

The background of the slide is a microscopic image of numerous blue, rod-shaped bacteria, likely probiotics, arranged in a dense, overlapping cluster. The bacteria are illuminated from the side, creating a strong blue glow and highlighting their individual shapes and textures.

Application of Innovative Technologies in Probiotic Dairy Foods Production

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Functional Foods

Functional Food is a natural or processed food that contains known biologically-active compounds which, in defined quantitative and qualitative amounts, provides a clinically proven and documented health benefit, and thus, an important source in the prevention, management and treatment of chronic diseases of the modern age



Probiotics and Prebiotics: Their impact in human health



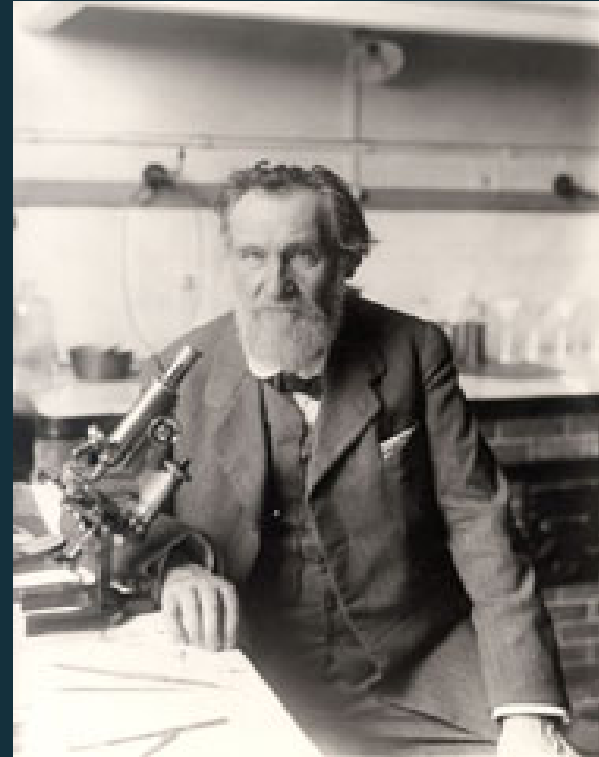
What are probiotics?

“live microorganisms which, administered in adequate amounts, confer a beneficial physiological effect on the host” (FAO/WHO, 2002)



Probiotics

In the beginning of the 20th century Elie Metchnikoff suggested that the longevity of Bulgarian peasants could be related to their large consumption of sour milk containing *Lactobacillus bulgaricus*



Elie Metchnikoff



Beneficial Effects of Probiotics



1. Metabolism: lactose digestion/intolerance, reduce cholesterol and triglyceride levels, etc.
2. Reduction of risk factors of infection: modulation of gut microbial flora, infectious diarrhea, necrotizing enterocolitis (infants), *Helicobacter pylori*, etc.
3. Inflammatory bowel diseases: Crohn's disease, irritable bowel syndrome, etc.
4. Allergic diseases: eczema, atopic dermatitis, etc.
5. Reduce cancer risk possibility.



Beneficial Effects of Probiotics

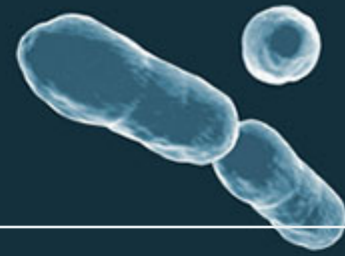


The beneficial effects will depend upon a number of factors including:

- a) Microbial strain,
- b) Level of consumption,
- c) Duration and frequency of exposure, and
- d) Physiological condition of individual.



Criteria for Probiotics



Safety criteria

- Be of human origin
- Non pathogenic in nature
- Generally recognized as safe (GRAS)

Functional criteria

- Be resistant to destruction by gastric acid and bile salts
- Adhere to intestinal epithelial tissue
- Be able to colonize the gastrointestinal tract, even in the short term
- Modulate immune responses
- Produce antimicrobial substances
- Influence human metabolic activities (i.e. cholesterol assimilation, lactase activity, vitamin production, etc)

Technological criteria

- Be resistant to destruction by technical processing
- Be subjected to scale-up processes



Most Common Probiotics



Lactobacilli

L. acidophilus

L. casei

L. rhamnosus

L. reuteri

L. plantarum

L. fermentum

L. johnsonii

L. helveticus

L. farciminis

L. curvatus

L. brevis

L. gasseri

L. salivarius

L. cellobiosus

Yeast

Saccharomyces boulardii

Saccharomyces cerevisiae

Bifidobacteria

B. bifidum

B. breve

B. infantis

B. longum

B. lactics

B. thermophilum

B. adolescents

B. animalis

Other bacteria

Enterococcus faecium

Escherichia coli Nissle 1917

Lactococcus lactis

Propionibacterium freudenreichii

Bacillus clausii

Bacillus oligonitrophilus



Prebiotics

- Prebiotics are typically non-digestible fiber compounds that stimulate the growth and/or activity of advantageous bacteria (probiotics) that colonize the large bowel.
- Most common prebiotics are inulin, galacto-oligosaccharides, etc, that usually are constituents of fruits, vegetables and cereals.



Cell Immobilization of Probiotics



Beneficial effects of probiotics

Crucial Prerequisite

Probiotics need to contain an adequate amount of live bacteria (at least 10^6 – 10^7 cfu/g), able to survive the acidic conditions of the upper GI tract and proliferate in the intestine, a requirement that is not always fulfilled.




Why Cell Immobilization?

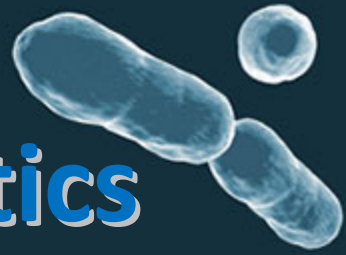


Problem: Severe conditions often employed during food processing and storage might lead to important losses in viability, as probiotic strains are very often thermally labile (on heating and/or freezing) and sensitive to acidity, oxygen or other food constituents (e.g. salts).

Solution: To overcome such deficiencies, immobilization techniques are usually applied in order to maintain cell viability, activity and functionality.



Cell Immobilization of Probiotics



- ❑ Starch (Mattila-Sandholm et al. 2002).
- ❑ Fruit pieces (Kourkoutas et al. 2005; 2006; Sidira et al. 2013).
- ❑ Cereals (Bosnea et al., 2009; Dimitrellou et al. 2013).
- ❑ Milk and whey protein (Dimitrellou et al. 2008; 2014).



Application in Dairy Technology



- **Fermented milk** (immobilization of probiotic cells on fruits, Kourkoutas et al. 2005)
- **Yogurt** (immobilization of probiotic cells on fruits and cereals, Sidira et al. 2013)
- **Cheese** (immobilization probiotic cells on fruits and whey protein, Kourkoutas et al. 2006; Dimitrellou et al. 2014)

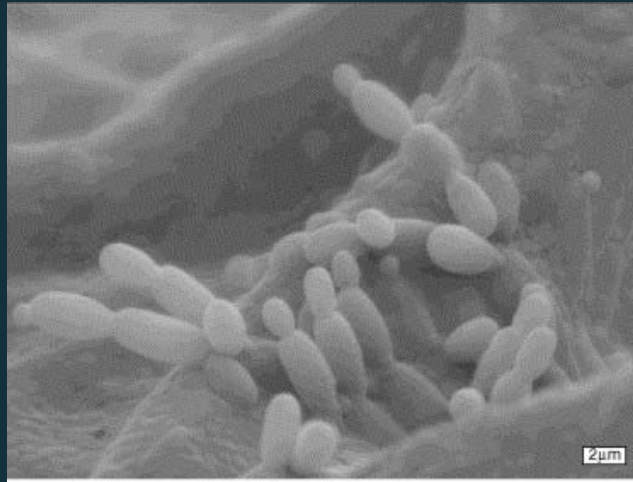
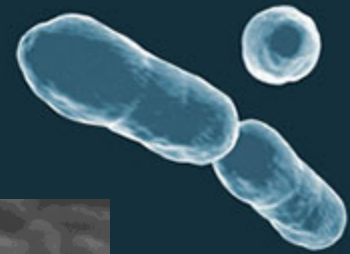
(Industrial use of wet or lyophilized immobilized lactic acid bacteria in production of dairy products. Patent No. 1005393, 2006, Hellenic Industrial Property Organization)

- **Ice-cream** (immobilization of probiotic cells on dry nuts and pastry products)

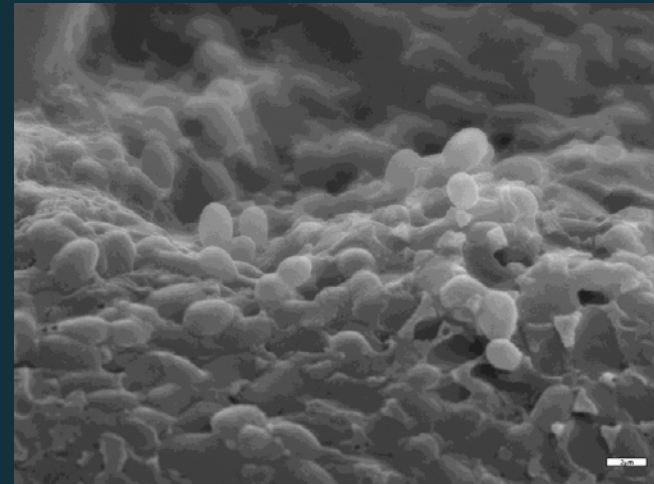
(Industrial use of wet or dried immobilized lactic acid bacteria on dry nuts and pastry products in probiotic ice-cream production. Patent No. 1008050, 2013, Hellenic Industrial Property Organization)



Probiotic Fermented Milk



(a)



(b)

Scanning electron microscopy micrographs of immobilized *L. casei* ATCC 393 on (a) apple, and (b) quince pieces.

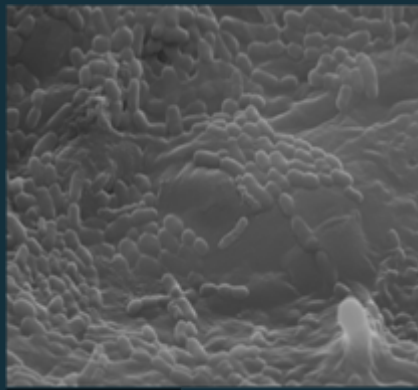
Preliminary sensory test ascertained the fruity distinctive aromatic potential of the fermented milk, even after storage for 129 days (Kourkoutas et al. 2005, Process Biochem 40:411-416; Patent No. 1005393, 2006, Hellenic Industrial Property Organization)



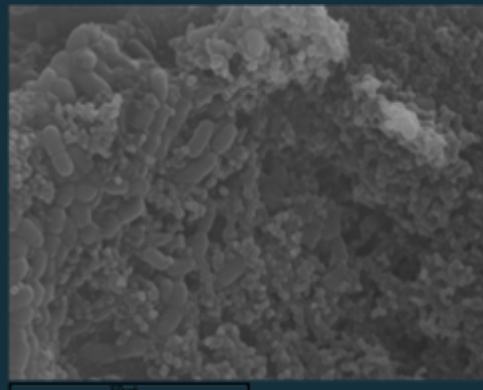
Probiotic Yogurt

Immobilization of *L. casei* ATCC 393 on fruits and cereals

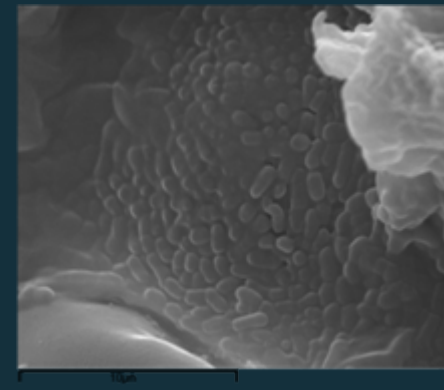
(Patent No. 1005393, 2006, Hellenic Industrial Property Organization; Sidira et al. 2013, J Dairy Sci 96:3369-3377)



(a)



(b)



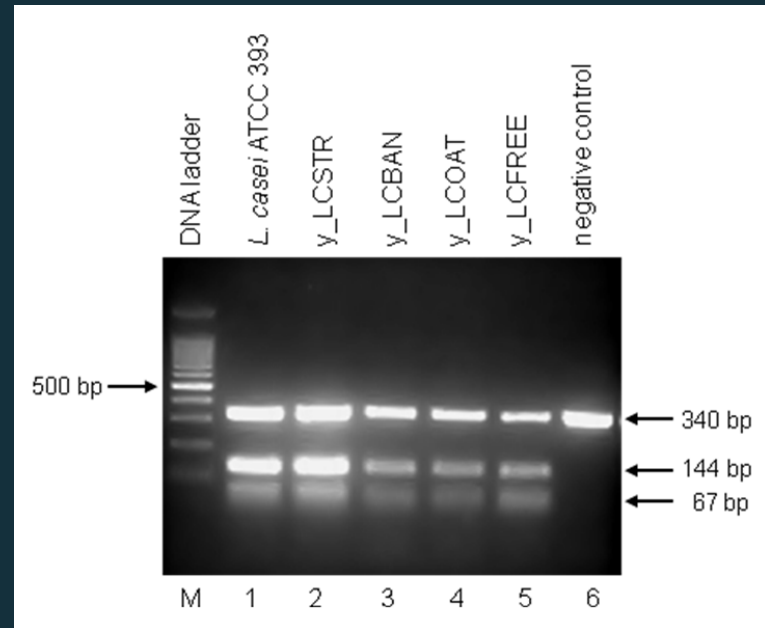
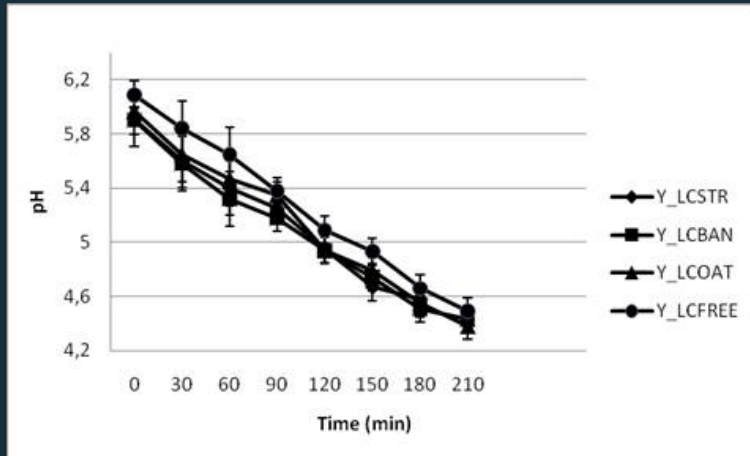
(c)

Scanning electron microscopy micrographs of immobilized *L. casei* ATCC 393 on (a) strawberry, (b) banana, and (c) oat pieces.



Probiotic Yogurt

Fermentation kinetics



Molecular identification of *L. casei* ATCC 393 at levels ≥ 6 logcfu/g by strain-specific Multiplex PCR in probiotic yoghurts after storage for 30 days at 4°C. LC: *L. casei* ATCC 393, STR: immobilized cells on strawberry, BAN: immobilized cells on banana, OAT: immobilized cells on oat, FREE: free cells

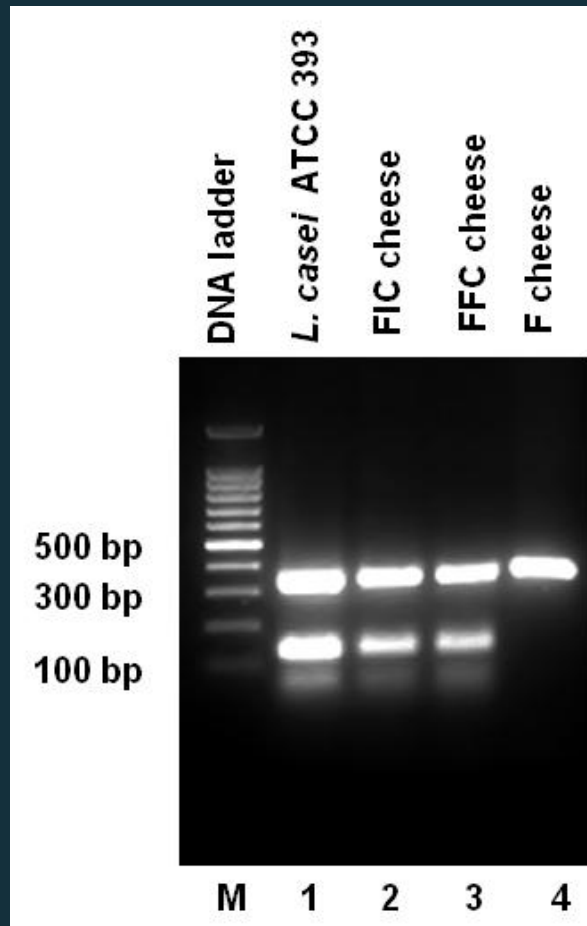
(Patent No. 1005393, 2006, Hellenic Industrial Property Organization; Sidira et al. 2013, J Dairy Sci 96:3369-3377)

Probiotic Feta-Type Cheese



Immobilization of *L. casei* ATCC 393 on whey protein for probiotic feta-type cheese production

(Dimitrellou et al. 2014, J Dairy Sci, 97: 4675-4685)



Molecular identification of *L. casei* ATCC 393 at levels ≥ 6 logcfu/g by strain-specific Multiplex PCR in probiotic cheese after ripening for 70 days. FIC: cheese with immobilized *L. casei* ATCC 393 on whey protein, FFC: cheese with free *L. casei* ATCC 393, F: cheese with no starter culture.



Probiotic Ice-Cream



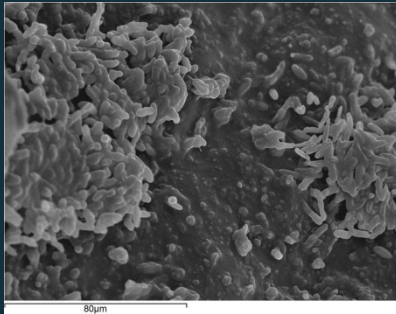
Production steps (briefly):

- Immobilization of *L. casei* ATCC 393 cells on dry nuts and pastry products (biscuits).
- Freeze- or thermal-drying of free or immobilized probiotic culture.
- Production of probiotic ice-cream using free or immobilized probiotic cultures.

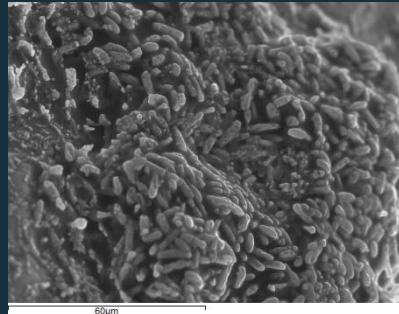
Patent No. 1008050, 2013, Hellenic Industrial Property Organization.



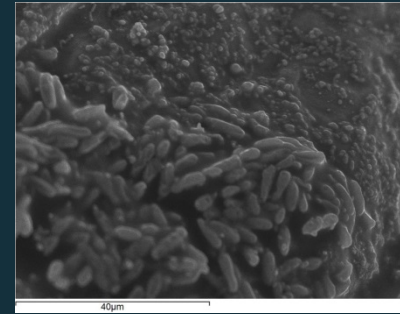
Probiotic Ice-Cream



(a)



(b)



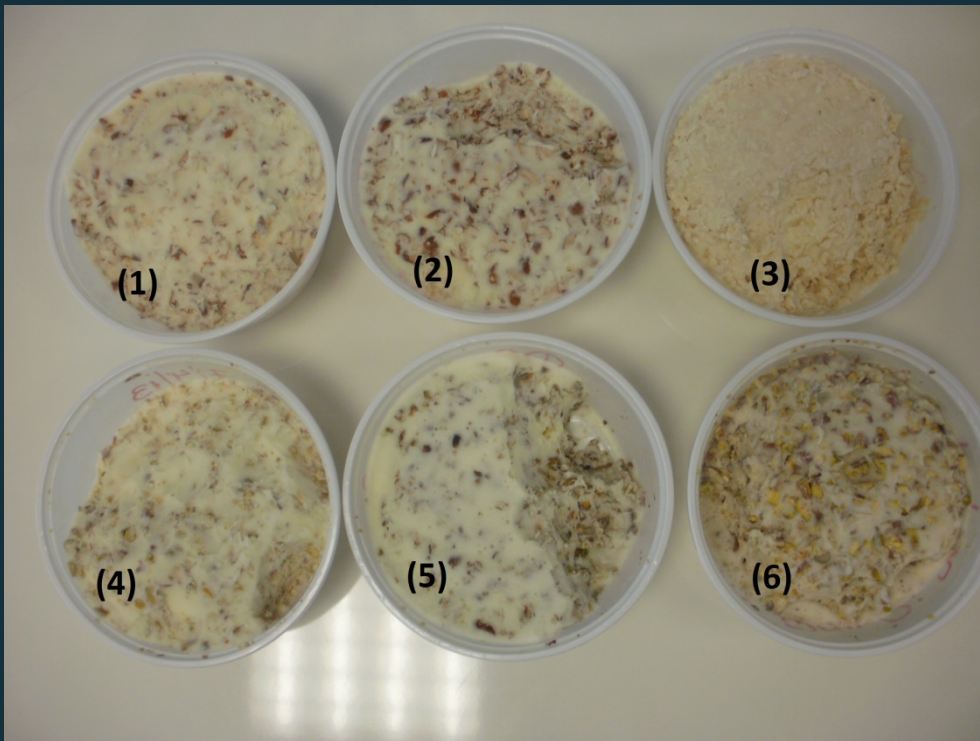
(c)

Scanning electron microscopy micrographs of immobilized *L. casei* ATCC 393 on (a) dried almond, (b) peanuts, and (c) biscuit pieces.



Probiotic Ice-Cream

Probiotic ice-cream production with immobilized *L. casei* ATCC 393 cells on dried nuts



Probiotic ice-creams with

(1) wet immobilized *L. casei* ATCC 393 on dried nuts,

(2) thermally-dried immobilized *L. casei* ATCC 393 on dried nuts,

(3) freeze-dried immobilized *L. casei* ATCC 393 on dried nuts,

(4) wet immobilized *L. casei* ATCC 393 on peanuts,

(5) thermally-dried immobilized *L. casei* ATCC 393 on peanuts,

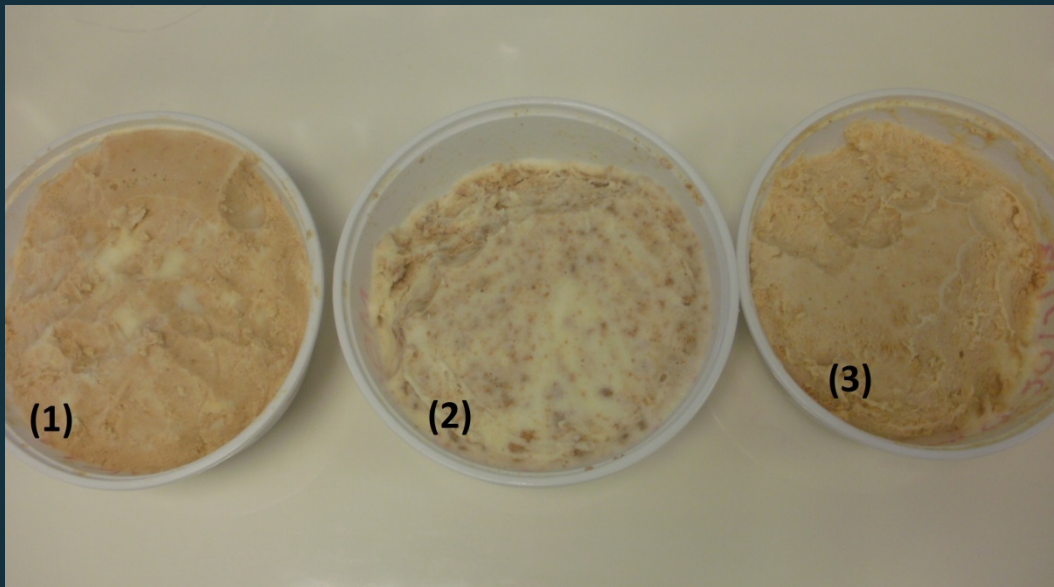
(6) freeze-dried immobilized *L. casei* ATCC 393 on peanuts.



Probiotic Ice-Cream



Probiotic ice-cream production with immobilized *L. casei* ATCC 393 cells on pastry products



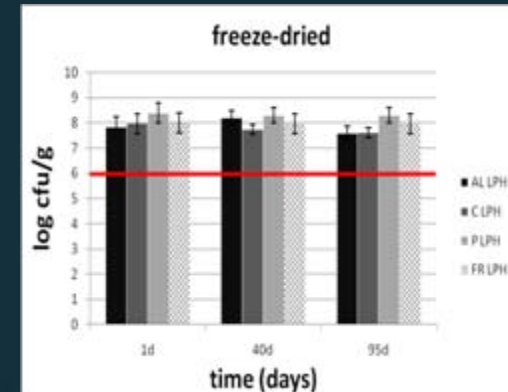
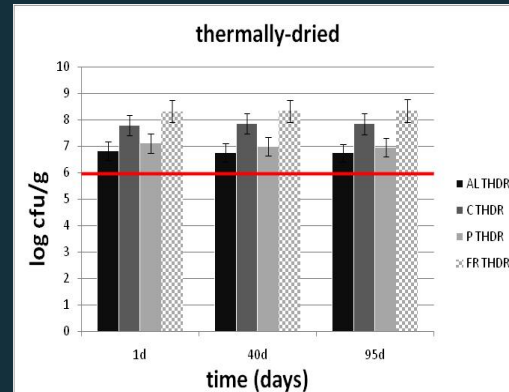
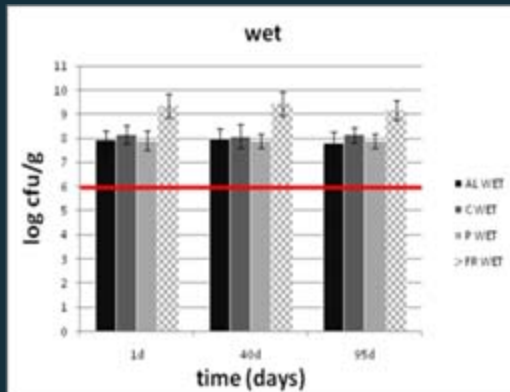
Probiotic ice-cream with
(1) wet immobilized *L. casei* ATCC 393 on biscuits,
(2) thermally-dried immobilized *L. casei* ATCC 393 on biscuits,
(3) freeze-dried immobilized *L. casei* ATCC 393 on biscuits.



Probiotic Ice-Cream



Effect of storage of probiotic ice-cream at -18°C on viability of *L. casei* ATCC 393 cells



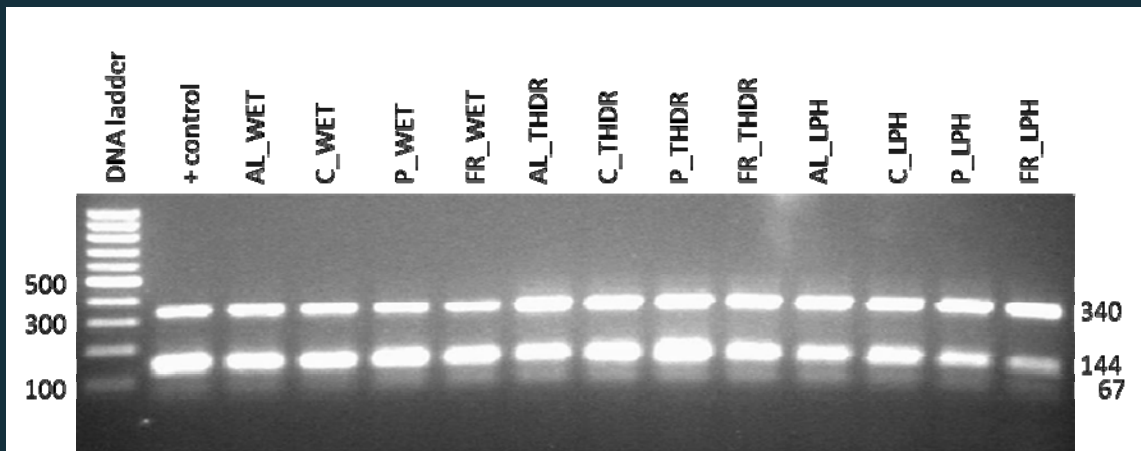
AL: immobilized cells on dried almond, C: immobilized cells on biscuits, P: immobilized cells on peanuts, FR: free cells, WET: wet cells, THDR: thermally-dried cells, LPH: freeze-dried (lyophilized) cells.



Probiotic Ice-Cream



Molecular identification of *L. casei* ATCC 393 at levels ≥ 6 logcfu/g by strain-specific Multiplex PCR after storage for 95 days at -18°C

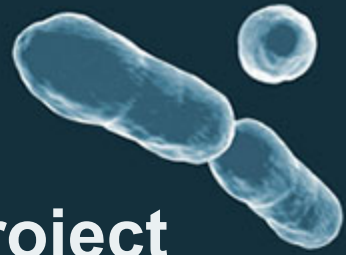


AL: immobilized cells on dried almond,
C: immobilized cells on biscuits,
P: immobilized cells on peanuts,
FR: free cells,
WET: free cells,
THDR: thermally-dried cells,
LPH: freeze-dried (lyophilized) cells.



Probiotic Ice-Cream

Presentation of 15NEW2009 Research Project Results at 79th HELEXPO (September 2014)



Relevant Publications



J. Dairy Sci. 97:4675-4685
<http://dx.doi.org/10.3168/jds.2013-7697>
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Free and immobilized *Lactobacillus casei* ATCC 393 on whey protein as starter cultures for probiotic Feta-type cheese production

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<http://dx.doi.org/10.3168/jds.2012-6343>
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Monitoring survival of *Lactobacillus casei* ATCC 393 in probiotic yogurts using an efficient molecular tool

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Short Communication

J. Mol. Microbiol. Biotechnol. 2010;18:156-161
Published online April 13, 2010
DOI: 10.1159/000308518

Rapid Detection and Identification of Probiotic *Lactobacillus casei* ATCC 393 by Multiplex PCR

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LWT - Food Science and Technology 42 (2009) 1896-1902

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Functionality of freeze-dried *L. casei* cells immobilized on wheat grains

Loulouda A. Bosnea^a, Yiannis Kourkoutas^b, Natalia Albantaki^a, Constantina Tzia^c, Athanasios A. Koutinas^a, Maria Kanellaki^{a,b,*}

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Process Biochemistry 43 (2008) 1323-1329

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio



Fermentation efficiency of thermally dried immobilized kefir on casein as starter culture

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J. Dairy Sci. 89:1439-1451

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Probiotic Cheese Production Using *Lactobacillus casei* Cells Immobilized on Fruit Pieces

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Process Biochemistry 40 (2005) 411-416

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Lactobacillus casei cell immobilization on fruit pieces for probiotic additive, fermented milk and lactic acid production

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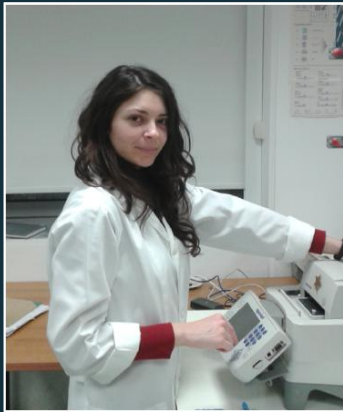
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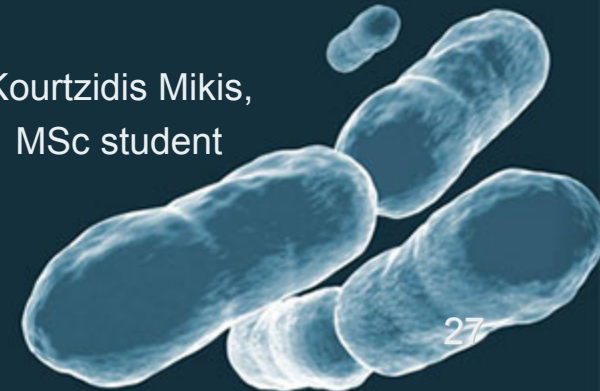
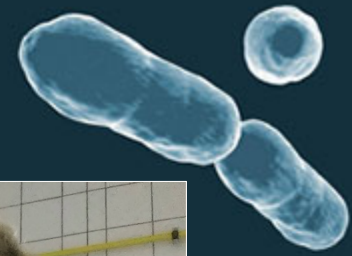
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Kourtzidis Mikis,
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Conclusion

**Innovation is the only way to
achieve sustainability and
financial development**





Thank you for your attention!



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