DATE: 21st October 1996

CRAFT
SYNTHESIS REPORT

PROJECT N°: CR-1265-91
CONTRACT N°: BRE2-CT94-1455

STARTING DATE: 01/12/1994 DURATION: 20 MONTHS

TITLE: *«Research on stable mix fermentation of lactic bacteria and on method to preserve this mix»*

PROJECT COORDINATOR: BIO ARMOR

PROPOSERS:	R&D PERFORMERS:
- BIO ARMOR	- CRITT CBB DEVELOPPEMENT
- MEDILABOR	- CENTRE DE MICROENCAPSULATION
- GOAVEC SA	- ISTAB (Université de Bordeaux)
- LANNOO	
- SODEVA	

FOR THE PERIOD FROM: 01/12/1994
TO: 30/06/1996

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1. Contents page

2. Summary page

2.1. Keywords

Feed additive, Lactobacillus, microencapsulation, coating, mix fermentation,

2.2. Abstract of the results and benefits of the project

The project is aimed at developping technologies and know-how adapted to the industrial production of new generation of zootechnical feed additives : **probiotic *lactobacilli***.
Two *Lactobacillus* strains : a homofermentary *Lactobacillus farciminis* **P222** and a heterofermentary *Lactobacillus fermentum P24* were selected on basis of their specific in vitro probiotic capacities.
We defined and optimized a medium and a batch fermentation process at laboratory level which allows the production of a sufficient lactobacilli biomass between 5.109 till 10.109 CFU/ml of broth medium.

We demonstrate feasibility of mix optimal fermentation on dextrose and lactose of both strains in a batch process, managing the inoculum rate : an equilibrated biomass was achieved with a biomass level superior to 10^9 CFU/ml for each strain.

We developed a specific microencapsulation process using a laboratory pilote spray-drying process. The bacteria, in acqueous suspension with specific low viscosity polymers and protestants, are sprayed in tiny droplets in a sparing temperature-scale air flow. The maximal biomass level achieved was 10.109 CFU/g.

The entire line (fermentation and microencapsulation-drying step) was then assembled and optimized on basis of the maximum revivifiability of the dried biomass and of stability of dried biomass during further storage.
This optimized process allowed a quite good revivifiability rate (between 40 to 70%) and a 3- to 6-month stability of achieved biomass.
Feasibility of scale up pilote production was tested on a 10 then a 20 times scale. The same results were achieved on stability and revivifiability. Nevertheless we do not succeed in collecting an important biomass in the powder (about 10^9 CFU/g). The use of multi-stage dryer will certainly permit us to optimize the final population recovery.
In addition, the scale up permits to generate productivity gain from 3 to 6 times because of drying tower height (laboratory pilote dryer course was short).

Industrial exploitation is planned in 1997 on piglets feed industry on basis of European directives 87/1 53 and 94/40. Experiment on weaned piglets (1 kg/ton of 10^9 CFU/g product) has demonstrated a 9 % improvement on average daily growth and 5 % improvement cm feed conversion (piglets from 4 to 10 weeks).

3. The consortium

3.1. Names and addresses of the partner organizations .....

SME PROPOSERS	R&D PERFORMERS
BIO ARMOR Mr COLAS - Managing Director Zone Industrielle de la Gare F-22940 PLAINTEL	**CBB DEVELOPPEMENT** MR BLANCHARD - Director 9, rue du Clos COURTEL F-357(N RENNES
MEDILABOR Dr Carlo ODORE - President, Owner. Via Cuneo 17 I- 12030 CAVAILLERMAGGIORE	**CENTRE DE MICROENCAPSILATION** **Mr RICHARD** - Director 8, rue Andre BOQUEL F - 4 9 1 00 ANGERS
GOAVEC Mr GOAVEC - Marketing Director 32, rue EIFFEL F-61OOO ALENCON	**[STAB - ADERA** **Mr** DESCHAMPS - Head of Laboratory University Bordeaux I Avenue des Facultés F-33405 TALENCE Cedex
VOEDERS LANNOO-MARTENS Mr LANNOO - Managing Director VAARTSTRAAT 30 B-9850 HANSBEKE-NEVELE	
SODEVA Mr VAXELAIRE - Technical Manager 26, rue des GLIERES F-74 100 ANNEMASSE	

3.2. Consortium description

The consortium was divided into two working groups: The fermentation group and the encapsulation group.
Each group has worked in the first period of the project in a separate way. Nevertheless, each phasis has been approached with special emphasis on collaboration between scientific and industrial managers.
The second step of the project was the adaptation of the entire line and the scale-up of the achieved pilote line.

The management Committee :

BIO ARMOR was the prime proposer of the project. BIO ARMOR know-how and products concern the animal biotechnology and feed dietetic. BIO ARMOR produces and

commercializes living lactic acid bacteria for pigs and poultry production and preparations from these products.
They have developed a recognized activity and know-how in manure treatment for nitrogen and phosphorus preservation of intensive rearing areas.
Its commercial activity represents a turnover of 2250000 ECU. Its direct markets are Brittany and France and about 20 % of its turnover is done on the export market: Eastern Europe, Benelux, South-east Asia and north Africa.

CBB Développement is the R&D performers in charge of the scientific management of the project and particularly of the relation and adaptation between scientific and industrial know-how.
CBB Développement know-how concerns technologies transfer between research and industrials in the area of biotechnology, environment and chemistry. Its engineers elaborated and participated to projects from the laboratory scale till the industrial scale. One of their missions, financed by the Region authorities, is animating a network of industrial and scientific abilities and of technologies lookout.

SME proposers :

MEDILABOR is an italian firm specialized in feed additives. They manufacture and commercialize vitamins, trace elements and medicinal premix particularly in the cattle rearing. An ultimate development allowed new activities in liquid vitamins and analysis laboratory. Its turnover amounts 1000000 ECU.

Among the consortium, its know-how concerns the distribution and market characteristics of feed additives such as Lactobacilli. Especially, Medilabor is in charge of the adaptation of acquired encapsulation know-how to other unstable feed additives (vitamins, trace element...).

LANNOO-MARTENS VOEDERS is a Belgian firm specialized in livestock feedstuff and particularly in horse feeding. Its turnover is progressing till 1570000 ECU.
Its function in the project concerns the final use of this kind of unstable additive in feedstuffs: market definition, respect of feed industry specifications and efficiency of Lactobaciili additives in piglets rearing.

GOAVEC is an equipment manufacturer for food and feed industry. Goavec has developed a special know-how in fermentation apparatus for lactic acid bacteria in milk fermentation.
During the project, the control of this SME was taken by the consortium SOGEPA-SIRAGA. The firm GOAVEC has preserved is SME status and the new management team confirmed its participation to the project.
GOAVEC worked with the fermentation group in order to develop a process to be scaled up in an easy way.

GOAVEC will assume the industrialization of the fermentation process for BIO ARMOR during the industrial step.

SODEVA is a firm dealing with ultrasonic technology in the spray drying processes and in metal technology. Its turnover amounts 700000 ECU.

During the project, we demonstrate that the ultrasonic nozzles technology has not improved the encapsulation process. SODEVA decided not to go on because the technology orientation did not involve SODEVA know-how.

Scientific Committee :

ISTAB is a laboratory of a superior food engineering school specialized in biotechnology and microbiology of lactic acid bacteria.
This laboratory has initiated the project with BIO ARMOR with the screening and the selection of lactic acid bacteria of specific probiotic activities.
The two selected strains have been registered in the National Collection of Microorganisms Cultures of Pasteur Institute.
This laboratory was in charge of metabolic studies of this microorganisms and adaptation of these information to fermentation and mix fermentation feasibility.

CENTRE DE MICROENCAPSULATION is a Center of technology transfer in all encapsulation technologies. Its activity concerns laboratory studies and scale-up on coating technologies in the area of food, cosmetics, pharmacology and agronomy.
Its activity consist in developing a laboratory pilote able to spray-dry lactobacilli bacteria and permit to preserve bacteria activities.
It was always in contact with CBB Développement during the optimization of the line : fermentation-encapsulation technology and during the scale up feasibilities experiments.

4. Technical achievements

Technical achievements could express themselves in two kinds of deliverables :
- Know-bows acquisition
- Active principle pilot production in a coated form

Two strains have been selected with bioregulators propriety:
Lactobacillus farciminis P222 : the homofermentary lactic bacteria
Lactobacillus fermentum P24 : the heterofermentary lactic bacteria

4.1. Fermentation process know-how

4.1.1. Batch medium improvement

A screening was organized to select complex sources of nitrogen and trace elements, the point of *major* importance is the absence of insoluble parts. Various nitrogen sources have been tested at equal total nitrogen concentration (2,3 g/l TN) :

The peptone and soluble extracts had the advantage of presenting no cloud in the medium what permitted the use of O.D. technique. With the other substances selected, a cloud occured. Nevertheless, the cost for these products was much lower.

➪ The soluble tested organic *nitrogenous* consist of the nutritional needs of the P24 strain (1,35.109 cfu/ml).
In return, for the P222 strain, this nitrogenous source does not seem very performant and has to be associated with complementary trace elements.

The initial bacteria inoculum is defined to be high not to induce lag time. The cell biomass increase immediately in exponential phasis. This phasis lasts about 4 to 5 hours and is defined by maximum growth rates of 0,63 h^{-1} for P24 and 0,69 h^{-1} for P222.
We **have to notice that in optimum conditions of temperature and of maximum growth rates were :0,72 and 0,90 h^{-1} for P24 and P222 respectively.**

The exponential phasis is followed by stationary and lysis phasis. In the case of the P24 strain, the stationary phasis is very short, since after 24 hours of culture, the bacteria concentration falls down to initial amount (10^7-10' cfu/ml) :

At the end of this preliminary tests, the medium presenting Minerals source has been selected. As a matter of fact, we have to remind that both strains will be produced in mix cultures. So. the culture medium has to comply with the nutritional requirements of both strains.

4.1.2. Optimization of the medium composition by experiences plans

The optimization has been achieved by a statistical method of experiences plans as described by Hadamard.
For the first experiences plan, a Hadamard matrix, with 7 factors and 8 experiences, has been used. The seven **medium A** components (except dextrose) are studied at levels -1 and +1.

4.1.3. Fermentation variables definition

pH, temperature and bases to be supplied in order to determine optimum conditions for the growth of each bacteria individually (Productivity during the batch culture).
The effect measured **was** the specific growth velocity for each strain :

	P24	P222
Optimal pH for growth	5,3	6,2
Optimal temperature for growth	40 'c	**40** °C

4.2. Homofermentary and heterofermentary bacteria mix-cultures know-how

It had been noted that P24 seems to be affected by the lactic acid produced by P222 strain in mix culture (heterofermentary bacteria).
The biomass and biomass yield from dextrose are less affected by lactic acid concentration at a pH of 5,8 than at a pH of 5,3 related to a less dissociated lactic acid (pKa 3,6) : it appears that for a concentration from 0 to 20 g/1 in the medium, the growth rate decreases in a range of 54 % at pH 5,3 and only 35 % at pH 5,8.

In accordance with the achieved results in simple cultures, they present a same growth optimal temperature (40°C) and specific growth rate of biomass production neighboring at pH 5,8.

4.2.1. Influence of the inoculation rate

An equal inoculation rate of each strain (50 % of P222 strain and 50 % of P24 strain) have not permitted generation of an equilibrated biomass (P24 :8,9 e8 and P222 :4,8 e9) because of the sensitivity of P24 strain to lactic acid.
We achieved a culture in the bases of an inoculation rate of 86 %-14 %. We could notice that both specific growth rates were enhanced (P24 :0,86 h-* and P222 : 1,38 h^{-1}) so we could provide a quasi optimal productivity and an equilibrated biomass (P24 : 1,6 e9 and P222 : 3,0e9 cfu/ml).

We could obtain a 5e9 CFU/ml population with an enhanced substrat consumption rate and we noticed a modified product profile with a special emphasis on ethanol production level.

4.2.2. **Influence of substrat addition in course of mix fermentation**

We made this experiment with two substrats : lactose arid dextrose. On lactose, the inoculum amount of both strain is of 50% of each strain because both growth rates are equal on that substrat ($\mu = 0,3\ h^{-1}$)

STRAINS	µ max. (h-')	Biomass CFU/ml	Dextrose consumed (g/l)	Lactic acid (g/l)	Acetic acid (g/l)	Ethanol (g/l)
P24 (10 %)	1,21	2.109	46,7	19,9	4,8	8,5
P222 (90 %)	1,48	1,3.109				

Mix culture of homofermentary and heterofermentary strains on dextrose

STRAINS	µ max. (h^{-1})	Biomass CFU/ml	Lactose consumed (g/l)	Lactic acid (g/l)	Acetic acid (g/l)	Ethanol (g/l)
P24 (50 %)	0,72	3,1.109	42,5	19,65	3,05	7,2
P222 (50%)	0,49	1>6.109				

Mix culture of homofermentary and heterofermentary strains on lactose

We could demonstrate that substrat injection in course of mix culture does not increase mix biomass production. However, it appears that this mix fermentation management permits better growth rates on lactose than the one achieved in single cultures.

4.3. **Bacteria coating know-how**

We made the choice of a water solution basis for the coating process regarding the natural occuring medium of bacteria (culture broth).

The process is based upon a gentle spray - drying process :

- Generation of the separate particles by spraying the liquid phasis containing high level of soluble polymers in tiny droplets.
- During the drying phasis, soluble polymers formed a **matrix structure** when bulkwater is eliminated : **the microsphere.**

To this purpose, CME used a simple-effect spray-drying laboratory tower with a 50 cm high evaporation chamber. The maximal inlet air flow is of 40 Nm^3/h and a pneumatic nozzle was used.

4.3.1, Calibration runs

The dry powder is normally recovered in the cyclone flask. It appears an area at the bottom of the evaporation chamber cone where the microparticles accumulate because they are not dried enough. Increasing the temperature of inlet gas could allow a better recovery rate of microparticles.

The bacteria harvested with an inlet air temperature of 60°C seemed to be as stable as the ones obtained with an inlet air temperature of 40°C. Indeed, bacteria collected in the atomization cone presented the same capacity to be stable after the coating process and during storage. So,

the longer residence time in the contact of airflow temperature (40 to 60°C) did not seem to be injurious for the bacteria stability.

It was not possible to obtain a good stability during normal storage conditions using coating polymers alone as support. Between 15 days, we lost 4 to 6 Log using various soluble polymers.

The use of some substances as protestants could enhance the stability of coated bacteria although it appeared that the recovery rate after the process was not influenced by these protestants. The loss of bacteria after 15 days of storage was of 2 Log and particularly 1 Log and less for the best results.

These results, in particular those obtained about stability, are in quite agreement with the o expected. Nevertheless, the bacteria lost during the process were too much drastic to allow a sufficient stability during the storage.

batch number	Protestant	coating products.	T O day	T + 60 days
SE 2-132-1	Protectant 1	soluble polymer	6,9E9	2,0E6
SE 2-144-1	Protectant 1 + C vit.	soluble polymer	2,8E8	6,1E5
SE 2-148-1	Protestant 1 + lactose	soluble polymer	1,2E1O	9,0E8
SE 2-150-1	**Protectant 2**	soluble polymer	7,6E9	**1,1E1O**
SE 2-151-1	**Protestant 3**	soluble polymer	9,6E9	3,4E9
SE 2-149-1	Lactose	soluble polymer	8,0E9	4,7E6
SE 2-154-1	Dextrose	soluble polymer	3,5E10	2,1E4

4.3.2. **The polymer/protectant rate**

Batch	Protectant 2	Protestant 3	Polymers / protestant rate	T0	T30	T45
SE 2-195-1	X		**> 1**	**5,9E8**	**8,5E6**	**8,5E5**
SE 2-197-1	X		1	8,3E8	1,2E6	8,1E6
SE 2-199-1	X		< 1	5,1E8	9,0E8	
SE 2-196-1		x	>1	**1,3E9**	**2,3E8**	**3,0E7**
SE 2-198-1		x	1	1,1E9	**6,7E7**	1,4E7

We notice that the amount of coating polymers could not be reduced fewer than 50% of the total dry material amount.

4.4. **In line fermentation and coating experiments know-how**

Biomass from the in line fermentation done on basis of the best productivity and the lower immobilizing time of fermentation tank was applied to the drying process

The production line would be as follows in optimal conditions defined previously :

2,5 ml inoculum frozen → **31** Culture 1 → **2°A** . **151** Culture 2 → **2 %** **750 I** Culture 3

Yields were drastically inhibited during the consecutive encapsulation steps.

COATING TRIALS	TO	T15	T30
SE 3-11-1	8,6 e8	2,8 e8	1,3 e7
SE 3-15-1	8,9 e7	8,3 e7	6,7 e6
SE 3-24-1	<3,0 e5	< 3,() e5	
SE 3-26-1	< 3,() e5		

After valuation the most directly accused is the fact that **the bacteria manufactured are stressed and do not seem to be able to suffer from the stress of the consecutively drying step.**

A more extensive analysis of the stability results obtained according to the used culture conditions enabled to emphasize several **risk factors :**

Optimal fermentation temperature very close to the lysis temperature
Appearance of a lysis stage at the time of deterioration of nutritive medium.
Difficulty of concentrating the biomass in stationary stage because risk of appearance of a lysis stage.

4.4.1. Stability optimization of the P222 strain

The stability of the dried biomass will be valued by :

- The estimation of the recovery rate after drying: stress viability.
- The study of its viability after exposure at room temperature (3 months) and at 40°C (3 weeks; accelerated maturing).

The parameters identified as being able to influence the stability of the produced biomass are :

growth temperature

A temperature of 40 °C is very close to the lethal temperature and especially presents an inhibition risk particularly in stationary stage (growth stopping at maximal speediness). Moreover the membrane composition changes according to the growth temperature.

- Collection stage:

The end of the exponential phase or the stationary stage are convenient stages for concentrating the collected biomass.

It seems to appear that the stationary phase is the most convenient stage for concentrating. Indeed, the cell structure and metabolism are different at this stage and more able to master a stand-by state.

The viability and stability of the biomass collected in such conditions are very satisfying and close to the best results we obtained. Temperature is the most important parameter included in the drying stability mastering.

4.4.2. Feeding in course of fermentation

- P222 fed-batch production

The general goal of this study is to identify growth limiting factors at the time of the appearance of a stationary stage.

When the nitrogen source is injected, the dextrose concentration is low (# 6 g/l). We injected dextrose one hour after organic nitrogen injection, at the moment of the biomass growth lead to the stationary phasis. This feeding mode enaMes us to produce 5.109 UFC/ml after 6 hours of culture, what represents an important improvement of cell production of about 180%.

The *conversion* rate of dextrose in lactic acid keeps increasing from3770 during first hours, to 73 % during growth stop stage.
The growth stop noticed in this case, can be due either to. a minerals deficiency as additions do not bring them, or to an inhibition by the produced lactic acid (L isomer of lactic acid).

- P24 fed-batch production

Biomass production has been increased to 7,2. 10^9 cfu/ml although the specific growth rate remains lower than in batch culture.
The parameters of great interest was the substrat conversion yield which present increasing factor of161 % and an extension of exponential phasis duration from 3 hours to 5 hours.

4.5. In line process scale-up know-how

Results expected after the laboratory scale experiments were fullfilled in terms of:

Biomass level between 3-5.10^9 CFU/ml
Duration of the line process
Viability yield after concentration process (70 to 100 %)
Stability of the concentrated biomass at 4 “C (> 1 month)

Some parameters specific to the industrial scale have to be applied in order to have an optimal run fermentation:

Dextrose sterilization in a special tank apart with a less stressing temperature/time thermic treatment.
Planning of the delay for sterilization, pH control and temperature regulation.
Control of Microbiological quality of the powder obtained after pilote line (<10^5 CFU/g of aerobic microorganisms)

This strain presents a fast production of lactic acid at the beginning of the fermentation run. Although the specific growth rate was low in comparison with aerobic strains, the risk of contamination is low. Indeed, the quick acidification of the medium allows a rapid protection of the *Lb farciminis* biomass.

Afterwards, during the collection, concentration, and drying of the biomass the risk for quality alteration is reduced.

4.5.1. Feasibility of scale-up of CME technology

The CME powder presenting an important hygroscopicity, we first work with a simple effect tower (turbine atomization). This represent a linear translation rate of the laboratory process of 10 folds (based on inlet drying air flow).
This first experiment permits to check that there is no nozzle or tower plugging during spray-drying process, to check the product flow and the temperature profile proposed.

Accelerate maturing at 40 'C	Viability yield	T0	T + 14 d.	T + 21 d.
E1	**3,3 %**	**6,0 e8**	**9,3 e7**	**3,9 e6**
E2	**2,3 %**	**4, I e8**	6,4 **e6**	**< 3 e 5**
E3	**9>4 %**	1,7 **e9**	**2,3** e8	1,4 **e7**

This simple tower allows a **#4** times higher than the one previously expected. The pilote process presents an important tower height in comparison with laboratory tower height which provides this productivity gain.
The low viability yield is due to the non-adapted spray-drying apparatus to sensitive product drying.

We use a multiple-effect tower in the 15-20 folds scale comparatively to CME apparatus (600-800 Nm^3/h).
This first experiment is used as a simple effect tower to first optimize the first stage of the process.

Accelerate maturing at 40 'C	Viability yield	T0	T + 14 d.	T + 21 d.
E1	**65 %**	.1,3 e 9	5,3 e5	< 1 e5
E2	50 %	1,0 e9	1,5 e?	< 1 e5
E3	41 %	8,1 e8	1,3 e6	< 1 e5
E4	50%	1,0 e9	< 2 e 4	< 1 e5

The gain of productivity is of 3 to 6 times the one previously expected.
We made the hypothesis, we could use this gain in order to enhance the viability yield. In this way, we tried to lower oulet air temperature.

4.6. Stable coated probiotic powder deliverable

Pilot in line process allows the production of some kg of coated powder. This first presentation of the product presents the following characteristics :

PROPERTY OF THE POWDER	RESULTS
Revivifiability level of coated bacteria	**10^9 CFU/g**
Storage stability at 25 "C	6 **months**
Zootechnical efficiency on weaned piglets	9 **% improvement on average daily growth** **4 % improvement on feed conversion**

Zootechnical results obtained on this pilot powder, in Belgium with Lannoo-Martens proposer, demonstrated a very good efficiency so that the bacteria preserves its *in vivo* properties which were selected on.

Gentle pelletization experiments demonstrated a one Log injury during this feed process at 60°C. These results are in agreement with the ones expected for the applied process.

Nevertheless some constraints were not resolved by the pilot process :

- 3 months stability in humid media : feed and premix
- Stability to vapor during pelletization process
- Hygroscopicity of the powder
- Dust emission occuring in powder working unit

5. Exploitation plans and follow-up actions

5.1. Fermentation process stability

- Short terms exploitation perspectives

The industrial activity will start with an industrial partner in order to calibrate the industrial aspect and to dispose of the efficiency parameters : productivity, recovery yields, time of each unitary phasis, necessity of thermic treatment...

The operation costs permit to determine the production strategy :

- Number of batch per month
- Occupation time of each tank
- Size and number of each tank
- Number of inoculum transfer

- Medium terms exploitation perspectives

After this phase, we design a complete fermentation - concentration industrial process with GOAVEC in order to transfer this technology in the BIO ARMOR industrial locus.
The correct management of this phasis will allow an important gain because the risk can be relatively easily mastered and the cost of the fermentation performance is very expensive and represents an important part in the product cost.

5.2. Drying and coating precess

The characteristic of this part of the process is the high technological possibilities it will authorize. All these useful characteristics were not explored in the case of the CRAFT project scope.
Particularly, the static fluidized bed part of the multiple effect industrial dryer used for the final drying step could be used for a one-step cooled-air coating phase.

Coating - This final step of the powder conditioning aims at :

- Balancing the hygroscopicity of the formulation
- Stabilizing the dried bacteria in a humid environment (feed : 12 % humidity)
- Optimizing thermic treatment stability of the additive.
- Defining an industrial packaging for the best preservation

- Short terms exploitation perspectives

Afterwards, the routine production will be achieved with industrial partner for at least one year.

This step will permit us to identify the risk factors of a routine use of such a dryer and the operative field (Bacteria, feed additives.. .).
With these results, we could make an exploitation plan of an industrial exploitation:

- Cost of the operation : time of function, time of immobilizing : size of the unit
- Cost of material and know-how acquisition
- Cost of production team training
- Level of production reorganization

- **Medium terms exploitation perspective**

The risk and the cost would permit us to determine whether this technological transfer could be achieved with a reasonable amortization scope.
Nevertheless, the technological feasibility was proven during the CRAFT research scope. We have to evaluate if this technology could be imported in BIOARMOR or has to be treated with a drying specialist.
Sodeva will not participate to the exploitation of the industrial drying phase.

5.3. Commercial exploitation

Lannoo-Martens exploits the stable probiotic additive and coated vitamin and mineral in Benelux.

Medilabor exploits the stable probiotic additive in Italia and coated vitamin and mineral in all countries excepted from France and Benelux.

Bio Armor exploits the exclusivity of the stable probiotic additive in all area except in Italia and Benelux. Coated vitamin and mineral were exploited by Bio Armor in France.

All these associated contracts are related to commercial objectives for each product. The non respect of these objectives would disengage productors from exclusivity clauses.

5.3.1. Apparatus manufacturer

Bio Armor will design and manufacture the industrial fermentation line equipments with Goavec thanks to the common know-how acquired during the scope of the project and thanks to the special know-how of Goavec in its industrial activity.
This agreement is understanding for a global cost minor or equal to other competitors proposals for the same technology level.

5.3.2. Know-how protection and exploitation

The know-how will be protected by the confidentiality to which all partners committed themselves.

Drying and coating group

Bio Armor will exploit the exclusivity of lactic acid bacteria dried form stabilization within the scope of animal nutrition.

CME preserves the know-how property and the industrial exploitation on all other industrial scopes for both bacterial and nutrient scopes (Agronomy, pharmaceutical scope,...)

Medilabor will exploit the exclusivity of vitamins or other nutrients stabilized . dried form developed within the scope of animal nutrition.

Fermentation group

ISTAB preserves the property with Bio Armor of bacterial strain isolated during the scope of the project. Bio Armor will financially compensate I STAB for commercial use of those strains.

CBB will preserve the know-how it engaged and the one it developed during the project. Exploitation of this know-how could be realized by CBB and Bio Armor between all industrial scopes.
Nevertheless CBB committed itself not to reveal development realized by its investigation team and the one of which it would be informed during its technical direction of the project.

5.4. Patents

The first point, the Soleau envelop, which concerns the coating results obtained by CME has been deposited under the number 55472 on April, 9th of 1996.
The title retained is « Coating of bacteria (lactica) with a spray-drying technic ».

The patent has been registered on 20th May 1996 under the registration number: 96 06215. The title retained is « Microparticles containing bacteria, using and production process of mentioned microparticles for seeds coating and agro-alimentary compositions ».

BA will dispose of the exclusive exploitation of this process in the animal nutrition area. CME preserve the use of this know-how in investigation area and in the commercial activities without relation with animals nutrition.
BA will dispose of possibilities for this process evolution to a better industrial adaptation or to an adaptation of this technology with other raw materials.