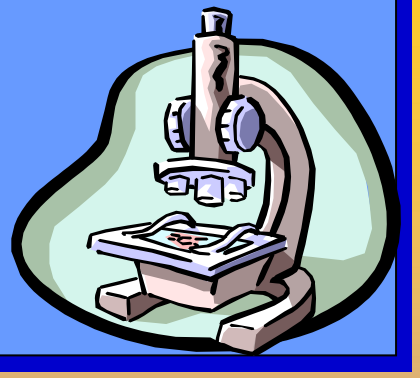
Gli alimenti probiotici visti dal microbiologo



L. MORELLI

Istituto di Microbiologia, U.C.S.C.
Via Emilia Parmense 84, 29100 Piacenza

In questa presentazione…

- Definizioni e aspetti regolatori
- Dalle regole al laboratorio
- Dal laboratorio alle applicazioni

Definizioni da parte del mondo della scienza

"Integratore alimentare di microrganismi vivi che esercitano un effetto benefico migliorando l'equilibrio microbico intestinale"

(Fuller - 1989)

"Microrganismi vivi che, ingeriti in una certa quantità, esercitano effetti benefici"

(Guarner e Schaafsma - 1998)

Ma i medici…

Biotherapeutic Agents

A Neglected Modality for the Treatment and Prevention of Selected Intestinal and Vaginal Infections

Gary W. Elmer, PhD; Christina M. Surawicz, MD; Lynne V. McFarland, PhD

Conclusions.—There is now evidence that administration of selected microorganisms is beneficial in the prevention and treatment of certain intestinal and, possibly, treatment of vaginal infections. In an effort to decrease the reliance on antimicrobials, the time has come to carefully explore the therapeutic applications of biotherapeutic agents.

(*JAMA*. 1996;275:870-876)

E gli enti regolatori…

Food and Agriculture Organization
of the United Nations

World Health Organization

Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria

Report of a Joint FAO/WHO Expert Consultation on
Evaluation of Health and Nutritional Properties of Probiotics in Food Including
Powder Milk with Live Lactic Acid Bacteria

Amerian Córdoba Park Hotel,
Córdoba, Argentina
1-4 October 2001

SCOPE

The Consultation agreed that the scope of the meeting would include probiotics and prebiotics in food, and exclude reference to the term BIOTHERAPEUTIC AGENTS, and beneficial microorganisms not used in food.

The Consultation has redefined probiotics for the purpose of this meeting as 'LIVE MICROORGANISMS WHICH WHEN ADMINISTERED IN ADEQUATE AMOUNTS CONFER A HEALTH BENEFIT ON THE HOST'

E il Ministero della Salute…

DEFINIZIONI.

PROBIOTICO.

Microrganismi vivi e vitali che conferiscono benefici alla salute dell'ospite[2] quando consumati, in adeguate quantità, come parte di un alimento[3] o di un integratore,. L'identificazione dei microrganismi probiotici deve avvenire secondo le raccomandazioni elencate nella Sezione "**Identificazione di specie e ceppi probiotici** " di questo documento.

ALIMENTO/INTEGRATORE CON PROBIOTICO.

Alimenti/integratori, che contengano, in numero sufficientemente elevato, microrganismi probiotici, in grado di raggiungere l'intestino, moltiplicarsi ed esercitare una azione benefica per lo stato di salute/benessere dell'uomo

La definizione di alimento o integratore con probiotico si applica ai prodotti che seguano il processo per la valutazione illustrato nell'All.1 di queste linee guida.

La definizione di alimento (integratore) con probiotico prevede anche le interazioni fra il microrganismo probiotico e la matrice alimentare o gli eccipienti o altri ingredienti; per tale ragione l'azione benefica deve quindi essere specificamente dimostrata utilizzando il prodotto pronto per il consumo (vedi All.1)

Dalle regole al laboratorio

5.2. Classification and identification of individual strains

Classification is the arranging of organisms into taxonomic groups (taxa) on the basis of similarities or relationships. Nomenclature is the assignment of names to the taxonomic groups according to rules. Identification is the process of determining that a new isolate belongs to one of the established, named taxa. The Consultation recommended that probiotics be named according to the International Code of Nomenclature to ensure understanding on an international basis. The Consultation strongly urged that for the sake of full disclosure, probiotic strains be deposited in an internationally recognized culture collection.

"*Lactobacillus sporogenes*" Is Not a *Lactobacillus* Probiotic

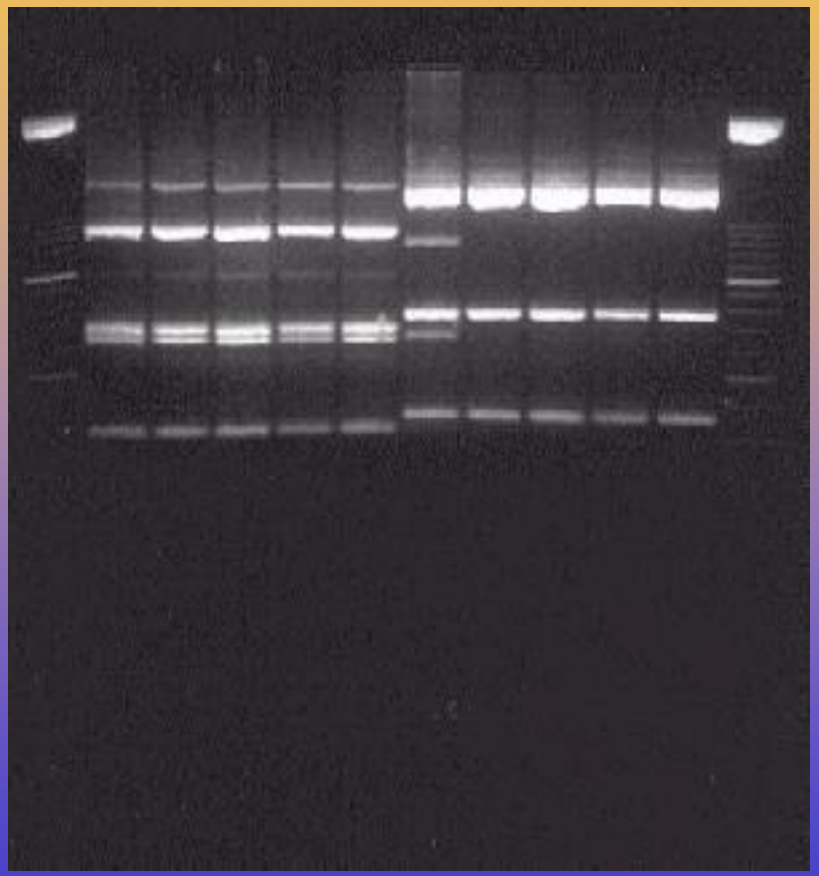
(M.E. Sanders, L. Morelli, S.Bush-ASM news August 2001)

The implications of the persistence of this mislabeling should be considered. The most important of these is safety. Since "Lactobacillus sporogenes" is not recognized as a species, a product labelled with this name confirms nothing about its contents. If the identity of the bacterium is in question, no conclusions about its safety can be made. Presumably the products on the market are consumed without undue risk. Erroneously calling this organism a lactobacillus incorrectly associates it with the same safety record as lactobacilli.

CLASSIFICAZIONE DI SPECIE

- **Analisi elettroforetica del DNA cromosomico amplificato e digerito (Metodologia ARDRA)**

Ventura, M., Casas, I. A.,Morelli,L., Callegari,M.L. (2000) Rapid amplified ribosomal DNA restriction analysis (ARDRA) of Lactobacillus spp. isolated from fecal and vaginal samples. Applied and Systematic Microbiology, ***23****,504-509.*



L B S N

List of
Bacterial names with
Standing in
Nomenclature

http://www.bacterio.cict.fr/

Dalle regole al laboratorio

Poiché le proprietà probiotiche sono legate al ceppo, appare evidente come sia importante selezionare il “ceppo giusto”.

A questo proposito la comunità scientifica ha elaborato un insieme di tests, a cui sottoporre i ceppi “candidati” per l’uso probiotico. Si è venuta così a creare una griglia di selezione per batteri probiotici

What about the safety:

- ❑ antibiotic resistance (phenotype/genotype)
- ❑ QPS status
- ❑ GRAS status

Safety assessment (Detailed in Section 3.3)

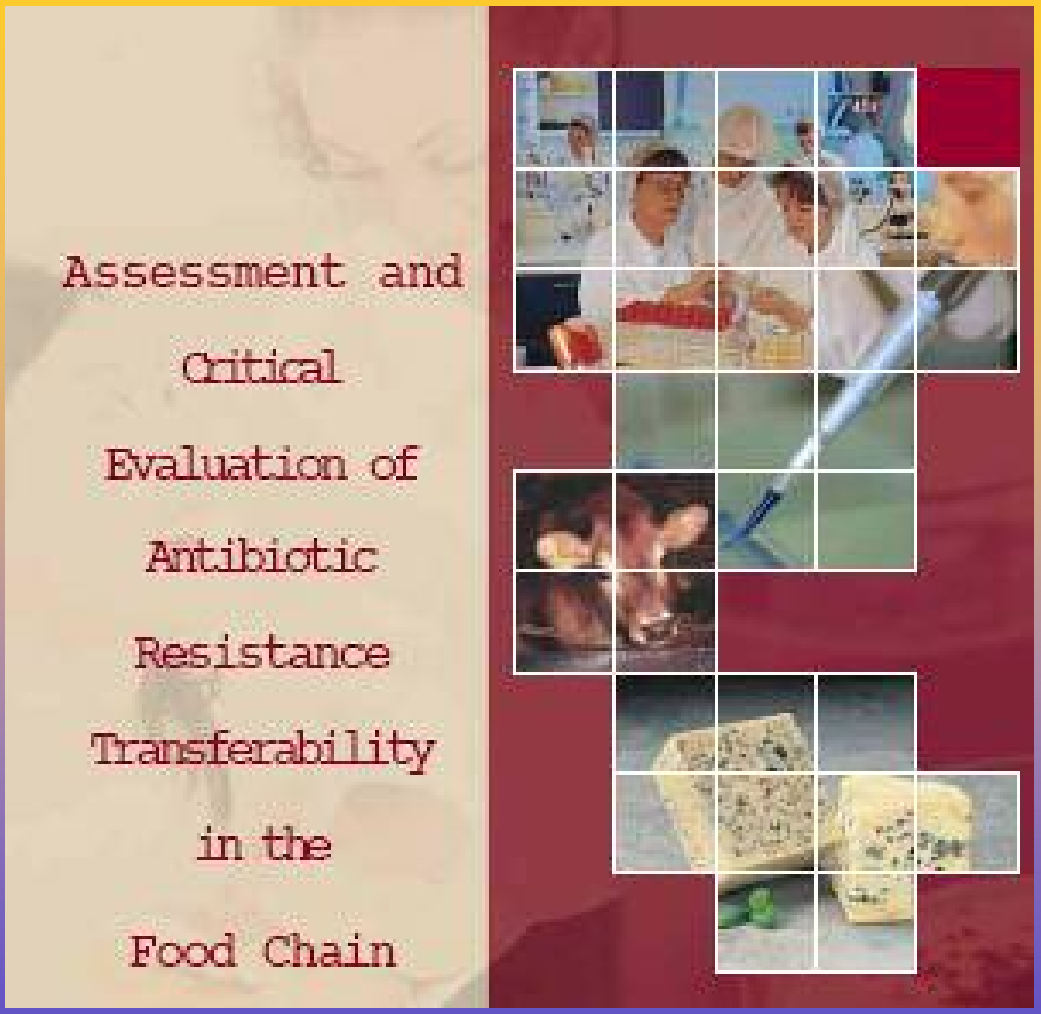
- *In vitro* and/or animal
- Phase 1 human study

E il Ministero della Salute…

Caratteristiche dei microrganismi probiotici.

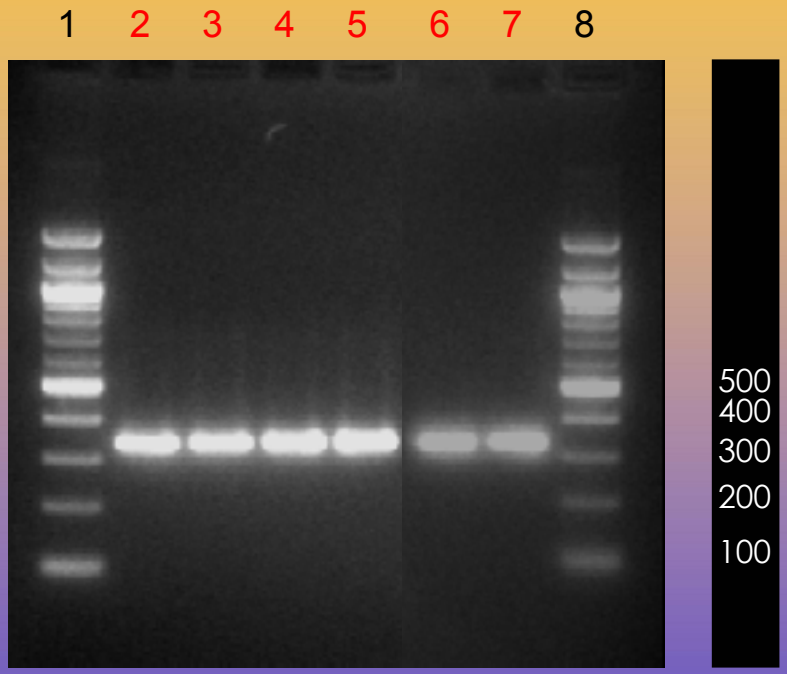
I microrganismi per essere considerati probiotici devono soddisfare i seguenti requisiti:

- essere sicuri per l'impiego nell'uomo; a tale proposito un utile riferimento potranno essere i criteri che verranno emanati a cura della European Food Safety Authority (EFSA) circa l'adeguatezza delle conoscenze disponibili. In ogni caso, oltre ad eventuali ulteriori criteri che EFSA considererà opportuno inserire, i microrganismi probiotici non devono essere portatori di antibiotico-resistenze acquisite e/o trasmissibili;
- essere attivi e vitali a livello intestinale in quantità tale da giustificare gli eventuali effetti benefici osservati in studi di efficacia;
- essere in grado quindi di persistere e moltiplicarsi nell'intestino umano;
- essere in grado di conferire un beneficio fisiologico dimostrato secondo i criteri riportati in All.1;



ACE-ART PROJECT: www.aceart.net

PCR to confirm the presence of *tetS* in *S.thermophilus*



1=ladder 100bp
2=E2
3=E13
4=E14
5=E15
6=E17
7=E18
8=ladder 100bp

NAME	SEQUENCE	Product SIZE	REFERENCE
tetS-1	5-'GGAGTACAGTCACAAACTCG -3'	335 bp	RIKILT
tetS-2	5'-GGATATAAGGAGCAACTTTG -3'		

ma anche le proprietà probiotiche sono ceppo-specifiche:

- bile/acido resistenza
- adesione (persistenza)
- proprietà probiotiche(immuno regolazione…)

Functional characterization
(Detailed in Section 3.2)

- *In vitro* tests
- Animal studies

Strain typing

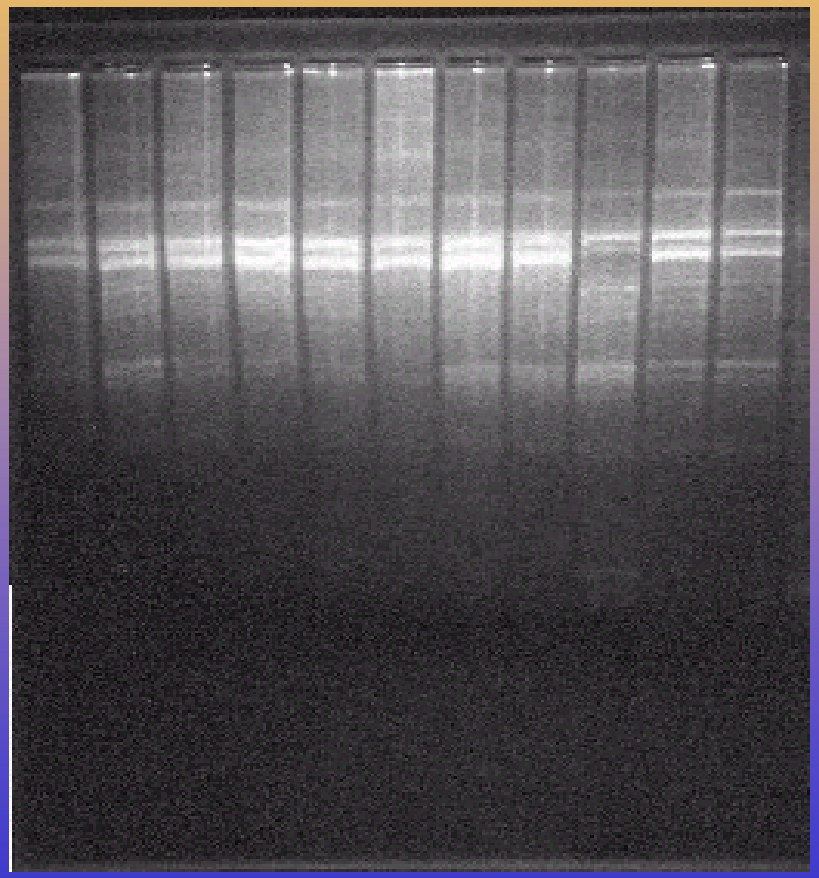
- Specific probiotic properties are strain-related
- If these properties are claimed, it seems mandatory to develop a strain typing method
- Several methods have been described, base mainly on genetic techniques
- PFGE, RAPD, Rep,multiplex PCR etc
- But also; protein profile, etc

Dalle regole al laboratorio

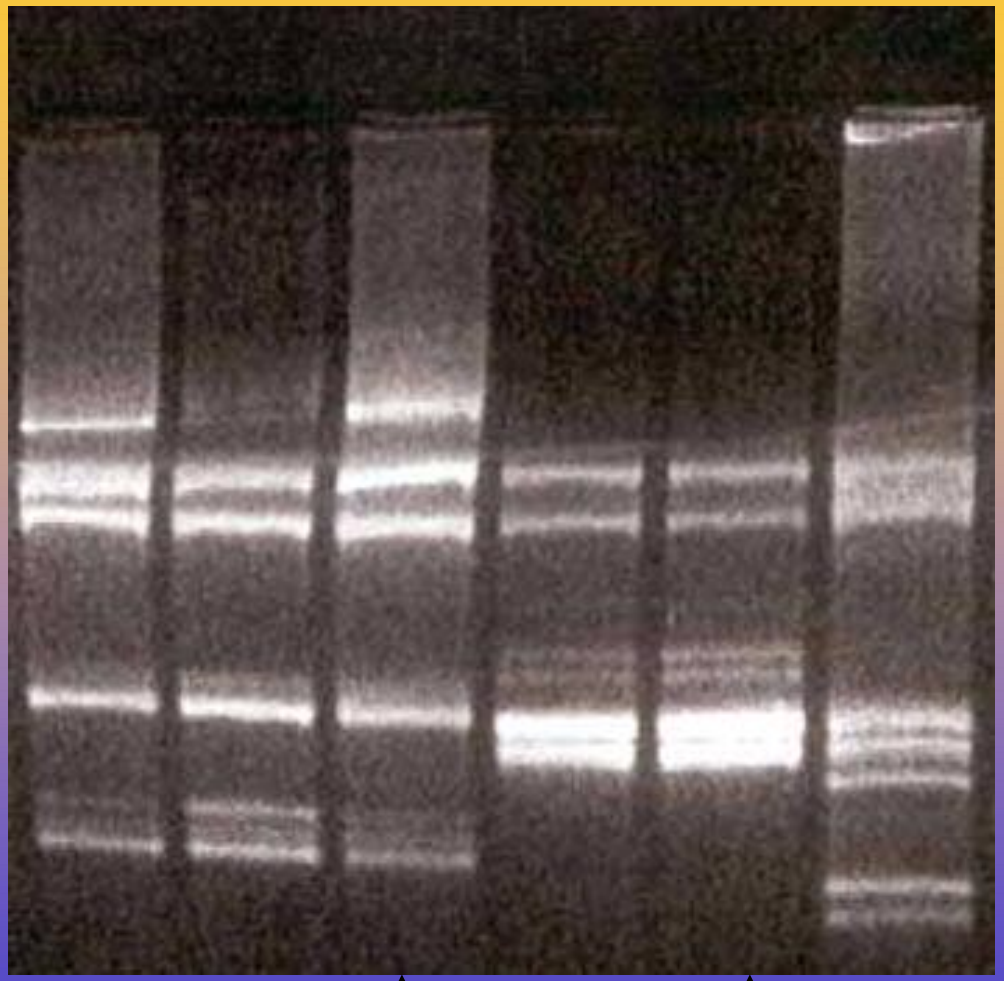
- ~~Origine umana~~
- Resistere all'ambiente gastrico e alla bile
- Aderire agli epiteli intestinali
- Persistere (colonizzare) l'intestino
- Svolgere dimostrate azioni positive per la salute/benessere

RAPD

1 2 3 4 5 6 7 8 9 10 11



REP



I-1794

I-1688

Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food
London, Ontario, Canada, April 30 and May 1, 2002

Food and Agriculture Organization
of the United Nations

World Health Organization

Guidelines for the Evaluation of Probiotics in Food

Double blind, randomized, placebo-controlled (DBPC) phase 2 human trial or other appropriate design with sample size and primary outcome appropriate to determine if strain/product is efficacious (Detailed in Section 3.4)

→ Preferably second independent DBPC study to confirm results

↓

Probiotic Food

Food and Agriculture Organization of the United Nations

"Proper *in vitro* studies should establish the potential health benefits of probiotics prior to undertaking *in vivo* trials."

6. Testing Methods for Establishing Health Benefits Conferred by Probiotic Microorganisms

World Health Organization

For *in vivo* testing, randomized double blind, placebo controlled human trials should be undertaken to establish the efficacy of the probiotic product.

Food and Agriculture Organization of the United Nations

6. Testing Methods for Establishing Health Benefits Conferred by Probiotic Microorganisms

World Health Organization

The Consultation recognized that there is a need for human studies in which adequate numbers of subjects are enrolled to achieve statistical significance.

Food and Agriculture Organization of the United Nations

6. Testing Methods for Establishing Health Benefits Conferred by Probiotic Microorganisms

World Health Organization

Dalle regole al laboratorio

Ogni ceppo probiotico deve avere una sua propria documentazione scientifica.

- **L'estrapolazione di dati da un ceppo ad un altro non è accettabile.**
- **Gli studi devono essere condotti su ceppi batterici e prodotti ben definiti.**
- **Dove possibile gli studi *in vivo* devono essere di tipo randomizzato, a doppio cieco e con un placebo di controllo.**

Microbiota in healthy/allergic subjects…
ex vivo assessment

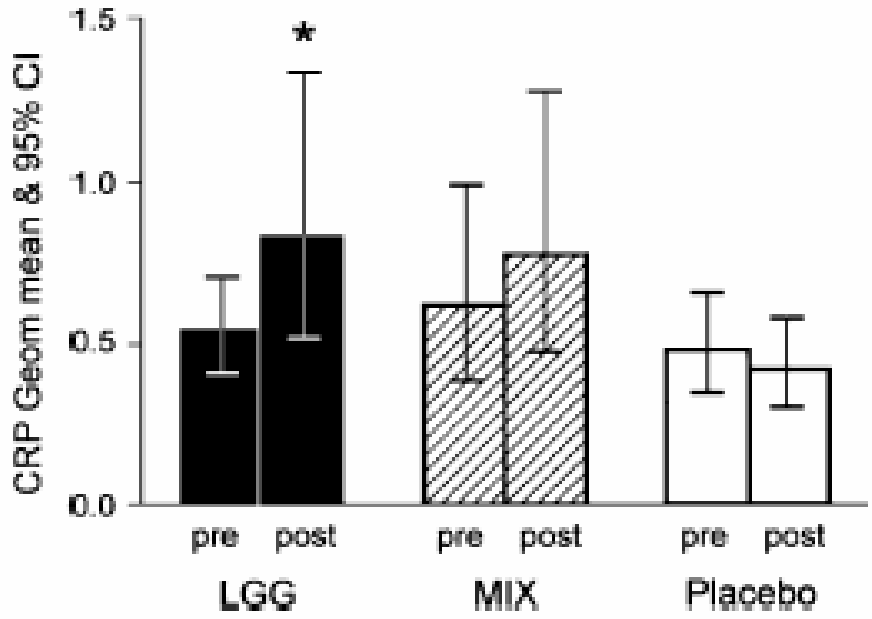


FIG 1. Unadjusted pretreatment and posttreatment serum CRP geometric means and 95% CIs in 3 treatment groups of infants with IgE-associated AEDS. Posttreatment serum CRP adjusted by pretreatment value. *Fisher least significant difference test: LGG versus placebo, $P = .021$.

Induction of inflammation as a possible mechanism of probiotic effect in atopic eczema–dermatitis syndrome

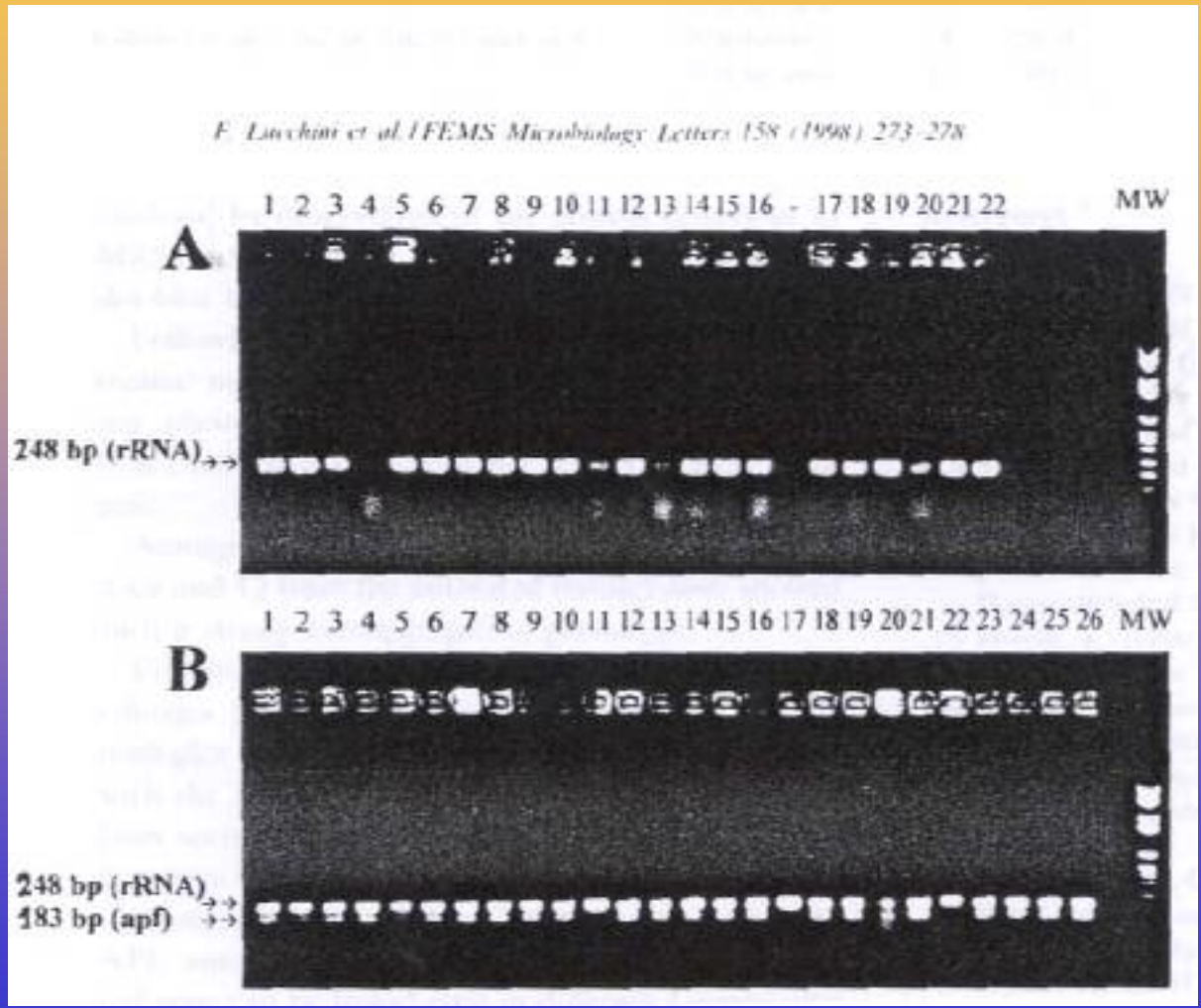
Minna Viljanen, MD,* Emma Pohjavuori, MD,* Tari Haahtela, MD, PhD,* Riitta Korpela, PhD,*# Mikael Kuitunen, MD, PhD,* Annikki Sarnesto, PhD,* Outi Vaarala, MD, PhD,† and Erkki Savilahti, MD, PhD*

Legenda:

AEDS:atopic eczema-dermatitis

CRP: C-reactive protein

Multiplex PCR



5.3.6. Use of probiotics in otherwise healthy people

Many probiotic products are used by consumers who regard themselves as being otherwise healthy. …The Consultation would like studies to be done to give credibility to the perception that probiotics should be taken on a regular basis by healthy men, women and children. It is currently unclear as to the impact of regular probiotic intake on the intestinal microflora. For example, does it lead to the depletion or loss of commensal microorganisms which otherwise have beneficial effects on the host? While there is no indication of such effects, the issue needs to be considered. Furthermore, the concept of restoring a normal balance assumes that we know what the normal situation in any given intestinal tract comprises. It was deemed important by the Consultation to further study the various contributions of gut microorganisms on health and disease.

Il riconoscimento internazionale

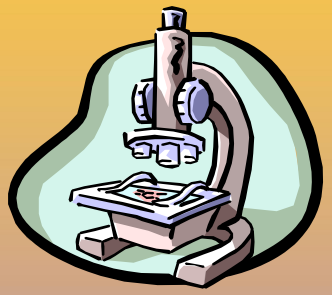
Conclusions

There is good evidence that specific strains of probiotics are safe for human use and able to confer some health benefits on the host, but such benefits cannot be extrapolated to other strains without experimentation.

Per concludere

- **Ogni ceppo probiotico deve avere una sua propria documentazione scientifica.**
- **L'estrapolazione di dati da un ceppo ad un altro non è accettabile.**
- **Gli studi devono essere condotti su ceppi batterici e prodotti ben definiti.**
- **Dove possibile gli studi *in vivo* devono essere di tipo randomizzato, a doppio cieco e con un placebo di controllo.**

Per concludere:



1. **Il microbiologo svolge un ruolo essenziale nella prima parte della ricerca di un nuovo probiotico**

2. **Il microbiologo ha funzione di supporto nei processi di validazione dell'efficacia di un probiotico**

GRAZIE PER

L’ ATTENZIONE