

# SC. Laboratoare Medica SRL.

Research - Production - Distribution



president of  
S.C. Laboratoare  
Medica S.R.L.  
Otopeni Romania



# In memoriam

I dedicate this work to our titans who created the premises for the development of beekeeping and apitherapy, both in Romania and worldwide.



**Veceslav Harnaj**  
1917-2017

Presedinte Apimondia  
1965-1985



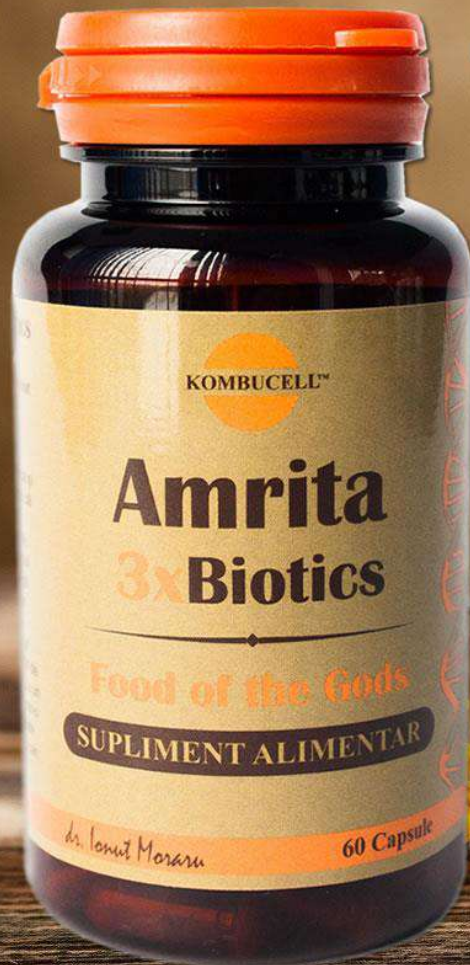
**Nicolae Ilieșiu**

Inventatorul  
Apilarnil-ului



# FOOD OF THE GODS

Fermented  
Polyfloral Pollen  
an original method





## Many thanks to our friends for their professional help and support:

- Professor Dr. **Florin Oancea** - National Institute for Research and Development for Chemistry and Petrochemistry Bucharest – Biotechnologies Department ICEHIM
- **Florina Matei** - Assoc. Prof. PhD University of Agricultural Sciences and Veterinary Medicine Bucharest, Faculty of Biotechnologies
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- Professor Dr. **Corneliu Moldovan** - Romanian National Institute of Integrative Medicine “Florin Bratila” Bucharest.
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- Dr. **Ciprian Ene Soma** - Quantum Clinic Bucharest.

Special thanks for the help and understanding of my wife PhD. **Angela Moraru** University of Agricultural Sciences and Veterinary Medicine Bucharest, Faculty of Biotechnologies

**The result of the studies are part of two european  
projects of SC. Medica Laboratories SRL. :  
ERA - Convert SI 2016-2018 continued with  
EUREKA – Tribiotics 2018-2019**

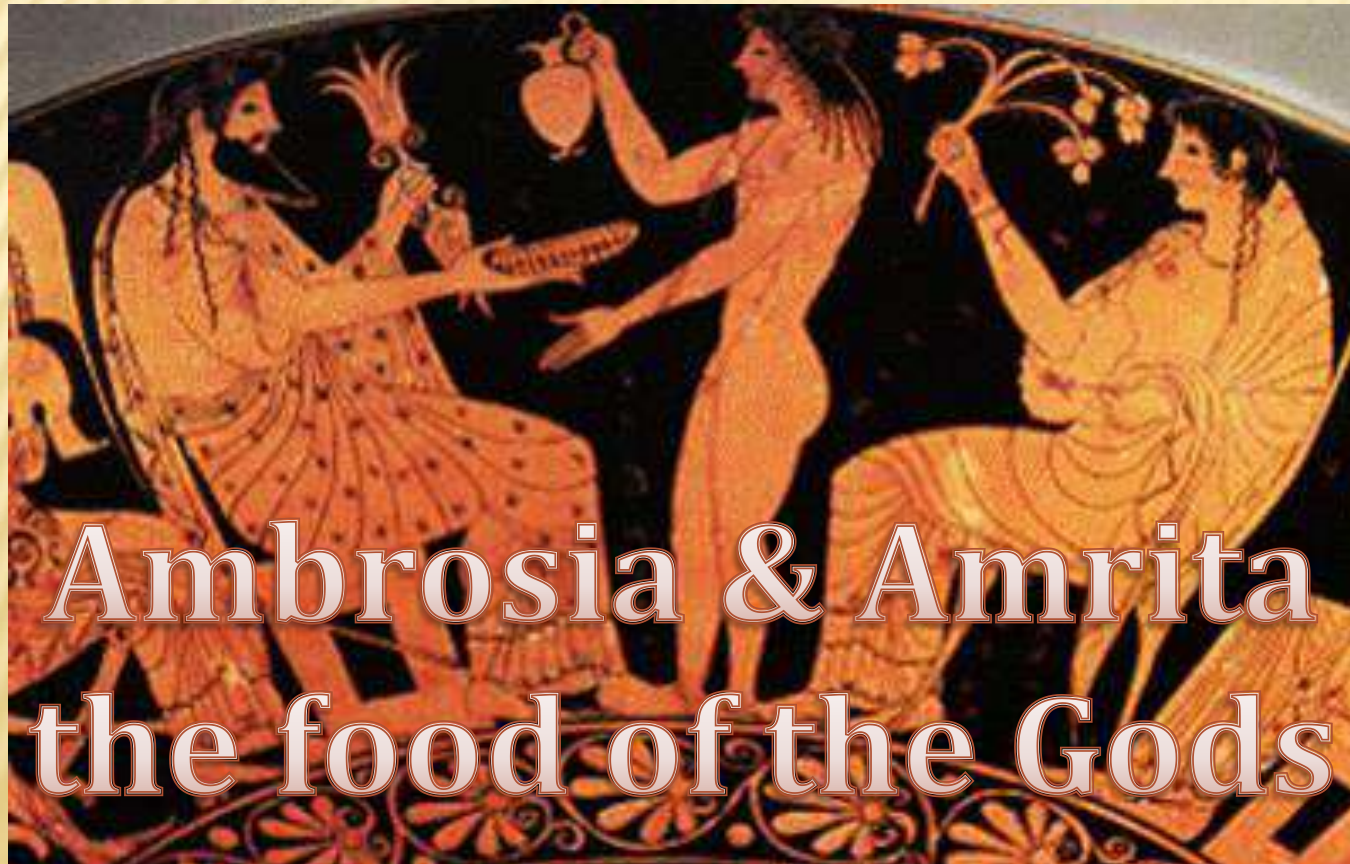


Most of the traditions have the following myths:

- I. Super foods of the Gods.
- II. Health comes from your intestines.
- III. From dust we came in dust we return.
- IV. Everything in the Univers is Energy.



I. The first chapter refers to the raw materials (polyfloral pollen & SCOBY consortium) used to obtain a super nutrient called Amrita.





Since ancient times, humanity was looking for a „panaceum” for health. It is named **Amrita** - in sanskrit or **Ambrosia** - in greek language; the significance is the same: “food of the gods” which keeps you young, healthy and influences also longevity (immortality) and well being. We wanted to prove that ancient myths from different traditions are of great value:

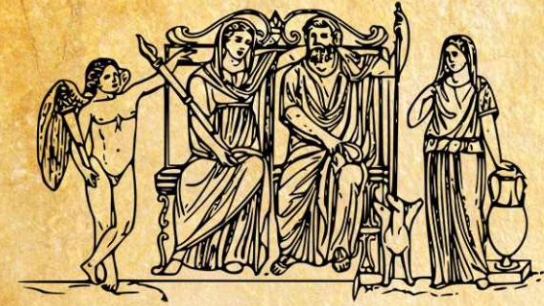
## I. First myth: Is it possible to obtain such a special food supplement like Amrita?

1+1≥2

Starting from the Holy Books of all religions which are saying that „for any disease God gave us many herbs as remedies”, we selected the polyfloral pollen as a raw material (the sperm cells of the herbs).

**Pollen** is considered the most complex food/food supplement. It brings a lot of energy, almost all nutrients required by the human body (bioactive compounds, vitamins, minerals, essential fatty acids, carotenoids, flavonoids) and also medicinal active principles. Polyfloral pollen can be considered as a “whole pharmacy”. **Clinical effects of pollen:** reduces acute and chronic inflammation; has strong antioxidant effect, superior liverprotector than silymarin antiviral, antimicrobial effect; anti-allergic effect by inhibiting mast cells; stimulates the immune system; fights anemia, increases appetite, useful in convalescence; combat the symptoms of menopause and breast cancer; increases mental capacity; one of the most powerful anti-stress products; has local analgesic and inflammatory effect; prevents infection; increases metabolism and regulates hormones.

### Ambrosia food of Greek Gods



### Amrita food of Hindu Gods

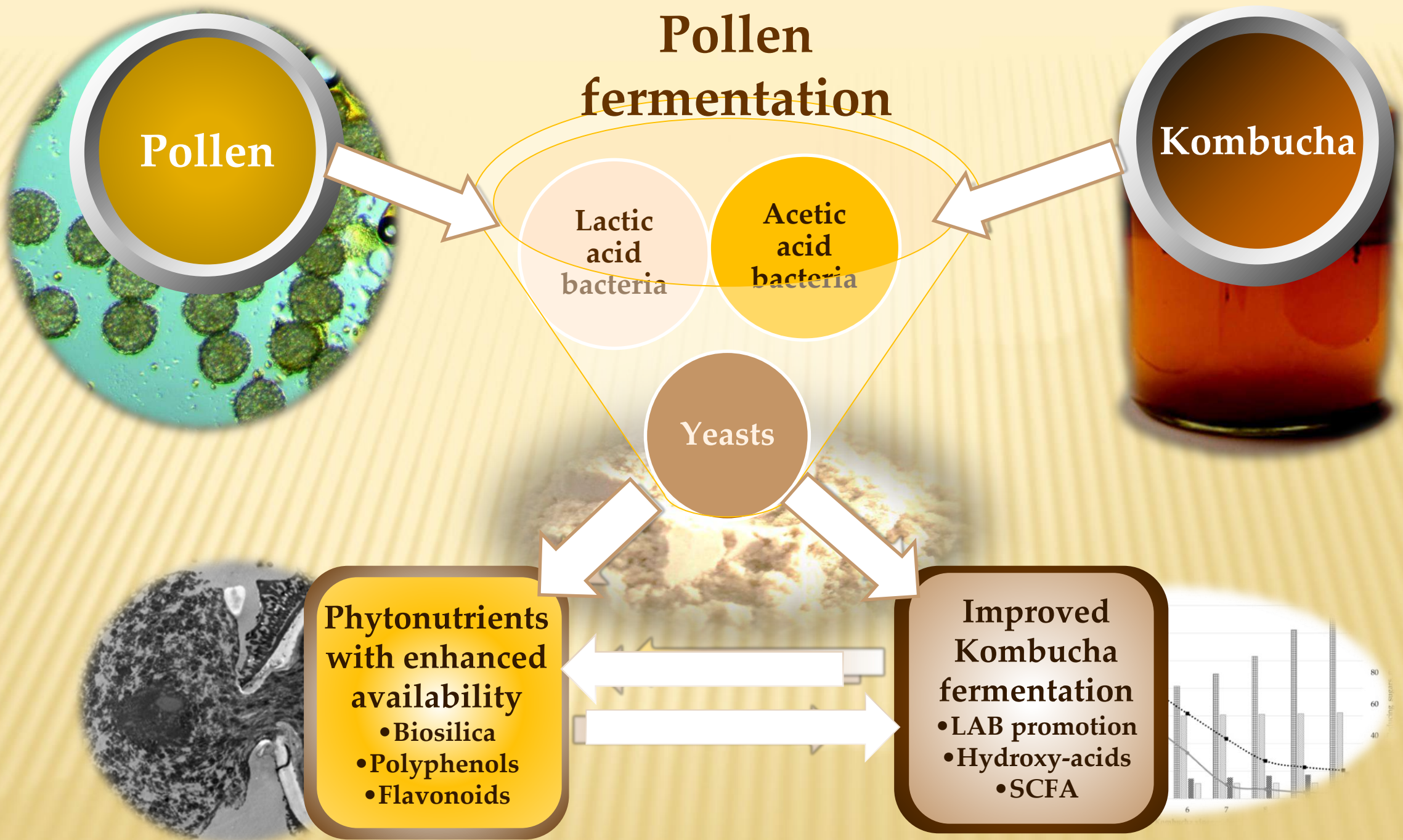


Lore & Recipe

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# Pollen fermentation





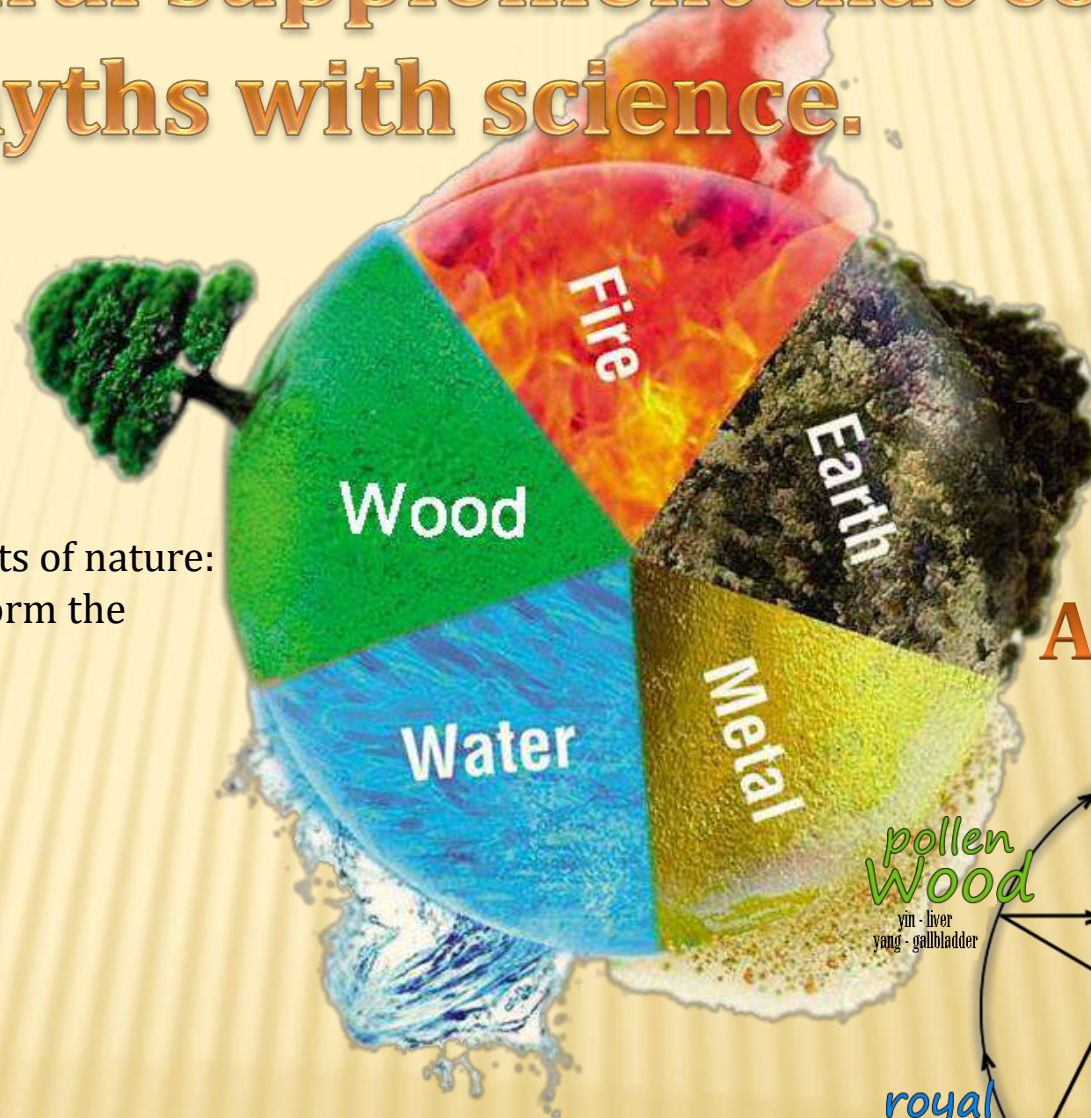
# Amrita, a natural supplement that combines myths with science.

## AMRITA 3xBiotics

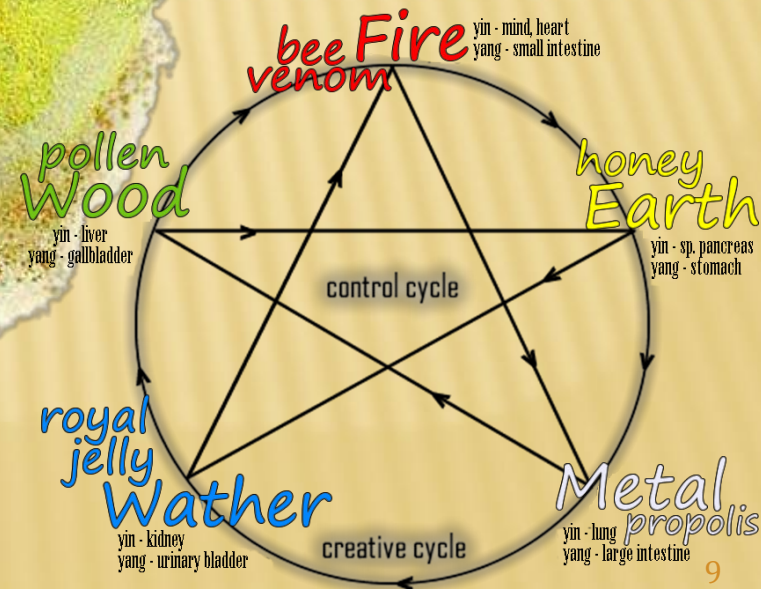
is a product acting on the whole body;  
"feeding" the five lodges of energy:  
kidney, liver, heart, spleen and lungs.

According to Traditional Chinese Medicine TCM  
"The theory of five elements" associates elements of nature:  
earth, metal, wood, water and fire energy that form the  
five lodges.

1. **Earth** - spleen, pancreas, stomach.
  2. **Metal** - lungs, large intestine
  3. **Water** - kidney, urinary bladder
  4. **Wood** - liver, gallbladder
  5. **Fire** - heart, mind, small intestine
- which are influencing each other through  
cycles of creation, destruction and control.

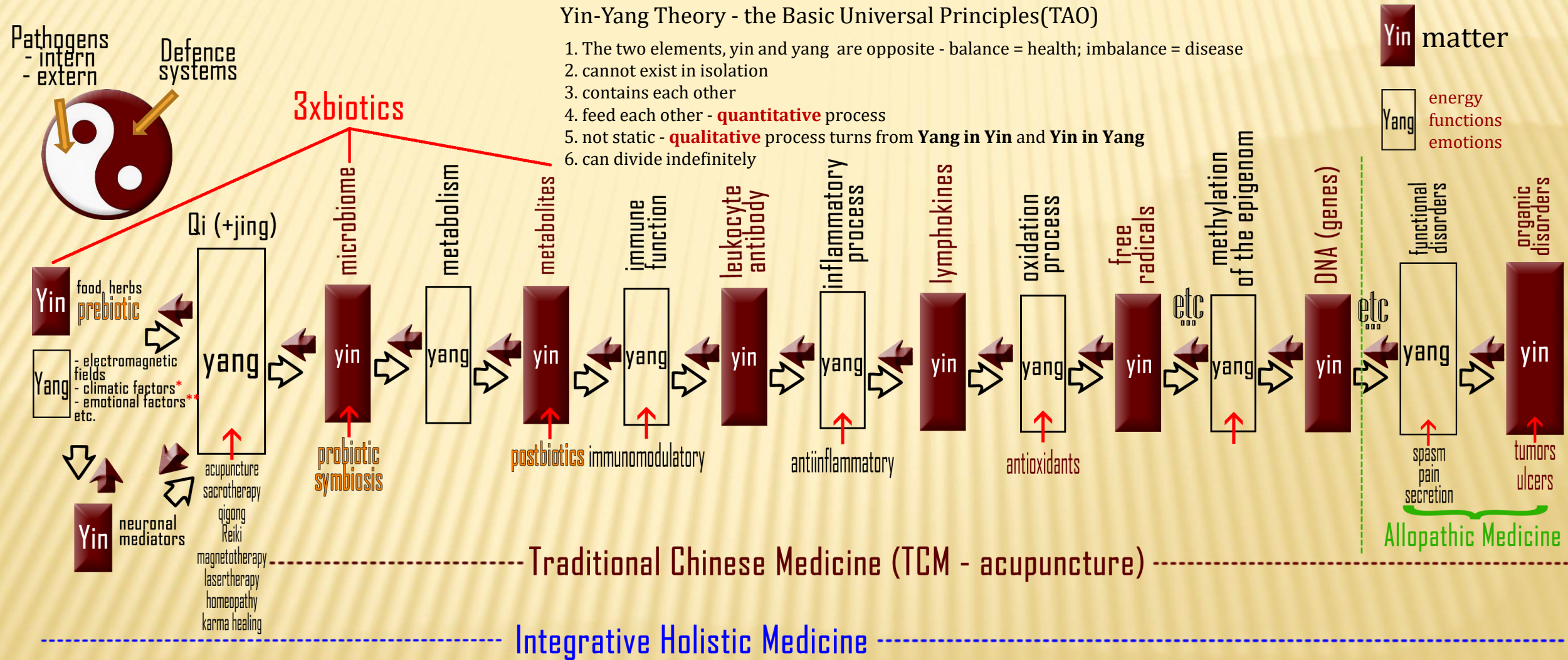


## Apitherapy





# We have highlighted the importance of tribiotics (pre/pro/post biotic) and holistic treatments in a new etiopathologic approach of chronic diseases



\***six climatic factors:** wind, extreme heat, humidity, dryness, cold

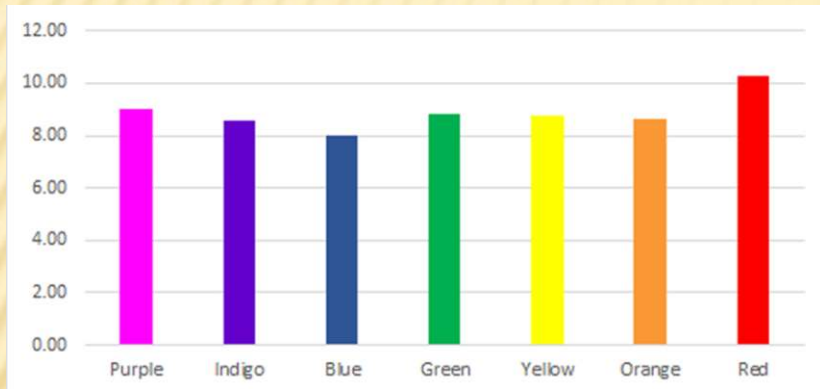
\*\***nine emotions:** anger/fury, excessive joy, anxiety/worry, melancholy/depression, fear/terror

**Conclusions:** Any disease is reversible if we manage each stage.

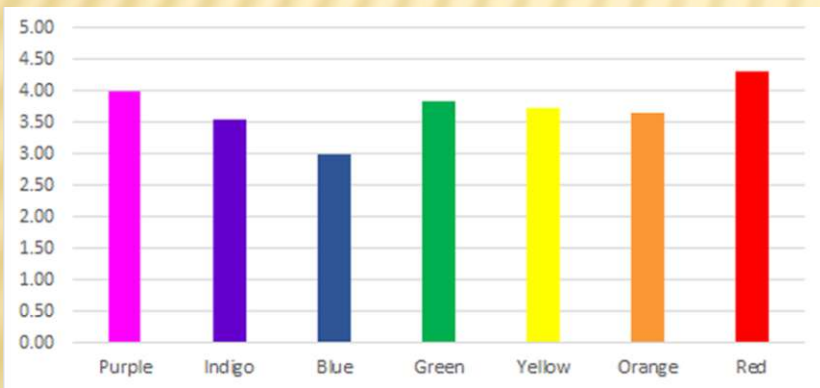


The influence of electromagnetic fields (polarized light) on two symbioses of bacteria and yeasts (kombucha and kefir) showed us the following, confirming the influence of electromagnetic fields on microbes :

## Kefir

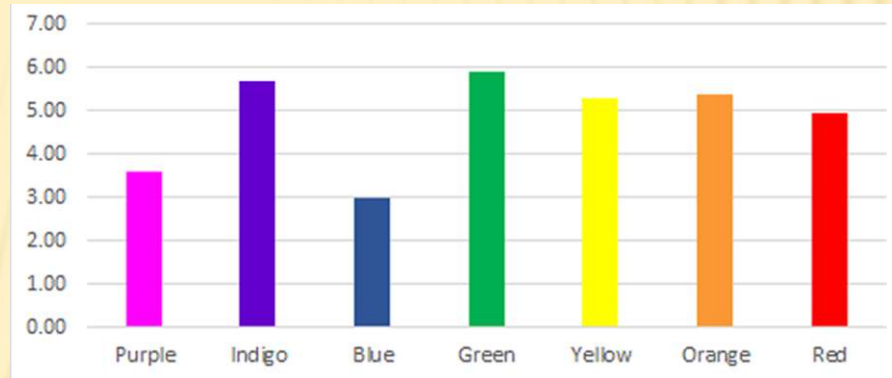


Lactic bacteria load  
log10 from the living cells number/ml



Yeasts load  
log10 from the living cells number/ml

## Kombucha



Lactic bacteria load  
log10 from the living cells number/ml

Simbiotic cultures  
fermented  
under the influence  
of polarized light

Maximum development of lactic bacteria occurred in:

- kombucha at stimulation with **green light**
- kefir at stimulation with **red light**.

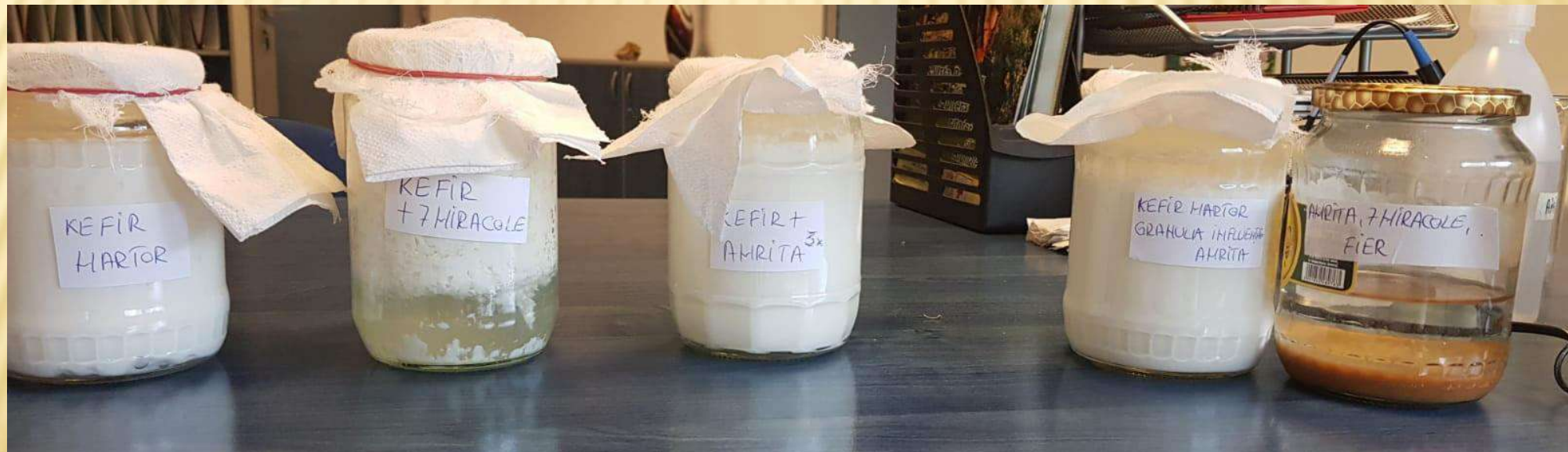




The development of kefir consortium (similar to kombucha = symbiosis of bacterias and yeasts) is totally different, confirming the influence of herbs on microbes:

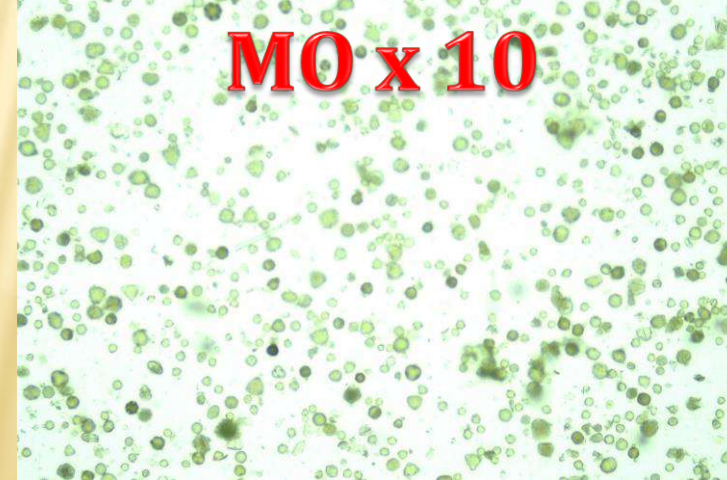
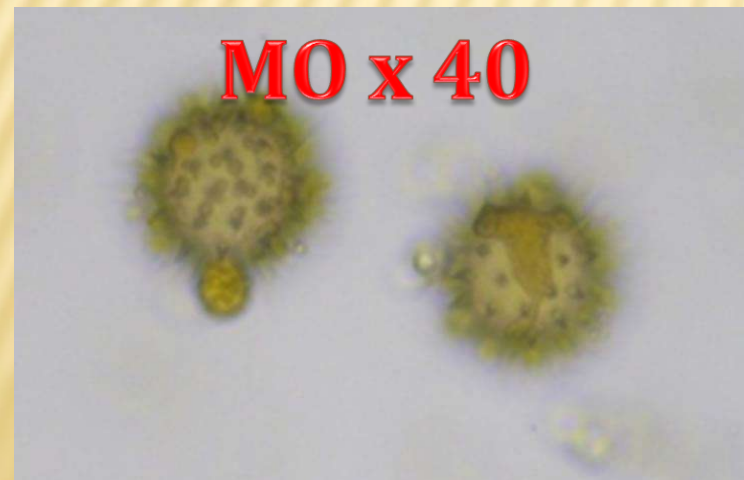
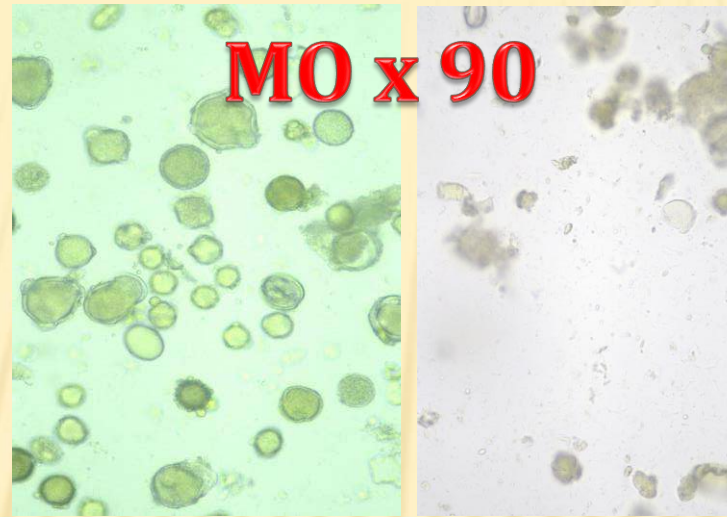
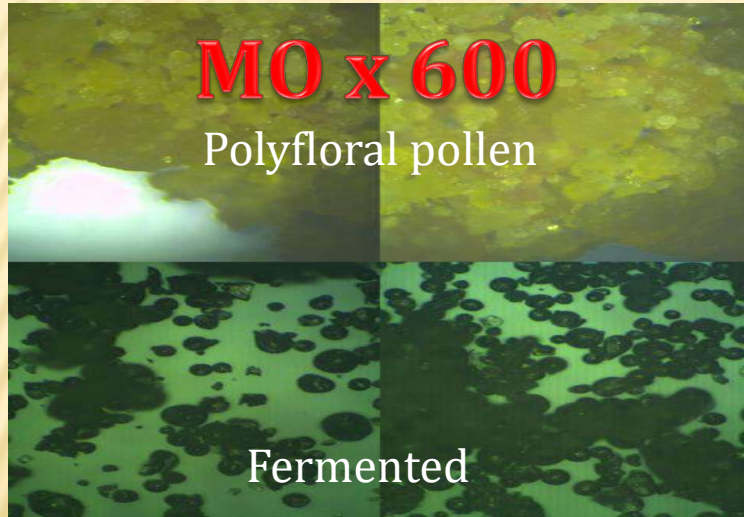
1. sample control of caucasian kefir.
2. sample control where fermented medicinal mushrooms in kombucha were introduced in caucasian kefir.
3. sample control where fermented pollen in kombucha (= amrita) was introduced in caucasian kefir.
4. indirectly influenced Caucasian kefir sample (energy imprinting) with amrita.

We noticed the different organoleptic development, but also from the point of view of microbial cultures.





The only problem was that we couldn't benefit of all these nutrients due to the Sporopollenin, a biopolymer with extremely high stability and very high resistance to biodegradation (including to the action of digestive enzymes and it is present in exine composition – the rigid outer layer of pollen) which is the indestructible wall of the pollen grains. Bacteria and yeasts fermentation biotechnology of the polyfloral pollen penetrated the sporopollenin and riched the content of the pollen grains as seen (in optical and electronic microscopy).



## Optic Microscopy with different lenses

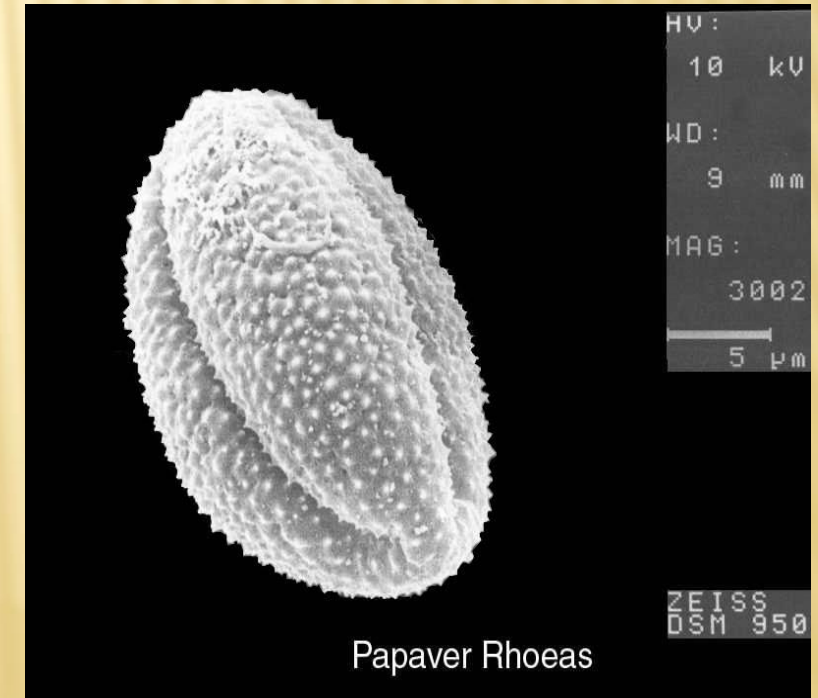
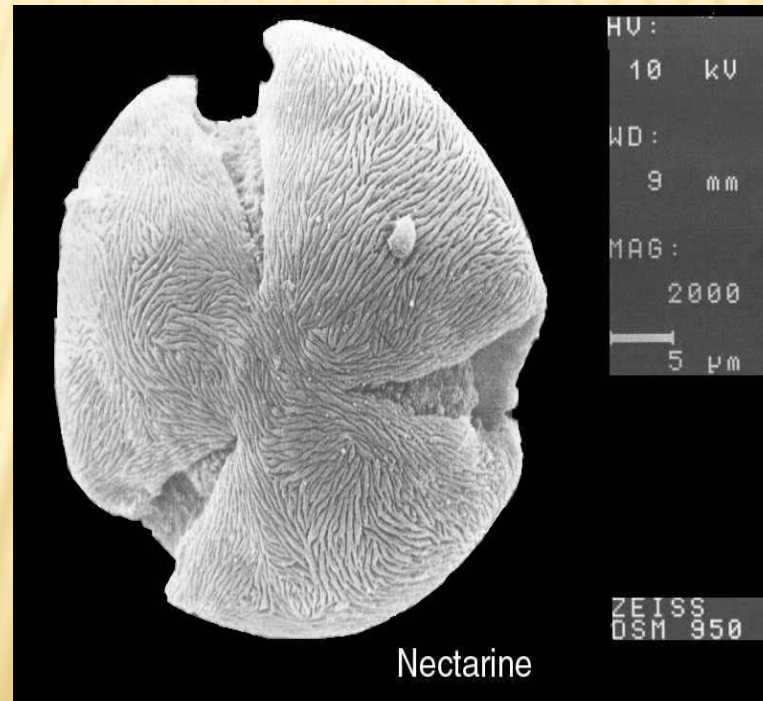
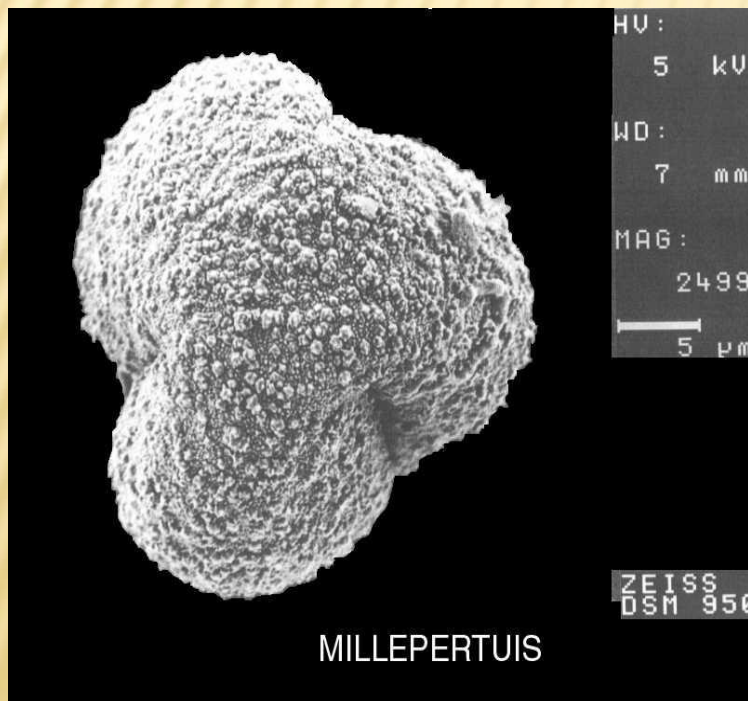
Images from the optical  
microscope on the fermented  
polyfloral pollen:



# Electronic Microscopy

a) **First study 2017:** For ultrastructural analysis of the polyfloral pollen fermented by bacterias and yeasts samples were investigated using a Philips EM 208S electronic transmission microscope. Fermented pollen grains morphological analysis were performed by scanning electron microscopy using an SEM-HITACHI SU-1510 microscope.

**Polyfloral pollen contains various varieties (images) of granules:**

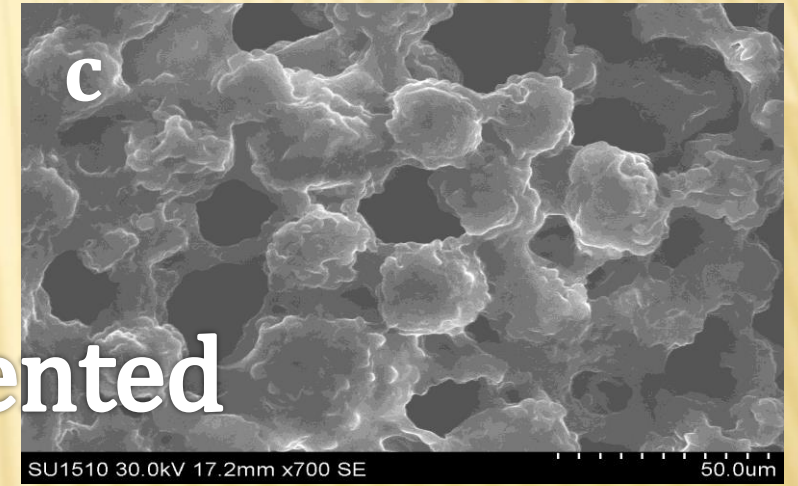




**Figure 1.** SEM images. (a) Apple pollen grain.

(b) Apple pollen grain „cracked” by bacteria and yeasts cells fermentation.

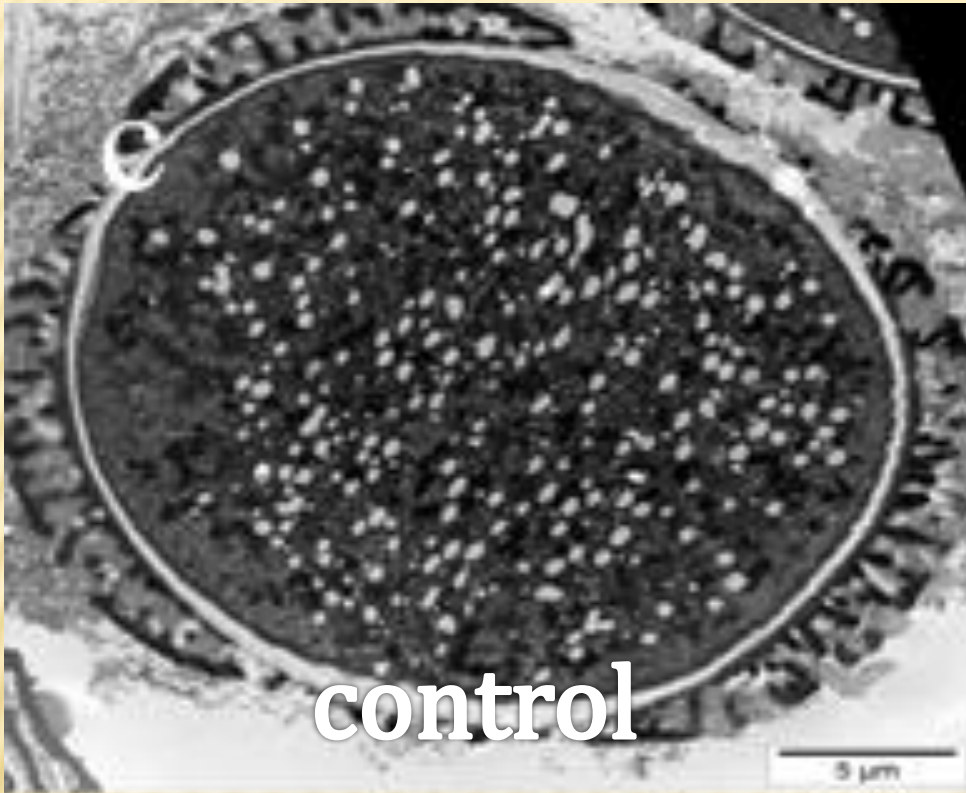
(c) Pollen grains fermented. It is observed the adherence bacteria and yeasts cells of consortium to pollen grains surface



The SEM images highlighted the adhesion of the bacteria and yeasts cells consortium to pollen grains forming a relatively compact structure (Fig.1). Also, after the fermentation process, morphological changes of the pollen grains (swelling, breaking, deformation) could be observed. TEM investigations showed differences between the structure of control pollen grains and those undergoing fermentation in (Fig.2). We observed the outer layer modification, the emptying of the germination pores, the breaking of the exine layer and the release of the grains contents in their environmental media.



**Figure 1.** TEM images. c) Apple pollen grain. d) bacteria & yeasts cells fermented apple pollen grain releasing its content.



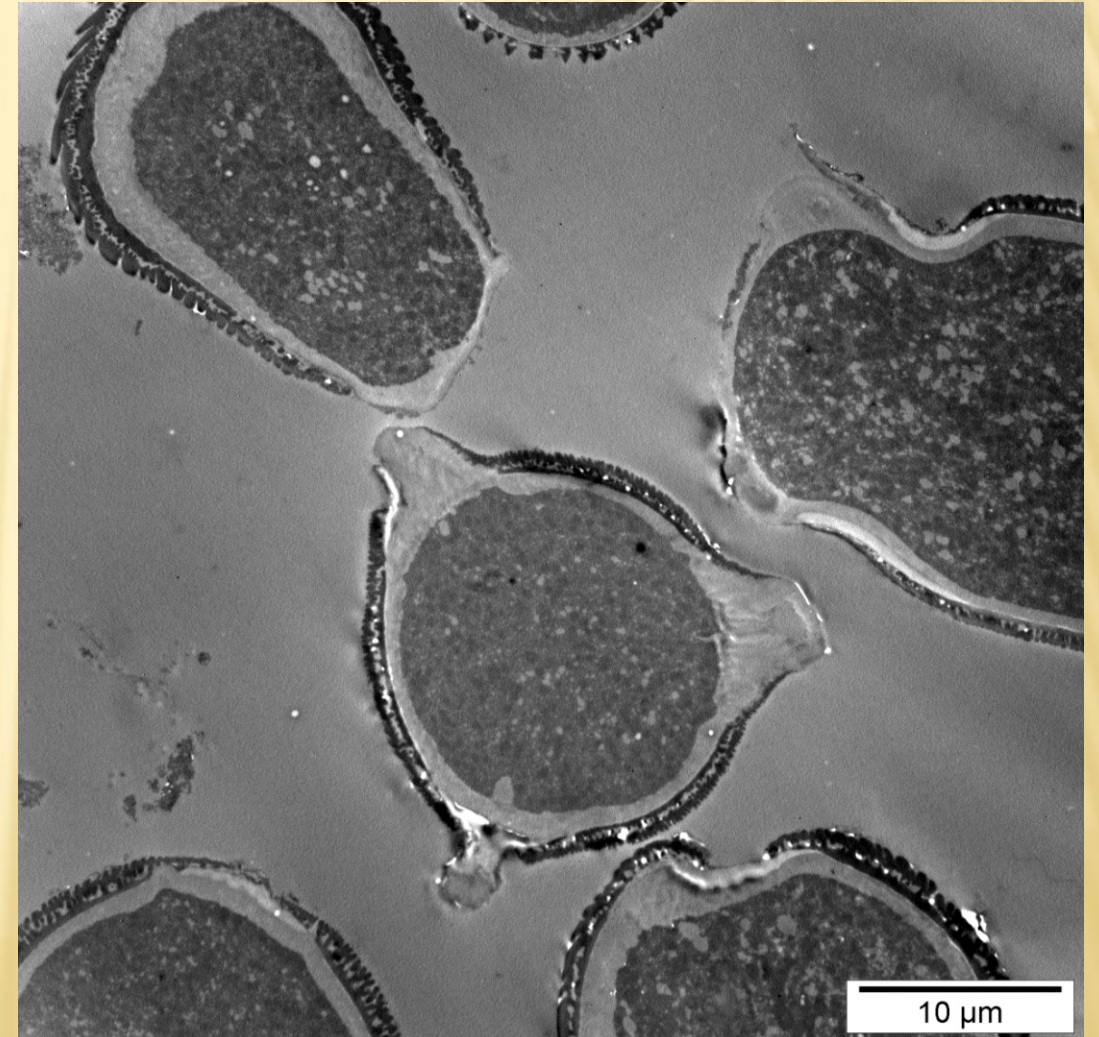
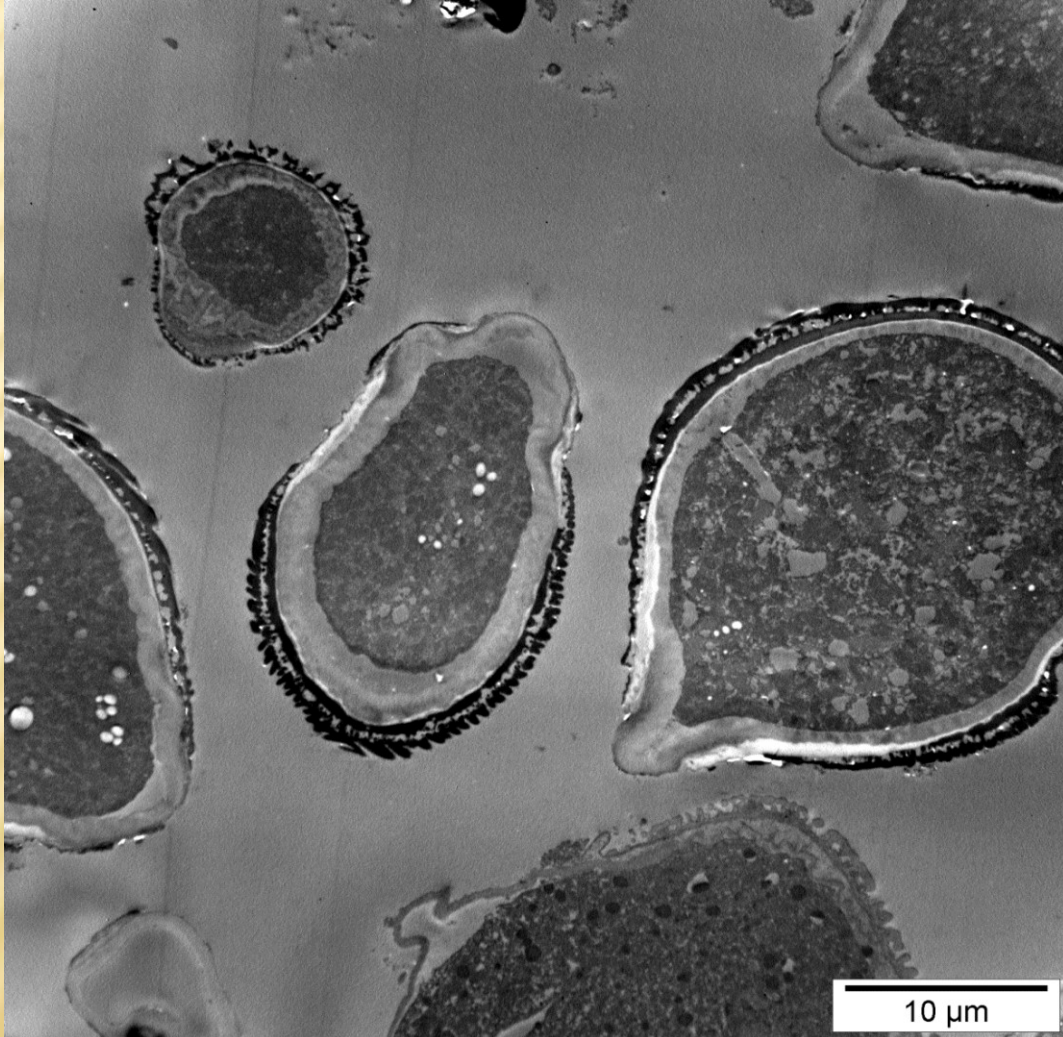
### Conclusions:

Bacterias and yeasts cells consortium, which are developing on clusters on the surface of pollen exine, most probably promote the formation of small pores, by oxidative degradation of sporopollenin matrix. Our morphological and ultrastructural analysis demonstrated that, through the pollen fermentation process in presence of bacterias and yeasts cells consortia, there are **modifications of external membrane and a release of pollen grains content**, increasing their bioavailability.

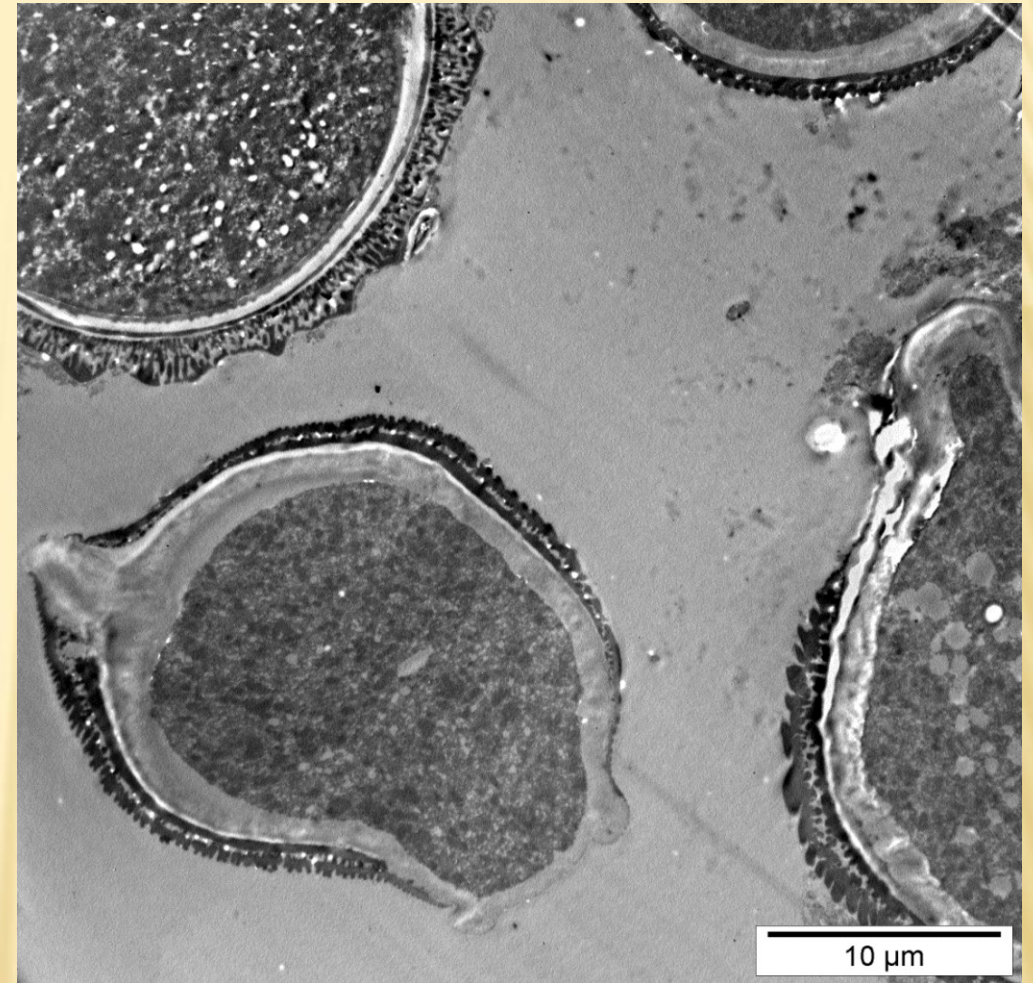
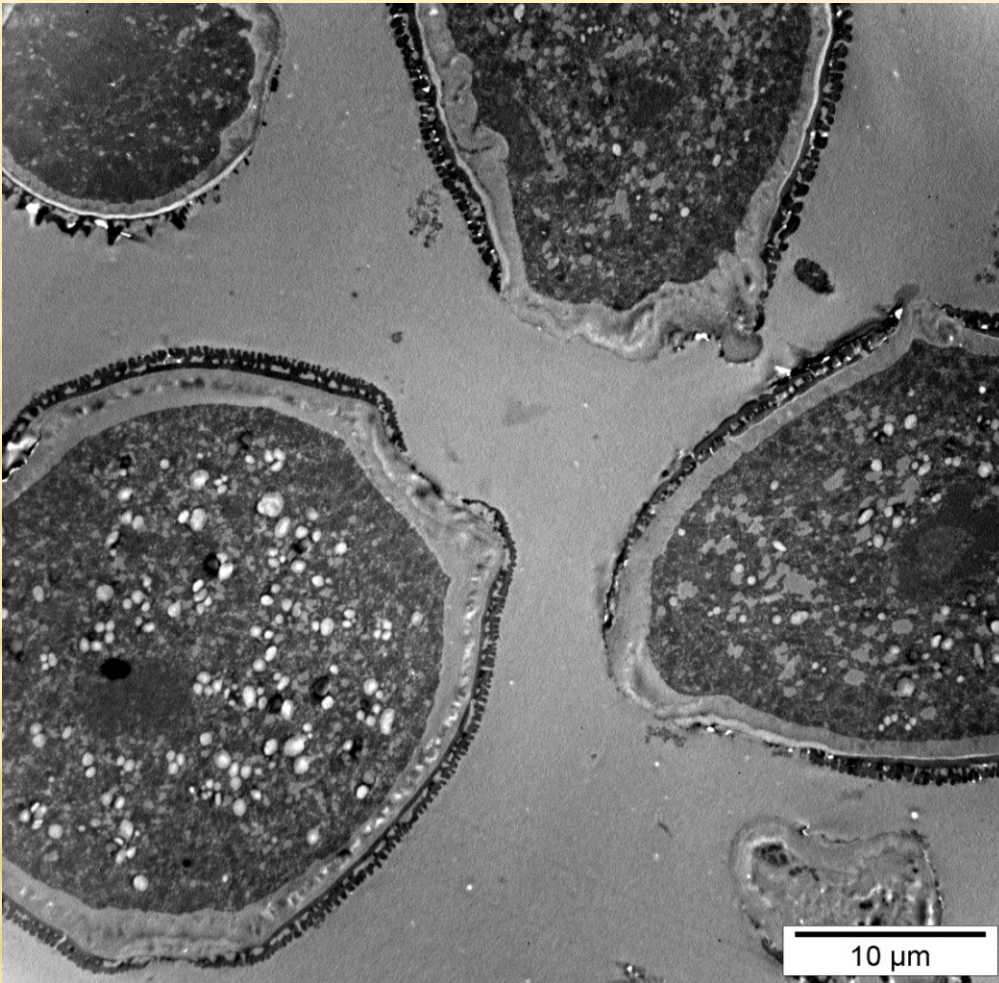


# Second study january 2018

a) Five days of fermentation of [monofloral prunus/plum pollen](#):







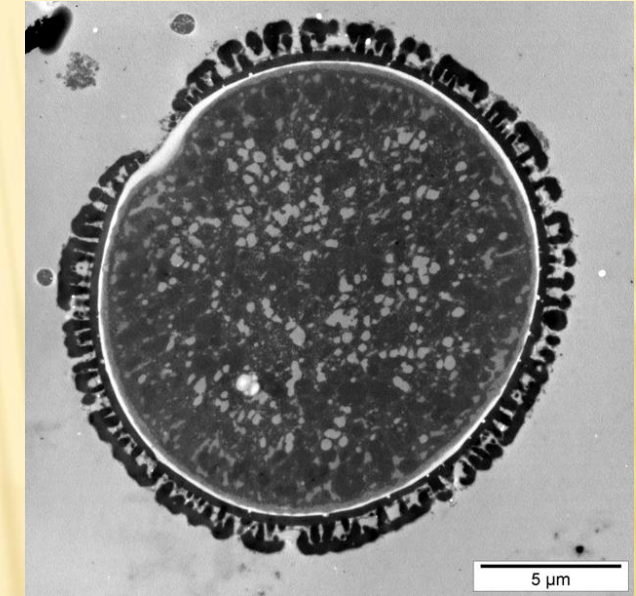
We can clearly see the changes in external membrane and the release of the content of the pollen grains.

National Research Development Institute for Biological Sciences Bucharest

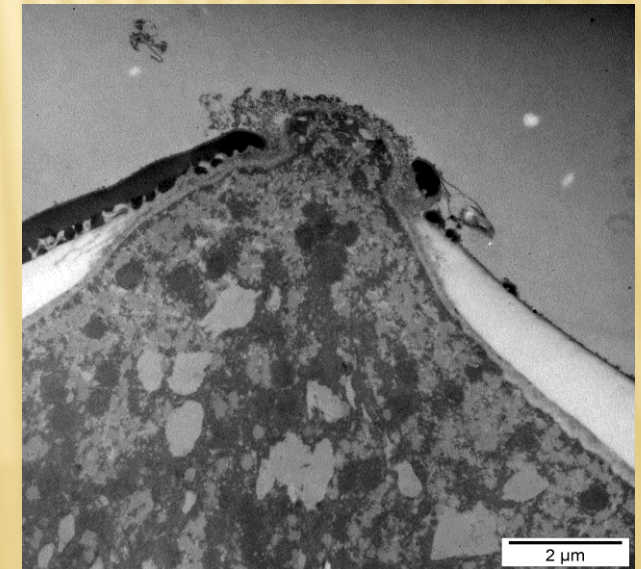
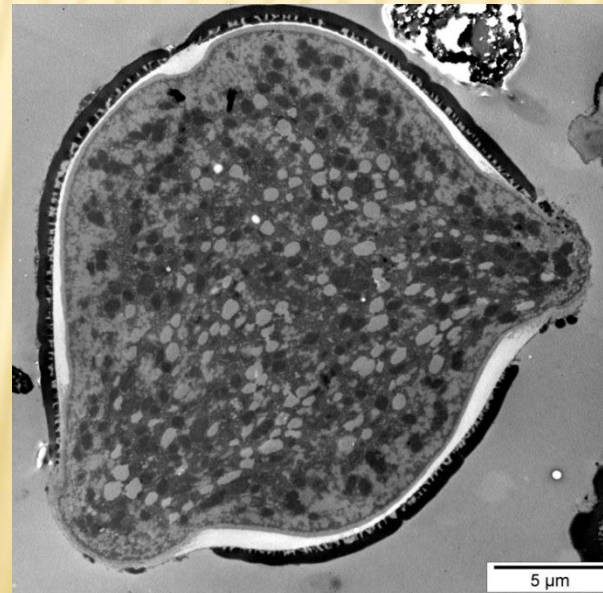
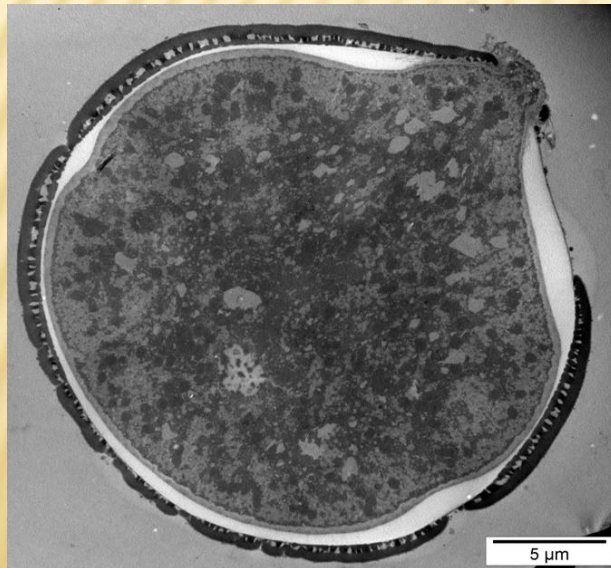


# Evolution in time of a fermented monofloral pollen grain:

Control (**unfermented pollen**)

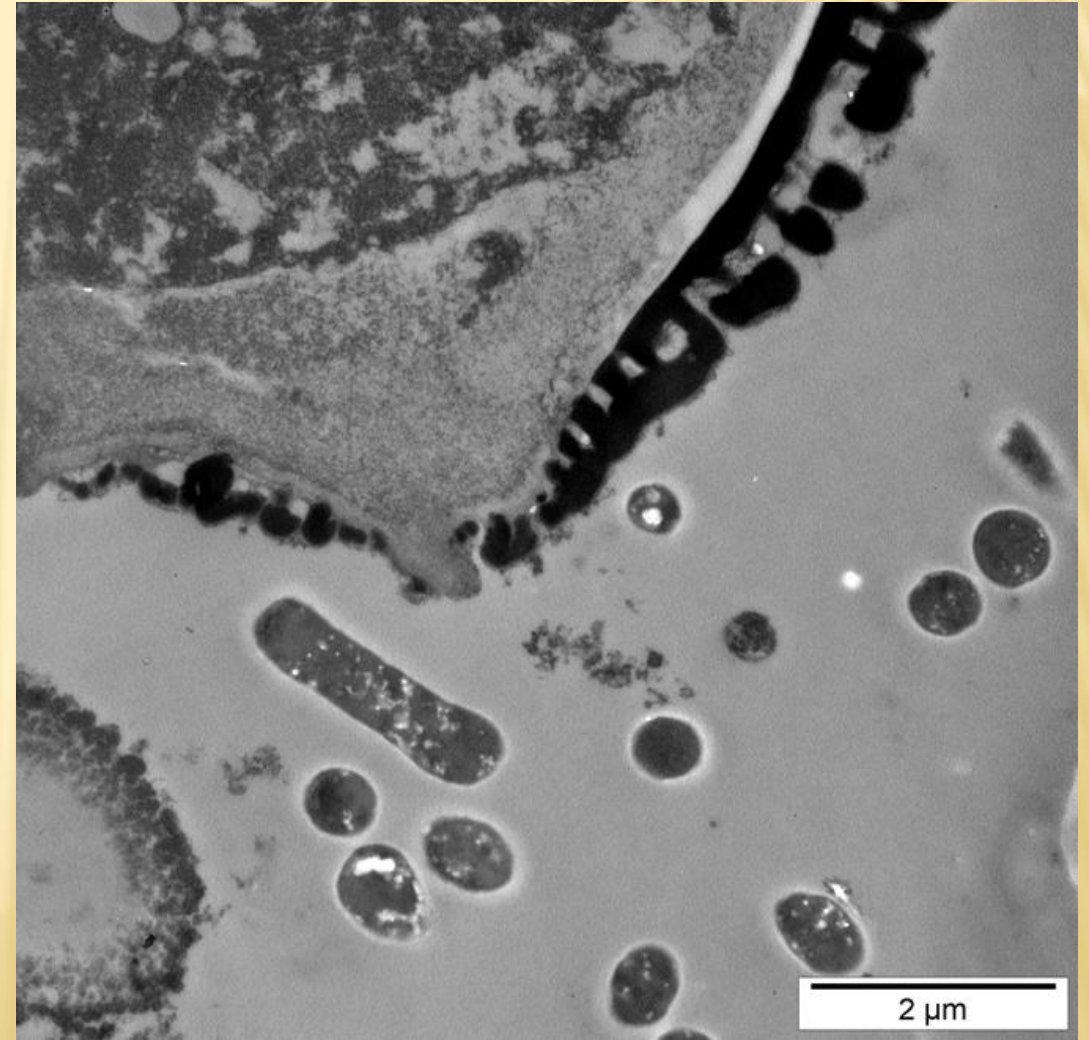
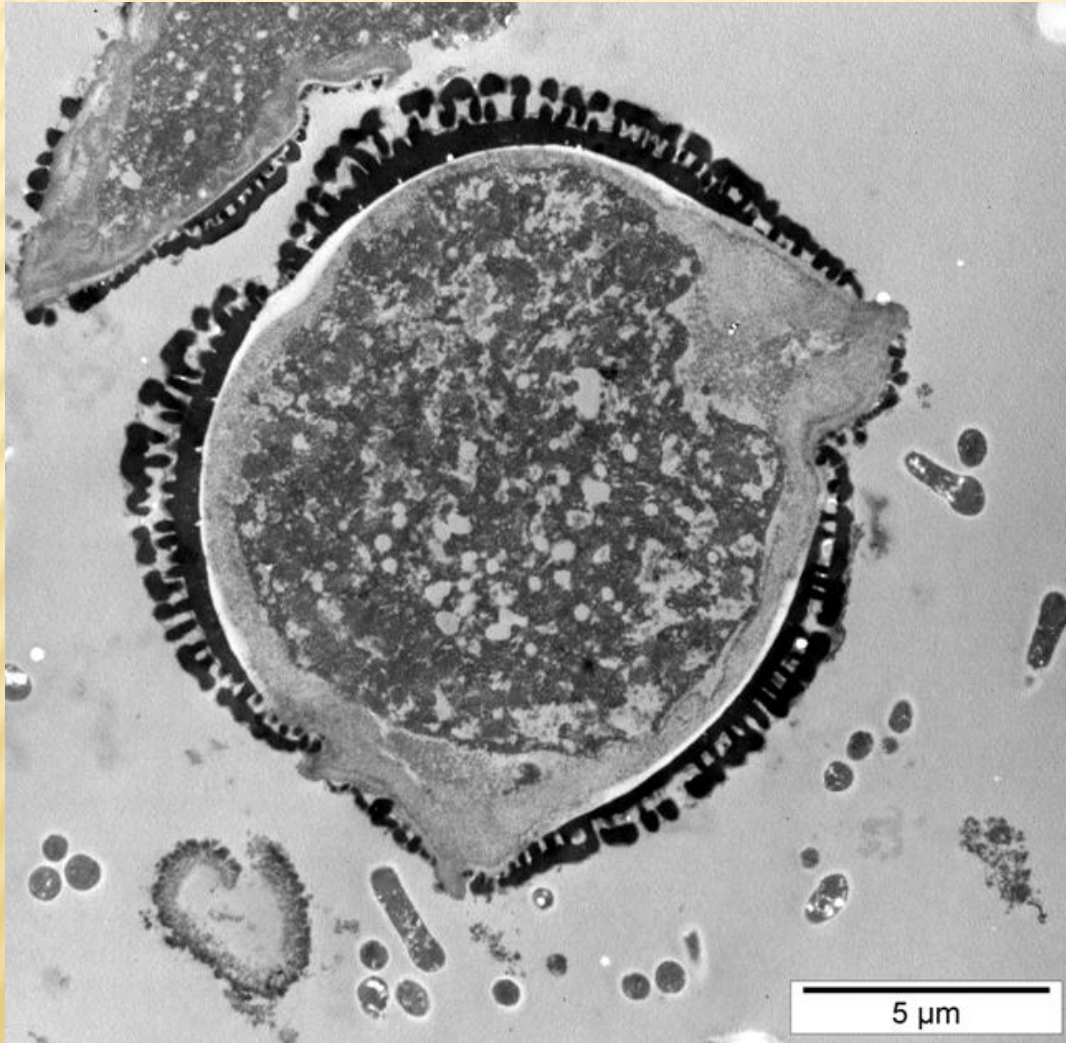


Pollen after **3 days** of fermentation



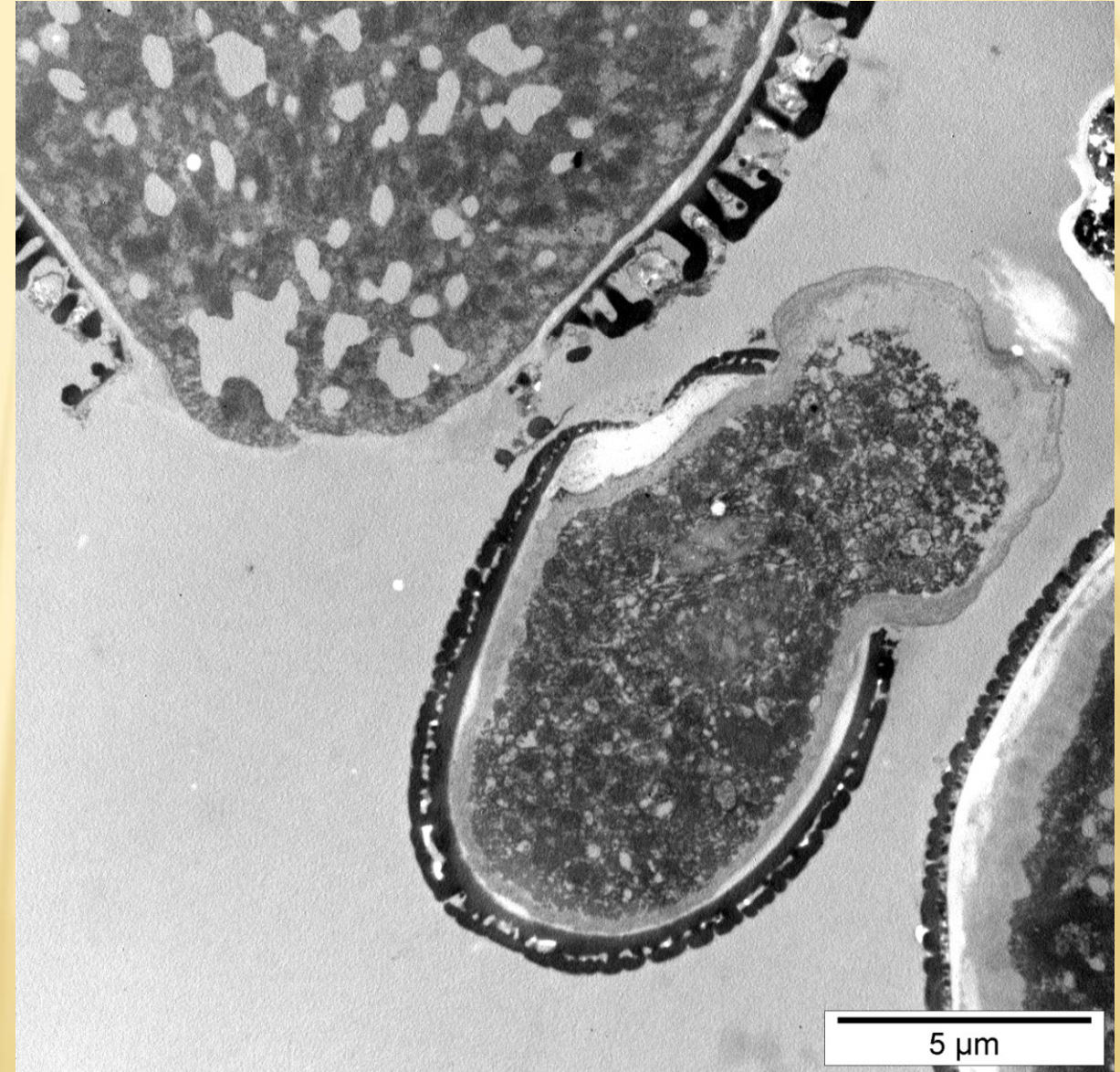


Pollen after **5 days** of fermentation



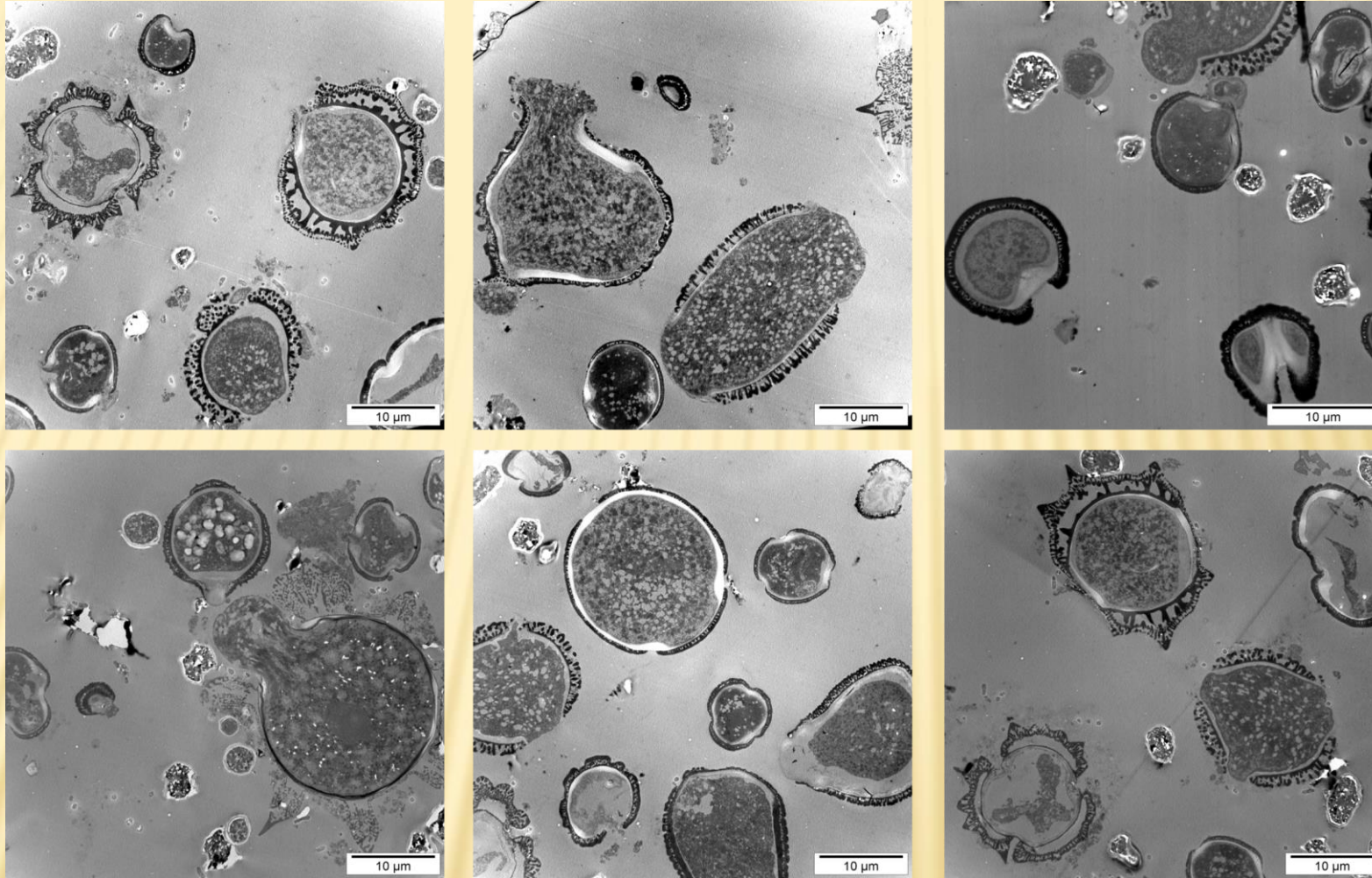


**5 days** of fermentation of polyfloral pollen grains:





## 5 days of fermentation of polyfloral pollen grains:



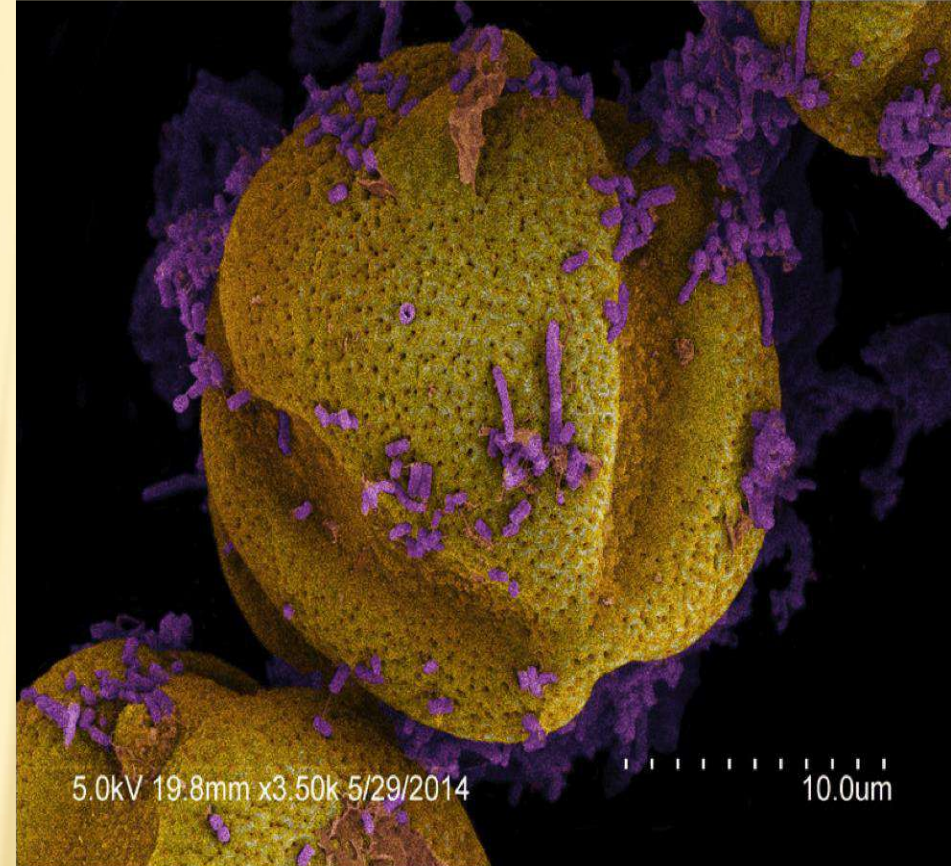
**Conclusion:** we have an unique innovative biotechnology for "breaking" the pollen grain wall which gives us access to the miraculous polyfloral pollens content



## Pollen For Preservation or Digestion? By Randy Oliver - ScientificBeekeeping.com



A scanning electron micrograph of dried beebread. The pink substance is dried simple sugars which constitute about 40-50% by weight. Note the distinct lack of bacteria. Electron micrographs courtesy Kirk Anderson.

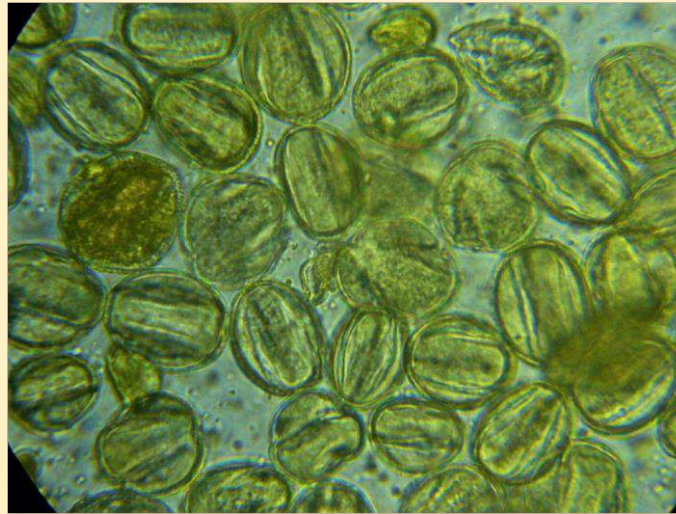


For comparison, these pollen grains from a **nurse bee hindgut** are covered with live bacteria (stained purple).

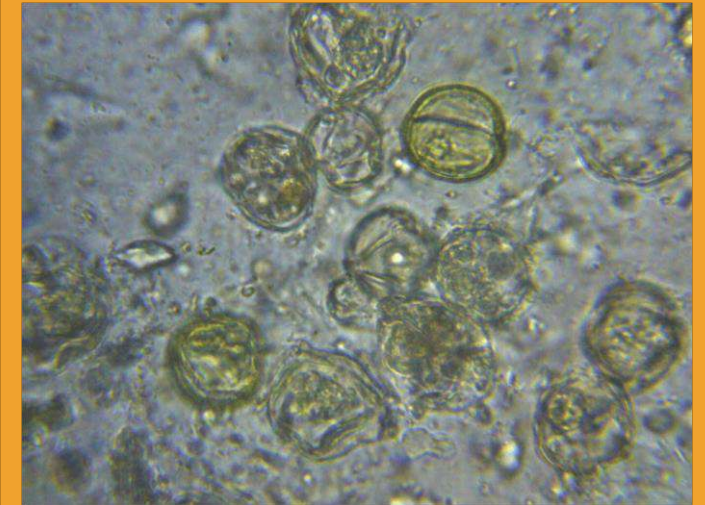




Pollen grains in aged beebread under light microscopy. Note the relative absence of yeast or bacteria, and the unbreached (not misshapen) exines and intact colored contents of the pollen grains.



Pollen grains from beebread that I diluted with a weak sugar solution and allowed to ferment for 10 days. Note the abundant yeast and bacterial cells in the background (unfortunately there was not enough depth of field to bring both the pollen and microbes simultaneously into focus). Despite being exposed to over a week of vigorous aerobic fermentation, these pollen grains remain intact and undigested. Compare these intact grains to those in the right.



A sample of digested pollen from the hindgut of a nurse bee. Note how most of the pollen shells are empty (no longer yellow inside) and distorted, indicating digestion of the contents.

Similar image with those obtained by the aerobic and anaerobic fermentation in kombucha.

*Relative to other plant material involving microbial digestion or extensive fermentation, hive-stored pollen contains very few microbes. The absolute number of bacteria in hive-stored pollen decreases with storage time, indicating that it is not a suitable medium for microbial growth. Regardless of sampled season or the taxonomic character of microbial communities, microscopic examination revealed no intermediate stage of pollen digestion in hive-stored pollen.*





Amrita is obtained through a thermal method (spray drying) thus being pre/pro/**parapro**/post – biotic product

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Review

**Paraprobiotics: Evidences on their ability to modify biological responses, inactivation methods and perspectives on their application in foods**

Caroline N. de Almada, Carine N. Almada, Rafael C.R. Martinez, Anderson S. Sant'Ana\*

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**A R T I C L E I N F O**

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Ghost probiotics  
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Postbiotics  
Functional foods  
Food technology  
Immune response  
Health benefits  
Gastrointestinal tract

**A B S T R A C T**

*Background:* The classical definition of probiotics indicates “they are live microorganisms which, when ingested in adequate amounts can provide health benefits to the host”. These benefits are provided due to interactions between the probiotics and the gastrointestinal microbiota and immunological system. On the other hand, non-viable probiotics have been known as “ghost probiotics”, “postbiotics” and “inactivated probiotics”, but recently the term “paraprobiotics” has been coined.

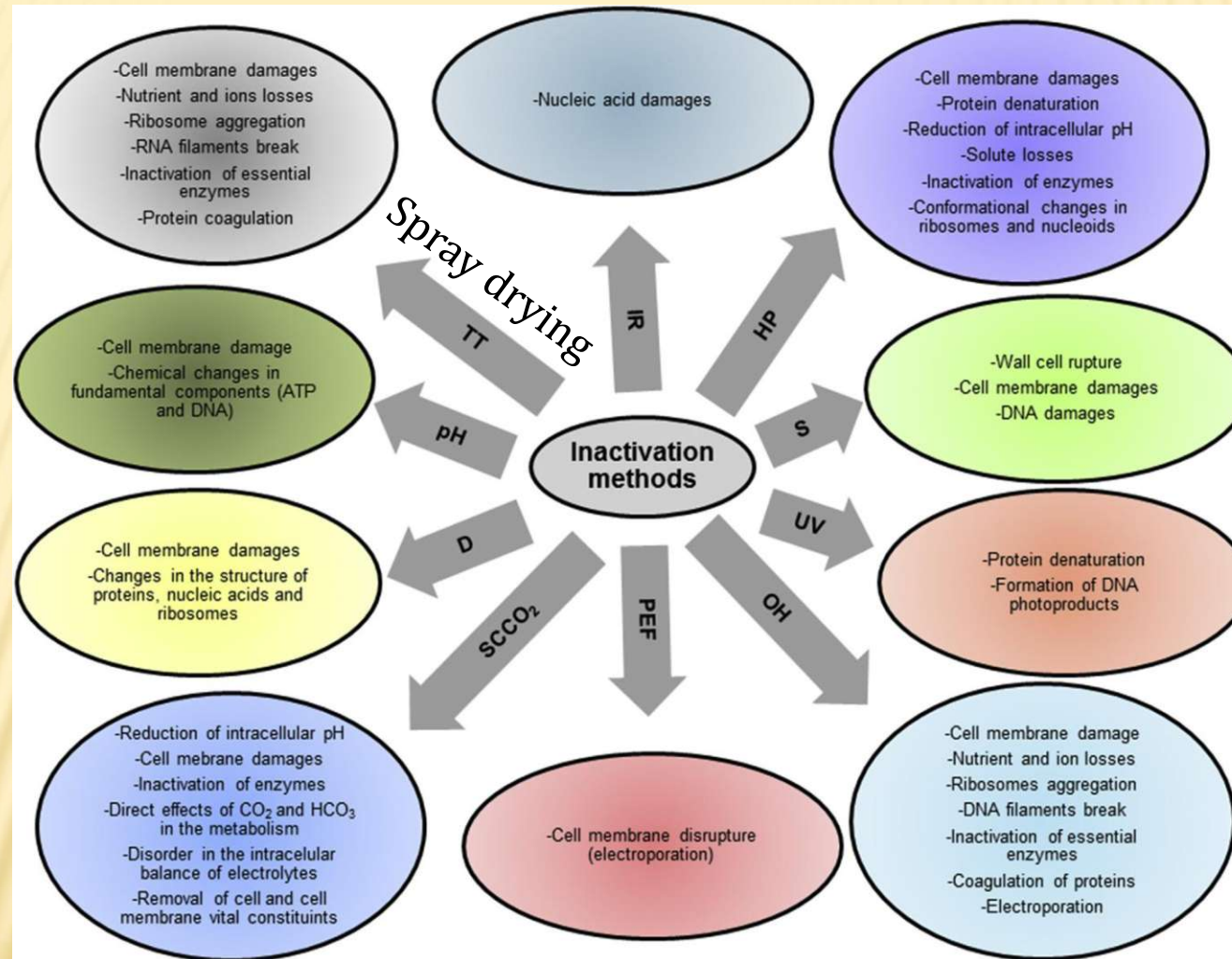
*Scope and approach:* In this study, the main methods used to inactivate probiotics to produce paraprobiotics, their role as modifiers of biological responses as well as their potential application in foods are discussed.

*Key findings and conclusions:* A number of biological effects have been associated with paraprobiotics, highlighting that they could constitute an **excellent option to improve health status and wellness**. Although health benefits have been associated to paraprobiotics, most data in literature show these effects are linked to their direct consumption. Therefore, the use of foods as carriers for paraprobiotics seems to constitute a field to be explored with several opportunities and challenges. Among them, are of special importance the selection of probiotic species and strains to be used for paraprobiotics production, the use of appropriate methods for inactivation and delivery, the evaluation of their stability and activity in foods during shelf life, and the use of adequate methods to assess their biological effects.

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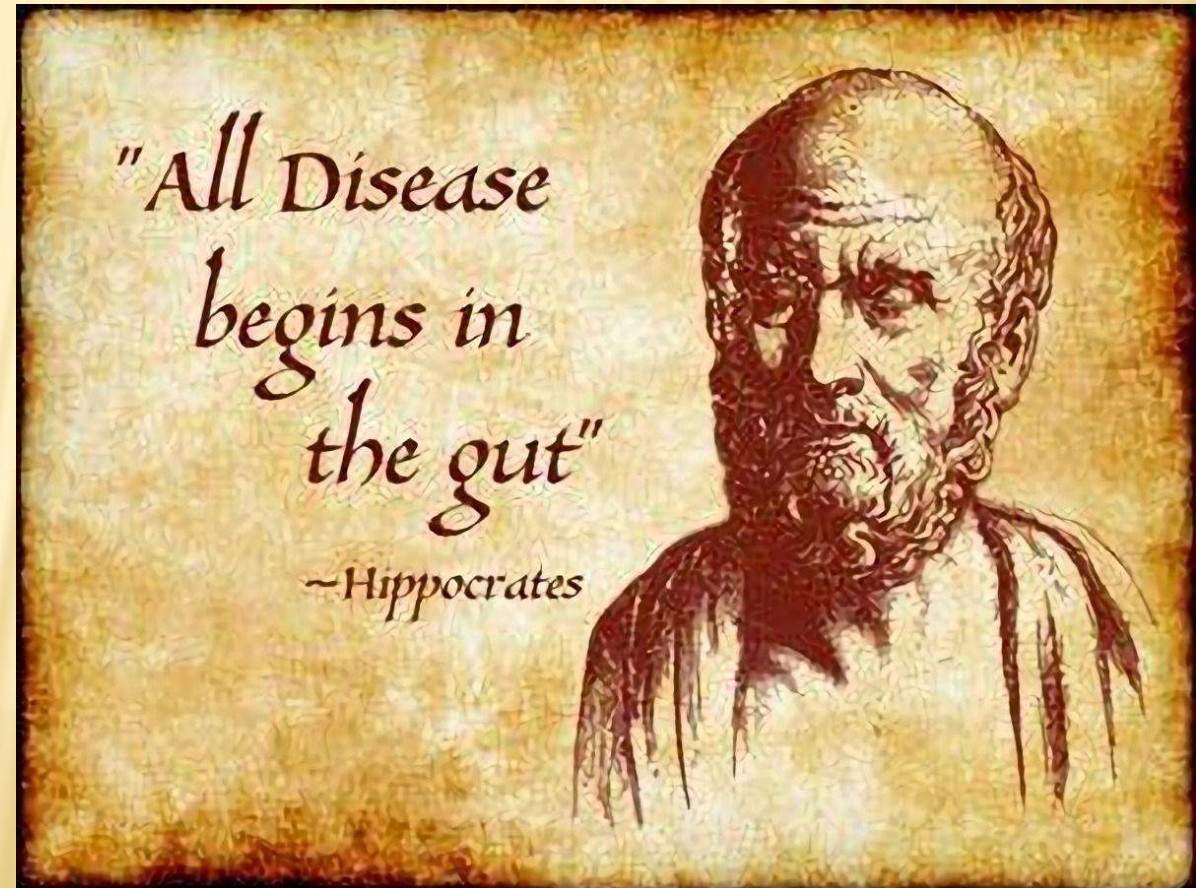
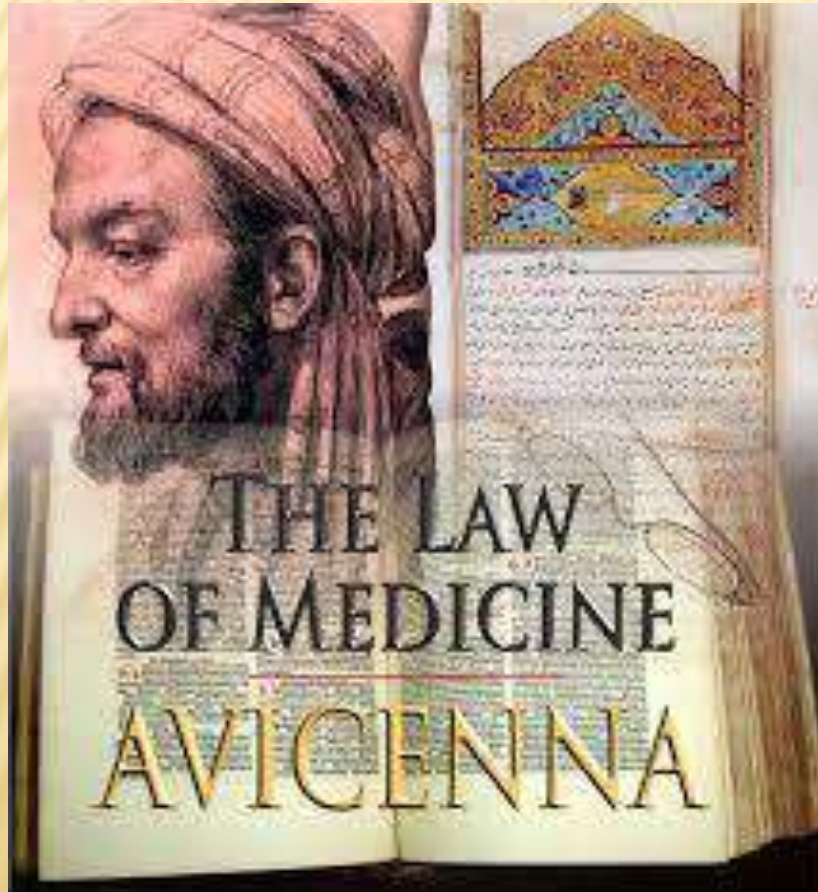
# Inactivation methods for obtaining paraprobiotics



\*paraprobiotic = dead bacterias and yeasts (due to: UV, ionizing radiation, high pressures, sonication, high voltage electric fields, supercritical CO<sub>2</sub>, high temperature & dehydration = spray drying) which have biological effects.



II. The second chapter refers to the importance of the digestive tract (especially gut microbiota ) in maintaining the health of the human body.





## II. Second myth. The current importance of tribiotics (pre/pro/post biotic\*) confirming allegations made by Ayurvedic, Chinese medicine, Hippocrates, Avicenna, which have stated that health is linked to the integrity of the digestive system:

Nowadays allopathic medicine has discovered that the human organism lives in a perfect symbiosis with its microbiome (germs especially in the digestive tract, but also in the nose, ears, vagina, respiratory and urinary tract, skin, etc.. ). This microbiome is nine times larger as the number of cells compared to human cells, and compared to the number of genes (= information), it has more than 50 - 100 times more genes. Also in the large intestine there are over one hundred billion **neurons** (more than in the spinal cord); that's why the gut is considered to be the "third brain" (the lower Dan Tien in Chinese medicine). At this level, 90% of human **serotoline** (one of the most important neurotransmitters) is secreted, the remaining 10% being secreted by the central nervous system. It is also known that an impulse sent from the brain to the intestine corresponds to **400 impulses** (information) that goes in the opposite direction. Changing the normal gut microbiota (= dysbiosis) leads to changes, in the intestinal transit, of the **immune system** (80% of the immune cells are located in the Peyer plaque and the lymph nodules), as well as the damage to other organs and systems, but also to **psycho-neurological problems**: anxiety, depression, autism, Parkinson, Alzheimer, etc ... It is known that emotional changes occur within the irritable colon through both a psychological and a material component. We also call gut microbiota "**the queen**" of the **endocrine glands** (due to the postbiotics which are passing directly into blood stream to the brain and then influencing the hypothalamic - pituitary endocrine tract).

**During the last 100 years, due to multiple factors, but especially those related to the chemical-pharmaceutical industry, there has been a change in the normal flora of the soil, plants, insects, animals and humans, creating negative effects therefore the need to apply tribiotic treatments for restoring the flora.**

### **\* Explaining 3x Biotics:**

- **Probiotic bacteria:** Probiotics is the term that refers to the friendly or beneficial bacteria that reside in the human gastrointestinal tract. It is now understood that a healthy microbiome, which consists of an overwhelming majority of beneficial probiotic bacteria, is one of the most fundamental aspects of good health.
- **Prebiotic Foods:** For your probiotic bacteria to thrive and survive, they must be fed well. The type of nourishment or nutrition that probiotic bacteria prefer and require are classified as prebiotic foods. The most important category of prebiotic foods are the fiber-rich, multi-colored vegetables, followed by leafy greens and multi-colored fruits. A higher diversity of different strains of probiotic bacteria in the intestinal tract equates to better health. The most important and most effective way to create and maintain a diverse microbiome is to feed your probiotic bacteria with a highly diverse, fiber-rich diet. It is not just the quantity of dietary fiber that is important. To maintain a highly diverse microbiome, you must supply your probiotic bacteria with a highly diverse, fiber-rich diet. This means it is best to consume many different kinds of fiber-rich vegetables, fruits and leafy greens every day.
- **Postbiotic Metabolites:** The metabolic processes of bacteria result in the production of a wide range of compounds referred to as postbiotic metabolites. Pathological bacteria are "bad" because they secrete postbiotic metabolites that are highly inflammatory and toxic to us. However, probiotic bacteria produce postbiotic metabolites that have a wide range of health-regulating effects. Examples of postbiotic metabolites include B-vitamins, vitamin K, amino acids, neurotransmitters, short-chain fatty acids, organic acids, enzymes, natural antibiotics, antioxidants, anti-inflammatory compounds, glutathione, fulvic acids and phenyllactic acid.



Kombucha cultures contains pre, pro and post biotics, which are changing dysbiosis in eubiosis of the digestive tract of bees, animals and humans. Its **composition** is the following:

**1/ Bacteria:** *Bacterium gluconicum, Bacterium xylinum, Acetobacter xylinum, Acetobacter xylinoides, Acetobacter Ketogenum, Acetobacter aerobic, Pediococcus anaerobic, Lactobacillus aerobic, Bifidobactereum*

**2/ Yeasts:** *Saccharomyces boulardi, Saccharomyces ludwigii, Saccharomyces apiculatus, Schizosaccharomyces pombe, Zygosaccharomyces, Saccharomyces cerevisiae, Brettanomyces.*

Bacteria and yeasts are probiotics essential for optimal digestion of food and absorption of nutrients, and they help your body produce vitamins, absorb minerals and aid in the elimination of toxins.

As we have demonstrated in the following studies, the harmonious symbiosis of these bacteria and yeasts has, besides the ability to **destroy polyfloral pollen grains membrane** with the release of active principles that will behave like a prebiotic, but will also significantly **increase the number of lactic bacteria** as well as their **increased adherence to intestinal mucosal epithelium**, ultimately leading to an improvement in the digestion process with an **increase in nutrients absorption**.



**3/ Organic acids** perform multiple roles in the body, such as:

- **Acetic acid:** regulates blood pressure, detoxifying action, liver protection, fights against liver steatosis, anticholesterolemia effect, antimicrobial and antiparasites effects, improves digestion; hemostatic effect; promotes "Qi" energy flow.
- **Gluconic acid:** adjusts Ph, antibacterial, antifungal;
- **Glucuronic acid:** anti-inflammatory effect;
- **Citric acid:** antibacterial, antifungal;
- **L-lactic acid:** improves digestion, antifungal, anticancer;
- **Malic acid:** reduces ischemic sequelae, reduces blood pressure, reduces pain caused by fibromyalgia;
- **Tartaric acid:** antioxidant, stimulates and improves digestion;
- **Malonic Acid:** antifungal effect;
- **Succinic acid:** increases stress resistance, improves nervous system function, anti-inflammatory effect, improves the immune system, antitumoral effect, reduces toxicity, prevents aging, intensifies the action of vitamins and drugs;
- **Piruvic acid:** an important role in the Krebs cycle;
- **Usnic acid:** is a powerful antibiotic, has antiviral, antimycotic, antiprotozoal, antiinflammatory and analgesic properties;



**4/ Enzymes** have multiple roles, such as:

- **glutathione-S-transferase**: antioxidant, anticancerogenic, improves digestion, detoxifier activity (produces a liver reaction that helps toxic compounds become more water soluble to be eliminated from the body);
- **glutathione peroxidase**: intracellular antioxidant, maximizes the role of vitamins;
- **glutathione reductase**: antioxidant, antitumoral;
- **catalase**: antioxidant;
- **superoxide dismutase**: antioxidant, anti-cancer, anti-inflammatory, slows aging and protects against UV radiation;

**5/ Minerals** have many beneficial effects as follows:

- **Soluble silicon**: Information-based semiconductors help to fix calcium in the bones, stimulate collagen synthesis, role in maintaining the health of bones, teeth, joints, skin, nails, hair, normalizing blood pressure, strengthens the immune system, regulates calcium metabolism;
- **Manganese**: antioxidant, increases the use of vitamin B1 and vitamin C, regulates glycemic level, can prevent osteoporosis and slows its progress, acts as a vasodilator, helps in good functioning of the liver, alleviates symptoms of premenstrual syndrome (PMS), improves digestion ;
- **Iron**: vital component of red blood cells, role in preventing anemia and in good functioning of human metabolism;
- **Copper**: antioxidant, increases the use of iron in the blood, reduces the action of free radicals on tissues, an important role in the prevention of allergies, AIDS, leukemia, osteoporosis and gastric ulcer;



- **Zinc:** anticancer (role in the prevention of malignant melanoma), hormonal balance, immunostimulant, antidiarrheal, prevents the occurrence of hyper-emotivity and hyperactivity, improves the intellectual performances, rejuvenates the skin, improves potency, prevents premature andropause, normalises sexual appetite stimulates the production of testosterone and insulin, increases spermatogenesis, anti-aging, psychic tonic, prevents heart rhythm disorders; fights against benign prostate adenoma; present in many enzymes.
- **Cobalt:** together with iron is an effective antianemic agent, vasodilating properties, maintains the integrity of the nervous system;
- **Chromium:** improves digestion, a role in the metabolism of carbohydrates, fat and protein, anti-cholesterol-lowering effects, helps to fix calcium in the bones;
- **Magnesium** plays a role in the metabolism of carbohydrates, lipids and proteins, of some hormones; antioxidant; role in the metabolism of vitamin C and minerals such as phosphorus, sodium and potassium; an important role in calcium fixation in the bones and muscle contraction, improves the functioning of the nervous system, participates in maintaining a healthy cardiovascular system, glycemic normalization, anticholesterolemia effects, influences the regulation of intestinal transit and blood coagulation, is a general tonic, is a growth factor, cellular, hepatic drencher, interferes with energy metabolism in active membrane transport;
- **Potassium** plays a role in: muscle contraction and nerve function, storage of carbohydrates used as fuel by the muscles, nervous system function, helps to prevent cerebral attack and correct contraction of the muscles and acts together with sodium to maintain the balance hydro-electrolytic in the body, is important for the chemical reactions inside the cells and helps maintain blood pressure, maintaining a healthy cardiovascular system.



## 6/ Vitamins:

- Thiamin (B1)
- Riboflavin (B2)
- Niacin & Niacinamide (B3)
- Pantothenic Acid (B5)
- Pyridoxine (B6)
- Biotin (B7)
- Folic Acid (B9)
- B-12
- Vitamin C

These vitamins have a much higher bioavailability compared to synthetic vitamins; even if the amounts are not very high (less vitamin C which is 6 times more than in orange juice), let's not forget that the whole is greater than the sum of its parts. The presence of the vitamin B complex (especially B12) is beneficial to those on a vegan diet.

**As you see the culture environment in which the fermentation of the pollen is taking place comes with an extraordinary wide variety of beneficial nutrients.**

We will see at the end that  $1 + 1 > 2$  (pollen plus kombucha = Amrita)



# Two microbiological studies at the USAMV Faculty of Biotechnology showd the following:

## 1. First study:

**Objective:** the main focus was to isolate and characterize lactic acid bacteria with probiotic properties from cell culture, used for the production of a 3-biotic functional product Amrita by Laboratoarele Medica S.A. Otopeni. Most probiotics in food are lactic acid bacteria (LAB). Probiotic LAB belongs mainly to the genera Lactobacillus, Bifidobacterium, Enterococcus, Lactococcus, Streptococcus and Leuconostoc. Within this group, Lactobacillus species are most commonly used group of microorganisms for their potential beneficial properties as probiotics. Several criteria are taken in account to call LAB as probiotic including activity antagonist against other microorganisms, resistance to acid, bile salt and phenol, adherence to gut epithelial tissue, cholesterol assimilation and lactose activity.

**Main conclusion:** in view of these different experiments, it is possible to conclude that bacteria and yeast culture contains lactic acid bacteria which **have probiotic potential**, beneficial for human health and the best probiotic strains which were discovered during this test are L3 and S1.



In 2018 a study which took place in the USAMV Faculty of Biotechnology, presented at Slovenian congress, shows the following antibacterial effects

Antibacterial activity of crude and lyophilized **symbiotic microorganisms** broth:

- Cultivation medium:
  - Black tea (BK)
  - Green tea (GK)
  - Oolong tea (OK)
- Lyophilization conditions of symbiotic microorganisms broth: vacuum, at  $-51 \div -53^{\circ}\text{C}/6$  hours
- Testing the antibacterial activity:
  - by "**drop agar diffusion**" method
  - pathogenic bacteria were spread using  $100\mu\text{l}$  of suspension containing  $10^8\text{CFU/ml}$  on nutrient agar and cultivation for 24 hours
  - $10\mu\text{l}$  of crude filtrate or rehydrated lyophilized has been added on sterile filter paper (5 mm)
  - results are appreciated by the inhibition halo (diameter)





# Inhibitory effect of symbiotic microorganisms broth and lyophilized on pathogenic bacteria

Pathogenic bacteria	symbiotic microorganisms culture					
	BKL	BKF	GKL	GKF	OKL	OKF
<i>Bacillus pumilus</i>	+	+	++	++	++	++
<i>Bacillus megaterium</i>	+	+	++	++	++	++
<i>Enterococcus faecalis</i>	+	+	++	+	++	+
<i>E. coli</i>	+	+	+++	++	+++	+++
<i>Salmonella tiphymurium</i>	+	+	+++	++	+++	++
<i>P. aeruginosa</i>	+	++	+++	+++	+++	+++

(-): non inhibitory halo  
 (+): low inhibitory activity  
 (++) : moderate inhibitory activity  
 (+++) : high inhibitory activity

- Black tea (BK)
- Green tea (GK)
- Oolong tea (OK)
- L – lyophilized
- F – crude filtrate



# Preliminary conclusions on antibacterial activity

- Crude broth and lyophilized broth cultivated on **Black tea** have low to moderate inhibitory activity on the pathogenic bacteria
- Crude broth and lyophilized broth cultivated on **Green tea** have:
  - moderate inhibitory effect on the following pathogenic bacteria: *Bacillus pumilus*, *Bacillus megaterium*, *Enterococcus faecalis*
  - high inhibitory effect on the following pathogenic bacteria: *E. coli*, *Salmonella*, *Pseudomonas*

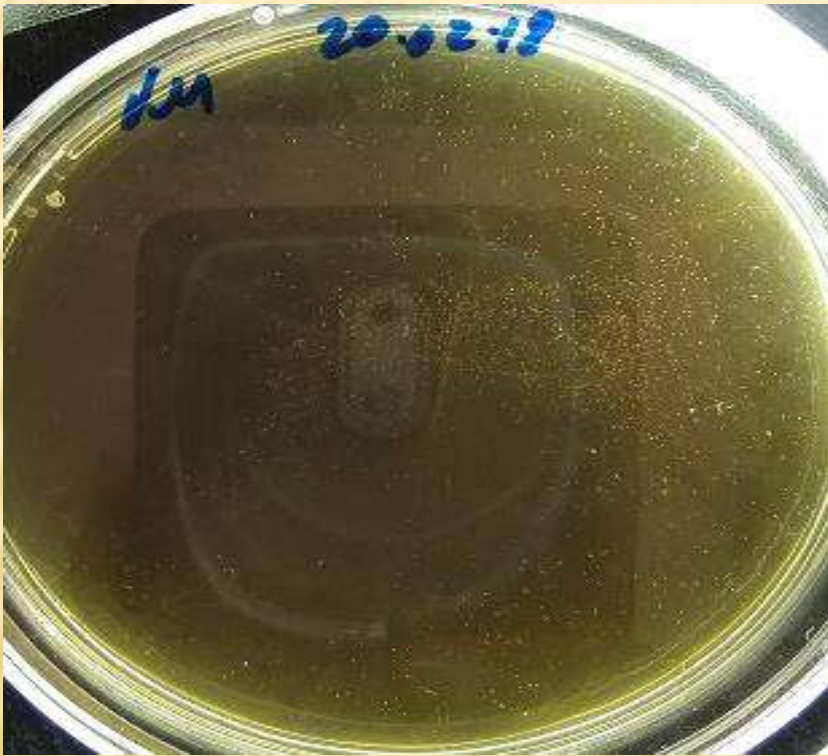
Nowadays we have a study of the use of medicinal herbs with antibiotic effects in the two tea cultures (green and black) aiming at highlighting the increase in the antibiotic potential of these cultures.



## 2. Second study: Evolution of lactic acid bacteria population during Amrita (tri-biotic) production process

**Objective:** The tests have been developed as part of the general study which intends to demonstrate the tri-biotic effect (pre/pro/post biotic) of a new product based on bacteria and yeasts cells soup and bee polyfloral pollen.

**Aspects of lactic acid bacteria population cultivated in MRS medium isolated from different sources :**



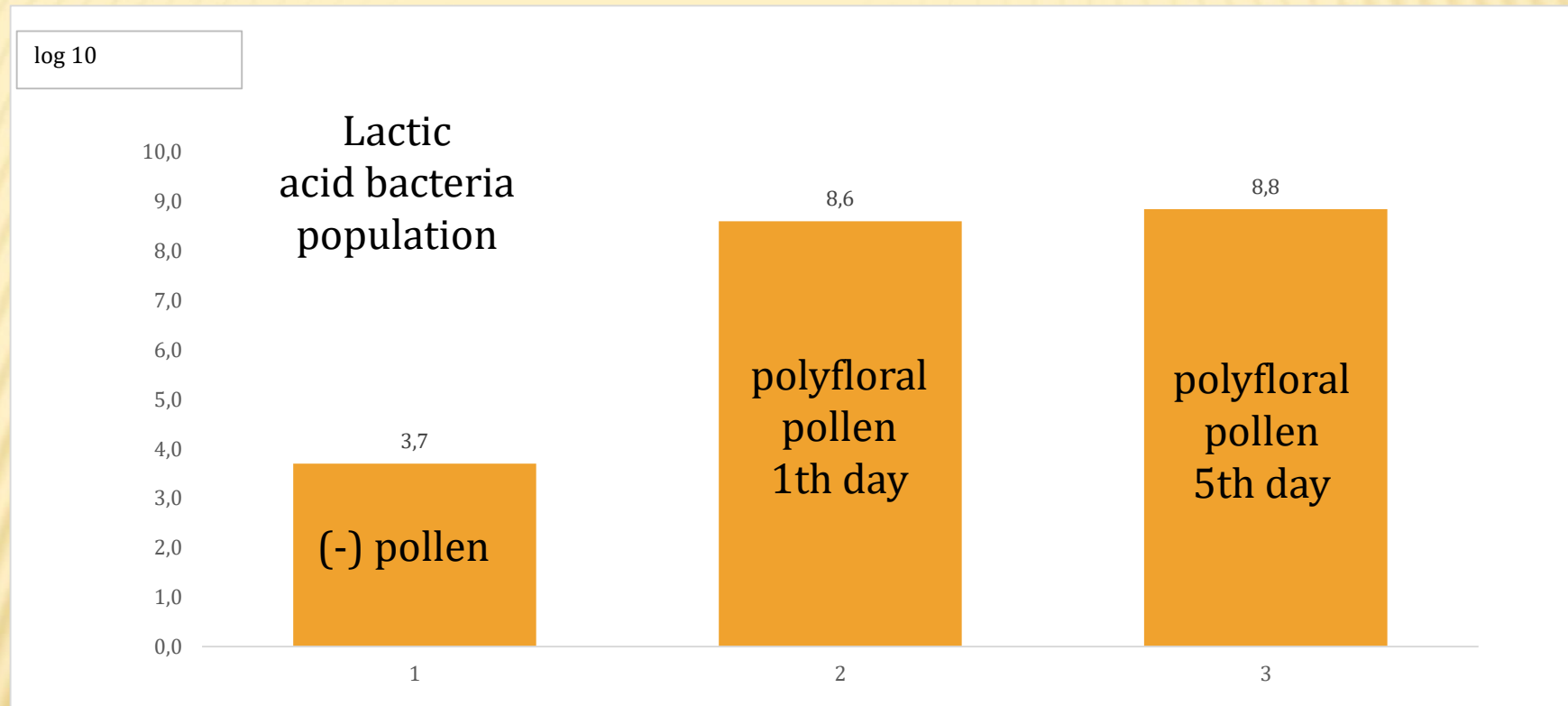
bacterias and yeasts cells soup fermented 21 days



mix of bacterias and yeasts cells soup and  
bee polyfloral pollen after 5 days of maceration



In the case of classic counting, it has been noticed an increase of the lactic acid population from an average of  **$0.5 \times 10^5$**  UFC/ml in fermented polyfloral pollen soup to  **$0.7 \times 10^9$**  UFC/ml after 5 days of maceration with bee polyfloral pollen (fig.1 and fig.2). It has not been noticed any significant difference of bacterial population level between first and last maceration day.

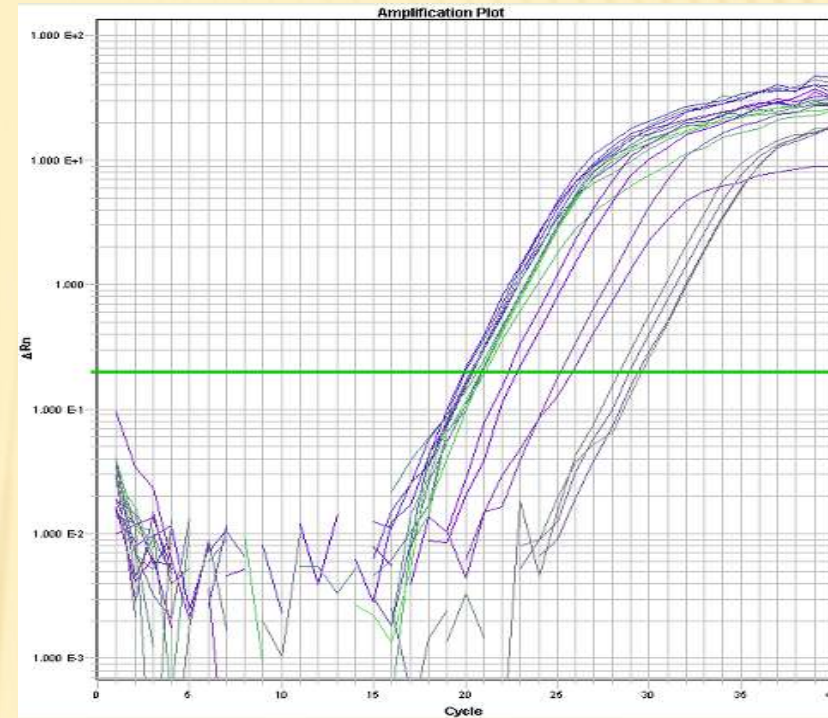
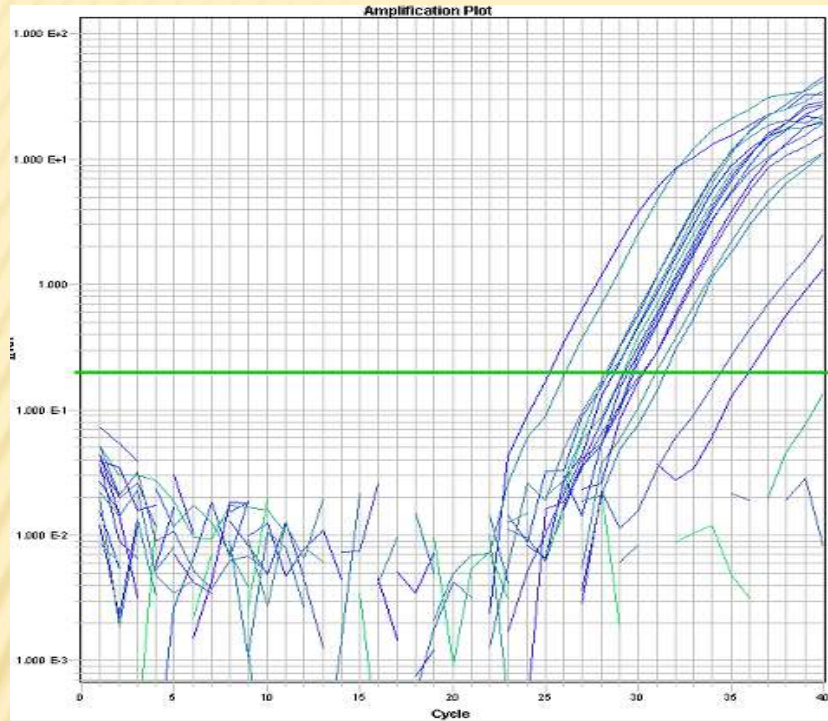


**Fig. 2 Lactic acid bacteria levels in different samples:**

- 1- Bacterias and yeasts cells soup fermented 21 days;
- 2- Mix of bacterias and yeasts cells soup and bee pollen in the first day of maceration;
- 3 - Mix of bacterias and yeasts cells soup and bee pollen after 5 days of maceration.



The data have been confirmed by molecular tool, Real Time q-PCR



**Fig.3 qPCR runs for two probes of lactic acid bacteria**

**left:** bacterias and yeasts cells soup fermented 21 days;

**right:** mix of bacterias and yeasts cells soup and bee pollen after 5 days of maceration.

Such important bounce of the lactic acid bacteria population could be caused by the pollen contribution, considering some reports (Vasquez, 2009; Vasquez 2012) which emphasize that transfer of lactic bacteria of genus *Lactobacillus* sp, *Bifidobacterium* sp. from the bees' stomach into the bee pollen.

**This is an important input in the final product, increasing its probiotic properties.**



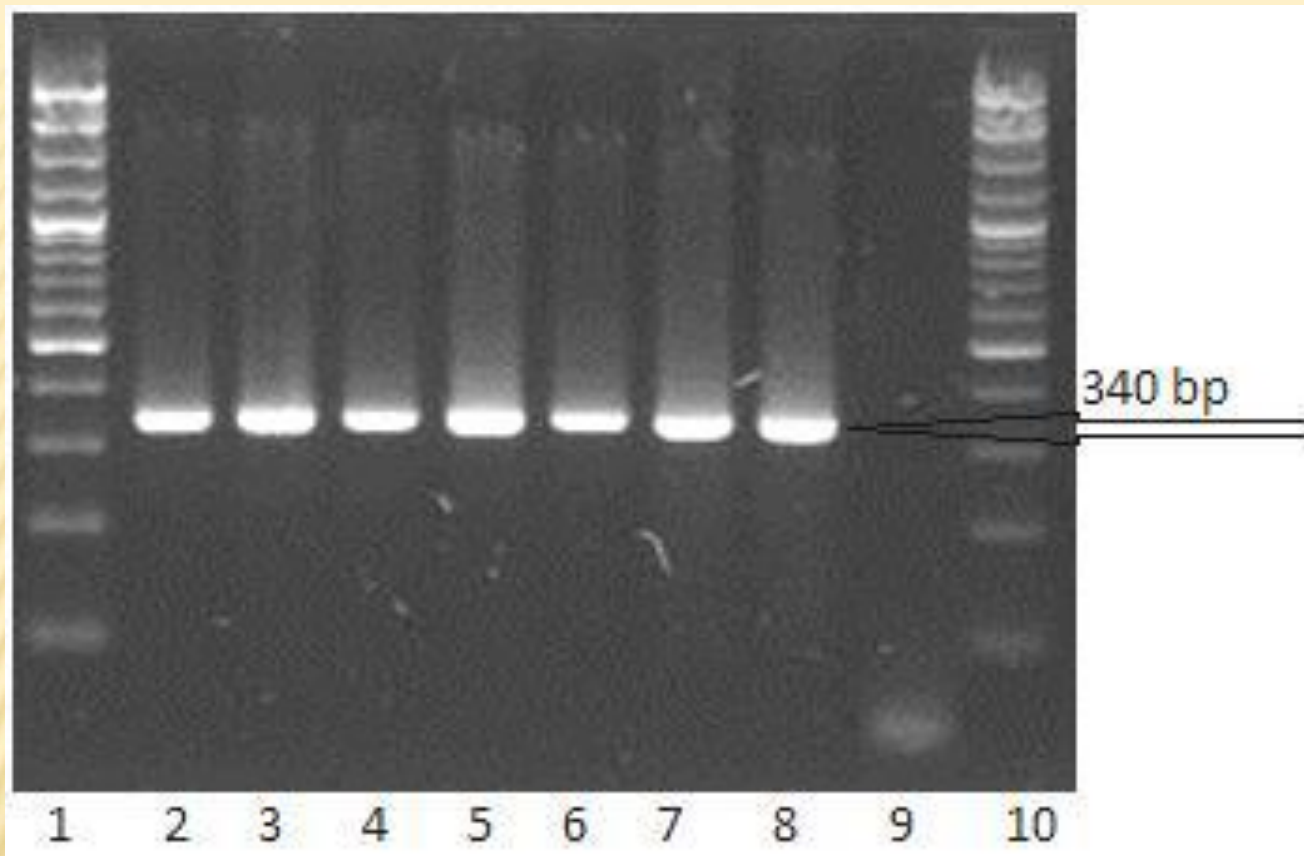
***12th Baltic Conference on Food Science and Technology “Food R&D in the Baltics and Beyond”, May 17-18, 2018, Kaunas, Lithuania***

**POLLEN FERMENTATION – A NEW METHOD TO OBTAIN A TRI-BIOTIC FOOD SUPPLEMENT**

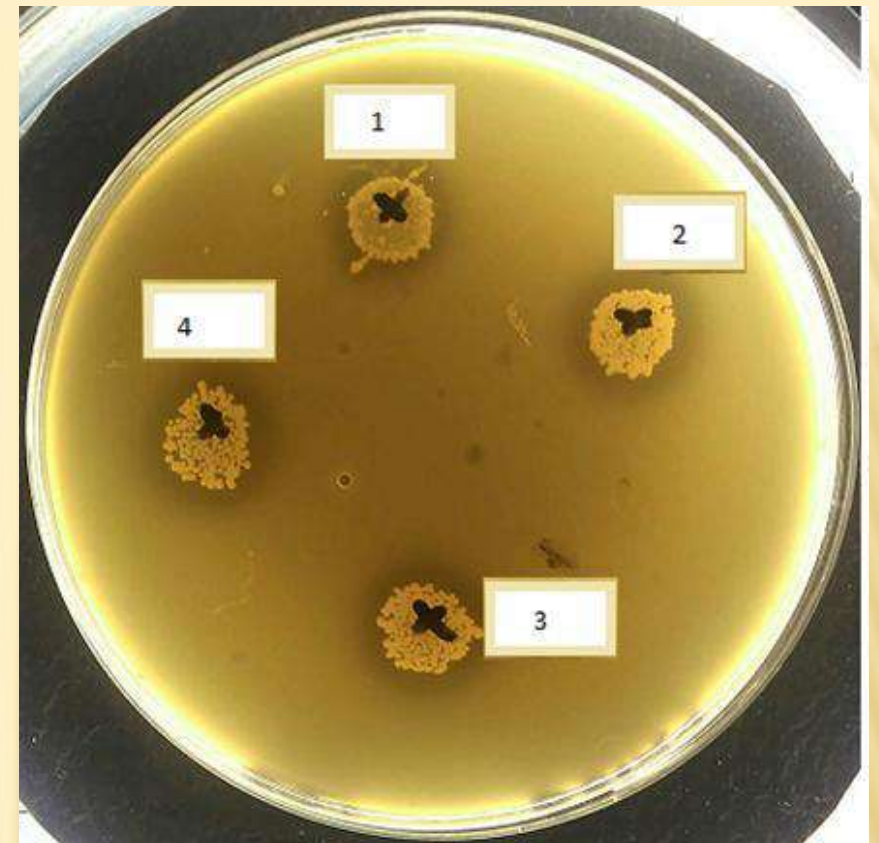
Angela Moraru<sup>1,3</sup>, Anca Oancea<sup>2\*</sup>, Petruta Cornea<sup>3</sup>, Florentina Matei<sup>3</sup>, Camelia Diguta<sup>3</sup>, Alexandra Gaspar – Pintiliescu<sup>2</sup>, Elena Utoiu<sup>2,3</sup>, Agnes Toma<sup>2</sup>, Viorica Coroiu<sup>2</sup>, Florin Oancea<sup>4</sup> Acknowledgments: This study was supported by Romanian National Program PN III, Contract 6BG/2016, 3 - BIOTIC *12th Baltic Conference on Food Science and Technology “Food R&D in the Baltics and Beyond”, May 17-18, 2018, Kaunas, Lithuania* 1 MEDICA LABORATORIES SRL, Romania 2 Department of Cellular and Molecular Biology, National Institute of Research and Development for Biological Sciences, Romania 3 Faculty of Biotechnologies, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania 4 Department of Bioresources – Bioproducts, National Institute of Research and Development in Chemistry and Petrochemistry, Romania.

Tri-biotic products represent a new trend in dietary supplement domain. The pollen is well known for its nutritional properties. However, due to the complex structure of its cellular wall (sporopollenin), the bioavailability of the active compounds from the pollen grains is low. The **synbiotic microorganisms cultures** consortium was used for the pollen fermentation process in order to increase the bioavailability of the bioactive ingredients and to obtain a complex nutraceutical product. To develop an innovative pollen fermentation technology, based on the cultivation and use in the pollen fermentation process of yeasts and bacteria symbiotic colonies, to use it to produce a new food supplement and to determine the active ingredients that offer the tri-biotic character of the obtained product.





Amplified fragments with Lac F and Lac R primers (340 pb) specific to lactobacilli group (1&10-DNA 100 bp ladder; 2- S1; 3-S2; 4-S3; 5- L3; 6-L5; 7- *L.acidophilus*; 8- *L.fermentum*; 9- negative control)

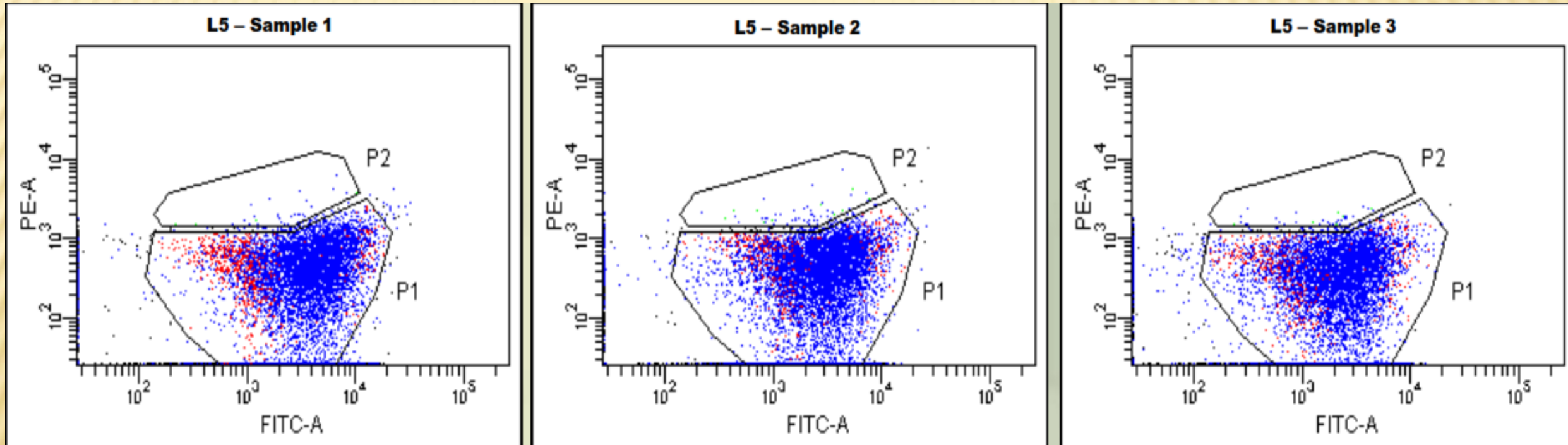


Aspects of bacteriocin screening on-plate. Positive strains are halo-forming. (1-S2; 2- *Lactobacillus acidophilus* - positive control; 3-S3; 4-L5)

Bacteria from *Lactobacillus*, *Leuconostoc* / *Weissella*, *Bifidobacterium* genus and some yeasts such as *Saccharomyces boulardii* were identified in synbiotic microorganisms cultures consortium. We have identified 7 **lactic bacteria** in the synbiotic microorganisms cultures sample utilized for pollen fermentation. We analyzed the *Lactobacillus* L5 strain that was subject to molecular identification.



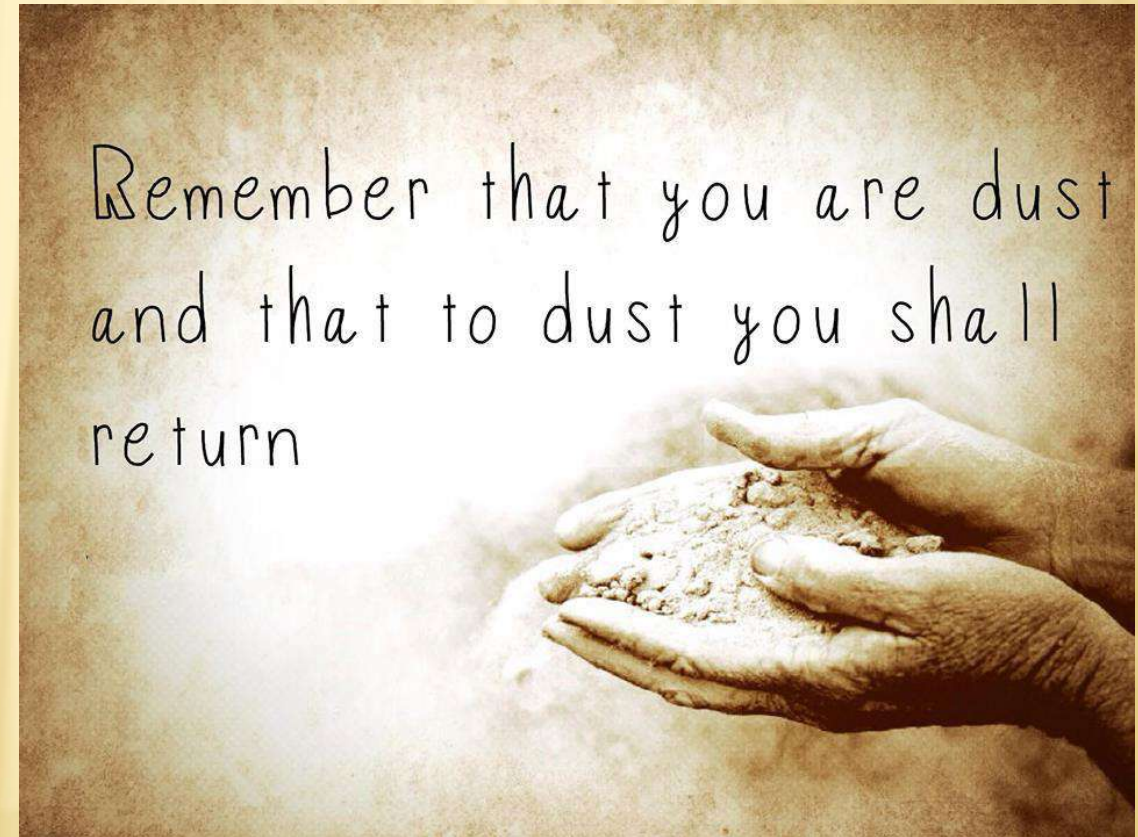
The LIVE/DEAD BacLight assay distinguishes between live **bacteria L5**, exhibiting green fluorescence and damaged membrane bacteria, exhibiting low green fluorescence and strong red fluorescence. Flow cytometry analysis showed that the tested sample (bacterial cells isolated from **synbiotic microorganisms cultures**) contains a significant **population of viable cells**, that exhibited a strong green fluorescence (91.2 – 93.4% from total cells counted) and a low percentage of membrane damaged cells (0.4 – 0.7%).



Flow cytometry analysis of bacterial L5 strain isolated from consortium using LIVE/DEAD BacLight assay. Bacterial cells (sample 1, 2 and 3) stained with Syto 9 and PI (A, B and C); P1 – population of untreated live bacterial cells and P2 – population of alcohol treated, dead bacterial cells



### III. The exceptional role of soluble silicon in promoting longevity and health.

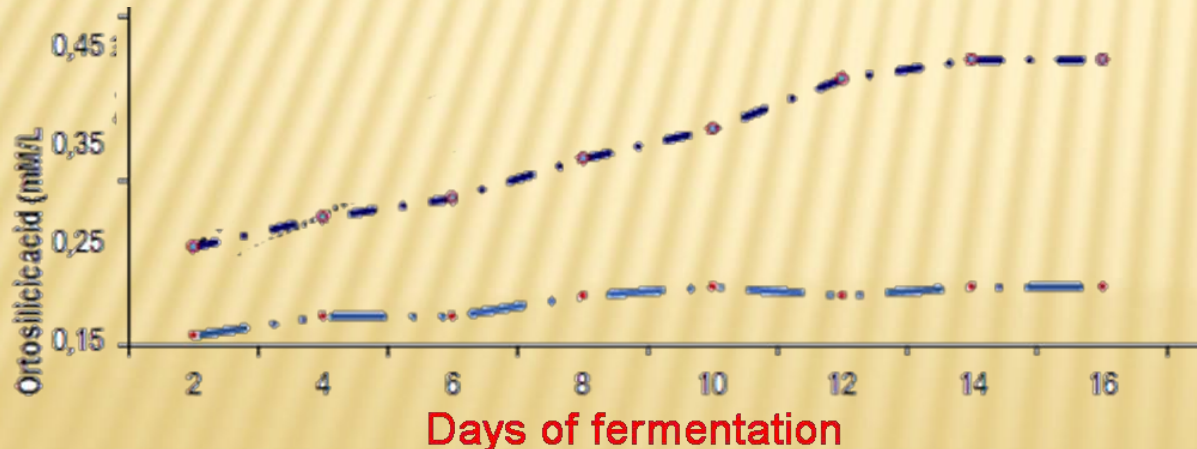







### III. Third mith. Recent discoveries demonstrating the essential role of soluble Organic Silicone in the harmonious development of the body, fact supported by most religions: “from the dust we come and in the land we return”.


The richest element of earth is silicon which has great properties:

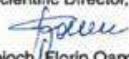
- Silicon, germanium and selenium are semiconductors with informational functions.
- Silicon increases calcium absorption in old age individuals and directs its deposit in the bones and teeth, unlocking the pathological deposits (atheroplaque, urinary and hepatobiliary tract-litiasis, osteophyts). Biological transmutation theory developed by Nobel candidate Louis Kervran goes even further saying that silicon can turn into calcium by atomic reactions at the cellular level.
- From our point of view the most important biological effect of silicon is stimulating synthesis of collagen (besides the role it has for bones, teeth, joints, skin, nails, hair, is found in the acupuncture meridians or at vascular walls, etc...); there is a correlation, it seems, that the ligaments status and senescence processes (in this case there is the possibility of using soluble silicon in slowing the aging process); are directly linked.
- Soluble silica according to scientific evidences is lowering blood pressure, increases immunity, regulates metabolism of calcium, etc...



- Silicic acid in fermented polyfloral pollen
- Silicic acid in symbiotic microorganisms cultures consortium

<div>  <div> <b>Institutul National de Cercetare - Dezvoltare pentru Chimie si Petrochimie</b>  <b>ICECHIM</b>  <small>060021 Bucuresti, Splaiul Independentei, nr. 202, Telefon 021-315.32.99, fax 021-312.34.93, www.icechim.ro</small> </div> <div>  </div> </div>		
TO: Laboratoarele Medica, str. Frasinului nr.11, 075100 Otopeni, jud. Ilfov Certificate of Analysis <div>  </div>		
Sample: multi-floral pollen, fermented by symbiotic colony of bacteria and yeast		
Parameter	Value	Method
Energy, KJ (100 g)	1496	Calculated
Energy, Kcal (100 g)	358	Calculated
Proteins (%)	5.09	Kjedahl, Total N x 5.6 ( Rabie et al. 1983)
Lipids (%)	1.44	Chloroform: methanol 2:1 extraction
Total carbohydrates	88.84	By difference
Dietary fibers	0.85	McCleary Method (AOAC 2009.01)
Ash (%)	3.78	ISO 928:1997
Minerals, trace element (mg/kg)		ICP-OES
Potassium	960.00	
Magnesium	280.00	
Phosphorus	810.00	
Zinc	21.70	
Copper	1.36	
Manganese	4.00	
Silicon		
Total	472.00	
Soluble	294.00	



Scientific Director,  
  
 Dr. Ioan Florin Oancea



# ENHANCEMENT OF NANOSILICA BIOSOLUBILITY BY KOMBUCHA FERMENTATION

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<sup>4</sup>National Institute for Research & Development in Chemistry and Petrochemistry – ICECHIM, 202 Spl. Independentei, 6<sup>th</sup> district, 060021 Bucharest, Romania

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**Introduction:** Nanosilica phytolith  $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ , resulted after orthosilicic acid polycondensation and precipitation into plant cell wall are the main source of soluble silicon species [1]. Soluble silicon species, and especially orthosilicic acid,  $\text{H}_4\text{SiO}_4$ , have been proved to exert several beneficial effects on humans, as maintenance of the optimal connective tissue function, including osteoporosis prevention, stimulation of the immune system and Alzheimer's disease prevention [2,3].

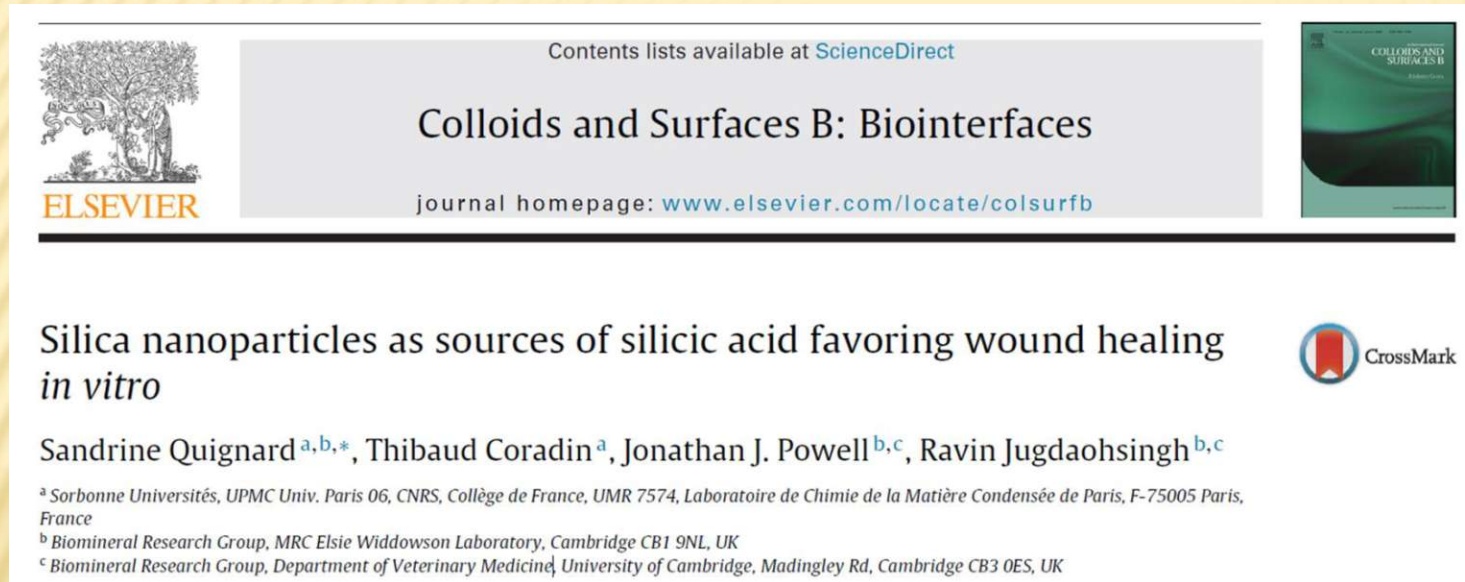
**Materials and methods:** We used a liquid fermentation by a symbiotic colony of bacteria and yeast (SCOBY) to enhance biosilica solubility. SCOBY is traditionally used to produce Kombucha, an Asian traditional beverage, produced traditionally from sweetened *Camelia sinensis* leaf tea by fermentation with SCOBY. We used rice husk, horsetail and honey bee collected pollen as biomaterial rich in biosilica. We monitored the evolution of soluble silicon species by the silicomolybdic acid spectrophotometric method [4].

**Results:** **SCOBY fermentation increases the soluble silicon** significantly, by 180%, 225% and, respectively, **280%** on rice husk, horsetail aerial part powder and **honey bee collected pollen**. The plant nanosilica solubilization was more significant after 18 days of Kombucha fermentation. Such solubilization it is probably a result of the accumulation of organic acids.

**Conclusions:** The fermentation of pollen with Kombucha releases important amounts of soluble silicon from nanobiosilica embedded into siliceous plant material. The resulted products represent a new source to produce dietary supplements and cosmeceuticals, wherein highly available soluble silicon is combined with Kombucha phytonutrients.



The use of silicon nanoparticles in wound healing was dismantled and published in ELSEVIER April 2017 (link); which explains the extraordinary effects of using amrita raw material as a dermatological product. At Medica Group we have been using with great success for 3 years a mixture of Amrita and honey in the treatment of dermatological lesions (wounds, burns, herpes, insect bites, etc...), as well as in skin nutrition. Our belief is that soluble silicon containing nanoparticles plays a role as a **nutrient carrier**, helping to penetrate them intracellularly.



**Abstract:** There is good evidence that certain silicon-containing materials promote wound healing and their common feature is the delivery of orthosilicic acid ( $\text{Si}(\text{OH})_4$ ) either directly or following metabolism. In this regard, amorphous silica nanoparticles (NP), which dissolve in aqueous environments releasing up to 2 mM orthosilicic acid, may be appropriate 'slow release' vehicles for bioactive silicon. Here we studied the impact of silica NP suspensions (primary particles  $\sim 10$  nm) in under saturated conditions (below 2 mM Si) with differing degrees of surface charge and dissolution rate on human dermal fibroblasts (CCD-25SK cells) viability, proliferation and migration in a cellular wound model.

Silica was shown to be non-toxic at the concentrations tested and whilst the anticipated stimulatory effect of orthosilicic acid was observed, the silica NPs also stimulated fibroblast proliferation and migration. In particular, the amine-functionalized particles promoted wound closure more rapidly than soluble orthosilicic acid alone. We suggest that this effect is related to easy cellular internalization of these particles followed by their intra-cellular dissolution releasing silicic acid at a faster rate than its direct uptake from the medium. Our findings indicate that amorphous silica-based NPs may favour the delivery and release of bioactive silicic acid to cells, promoting wound healing. © 2017 Elsevier B.V. All rights reserved.





**24 weeks** after changing  
breasts implants, under topic  
treatment with different  
products



**Evolution of scar surgery breasts  
implants, under external  
treatment with Amrita & Honey  
in 7 days**



# Certificate of Analysis Amrita

Due to the fact that polyfloral pollen shows slightly modified compositions depending on the place and time of collection, kombucha is also slightly different on every continent, the analysis bulletins may have small differences, as any raw material in nature.



## No.1

First of all, conform to Hippocrates' first amendment "primum non nocere" we have proved that **Amrita has no toxicity**:



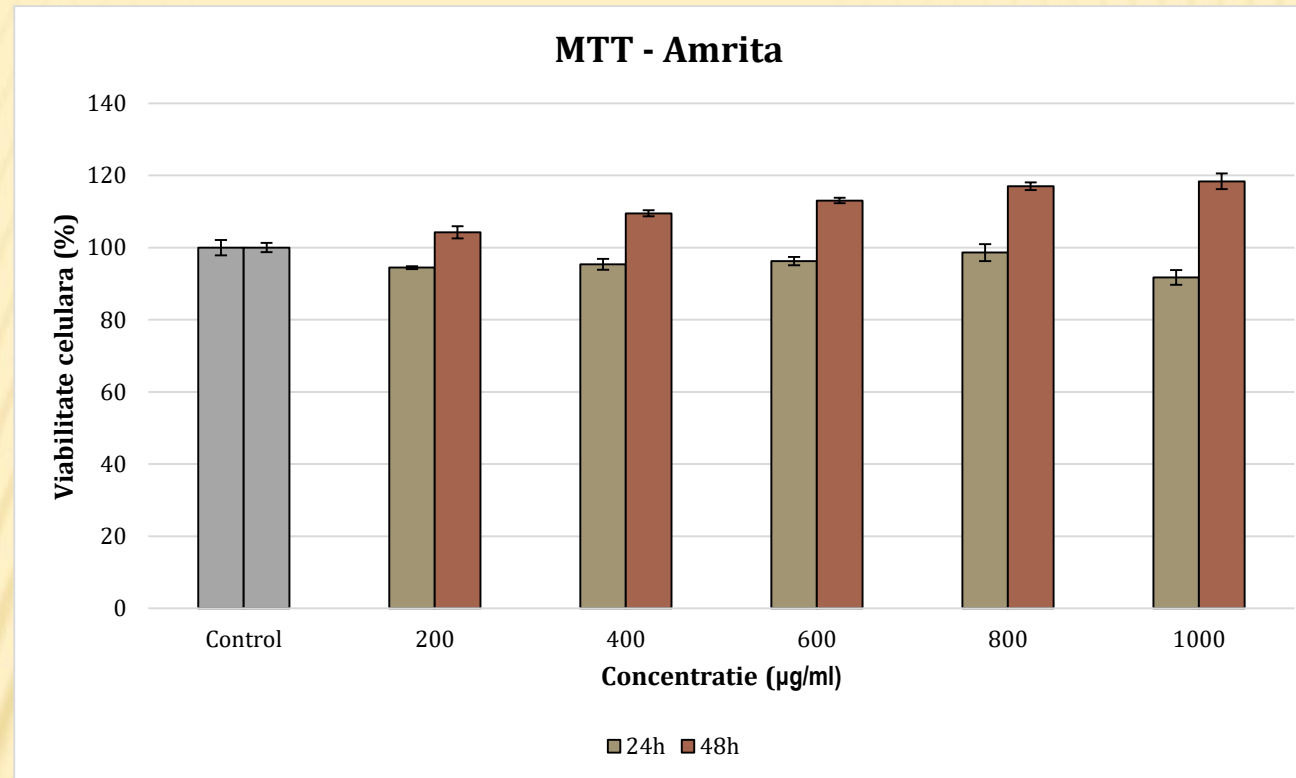
The cytotoxicity of the product was analyzed on the stabilized Caco-2 cell line by the MTT assay, which evaluates cellular metabolic activity by the ability to reduce a tetrazolium colorant by the mitochondrial dehydrogenases. The values obtained are directly proportional to the number of viable cells present at the end of the incubation. The results were reported as percentage of viability versus control (untreated cells), considered to have a 100% viability.

Amrita	200µg/ml	400µg/ml	600µg/ml	800µg/ml	1000µg/ml
24h	94.47	95.36	96.25	98.62	91.70
48h	104.25	109.49	113.06	117.01	118.38

Cell viability, reported in percent, after 24h and 48h of Caco-2 cells, respectively, in the presence of different concentrations of Amrita product.



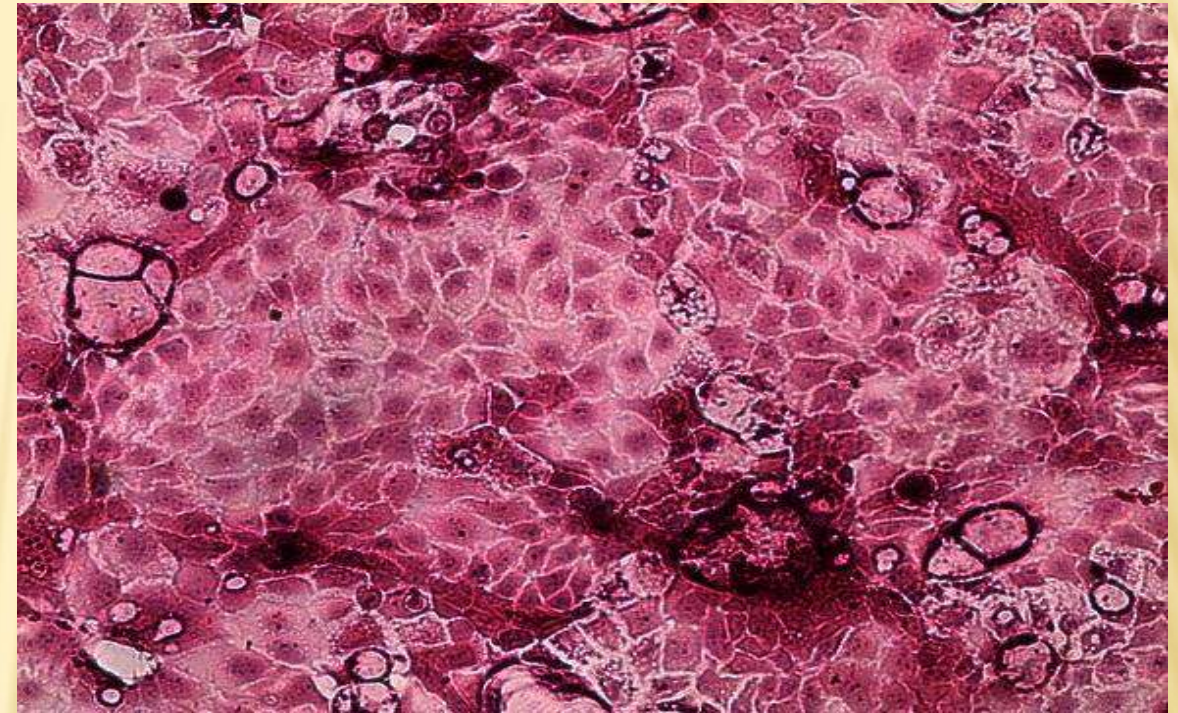
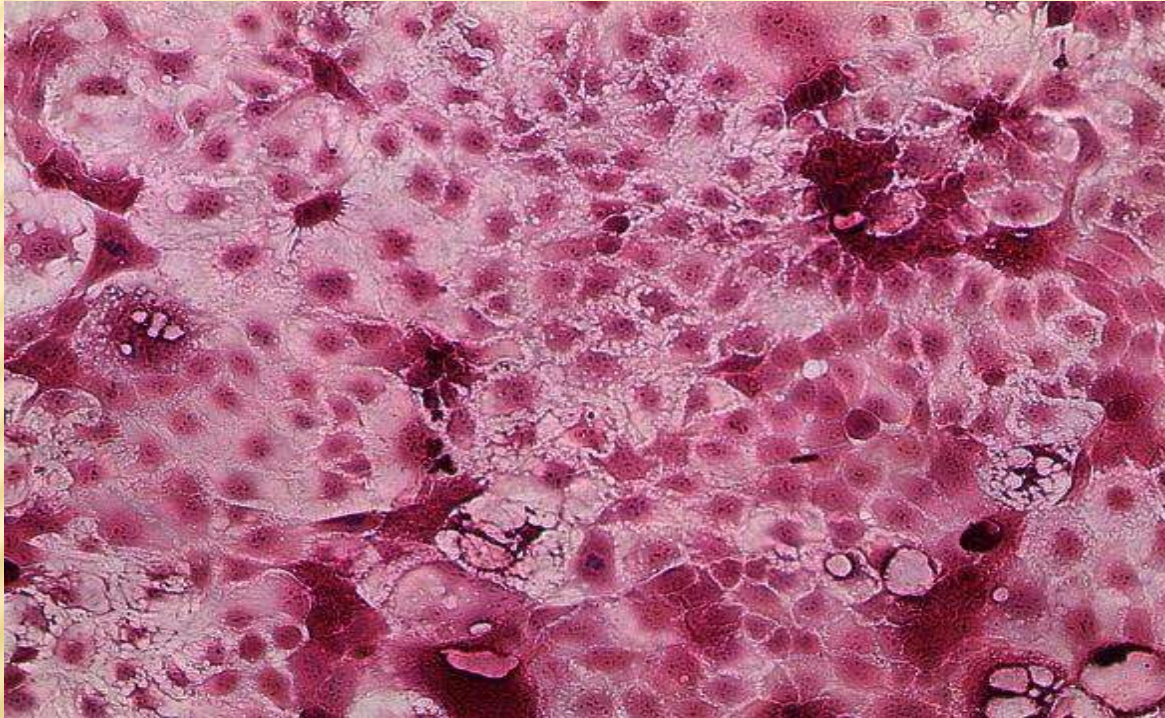
Effect of Amrita on Caco-2 stabilized cell line after 24h and 48h of culture, assessed by the MTT method. Control (cells grown in the absence of the product) is considered to have a 100% viability.



The results of the MTT test showed that in 24 hours after cell inoculation in the presence of Amrita, cell **viability was greater than 80% (non-cytotoxic effect)** across the range of test concentrations (200-1000 µg / ml) , indicating that this product is biocompatible. After 48h treatment, cell viability increased significantly over 100%, the test product stimulated cell proliferation compared to cultured. In terms of cell morphology analysis, at the maximum test concentration, 1000 µg / ml for Amrita , the cell culture retained normal morphology, Caco-2 cells having a cuboidal appearance similar to intestinal enterocytes and domination specific for epithelial cell culture, **morphology similar to that of untreated culture**. Cells also showed a high degree of proliferation, with a single cell monolayer (100% confluent cells) being visible.



Optical microscopy images of Caco-2 intestinal cells grown in the presence of Amrita samples (1000  $\mu\text{g} / \text{ml}$ ), (B) for 48 hours. The untreated cell culture was used as control (A); Hematoxylin-Eosin staining, 20X lens.



In terms of cell morphology analysis, at the maximum test concentration, 1000  $\mu\text{g} / \text{ml}$  for Amrita, the cell culture retained normal morphology, Caco-2 cells having a cuboidal appearance similar to intestinal enterocytes and domination specific for epithelial cell culture, morphology similar to that of untreated culture. Cells also showed a high degree of proliferation, with a single cell monolayer (100% confluent cells) being visible.



## No.2

Concerning the **dietary fibers** - a significant increase in total dietary fibers (TDF) and in insoluble dietary fibers (IDF) content during 10 days fermentation process was observed.

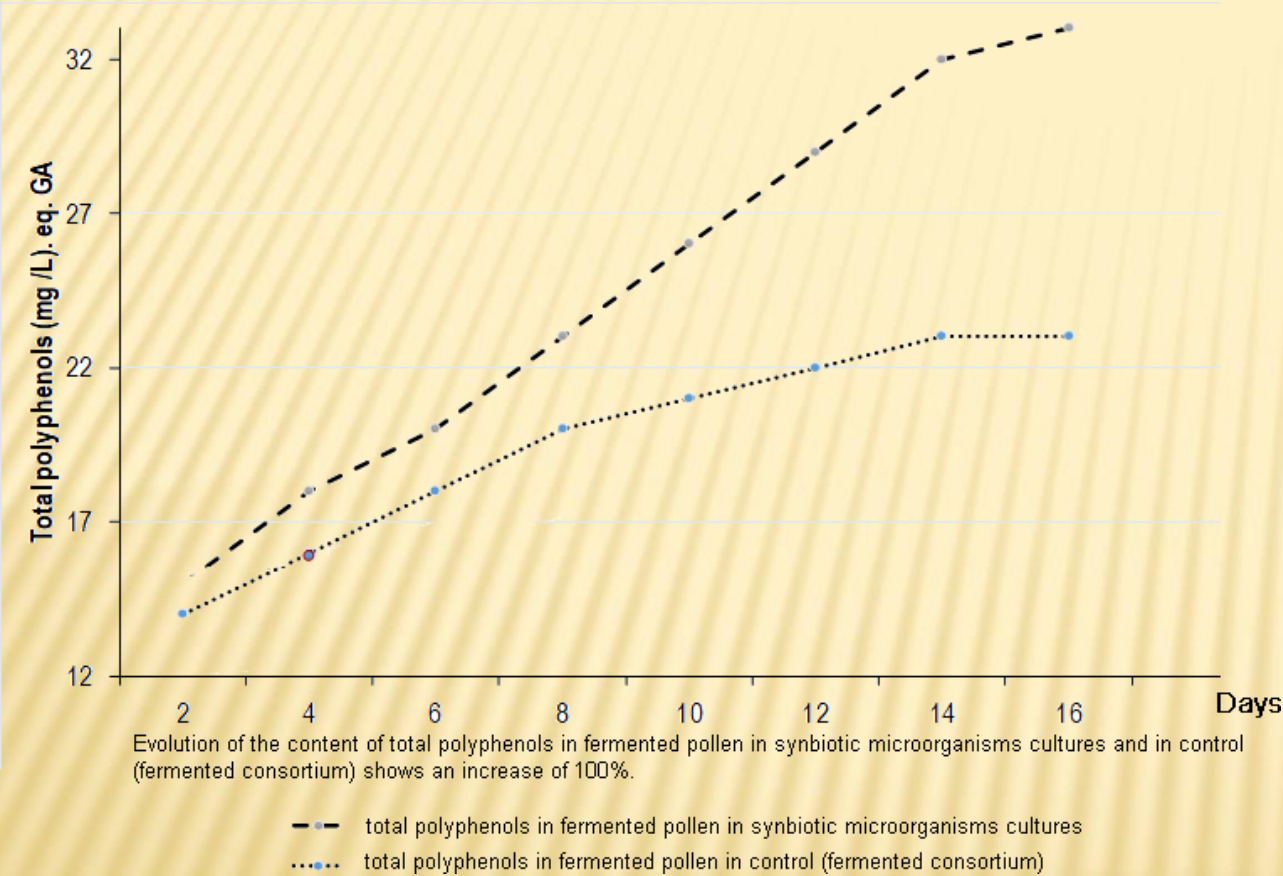
<b>Dietary fibers g/100g</b>	<b>Unfermented pollen</b>	<b>Fermented pollen in microorganisms cultures</b>
	<b>Time intervals (days)</b>	<b>Time intervals (days)</b>
	<b>0</b>	<b>10</b>
IDF (insoluble dietary fibers)	11.69	17.56
SDF (soluble dietary fibers)	1.94	2.72
TDF (total dietary fibers)	13.57	19.88

Dynamics of dietary fiber content of unfermented and synbiotic microorganisms cultures fermented pollen.



No.3

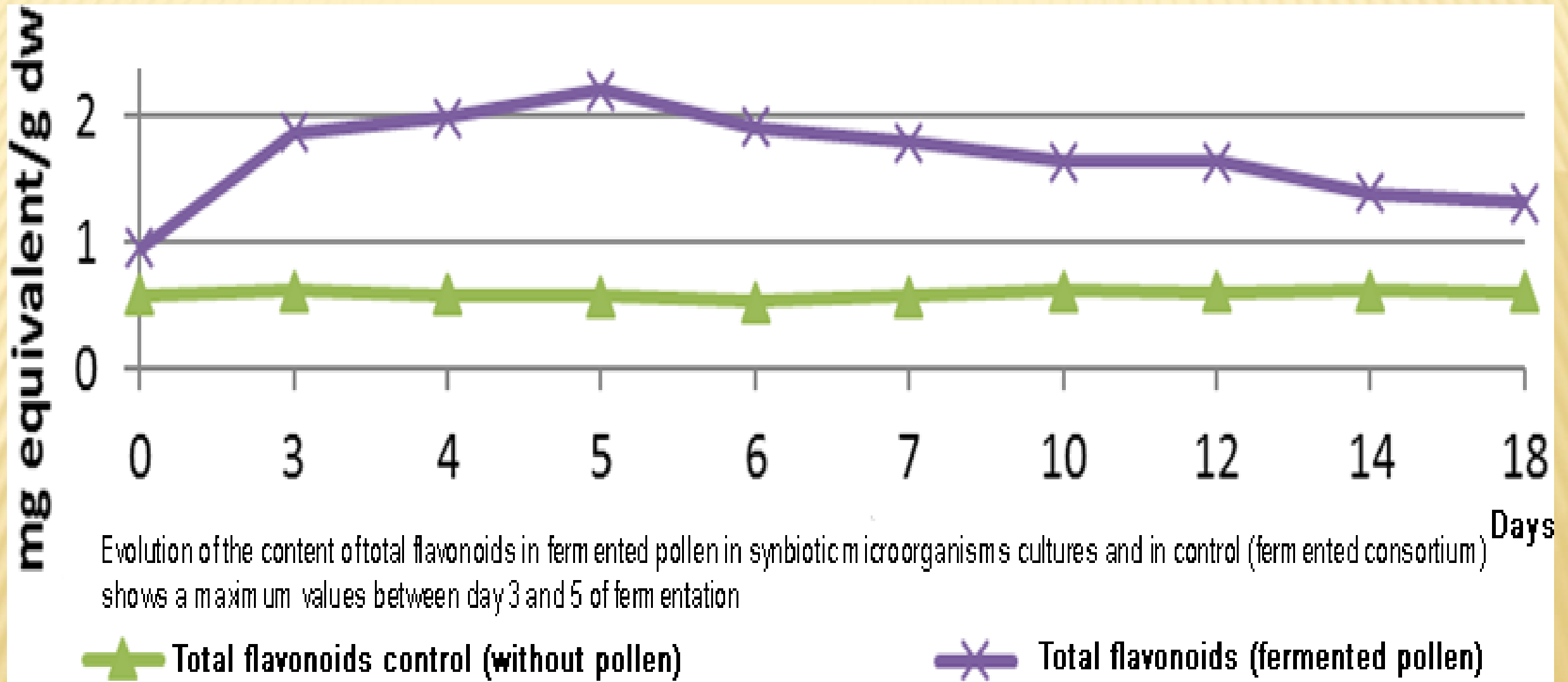
Polyfloral fermented pollen studies have also shown an increase in **flavonoids** and **total phenols** as well as **antioxidant capacity** on the fermentation pathway in the synbiotic microorganisms cultures consortium which suggested a membrane permeabilisation of the pollen granules .



HPLC determination of main polyphenolic and phenolic compounds

Compounds	pollen	control
Gallic acid	18.8±0.2	8.7±0.1
Chlorogenic acid	1.2±0.2	0.4±0.05
Caffeic acid	2.4±0.1	1.3±0.07
Coumaric acid	1.2±0.2	0.7±0.05
Ferulic acid	3.1±0.2	2.1±0.1
Rutin	2.8±0.1	1.1±0.05
Myricetin	ND	ND
Luteolin	8.0±0.2	3.7±0.2
Quercetin	1.13±0.1	0.42±0.03
Apigenin	0.06±0.01	0.03±0.004
Kampferol	2.88±0.05	1.27±0.03







## No.4

We also observed that pollen fermentation determined a considerable increase in its **antioxidant activity**, measured by DPPH and ABTS, indicating two times higher values than control. Maximum values were recorded within 5 to 7 days of fermentation.

Fementation day	TEAC (mg/gdw)*			
	ABTS		DPPH	
	Control (symbiotic microorganisms cultures consortium without pollen)	Pollen fermented in symbiotic microorganisms cultures consortium	Control (symbiotic microorganisms cultures consortium without pollen)	Pollen fermented in symbiotic microorganisms cultures consortium
0	4.20813	4.94927	0.91440	1.23189
3	3.95939	6.13069	0.92029	1.87357
4	4.20546	6.79568	0.92157	1.88122
5	<b>4.57909</b>	<b>8.16283</b>	<b>0.92825</b>	<b>2.23893</b>
6	<b>4.32401</b>	<b>7.42724</b>	<b>0.95735</b>	<b>2.02625</b>
7	<b>4.14467</b>	<b>7.37302</b>	<b>0.96451</b>	<b>1.97859</b>
10	4.02925	7.36143	0.89081	1.97774
12	3.89905	7.23261	0.86945	1.75884
14	4.01998	7.05731	0.83234	1.73515
18	4.11979	6.98437	0.81573	1.71537



No.5		Monosaccharide content (µg/ mg d.w.)						
Sample	Fermentation time (days)	D-(-)Ribose ↑	D-(+)Xylose ↑	D-(-)Arabinose ↑	D-(-)Fructose ↓	D-(+)Manose ↑	D-(+)Glucose ↓	D-(+)Galactose ↑
Fermented pollen in Kombucha vinegar	0	3.753	0.694	1.887	189.981	ND	201.271	ND
	1	2.702	1.153	2.294	172.841	ND	191.523	ND
	2	4.459	1.289	2.741	145.569	ND	185.613	ND
	3	4.637	1.047	2.918	81.100	ND	145.901	ND
	4	4.032	2.101	4.632	5.943	ND	17.784	4.615
	5	5.324	2.597	5.779	4.558	4.768	13.012	5.205
	6	5.610	2.603	5.602	3.888	9.867	12.092	5.990
	7	6.692	17.070	4.880	3.711	9.263	13.368	5.948
	8	6.508	2.672	5.282	4.371	11.934	12.362	7.310
	9	6.865	2.895	4.765	3.934	9.210	11.614	6.333
	10	9.333	2.616	4.457	3.948	11.509	11.026	6.079
Control 1 - Kombucha culture (20 days)		5.15	ND	4.76	221.84	ND	157.96	ND
Control 2 - Pollen in water (1 hour)		2.28	0.65	1.76	142.03	ND	89.73	ND
Control 2 - Pollen in water (3 days)		1.94	0.64	2.76	139.98	ND	84.52	16.58

**Ribose** 3,75 -> 9,33

Is a kind of sugar produced by the body, improving athletic performance by boosting muscle energy; improves symptoms of chronic fatigue syndrome, fibromyalgia, and coronary artery disease; prevents symptoms such as cramping, pain and stiffness after exertion in the inherited disorder (AMD deaminase deficiency, McArdie's disease).

**Xylose** 0,69 -> 2,61

Unmetabolized to a significant degree.

**Arabinose** 1,88 -> 4,45

Originally commercialized as a sweetener, arabinose is an inhibitor of sucrase, the enzyme that breaks down sucrose into glucose and fructose in the small intestine, therefore arabinose could be used in foods to attenuate the peak of glycemic response after consumption of sucrose.

**Manose** ND -> 11,50

Is used for preventing urinary tract infections UTIs

**Galactose** ND -> 6,08

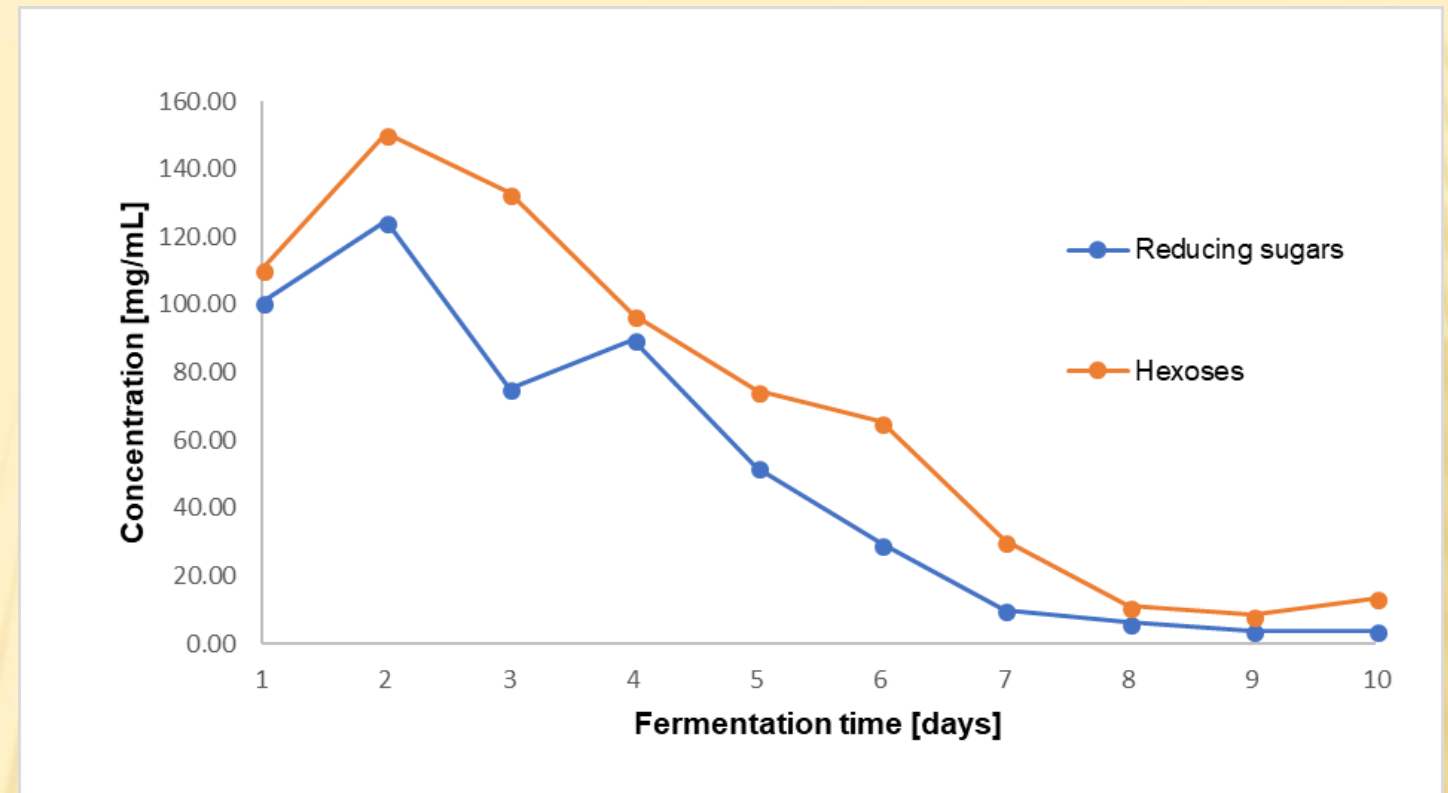
Has a role in treatment of focal segmental glomerulosclerosis.



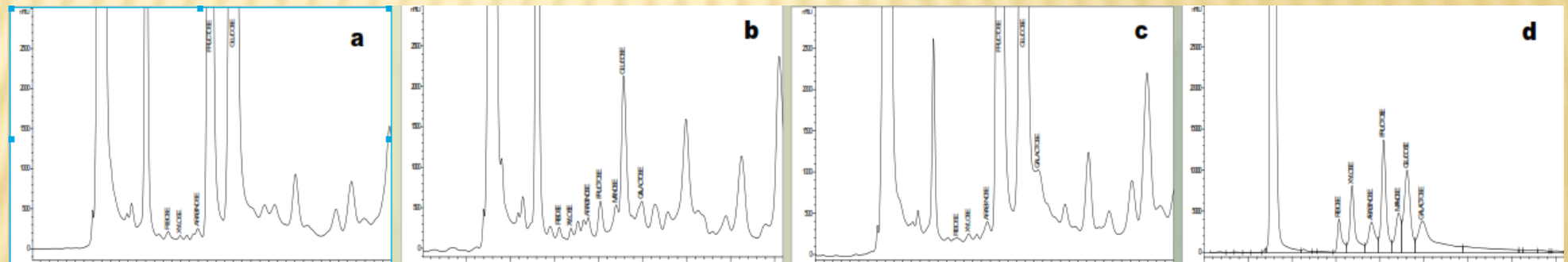
## No.6

### Evolution of the **carbohydrates** level during pollen fermentation

The carbohydrate content in the fermented pollen samples was monitored during the fermentation process and we observed a quantitative decrease of both reducing sugars and hexoses of over 90% from the start of the fermentation (day 1) until its end (day 10), due to yeast and bacteria **metabolism** from the fermented broth.



### HPLC identification of monosaccharides:



Chromatographic sample profile: a) pollen fermented in **synbiotic microorganisms cultures** (after 7 days); b) monosaccharide analytical standards (2.5 mg/mL); c) pollen fermented (after 3 days); d) pollen in water (after 3 days). Through pollen fermentation process we observed that fructose and glucose content decreased during the 10 days of experiment, that can be explained by the consumption of these monosaccharides during the consortium microorganisms metabolism.



# Certificate of Analysis Amrita IBNA Balotesti

**No.7** - The results of the **macroelement determinations** obtained for the analyzed samples.

No crt.	Nr. PRB.	Name of the sample	Ca %	Na %	K %	Mg %
1.	2095	Amrita (atomized fermented polyfloral pollen)	0.17	0.042	0.083	0.012

**No.8** - The results of the **microelement determinations**, obtained for samples under analysis.

No crt.	Nr. PRB.	Name of the sample	Fe ppm	Cu ppm	Mn ppm	Zn ppm
1.	2095	Amrita (atomized fermented polyfloral pollen)	23.69	1.69	14.06	19.39

Observations:  
Results  
expressed per  
100 g sample as  
such.

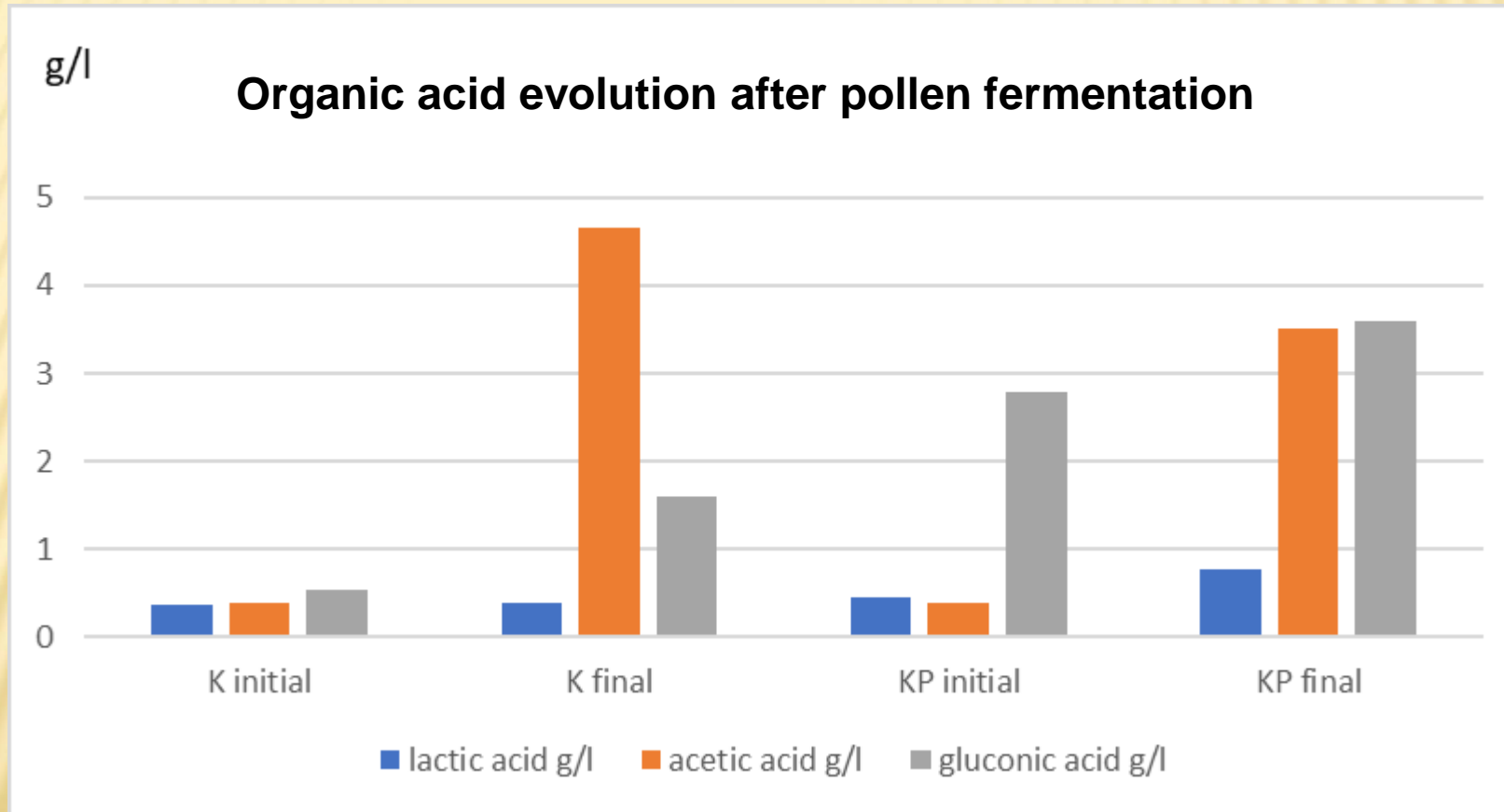
**No.9** - Profile of **amino acids** in analyzed samples g/100g.

Amino acids determined	U.M.	Analysis method	Name of the sample
			2095
			Amrita
Ac. aspartic	g/100g	HPLC-Thermo Electron Amino Acid Analyzer	0.097
Ac. Glutamic			0.091
Serina			0.060
Glicina			0.027
Treonina			0.105
Arginina			0.041
Alanina			0.081
Tirozina			0.019
Valina			0.107
Fenilalanina			0.092
Izoleucina			0.099
Leucina			0.127
Lizina			0.056
Cistina			0.010
Metionina			0.030
Total amino acids determined			1.042



## No.10

Post-biotic compounds: short-chain **fatty acids** (lactic, gluconic or acetic acid) were identified in **synbiotic microorganisms cultures** fermented products. The dynamic of short chain fatty acids generation during pollen fermentation in **synbiotic microorganisms cultures** compared to the control (**synbiotic microorganisms cultures** without pollen) is presented in the chart below. Adding pollen in **synbiotic microorganisms cultures** fermentation medium determined changes especially in the case of **lactic acid** which has increased from an average of 0.46 g/l to 0.77 g/l (almost double) due to the high level of lactic bacteria cells. The initial content in gluconic acid was higher when adding pollen (2.79 g/l), but the final content did not increased dramatically (3.59 g/l).



Evolution of different organic acids content during **synbiotic microorganisms cultures** (K) fermentation (18 days) and cultures and Pollen (KP) maceration (7 days)



**No.11** - Quantitative determination of fatty acids (expressed in g/100 g of fat).

Specification Fatty acids		Amrita (atomized fermented polyfloral pollen)
	U/M g% g GB	
Capric acid C10:0		1.72
Caprylic acid C10:0		1.66
Myristic acid		3.16
Pentadecanoic C15:0		1.27
Palmitic acid C16:0		22.49
Palmitoleic acid C16:1		0.54
Stearic acid C18:0		7.86
Cis oleic acid C18:1n9		22.32
Linoleic acid a C18:4n3		36.26
Linolenic acid a C18:3n3		1.71
Octadecatetraenoic C18:4n3		1.01
Other fatty acids		0.00

**No.12** - The coupling of the analytical results on the groups of **saturated fatty acids (SFA)**, **monounsaturates (MUFA)**, **polyunsaturates (PUFA)** and **total unsaturated fatty acids (UFA)** .

Specification	<u>SFA</u> Saturated fatty acids g% g GB	<u>MUFA</u> Monounsaturated fatty acids g% g GB	<u>PUFA</u> Polyunsaturated fatty acids g% g GB	<u>UFA</u> Total unsaturated fatty acids g% g GB	SFA/UFA	PUFA/MUFA
Amrita (atomized from polyfloral pollen)	38.16	22.86	38.98	61.84	0.617	1.705

**No.13** - The composition in **Ω 3** and **Ω 6** as well as their **ratio Ω6/Ω3**

Specificatie	Ω3 Polyunsaturated fatty acids	Ω6 Polyunsaturated fatty acids	Ω6/Ω3
Amrita (atomized from polyfloral pollen)	2.72	36.26	13.33



## No.14 Organic acid chemical profile in Kombucha fermented pollen

*Chromatographic determination of organic acids by HPLC.* For this determination we used an Agilent HPLC 1200 system (Agilent USA), comprising a DAD detector, quaternary pump, and a thermostatic auto sampler. A Luna 2 Phenomenex (size:  $\Phi$  4.6 x 150 mm) column served as a stationary phase at 25°C. The used mobile phase was: (A) 10mM  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  (pH  $2.50 \pm 0.02$ ) and (B) acetonitrile. The elution was performed using an isocratic method with 90% (A) and a variable flow:  $0.6 \text{ ml min}^{-1}$  until 1.5 min,  $0.4 \text{ ml min}^{-1}$  at 2 min,  $0.25 \text{ ml min}^{-1}$  from 3 to 15 min,  $0.3 \text{ ml min}^{-1}$  at 16 min,  $0.6 \text{ ml min}^{-1}$  to 17 min,  $1 \text{ ml min}^{-1}$  from 18 to 23min,  $0.6 \text{ ml min}^{-1}$  until the 25<sup>rd</sup> minute in order to establish an optimum separation. The injection volume was 10  $\mu\text{l}$  and the absorbance was monitored at 220nm. Collected samples were centrifuged at 10000 rpm and then filtered (with  $0.45 \mu\text{m}$  RC membrane). All samples were injected using a 1:10 (v:v) dilution. The separation of organic acids was achieved in 25 minutes and identification was made by comparing their retention times with those of standards using a Chemstation software. The elution order and the content of organic acids in fermented pollen is summarized in Table 1) Organic acid content in fermented pollen.

Organic acids [g/l]	Fermentation time [days]										
	0	1	2	3	4	5	6	7	8	9	10
Oxalic	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tartaric	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Malic	nd	1.661	2.132	2.125	2.328	1.896	1.869	1.815	1.512	1.616	1.327
<b>Formic</b>	0.518	0.620	0.768	1.093	<b>1.361</b>	0.928	0.793	0.920	0.886	0.956	0.824
Lactic	0.32	0.93	1.13	1.12	0.51	0.80	0.44	0.43	0.67	0.44	1.15
Citric	0.318	nd	nd	0.727	0.809	nd	nd	nd	nd	nd	nd
Maleic	0.000	0.663	0.919	0.652	0.831	0.904	0.873	0.957	0.971	1.159	1.033
<b>Acetic</b>	1.948	1.929	2.031	2.017	2.052	2.018	1.983	1.992	2.027	<b>2.089</b>	2.002
Succinic	0.514	0.883	1.318	1.222	1.678	1.667	1.482	1.847	1.686	1.948	1.489
<b>Propionic</b>	0.276	0.094	0.233	0.429	0.524	0.189	0.661	0.579	0.303	<b>1.123</b>	0.180
<b>Butiric</b>	1.785	1.088	1.213	1.150	1.252	1.179	1.205	1.244	1.118	<b>1.764</b>	1.130
<b>TOTAL</b>	6.271	10.702	13.315	<b>14.083</b>	12.622	11.942	10.344	10.755	11.045	12.138	12.766

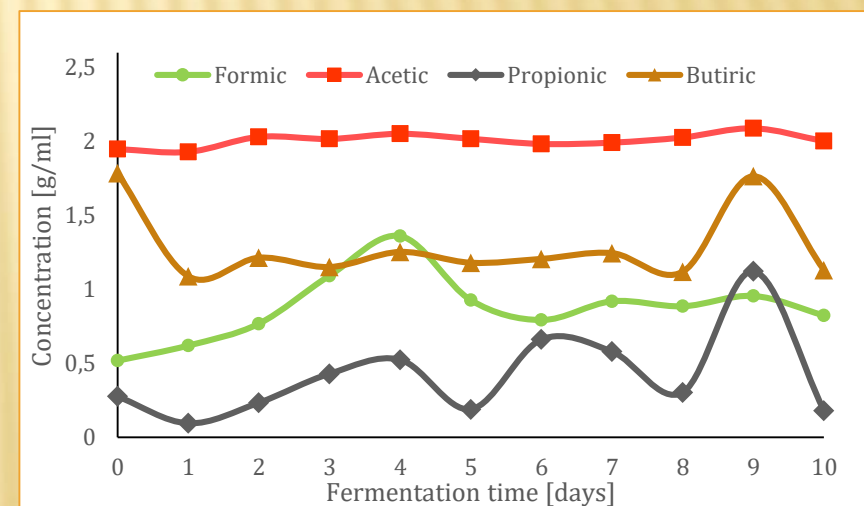


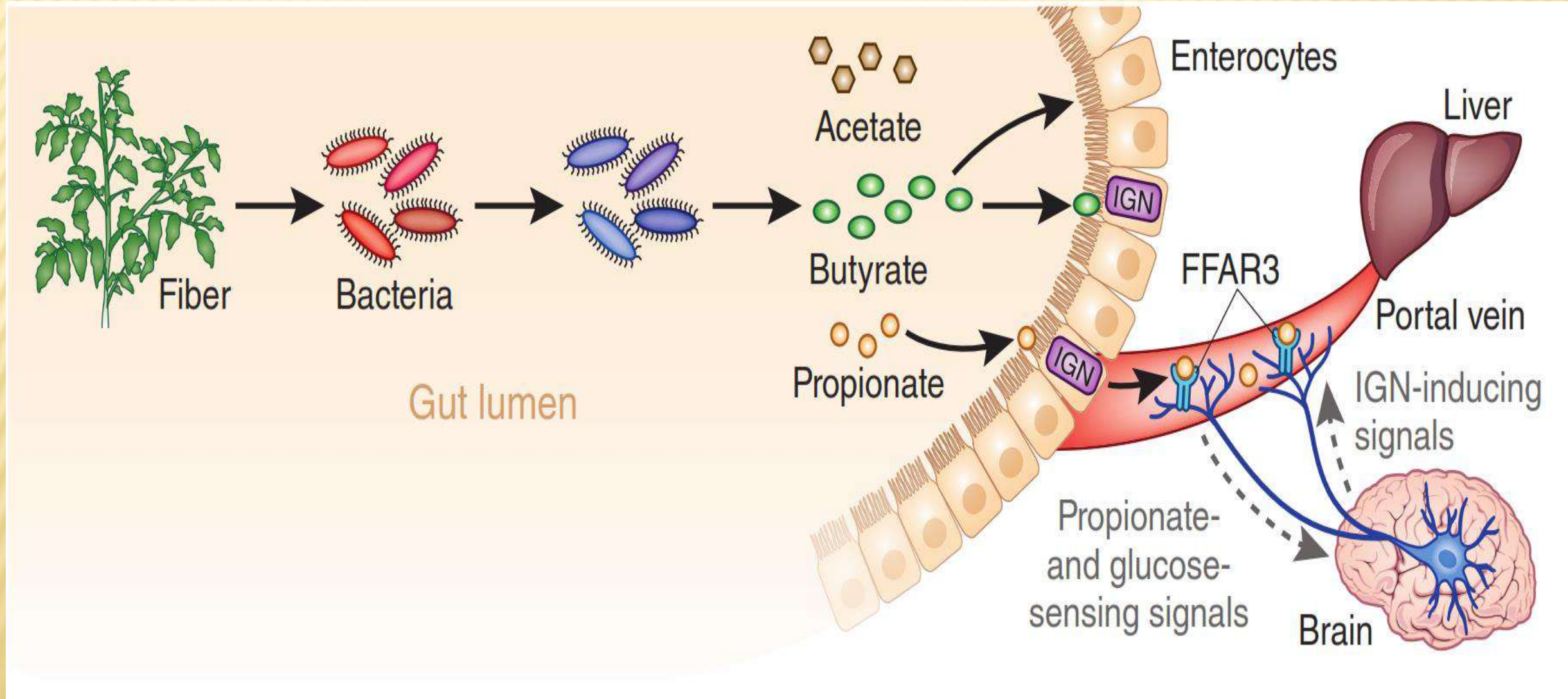
Figure 1. Principal short chain fatty acids dynamics during fermentation process



Very important are acetic, butyric and propionic acids as a result of the fermentation of the vegetal fibers by the bacteria in the gut lumen.

**Nature Biotechnology** - You are what you eat - Scott J. Parkinson

Fibers promote health by changing the composition and metabolic products (postbiotics=short chain fatty acids - SCFA) of gut bacteria.





Prebiotics (plant fibers) are manipulating gut microbiota through fermentation of the oligosaccharides into SCFA (acetate, propionate and butyrate); SCFA influence lipogenesis and cholesterol biosynthesis and gluconeogenesis and also they act like signaling molecules; they improve glucose tolerance. SCFA involves gut-brain neural communication and gut-lung axis (reducing inflammation in allergies) SCFA for sure exert beneficial effects on tissues other than the gut and the brain; intentional modulation of the gut microbiota is a valid strategy for improving human health.

**Gut microbiota (GM) can be considered an "interface" between human body and the Universe/God; GM leads us physically and mentally.**



# Conclusions:

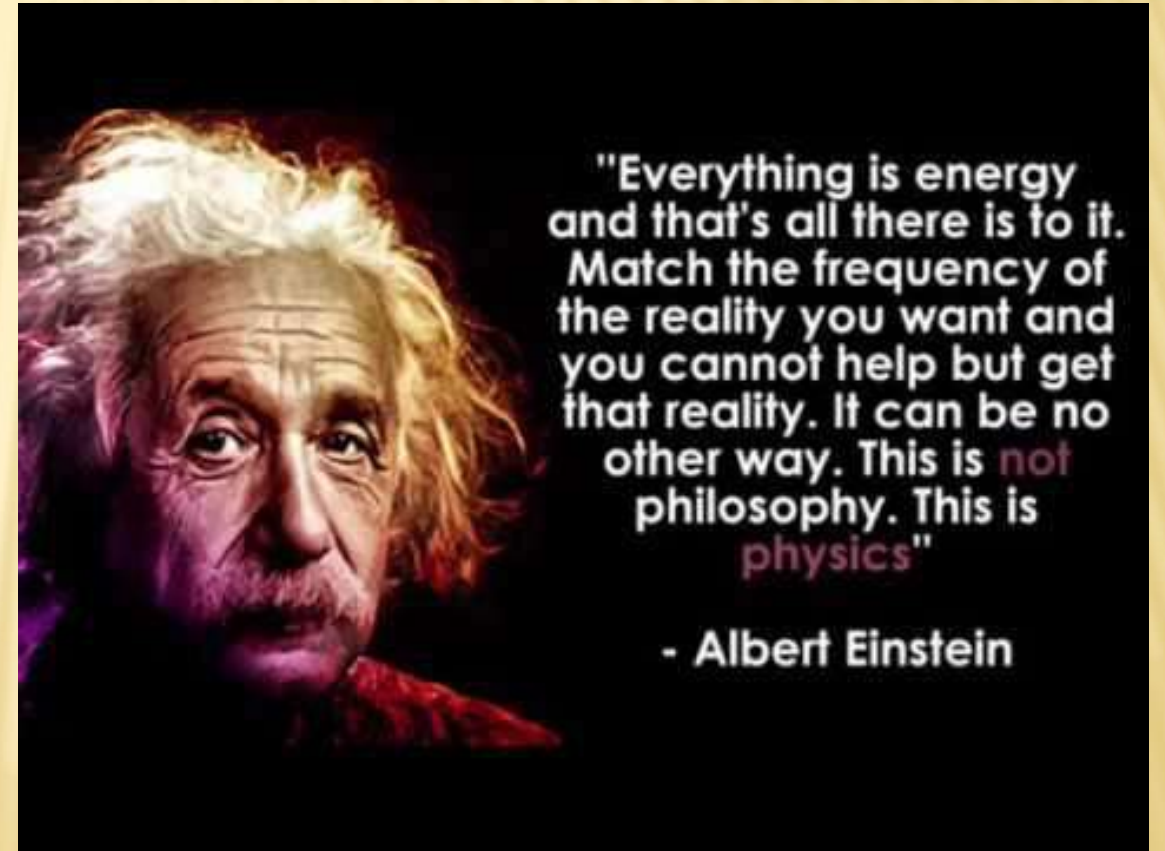
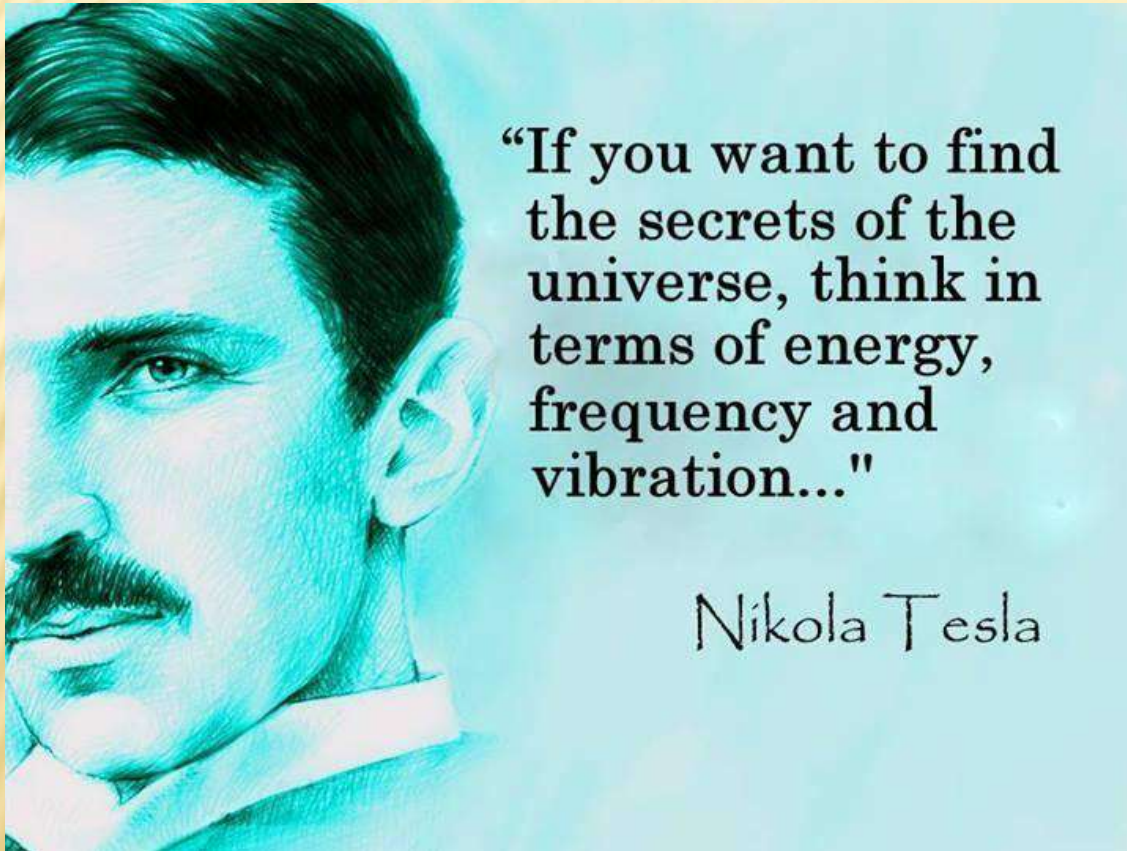
The results of our studies prove the tri-biotic potential effect of fermented pollen in **synbiotic microorganisms cultures** the **prebiotic** effect conferred by the bioactive carbohydrates and fibers (especially insoluble fibers) content, the **probiotic** effect due to the live beneficial microorganisms from **synbiotic microorganisms cultures** and the **postbiotic** effect that is determined by the probiotic microorganisms metabolism products (short chain fatty acids).

**Bee pollen fermentation biotechnology with synbiotic microorganisms cultures is a dynamic process which increases the bioavailability of pollen compounds and adds specific bioactive compounds of the cultures in the final product, generating a complex nutraceutical and dermatological product.**

Acknowledgments: This study was supported by Romanian National Program PN III, Contract 6BG/2016, 3 - BIOTIC



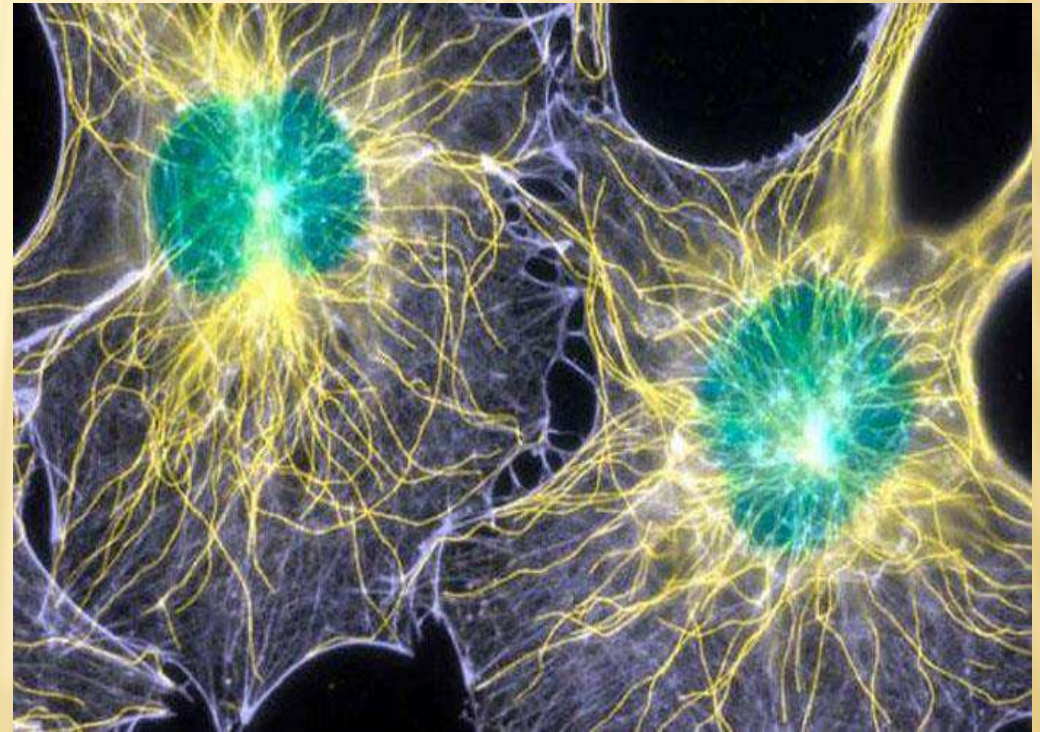
## IV. The Energetic characteristics of the Amrita product.





# Everything in the universe is energy

Energetic medicine gains ground, both in diagnosing patients and treating them. Current technologies increasingly use the theories of quantum physics, and new research shows that including neural communication is through bio-photons emissions.





That is why we made determinations of the energy of Amrita raw material and of the patients who consume it

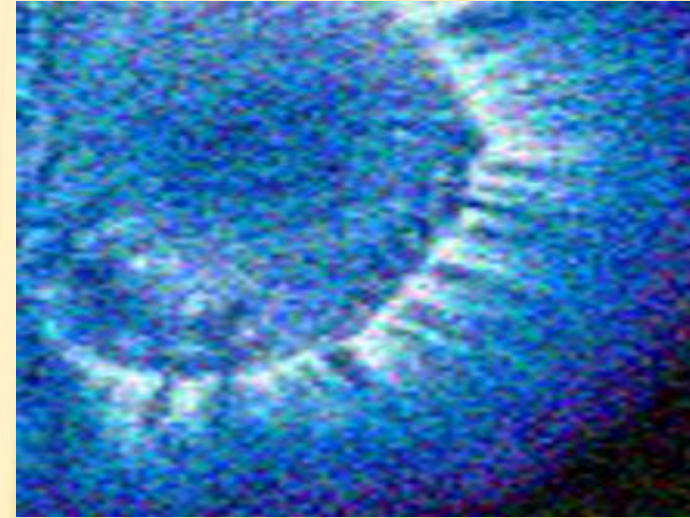
## Energy proved by Kirlian Electrophotography

we used Amrita powder ("living matter") and we compared it with talc ("dead matter").

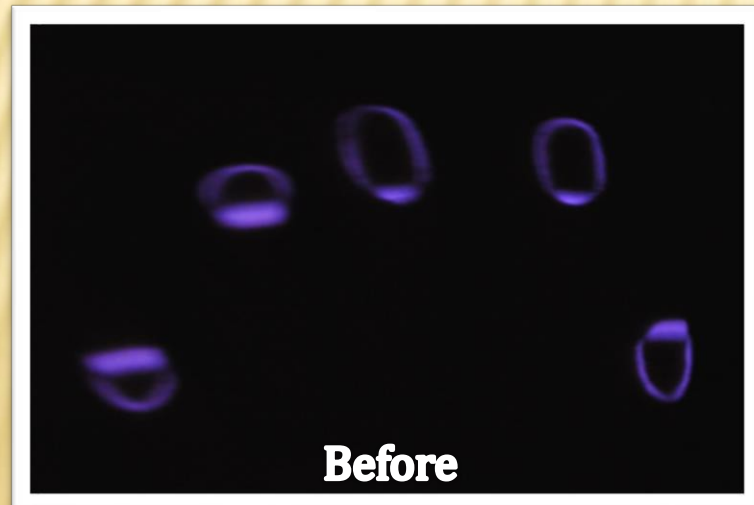
Solution 72% powder of Talcum



Solution 72% powder of Amrita®

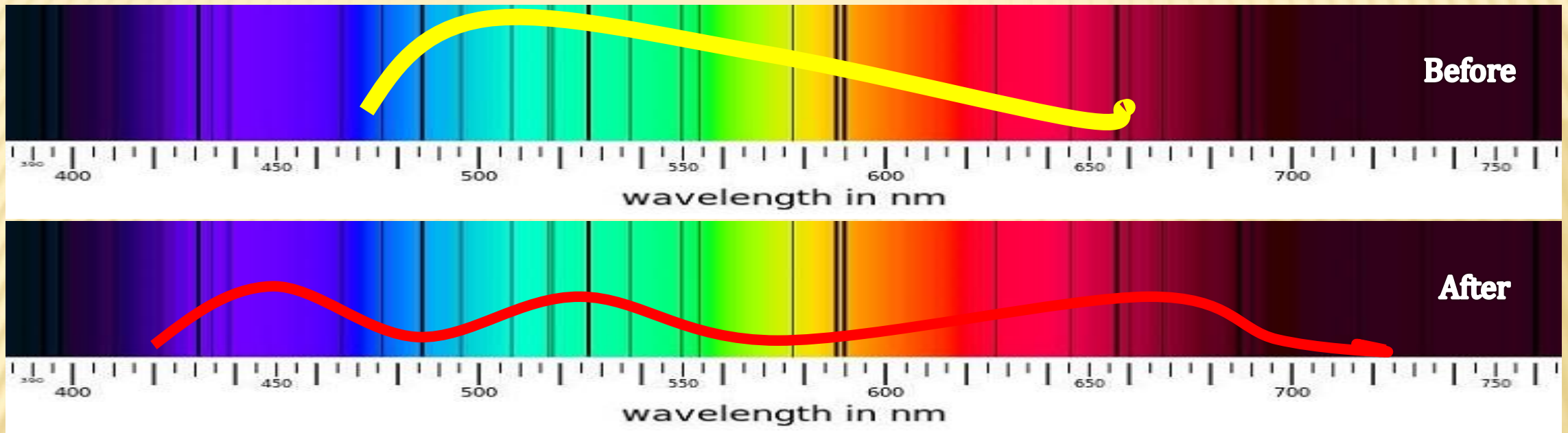


patient M.A. 36 years old, M,  
adm 2cps Amrita





## Spectrometry Kirlian, patient A.E. 48 years old, F, adm. of 2 cps of Amrita®



We have a different emission as waveform and a higher range of wavelengths.

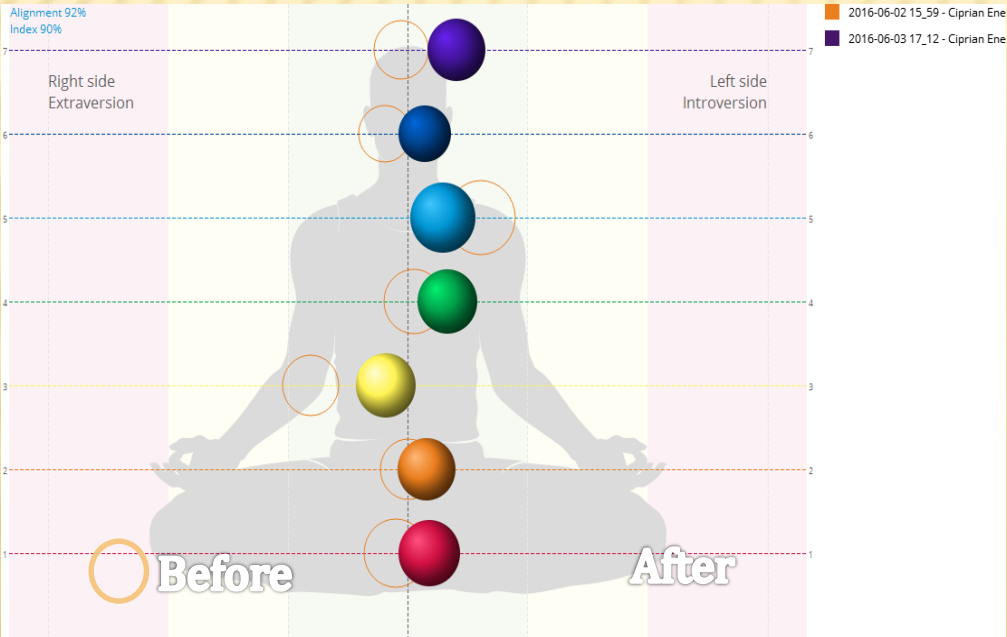


Bioresonance with the Bio-Well, ORMA and SYSTEM NLS METATRON in different clinics, shows after the administration of Amrita a control of entropy at all levels (DNA, mitochondria, cell membrane, tissues, organs) with a percentage varying between 67-82% :

a) the improving of the gut microbiota.

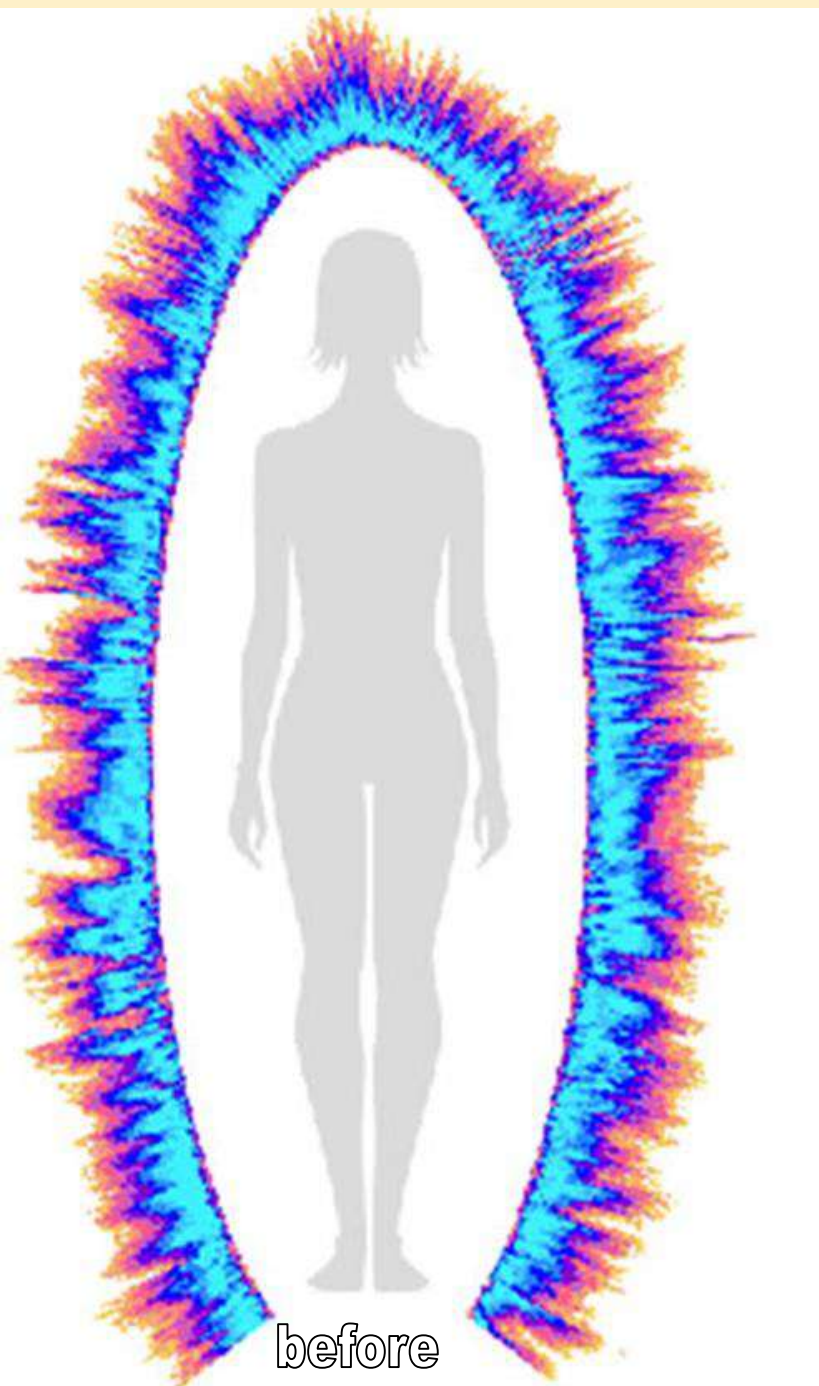
(Large Intestine Function) Analysis Report Card			
Name: Zmaranda Elena		Sex: Female	Age: 58
Figure: Slightly heavier than the(158cm,60kg)		Testing Time	2016-07-05 0:45
Actual Testing Results			
Testing Item	Normal Range	Actual Measurement Value	Testing Result
Intestinal bacteria coefficient	1.734 - 2.621	1,35	<div><div></div></div>
Intraluminal pressure coefficient	1.173 - 2.297	3,121	<div><div></div></div>

(Large Intestine Function) Analysis Report Card			
Name: Zmaranda Elena		Sex: Female	Age: 58
Figure: Severe partial fat(158cm,65kg)		Testing Time	2016-08-08 0:54
Actual Testing Results			
Testing Item	Normal Range	Actual Measurement Value	Testing Result
Intestinal bacteria coefficient	1.734 - 2.621	2,04	<div><div></div></div>
Intraluminal pressure coefficient	1.173 - 2.297	2,118	<div><div></div></div>

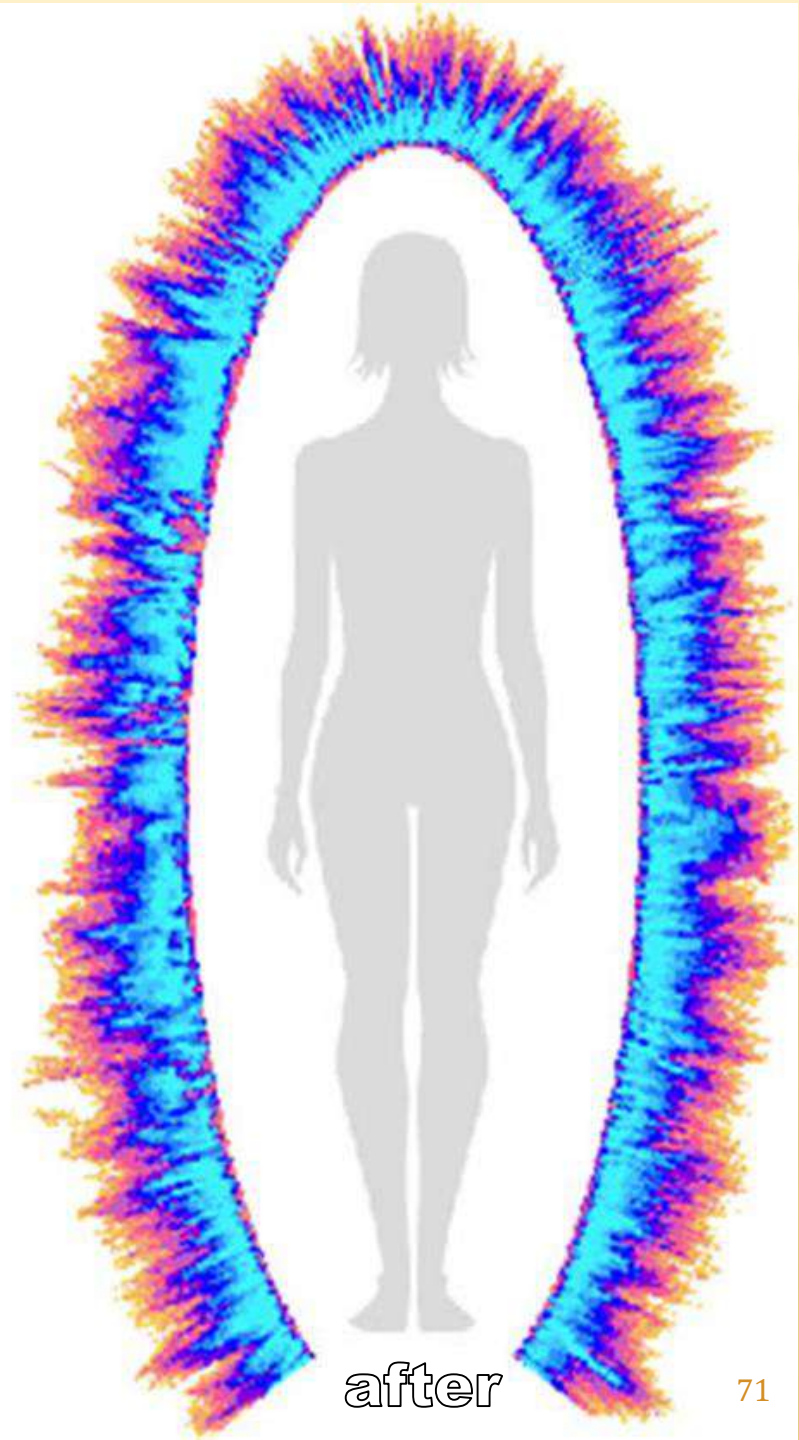


b) better alignment of Chacras





c) Improvement of the human aura.





d) Improvement of the energetic status especially in **nervous** and **immune system**

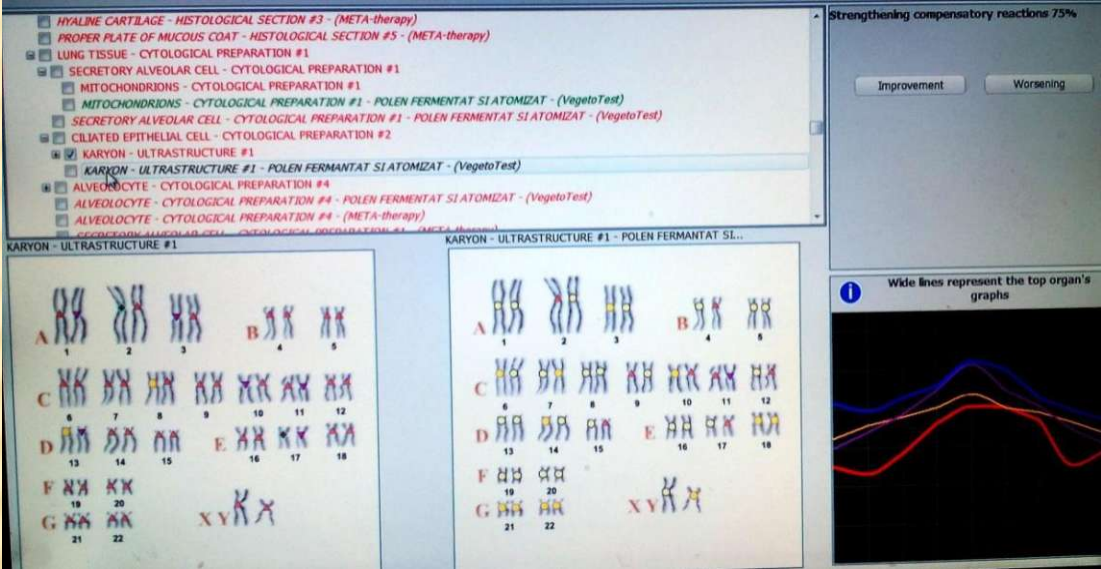
Organs and systems	
▶	Head energy (4.61)
▶	Cardiovascular system energy (4.00)
▶	Respiratory system energy (4.93)
▶	Endocrine system energy (4.32)
▶	Musculoskeletal system energy (4.80)
▶	Digestive system energy (4.49)
▶	Urino-genital system energy (5.04)
▶	Nervous system energy (3.68)
▶	Immune system energy (3.66)

Organs and systems	
▶	Head energy (4.75)
▶	Cardiovascular system energy (4.46)
▶	Respiratory system energy (4.73)
▶	Endocrine system energy (4.71)
▶	Musculoskeletal system energy (4.66)
▶	Digestive system energy (4.70)
▶	Urino-genital system energy (4.80)
▶	Nervous system energy ( <u>4.83</u> )
▶	Immune system energy ( <u>4.93</u> )

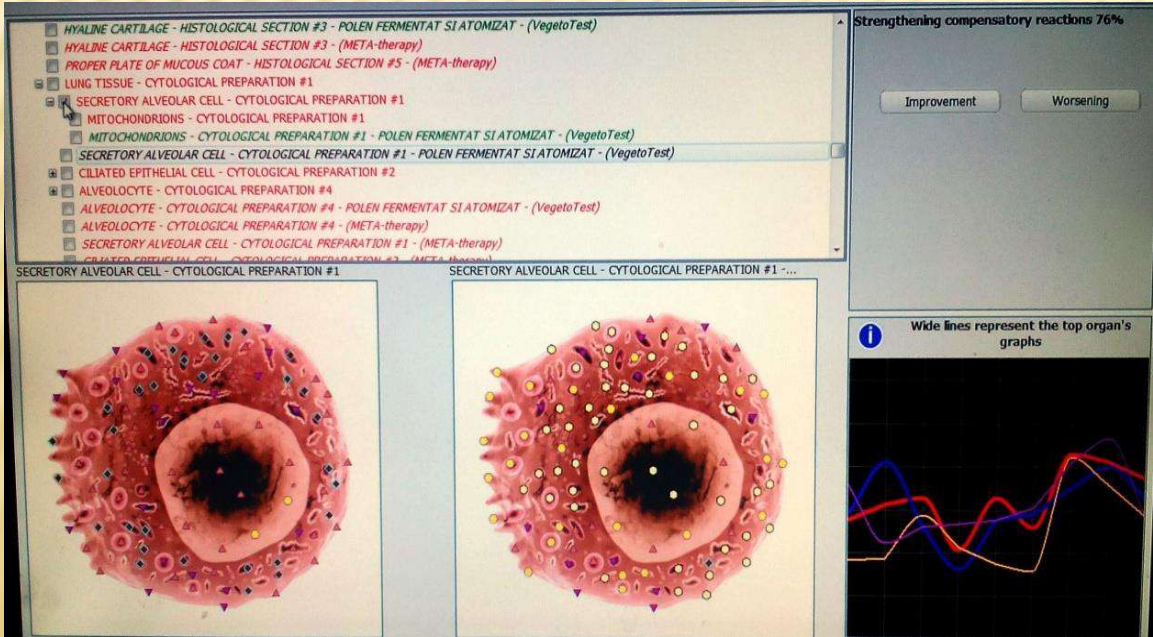
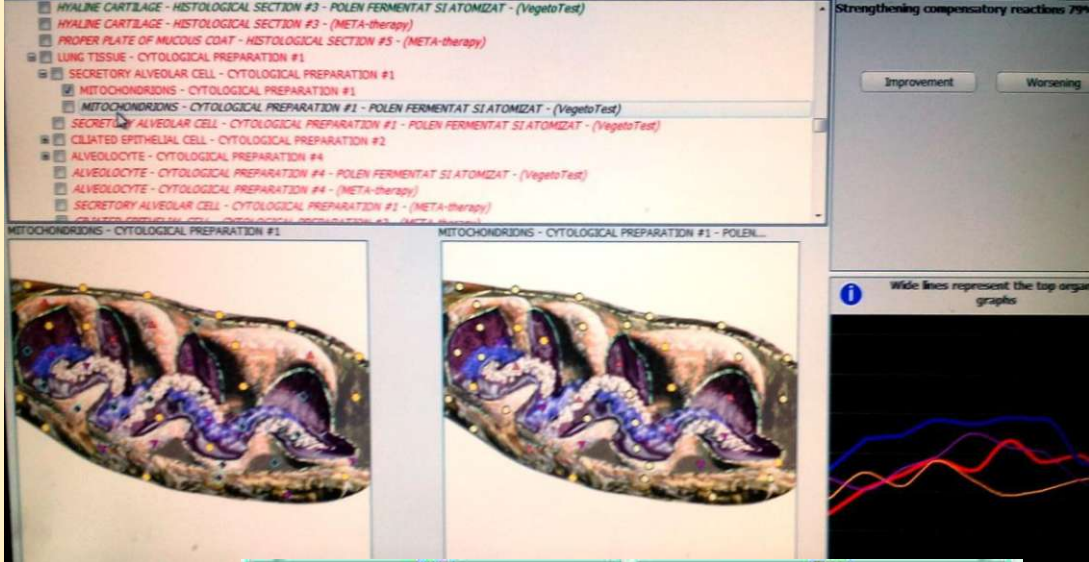


e) Amrita is beneficial on all organs and structure levels examined (improvement from 67-82%), concerning the entropic status.

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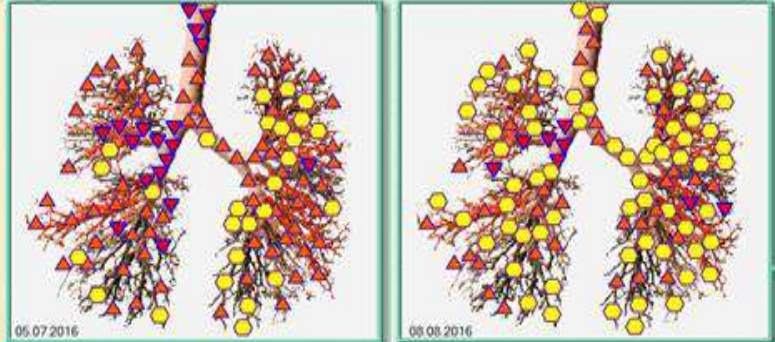


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**Abstract:** The bioavailability for humans of the pollen bioactive compounds is limited. Our aim was 19 to enhance the health-related benefits of the pollen by fermentation with a Kombucha / SCOBY 20 (symbiotic culture of bacteria and yeasts) consortium. We performed the fermentation of pollen 21 suspended from the beginning with SCOBY on sweetened green tea or on Kombucha vinegar, by 22 adding pollen after 20 days of Kombucha fermentation. We analyzed: formation of bioactive 23 compounds (anti-oxidant polyphenols, soluble silicon, hydroxy-acids, short chain fatty acids - 24 SCFA); parameters related to Kombucha fermentation (dynamics of lactic acid bacteria - LAB, 25 formation of organic acids, soluble sugar evolution on Kombucha vinegar); the influence of 26 Kombucha fermentation on pollen morphology and ultrastructure; *in vitro* cytotoxic and 27 antitumoral effects of the Kombucha fermented pollen. *The pollen addition increases LAB 28 proportion in the total number of SCOBY microbial strains. SEM images highlight the adhesion of 29 the bacteria from SCOBY to pollen. Ultrastructural analysis reveals the release of the pollen content. 30 The content of bioactive compounds (anti-oxidant polyphenols, soluble silicon species and SCFA) 31 is higher in the fermented pollen and the product shows a slight antitumoral effects on Caco-2 cells. 32 The health benefits of pollen are enhanced by fermentation with a Kombucha consortium.*

**Conclusions:** The pollen fermentation with Kombucha leads not only to enhanced bioavailability of pollen 632 phytonutrients. Pollen was already demonstrated to be a good fermentation activator for mead and 633 white wine. Our data presented here show that pollen is also a good activator of Kombucha / SCOBY 634 fermentation. Addition of the bee collected pollen improves Kombucha fermentation and the 635 formation of Kombucha health-related compounds. At the end of the pollen fermentation with 636 Kombucha consortium, a product with enhanced health benefits is formed, with complementary 637 bioactive ingredients.



**Amrita is already used  
in various nutritional  
supplements and  
veterinary products**





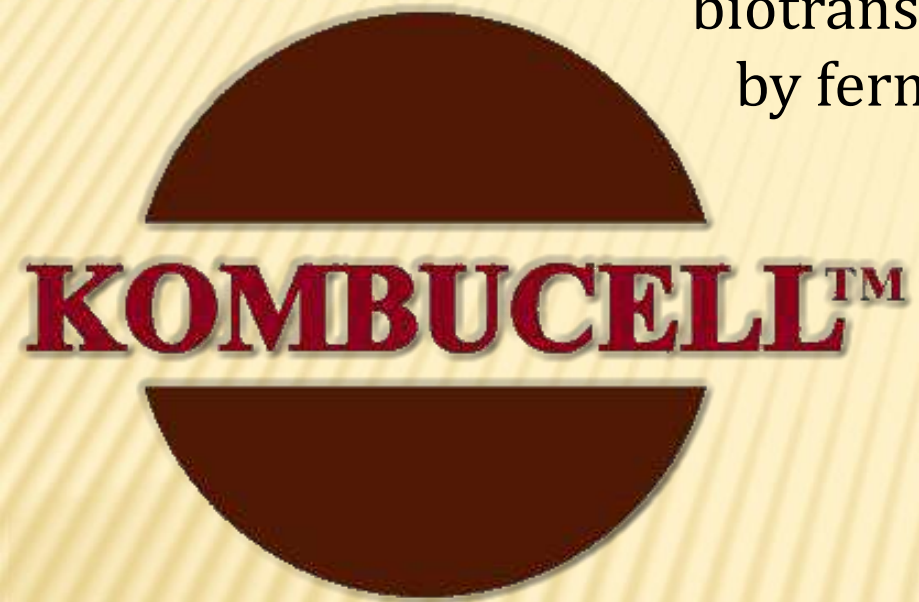
Finally we will present the range of human & veterinary products (for external and internal use) containing Amrita.

## Veterinary Supplements





Range of food supplements  
3xbiotics, containing amrita and  
other combinations of  
biotransformed medicinal plants  
by fermentation in kombucha







# Thank you!

**For further information please contact us:**

**Tel: +4021.351.47.77 ~ e-mail: [office@pro-natura.ro](mailto:office@pro-natura.ro) ~ [www.pro-natura.ro](http://www.pro-natura.ro)**