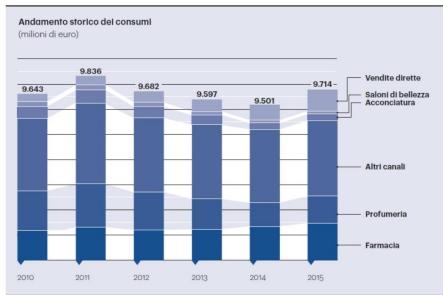
'Conference and Exhibition for the Sustainable Aquaculture and Fishing Industry. Focus also on algae cultivation and vertical farming' Pordenone 26-27 gennaio 2017

### European cosmetics market



## In Italy.





Cosmetics Europe-The Personal Care Association, 2016

#### FUNCTIONS OF COSMETIC PRODUCT

#### **TOILET FUNCTION**

It aims to remove dirty from the surface of the skin respecting the physiological characteristics (Cleaning, Deodoration).

#### **EUTROPHIC FUNCTION**

supports the state of the tissues, on which the cosmetics are applied under the best conditions. Cosmetic eutrophic: it maintains proper skin tropism generating substances that support normal physiological events that occur in a healthy skin (Protection, Normalisation)

#### COSMETIC FUNCTION

It aims to positively influence the sensory functions of sight and smell. And 'the link of igiene- circle health- beauty (Decoration, Treatments, Scent)













## Reference books on cosmetic commodities

- International Cosmetic Ingredient Dictionary
   Cosmetic, Toiletry and Fragrance Association USA (Personal Care Products Council) XVI Ed, 2016
- European Inventory of Cosmetic Ingredients (2006)





#### the same with some exceptions

**Dyes** – similar names to FDA in USA, Colour Index in EU Eg. Blue 1 (USA), CI 42090 (EU)

**Vegetable extracts** – In USA It is most often used English common name, in EU botanical name, eg. Shea Butter (USA), *Butyrospermum parkii* Butter (EU)

Some conventional products, eg. Beeswax (USA), Cera alba (EU)

## Cosmetic Ingredients classification

Lipids, Emulsifiers, surfactants, solubilizing, rheology modifiers, Matting / pearling for tensiolitics, Preservatives and antimicrobial skin, sequestering, antioxidant, Humectants, coloring matter, conditioning, Film-forming and fixatives, texturizers, solvents, propellants, silicone, Flavor and fragrance, functional ingredients



## Microalgae: which opportunities

 Thousands of species that can be screened for the different needs

 Flexible metabolism that can be addressed towards the production of lipid, proteins or phytochemicals

 Advantages and disadvantages compared to terrestrial plants



# Microalgae in Personal Care Products





Body and face Care, Scrubs, Masks, Creams, Cleansers, Shampoos, ....



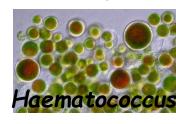


European Commission database for information on cosmetic substances and ingredients





Haematococcus pluvialis EXTRACT as antioxidant
Chlorella EXTRACT as skin conditioning
Spirulina maxima EXTRACT as smoothing
Porphyridium cruentum EXTRACT as skin conditioning













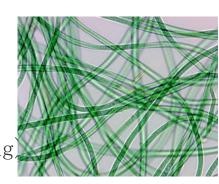




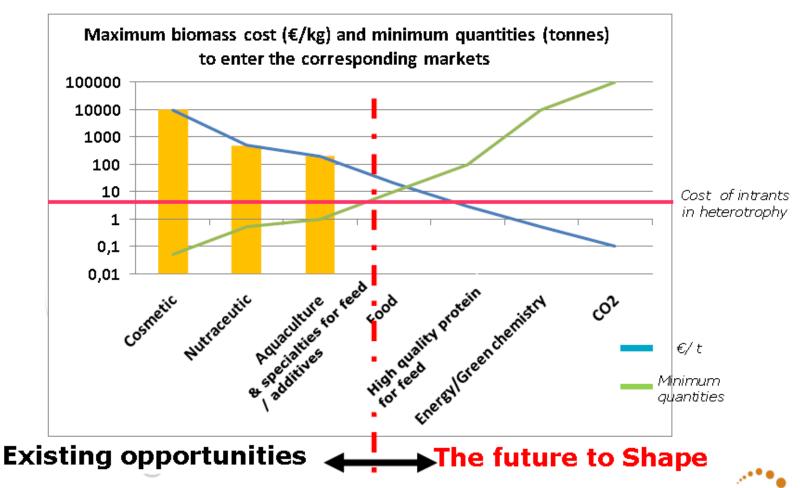


## THERMAE ABANO MONTEGROTTO

Phormidium e il ceppo ETS-05 (da Euganean Thermal Spring)



#### Microalgae and market access



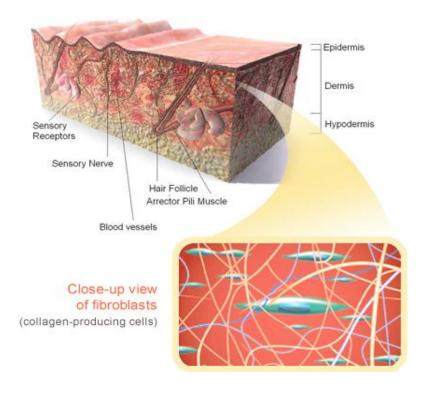
Estimated world microalgae production: 15.000-20.000 tonn



# Biological activities of cosmetic interests

Studies based on cell biology using reliable in vitro assays and suitable cell lines are required.

The effect of putatively active algae extracs should be tested on:



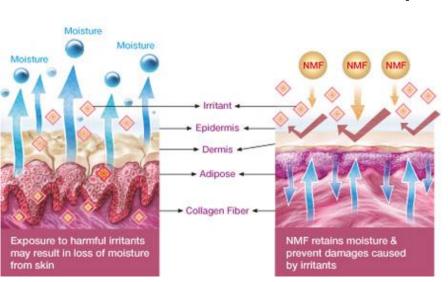
Metabolic pathways which are known to interfere with the health of skin tissue

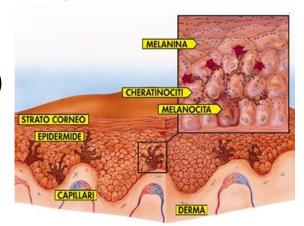
 Expression of genes and activity of proteins correlated to this pathways



## Skin cell biology: cell lines

- Keratinocytes (HaCaT)
- Murine Fibroblasts (NIH 3T3)
- Human Dermal Fibroblasts (primary cells)
- Melanocytes (B16F1)
- Adipocytes precursors (hMSC)
- Differentiated Adipocytes



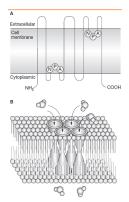


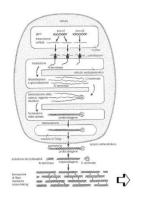
#### **Assay conditions**

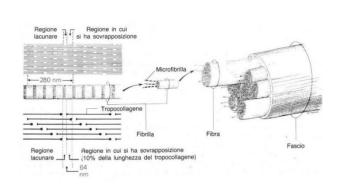
- Oxidative Stress
- UV Radiations
- Heavy Metals Stress

## Skin Cell Biology: cell-based Assays

- Collagen synthesis & degradation (Coll, Col III MMP's)
- Hydration capacity (Aquaporin3, Filagrin, Involucrin)
- Anti-inflammation assay (NO radical, COX2 iNOS)
- DNA repair and longevity (Comet Assay, SIRT1 expression and activity)
- Heavy Metal protection (Heat shock proteins hsp70)
- Repulping, anti-cellulite, anti-fat (cAMP, Lipase activity, Adipocyte formation)
- Skin rejuvenating activity (mesenchymal stem cell differentiation assay; Fibroblast and keratinocyte induction)







Struttura delle acquaporine

Sintesi e struttura del collagene

#### ORIGINAL PAPER

Biological activities of dermatological interest by the water extract of the microalga *Botryococcus braunii* 

Silvia Buono · Antonio Luca Langellotti · Anna Martello · Marida Bimonte · Annalisa Tito · Antonietta Carola · Fabio Apone · Gabriella Colucci · Vincenzo Fogliano

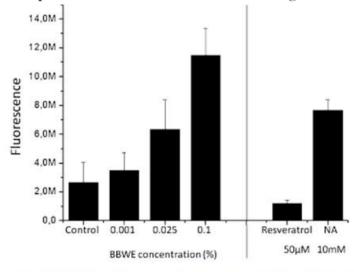


Fig. 1 Adipocyte differentiation assay. Fluorescence measured on human mesenchymal stem cells differentiated in adipocytes treated with BBWE at different concentrations and with nicotinamide (NA positive control) and resveratrol (negative control)

At concentrations ranging from 0.1 to 0.001 % (w/v) BBWE promoted adipocytes differentiation by inhibiting hormonesensitive lipase, thus promoting triglyceride accumulation in the cells.

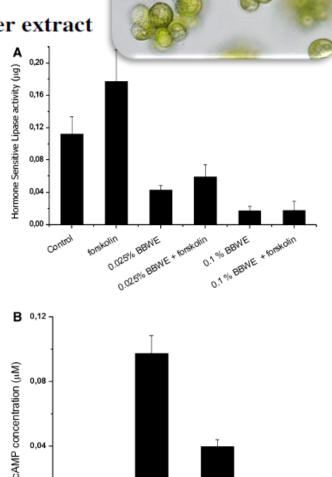


Fig. 2 a Effect of BBWE of hormone lipase activity in combination with forskolin (5  $\mu$ M), a well-known activator of the adenylate cyclase. b Effect of BBWE in combination with forskolin (5  $\mu$ M) on cAMP concentrations measured in adipocytes

O.Tolo BEINE

#### Effects of BBWE at different concentrations on the synthesis of collagen, expression of genes related to cell hydration, production of reactive oxygen species

Synthesis of collagen type I and type III (control was set as 100 %). NIH3T3 cells were treated with BBWE (range from 0.001 to 0.1 %). Ascorbate was used as positive control

I Collagen production (% ± SD)	III Collagen production (% ± SD)		
131.5 ± 3.2	120.9 ± 15.1		
$137.1 \pm 13.2$	119.7 ± 20.5		
$159.4 \pm 18.9$	$122.6 \pm 17.6$		
$180.6 \pm 2.1$	143.5 ± 15.1		
$166.9 \pm 20.3$	$154.2 \pm 18.3$		
,	$131.5 \pm 3.2$ $137.1 \pm 13.2$ $159.4 \pm 18.9$ $180.6 \pm 2.1$		

Expression of AQP3, FLG and INV genes (control was set as 100 %). Human keratinocytes (HaCaT) treated with BBWE at different concentrations

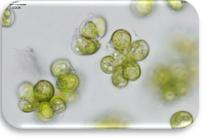
	AQP3 (% $\pm$ SD)	FLG (% ± SD)	INV (% ± SD)
BBWE 0.001 %	133 ± 6	122 ± 3	171 ± 8
BBWE 0.05 %	$242 \pm 10$	139 ± 5	175 ± 7
BBWE 0.1 %	$266 \pm 9$	$149 \pm 5$	$188 \pm 8$

ROS production (control was set as $100\%$ ) measured on NIH3T3 treated with BBWE at different concentrations. No stress and stress ( $H_2O_2$ 150 $\mu$ M treatment) conditions are shown				
<del>70</del>	ROS production (% ± SD) No stress	ROS production (% $\pm$ SD) H <sub>2</sub> O <sub>2</sub> (150 $\mu$ M)		
Control	$100 \pm 20.1$	794.7 ± 71.3		
BBWE (0.05 %)	$97.6 \pm 64.3$	$521.7 \pm 75.4$		
BBWE (0.1 %)	$98.6 \pm 13.9$	$489.7 \pm 66.4$		

 $289.4 \pm 25.6$ 

 $94.8 \pm 8.0$ 

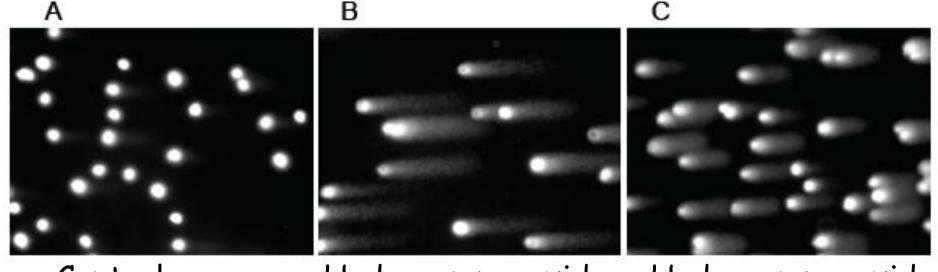
Ascorbate (250 µM)



#### DNA damage - COMET assay

Length wake of cellular nucleus (μm ± SD) measured for each treatment (control, H<sub>2</sub>O<sub>2</sub>, B. braunii extract 0.02-0.1 % in the presence of H<sub>2</sub>O<sub>2</sub>)

	Tail length (±SD)	
Control	$1.4 \pm 1.0$	
$H_2O_2$ (175 $\mu$ M)	$25.0 \pm 4.5$	
BBWE (0.02 %) + H <sub>2</sub> O <sub>2</sub>	$21.8 \pm 5.0$	
BBWE (0.1 %) + $H_2O_2$	$18.7 \pm 3.5$	



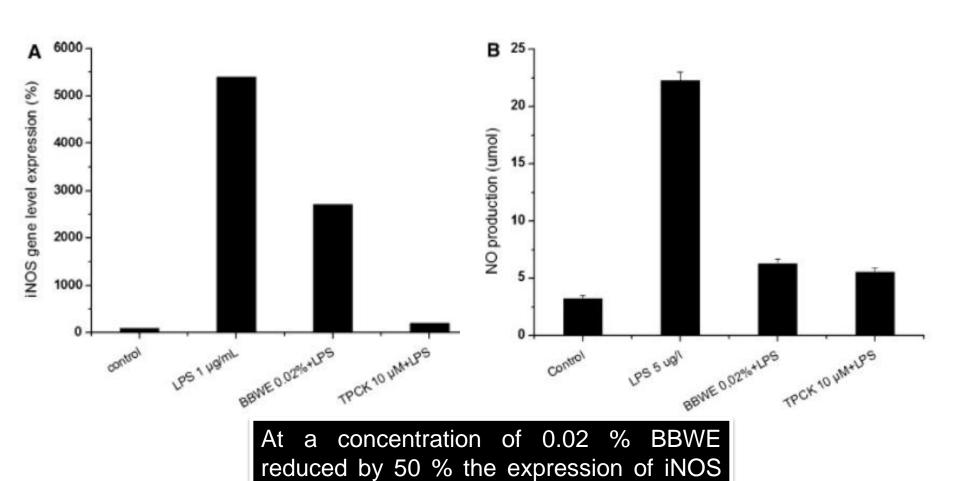
Control

Hydrogen peroxide

Hydrogen peroxide

alga extract

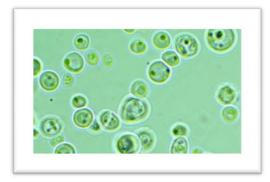
BBWE inhibited the inducible nitric oxide synthase (iNOS) gene expression and the consequent nitrite oxide (NO) production under oxidative stress.



and by about 75 % the NO production.







**Patent** 

cosmetic compositions containing extracts derived from microalgae *Galdieria sulphuraria*, particularly suitable to reduce the harmful effects caused by acne

TRICHOLOGY AND COSMETOLOGY



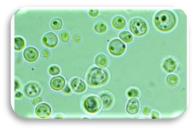
— Open Journal∂

http://dx.doi.org/10.17140/TCOJ-1-103

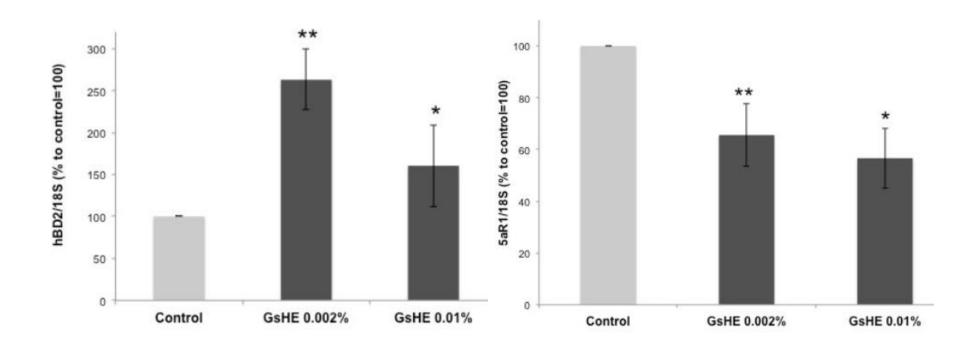
Galdieria sulphuraria Relieves Oily and Seborrheic Skin By Inhibiting the 5-α Reductase Expression in Skin Cells and Reducing Sebum Production *In Vivo* 

M. Bimonte, PhD¹; A. De Lucia, PhD¹; A. Carola, PhD¹; A. Tito, PhD¹; S. Buono, PhD²; A. L. Langellotti, PhD²; V. Fogliano, PhD³; G. Colucci, PhD⁴; Fabio Apone, PhD⁴\*

#### Results



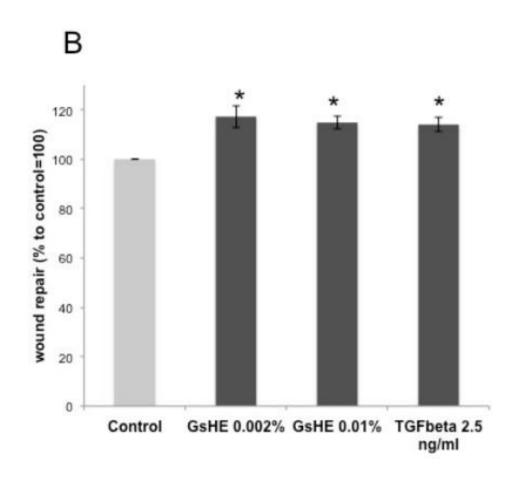
From the extremophile microalga *G. sulphuraria the* water-soluble extract was capable of inhibiting the enzyme  $5-\alpha$  Reductase, inducing the expression of the  $\beta$ -defensins.



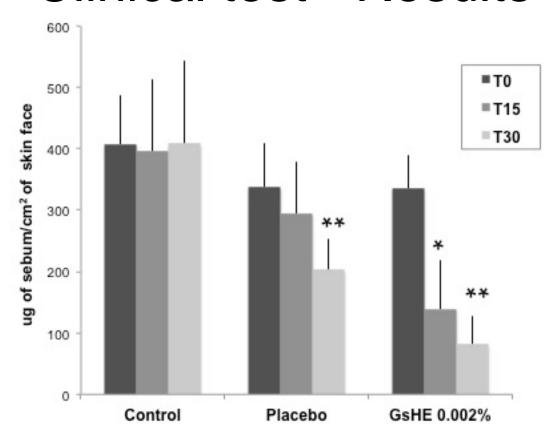
#### Results

GsHE increased the capacity of the cells to repair the wound: it accelerated the cell migration and the healing process by 17% at the concentration of 0.002%, compared to the untreated control, similarly to the Transforming Growth Factor-β (TGF-β), known for its ability to accelerate the wound repair.

Α Control (T0) Control (T7) GsHE 0.002% (T0) GsHE 0.002% (T7) TGFB 2.5 ng/ml (T0) TGFβ 2.5 ng/ml (T7)



#### Clinical test - Results



Cream without the active (placebo);

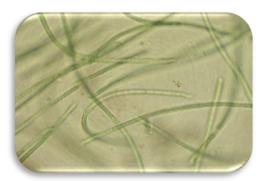
Cream containing GsHE (0.002%);

Untreated skins (Control).

Treatment: twice a day for 28 consecutive days.

The sebum level on the skin was measured by the instrument Sebumeter® SM815.

## Cyanobacterium unknown from Ischia island: *Phormidium*-like

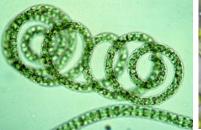


Isolated from a thermal pond in **Ischia** island. It grows in alkaline waters and at high temperature (60 -70 °C)



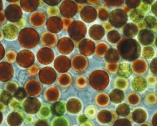
**Hydrosoluble extract** was effective in inducing the formation of the skin hydro-lipidic layer (hydration and water retention), inhibiting proinflammatory cytokinin production (anti-inflammation), inducing Kallicrein synthesis (exfoliation).

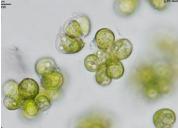
	Hydration		Anti	Anti-Inflammation		Exfoliation	
	GBA	SMPD1	IL1 alpha	IL1 beta	IL8	KLK5	
0.000032 %	24%	38%	-	-	-		33%
0.00016 %	36%	76%	-35%	-34%	-28%		51%
	Ha	Cat		HaCat		NHEK	











### Conclusion

- Microalgae has great potentiality in cosmetics (largely unexplored)
- Well designed experiments allows to define the specific activity for each algae-based bioactive preparations
- Beside in vitro and cells assay there is a need of tools to assess activities on tissue (artificial skin)









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#### Acknowledgments

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## Thank you for your attention

silvia.buono@unina.it www.acquacoltura.unina.it