



# «Προβιοτικά τρόφιμα»

Ευανθία Λιτοπούλου – Τζανετάκη  
Ομότιμος Καθηγήτρια

Τομέας Επιστήμης και Τεχνολογίας Τροφίμων  
Γεωπονική Σχολή ΑΠΘ



# Προβιοτικά = προς + βίος (για τη ζωή)

FAO/WHO 2001

Προβιοτικά είναι «οι ζωντανοί οργανισμοί (βακτήρια και ζύμες) που όταν καταναλωθούν σε κατάλληλες ποσότητες ασκούν ευεργετική δράση στον ξενιστή»

Ικανοποιητικός αριθμός:  $10^8$ - $10^9$  (cfu/g) την ημέρα (Speck, 1978)

Δηλαδή:  $10^6$  cfu/g σε ζυμούμενο γάλα x 100g (ημερήσια κατανάλωση)

$10^7$  cfu/g σε τυρί x 10g (ημερήσια κατανάλωση)



## Κυριότερα είδη προβιοτικών μικροοργανισμών

### ΒΑΚΤΗΡΙΑ

Lactobacillus	<i>L. acidophilus</i> , <i>L. casei</i> , <i>L. johnsonii</i> , <i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>L. helveticus</i> , <i>L. paracasei</i> subsp. <i>paracasei</i> , <i>L. gasseri</i> , <i>L. plantarum</i> , <i>L. rhamnosus</i> , <i>L. curvatus</i> , <i>L. brevis</i> , <i>L. fermentum</i> , <i>L. reuteri</i> , <i>L. cellobiosus</i>
Lactococcus	<i>Lc. lactis</i> subsp. <i>lactis</i> , <i>Lc. lactis</i> subsp. <i>cremoris</i>
Streptococcus	<i>Str. thermophilus</i>
Enterococcus	<i>Ent. faecium</i> , <i>Ent. faecalis</i>
Pediococcus	<i>P. acidilactici</i>
Bifidobacterium	<i>B. bifidum</i> , <i>B. infantis</i> , <i>B. longum</i> , <i>B. breve</i> , <i>B. adolescentis</i>
Propionibacterium	<i>Pr. freudenreichii</i>

### ZYMEΣ

Saccharomyces	<i>S. cerevisiae</i> , <i>S. boulardii</i>
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## Κριτήρια επιλογής προβιοτικών μικροοργανισμών (FAO/WHO 2002)

- ✓ Να έχει γίνει η ταυτοποίησή τους σε επίπεδο είδους (ή ακόμη και στελεχούς)
- ✓ Να είναι ασφαλή για χρήση σε τρόφιμα και φαρμακευτικά σκευάσματα (έλεγχος αιμολυτικής και τοξικής δράσης, επιδημιολογικές μελέτες)
- ✓ Να επιβιώνουν και να διατηρούν τις ιδιότητές τους στο γαστρεντερικό σωλήνα (ανθεκτικότητα στο γαστρικό οξύ-χαμηλό pH και στα χολικά άλατα)
- ✓ Να έχουν ικανότητα προσκόλλησης ή και πολλαπλασιασμού σε επιφάνειες βλεννογόνου και ιδιαίτερα στον εντερικό βλεννογόνο.
- ✓ Να παρεμποδίζουν ή να μειώνουν την προσκόλληση παθογόνων μικροβίων στον εντερικό βλεννογόνο (ανταγωνιστική ή αντιμικροβιακή δράση)
- ✓ Να παρουσιάζουν ανοσοτροποποιητική δράση
- ✓ Να είναι ανθεκτικά σε σπερμοκτόνες ουσίες (εφόσον προορίζεται για κολπική χρήση)
- ✓ Να παρουσιάζουν καλές τεχνολογικές ιδιότητες εφόσον χρησιμοποιηθούν σε λειτουργικά τρόφιμα (σταθερότητα, να έχουν μεγάλη διάρκεια ζωής, να πολλαπλασιάζονται σε μεγάλη κλίμακα, να μην έχουν επίδραση στη γεύση του προϊόντος).



ORIGINAL ARTICLE

# Characterization of *Lactobacillus* isolates from infant faeces as dietary adjuncts

V. Xanthopoulos, E. Litopoulou-Tzanetaki\* and N. Tzanetakis

*Lactobacillus paracasei subsp. paracasei (six strains), Lb. rhamnosus (six strains), Lb. acidophilus (two strains), Lb. gasseri (three strains) and Lb. reuteri (three strains) isolates from new-born infants were tested for their ability to grow and metabolize in milk and to resist specific conditions of the gastrointestinal tract. Many of the tested strains had desirable properties concerning their ability to withstand adverse conditions of the gastrointestinal tract, and produce carbonyl compounds. In general, strains of Lb. paracasei subsp. paracasei and Lb. rhamnosus were more resistant to low pH of the stomach than all the other strains. On the other hand, Lb. gasseri and Lb. reuteri strains did not acidify milk. Thus, Lb. paracasei subsp. paracasei, Lb. acidophilus and Lb. rhamnosus strains could be preferably used as starters to produce fermented milks with possibly interesting organoleptic properties, as well as dietary and possible therapeutic importance.*



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## Some probiotic properties of yeast isolates from infant faeces and Feta cheese

E. Psomas<sup>a</sup>, C. Andrighetto<sup>b</sup>, E. Litopoulou-Tzanetaki<sup>a,\*</sup>, A. Lombardi<sup>b</sup>,  
N. Tzanetakis<sup>a</sup>

<sup>a</sup> *Laboratory of Food Microbiology and Hygiene, Faculty of Agriculture, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece*

<sup>b</sup> *Veneto Agricoltura, Istituto per la Qualità e le Tecnologie Agroalimentari, Via S. Gaetano 74, 36016 Thiene, Italy*

### Abstract

Yeast isolates from infant faeces and Feta cheese were characterized to species level by phenotypic criteria, Randomly Amplified Polymorphic DNA (RAPD)-PCR and mitochondrial DNA (mt-DNA) restriction analysis. Results suggested that there is a good agreement between phenotypic characterization of yeasts and RAPD-PCR at species level; in addition, RAPD-PCR as well as mt-DNA restriction analysis provided good discrimination at strain level. Some technological and probiotic properties of selected strains were also investigated. The test strains exhibited lipolytic and proteolytic activities. They also tolerated low pH and survived satisfactory in gastric juice in vitro as well as in the presence of bile. In general, the isolates from faeces were more resistant to low pH and bile than those from Feta cheese. Selected strains could be used as starter supplements for industrial fermentations. © 2001 Elsevier Science B.V. All rights reserved.

## Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties

E. Papamanoli, N. Tzanetakis, E. Litopoulou-Tzanetaki, P. Kotzekidou\*

*Laboratory of Food Microbiology and Hygiene, Department of Food Science and Technology, Faculty of Agriculture, Aristotle University of Thessaloniki, P.B. 250, 54124 Thessaloniki, Greece*

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### Abstract

A total of 147 lactic acid bacteria was isolated from two types of naturally fermented dry sausages at four different stages of the ripening process studied in order to select the most suitable strains according to their technological characteristics including probiotic properties and antimicrobial activity against food-borne pathogens. Identification of the isolates revealed that 90% were lactobacilli, 4% enterococci, 3% *Pediococcus* sp. and sporadic isolates of *Weissella viridescens*, *Leuconostoc pseudomesenteroides*, and *Leuconostoc* sp. The isolated strains of *Lactobacillus sakei* (49 isolates), *Lactobacillus curvatus* (24 isolates) and *Lactobacillus plantarum* (7 isolates) were further characterized. All strains could grow at 15 °C, whereas the majority of the strains was able to grow in the presence of 6.5% NaCl and on acetate agar. The enzymatic potential of the strains was evaluated using the API ZYM system. During in vitro investigations all strains exhibited high leucine and valine aminopeptidase activities and moderate acid phosphatase and phosphohydrolase activities. Some strains showed very weak lipolytic activity. The enzyme profiling is an important factor for selection of strains as starter cultures. A large majority of the strains tolerated 0.1% bile salts whereas 58% of *Lactobacillus curvatus* strains and all *Lactobacillus plantarum* strains were resistant to 0.3% bile salts. All *Lactobacillus sakei* strains and the majority of *Lactobacillus curvatus* and *Lactobacillus plantarum* strains exhibited an anti-listerial activity against three *Listeria monocytogenes* strains. A percentage of 75, 50 and 29% of *Lactobacillus sakei*, *L. curvatus* and *L. plantarum* strains, respectively, could inhibit two *Staphylococcus aureus* strains. The contribution of the selected strains to a possible inhibition of *Listeria monocytogenes* and *S. aureus* in situ on fermented meats would be of considerable interest to enhance the hygienic quality of these products.

# Probiotic and Technological Properties of Facultatively Heterofermentative Lactobacilli from Greek Traditional Cheeses

George Floros, Magdalini Hatzikamari,  
Evanthia Litopoulou-Tzanetaki, and Nikolaos Tzanetakis

Laboratory of Food Microbiology and Hygiene, Aristotle University of Thessaloniki, Greece

Nineteen isolates of facultatively heterofermentative lactobacilli from Feta, Graviera, and Kasseri cheeses were identified by SDS-PAGE of whole-cell proteins as *L. paracasei* subsp. *paracasei* (12 strains) and *L. plantarum* (7 strains) and differentiated at strain level by RAPD-PCR. Properties of technological interest, such as acidification ability, proteolytic activity, and enzyme activities, were also studied. The test strains exhibited a low acidification activity, with significant interstrain differences after growth in milk for 24 h. They were also characterized by different casein breakdown ability, with around 50% of them accumulating amino acids at low amounts in the milk. Lactobacilli isolates differed in respect of enzyme activities, with  $\beta$ -galactosidase being the strongest activity found. Their probiotic potential was evaluated with *in vitro* studies on the resistance to low pH, bile salts, and pancreatin. The isolates from Feta showed a better survival than those from Kasseri and Graviera at low pH and viable cells were detected even after 3 h at pH 2.0. All strains tolerated bile salts at 0.3% and retained viability in the presence of pancreatin at 0.1%. Different patterns of antibacterial activities were recorded. The strains inhibited preferentially LAB species and some of them clostridia, *E. coli* O44 and *B. cereus*. Distinguished strains are promising probiotic candidates as adjuncts and deserve further studies.



# Selection of Dominant NSLAB from a Mature Traditional Cheese According to their Technological Properties and *in vitro* Intestinal Challenges

Zacharias Papanikolaou, Magdalini Hatzikamari, Panayiotis Georgakopoulos, Minas Yianguou, Evanthia Litopoulou-Tzanetaki, and Nikolaos Tzanetakis

**Abstract:** Isolates (47) of lactobacilli from 5 different productions of Melichloro cheese were examined for potential use as adjunct cultures. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of whole-cell proteins classified 29 isolates as *L. paraplantarum* and 18 as *L. paracasei* subsp. *paracasei*. Randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis differentiated the *L. paraplantarum* and *L. paracasei* subsp. *paracasei* isolates at strain level and both, RAPD analysis and whole-cell protein profiling provided useful information about the diversity of nonstarter lactic acid bacteria (NSLAB) in the different cheese productions. The isolates were slow acidifiers and about 70% of them degraded, preferentially  $\alpha_1$ -casein. The amounts of amino acids accumulated in the milk increased with the incubation time. A similar enzyme profile was exhibited by strains of both species, except for  $\alpha$ -mannosidase and  $\alpha$ -fucosidase, which were not detected in the *L. paracasei* subsp. *paracasei* strains. All strains grew in the presence of bile at 0.3% and the majority was able to withstand pH 2.5 and pancreatin at 0.1%. Moreover, all strains reduced cholesterol *in vitro*, with higher removal ability recorded for strains of *L. paraplantarum*. A narrow spectrum of antibacterial activity was recorded for 88% of the strains. Selected isolates with appropriate technological and interesting *in vitro* intestinal challenges could be used as adjuncts and deserve further studies.



## Προβιοτικά γαλακτοκομικά προϊόντα

<i>Product</i>	<i>Country of origin</i>	<i>Starter culture</i>
AB milk products	Denmark	<i>L. acidophilus</i> , <i>B. bifidum</i>
A38 fermented milk	Denmark	<i>L. acidophilus</i> , mesophilic lactic culture
Acidophilus milk	Many countries	<i>L. acidophilus</i>
Acidophilus yoghurt	Many countries	<i>L. acidophilus</i> , yoghurt starter bacteria
Acidophilus bifidus yoghurt	Germany	<i>L. acidophilus</i> , <i>B. bifidum</i> (or <i>B. longum</i> ), yoghurt starter bacteria
ACO-yoghurt	Switzerland	<i>L. acidophilus</i> , yoghurt starter bacteria
Arla acidophilus	Norway	<i>L. acidophilus</i>
BA	France	<i>B. longum</i> , yoghurt starter bacteria
Bifidus milk	Germany	<i>B. bifidum</i> (or <i>B. longum</i> )
Bifidus yoghurt	Many countries	<i>B. bifidum</i> (or <i>B. longum</i> ), yoghurt starter bacteria
Bifighurt	Germany	<i>B. longum</i>
Bifilact	Former Soviet Union	<i>Lactobacillus</i> spp., <i>Bifidobacterium</i> spp.
Biobest	Germany	Bifidobacteria, yoghurt starter bacteria
Biogarde	Germany	<i>L. acidophilus</i> , <i>B. bifidum</i> , <i>Streptococcus thermophilus</i>
Bioghurt	Germany	<i>L. acidophilus</i> , <i>B. bifidum</i> , <i>Streptococcus thermophilus</i>
Biokys	Former Czechoslovakia	<i>B. bifidum</i> , <i>L. acidophilus</i> , <i>Pediococcus acidilactici</i>
Biomild	Germany	<i>L. acidophilus</i> , <i>Bifidobacterium</i> spp.



<b>Product</b>	<b>Country of origin</b>	<b>Starter culture</b>
Cultura	Denmark	<i>L. acidophilus</i> , <i>B. bifidum</i>
Diphilus milk	France	<i>L. acidophilus</i> , <i>B. bifidum</i>
Kefir	Many countries	<i>L. acidophilus</i> , lactic acid bacteria, yeasts
Mil-Mil E	Japan	<i>L. acidophilus</i> , <i>B. bifidum</i> , <i>B. breve</i>
Miru-Miru	Japan	<i>L. acidophilus</i> , <i>B. breve</i> , <i>L. casei</i>
Ofilus	France	<i>Streptococcus thermophilus</i> (or <i>Lactococcus lactis</i> subsp. <i>cremoris</i> ), <i>L. acidophilus</i> , <i>B. bifidum</i>
Progurt	Chile	<i>Lactococcus lactis</i> subsp. <i>cremoris</i> biovar <i>diacetylactis</i> , <i>L. acidophilus</i> , <i>B. bifidum</i>
Smetana	Eastern Europe	<i>L. acidophilus</i> , <i>Lactococcus lactis</i> biovar. <i>diacetylactis</i>
Sweet acidophilus bifidus milk	Japan	<i>L. acidophilus</i> , <i>B. longum</i>
Sweet bifidus milk	Japan, Germany	<i>Bifidobacterium</i> spp.
Vita Fresh	Greece	<i>B. bifidum</i>
Vitalia	Greece	<i>Bifidobacterium lactis</i>



## Παραδοσιακά ζυμούμενα προϊόντα που παράγονται σε διάφορα μέρη του κόσμου (63 προϊόντα)

<b>Product</b>	<b>Country</b>	<b>Type of milk</b>	<b>Microflora</b>
Amasi	Zimbabwe	Bovine	<i>Lactobacilli (helveticus, plantarum, paracasei</i> subsp <i>paracasei, delbruecki</i> subsp <i>lactis)</i> , <i>lactococci (lactis</i> subsp <i>lactis, lactis</i> subsp <i>lactis biov. diacetylactis)</i> , <i>leuconostocs (mesenteroides</i> subsp <i>mesenteroides)</i> , <i>enterococci (faecalis, faecium)</i> .
Ayran	Turkey	Ewe, goat, cow	Yoghurt microorganisms
Chakka	India	Mixed buffalo and cow	<i>Lactococcus lactis</i> subsp. <i>lactis</i>
Dahi	India	Buffalo, goat	<i>Lactococcus lactis</i> subsp. <i>lactis, cremoris</i> , biovar <i>diacetylactis, Leuconostoc</i> spp., yoghurt microorganisms
Dough	Iran, Afghanistan	Ewe	Yoghurt microorganisms
Jamid	Jordan	Goat	
Katyk	Uzbekistan	Buffalo	<i>Streptococcus thermophilus, Thermobacterium</i> spp.
Kefir	Former Soviet Union	Ewe, cow	<i>Lactococcus lactis</i> subsp. <i>lactis, cremoris</i> , <i>Leuconostocs, Acetobacter aceti</i> , yeasts (lactose-positive or -negative)
Koumiss (Airag)	Mongolia, Kazakhstan, Kyrgyzstan, Russia	Mare, camel	<i>Lactobacilli (plantarum, paraplantarum, pentosus, rhamnosus, helveticus, kefigranum, delbruecki</i> subsp <i>bulgaricus)</i> , <i>Lactococcus lactis</i> , alcoholic-fermenting yeasts, <i>enterococci, leuconostocs (mesenteroides, psedomesenteroides)</i> , <i>Streptococcus parauberis, Str. thermophilus</i>



<b>Product</b>	<b>Country</b>	<b>Type of milk</b>	<b>Microflora</b>
Laban (leben)	Middle East	All types	<i>Streptococcus thermophilus</i> , <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> , <i>L. acidophilus</i> , <i>Leuconostoc lactis</i> , <i>Kluyveromyces marxianus</i> subsp. <i>marxianus</i> , <i>Saccharomyces cerevisiae</i>
Lactofil	Sweden	Cow	<i>Lactococcus lactis</i> subsp. <i>lactis</i> , <i>cremoris</i> , biovar <i>diacetylactis</i> , <i>Leuconostoc citrovorum</i>
Skyr	Iceland	Ewe, cow	Yoghurt bacteria, yeasts
Tarag	Mongolia	All types	<i>Lactobacilli</i> (predominantly: <i>casei</i> , <i>delbruecki</i> subsp. <i>bulgaricus</i> , <i>fermentum</i> , <i>helveticus</i> , <i>kefiranoformis</i> ), <i>Streptococcus thermophilus</i> , <i>Enterococcus faecium</i> , yeasts ( <i>Candida</i> , <i>Debaryomyces</i> , <i>Issatchenkia</i> , <i>kazachstania</i> , <i>Kluyveromyces</i> , <i>Pichia</i> , <i>Saccharomyces</i> , <i>Torulasporea</i> , <i>Yarrowia</i> )
Xynogalo	Greece	Ewe	<i>Lactococcus lactis</i> subsp. <i>lactis</i> , <i>cremoris</i> , <i>Lactobacillus plantarum</i> , <i>L. maltaromicus</i> , <i>L. casei</i> , <i>Leuconostoc lactis</i> , <i>L. mesenteroides</i> , <i>L. paramesenteroides</i> , <i>Enterococcus faecalis</i> , <i>E. faecium</i> , <i>E. durans</i>
Yiaourti	Greece	Ewe, goat, cow	<i>Streptococcus thermophilus</i> , <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> , <i>L. paracasei</i> , <i>Leuconostoc</i> spp., <i>pediococci</i> , <i>enterococci</i>
Zabady	Egypt	All types	Yoghurt microorganisms



# Kefir (από όλα τα είδη γάλακτος) προϊόν Καυκάσου

<b>Microbial group</b>	<b>Kefir grains (cfu/g)</b>	<b>Kefir starter (cfu/ml)</b>	<b>Kefir (cfu/ml)</b>
<i>Lactococci</i>	$10^6$ ( <i>cremoris</i> )	$10^8-10^9$	$10^9$
<i>Leuconostocs</i>	$10^6$	$10^7-10^8$	$10^7-10^8$
Thermophilic lactobacilli	$10^8$	$10^5$	$10^7-10^8$
Mesophilic lactobacilli	$10^6-10^9$	$10^2-10^3$	
Acetic acid bacteria	$10^8$	$10^5-10^6$	$10^4-10^5$
Yeasts	$10^6-10^8$	$10^5-10^6$	$10^4-10^5$



- Koumiss (Airag): από γάλα καμήλας, προϊόν Μογγολίας, Θιβέτ, Ρωσίας
- Παραδοσιακό ελληνικό γιαούρτι
- Παγωτό: Προσθήκη γιαουρτιού με προβιοτικά σε μίγμα παγωτού. Ζύμωση μίγματος παγωτού με καλλιέργεια γιαουρτιού που περιέχει προβιοτικά
- Παγωτό γιαούρτι HB στην Ιρλανδία
- Παγωτό Sia στη Σουηδία
- Τα προβιοτικά συνήθως δεν μειώνονται κατά τη συντήρηση (Korslund & Søndergaard, 2005)



# Τυριά

- ❖ Τυρί Cheddar με *Bifidobacterium*. Καλή επιβίωση και οργανοληπτικές ιδιότητες (Mc Brearty et al., 2001).
- ❖ Τυρί Gouda με *Bifidobacterium* και *Lb. acidophilus* (Gomes et al., 1995).
- ❖ Τυρί Fresco (Αργεντινή) με *Bifidobacterium*, *Lb. acidophilus* και *Lb. casei* (Vinderola et al., 2000)
- ❖ Τυριά Crescenta (Gobetti et al., 1998), Cottage (Blanhette et al., 1996), white brined (Ghoddusiand and Robinson, 1996), hard pressed (Daigle et al., 1999), **κατσικίσιο** (Gomes & Malcata, 1998), fresh (Roy et al., 1997) με *Bifidobacterium*.
- ❖ Τυριά τύπου Κεφαλοτύρι (Kalavrouzioti et al., 2005)





# Ποτά

- Γάλα
  - Γάλα σόγιας
  - Μίγματα με φρούτα
- Με προβιοτικά
- Σουηδία (ProViva)
  - Φινλανδία (Chiandtundo)
  - Αγγλία (Pet and Johnny)
  - Γερμανία (ποτά από ορό με LA-5 και BB-12)
  - Ιταλία (αποβουτυρωμένο γάλα με προσθήκη προβιοτικών)



# Δημητριακά

Για όσους πάσχουν από δυσανεξία στη λακτόζη

Βρώμη: β-glucans (μείωση χοληστερόλης)

- Ζύμωση με: *Lb. acidophilus*, *Lb. reuteri*, *B. bifidum* με ή χωρίς καλλιέργεια γιαουρτιού (Martensson et al., 2002).

- ❖ Υπάρχουν διάφορα προϊόντα στις αγορές της Σκανδιναβίας (ProViva και Yosa) και της Γερμανίας (Primavita).
- ❖ Γερμανία: muesli με προβιοτικά



## ■ Προϊόντα μαγιονέζας

- Με προβιοτικά έγκλειστα σε αλγινικό ασβέστιο (Khalil and Mansour, 1998).

## ■ Προϊόντα βουτύρου και spreads

- Γερμανία: γιαούρτι με βούτυρο
- Ελβετία: Γιαούρτι με βούτυρο
- Ιταλία: Cheese spread
- Αγγλία: spread λαχανικών με ελαιόλαδο

*Όλα με προβιοτικά*



# Κρέας

- ❖ Προστατεύει τα προβιοτικά από τα χολικά άλατα
- ❖ Τα προβιοτικά χρησιμοποιούνται με εγκλεισμό σε αλγινικό Ca και ενσωματώνονται σε ζυμούμενα προϊόντα κρέατος.
- ❖ Επιβιώνουν καλά, όμως η αντιβακτηριακή τους δράση μειώνεται (Muthukumasara & Holley, 2006 & 2007).



***Σας ευχαριστώ θερμά για την  
προσοχή σας!***

**«Προβιοτικά τρόφιμα»**

Ευανθία Λιτοπούλου – Τζανετάκη

e-mail: [ganet@agro.auth.gr](mailto:ganet@agro.auth.gr)

Τηλ/fax: 2310 991674