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FIRST DRAFT

PROPOSAL FOR SELECTION AND CHARACTERIZATION OF STARTER
CULTURES FOR THE DAIRY INDUSTRY IN BRAZIL THROUGH
INTERNATIONAL COOPERATIVE R&D PROGRAMMES

1985

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1. Special considerations

The Section of Dairy Science and Technology from ITAL - Instituto de Tecnologia de Alimentos - the Institute of food Science from the Agricultural Department of State, Government of Sao Paulo, has formulated a lactic acid bacteria collection project objecting the isolation, selection and mass production of lactic starters to supply the Brazilian dairy industry. Actually, all the starters used in Brazil are imported. Furthermore, these cultures function under their manufacturing conditions. Therefore, the strain native to that country need to be isolated and made available to Brazilian cheese-makers. To achieve this, either basic techniques of microbial genetics, such as the recombinant DNA will be necessary. For these reasons, it is important to set up a project to establish a cooperation between the Section of Dairy Science and Technology from ITAL and the laboratory of Prof. L. McKay, for technical support, in order to accomplish preplanned goals with a higher degree of success.

2. Objectives

A. Development objectives

This project is part of UNIDO's programme to develop and/or enhance the capability of researchers in developing countries to perform advanced biotechnology R&D and for industrialists to establish a bioscience-based industry, able to capitalize on its results.

Accordingly, as a first step, researchers from ITAL will initiate the isolation, identification and screening of mesophilic cultures from raw milk in Brazil. They will also visit dairy industries to collect cheese whey regarding the isolation of phages. Then, having a selected group of phage resistant strains, the researchers will be able to begin with the genetic improvement.

Therefore, they have to become familiar with the genetic engineering techniques; a strategy which enables the manipulation of the genetic information contained within bacterial cells in order to achieve the expected lactic acid fermentation. At a later stage, the genetically selected and improved strains will be produced, preserved and distributed to the Brazilian cheese factories.

B. Immediate objectives

The following activities have to be undertaken:

- (a) To isolate, identify and screen mesophilic cultures from raw milk in Brazil;
- (b) To establish a working relationship between the research group of Prof. L. McKay and ITAL with the purpose of transferring the knowledge and know-how of plasmid biology and genetic engineering techniques to ITAL researchers;
- (c) To produce and preserve the lactic starters for commercial use in Brazil.

3. Project output

The project is expected to isolate, and make a technological and genetic selection of mesophilic lactic acid bacteria from raw milk in Brazil. Simultaneously it will isolate bacteriophages from whey collected in Brazilian cheese making factories. The final purpose is to produce selected and genetically improved starters, making them available for the Brazilian dairy industry.

This project also enables the researchers from ITAL to get acquainted with applying genetic engineering techniques. The procedures and results obtained will be reported for the benefits of other Brazilian researchers and for the dairy industry.

4. Project activities

The laboratory of dairy microbiology of ITAL is well-equipped to undertake studies on the selection and short-scale mass production of lactic acid bacteria. ITAL researchers have isolated several wild strains from raw milk and classified some of them. The work is mainly done with mesophilic cultures as they are the principal starters for most of the Brazilian cheeses. On the other hand, the work in the laboratory of Prof. McKay, at the University of Minnesota involves plasmid biology and genetic manipulation of lactic acid bacteria. The staff includes pioneers who have successfully achieved the DNA transformation and cloning of these bacteria.

It is also proposed to carry out the part of the project on plasmid biology and genetics identification of the selected Brazilian strains with emphasis on phage resistance and improving the capability of fermentation in this laboratory for a period of one year. The programme of this project can be divided as follows:

(1) Isolation, identification and screening of mesophilic cultures from raw milk in Brazil

The screening of the identified strains involves the methods utilized in New Zealand for the same purpose, such as (a) growth at 22° C for 18 hours; (b) determination of the acid production for the selection of cultures which can produce the proper rate of lactic acid under the cheese-making conditions in Brazil; (c) salt tolerance; (d) proteinase activity; (e) production of proper flavour for the main different kinds of Brazilian cheeses; and (f) phage sensitivity.

(2) Isolation and development of a phage collection and phage resistant strains

The phages will be isolated from whey, collected in several cheese factories by testing them against suitable sensitive cultures. All the strains screened above will be tested against this pool of isolated phages.

(3) Plasmid biology identification and genetic improvement

The knowledge of plasmid biology, necessary to further genetic manipulations will be developed at the laboratory of Prof. McKay including: determination of plasmid sizes and determination of their functional properties regarding mainly the phage resistance, lactose utilization and proteinase activity.

Attempts regarding the recombinant DNA technology will be made in those strains who have the best characteristics for making cheese and other dairy products in Brazil.

(4) Mass production, preservation and distribution of such cultures to the Brazilian cheese factories

Both fermentation technologies will be used to propagate cells. Attempts to develop the most adequate kind of preservation will be made. Generally, the frozen or liquid starters can be used because of the clima conditions.

5. Project inputs

A. ITAL should provide the following inputs:

- (a) Travel and per diem of the project leader in Brazil;
- (b) Laboratory supplies and equipments to carry out experiments (Annex I);
- (c) Laboratory space (200 m²), cost of rent: US \$4,000

B. The following staff would be required to undertake the activities:

- (a) One senior researcher (microbiologist; total cost of the salary, which will amount to US \$6,000, is to be provided by ITAL);
 - (b) One junior scientists (total cost of the salary, which will amount to US \$12,000, is to be provided by an international funding source);
 - (c) One technician (total cost of salaries will amount to US \$3,000, to be provided by international funding source).
- C. Travel for international staff (total cost of US \$20,000, to be provided by an international fundins source).
- D. Consultant's travel (total cost of US \$10,000, to be provided by an international funding source).
- E. It is envisaged to have four group coordination meetings; one per year and two in the first year, with a total cost of US \$12,000 to be provided an international funding source.

Annex I

List of consumable supplies and equipment

A. Consumable supplies

concept	Source of funding	Cost US \$
Beekman ultra centrifuge tubes	International	1,100
Micro-centrifuge tubes	International	1,000
CsCl for ultracentrifugation	International	1,500
Agarose for gel electrophoresis	International	1,500
✓ Bacterial media	International	3,000
Other chemicals for electro phoresis	International	1,500
Other general laboratory chemicals	International	3,000
Restriction enzymes	International	4,500
Ligases	International	3,000
Other enzymes and reagents for genetic experiments	International	1,000
Radioactive materials	International	4,000
Millipore filters	International	1,000
Glassware and plasticware material	International	3,000
Photographic material	International	700
Total funds requested for consumable supplies		<u>26,800</u>

B. Equipment

concept	Source of funding	Cost US \$
Microscope	ITAL	5,000
Spectrophotometer	ITAL	5,000
Ecologen	ITAL	5,000
Beekman centrifuge	ITAL	5,000
Liofilisator	ITAL	5,000
Freezer	ITAL	5,000
Refrigerator	ITAL	5,000
Batch fermentator	ITAL	5,000
Memo-.....	ITAL	5,000
Stomacher	ITAL	5,000
.....flow chamber	ITAL	5,000
Autoclave	ITAL	5,000
Growth incubator	ITAL	5,000
Sterilization incubator	ITAL	5,000
Eppendorf centrifuge	International	1,500
Ultracentrifuge with rotors	International	50,000
Sequencing gel	International	1,900
Electrophoresis system with accessories	International	1,000
Horizontal gel electrophoresis apparatus	International	1,000
Assorted combs, spacers and small electrophoresis equipment	International	800
Polaroid photographic equipments	International	5,000
Microwave oven	International	1,500
Power supplies	International	5,000
Total funds requested for equipment		70,800
Total supplies and equipment		97,600