PRODUCTION OF PHB FROM GLYCEROL WASTE BY *B. xenovorans* LB400

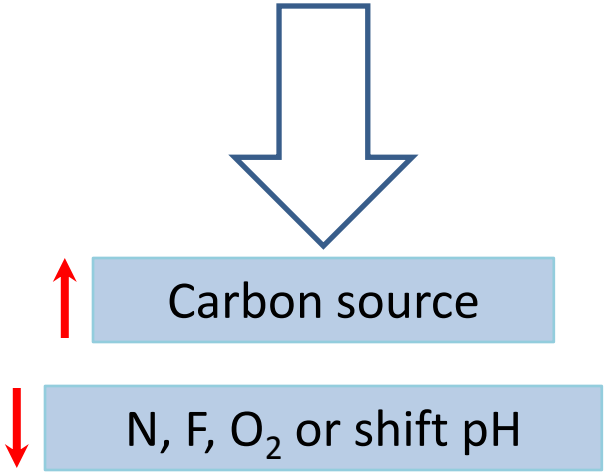
Pamela Villegas[1,2], Viviana Urtuvia[1], Myriam González[1], Gregorio Gomez[2] & Michael Seeger[1]

[1]Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Departamento de Química, Centro de Biotecnología & Center for Nanotechnology and Systems Biology, Universidad Técnica Federico Santa María, Valparaíso, Chile. [2]Facultad de Biología, Universidad de La Habana, Cuba. [3] Bioproducts Laboratory, Sao Paulo University, Sao Paulo (SP), Brazil.

POLYHYDROXYALKANOATES

Polyhydroxyalkanoates are polyesters synthesized by different bacteria as intracytoplasmic granules

The PHA synthesis occurs either constitutively or under unbalanced nutritional conditions



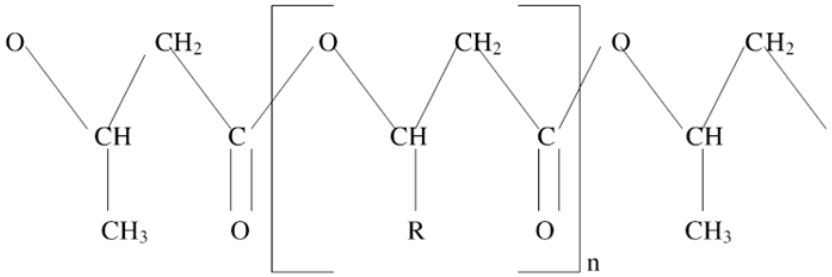
(Urtuvia *et al*., 2014 , Int J Biol Macromol; da Silva *et al.*, 2014, Int J Biol Macromol ; Hazer & Steinbüchel, 2007, Appl Microbiol Biotechnol.)

POLYHYDROXYALKANOATES

Short chain length: 3-5 carbons atoms

Medium chain length: 6-14 carbons atoms

More than 150 different monomers has been reported



n varies from 600 to 35000

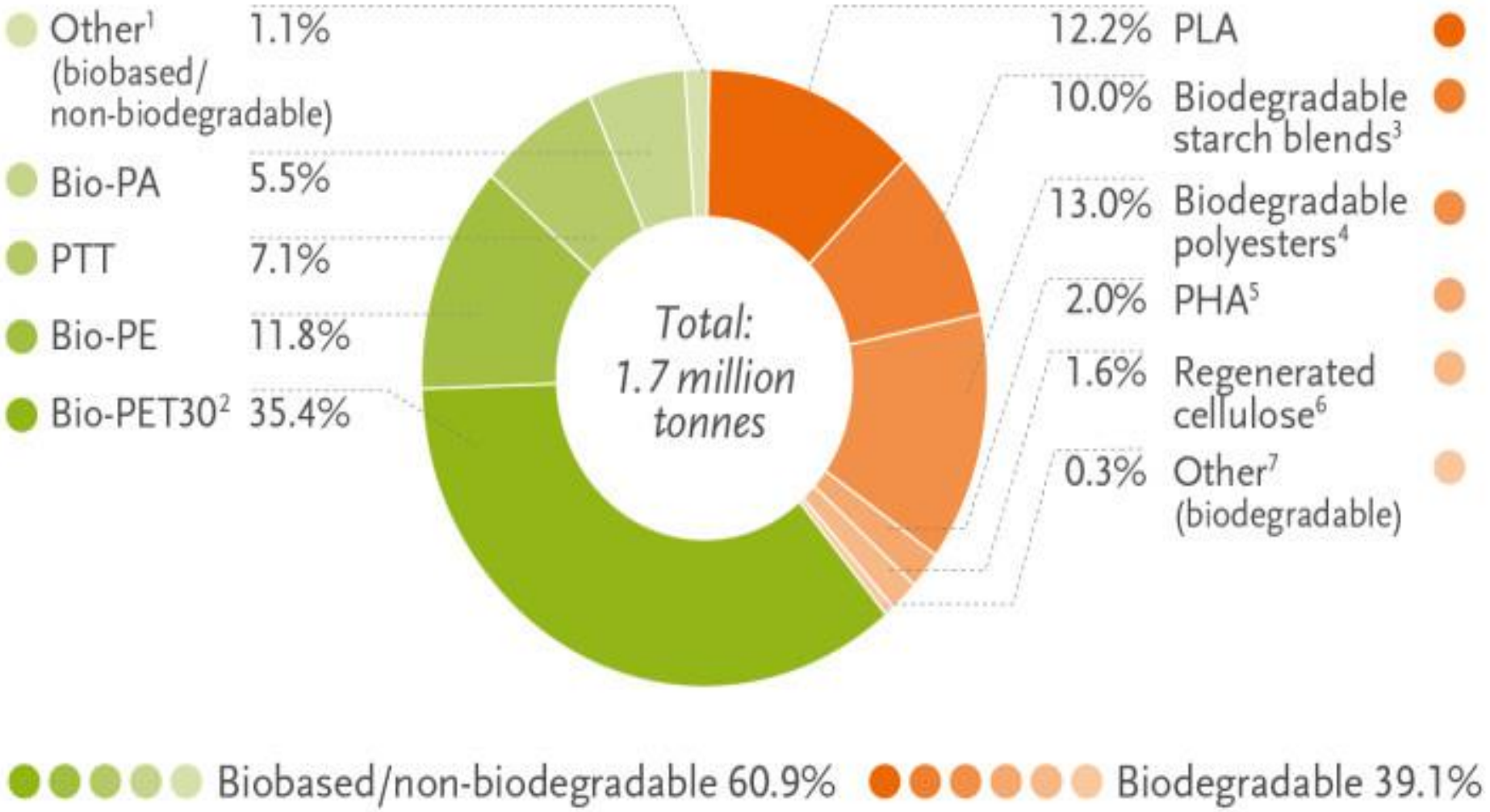
R= hydrogen	Poly(3-hydroxypropionate)
R=methyl	Poly(3-Hydroxybutyrate)
R=ethyl	Poly(3-hydroxyvalerate)
R=propyl	Poly(3-hydroxyhexanoate)
R=pentyl	Poly(3-hydroxyoctanoate)
R=nonyl	Poly(3-hydroxydodecanoate)

- ✓ PHAs have similiar physico-chemical properties than polypropylene
- ✓ PHAs have different composition depending on the microorganism, the carbon source and culture conditions
- ✓ 3-hydroxybutyrate and 3-hydroxyvalerate are two main monomers

http://www.biocycle.com.br/fotos_produtos.htm

(Urtuvia *et al*., 2014 , Int J Biol Macromol; da Silva *et al.*, 2014, Int J Biol Macromol ; Hazer & Steinbüchel, 2007, Appl Microbiol Biotechnol.)

Global production capacities of bioplastics 2014 (by material type)



http://en.european-bioplastics.org/

ADVANTAGES

- ✓ Biodegradable
- ✓ Biocompatible
- ✓ Synthesized from renovable sources (sugars and organic acids).
- ✓ Specific PHA composition for different applications

DISADVANTAGES

- ✓ 10 fold more expensive than plastics derived from petroleum, mainly due to:
  - i. carbon source costs
  - ii. extraction technologies
  - iii. volumen of production

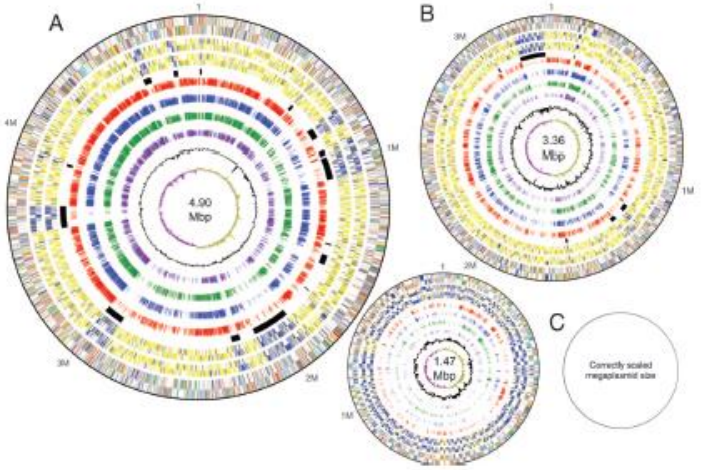
To decrease PHA cost, cheap carbon sources may be used:

- i. crude glycerol
- ii. hemicellulose hydrolysate
- iii. whey
- iv. alpechin
- v. olive oil

(da Silva *et al.*, 2014, Int J Biol Macromol ; Du, Ch. *et al*., 2012, Current Chemical Biology)

BACTERIUM

B. xenovorans LB400



- ✓ β- proteobacteria and non pathogenic
- ✓ Genome size 9,73 Mbp
- ✓ Model bacterium for degradation of polychlorobiphenyls and aromatics compounds

(Chain, *et* al., 2006, PNAS; Romero-Silva, *et a*l., 2013, PloS one; Mendez , *et al*., 2011, PloS one)

GROWN ON DIFFERENT CARBON SOURCES

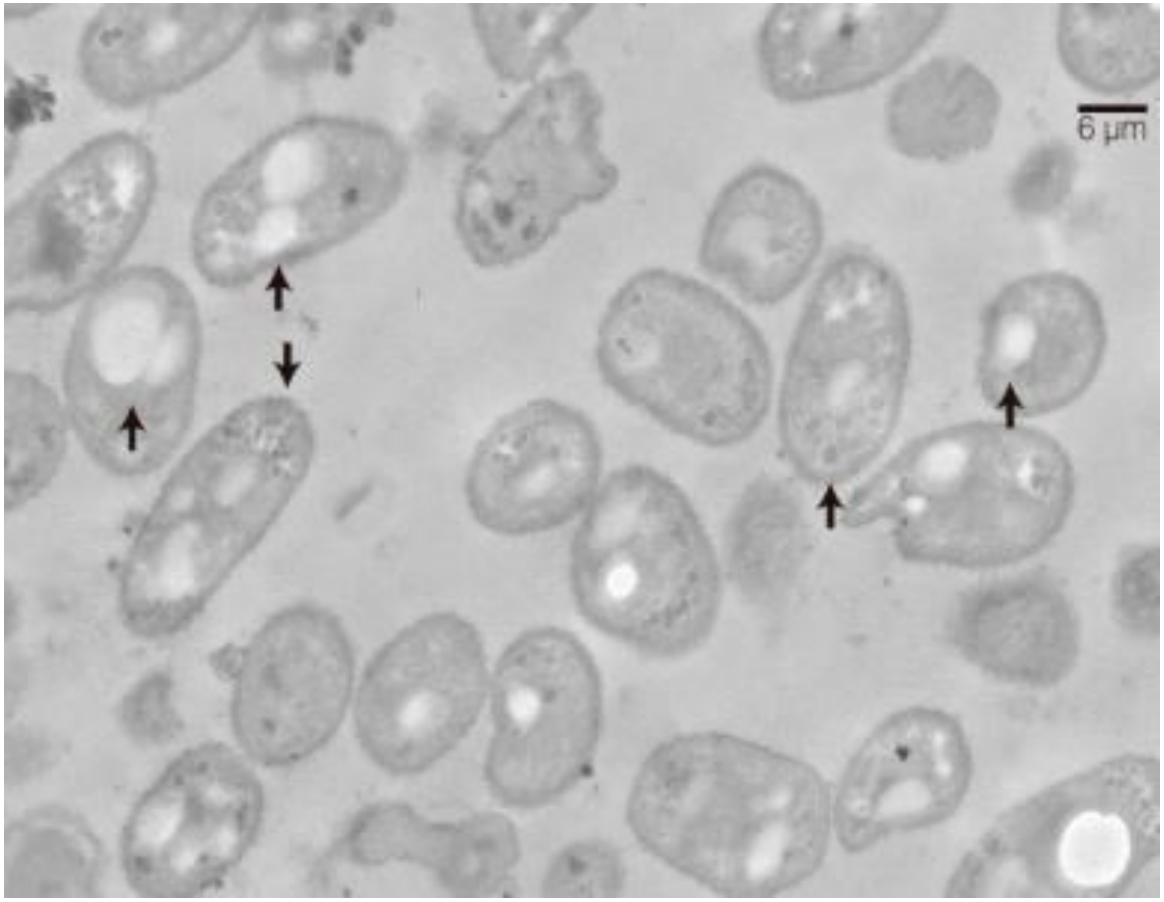
Carbon sources	
Glucose	++
Gluconate	++
Mannitol	++
Xylose	++
Maltose	-
Ramnose	++
Sucrose	-
Lactose	-

Carbon sources	
Arabinose	-
Valerate	+
Octanoate	+
Nonanoate	+

B. xenovorans LB400 is capable to growth on different carbon sources.

(Villegas , *et al*., unpublished results)

PHA PRODUCTION BY *B. xenovorans* LB400

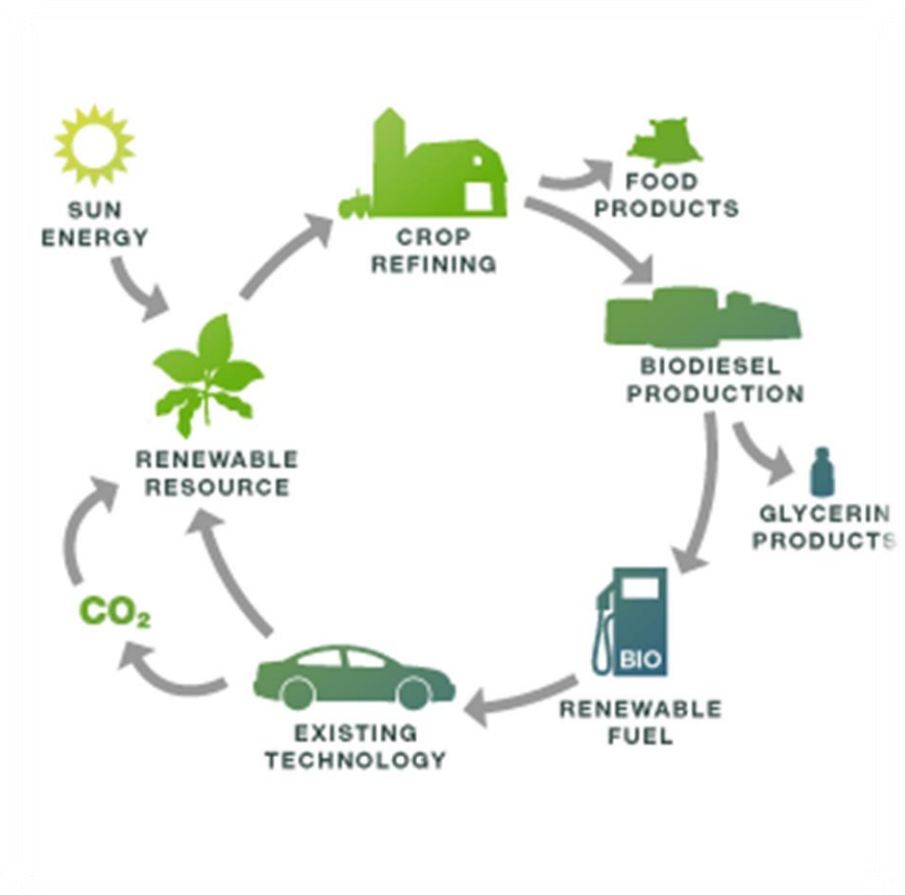


B. xenovorans LB400 grown on xylose (10 g L^{-1}) under N limitation (0.1 g L^{-1})

(Urtuvia, *et* al., 2014)

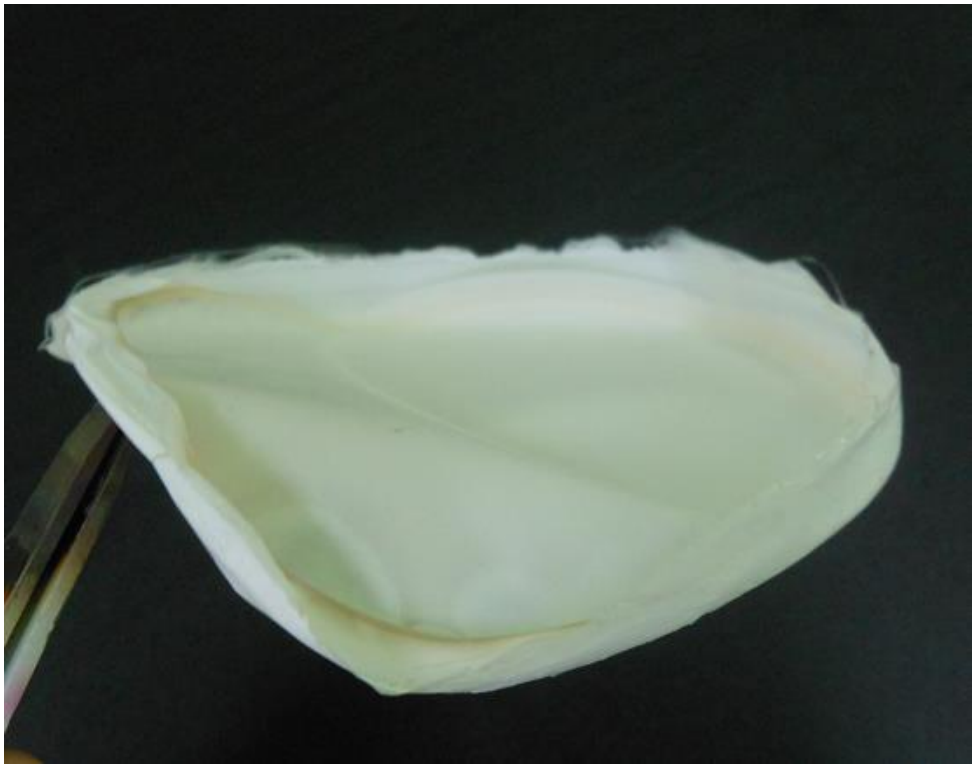
GLYCEROL AS CARBON SOURCE

- ✓ Biodiesel production generates 10% glycerol as sub-product
- ✓ From 1999 to 2013, biodiesel production increased from 500.000 to 1800 millions of gallons
- ✓ Glycerol costs has decreased significantly during the last years



(Silva ,*et al.*, 2009)

GLYCEROL CRUDE AS CARBON SOURCE



PHBV film extracted from *B. xenovorans* LB400 culture grown on glycerol waste and valeric acid

- ✓ PHB produced from glycerol possess higher MW than other PHB
- ✓ No significant differences were found in the PHB production from pure glycerol, crude glycerol or xylose.
- ✓ Therefore crude glycerol is an attractive low cost carbon source.
- ✓ Next steps will include high density cultures towards the scaling up of PHB and PHBV production.

ACKNOWLEDGEMENTS

Ph.D. Michael Seeger

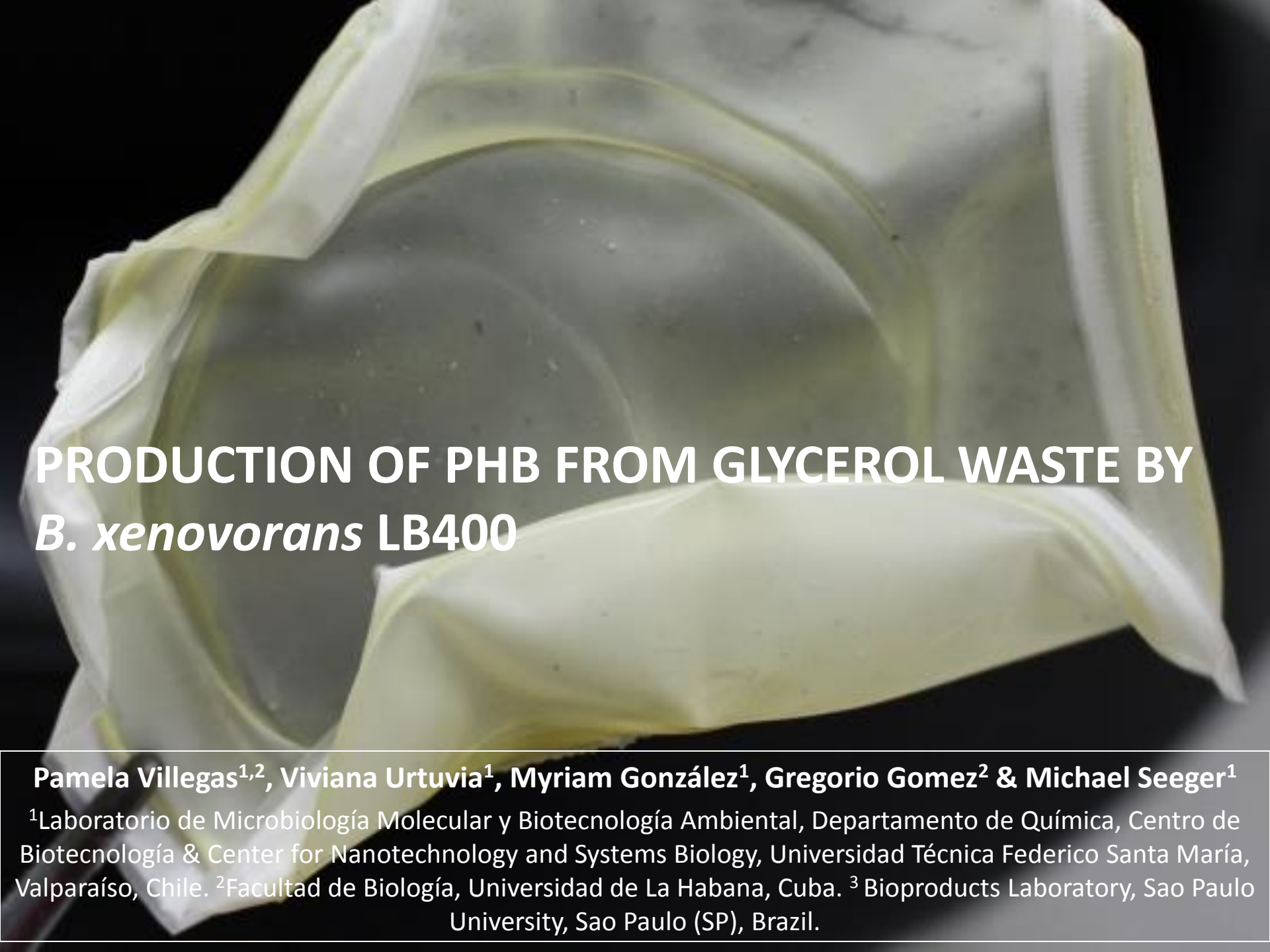
Ph.D. Viviana Urtuvia

Ing. Myriam González

Scientific networking
Ph.D Gregorio Gómez Bioproducts Laboratory, Sao Paulo University, Sao Paulo (SP), Brazil
Ph.D. Luziana da Silva, Bioproducts Laboratory, Sao Paulo University, Sao Paulo (SP), Brazil
Ph.D. Maria Elena Carballo, Facultad de Biología, Universidad de La Habana, La Habana, Cuba
Ph.D. Marcia Rojas, Facultad de Biología, Universidad de La Habana, La Habana, Cuba

Financial support from Conicyt, CD FSM1204 and Cyted PRIBOP fellowships (VU), Red RIABIN, FONDECYT (1110992, 1151174), USM (131109, 131342, 131562), CNBS and CYTED-PRIBOP grants (MS,MG).

LABORATORIO DE MICROBIOLOGÍA MOLECULAR Y BIOTECNOLOGÍA AMBIENTAL
UNIVERSIDAD FEDERICO SANTA MARÍA, VALPARAISO, CHILE.



PRODUCTION OF PHB FROM GLYCEROL WASTE BY *B. xenovorans* LB400

Pamela Villegas[1,2], Viviana Urtuvia[1], Myriam González[1], Gregorio Gomez[2] & Michael Seeger[1]

[1]Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Departamento de Química, Centro de Biotecnología & Center for Nanotechnology and Systems Biology, Universidad Técnica Federico Santa María, Valparaíso, Chile. [2]Facultad de Biología, Universidad de La Habana, Cuba. [3] Bioproducts Laboratory, Sao Paulo University, Sao Paulo (SP), Brazil.