Antimicrobial activity of lactic acid bacteria on pathogens in foods
Why succesful?
How succesful?

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- Microorganisms especially bacteria can be used for a number of beneficial purposes. Among them some are more prominent like Lactic acid bacteria (LAB) and bifidobacteria.
- Lactic acid bacteria have been used as natural food-grade preservatives against a variety of undesirable microorganisms.
- LAB has been used for production of fermented foods for many years. As a matter of fact fermented foods existed long before mankind discovered microorganisms.
- During their activity in production of fermented foods their inhibitory potential on pathogenic bacteria have been recognised and they gained increasing interest in the scientific community.
- Thus LAB has been used as a tool to produce antimicrobial compounds and to control undesirable microorganisms.

Antibiotics;

- On the other hand, acute diarrhea due to the loss of normal intestinal microbiota, drug-resistant strains and chronic toxicity due to widespread use of antibiotics are well known negative effects of antibiotics.

Pesticides;

- **The risks of toxic residues in foods**, as well as contamination of soil and water due to the **use of xenobiotics in food production chain** are considered negatively by the public.
- Lab producing a wide range of antimicrobial metabolites thus, the last two decades have seen pronounced advancements in using LAB and their metabolites for natural food preservation, as well as crop protection, and health protection.

Content:

- **The role of lactic acid bacteria**
  - Bio preservative agent
  - Probiotic potential
  - Antimicrobial agent
  - **B**iopestiside
  - Chemotherapeutic agent / infection control agent

  } **Why succesful**
- **Case studies; Antimicrobial activities against**
  - Listeria monocytogenes (the method we used and the
  - Staphylococcus aures results we obtained)
  - Salmonella typhimurium
- **Industrial applications of LAB** **How succesful**

LACTIC ACID BACTERIA

The term lactic acid bacteria (LAB) has no strict taxonomic significance, although the LAB have been shown by serological techniques and 16S ribosomal RNA cataloguing to be phylogenetically related.

They share a number of common features:

- they are Gram-positive
- non-sporeforming rods or cocci
- non-motile
- resistant to acid and
- high fermentative ability

The most are aerotolerant anaerobes which lack cytochromes and porphyrins and are therefore **catalase- and oxidase-negative**.

LAB compose a heterogeneous bacterial group, their common characteristic is the ability to ferment sugars.

Table 9.4 *Principal genera of the lactic acid bacteria*

Genus	*Cell Morphology*	*Fermentation*	*Lactate isomer*	*DNA (mole %GC)*
Lactococcus	cocci in chains	homo	L	33–37
Leuconostoc	cocci	hetero	D	38–41
Pediococcus	cocci	homo	DL	34–42
Lactobacillus	rods	homo/hetero	DL, D, L	32–53
Streptococcus	cocci in chains	homo	L	40[a]

[a] *S. thermophilus*

(Other genera that are currently included in the lactic acid bacteria, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Vagococcus*, *Aerococcus*, *Tetragenococcus*, *Alloiococcus*, *Weissella*)

- Lactic acid bacteria, which convert fermentable sugars to lactic acid and other organic acids depending on their metabolic pathways.
- They are the most important group of bacteria in fermented foods. Thus, due to the acid production the pH of the environment reduces to a pH 3.5.
- However, Lactobacillus spp. plays a major role in the process and Leuconostoc and Pediococcus to a lesser extent.

- **Heterofermenters** produce roughly equimolar amounts of **lactate, ethanol/acetate, and carbon dioxide from glucose**.
- Some authors also include **Bifidobacterium** among the lactic acid bacteria although this has less justification as they are quite distinct both phylogenetically and biochemically.
- For example, hexose fermentation by bifidobacteria follows neither the EMP glycolytic pathway nor the phosphoketolase pathway but produces a mixture of acetic and lactic acids.

The role of lactic acid bacteria Biopreservative agent

- LAB are well known for their activity as starter cultures in the manufacture of fermented foods for a number of industries.
- Lactic fermentation is one of the oldest forms of preparation and preservation of foods (Charlier et al.,2009). LAB are essential to the production of fermented products.

Raw materials for HARDALIYE

Table 1. *Some fermented foods*

Food	*Ingredients*	*Geographical Distribution*
Boza	[illegible]	Turkey
Beer	[illegible]	Widespread
Cheese	Milk	Widespread
Chicha	[illegible] others	S. America
Dawadawa	Locust beans	W. Africa
Gari	Cassava	Nigeria
Idli/dosa	Rice and black gram	India
Injera	Tef	Ethiopia
I-sushi	Fish	Japan
Kefir	[illegible]	Eastern Europe
Kenkey	Maize, sorghum	Ghana
Kimchi	Vegetables	Korea
Koko	Maize, sorghum	Ghana
Leavened bread	Wheat	Europe, N. America
Lambic beer	Barley	Belgium
Mahewu	Maize	S. Africa
Nam	Meat	Thailand
Ogi	[illegible]hum, millet	Nigeria
Olives	[illegible]	Mediterranean Area
Palm wine	Palm sap	Widespread
Poi	Taro	Hawaii
Puto	Rice	Philippines
Salami	Meat	Widespread
Salt stock, cucumbers	[illegible]	Europe, N. America
Sauerkraut	Cabbage	Europe, N. America
Sorghum beer	Sorghum	S. Africa
Sourdough bread	Wheat, rye	Europe, N. America
Soy sauce, miso	Soy beans	S.E. Asia
Tempeh	Soy beans	Indonesia
Tibi	Fruit	Mexico
Yoghurt	Milk	Widespread

Cereal based

Milk based

Fish based

Fruit based

Meat based

(Adams and Moss, 2008)

Health benefits

Since the trends towards

- natural (minimally processed or without additives)
- high nutritional value
- health-promoting
- flavor rich products

Fermented food play an important role in human diet around the world due to their health benefits.

Role of LAB Biopreservative Agent (Food preservation and safety)

Food preservation

Their ability to promote food preservation is summerized below;

- they cause a decrease in pH as a result of **lactic acid** production, and additionally,
- they produce a number of **antimicrobial agents** and thus play a role in the inhibition of pathogenic / spoilage microorganisms during fermentation process in foods.
- **A combination of these factors limits** the proliferation of undesirable microorganisms.
- LAB therefore undoubtedly play a role in promoting food safety.

2) Probiotic characteristics of lactic acid bacteria

Probiotic potential is another significant character of a LAB. The most commonly used species, in probiotic preparations are *Lactobacillus* ssp., *Bifidobacterium* ssp. and *Streptococcus* ssp. Probiotic strains have several beneficial properties such as

- Improving intestinal tract health
- producing antimicrobial substances
- enhancing the immune response
- reducing symptoms of lactose intolerance
- enhancing the bioavailability of nutrients, and
- decreasing the prevalence of allergy in susceptible individuals (Parvez et al., 2006; De Bellis et al., 2010: Mena and Aryana, 2012).

Probiotic characteristics of lactic acid bacteria
Beneficial role of LAB (Charlier et al.,2009)

- The current definition of a probiotic is a “live micro-organism which when administered in adequate amounts confers a health benefit on the host” (FAO/WHO report, October, 2001), which does not imply that microorganisms have to be orally ingested.
- The well known species of probiotics are lactobacilli and bifidobacteria (Lb. casei, Lb. rhamnosus, Lb. acidophilus, Bf. bifidum).

3. Antimicrobial metabolites

- LAB have antimicrobial effects against undesirable microorganisms through **producing various metabolites** or through **inhibiting the cell adhesion of pathogenic organisms in vitro**.

Antimicrobials produced by lactic acid bacteria

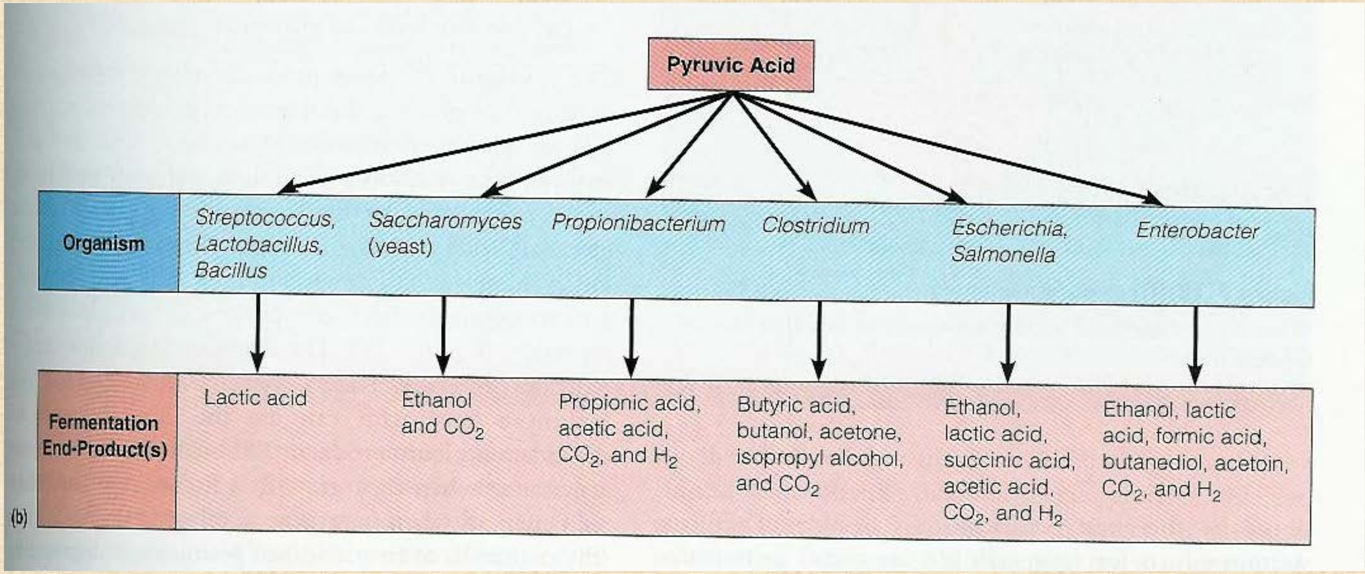
Organic acid	Lactic acid	Major metabolite of LAB fermentation. Active against spoilage and Gram-negative bacteria, some fungi
	Acetic and propionic acids	More antimicrobially effective than lactic acid. Active against spoilage bacteria, clostridia, some yeasts and fungi
Other	Hydrogen peroxide	Active against pathogens and psychotropic spoilage organisms e.g. Staphylococcus aureus, Pseudomonas sp.
	Carbon dioxide	Active against Gram positive and specially Gram-negative psychrotrophic bacteria e.g. Enterobacteriaceae and Listeria

Bacteriocins and bacteriocin-like peptides	Active against broad spectrum of Gram-positive and Gram-negative bacteria, yeast, fungi and protozoa e.g. species of Salmonella, Shigella, Clostridium, Staphylococcus, Listeria, Candida, and Trypanosoma
Reuterin	Active against broad spectrum of Gram-positive and Gram-negative bacteria, yeast, fungi and protozoa e.g. species of Salmonella, Shigella, Clostridium, Staphylococcus, Listeria, Candida, and Trypanosoma
Diacetyl	Active against Gram positive and Gram-negative bacteria e.g. Listeria, Salmonella, Yersinia, E. coli, and Aeromonas
Fatty acids, Fenil lactic acids	Active against Gram-positive bacteria and some fungi

Organic acids

Organic acids have been used as food additives and preservatives in food industry. It is used for preventing food spoilage and extending the shelf life of foods.

The type of organic acid depends on the type of producer species.



Homofermenters produce **lactic acid** as a single product from the fermentation of glucose. Whereas heterofermenters produce acetic acid as well.

depending on the **physiological status** of the organism and the **physicochemical characteristics** of the environment (Ricke, 2003).

Organic acids are capable of exhibiting **bacteriostatic and bactericidal properties**

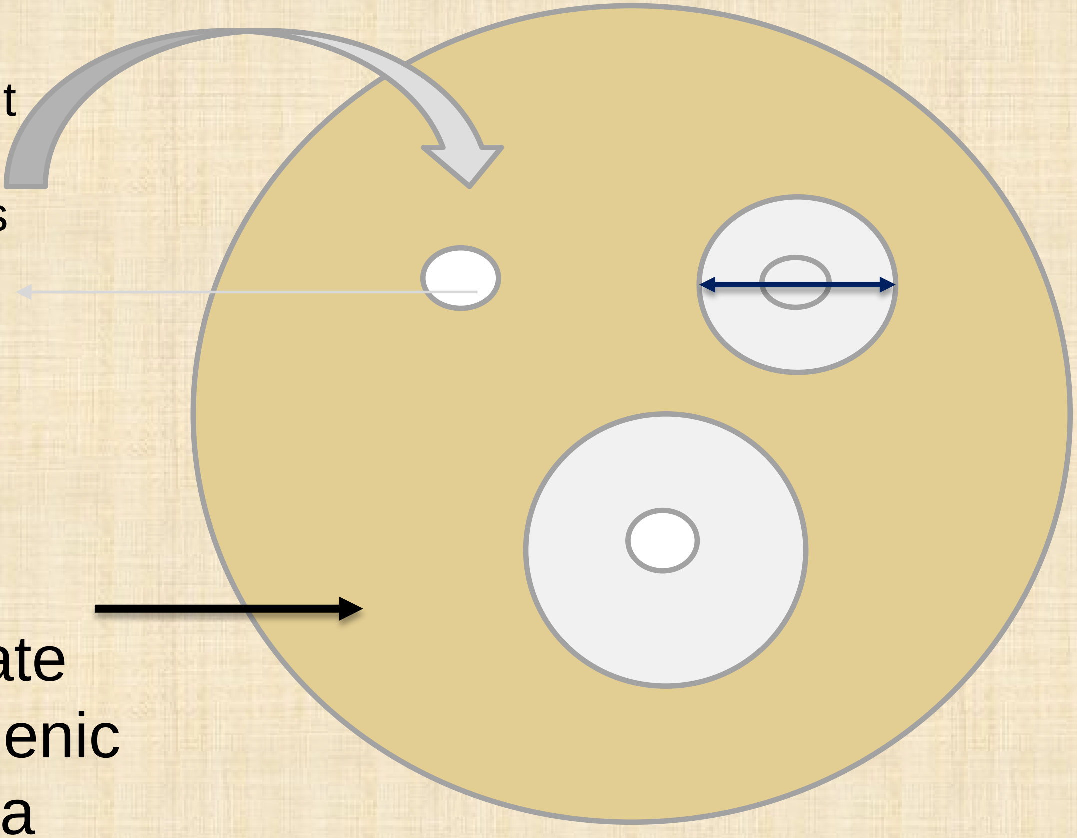
There are a number of research conducted to investigate the effect of different organic acids on inhibiting the growth of pathogens in laboratory media and liquid foods.

Propionic acid and acetic acid showed the strongest inhibitory effect against E. sakazakii in laboratory broth media (Back et al., 2009).

How can we test antimicrobial efect of LAB against pathogens?
(Agar overlay, disc diffusion, well diffusion)

Upload the supernatant containing metabolites

Sterile disk



Inoculate pathogenic bacteria

Incubation
(37 C for 24 h)

Measure the inhibited zone around the sterile disc (diameter, mm or cm)

Hydrogen peroxide (H_2O_2)

- **Lactic acid bacteria produce** H_2O_2 as a low molecular weight antimicrobial compound. **Hydrogen peroxide** showes bacterostatic effect against gram positive bacteria (including LAB) and bactericidal effect against a number of gram negative bacteria in general (Yang, 2000).

Mechanism

- **Hydrogen peroxide** oxidizes the sulphidril groups in a molecule and thus cause denaturation of a variety of enzymes.
- increases the permeability of the membrane by peroxidizing the lipids in the plasma membrane.
- has a role on production of superokside (O^{2-}) and hydroxil (OH*) free radicals and causing damage in DNA (bactericidal effect) (Ammor ve diğ., 2006).

- However, t**he actual role of hydrogen peroxide** produced by LAB **in the inhibition of pathogens has been difficult to demonstrate and thus is still controversial.**

Bacteriocins and bacteriocin-like inhibitory substances

- Many species of LAB produce bacteriocins with fairly large spectra of inhibition thus they considered as promising agents for use in food preservation.

There is an increasing interest in the literature which mainly focused on

- The isolation and identification of bacteriocin producing LAB
- Their antimicrobial / antagonistic effects to different pathogens
- Characterisation of the active metabolite and finally
- Possibility to use them as a biopreservative in foods as well as other area of interest.

Bacteriocins and bacteriocin-like inhibitory substances

- Numerous strains of LAB associated with foods produce bacteriocins, defined as **proteinaceous compounds** with activity against related species.
- Bacteriocins are ribosomally-synthesized peptides or proteins secreted by certain strains of bacteria.

Mechanisms

- The antagonistic activity of LAB to pathogens may affect the growth rate and/or survival depending on the type and the concentration of bacteriocin.
- Most bacteriocins kill target cells by permeabilization of the cell membrane, and the activity is often very specific, since they employ specific receptors on the target cell surfaces (Kjos et al., 2011).

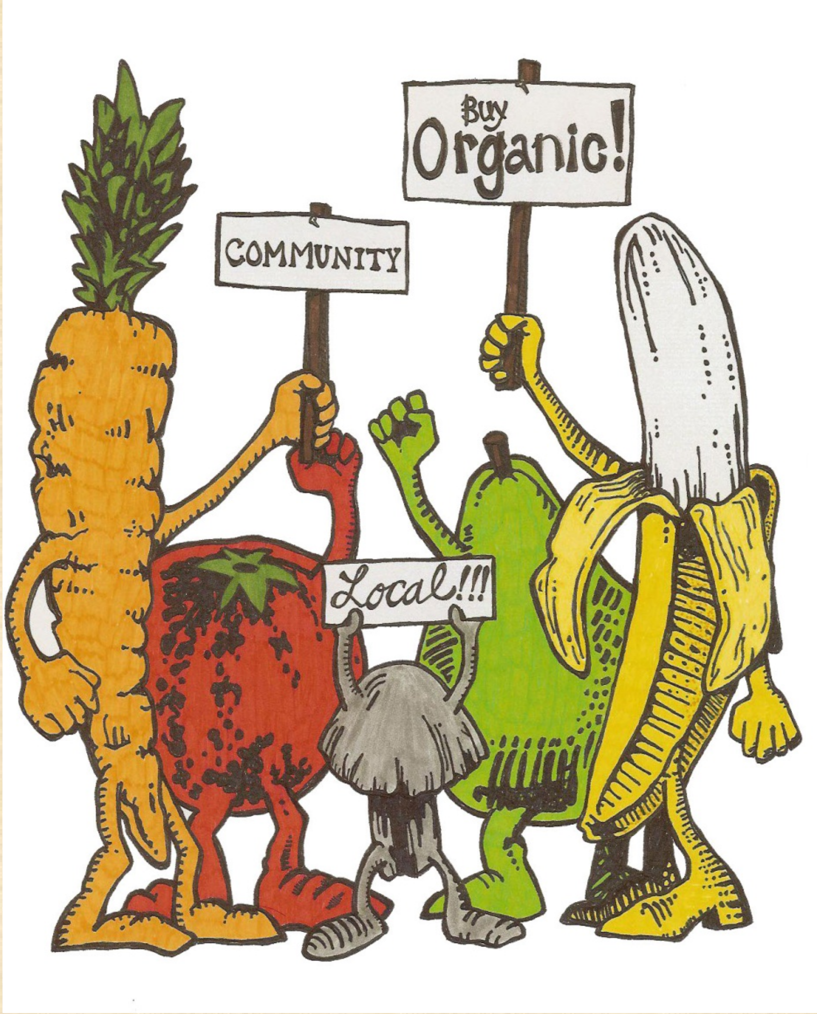
- **Nisin** (L. lactis) **and pediocin** (Pediococcus acidilactici) are among the **most well characterised bacteriocins** and the most frequently used in fermented products.

4. Biopestisides environmentally friendly biocontrol systems

- Several species of lactic acid bacteria have been recognised **as producers of bioactive metabolites** which are functional against a broad spectrum of undesirable microorganisms.
- They are effective to fungi, oomycetes and other bacteria.
- **Consumers in developed countries have become more critical and more fragmented in their food choices,** leading to situations where quality differentiation of food products, has become necessary in order to satisfy consumers.

Consumer pressure **against chemicals in food production** and the **risks of toxic residues**

G**eneral public have become interested** and often critical with regard to certain ways of producing food- both **at the farm level** and **at the processing level**.
As a result, discussions on **organic production, reduce the application of pesticides on crops, animal welfare,** and the use of **genetically modified organisms (GMOs) in food production**.



Consumer pressure

- Public fear of **using chemicals in food production**, the perceived **risks of toxic residues in treated products**, as well as contamination of soil and water, pesticides are looked upon negatively by the public.
- The necessity **to move away from traditional chemical treatments,** many studies have focused on finding **alternative biocontrol systems.**
- In general, due to the different modes of actions (**i.e. antagonistic effects or induction of plant defence mechanisms**),**the use of** LAB **as preservatives** has a definite potential (Axel et al., 2012).
- Thus, they may represent **an interesting tool for the development of novel concepts in pest management.**

5)Antimicrobial chemotherapeutic agents

- **Antimicrobial chemotherapeutic agents** have been widely used **to control gastrointestinal infections**. However, the widespread use of antibiotics is now being discouraged due to problems including the emergence of drug-resistant strains and chronic toxicity (Mody et al., 2003).
- In addition, antibiotics are often responsible for acute diarrhea due to the loss of normal intestinal microbiota as well as pathogenic organisms (Van der Waaij et al., 1982).
- **As alternatives, lactic acid bacteria or their derivates have been administered. LAB have been used or planning to use against pathogens not only in food and plant protection industry but also in medical industry as well.**
- Bacteriocin producer LAB strains which are active against S. aureus and other pathogenic bacteria are also screened in order to develop probiotic for the human body (Voravuthikunchai et al., 2006).

Why succesful ?

- LAB have been widely studied for their antimicrobial activity, and several antibacterial and fungicidal compounds have been isolated and characterised to date.
- Amongst the antibacterial compounds, nisin (Lactococcus lactis) has been used successfully as an effective biopreservative in some dairy products for decades. Nisin is currently used commercially as a food preservative in around 50 countries.
- ıt is registered as a food preservative E234 (No 95/2/EC 1995).
- Among other pthogenic bacteria the growth of S. enteritidis was also effectively inhibited by the presence of the (lactic acid bacteria culture condensate mixture) LCCM.
- I**n vitro and in vivo experiments showed that the LCCM has antimicrobial effect against S. enteritidis. Ingestion of the LCCM after a meal will be helpful for preventing S. enteritidis infection** (Park et al., 2005) **.**

Why succesful

LAB play a crucial role in every part of **nutrition safety** such as

- food preservation
- food safety and quality
- to a lesser extent in nutrition
- Majority of fermented foods is produced by the activity of lactic acid bacteria
- **environmentally friendly biocontrol systems (biopestiside)**
- **Antimicrobial chemoterapeutic agent/disease control on skin infections**

S.aureus

- Staphylococcus aureus is one of opportunistic pathogen, involved in food poisoning, toxic shock syndrome (TSS) and a wide range of infections.
- The natural habitat of this species are the nasal cavity and the skin of warm-blooded animals. Because of its importance, S. aureus is one of the most studied pathogen.
- S.aureus is the causative agent of skin-releated infections and superficial lesions to life-threatening septicaemia (Charlier et al.,2009).

Case studies
Material and Methods

A total of 47 strains were isolated from 30 tulum cheese samples obtained from east Anatolia. This cheese is a traditional semi- soft cheese.

- Species were identified by 16S rRNA using PCR.
- Antimicrobial activities of strains were tested using well diffusion assay
- **The antimicrobial activity of each strain were confirmed by classical microbiological analyses using selective media for each pathogen.**

- LAB have antimicrobial effects against pathogens such as (Park et al., 2005)
- Escherichia coli O157:H7
- Vibrio cholerae
- Salmonella enteritidis
- **Salmonella *typhimurium***ATCC 14028
- **Listeria monocytogenes**
- **Staphylococcus aureus** ATCC25923
- **E. sakazakii**
- **Aspergillus niger**
- **Yeasts**

Table 1. The number of species isolated from cheese

Species	Number of species
Lactobacillus brevis	27
Lactobacillus plantarum	20
Enterococcus faecium	1

Supernatants obtained from LAB tested for their total antimicrobial capasities after 24 and 48hrs.

Bacterial metabolite production

- Supernatant

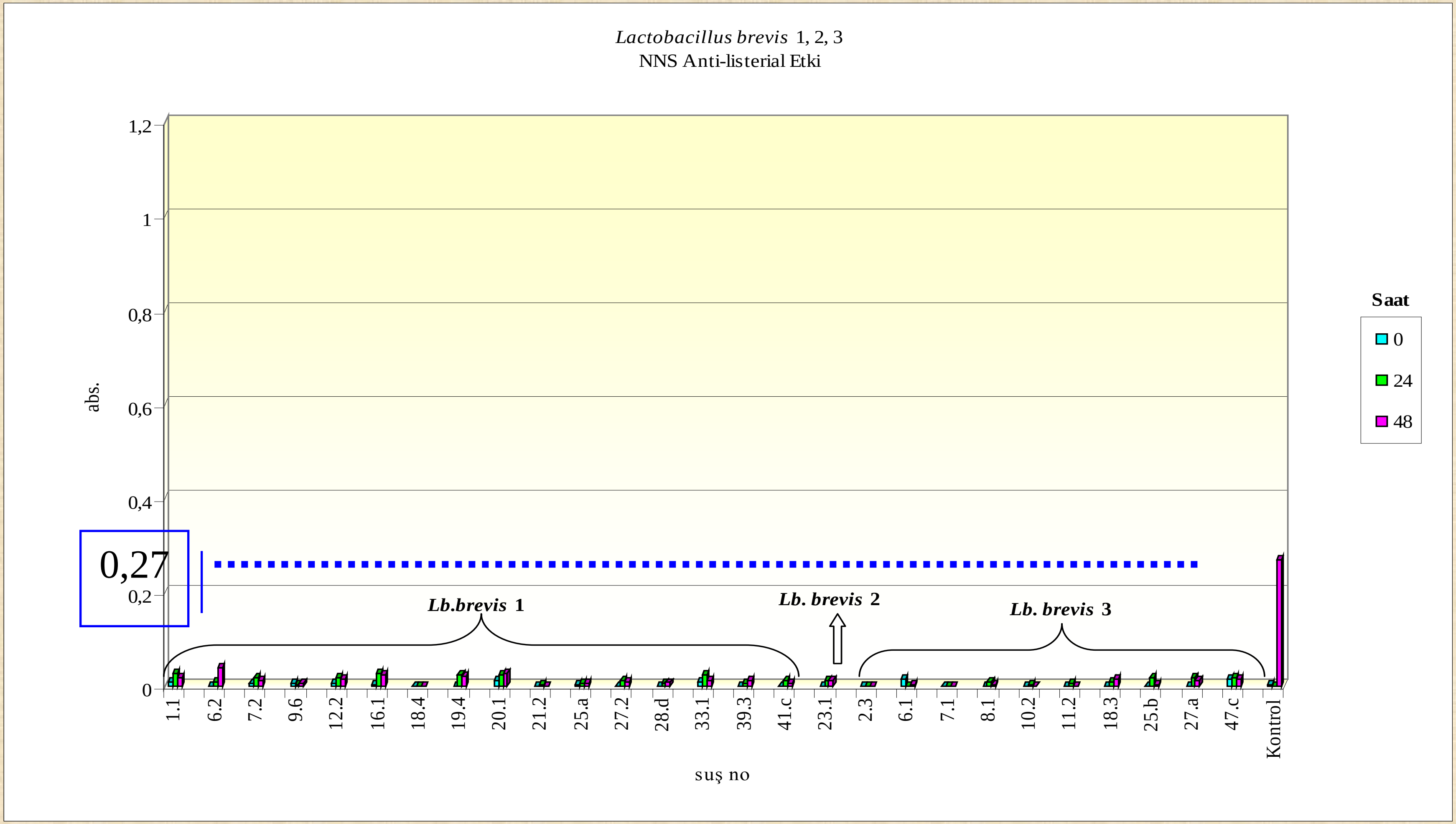
- Neutralized (NaOH)

- Neutralized (NaOH)
- Catalase added

Table 2. pH of supernatants after incubation

Species	Number of strains	pH of supernatants
Lactobacillus brevis	27	3,81-4,37
Lactobacillus plantarum	20	3,77-5,55
Enterococcus faecium	1	4,33

Figure1. Antagonistic effects of organic acids (non-neutralized supernatant) of *Lactobacillus brevis* against *Listeria monocytogenes*



23.1: Enterococcus faecium

Figure 2. Antagonistic effects of neutralized supernatant of *Lactobacillus brevis* against *Listeria monocytogenes*

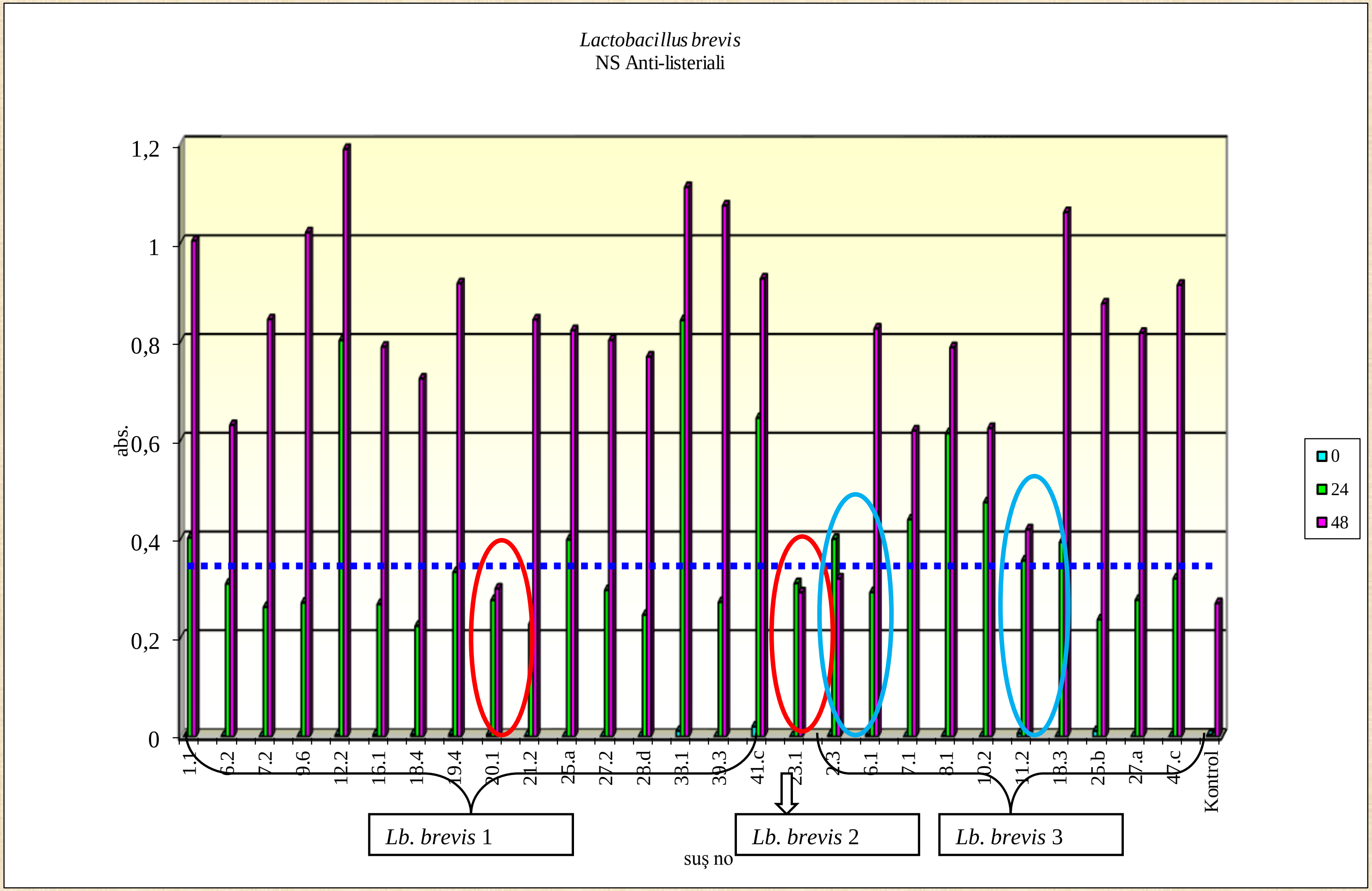
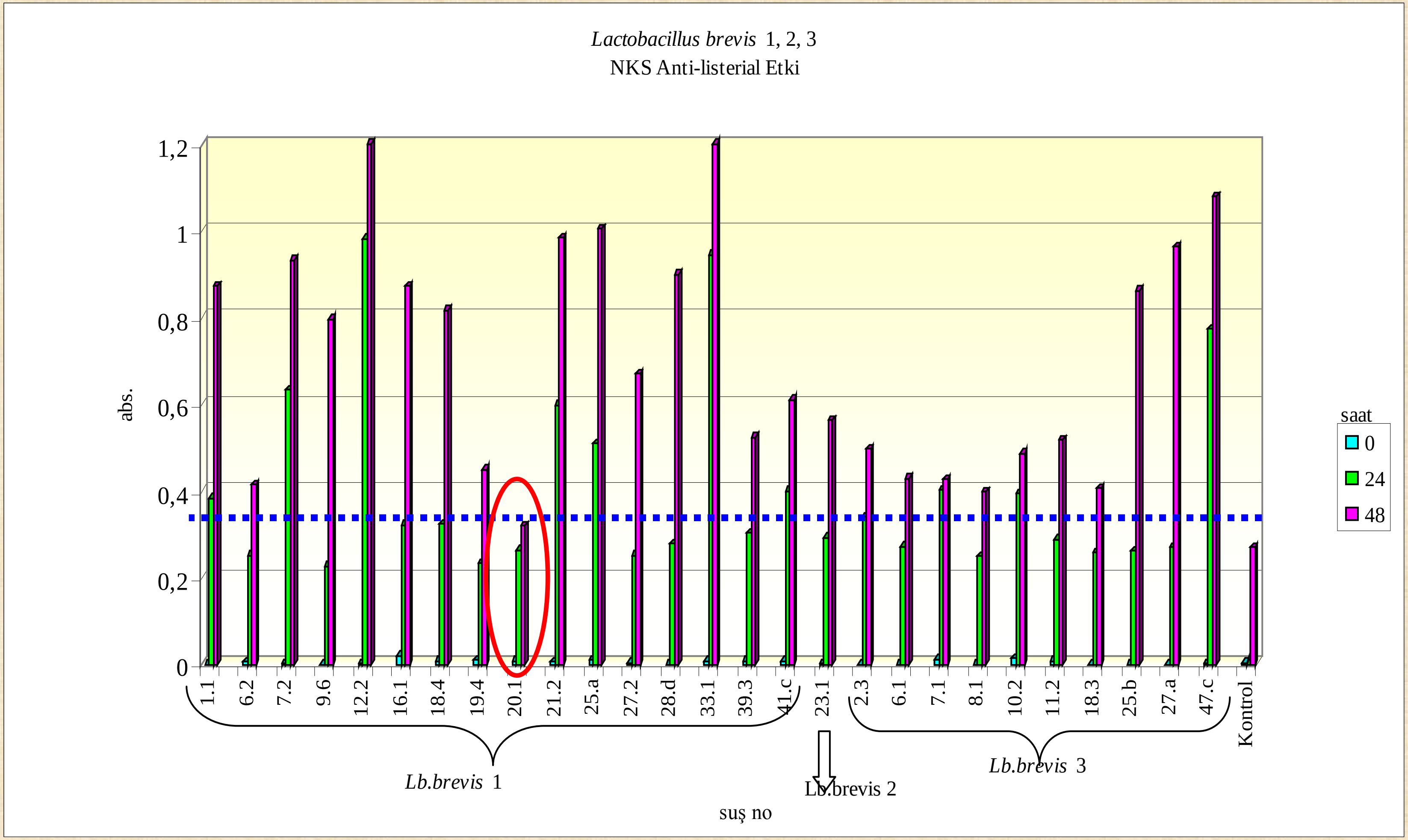


Figure 3. Antagonistic effects of **neutralized** supernatant **containing** catalase of *Lactobacillus brevis* antimicrobials against *Listeria monocytogenes*

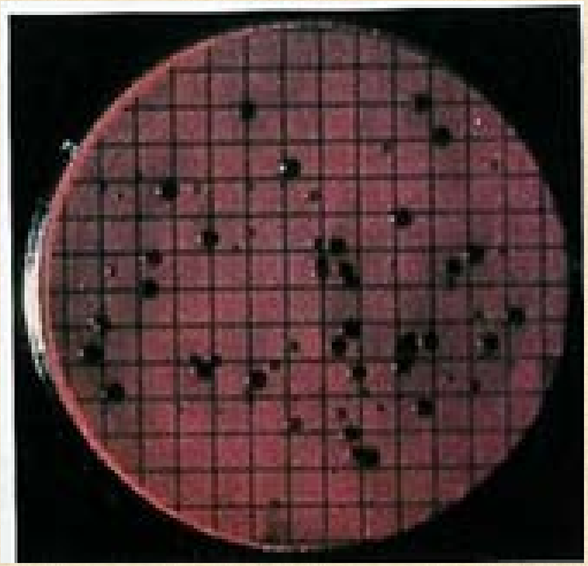


- Confirmation by inoculating supernatant with pathogen on PALCAM agar showed complete inhibiton by organic acids but not neutralized and neutralized supernatant with catalase.

- Thus the inhibitory effect of

  L. brevis associated with the

organic acid.



The results of this study were in agreement with the literature

the antagonistic effects of *Lb. brevis against*;

- *S. aureus* (Aslim B. 2005; Banerji 2011)
- *E. coli* (Aslim B. 2005; Banerji 2011)
- *Y. enterocolitica* (Aslim B. 2005)

- Aslim B. (2005) organic acid
- Banerji (2011) ise bacteriyocin-like compounds

Figure 4. Antagonistic effects of organic acids (non-neutralized supernatant) of *Lactobacillus plantarum against L. monocytogenes*

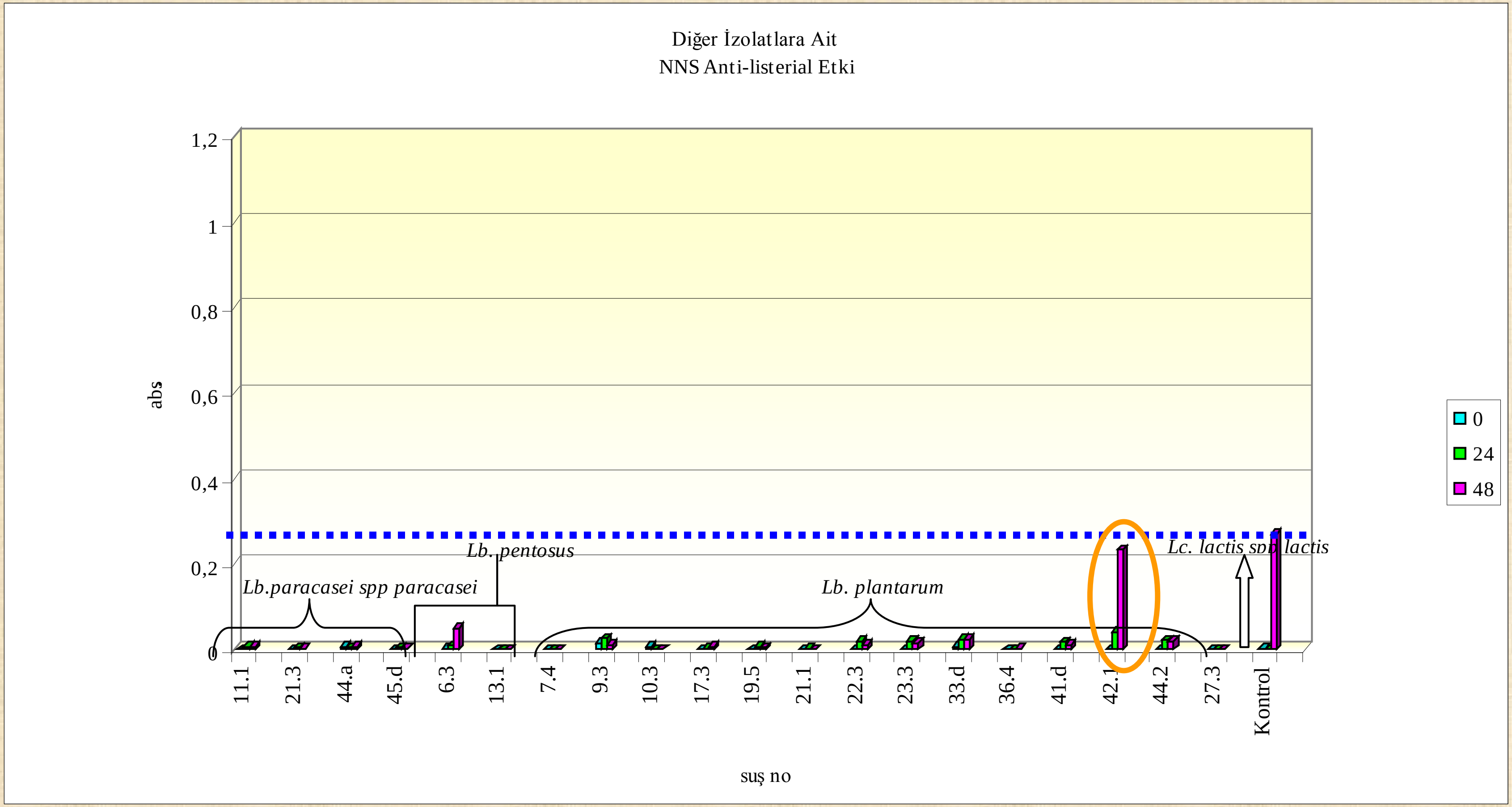


Figure 5. Antagonistic effects of neutralized supernatant of *Lactobacillus plantarum against L. monocytogenes*

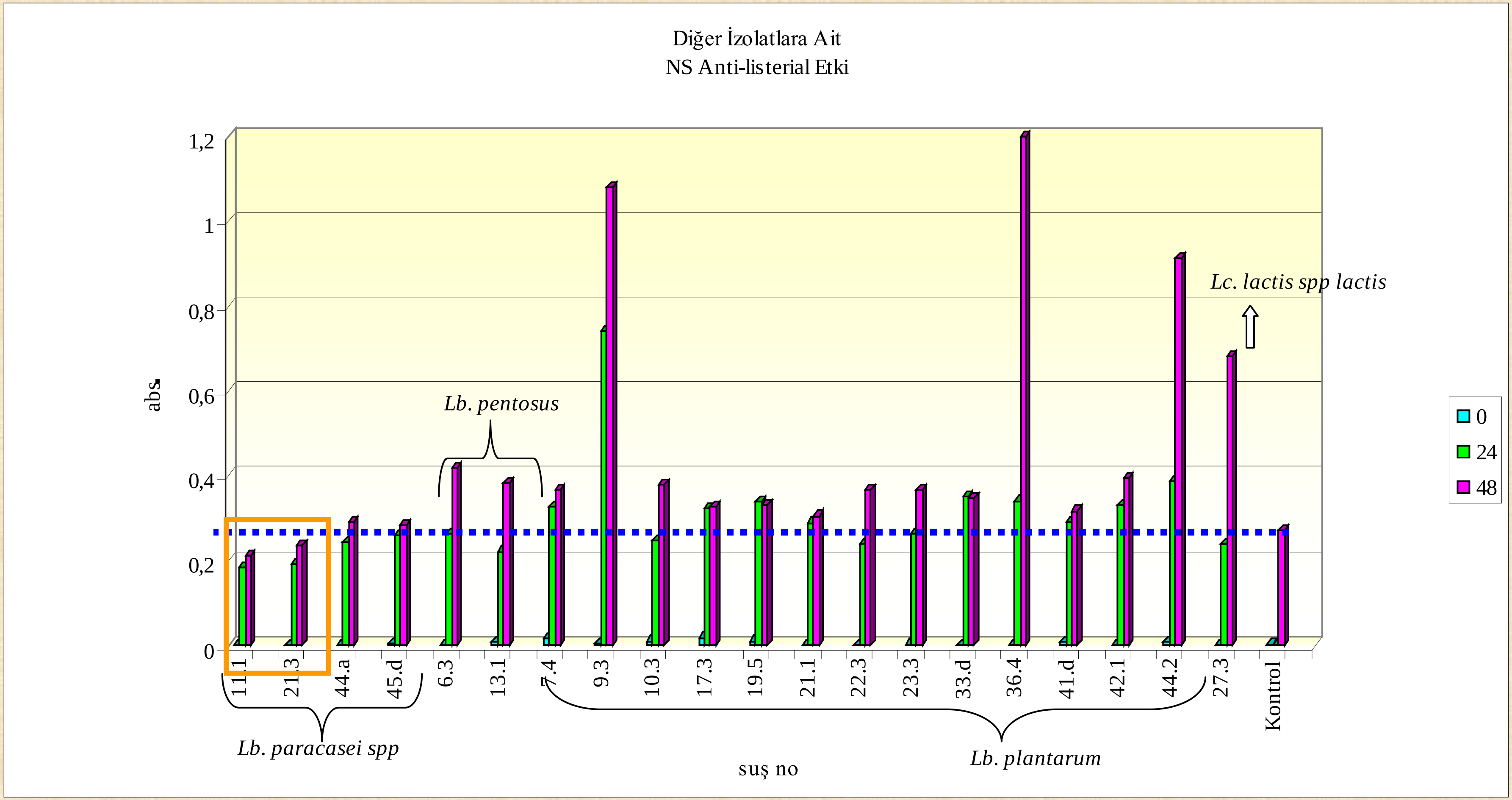
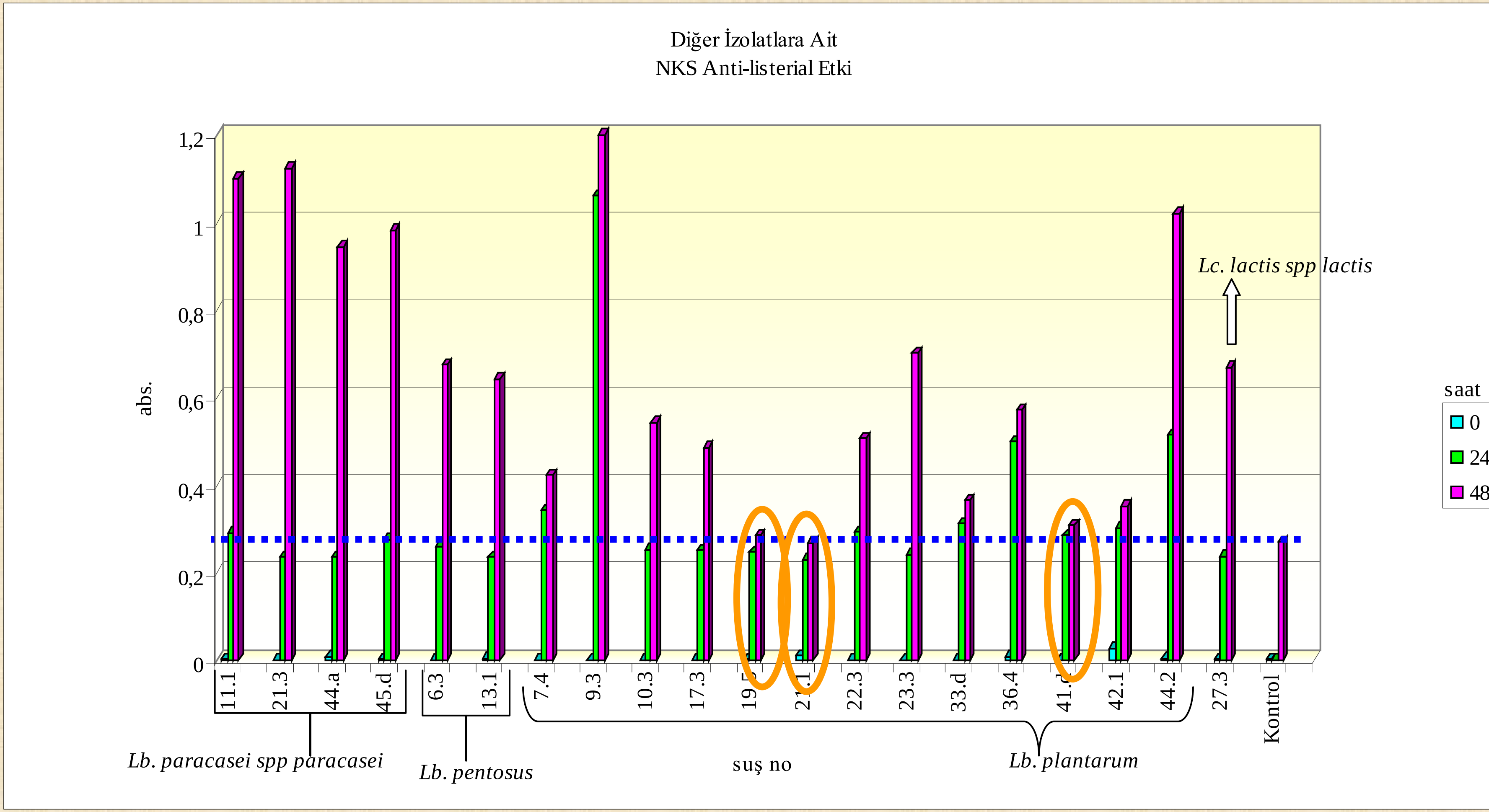


Figure 6. Antagonistic effects of **neutralized supernatant containing catalase** of *Lactobacillus plantarum against L. monocytogenes*



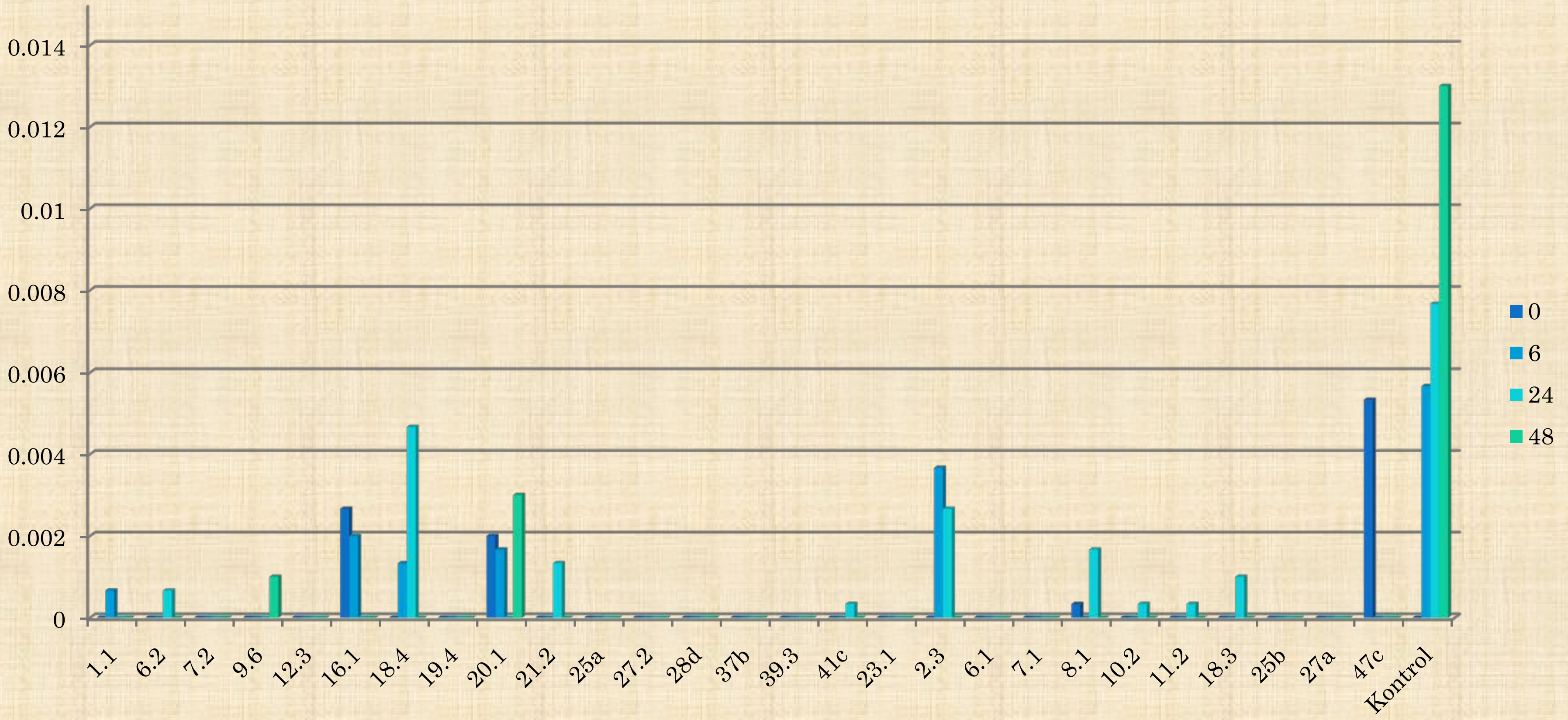
The results of this study were not inagreement with the literature

- The antagonistic effects of *Lb. plantarum* Gonzalez et al., (2007) organic acids only
- Neutralized and neutralized containing catalase 3 supernatants were effective *against L. monocytogenes*

Staphylococcus aureus
Salmonella typhimurium

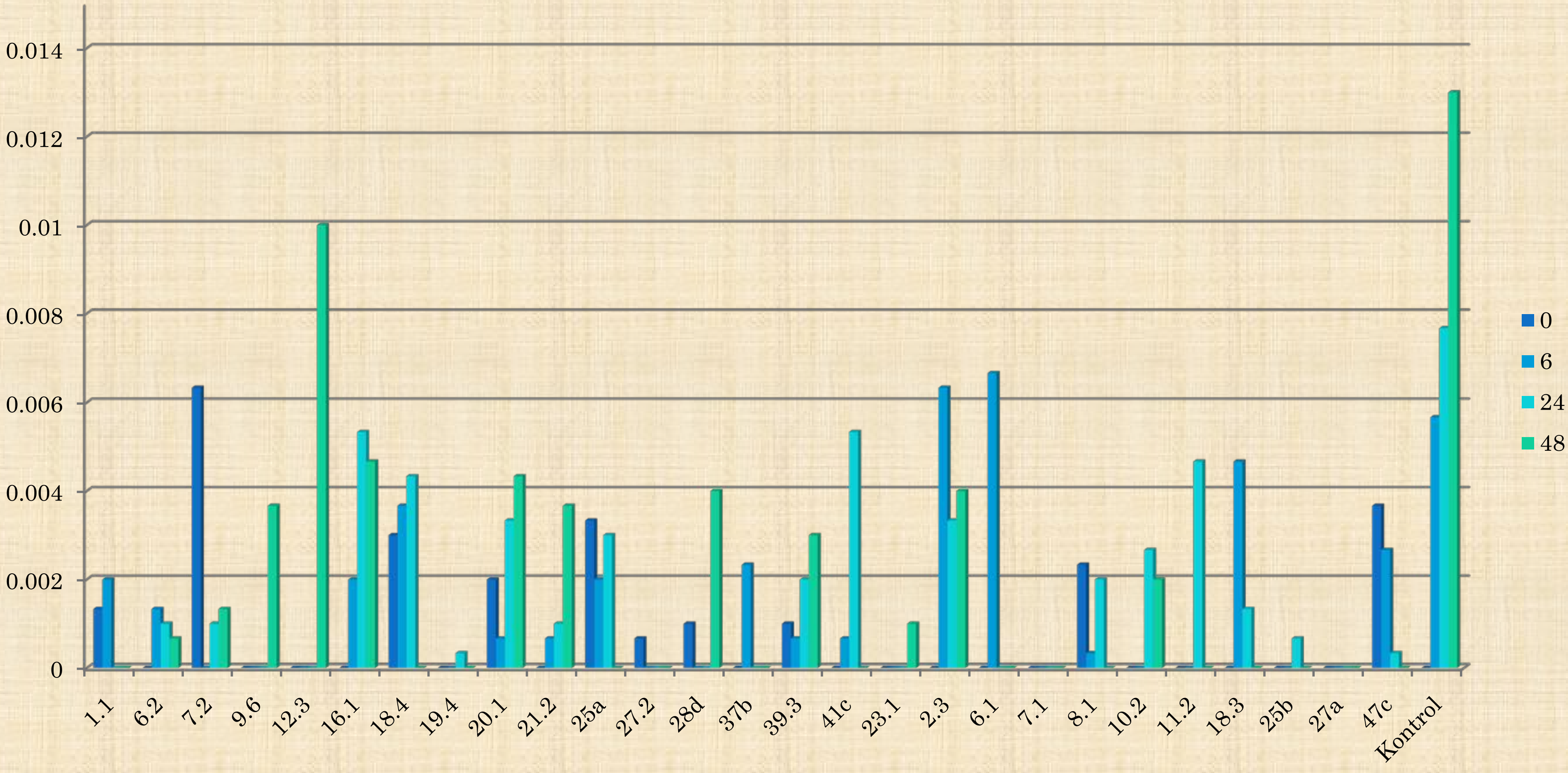
- Well diffusion
  - 0, 6, 24 and 48hrs
  - 640nm
- confirmed
  - After 24 and 48hrs
  - *Staphylococcus aureus* - BP agar
  - *Salmonella typhimurium* – XLD agar

Figure 7. Antagonistic effects of organic acids (non-neutralized supernatant) of *Lactobacillus brevis* against *Staphylococcus aureus*



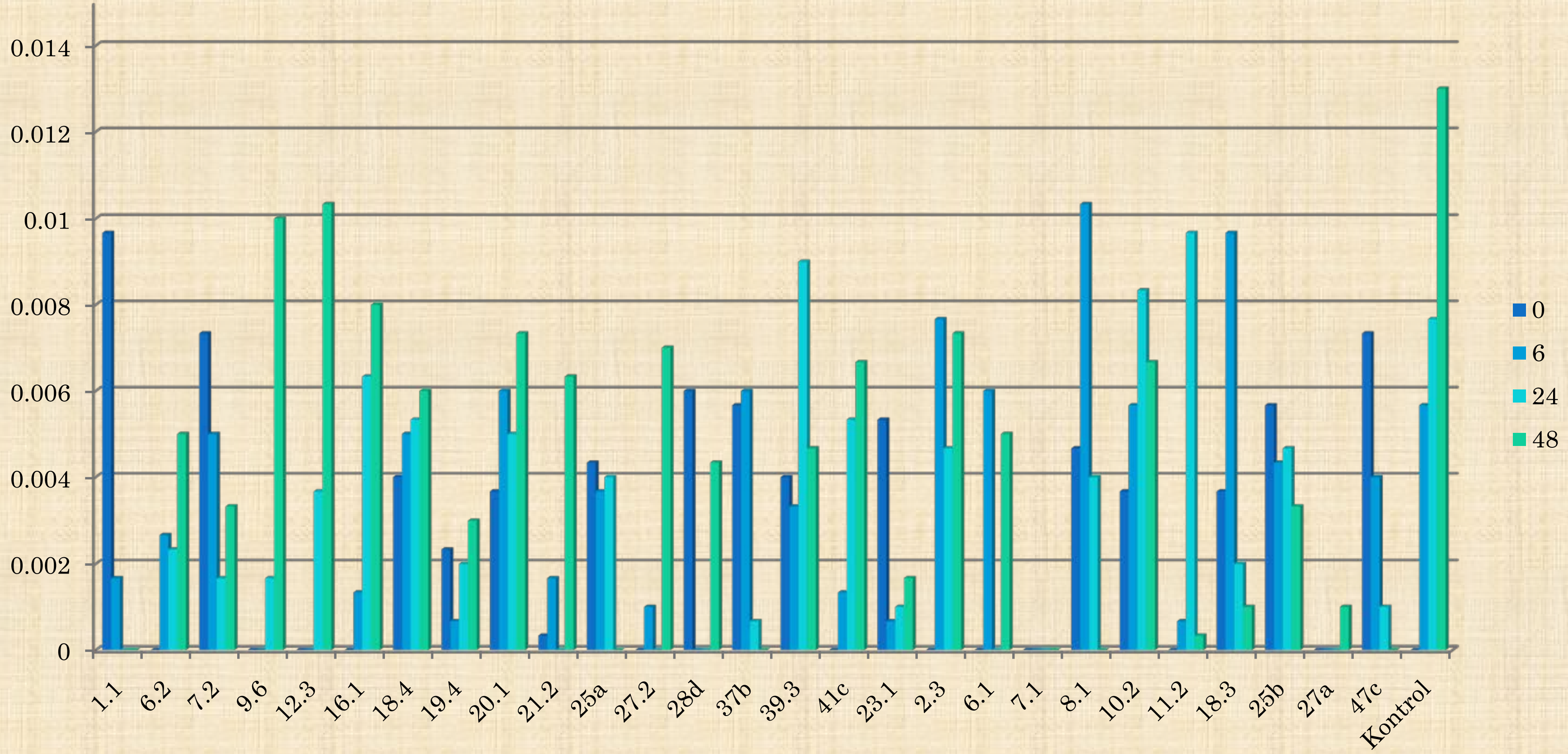
*23.1: *Enterococcus faecium*

Figure 8. Antagonistic effects of neutralized supernatant of *Lactobacillus brevis* against *Staphylococcus aureus*



*23.1: *Enterococcus faecium*

Figure 9. Antagonistic effects of neutralized supernatant **containing** catalase of *Lactobacillus brevis* antimicrobials against *S. aureus*



*23.1:*Enterococcus faecium*

Lactobacillus brevis against *Staphylococcus aureus*

Organic acids

- The absorbans of supernatants were below the the control

Thus

- Organic acids were effective against *S. aureus.*
- H_2O_2 were effective against *S. aureus.*
- Bactericin and bacteriosin like metabolites
- were effective against *S. aureus*

Figure 10. Antagonistic effects of organic acids (non-neutralized supernatant) of *Lactobacillus plantarum* against *S. aureus*

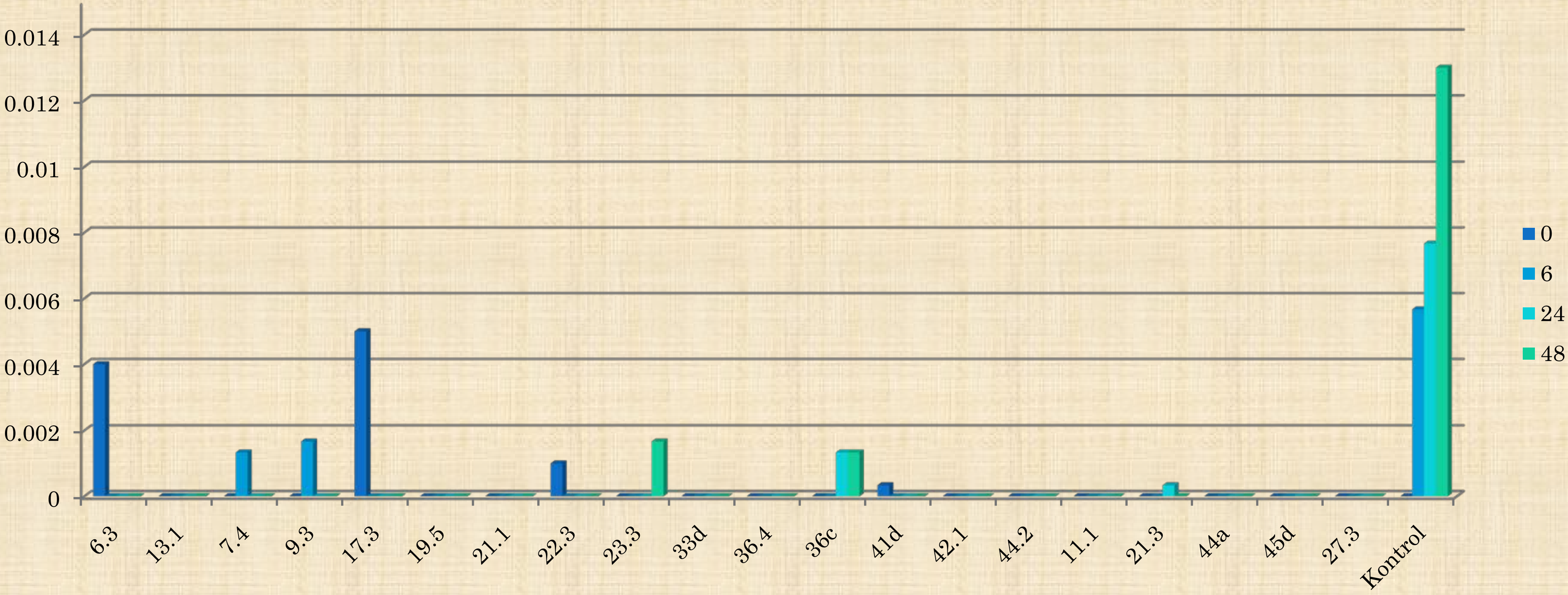


Figure 11. Antagonistic effects of neutralized supernatant of *Lactobacillus plantarum* against *S. aureus*

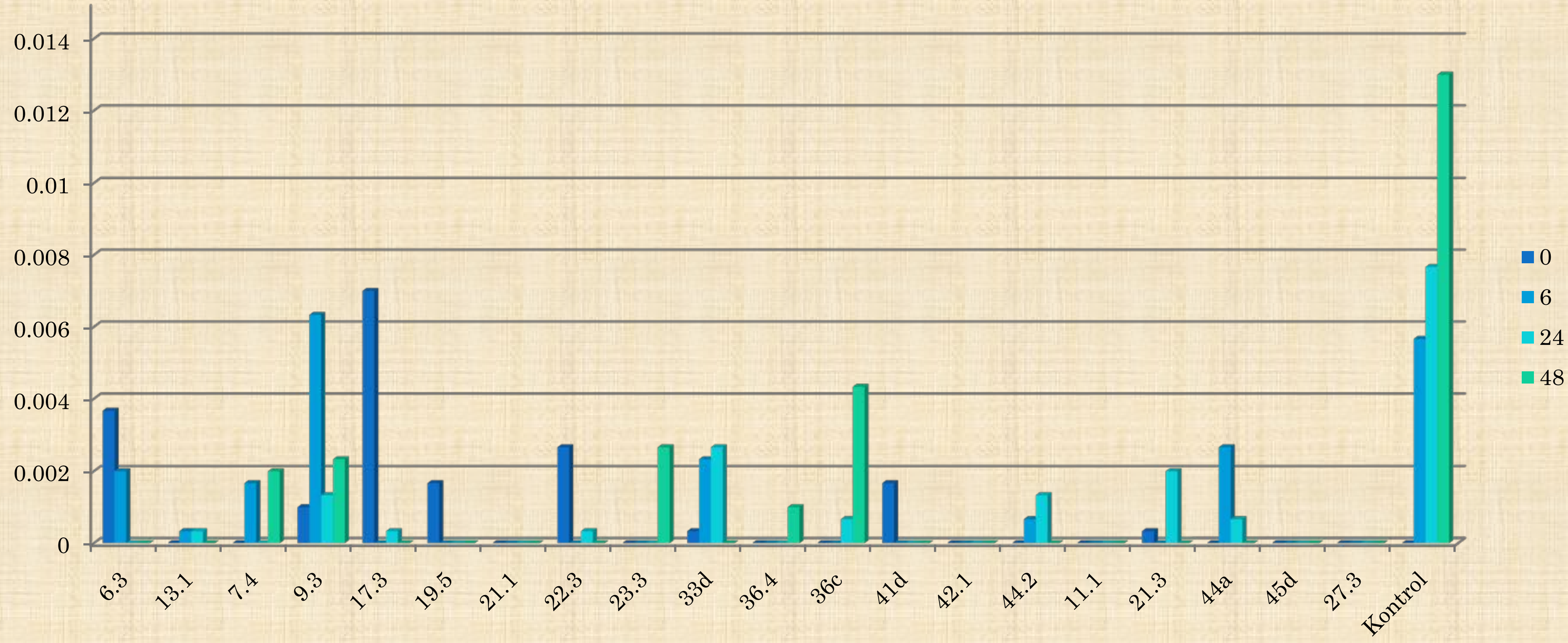
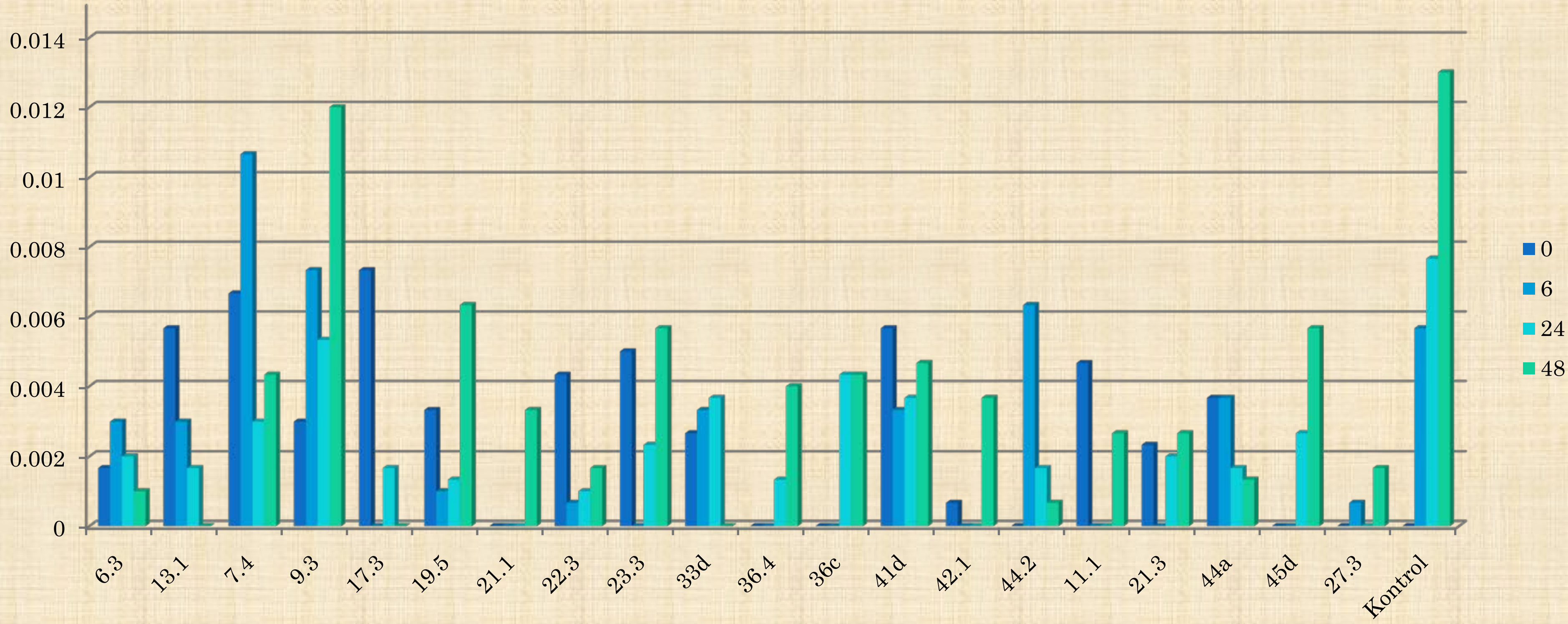


Figure 12. Antagonistic effects of neutralized supernatant **containing** catalase of *Lactobacillus plantarum* antimicrobials against *S. aureus*



Lactobacillus plantarum against *Staphylococcus aureus*

Organic acids

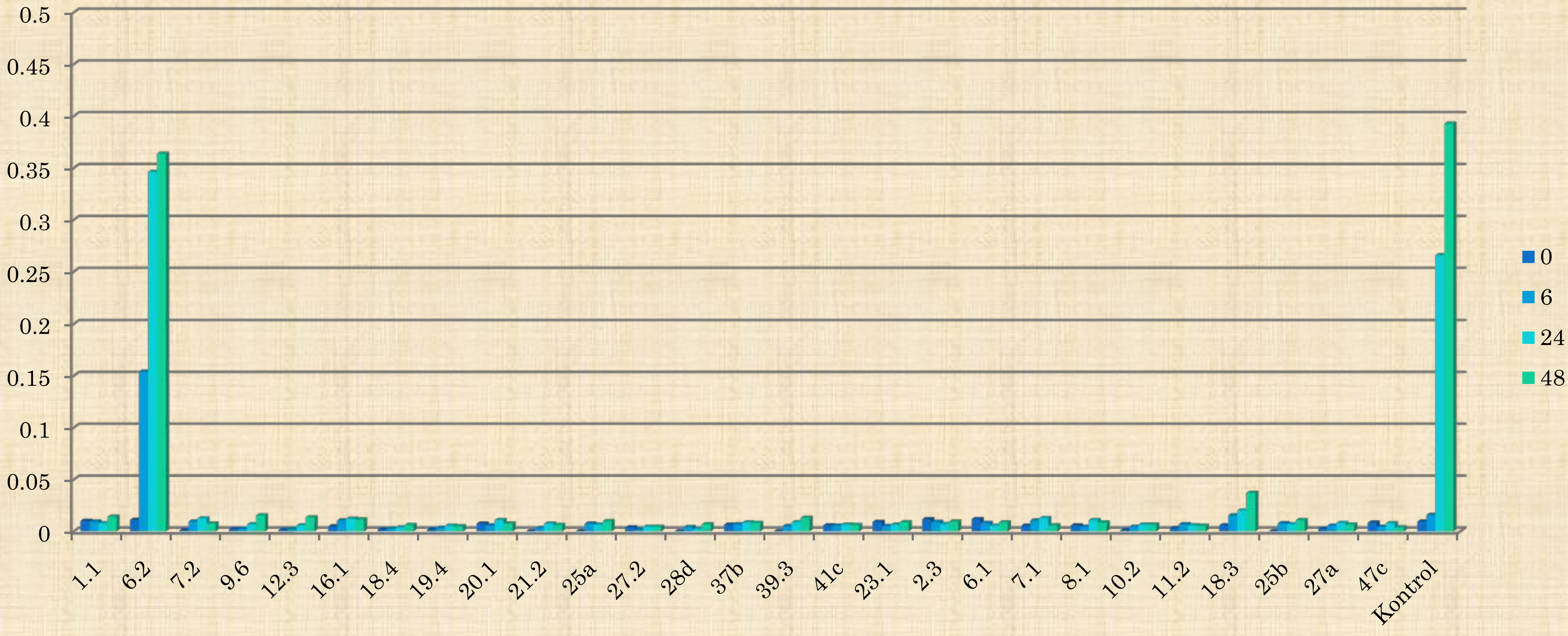
- The absorbans of supernatants were below the the control

Thus

- Organic acids
- H_2O_2
- Bactericins and bacteriosin like metabolites

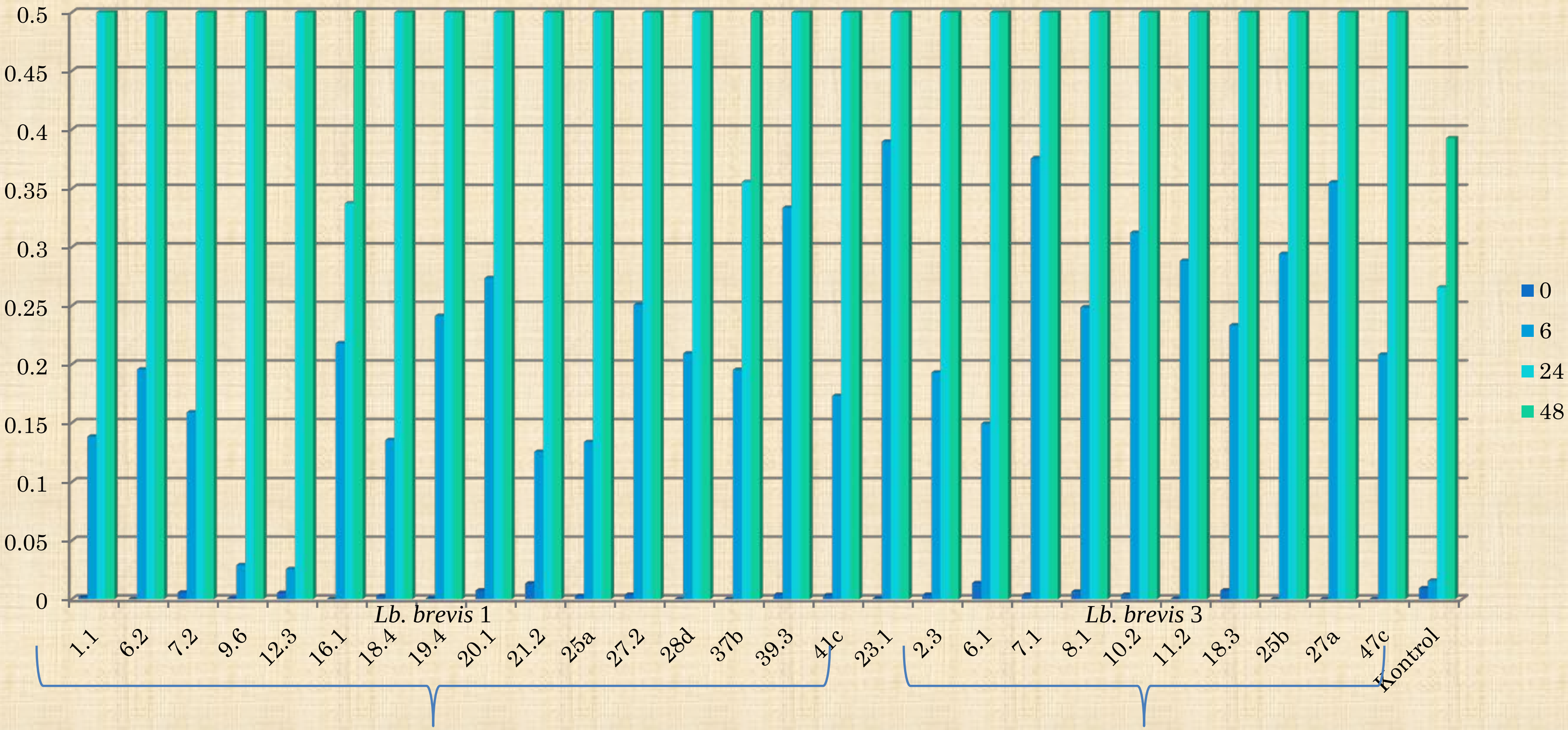
were all effective against *S. aureus.*

Figure 13. Antagonistic effects of organic acids (non-neutralized supernatant) of *Lactobacillus brevis* against *Salmonella typhimurium*



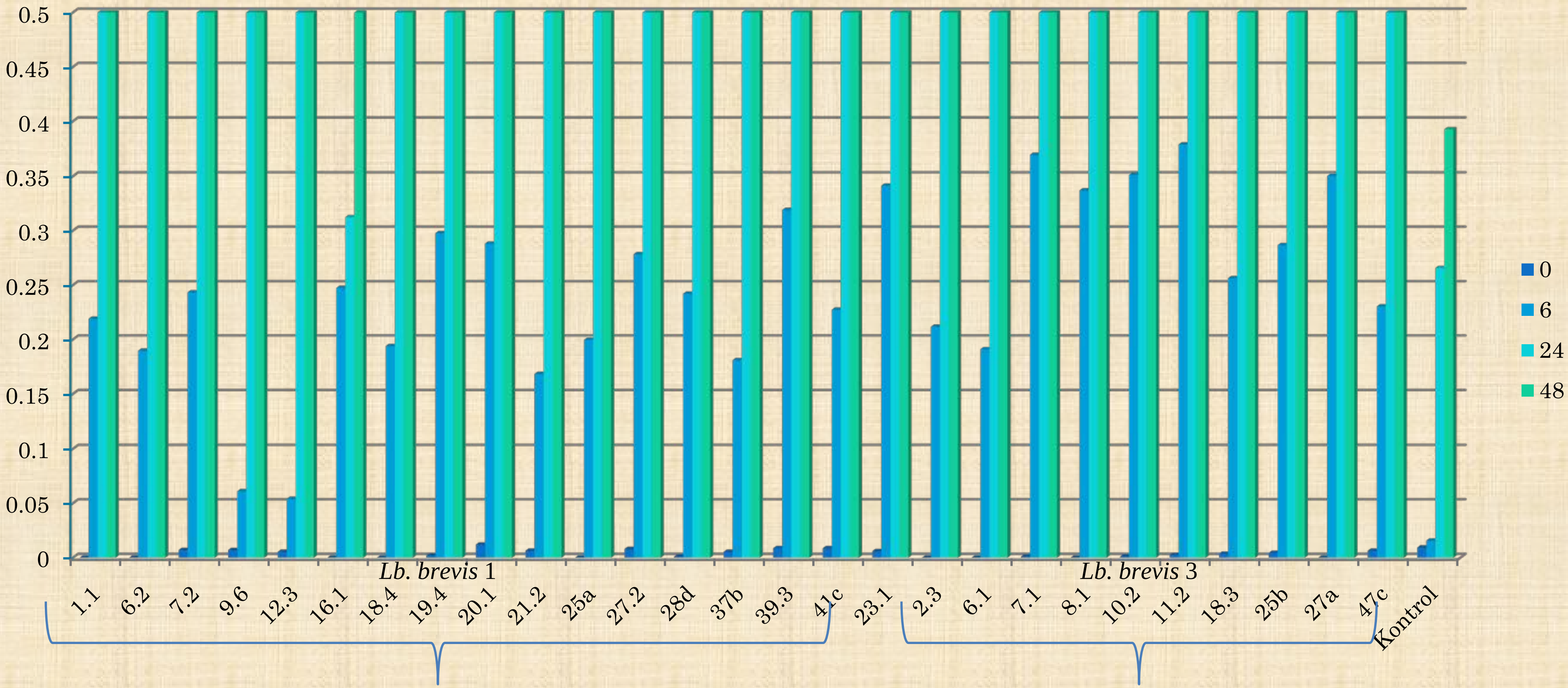
*23.1: *Enterococcus faecium*

Figure 14. Antagonistic effects of neutralized supernatant of *Lactobacillus brevis* against *Salmonella typhimurium*



*23.1: *Enterococcus faecium*

Figure 15. Antagonistic effects of neutralized supernatant **containing** catalase of *Lactobacillus brevis* antimicrobials against*Salmonella typhimurium*



*23.1: *Enterococus faecium*

Lactobacillus brevis against *Salmonella typhimurium*

Organic acids

- The absorbans of supernatants were below the the control
- The absorbans of supernatants were higher than the control for H_2O_2 and bactericin and bacteriosin like metabolites

Thus

- Organic acids were effective except 6.2
- H_2O_2 and were bactericin and bacteriosin like metabolites were ineffective

Figure 16. Antagonistic effects of organic acids (non-neutralized supernatant) of *Lactobacillus plantarum* against *Salmonella typhimurium*

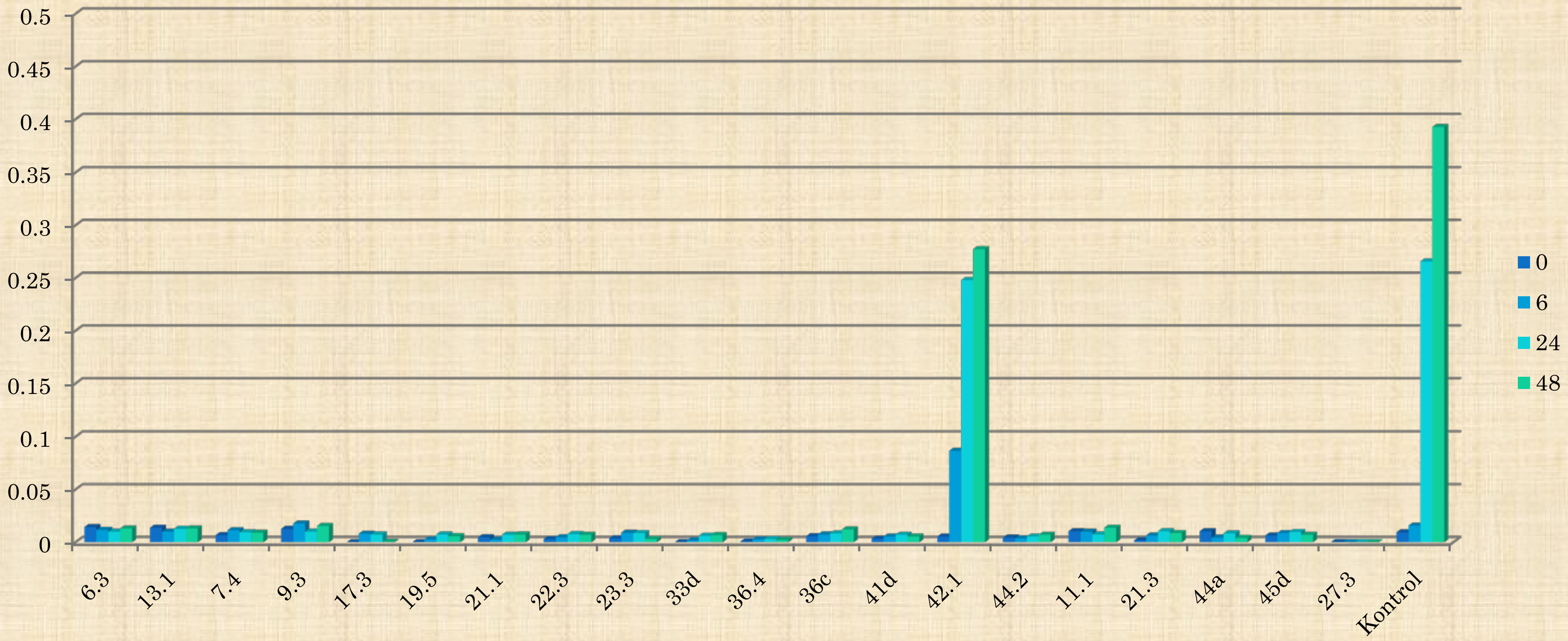


Figure 17. Antagonistic effects of neutralized supernatant of *Lactobacillus plantarum* against *Salmonella typhimurium*

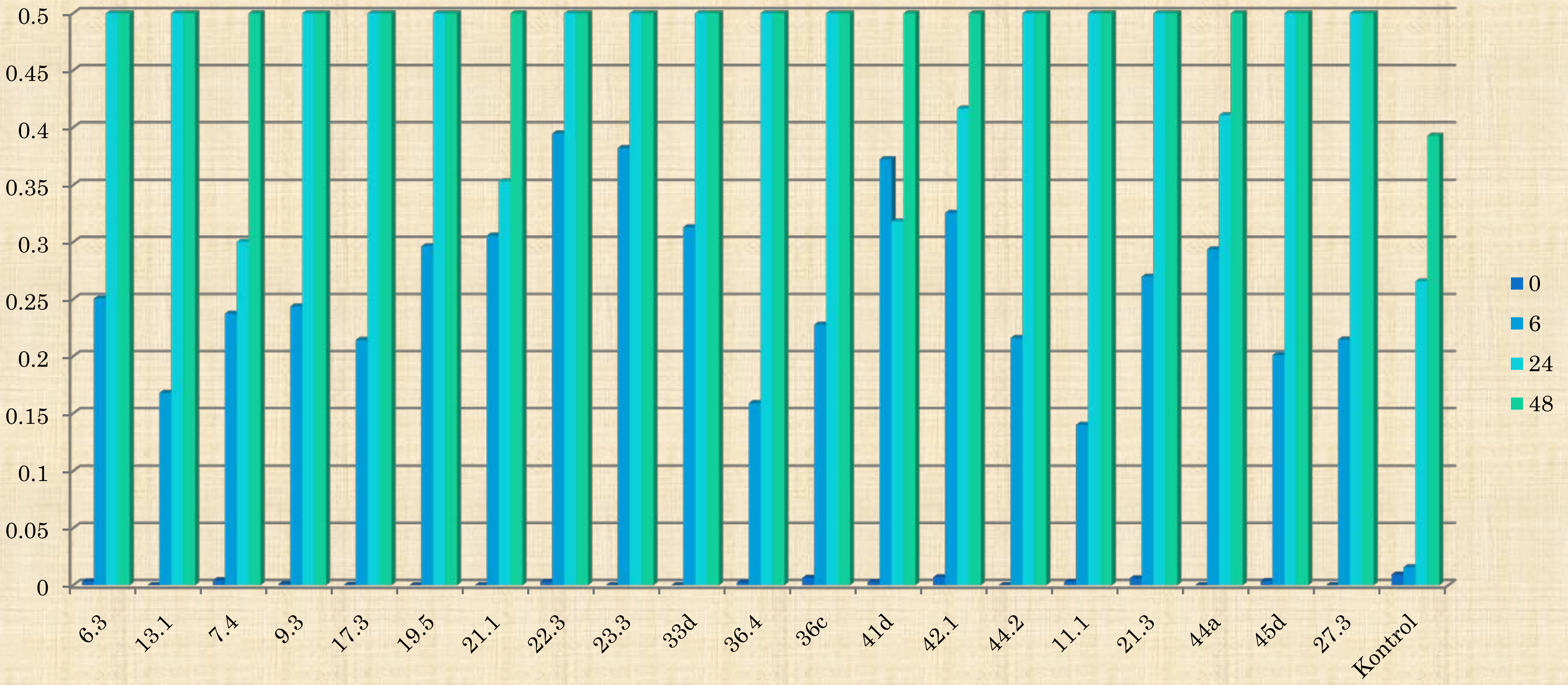
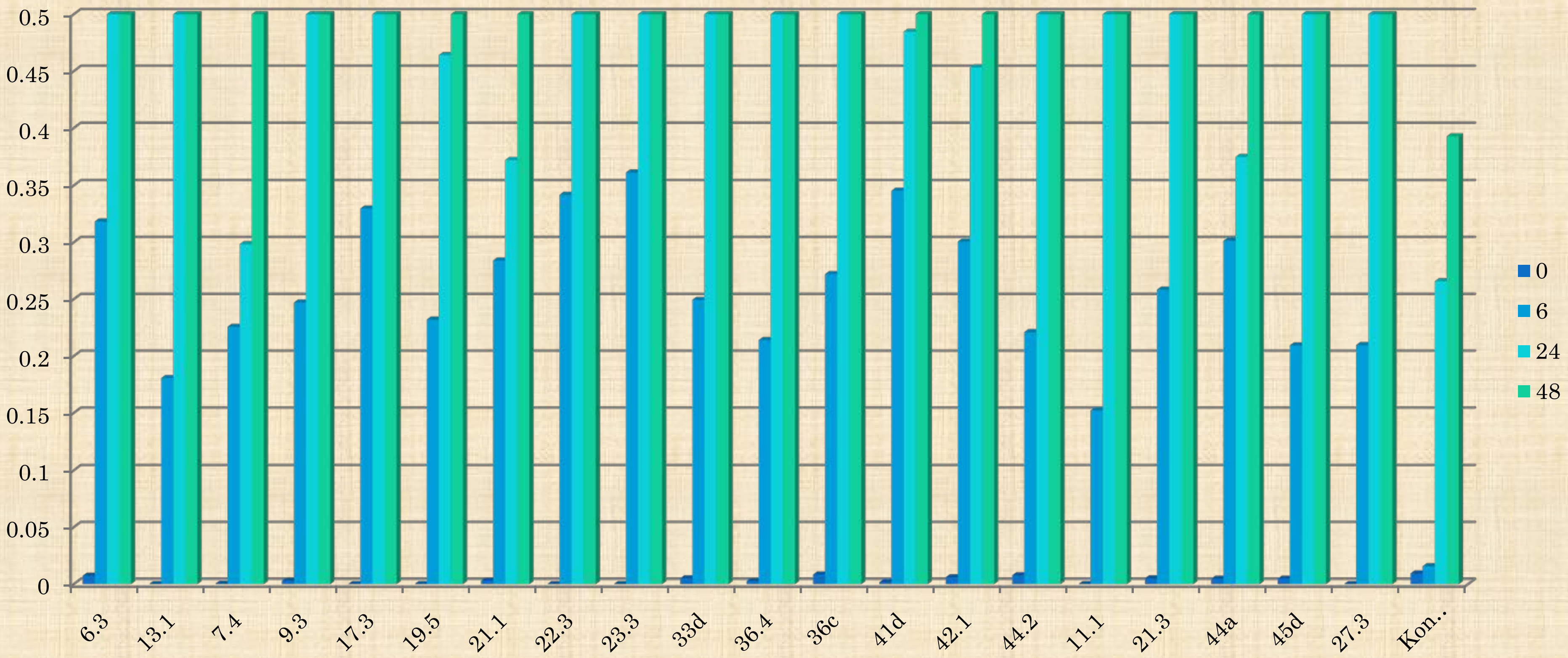


Figure 18. Antagonistic effects of neutralized supernatant **containing** catalase of *Lactobacillus plantarum* antimicrobials against *Salmonella typhimurium*



The results of this study were in agreement with the literature for *Staphylococcus aureus* and for *Salmonella typhimurium* (Todorov ve diğ., 2010; Zago ve diğ.,2011)

We also almost completed the characterization of metabolite that were effective against tested pathogens bu using spectroscopic methods and SDS-PAGE and.

How succesful ?

Many studies have focused on the investigation of **growth parameters** on the production of antimicrobials.

- Optimisation of antimicrobial production by manipulating **growth parameters such as** growth media composition, temperature or pH.
- Investigation of alternative carbon, nitrogen and mineral sources to increase antimicrobial and/or bacteriocin yields or more cost effective production.
- the inclusion of adjunct cultures to induce bacteriocin-production.

Another strategies have been (O' Shea et al., 2013)

- Use of conjugation to transfer a bacteriocin producing phenotype
- Subcloning and expression of bacteriocin genes or gene clusters
- Bioengineering of bacteriocin peptides

While genetic manipulation by recombinant and bioengineering-based approaches offer great promise, only strains which have been modified through non-recombinant approaches can be directly added to food.

How succesful in crop protection ?

- There are a number of studies in the literature that shows the potential of LAB against pathogen in crops.
- One of them is
- The control of Phytophthora infestans by biocontrol agents. It has been reviewed by Axel et al (2012) recently.
- There are two LAB-containing commercial products currently available which have been tested against potato blight.

How succesful as biopesticide?

- Effective microorganism (EM), applied as a liquid microbial inoculant to soil expecting to become dominant in the soil and improve the soil quality and enhancing crop production (Higa and Parr 1994).

•Although these applications improved the soil and crop quality and higher crop yields obtained the final evaluation was generally **ineffective / insufficient in the field** (Becktell et al. (2005); Dorn et al. (2007)

Similar reduction rate was obtained by (oregano) plant extract too (20 to 38 %)

Cereal-based products

- However, the news from many other more promising. For example, LAB bioprotection retards the development of fungal diseases in the field and inhibit pathogens and spoilage fungi in cereal-base products (Oliveira et al.i 2013).
- In addition to the health safety improvement, LAB metabolites also enhance shelf-life, organoleptic and texture qualities of cereal-base foods.
- The application of antimicrobial LAB during malting and brewing can be successfully applied as a hurdle to spoilage microorganism growth (Rouse and van Sinderen, 2008; Wolf-Hall, 2007).

how succesful

- It is estimated that the agricultural chemical industry produces over 45,000 different artificial pesticides/ fertilizers worldwide (Oliveira et al.i 2013)**.**
- **Current late blight control systems are primarily based on the application of pesticides** (Cooke et al. 2011).
- Environmentally friendly products for plant protection, still represent an insignificant portion of the overall pesticide market, (Glare et al. 2012).
- More research is needed in this area.
- Amongst the many trials conducted so far, biological control agents were mostly screened for their activity in vitro and in vivo, with assays generally being performed under very simplified conditions **(Axel et al 2012)**.
- Effective application measures in field still need further optimization (Leblanc et al., 2005).

How succesful as food biopreservation?

- When using live microbial antagonists in food biopreservation, there are a number of criteria and requirements, which must be taken into account;

Criteria and requirements:

- **Consumer protection is the most important aspect, in particular in terms of ready-to-eat food as well as other food products,**

Requirements of biopreservative agents

SAFETY: Consumer protection
Environmental friendly

COMPATIBILITY: protective cultures should not cause any detrimental effects

EFFECTIVITY
Antimicrobial / Antagonistic

STABILITY / SURVIVAL

APPLICIBILITY

Should meet REGULATORY CRITERIA

How succesful as seafood biopreservation?

- **Biopreservation of fish and seafood products is an alternative to meet safety standards and to control microbial deterioration without negative impact on the sensory quality of the product**
- Thus, LAB is effective and usually meets most of the necessary requirements for biopreservation of seafood products. **there is again some problems in their applicibility.** However, **since** they may be efficient only in a narrow range of food environment (pH, fat content, etc.) and this limits their application in many seafood products.

LAB-prosess combinations

- The weakness of LAB / antimicrobials observed in particular foods overwhelmed by combining biopreservative and food processes and more promising results obtained by these combinations. Some of the examples of these combinations in different foods.
- LAB-high hydrostatic pressure
- LAB-pulsed electrical field
- LAB-mild heat treatment
- LAB-ultrasound etc.

promising results were obtained like

In conclusion

- Lactic acid bacteria (LAB) possess a major potential for use in biopreservation **because most LAB are generally recognized as safe, and they naturally dominate the microflora of many foods.**
- **LAB bioprotection retards the development of fungal diseases like Fusarium head blight (**FHB) **in the field and inhibit pathogens and spoilage fungi in food products.**
- FHB is of growing international importance in recent years leading to significant economic losses across the value chain by reducing grain yield and quality of barley and wheat in cultivation sites worldwide (Gilbert and Tekauz, 2000).

In conclusion

- However, to date, all biopesticides tested against Phytophthora infestans showed inconsistent field performance, although some could inhibit the pathogen in vitro (Axel et al 2012).
- **Continued screening of bacteria and their metabolites is necessary to select potential candidates for further specific evaluation as P. infestans control agents.**

In conclusion

- The application of LAB starter cultures in food systems provides an excellent control system regarding the growth of typical food-associated pathogenic/spoilage microorganisms.
- The application of bacteriocin producing LAB, alone or in combination with processes, could be more widely applied by the food industry.
- Thus, lactic acid bacteria have been used as natural food-grade preservatives against a variety of undesirable microorganisms.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