Biofilms in biotechnology

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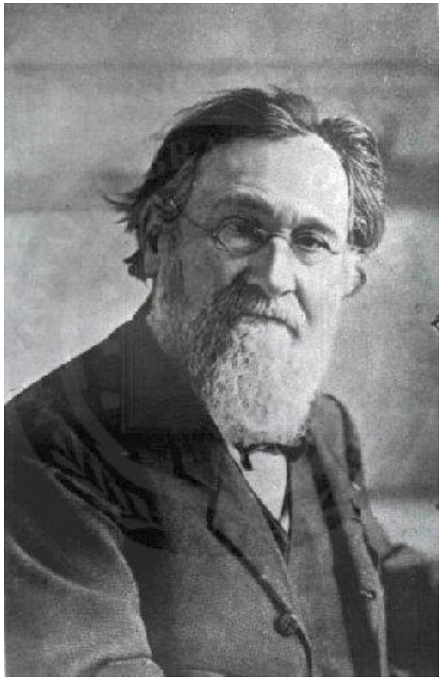
Probiotics - Definitions

Published Definition	Reference
... alter the microflora (by [illegible]) in a compartment of the host and by that exert beneficial health effects	... de Vrese 2001
Live microorganisms which when administered in adequate amounts confer a health benefit on the host.	FAO/WHO report, October 2001

Live microorganisms which when administered in adequate amounts confer a health benefit on the host.



- Scientific basis of probiotics’ application was created by Ilya Iljich Mechnikov (NP 1908).

  The probiotic yogurt featuring his portrait is marketed in Russia.

- The major factor limiting broad use of probiotics in agriculture is the high cost of manufacturing, which is determined by several features of the modern "western" technology.
- One of the major cost-building factors is the growth of microorganisms in sterile liquid medium followed by lyophilization.

Research team 2015



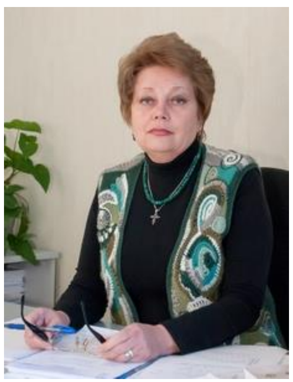
Russia

Georgia

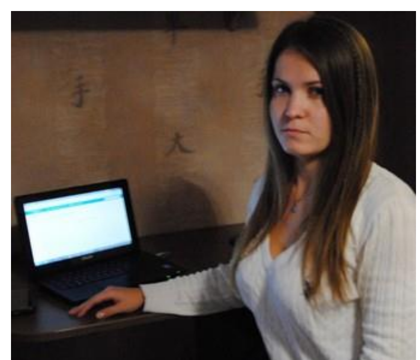
USA

Research team 2017

Russia



Georgia



USA

Everything new is well forgotten old

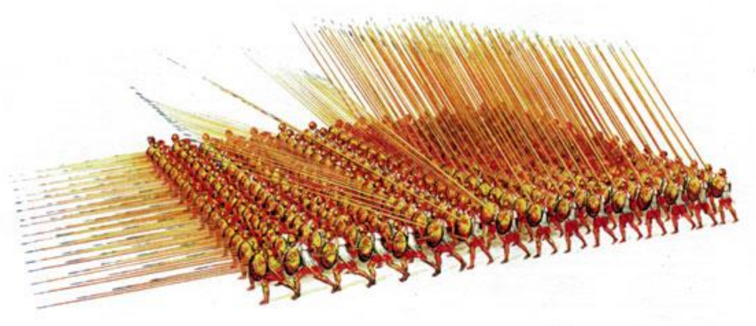
- Centuries-old experience of “traditional” biotechnology of Southeast Asia convinces, that products with high titres of probiotic microorganisms can be received with the help of less power-intensive and expensive technological decisions.

Everything new is well forgotten old

- In warmer and humid climate countries, most products contain predominantly Gram-positive bacteria such as *Bacillus subtilis* and *Bacillus licheniformis*.

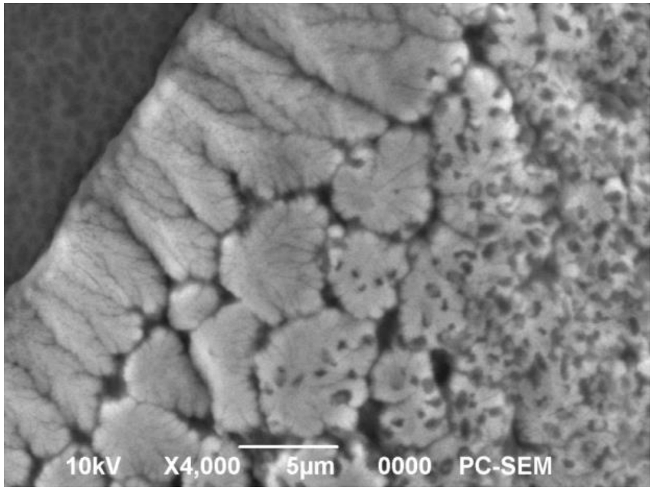
- Japanese ethnic product Natto, for example, contains monoculture of *Bacillus subtilis*.

Everything new is well forgotten old

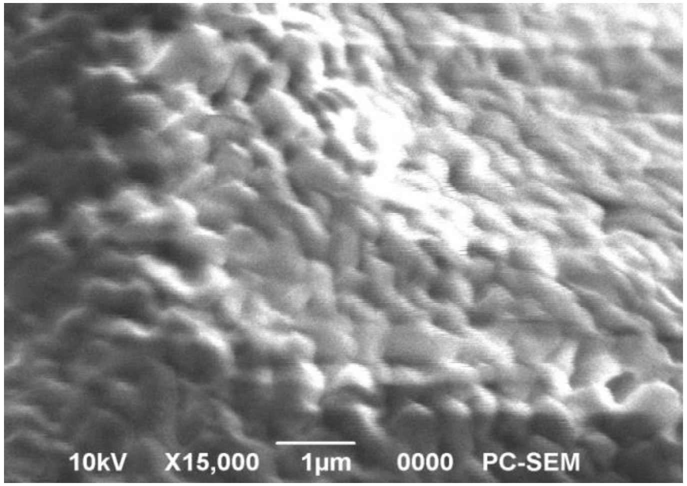


Utilization of biofilm-associated growth is the key biotechnological approach determining efficiency of “traditional” technologies largely overlooked by the modern industry.

Biofilms



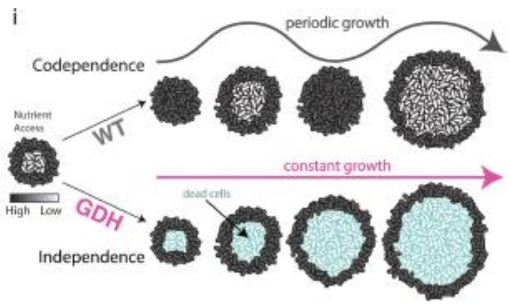
a



b

a – generation of *mushroom-like structures and channels by Vibrio fischeri NB15*

b - ordered location of bacteria in biofilm



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Metabolic codependence gives rise to collective oscillations within biofilms

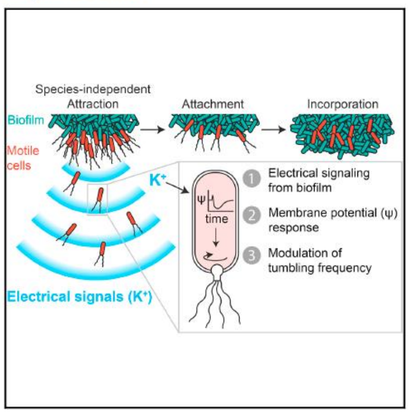
Jintao Liu[1], Arthur Prindle[1,*], Jacqueline Humphries[1,*], Marçal Gabalda-Sagarra[2,*], Munehiro Asally[3,*], Dong-yeon D. Lee[1], San Ly[1], Jordi Garcia-Ojalvo[2], and Gürol M. Süel[1]

Nature. 2015 November 5; 527(7576): 59–63. doi:10.1038/nature15709.

Ion channels enable electrical communication within bacterial communities

Arthur Prindle[1], Jintao Liu[1,*], Munehiro Asally[2,*], San Ly[1], Jordi Garcia-Ojalvo[3], and Gürol M. Süel[1,#]

Graphical Abstract



Highlights

- Electrical signaling within biofilms attracts distant motile cells
- Attraction is caused by membrane-potential-dependent modulation of tumbling frequency
- Electrical signaling is generic, resulting in species-independent attraction
- Attraction leads to incorporation of diverse species into a pre-existing biofilm

Cell 168, 200–209, January 12, 2017

Jacqueline Humphries,[1] Liyang Xiong,[2] Jintao Liu,[1] Arthur Prindle,[1] Fang Yuan,[1] Heidi A. Arjes,[3,4] Lev Tsimring,[5,6] and Gürol M. Süel[1,5,6,7,8,*]

Species-Independent Attraction to Biofilms through Electrical Signaling

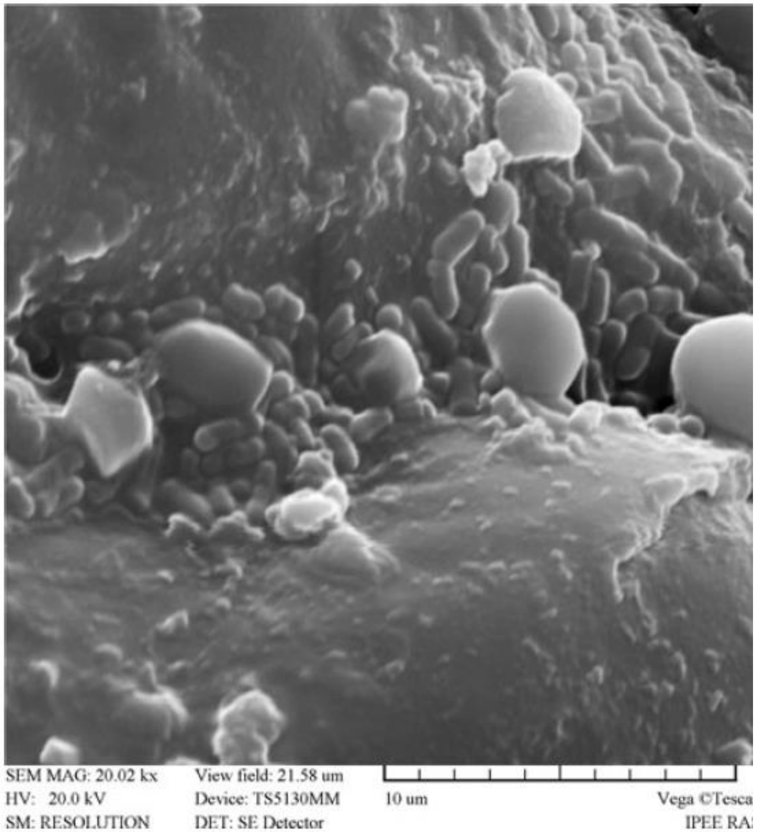


Fig. 4 Inoculated wheat bran, 48-h cultivation, ×20,000. Visib biofilm of *Lact. plantarum* 8-RA-3

Biofilm formation of pathogenic bacteria is a serious problem.

However, as the research carried out by Vyacheslav Melnikov and his colleagues has shown, probiotic microorganisms combined in a biofilm are able to fight against pathogens much more efficiently as compared to the probiotics grown in a liquid culture.

Probiotics & Antimicro. Prot.
DOI 10.1007/s12602-012-9106-y

Properties of the Probiotic Strain *Lactobacillus plantarum* 8-RA-3 Grown in a Biofilm by Solid Substrate Cultivation Method

Nina A. Ushakova · Vyacheslav M. Abramov · Valentin S. Khlebnikov · Alexandr M. Semenov · Boris B. Kuznetsov · Anna A. Kozlova · Alexey V. Nifatov · Vadim K. Sakulin · Igor V. Kosarev · Raisa N. Vasilenko · Marina V. Sukhacheva · Vyacheslav Melnikov

Probiotics species

Lactobacillus species

- *Lb. acidophilus*
- *Lb. amylovorus*
- *Lb. brevis*
- *Lb. casei*
- *Lb. casei ssp. rhamnosus*
- *Lb. crispatus*
- *Lb. delbrueckii* ssp. *bulgaricus*
- *Lb. fermentum*
- *Lb. gasseri*
- *Lb. helveticus*
- *Lb. johnsonii*
- *Lb. lactis*
- *Lb. paracasei*
- *Lb. plantarum*
- *Lb. reuteri*

Bifidobacterium species

- *Bf. adolescentis*
- *Bf. animalis*
- *Bf. bifidum*
- *Bf. breve*
- *Bf. infantis*
- *Bf. lactis*
- *Bf. longum*

Other species

- *Bacillus cereus*
- *Clostridium botyricum*
- *Enterococcus faecalis*
- *Enterococcus faecium*
- *Escherichia coli*
- *Lactococcus lactis ssp. cremoris*
- *Lactococcus lactis ssp. lactis*
- *Leuconostoc mesenteroides ssp. dextranicum*
- *Pediococcus acidilactici*
- *Propionibacterium freudenreichii*
- *Saccharomyces boulardii*
- *Streptococcus salivarius ssp. thermophilus*

Cited by W.H. Holzapfel and U. Schillinger, Introduction to pre- and probiotics, Food Research International 35 (2002), pp. 109–16

Why Bacilli?

- Adaptability to diverse conditions
- Long shelf life
- Found in the normal intestinal flora [1, 2, 3]
- Capable of germinating and re-sporulating in the gastrointestinal tract
- Becoming more prevalent in livestock applications, especially in the poultry industry[2, 4, 5]

[1]Barbosa et al., 2005. Appl Environ Microbiol 71:968–978
[2]Cartman et al., 2008. Appl Environ Microbiol 74:5254–5258
[3]Tam et al., 2006. Bacteriol 188:2692–2700
[4]Cutting, 2011. Food Microbiol 28:214-220
[5]Hong et al., 2005. FEMS Microbiol Rev 29:813–835

May/June 2014 Volume 2 Issue 3 e00633-14 Genome Announcements

Draft Genome Sequence of *Bacillus amyloliquefaciens* B-1895

Andrey V. Karlyshev,[a] Vyacheslav G. Melnikov,[b] Vladimir A. Chistyakov[c]

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May/June 2014 Volume 2 Issue 3 e00619-14 Genome Announcements

Draft Genome Sequence of *Bacillus subtilis* strain KATMIRA1933

Andrey V. Karlyshev,[a] Vyacheslav G. Melnikov,[b] Michael L. Chikindas[c,d]

School of Life Sciences, Faculty of Science, Engineering and Computing, Kingston University, Kingston upon Thames, United Kingdom[a]; International Science and Technology Center, Moscow, Russia[b]; School of Environmental and Biological Sciences, Rutgers State University, New Brunswick, New Jersey, USA[c]; Astrabiol LLC, Highland Park, New Jersey, USA[d]

Solid-phase cultivation of probiotic cultures

- **The soybeans covered with *Bacillus amyloliquefaciens* B-1895 biofilm.**

The virtue of simplicity

Simple process of inoculation and growth of microbial biofilm.

Soybean coated with B1896 biofilm

Soy-beans inoculated with the bacilli starter

Finished product

Study of the formulation's efficacy

Note Bioscience of Microbiota, Food and Health Vol. 34 (1), 25–28, 2015

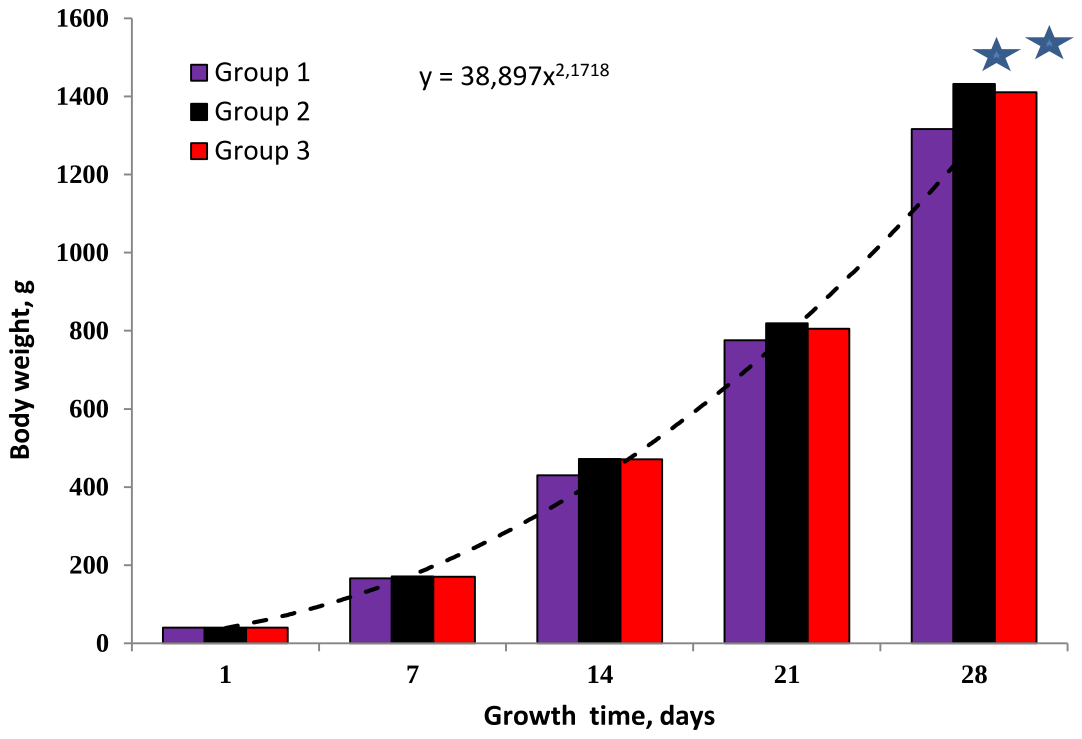
Poultry-beneficial solid-state *Bacillus amyloliquefaciens* B-1895 fermented soybean formulation

Vladimir CHISTYAKOV[1], Vyacheslav MELNIKOV[2], Michael L. CHIKINDAS[3, 4*], Maiko KHUTSISHVILI[5], Avtandil CHAGELISHVILI[5], Angelika BREN[1], Natalia KOSTINA[1], Veronica CAVERA[6] and Vladimir ELISASHVILI[5]

33 broiler chickens (11 birds per group), 28 days

Group 1: control (antibiotic cloxacillin, SYVA, Leon, Spain);
Group 2: antibiotic + probiotic *B. amyloliquefaciens* B-1895;
Group 3: probiotic *B. amyloliquefaciens* B-1895.

Growth Dynamics of Broiler Chicken



Group 1 – control (antibiotic); group 2 – antibiotic + probiotic; group 3 - probiotic.

★ **- Statistical significance of differences from group 1, P<0,05**

Feed Consumption by Broiler Chickens

Index	Unit	Group		
		1	2	3
Feed flow rate on 1 bird	kg	2.60	2.60	2.65
Feed flow rate for 1 kg growth	kg	1.97	1.81	1.87

Experiments in Volgograd, Russia

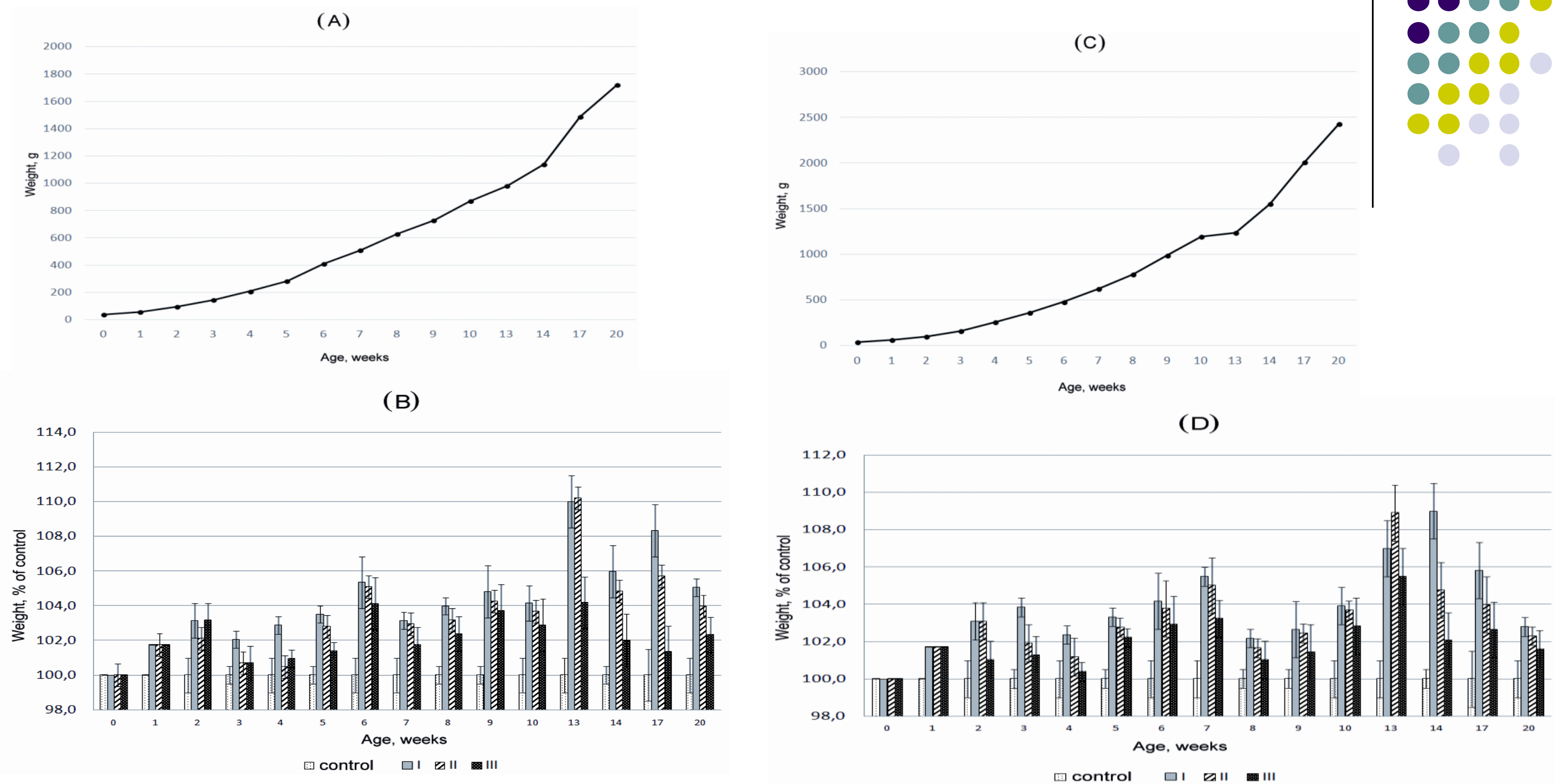
Study of the formulation's efficacy 2016 – 2017

308 birds, commercial layers hybrids "Highsex brown"
(70 hens & 7 roosters per group) 36 months, work in progress

Control – feed without antibiotics

I. **Probiotic preparation based on *Bacillus subtilis* KATMIRA 1933;**
II. **Probiotic preparation based on *Bacillus amyloliquefaciens* B-1895 ;**
III. **Probiotic preparation based on *Bacillus subtilis* KATMIRA1933 and *Bacillus amyloliquefaciens* B-1895**

Experiments in Volgograd, Russia



Living mass growth of chicken. A – Control, hens; B – Experimental groups, hens; C – Control roosters; D – Experimental groups, roosters

I. Probiotic preparation based on *Bacillus subtilis* KATMIRA 1933;
II. Probiotic preparation based on *Bacillus amyloliquefaciens* B-1895 ;
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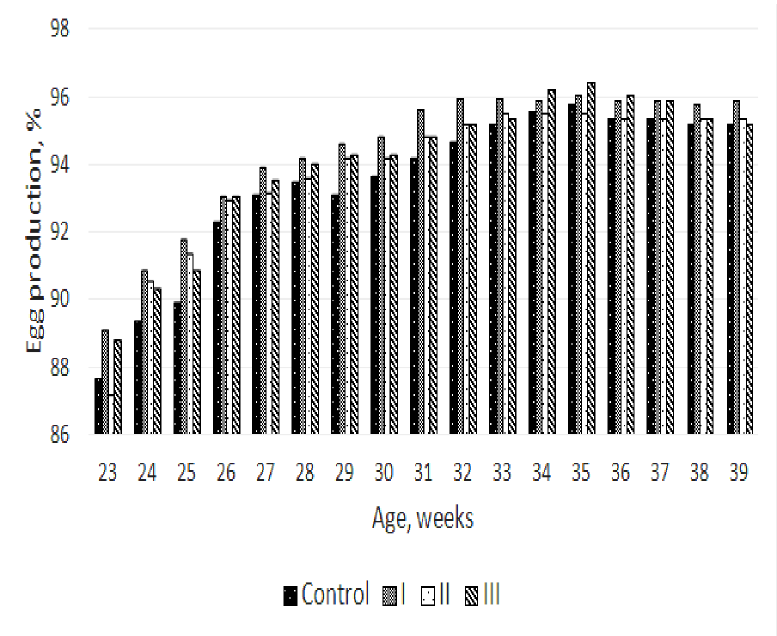
Number of eggs laid by the control and test groups up to the age of 39 weeks

	Control	I	II	III
Number of hens from weeks 19* to 21	64	64	64	64
Number of hens from weeks 21 to 39	61	61	61	61
Number of eggs, pieces.	7419	7538**	7469**	7482**
Difference from control, pieces	-	119	50	63
Difference from control, %	-	1,6	0,7	0,8

* - beginning of eggs laying
** Differences are statistically significant, paired *t*-test (www.r-project.org) , p<0,01



I. probiotic preparation based on *Bacillus subtil*is KATMIRA 1933;
II. probiotic preparation based on *Bacillus amyloliquefaciens* B-1895 ;
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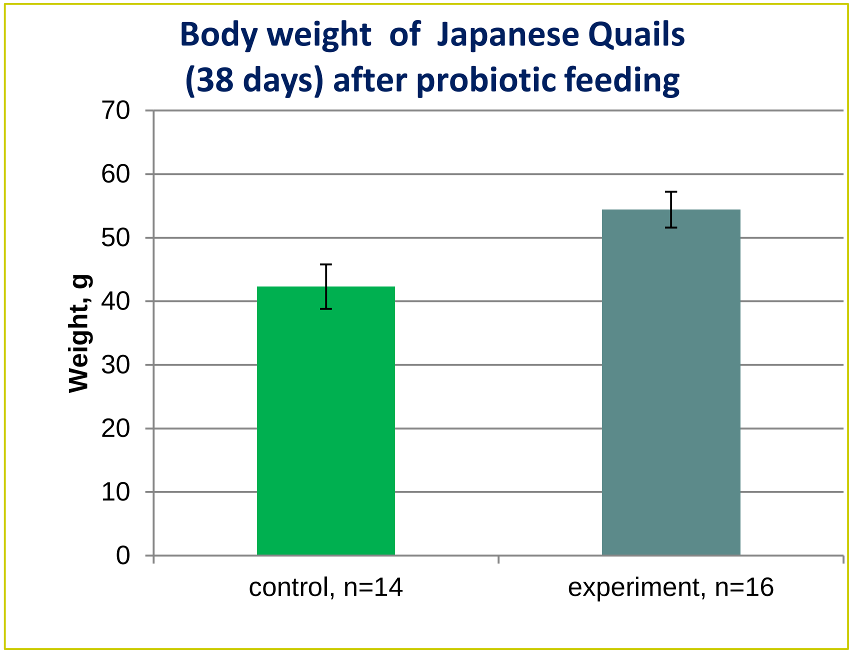
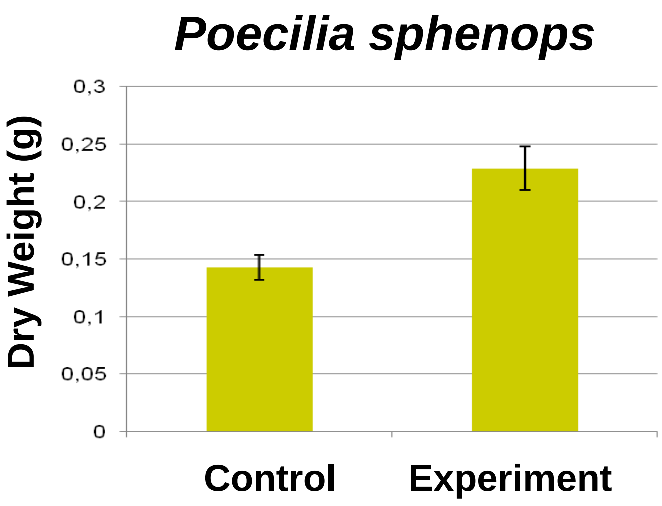
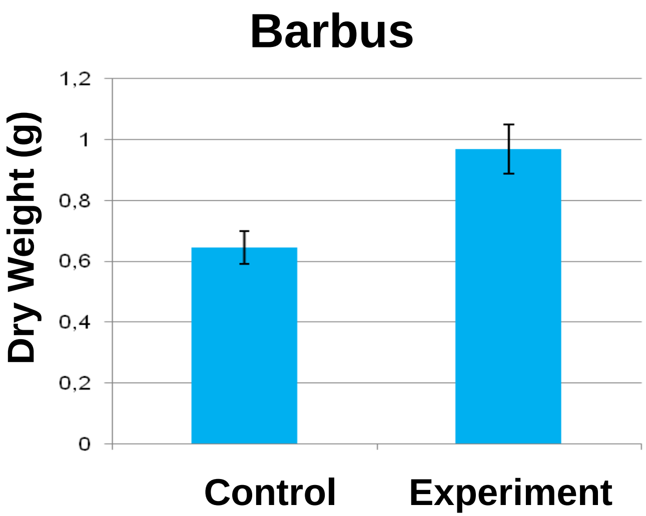


Poults of Japanese Quail (*Coturnix japonica*) (38 days) received in experiment a forage with addition of probiotic preparation based on B-1895. Difference between control and experimental groups is 26%. Also, better quality feathers was observed in experimental group.

Growth Improvement in Tank Fish

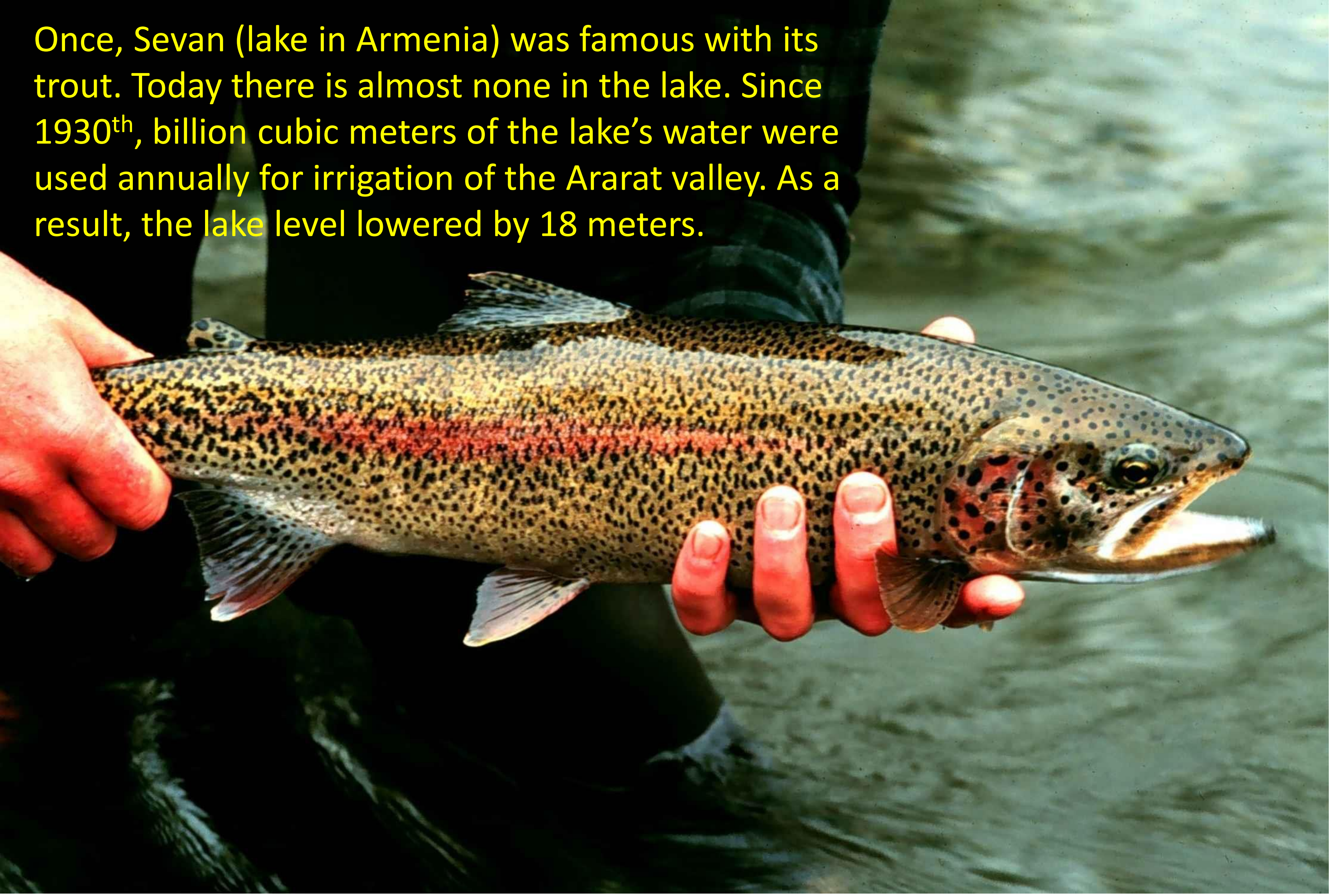




Bacilli probiotics application in aquaculture

- Use of probiotic formulation for farming of *Chalburnus chalcoides* (Caspian shemaya) and *Acipenser gueldenstaedtii* (Russian sturgeon) assists in controlling pathogenic bacteria such as *Salmonella sp*., *Klebsiella sp.,* and *Citrobacter freundii* in the fish digestive tract.
- Productivity of fish farms increases 30 – 40 % when these preparations are used as a feed additive

Once, Sevan (lake in Armenia) was famous with its trout. Today there is almost none in the lake. Since 1930th, billion cubic meters of the lake's water were used annually for irrigation of the Ararat valley. As a result, the lake level lowered by 18 meters.



Our recently initiated international (Armenia, Russia, Georgia, and the U.S.) effort is aimed at the use of probiotics for recovery and growth of the Armenian trout population.

https://travelarmenia.org/travel-to-armenia-and-taste-trout/

Not just birds and fish…

Use of Sporeforming Probiotic Supplement in Service Dogs

The use of the synbiotic formulation (1 g per 100 g of the daily diet) had a therapeutic effect on intestinal dysbiosis in German Shepherds.

Acknowledgements

- Russian Science Foundation

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RSF | Russian Science Foundation

- Teams from Russia, Georgia, and U.S.

Thank you!