


Biotechnological applications of the dairy industry

OPPORTUNITY FOR SUSTAINABILITY

22 Septiembre 2016.

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Director General de Biopolis S.L.

BIOPOLIS

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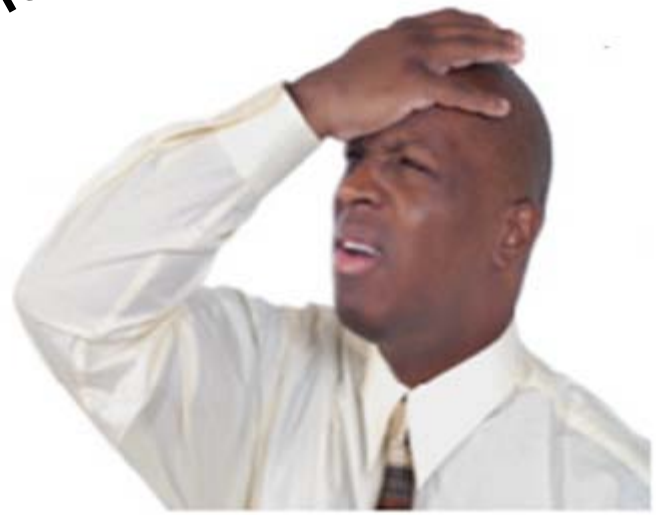
Technological concepts

PROBIOTICOS

TRANSGENICOS

GENES

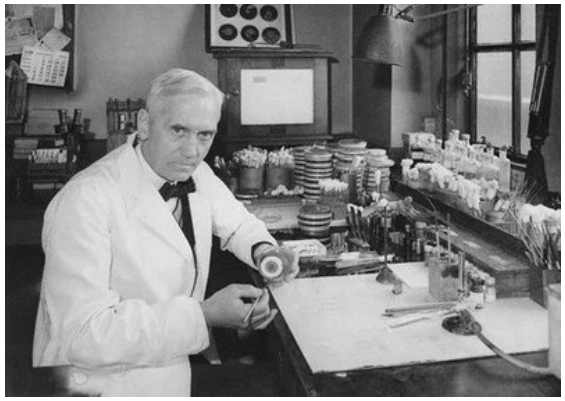


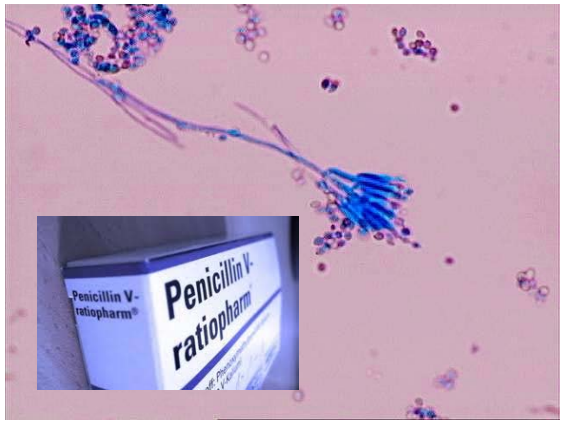
BIOSENSORES

FENOTIPO

MUTANTE

What is food biotechnology ?





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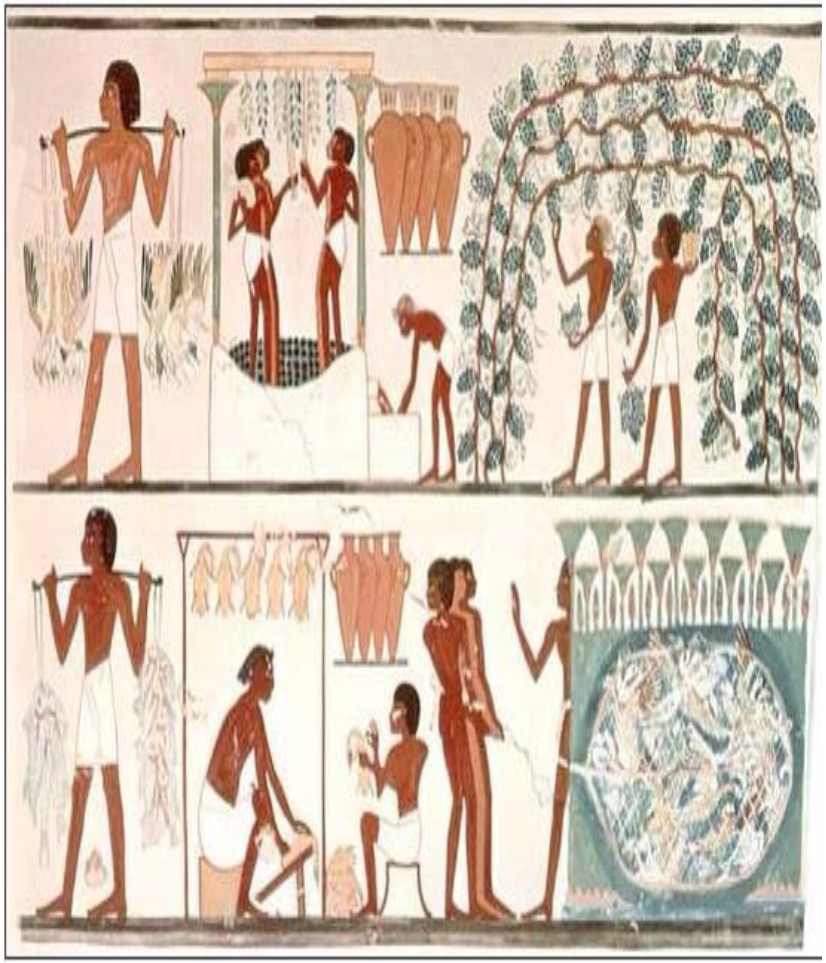
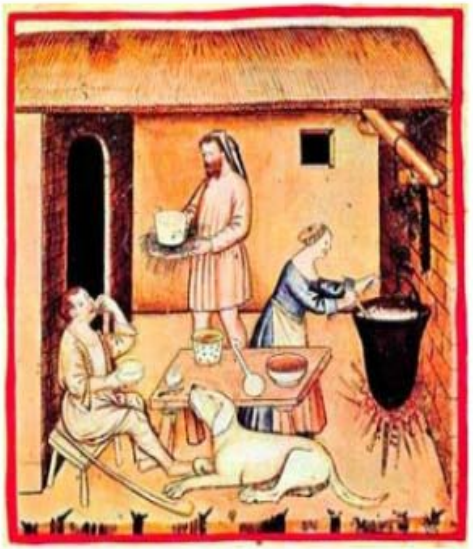
Background

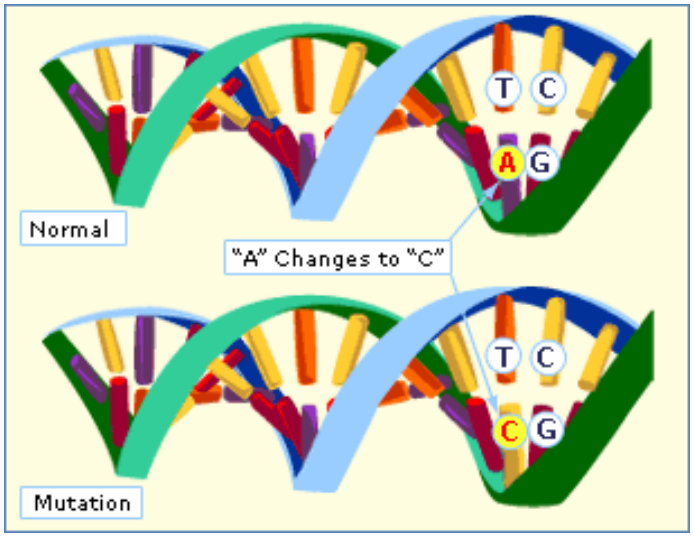


HISTORIA DE LAS LECHES LA FERMENTADAS

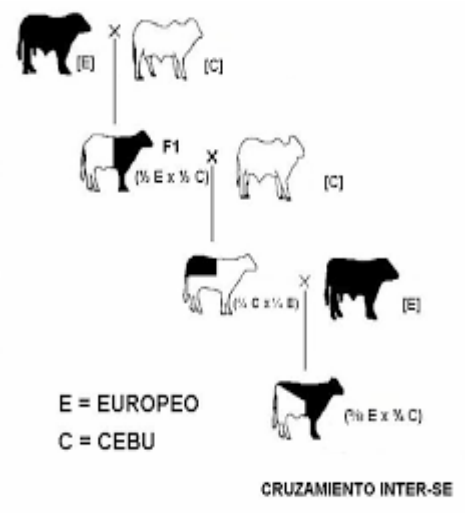


- Método mas antiguo para conservar los componentes nutritivos de la leche.
- Origen mas probable fue en Medio Oriente época Fenicia.
- Productos como *rayeblaban y khadlaban* se producian en el 5000 a.c, kumis 2000 a.c

Background



MUTACIÓN



CRUCE SEXUAL

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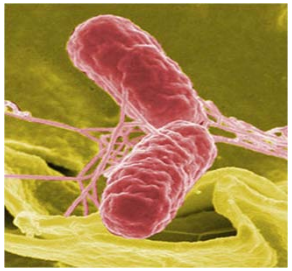
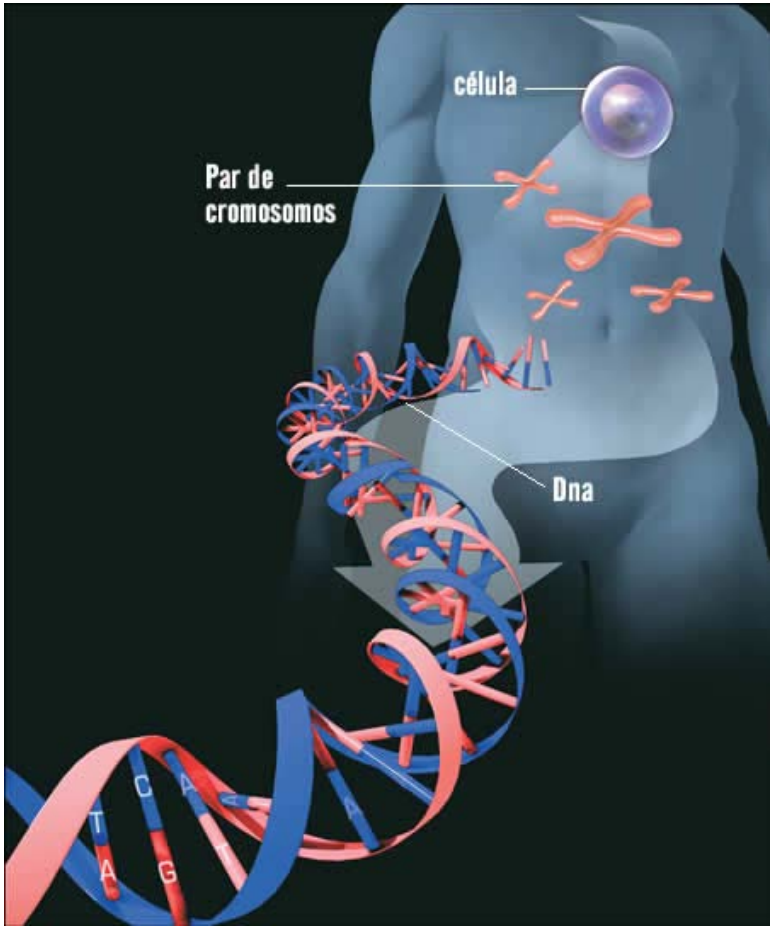
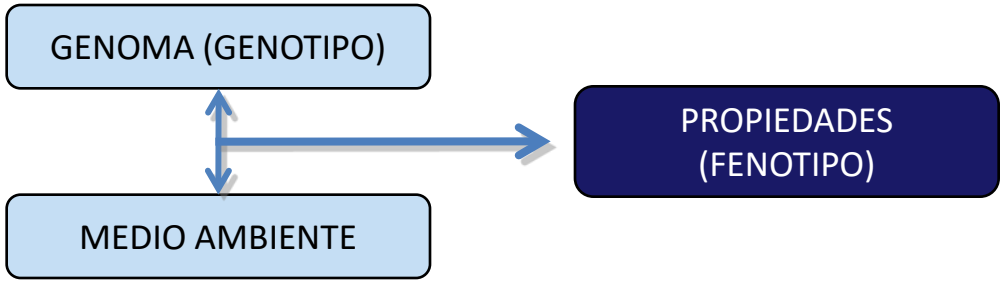
Molecular essence of life

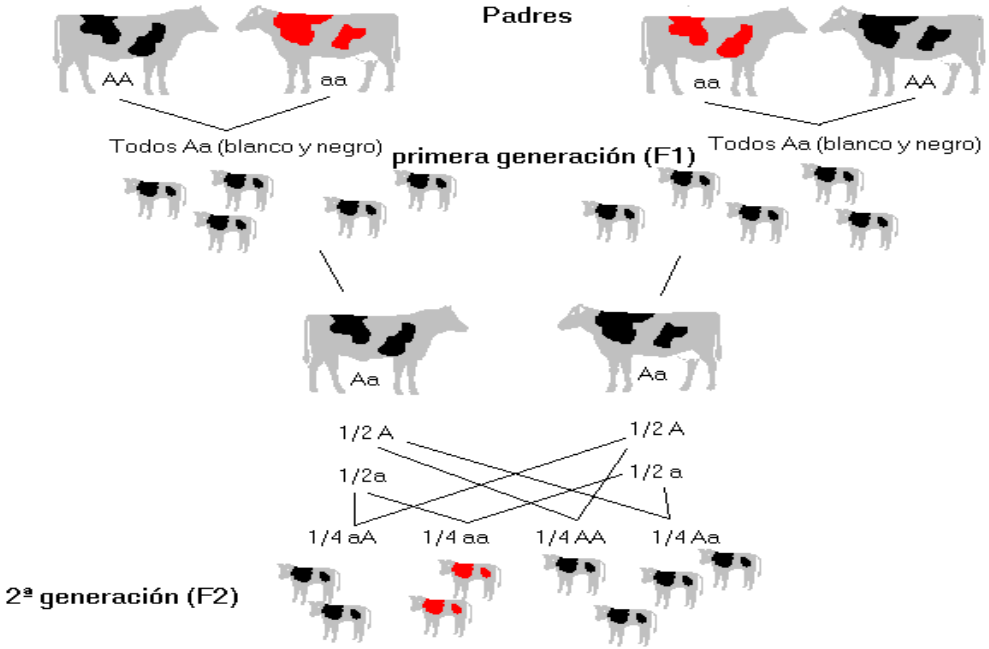


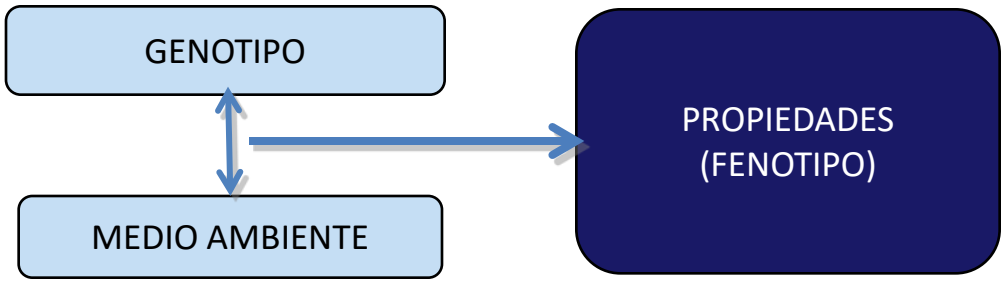


Genotypes and phenotypes

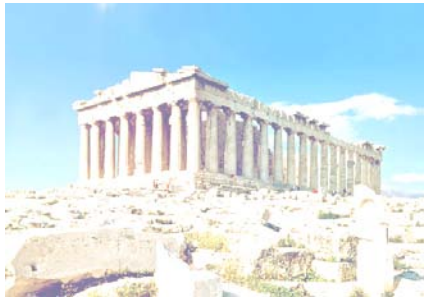


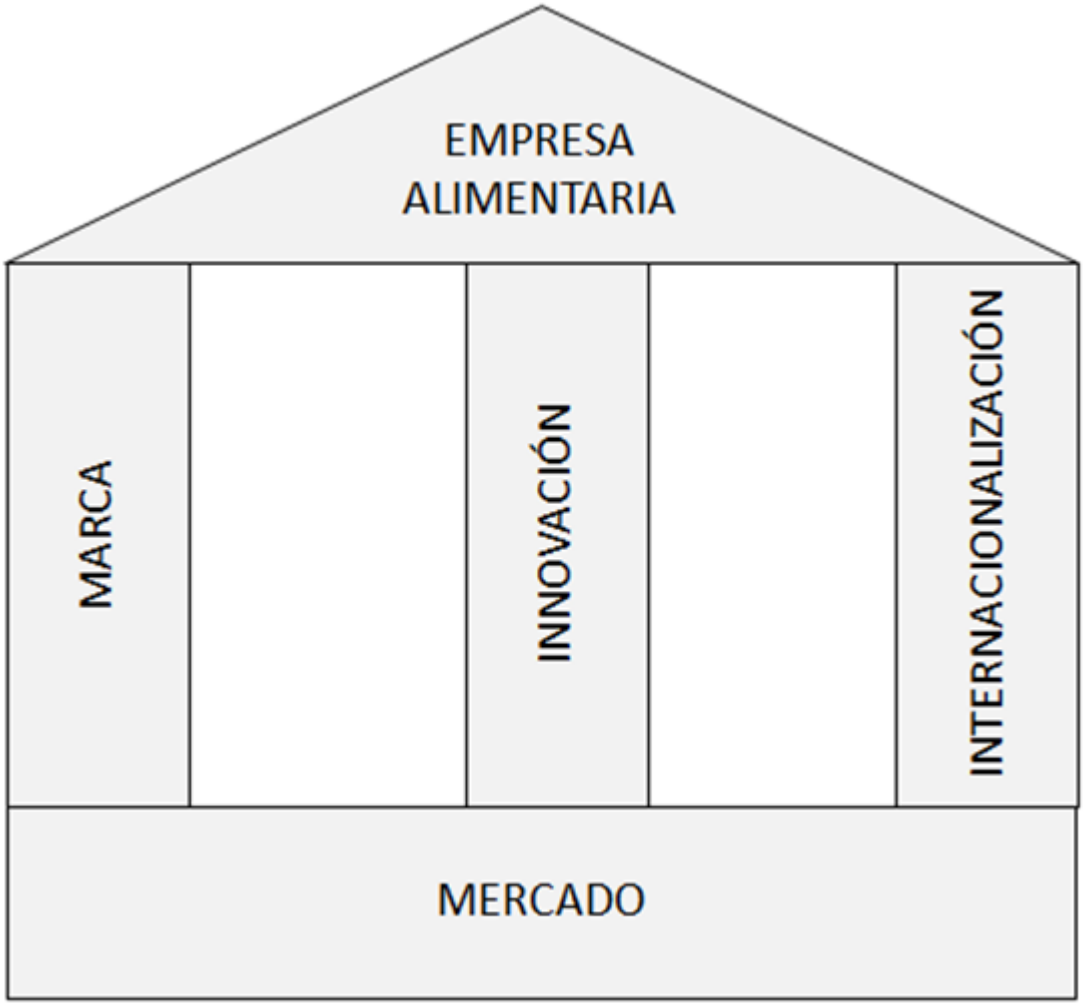
Genes and food





Innovation in the food industry





Dairy products

Type of dairy products:

1. Liquid milks

Short life

Long life

2. Fermented milk

Short life

Long life

3. Concentrated and evaporated mik

Dairy packaging

4. Cheese:

Dairy products

5. Butter:

6. Fat milk:

Dairy products

7. Powder milk:

8. Whey an casseine:

9. others

Biopolis

- Biopolis SL is located in the Cientific Part at the Universitat de València. Builsing of 1500 m^2
- It has 11 laboratories and 2 production plants (GMO and no-GMO)
- The buildings are recognised by the National Commission of Biosafety

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Eating mutants

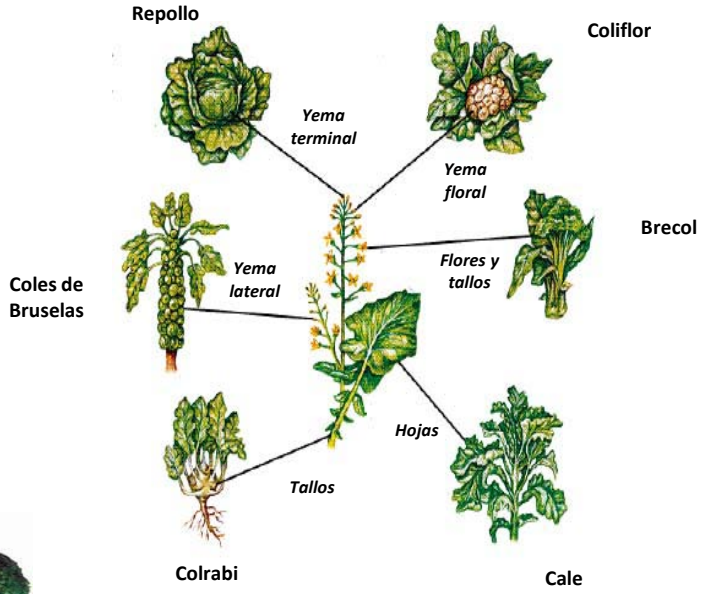
Col

Coliflor

ANCESTRO

Col de Bruselas

Brócoli



Mutants radiation

Naranja dulce

Pummelo

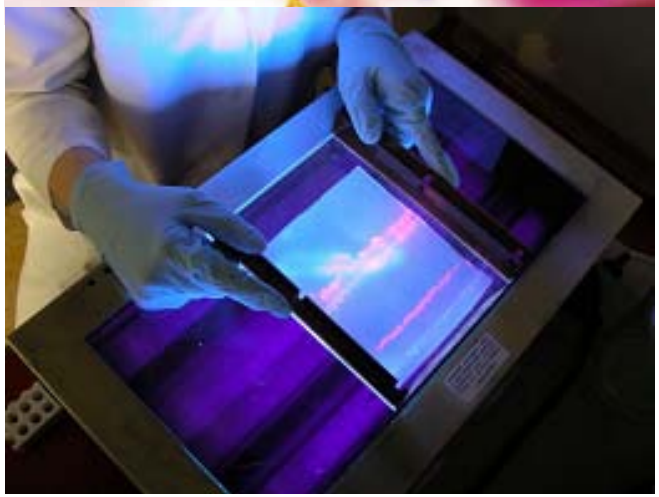
Pomelos ancestrales

Pomelo Hudson

Pomelo Star Ruby

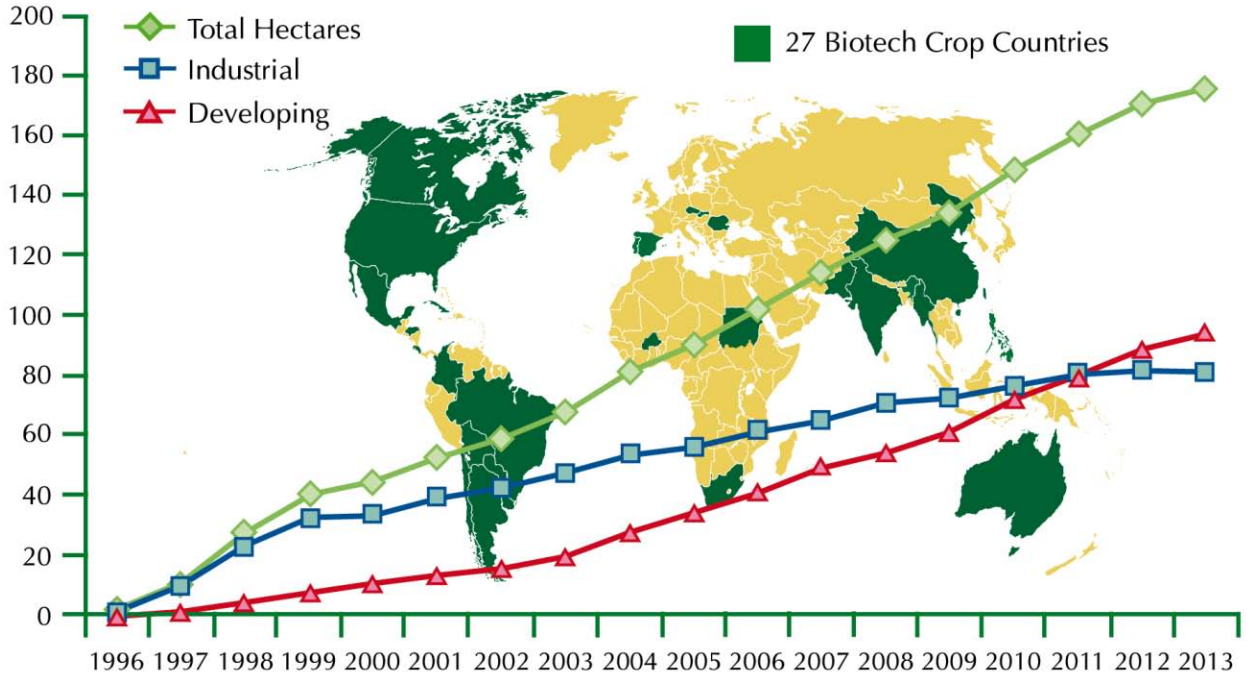
Genetic improvements

Genetic engineering



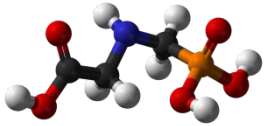
- You can work with isolated genes and not with complete genomes
- Improvements are controlled: there is no risk
- As a result the molecular understanding of the genetic modification is much higher
- You can cross the species barrier

Cultivos transgénicos 2013



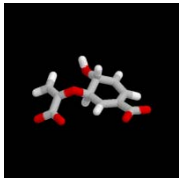
- Se plantaron 175.2 millones de hectáreas de plantas transgénicas (un incremento del 2.7% con respecto al año anterior); usaron semillas transgénicas 18 millones de agricultores; el 90% de estos agricultores viven en países pobres
- Se cultivaron en 27 países, sobre todo Estados Unidos (70.1 MHa), Brasil (40.3 MHa), Argentina (24.4. MHa), India (11 MHa)(, Canadá (10.8 MHa) y China (4.2 MHa); en la UE ocho países cultivaron transgénicos; por quinto año consecutivo impresionante crecimiento en Brasil
- Desde su uso en 1996, se han tomado más de 100 millones de decisiones de uso de esta tecnología por parte de los agricultores (su porcentaje de reuso está próximo al 100%), se han ahorrado497 millones de kg de pesticidas y reduciendo la emisión de CO_2 en 2.67 billones de kg
- Sobre todo se cultiva soja (50%), maíz (31%) y colza (8%)

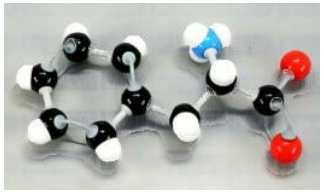
Genetically modified soybeans

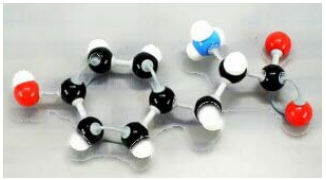


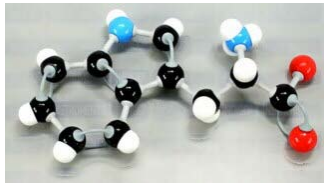
Glifosato

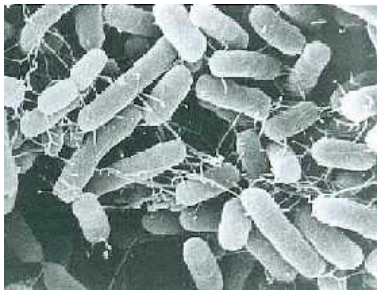
PEP
+
siquimato-3P











Golden Rice (letter 109 N)

NARCISO

Erwinia uredovora

IPP

↓

GGPP

↓ Fitoeno sintasa

FITOENO

↓ Fitoeno desaturasa

ε-CAROTENO

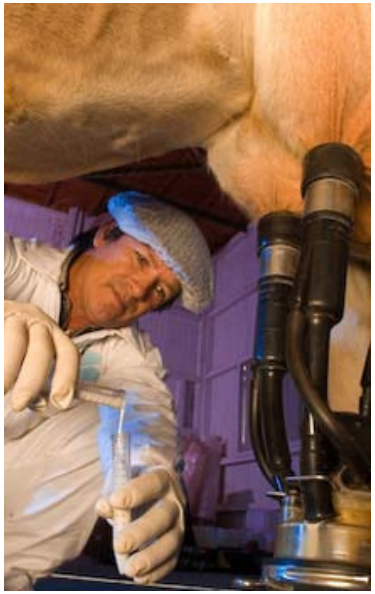
↓

LICOPENO

↓ Licopeno ciclasa

β-CAROTENO

Transgenic cows



BIO SIDUS

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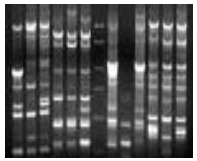
4. Applicatios in food industry

- a. Raw Materials
- b. Food Processing
- c. Food Safety
- d. Waste Revaluation

Food processing

Dairy Processing

Collection of functional probiotics



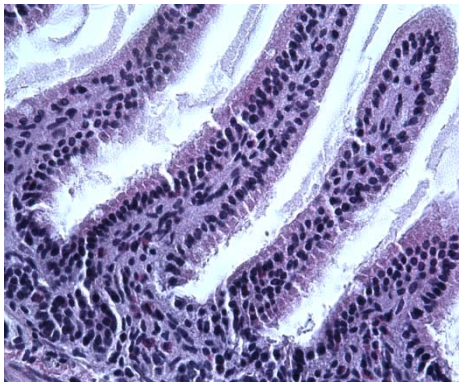

Selection → Identification → Evaluation → Safety → Scale up → Production → **Trials**

- Inflammatory bowel syndrome *Bifidobacterium longum* ES1
- Immune system: *Bifidobacterium breve* I-4035, *Lactobacillus paracasei* I-4034, *Lactobacillus rhamnosus* I-4036
- Rotavirus: *Bifidobacterium longum* subsp. *infantis* CECT 7210

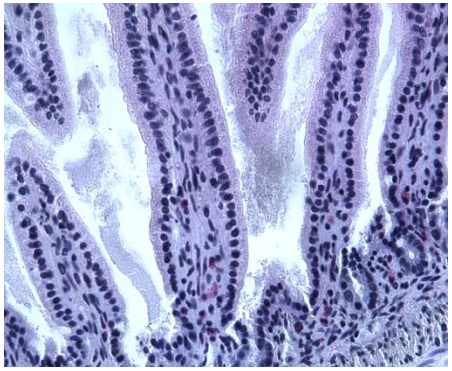
- *Helicobacter pylori: Bifidobacterium bifidum* CECT 7366
- Metabolic syndrome: *Bifidobacterium animalis* subsp. *lactis* CECT 8145
- Vaginosis: *Lactobacillus rhamnosus*

Products on the market

Probiótico frente a celiaquía



Placebo



B. longum ES1

Biopolis SL

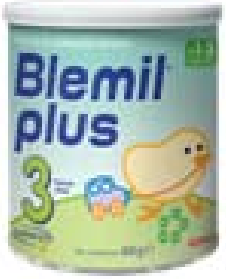
Probióticos en leches infantiles

SPAIN

SAUDI ARABIA

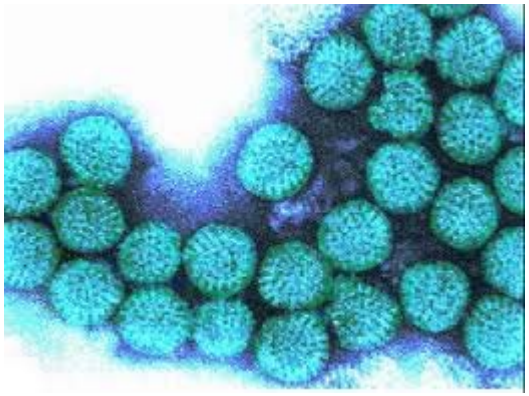
ITALY

MEXICO



Follow on formulas, growth formulas and Infant cereals containing *B. Longum infantis* CECT7210

Probiótico frente a rotavirus



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Probióticos para el sistema inmune

BIOPOLIS

BIOPOLIS
Tailor-made Biotechnology

Lactobacillus paracasei CNCM I-4034

Isolation and taxonomical identification. The probiotic strain CNCM I-4034 was isolated by the group of Dr. Angel Gil (Department of Biochemistry and Molecular Biology II; Institute of Nutrition and Food Technology José Mataix; University of Granada) from faeces of healthy babies under breast-milk feeding. Using 16S rDNA sequencing, the strain has been identified on the basis of highest scores as a member of the species *Lactobacillus paracasei* and has been deposited at the Collection Nationale de Cultures de Micro-organismes of the Institute Pasteur (CNCM) under the accession number I-4034.

Functional properties. The *Lactobacillus* I-4034 strain *in vitro* inhibited the growth of *Listeria monocytogenes* and *Salmonella typhi* and also *in vitro* replication of Va70 and Wa rotaviruses.

Safety evaluation. The *Lactobacillus paracasei* I-4034 strain is included in GRAS and QPS lists. Nevertheless, this probiotic strain has been evaluated *in vitro* and *in vivo* following the FAO/WHO guidelines and neither mortality nor clinical signs were observed during the oral toxicity animal study. There was not translocation of bifidobacteria in blood, liver, spleen, or mesenteric lymph node. Also the genome of probiotic I-4034 has been fully sequenced. Genome size was estimated at 3.1 Mb. Neither virulence factors nor antibiotic resistance genes were detected in areas with horizontal gene transfer risk.

Clinical trial. A multicenter, randomized, double-blind, placebo-controlled trial has been done in 100 volunteers enrolled in three Spanish cities. Gastrointestinal symptoms, defecation frequency and stool consistency were not altered by probiotic intake. No relevant changes in blood and serum parameters have been detected. The administration of I-4034 modified bacterial populations in the volunteers' feces.

Industrial production of I-4034. Industrial production has been done at the level of 1500 and 3000 L fermentors. At this scale, lyophilisation process has been optimized. Actually it is possible to produce industrial batches of I-4034 strain at a cell density of $1X10^{14}$ ufc/kg and with more than 18 months stability at 4°C.

BIOPOLIS
Tailor-made Biotechnology

Bifidobacterium breve CNCM I-4035

Isolation and taxonomical identification. The probiotic strain CNCM I-4035 was isolated by the group of Dr. Angel Gil (Department of Biochemistry and Molecular Biology II; Institute of Nutrition and Food Technology José Mataix; University of Granada) from faeces of healthy babies under breast-milk feeding. Using 16S rDNA sequencing, the strain has been identified on the basis of highest scores as a member of the species *Bifidobacterium breve* and has been deposited at the Collection Nationale de Cultures de Micro-organismes of the Institute Pasteur (CNCM) under the accession number I-4035.

Functional properties. The *Bifidobacterium* I-4035 strain *in vitro* inhibited the growth of *Listeria monocytogenes, Salmonella typhi, Salmonella thyphimurium* and *Shigella sonnei.* Co-incubation of this probiotic strain with dendritic cells decreased the production of pro-inflammatory cytokines such IL-8 and TNF-α and chemokines like CCL2 and CCL5. In this model, using microarrays a strong induction of the expression of genes related with innate immunity such as *TLR2* and *TLR9* have been detected.

Safety evaluation. The *Bifidobacterium breve* I-4035 strain is included in GRAS and QPS lists. Nevertheless, this probiotic strain has been evaluated *in vitro* and *in vivo* following the FAO/WHO guidelines and neither mortality nor clinical signs were observed during the oral toxicity animal study. There was not translocation of bifidobacteria in blood, liver, spleen, or mesenteric lymph node. Also the genome of probiotic I-4035 has been fully sequenced. Genome size was estimated at 2.2 Mb. Neither virulence factors nor antibiotic resistance genes were detected in areas with horizontal gene transfer risk.

Clinical trial. A multicenter, randomized, double-blind, placebo-controlled trial has been done in 100 volunteers enrolled in three Spanish cities. Gastrointestinal symptoms, defecation frequency and stool consistency were not altered by probiotic intake. No relevant changes in blood and serum parameters have been detected. The administration of I-4035 modified bacterial populations in the volunteers' feces. Also a significant increase in fecal secretory IgA was detected.

Industrial production of I-4035. Industrial production has been done at the level of 1500 and 3000 L fermentors. At this scale, lyophilisation process has been optimized. Actually it is possible to produce industrial batches of I-4035 strain at a cell density of $1X10^{14}$ ufc/kg and with more than 18 months stability at 4°C.

BIOPOLIS
Tailor-made Biotechnology

Lactobacillus rhamnosus CNCM I-4036

Isolation and taxonomical identification. The probiotic strain CNCM I-4036 was isolated by the group of Dr. Angel Gil (Department of Biochemistry and Molecular Biology II; Institute of Nutrition and Food Technology José Mataix; University of Granada) from faeces of healthy babies under breast-milk feeding. Using 16S rDNA sequencing, the strain has been identified on the basis of highest scores as a member of the species *Lactobacillus rhamnosus* and has been deposited at the Collection Nationale de Cultures de Micro-organismes of the Institute Pasteur (CNCM) under the accession number I-4036.

Functional properties. The *Lactobacillus* I-4036 strain *in vitro* inhibited the growth of *Escherichia coli, Listeria monocytogenes* and *Salmonella typhi* and also *in vitro* replication of Ito, Va70 and Wa rotaviruses.

Safety evaluation. The *Lactobacillus rhamnosus* I-4036 strain is included in GRAS and QPS lists. Nevertheless, this probiotic strain has been evaluated *in vitro* and *in vivo* following the FAO/WHO guidelines and neither mortality nor clinical signs were observed during the oral toxicity animal study. There was not translocation of bifidobacteria in blood, liver, spleen, or mesenteric lymph node. Also the genome of probiotic I-4036 has been fully sequenced. Genome size was estimated at 3.05 Mb. Neither virulence factors nor antibiotic resistance genes were detected in areas with horizontal gene transfer risk.

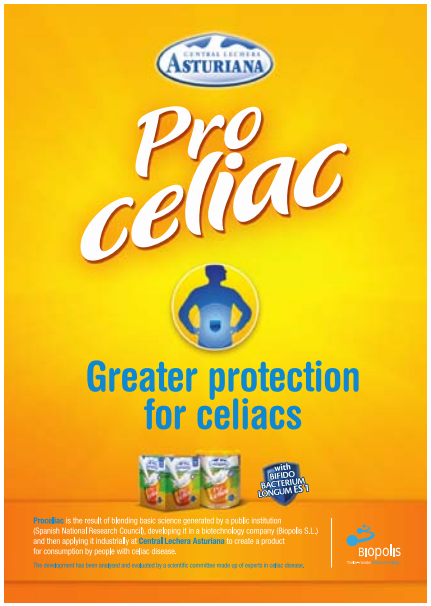
Clinical trial. A multicenter, randomized, double-blind, placebo-controlled trial has been done in 100 volunteers enrolled in three Spanish cities. Gastrointestinal symptoms, defecation frequency and stool consistency were not altered by probiotic intake. No relevant changes in blood and serum parameters have been detected. The administration of I-4036 modified bacterial populations in the volunteers' feces.

Industrial production of I-4036. Industrial production has been done at the level of 1500 and 3000 L fermentors. At this scale, lyophilisation process has been optimized. Actually it is possible to produce industrial batches of I-4036 strain at a cell density of $1X10^{14}$ ufc/kg and with more than 18 months stability at 4°C.

Biopolis SL

Hero

Products on the market

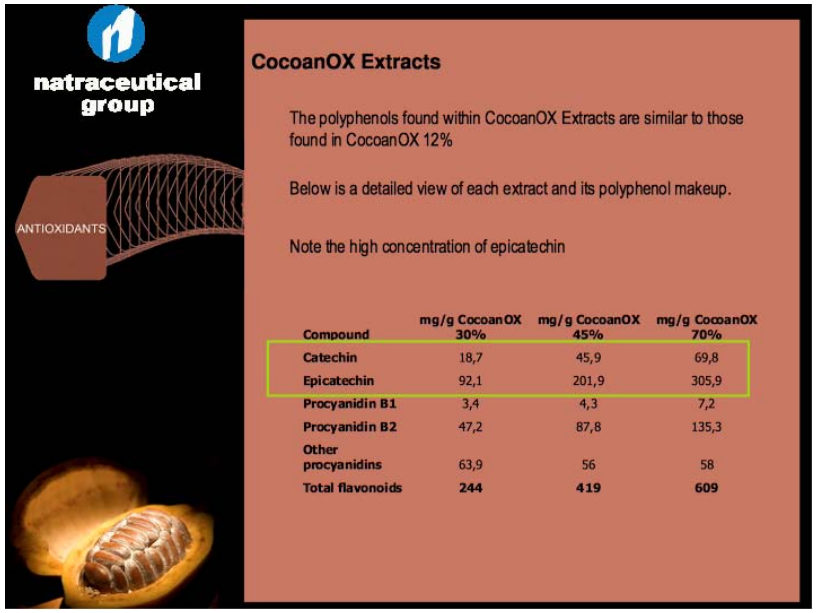


Funtional cocoa powder



- There are many references about the effect of cocoa polyphenols in the prevention of cardiovascular risk
- A conventional cocoa powder containing 3-4% polyphenols
- A product with higher content of polyphenols has ligh commercial value
- The key: inhibition of polyphenol oxidase during post-harvest processing

Products on the market



Compound	mg/g CocoanOX 30%	mg/g CocoanOX 45%	mg/g CocoanOX 70%
Catechin	18,7	45,9	69,8
Epicatechin	92,1	201,9	305,9
Procyanidin B1	3,4	4,3	7,2
Procyanidin B2	47,2	87,8	135,3
Other procyanidins	63,9	56	58
Total flavonoids	**244**	**419**	**609**

Indice

4. Applicatios in food industry

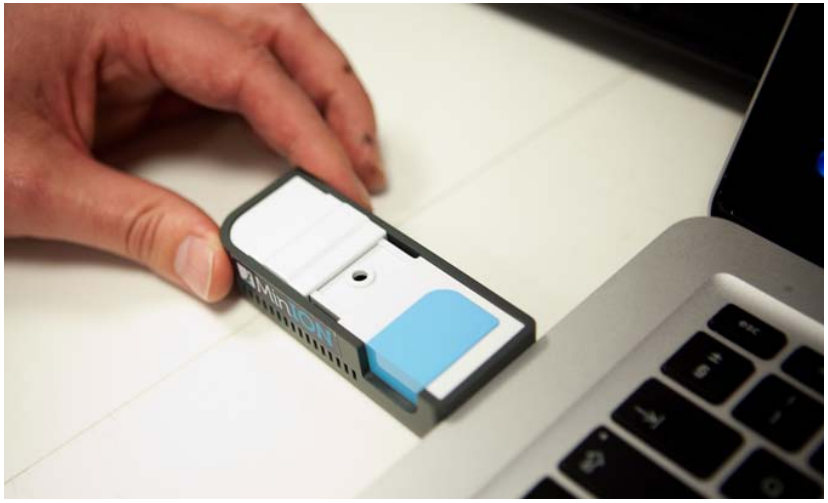
- a. Raw Materials
- b. Food Proccesing
- c. Food Safety
- d. Waste Revaluation

FOOD SAFETY

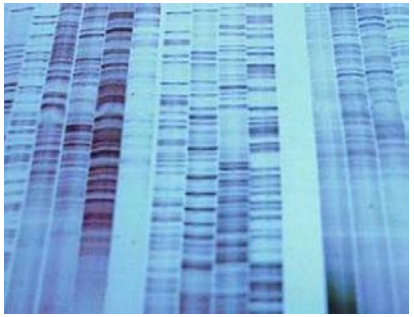
- Authorized GMOs are the most tested food in the entire history
- Following the OMS criteria, in all authorized GMO food the nutritional composition, allergenicity and toxicity have been analysed.
- There is no scientific evidence to conclude that any of these GMO foods are better or worse for the health of consumers than the corresponding conventional ones

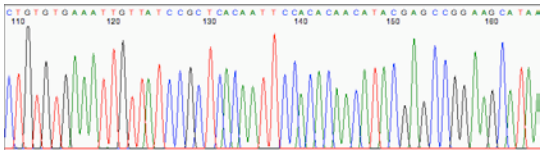
Massive Sequencing

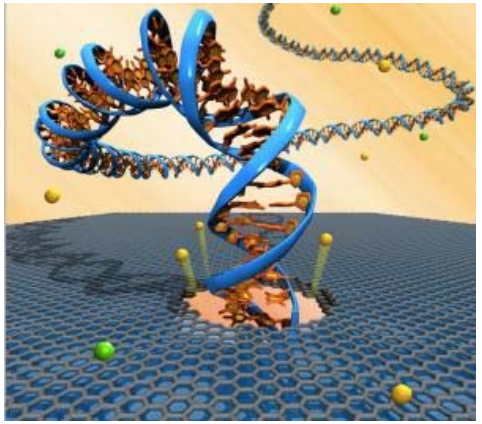
The future



9 minutos
100 $
¿1/4 técnico FP?







1970 1990 2014

Information

- We currently have 75000 genomic data of different organisms
- Of these, nearly one thousand are archaea, more than 39,000 are bacteria and tne rest are eukaryotic microorganisms.

. Microbiomes of many biological and environmental have also been sequenced.

- We've never had biological information of this magnitude

http://www.genomesonline.org/cgi-bin/GOLD/index.cgi

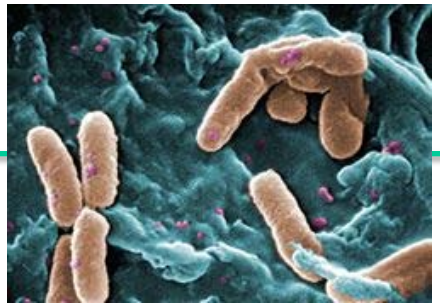
Indice

4. Applicatios in food industry

- a. Raw Materials
- b. Food Procesing
- c. Food Safety
- d. Waste Revaluation

Biorefineries

- Wheat straw
- Bagasse
- Vegetable solid waste
- Lignocellulosic hydrolysates
- Cheese whey
- Glycerol rich phases from biodiesel
- Slaughterhouse waste
- Butter byproducts
- Waste from olive oil production
- Frying oils
- Municipal solid waste
- Sewage sludge
- Syngas ($CO + H_2$)
- Biogas (methane + CO_2)



- Methanol
- Ethanol
- 1,3-propanediol
  2,3-butanediol
- Butanol
- Iso-butanol
- Dihydroxyacetone
- Poly-3-hydroxybutyrate
- Poly-3-hydroxybutyrate-co-valerate
- mcl-PHA
- (R)-3-hydroxy alkanoic acids

Biorefinery










Usuario final


Raw material characterization(I)

- Restos lignocelulósicos
- Bagazo
- Suero lácteo
- Mazada
- Glicerina de biodiesel
- Restos grasos de mataderos
- Residuos de almazara
- Aceites de fritura
- Lodos de depuradora
- Residuos municipales
- Gas de síntesis ($CO + H_2$)
- Biogás (metano + CO_2)

Raw material characterization(II)

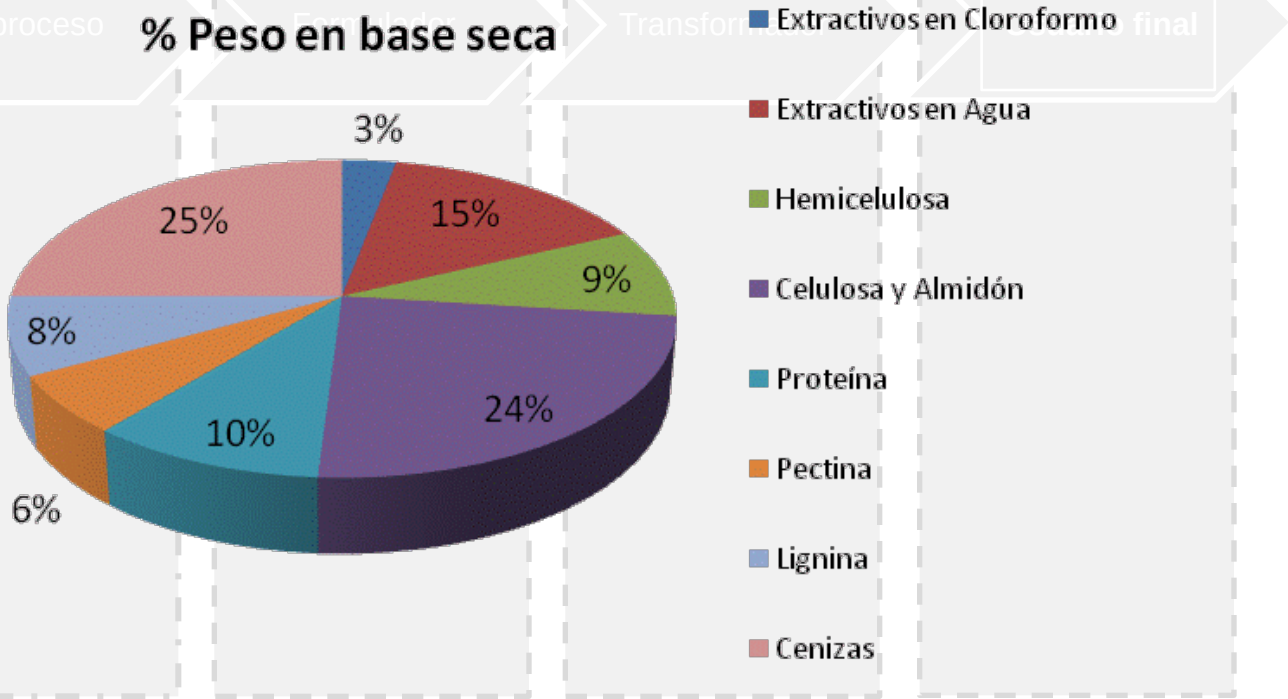
Materias primas

Bioproceso

Formulador

Transfor

final



Process development

Bioproceso

- Cepa microbiana
- Enzimas
- Condiciones de operación
- Consumos
- Rendimiento
- Productividad
- Especificaciones producto crudo

Final product definition

Polímeros en bulk

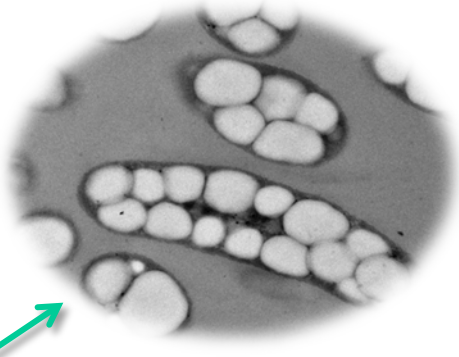
Intermediarios de síntesis

Formulador

- Metanol
- Etanol
- 1,3-propanodiol
- 2,3-butanodiol
- Ácido L-láctico
- Butanol
- Iso-butanol
- Dihidroxiacetona
- Poli-3-hidroxibutirato
- Poli-3-hidroxibutirato-co-valerato
- mcl-PHA
- Ácidos (R)-3-hidroxi alcanoicos

Biopolis-CAPSA: PHA production from whey

- Biopolis and CAPSA have developed a bioprocess to transform whey into bioplastic (Patent application EP 14382320.1).
- Whey can be used as culture media without processing
- Lactose in whey is converted in a polihydroxybutyrate (PHB)



Bioplastics

Definition: polymers that have been produced from renewable sources (plants or animals) and/or polymers obtained by fermentation of different microorganisms substratum.

Examples :

a) Polylactic acid

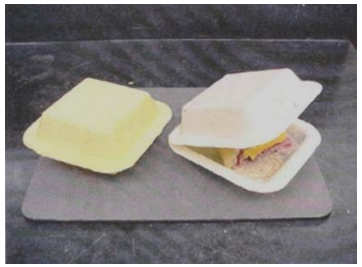
b) Polihidroxialcanoatos (PHAs)

Bioplastics – a sustainable alternative

BIOPOLYMERS

Natural origin

BIOPLASTICS



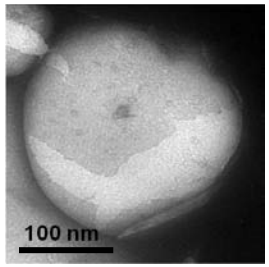
Semi-synthetic

- Biobased plastics

- Biodegradable

plastics

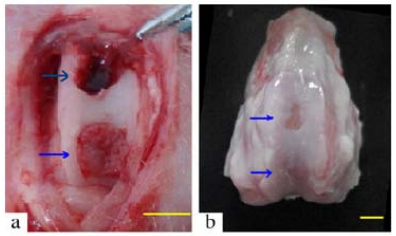
BIOMATERIALS



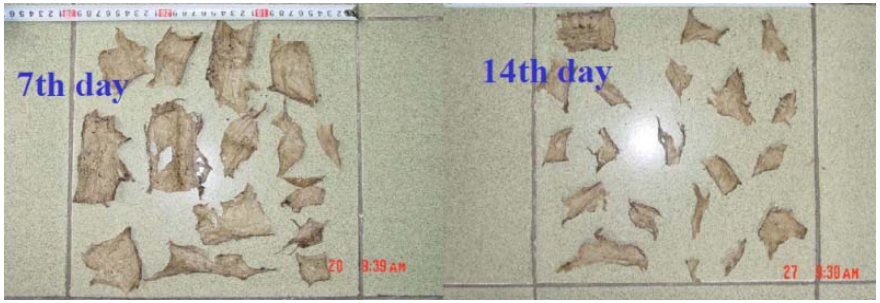
CIB-CSIC. Environmental Biotechnology Group

Biocompatibility

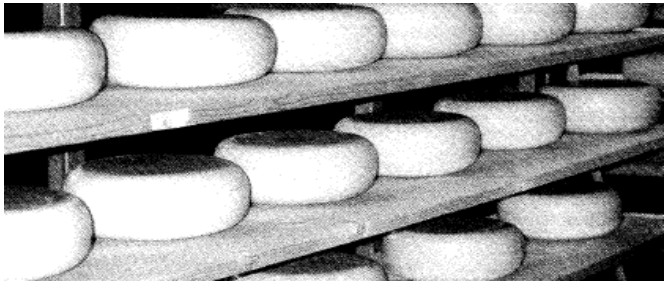
Degradability?



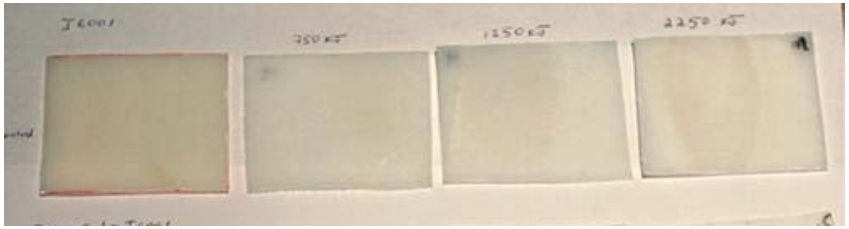
Bioplastics – a sustainable alternative



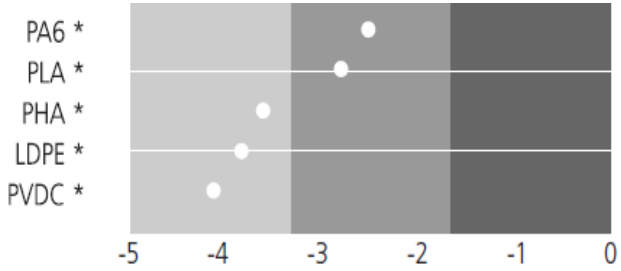
BIODEGRADABILITY



ANTIMICROBIOLOGICAL PROPIETIES



UV RESISTANCE

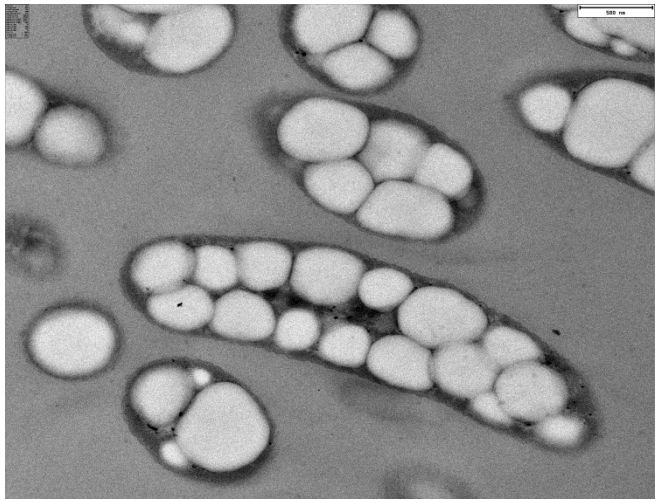


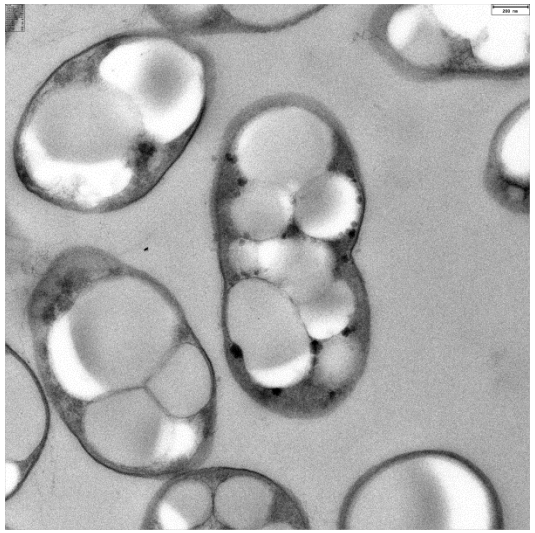
BARRIER PROPERTIES

Adv. Biochem. Eng. Biotechnol, 2001; Metabolix, 2013

Weber, 2000, Biobased Packaging Materials, ISBN 87-90504-

Bioplastic production from whey





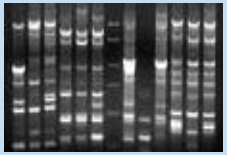
Biopolis SL

Bioquímica

Cultivos celulares

Microbiología

Organismos modelo



Biología molecular

Escalado

Fermentación

Genómica

life sequencing

华大基因 BGI

genometra

Metabolómica

METABOLON

Modelos murinos

DANONE RESEARCH

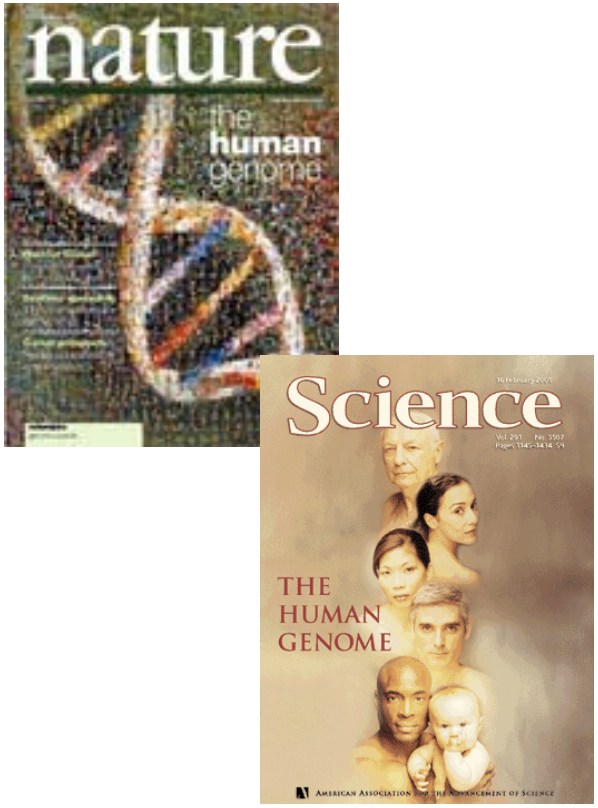
INSTITUT PASTEUR

INDEX

THE CONSUMER

- The problem of world hunger is not solved by genetics alone, it requires political a and social action
- There are no scientific data to support the positions opposing to the use of GMOs
- High concern for the environment and the future

The human genome



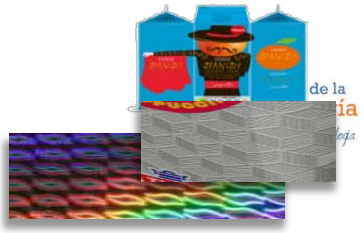
- Since 2001 we have the complete sequence composed of about 23000 genes the human genome; functionality is know of about half of these.
- It cost 3000 million dollars and almost ten years of work by more than 3,000 scientists
- In many cases we know that genes of our genome are associated with metabolic disorders, which have with possible nutritional prevention .
- With these studies we have discovered the metagenomics

Trazability

Advanced

printing

technique



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combibloc EcoPlus

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SUMMARY

1 Opportunity for nutrition

2 Opportunity for industry

3 Opportunity for society

INDEX

Final reflection

Nothing in life is to be feared, it is only to be understood

(Marie Curie, 1867-1934)

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