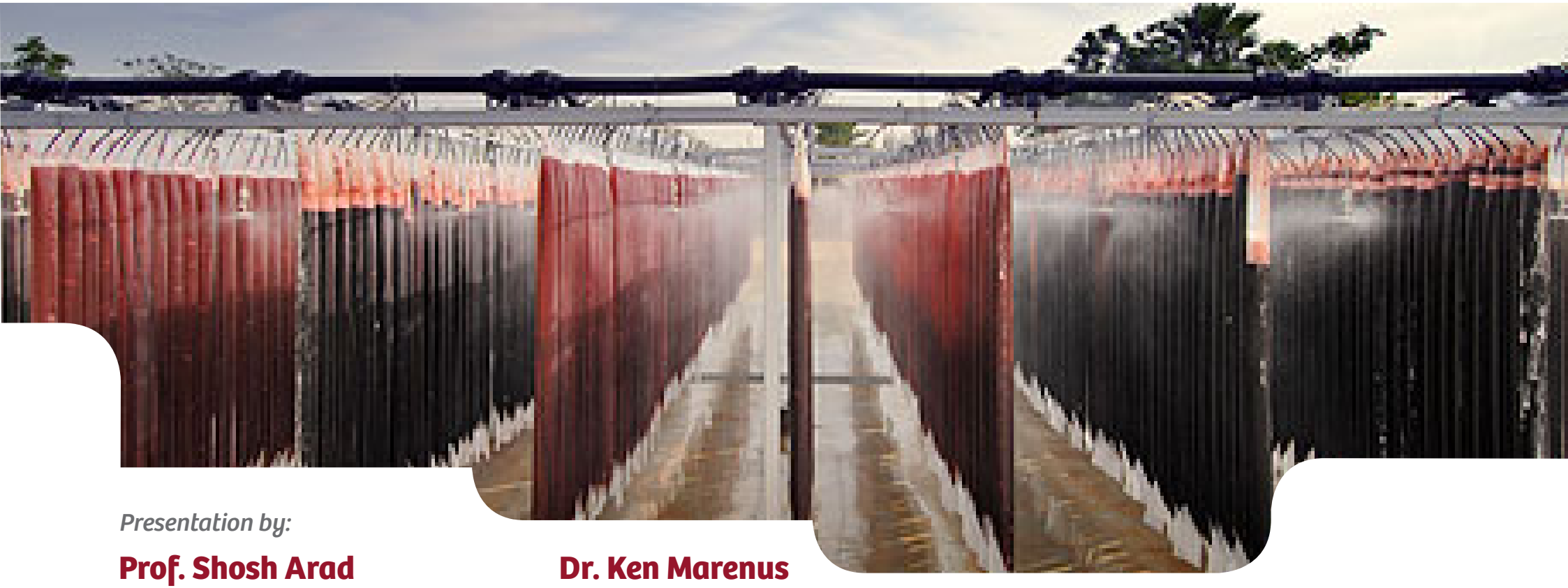
The Biotechnology of Algae Cultivation in the Negev Desert:
The Estée Lauder Project



Presentation by:

Prof. Shosh Arad
Senior Biotechnology Researcher
President
Ruppin Academic Center

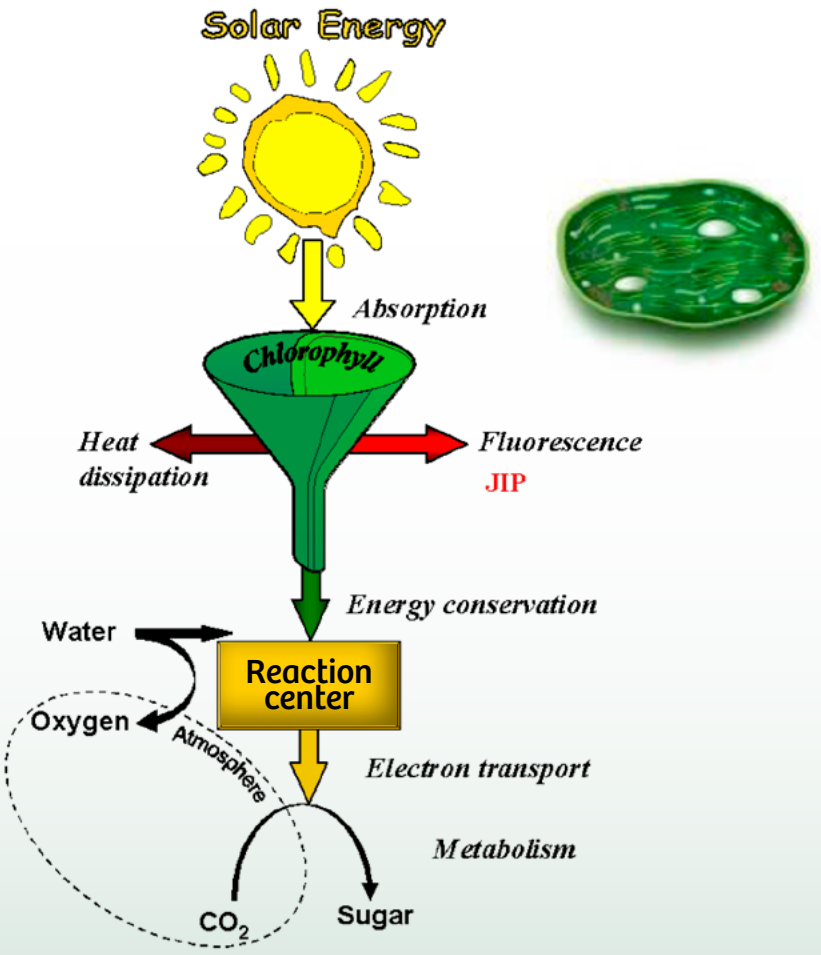
Dr. Ken Marenus
Senior Vice President
The Estée Lauder Companies

Rationale

EFFICIENT BIOCONVERSION OF SOLAR ENERGY

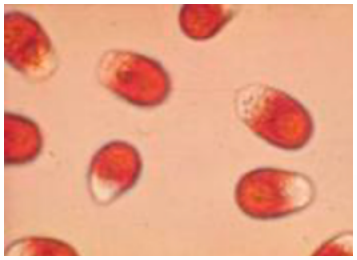
Using the sun as the most abundant energy source available

Harnessing the efficient photosynthetic machinery of micralgae



$$6H_2O + 6CO_2 \rightarrow C_6H_{12}O_6 + 6O_2$$

Beta-Carotene Production in Eilat



NBT Ltd. Eilat

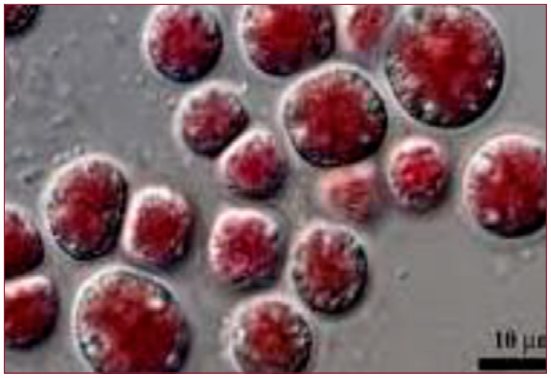
Astaxanthin Production in the Negev

Modular tubes system

Haematococcus

Algatechnologies Ltd., Kibbutz Ketura

Red Microalgae



Unicells:

10-20 µM DIAMETER

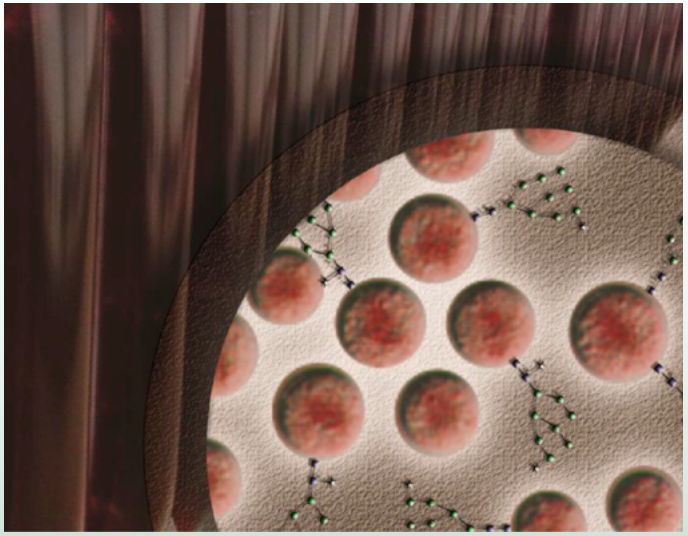
Habitat:

FRESH WATER

BRACKISH WATER

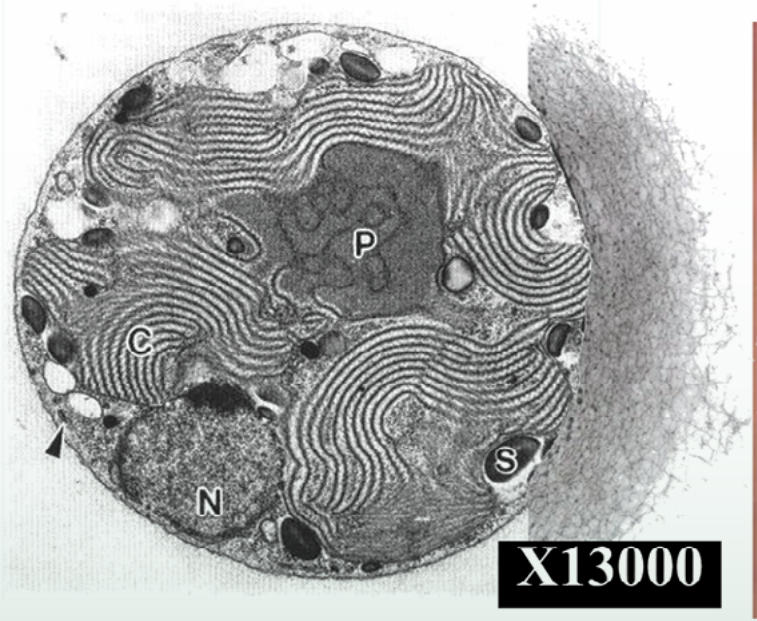
SEAWATER

SOIL



Red Microalgae Cells

The External Part of the Polysaccharide Dissolves in the Medium



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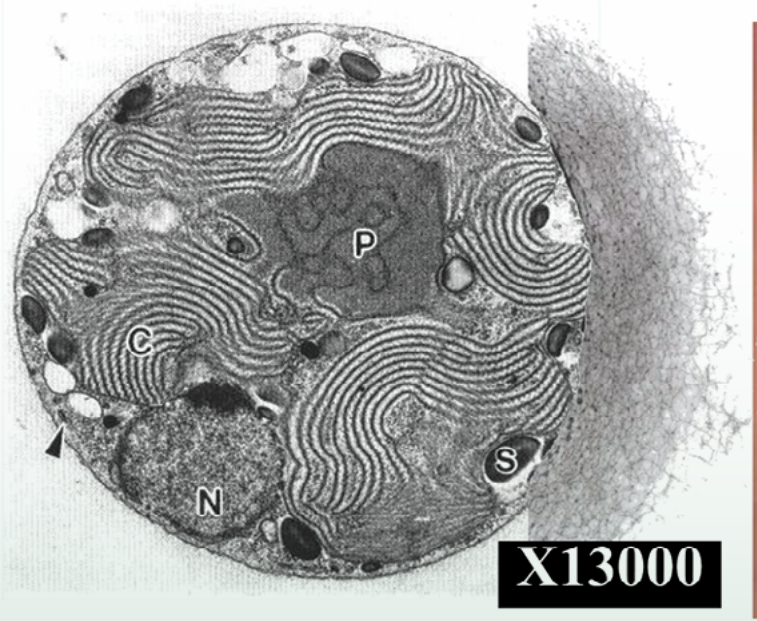
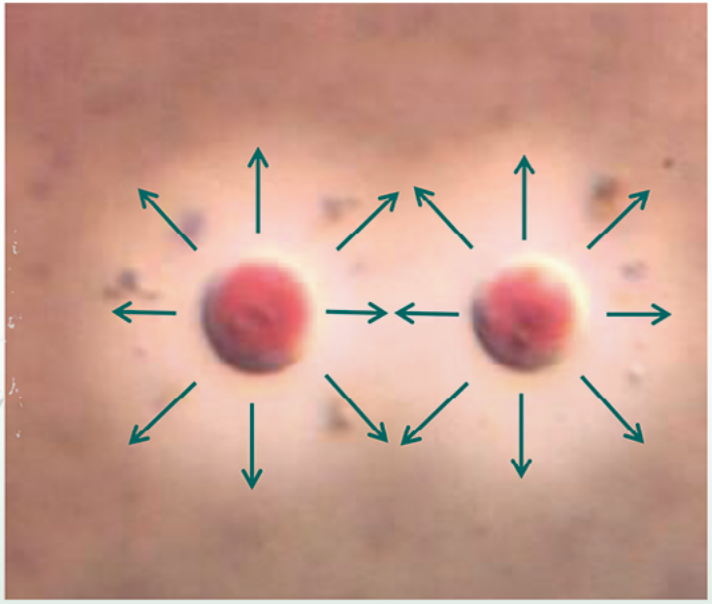




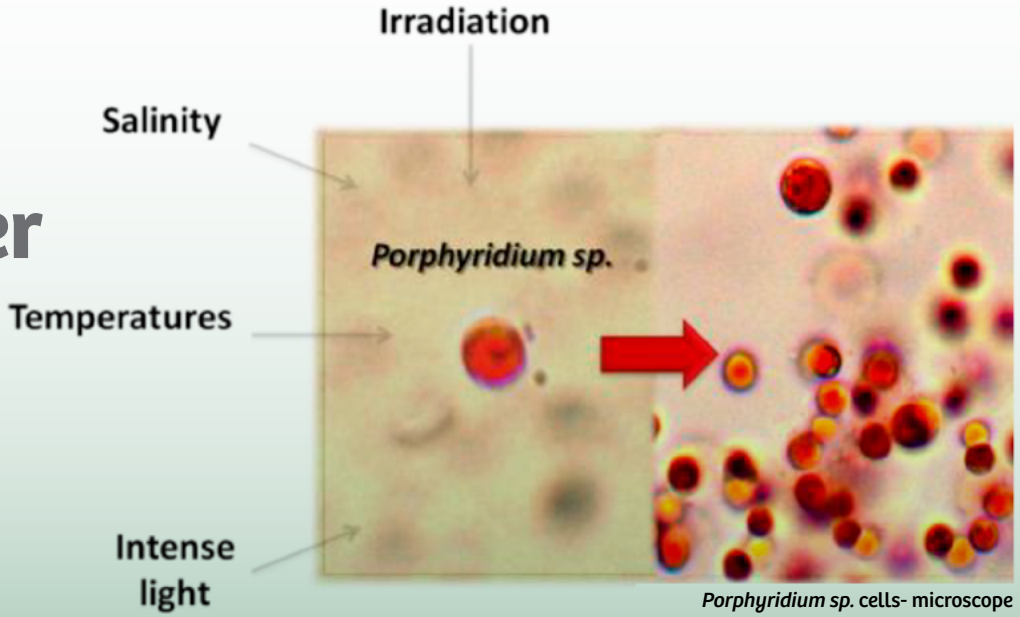
Biological Function

Cell Wall Sulfated Polysaccharide

- ***AGAINST BIOLOGICAL ATTACK***
  bacteria, fungi, viruses
- ***BUFFER LAYER AGAINST EXTREME ENVIRONMENTS***
  temperature, pH, salinity
- ***AGAINST SEA-SAND CONDITIONS***
  drought, light

Free Radical Scavenger

Antioxidant Activity



Porphyridium sp. cells- microscope

GOAL

TO DEVELOP THE BIOTECHNOLOGY FOR THE PRODUCTION OF BIOACTIVE PRODUCTS (SULFATED POLYSACCHARIDES) FROM RED MICROALGAE

The Biotechnology

COMMERCIAL CULTIVATION
LARGE SCALE

BIOACTIVE APPLICATIONS

CHEMISTRY
COMPOSITION
STRUCTURE
RHEOLOGY

MULTIDISCIPLINARY APPROACH

MOLECULAR GENETICS
GENOMIC
CELL FACTORIES

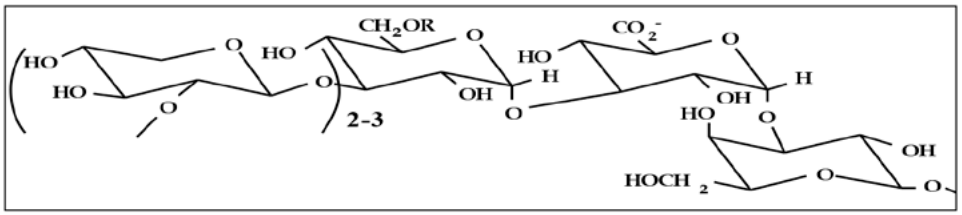
ENVIROMENTAL CONDITIONS
PHYSIOLOGY

CELL WALL FORMATION
BIOSYNTHESIS

The Polysaccharide

- *Negatively Charged*
- *Sulfate – 9%*
- *Glycoprotein – 66kDa*
- *M.W. – 3-5X10^{6}Da*
- ***Resistant to enzymatic degradation***

β–D-Xylp (1 →3) – α–D-Glup (1 → 3) – α–D-GlucA (1 →3) – α+βL-Galp



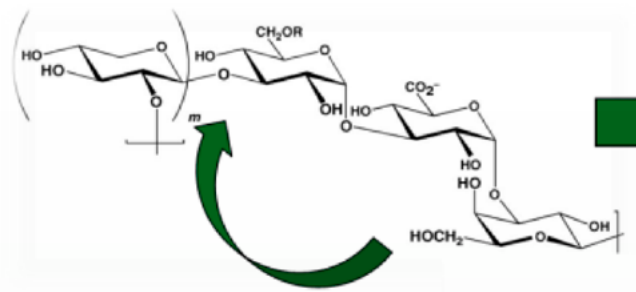
Levy-Ontman *et al. J.Biol.Chem.* 286:(24)21340–2135 (2011)
Geresh *et al. Carbohydr. Res.* 344: 343-349 (2009)

COMPOSITION

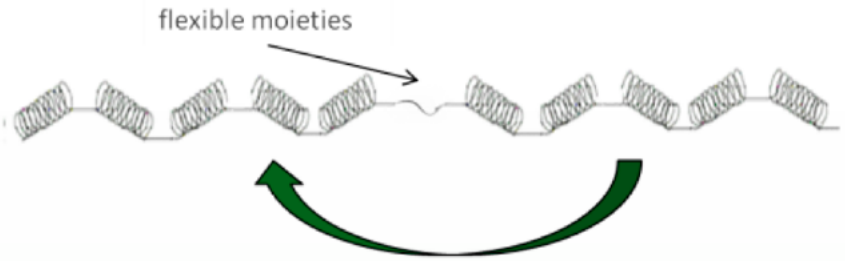
Sugar	% of sugars
XYLOSE	40.9
GLUCOSE	23.8
GALACTOSE	14.5
Glucuronic acid	7.0
3-MeO-pentose	0.06
Rhamnose	0.49
Arabinose	0.52
Mannose	1.1
4-MeO-hexose	1.3
Methyl galactose	1.4

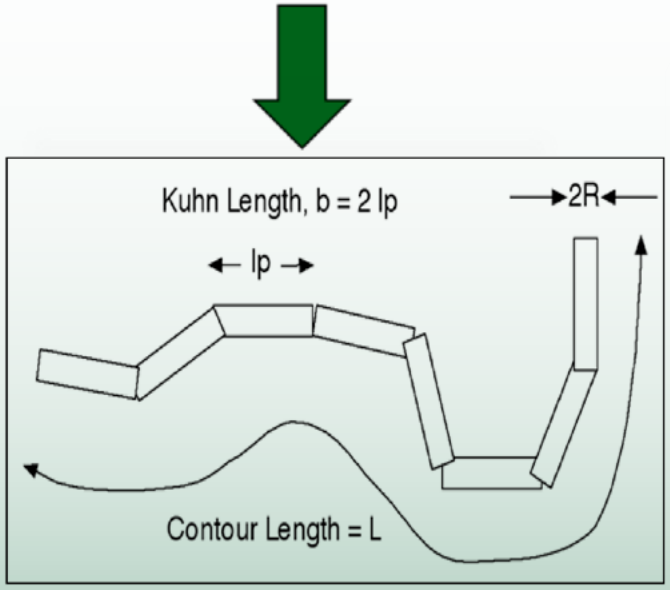
3D Structure of the Polysaccharide Gel

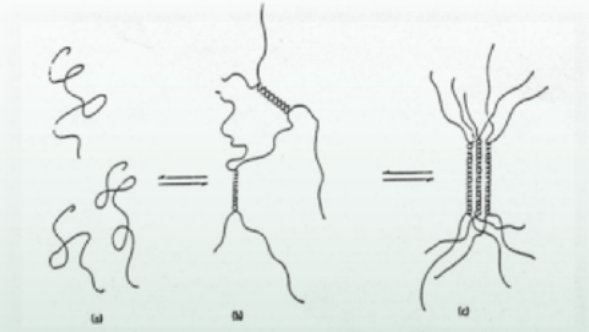
ENVISAGED, based on Rheological studies & SAX Analysis



b-D-Xylp (1 →3)–a-D-Glup (1 → 3)–a-D-GlucA (1 →3)–a+bL-Galp



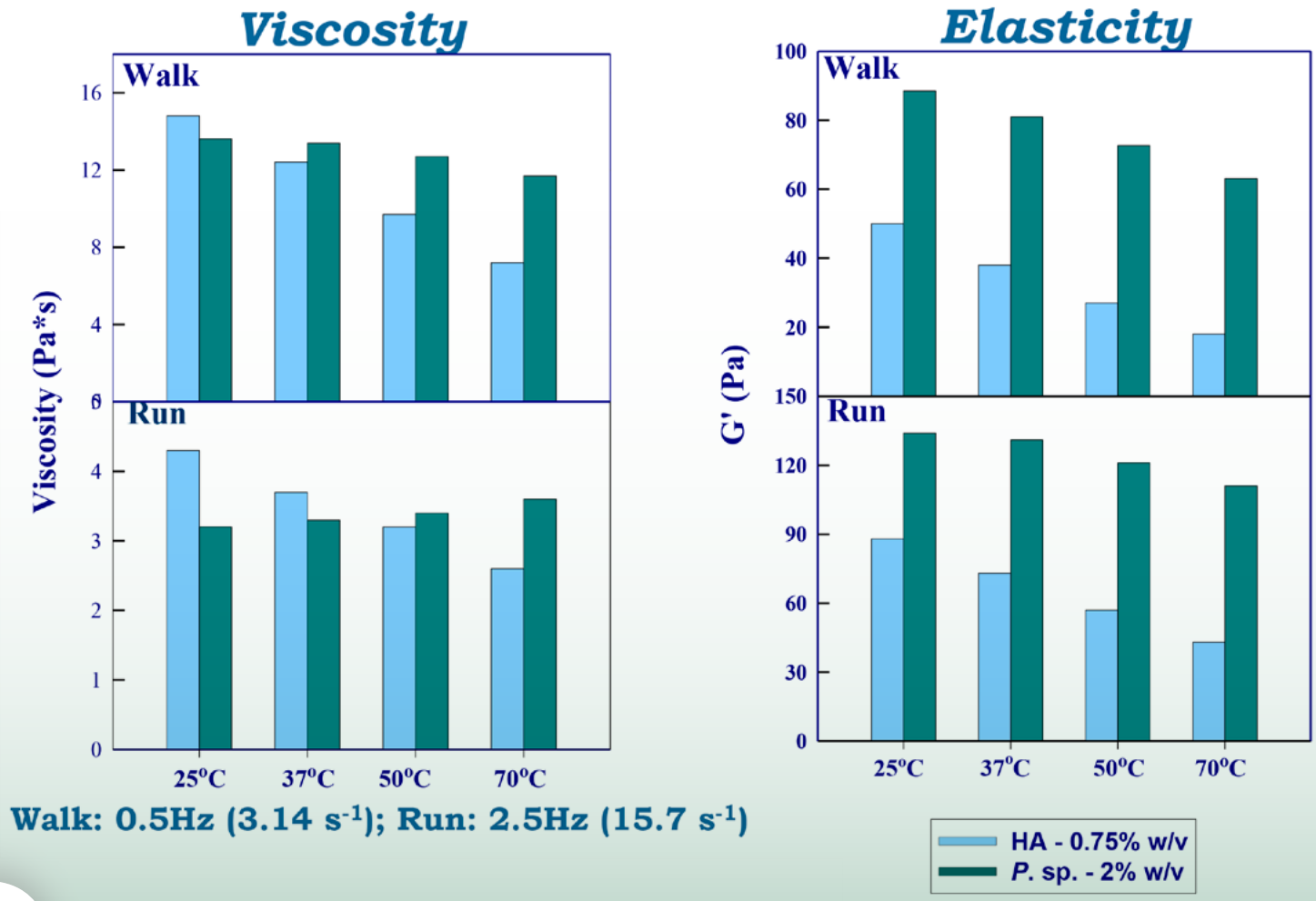




Chain association

Geresh *et al. Carbohyds. Research.* 344:343-349. (2009)

Viscoelasticity of the Polysaccharide



Walk: 0.5Hz (3.14 s^{-1}); Run: 2.5Hz (15.7 s^{-1})

Arad *et al. Langmuir* 22: 7313-7317 (2006)

Novel Polysaccharide

- Lubrication
- Adsorption
- Friction Reduction
- Bioactivities
- Resistance
  - Temperatures
  - pH
  - Enzymatic degradation

Research Approaches

Polysaccharide Production

Physiological aspects affecting quality & quantity:

Temperature; Salinity;
Nutrient *Starvation/ Enrichment: N,S,*CO_2

Biochemical Aspects

Cell wall glycoproteins; Inhibitors (cell wall modified mutants);
Golgi involvement; Sulfation; CO_2 **distribution**

Mode of Operation

Continuous; Batch; Harvesting time

Bioactivities

Algal Polysaccharide

- Anti Viral
- Anti Inflammatory
- Soothing
- Anti Oxidant

Anti-Aging

- Pharmaceutics
- Dermatology
- Cosmetics

Bioactivities

Polysaccharide Biolubrication

ORTHOPEDICS - JOINT LUBRICATION

Osteoarthritis of the Knee

OPTHALMICS

Eye Lenses

Anti Herpes Activity

- *Prevent further development*
- *Reduce time of recovery*
- *Reduce pain*

Control
(not infected and not treated)

HSV-1 Infected
(7 days post infection)

HSV-1 Infected
and treated with polysaccharide (7 days post infection)

Arad *et al. Marine Biotechnology: Biomaterials from Aquatic & Terrestrial Organisms.* 2006,p.37-62.

Anti-Inflammatory Activity

SUMMARY

In vitro

- *The polysaccharide inhibited two aspects of inflammation: recruitment and adhesion of polymorphonuclear leukocytes, which at least partly explains the mechanism of action of its anti-inflammatory effect.*

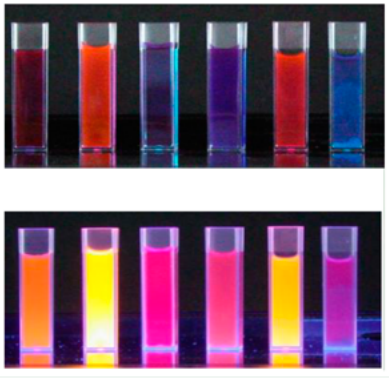
In vivo

- *Pre-treatment with native polysaccharide resulted in a 55% decrease in skin redness.*
- *In oxazolone-induced edema of the ear the polysaccharide exerted 52-75% inhibition.*

Industrial Applications

Cosmetics & Food

Natural Colors



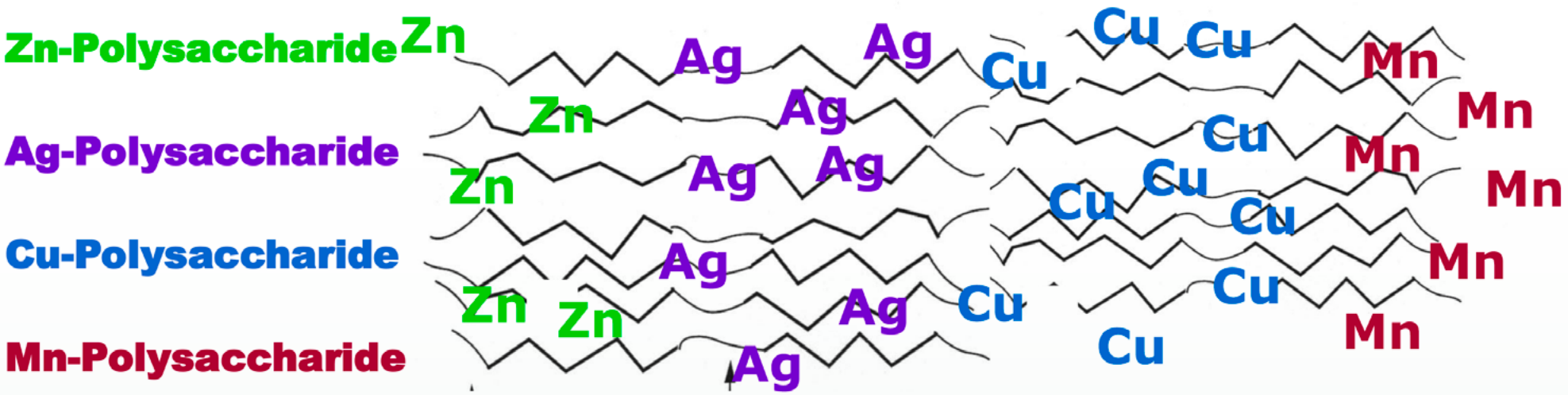
Diagnostics

Chemical Modifications

Tailor Made Hydrogels

QUATERNIZATION	→	Positive charge
CROSS LINKING	→	Higher M.W.
SULFATION	→	Higher sulfate content
METAL COMPLEXATION	→	**Synergistic Activities**

The Polysaccharide as a Platform for Metal Complexes



Patent No. : US 8,647,635 B2

Metal Complexes Advantages

NUTRACEUTICALS

- *Nutritional & gastrointestinal disorders*
- *Slow release of Zn*

PHARMACEUTICS

- *Burns*
- *Chronic wounds*
- *Skin infections*

COSMETICS

- *Preservatives*
- *Acne*
- *Skin soothing*
- *Hair treatments - dandruff*

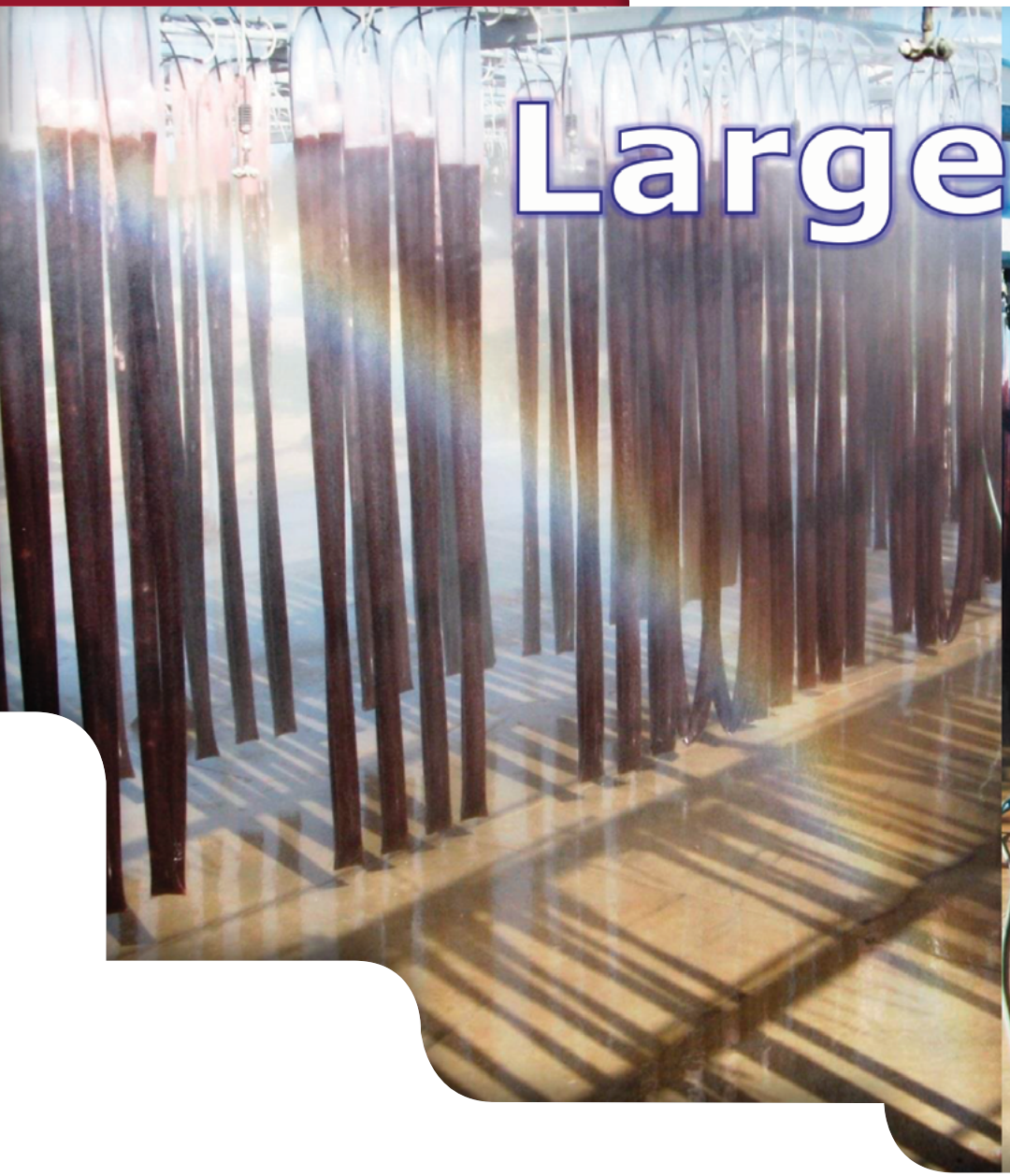
Cultivation

Commercial Cultivation

Closed Bioreactors-Polyethylene Sleeves

Cultivation

Large Scale



Closed Bioreactors - Polyethylene Sleeves

Advantages over open ponds:

- ***DISPOSABLE***
- ***IMPROVED LIGHT AVAILABILITY: higher surface to volume ratio***
- ***IMPROVED TURBULENCE : Better Light/Dark Cycles***
- ***BETTER CORRELATION BETWEEN: Temp. & Light Fluctuations***
- ***COOLING BY WATER SPRAYING***
- ***PREVENTION OF CONTAMINATION***

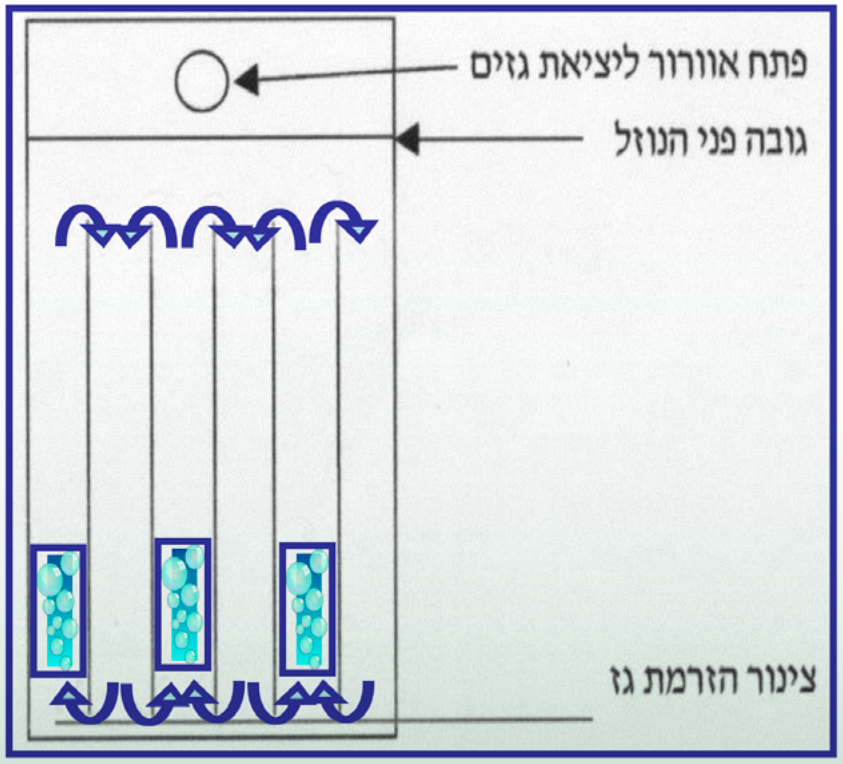
Large Scale Cultivation

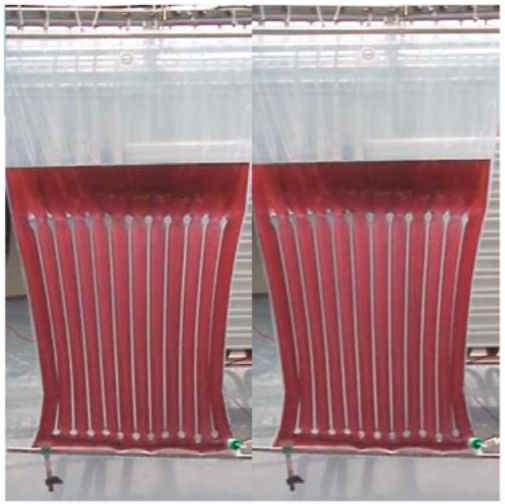
- *Outdoor growth –*

  Nature controls polysaccharide production
- *Natural U.V & light exposure to maximize production of polysaccharide*
- *Patented bioreactors growth system*

Future Potential

Schematic presentation





Polysaccharide Downstream Processing

Algal Culture

↓ Centrifugation

Algal Cells
(Biomass)

Supernatant
Soluble polysaccharide

↓ Filtration

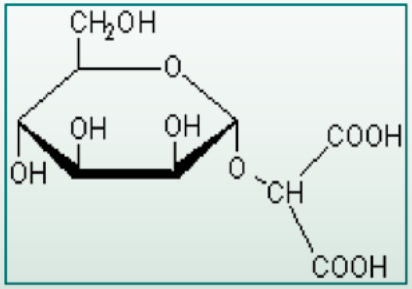
↓ Autoclaving

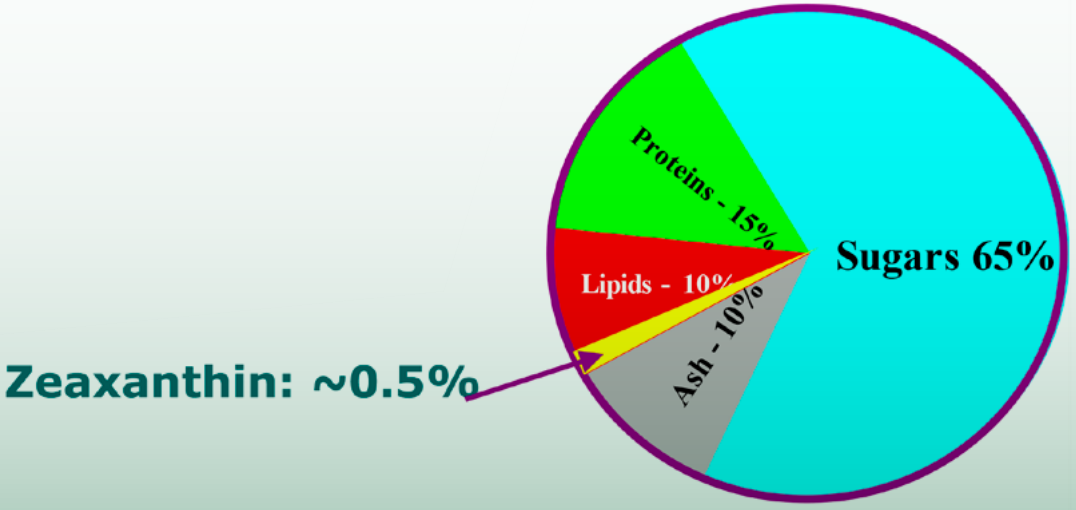
Polysaccharide

Algal Biomass for Health Food

Unique Combination

- ***DIETARY FIBERS (SOLUBLE & INSOLUBLE)***
- ***UNSATURATED FATTY ACIDS (AA, EPA)***
- ***MINERALS (Ca,K,Zn,Se)***
- ***ZEAXANTHIN***
- ***FLORIDOSIDE***

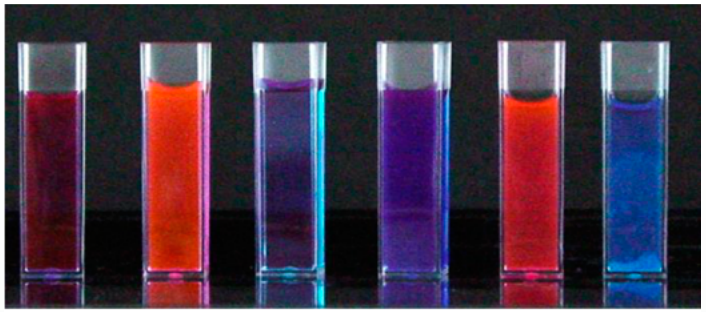


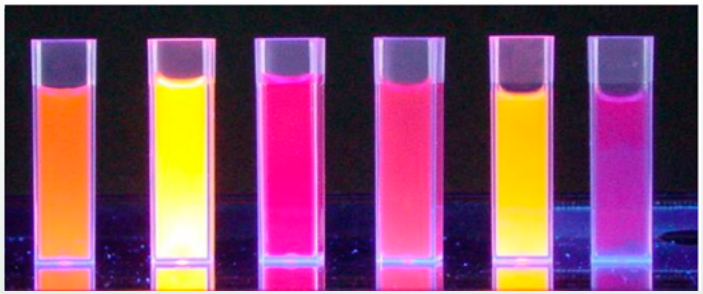


Algal Colors

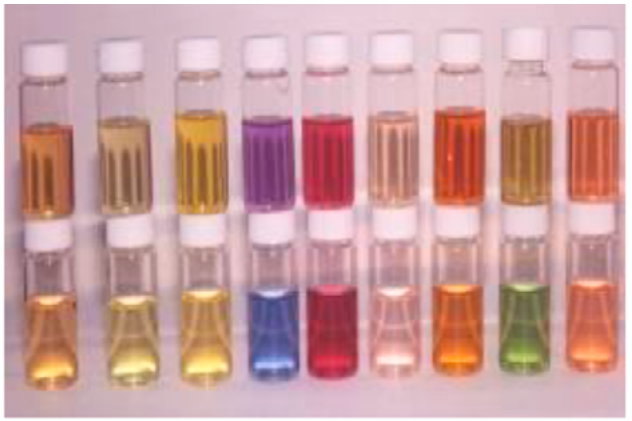
Cosmetics & Food

Colors





Fluorescence



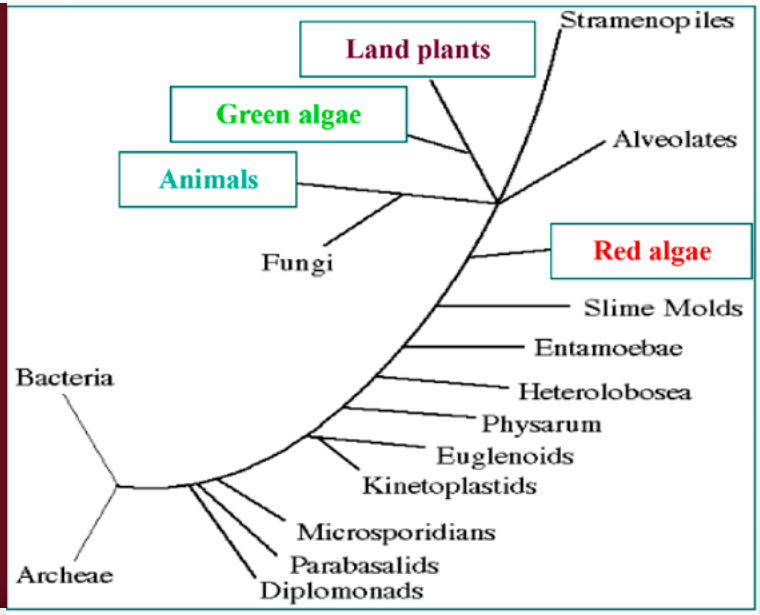
Red Microalgal Genome Project

UNIQUE METABOLIC PATHWAYS:
Polysaccharide, Sulfation, Fatty Acids

Novel genes

Proteins
pharmaceuticals

Evolution



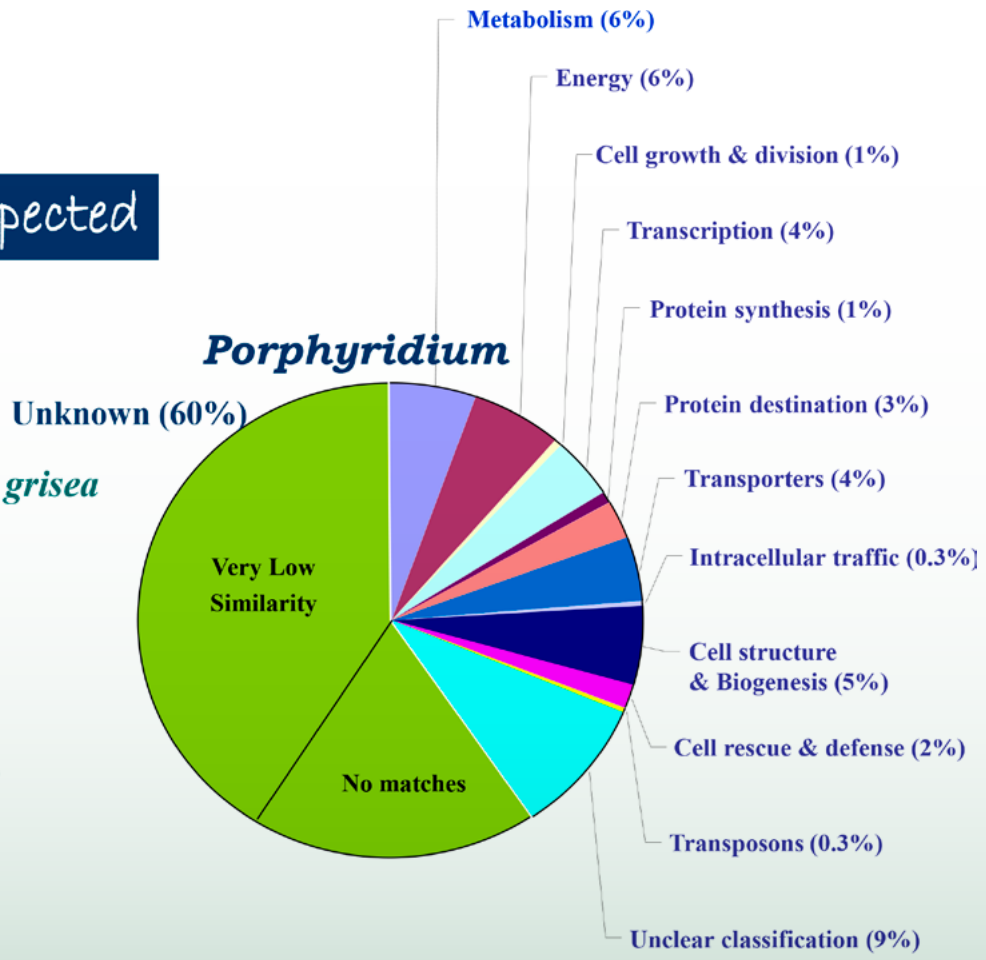
Phylogeny tree Based on ssu rDNA gene, 2002.

http://www.actionbioscience.org/evolution/dacks.html

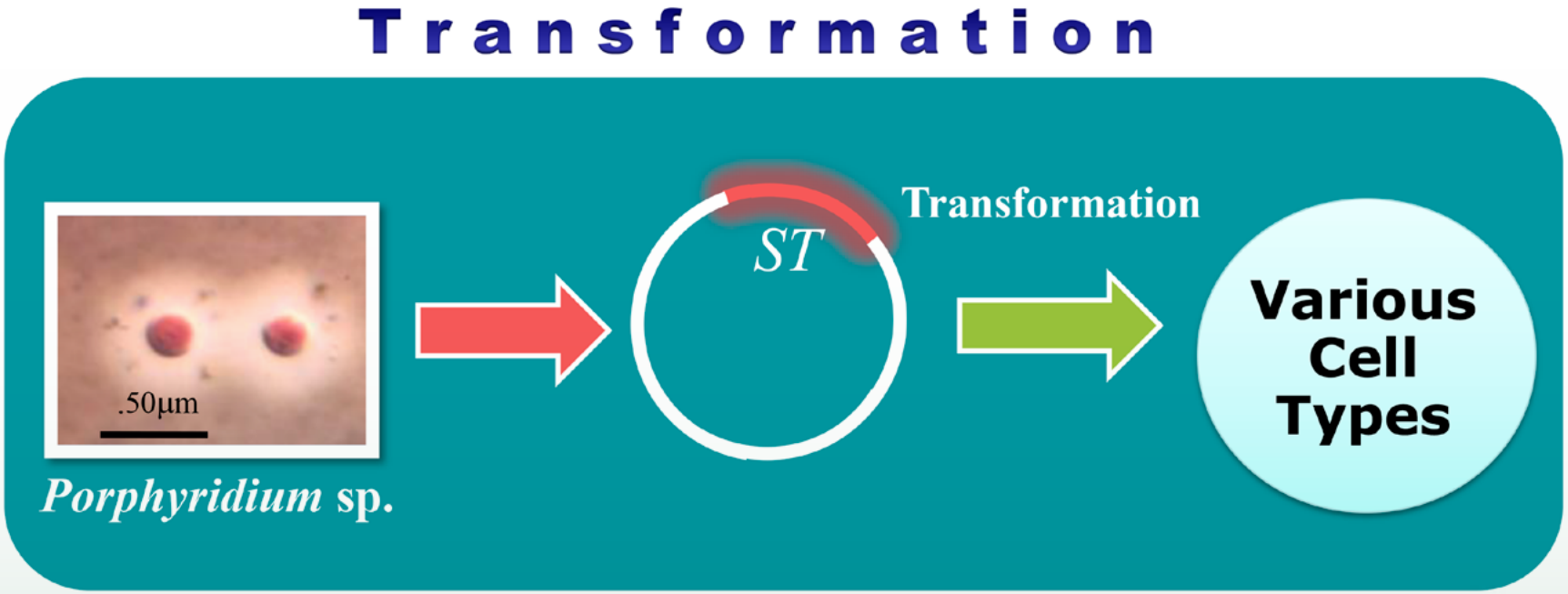
Unique Algal Genes as a Resource

~6000 genes expected

Total ESTs	*P. sp.*	*Dixoniella grisea*
Sequenced	*9,000*	*9,000*
Assembled	*2,089*	*1,980*
Contigs	*640*	*647*
Singlets	*1,449*	*1,333*



Algal Genomes as a Resource of Novel Genes

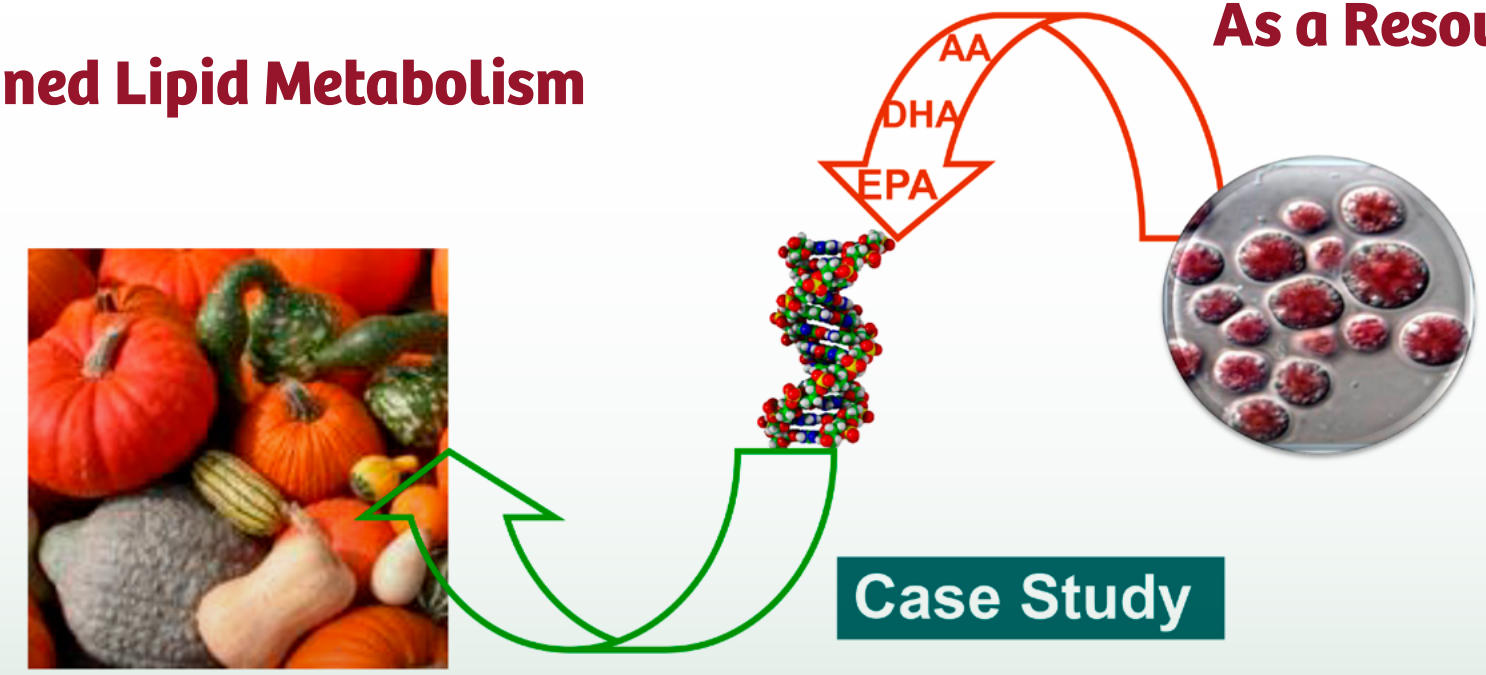


From Algae to Plants

Transformation of Genes

Algae Genes-
As a Resource

Newly Designed Lipid Metabolism



Microalgae as cell Factory

Platform for Protein Production in Red Microalgae

Pharmaceuticals

Vaccines → Animals, Humans

Antibodies

Hormones

Industry

Enzymes

Growth Factor

Edible Vaccines: Advantages

Development of non-invasive vaccines:

The new trend

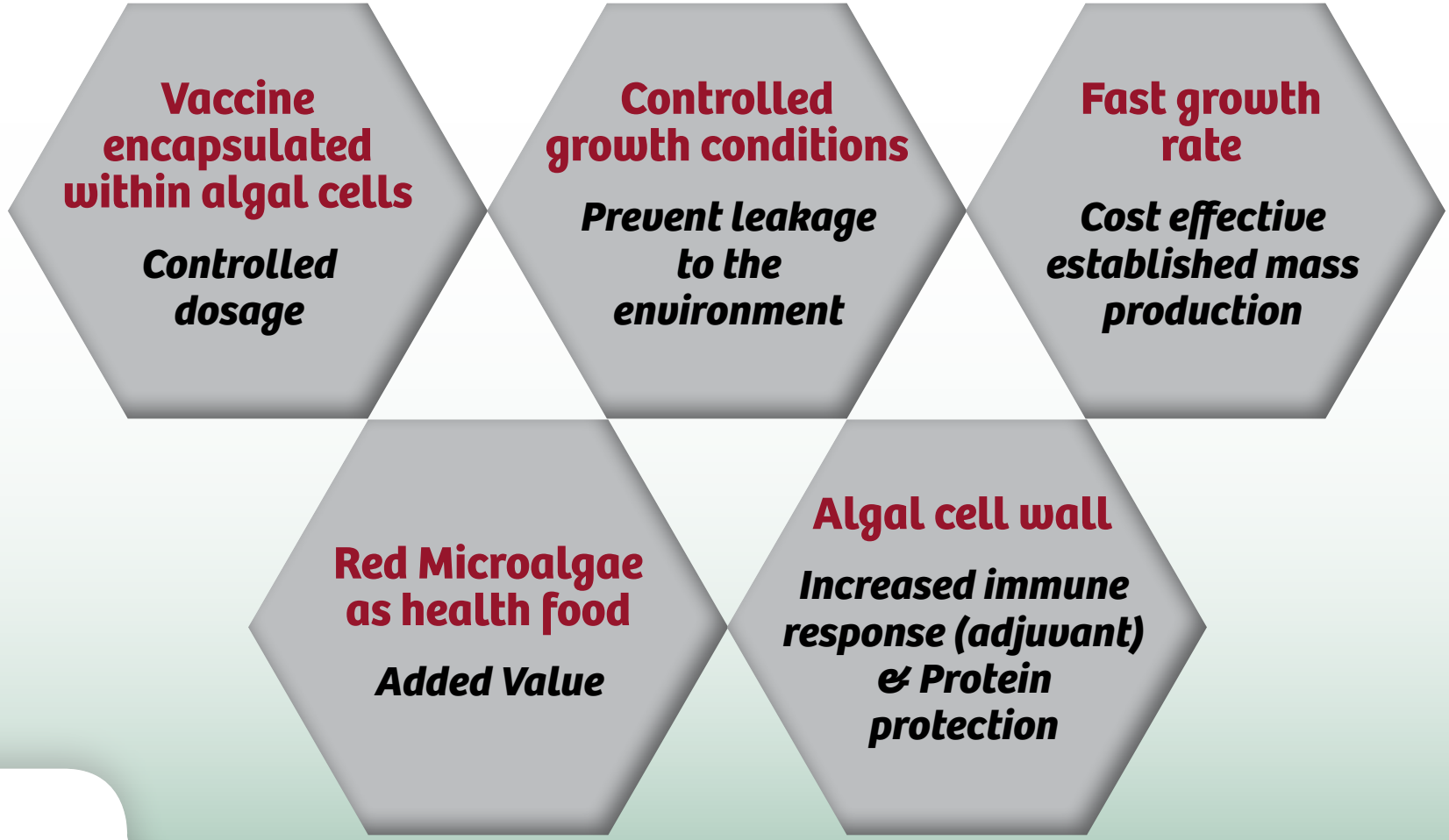
Advantages:

- *Inexpensive*
- *High availability*
- *Safe*
- *Fast mass immunization (The Third World)*
- *Efficient immunization against gastrointestinal & respiratory diseases*

No need for:

- *Sterile needles*
- *Vaccine purification*
- *Skilled manpower*

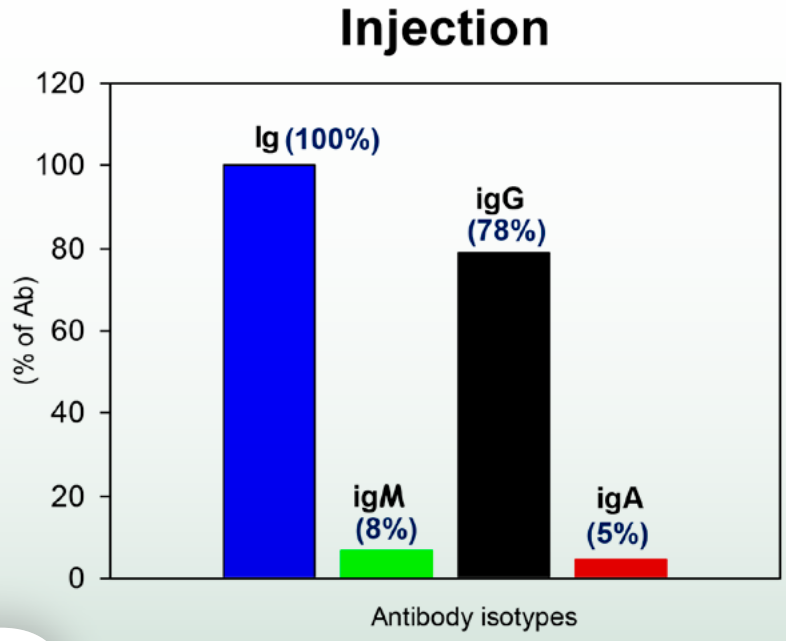
Oral Vaccines in Red Microalgae

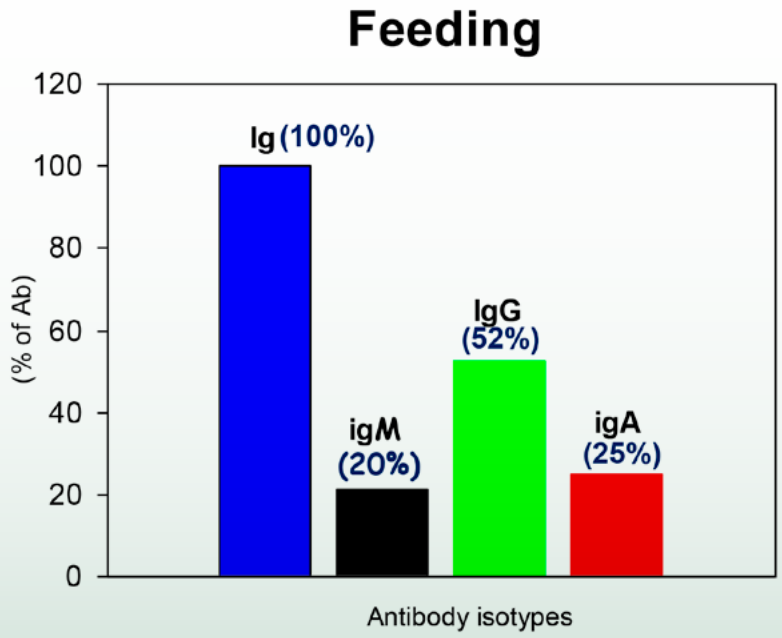


Feeding with Transformed Algae

Proof of Concept

Generation of anti-ovalbumin antibodies by feeding mice with transgenic algae





Fish Oral Vaccination

Using Red Microalgae Against Fish Diseases

Microalgae are Ideal: Part of the Natural Food Chain

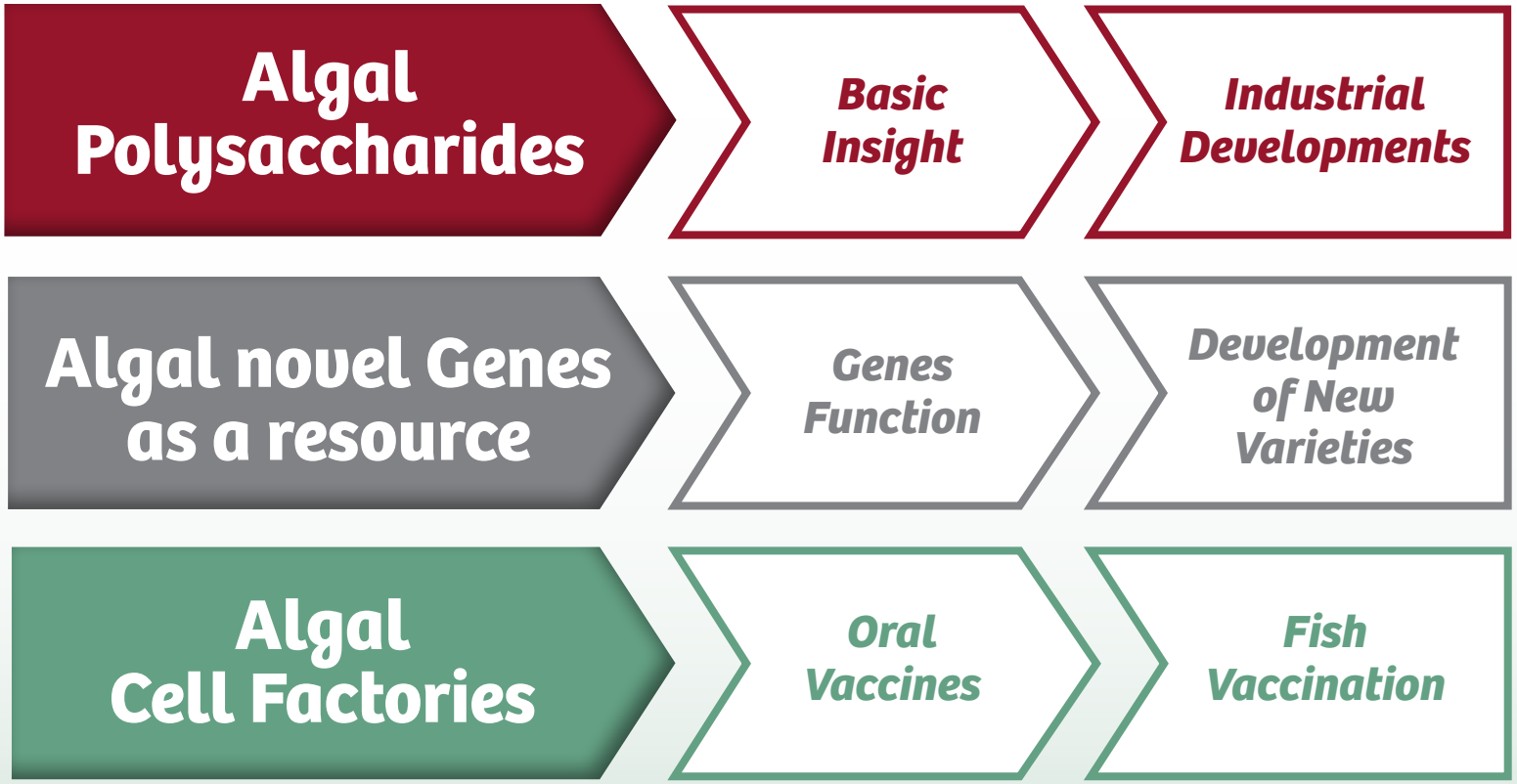
Viral nervous necrosis is a disease, caused by Betanodavirus (BTN)
The virus attacks over 30 species of fish

World aquaculture productionis valued > $70 billion
Increase for world demand for fish

High disease spread potential
with no treatment available

Future Perspectives

The Interplay Between Genes & Products



Industrial Production from Algae