Cite as: Rodríguez-Vaquero, M. J. (2020) Lactic Acid Bacteria as Alternative in the Control of Phytopathogenic Microorganisms, vol. 3, pp. 29-34, Journal Emerging Environmental Technologies and Health Protection, ISSN 2623-4874, e-ISSN 2623-4882 https://www.telegeco.gr/3_2.pdf

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Lactic Acid Bacteria as Alternative in the Control of Phytopathogenic Microorganisms

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Abstract

Lactic acid bacteria are ubiquitous members of many plant microbiomes and several members of the lactic acid bacteria are known to produce antibacterial substances.On the other side, plantsandcropscare is a big challengeandahard mission, which must be constantly improved.The status of food security is critical, and protection against losses caused by crop pestsandplant diseasesis necessaryto improvefood security. Actually,researchers are looking for new natural and non-toxic antibacterial agents as alternative to control plant diseases and the use of lactic acid bacteria could be a good alternative. Many agricultural groupsadopted lactic acid bacteria, as component of sustainableagriculture, to controlplantpestsand stimulate plant growth.

Keywords: Lactic acid bacteria; antibacterial; plant diseases; phyto-pathogenic microorganisms control.

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1. INTRODUCTION

In 1873,the first pure culture of a lactic acid bacterium ("*Bacterium lactis*") was obtained by JosephLister., ten years after Louis Pasteur studied lactic acid fermentation.[1,2]. Lactic acid bacteria are gram-positive, non–spore-forming, catalase-negative, aerotolerant, acid-tolerant, and fermentative, with lactic acid as the major end product during sugar fermentation. Lactic acid bacteria encompass a wide group of microorganisms belonging to the genera *Lactobacillus, Lactococcus Leuconostoc,Pediococcus*, among others [3].

Lactic acid bacteria are ubiquitous members of many plant microbiomes, they have complex nutritional requirements for growth and can survive under harsh environmental conditions. Lactic acid bacteria are generally associated with habitats rich in nutrients, such as various food products (dairy products, meat, wines, vegetables), but some are also members of the normal flora of the mouth, intestine, and vagina of mammals [4, 5, 6, 7].

Lactic acid bacteria have been used for centuries in the preparation and preservation of foods of meat, milk, and vegetable origin and aregenerally recognized as safe (GRAS).A substanceor microorganismmay be GRAS only if its general recognition of safety is based on the views of experts qualified to evaluate the safety of the substance. Starter cultures for cheese and sour milk production were introduced in 1890, while fermented food has been used by man for more than 5,000 years [2, 8].

Several members of the lactic acid bacteria are known to produce antibacterial substances. The antibacterial effect has been ascribed to the production of antibiotic-like substances, hydrogen peroxide production or lactic acid production, for example.

The interactions of lactic acid bacteria with other bacteria have been widely researched in food products and especially in fermented foods and actually, researcher are studying the possibility to use lactic acid bacteria against plant pathogens that produce economiclosses.

On the other hand, during the processing of agricultural products, significant economic losses occurdue to the action of deleterious microorganisms. So, the protection against losses caused by crop pests, plant diseasescan play a critical role in improving food security.

Plant pathogens produce an array of enzymes capable of degrading plant cell wall components. The plant pathogens can produce a disease in seeds, during plant growth or postharvest. Small wounds or cuts occurring during harvesting and transportation provide easy access for potential pathogens.

For example, an important problem in citrus plantsis citrus canker diseasecaused by *Xanthomonas citri*subsp c*itri*. Canker symptoms in leaves and fruits are characterized by surface-penetrating necrotic lesions surrounded by oily, water-soaked margins and yellow chlorotic rings. Citrus canker can lead to defoliation and immature fruit fall, in severe cases [9].The most important economicimpact is restriction of market access for fresh fruit entry into the canker-free European Union because *Xanthomonas citri*subsp c*itri*is a quarantine pathogen.

During the postharvest stage, fruits are susceptible to being attacked by various pathogens, such as viruses, viroids, phytoplasmas, nematodes, bacteria, and fungi[10].Anthracnose is the most frequent disease of fruit postharvest. The main causative agent of anthracnose is *Colletotrichum gloeosporioides* [11].

The blue mold rot, caused by fungi including various species of *Penicillium*, *Botrytis cinerea*, and *Monilinia laxa*, as well as other fungi that produce mycotoxins, and bacteria such as *Erwinia carotovora* and *Xanthomonas vesicatoria* have been described as common spoilage microorganisms of fresh fruits and vegetables [12].

At beginning of the 1930s, a lot ofalternatives of agricultural movements were initiated around the world. Many of these alternative agricultural movements adopted lactic acid bacteria, and especially Lactobacillus, as an indispensable component of sustainable agriculture, to control pests, condition soils, and stimulate plant growth[13, 14, 15].

So, the greatest challenges that researchers have is the search of new natural and non-toxic antibacterial agents as alternative to control plant diseases and the use of lactic acid bacteria could be a good alternative.

2. EXPERIMENTAL SETUP AND RESULTS

2.1. Isolation and preliminary identification of lactic acid bacteria from vine stalks of white grapes with antibacterial activity.

Lactic acid bacteria were isolated from vine stalks of white grapes obtained from a winery from Cafayate, Salta, Argentina). The samples were placed in sterile bags and immediately transported to the laboratory. For isolation of lactic acid bacteria, vine stalks were washed three times with sterile distilled water and water washing was collected under sterile conditions. An aliquots of water washing samples were placed on MRS agar medium supplemented with cycloheximide 1.0 %, then plates were incubated anaerobically for 72 h at 30 °C. The isolates lactic acid bacteria were routinely propaged in MRS broth and incubated at 30 °C. The phenotypic characterization of lactic acid bacteria isolates were carried out considered their results for Gram staining, cell morphology, motility, spore formation, ability of growth at different NaCl concentration and pHand catalase reaction were tested for fermentative catabolism of glucose, gas and D- or L-lactic acid isomers production from glucose in Gibson medium.

The antibacterial activity of lactic acid bacteria against *Xanthomonas citri*subsp c*itri* was determined. *Xanthomonas citri*subsp c*itri* was isolated from citric fruit by the NationalInstituteof Agricultural Technology (INTA). Before experimental use, cultures from solid medium were sub-cultured in liquid media, incubated for 24 h and used as the source of inocula for each experiment. The screening of the antibacterial activity of bacterial supernatants in solid media was carried out according Rodríguez-Vaquero et al. [16]. The agar diffusion test was used to investigate antibacterial effects of supernatants. Soft agar medium was inoculated with liquid overnight culture of *Xanthomonas citri*subsp c*itri* to a cell density of 2.0 X 10^8 cfu/ml, and plates containing 10 ml of agar media were overlaid with 10 ml of this inoculated soft agar. Equidistant holes were made in the agar and 30-µl of each supernatant was pipetted into the agar wells. Copper oxychloride (3 ppm) was used as a positive control and the negative control was the culture media. After 24h of incubation the inhibition zone diameters were measured with an accuracy of 0.5 mm.

Results of these investigations demonstrated that from 25 colonies picked up from MRS agar plates, a total of 9 strains were assignedas presumed lactic acid bacteriagroup because they were Gram positive, catalase negative, non-spore forming, non-motile bacteriaandthe ability of growth at different pH and NaCl concentrations.

The results of the screening of antibacterial activity of supernatants of lactic acid bacteria are presented in table 1.

Table 1. Antibacterial activities of supernatants of lactic acid bacteria isolated from vine stalks of white grapes against *Xanthomonas citri* subsp c*itri*

	Xanthomonas citri
VS 1	-
VS 2	++
VS 3	+
VS 4	-
VS 5	++
VS 6	+++
VS 7	+
VS 8	-
VS 9	-
Positive control	++++
Negative control	-

(-) no inhibition, (+) Inhibition zone between 1-3 mm, (++)Inhibition zone between 4-10 mm, (+++) Inhibition zone between 11-19 mm, (++++), Inhibition zone between 20 -25 mm.

In this work, the five lactic acid bacteria supernantants were effective against *Xanthomonas citri*subsp c*itri,* indicating that this could be a good natural alternative to control canker of citrus, however further studies are carried out to confirm this preliminary results.

In a previous work, Perez Merello *et al*. [17]isolated and identified lactic acid bacteria from vinasse obtained from Argentinean mills. The authors determine the exopolysaccharide production by isolated lactic acid bacteria and the antibacterial activity of lactic acid bacteria, their supernatants and the exopolysaccharide produced by them against *Xanthomonas citri*subsp c*itri*. The results showed that isolated lactic acid bacteria and their exopolysaccharides possess antibacterial activity.

Other authors evaluated the efficacy of lactic acid bacteria isolated from fresh fruits and vegetables as biocontrol agents against the phytopathogenic and spoilage bacteria and fungi*, Xanthomonas campestris, Erwinia carotovora, Penicillium expansum, Monilinia laxa,* and *Botrytis cinerea* [18]. The antagonistic activity of lactic acid bacteria strains was tested *in vitro* and all tested microorganisms except *Penicillium expansum* were inhibited by at least one isolate. The isolates were also analyzed for the inhibition of *Penicillium expansum* infection in wounds of Golden Delicious apples. Four strains reduced the fungal rot diameter of the apples by 20%. The author's results support the potential of lactic acid bacteria as biocontrol agents against postharvest rot.

Barrios-Robledo [19] reported that lactic acid bacteria isolated from fermented products have strong antifungal activity against the phytopathogenic fungus *Colletotrichum* gloeosporioides, ethiologic agent of anthracnoseinpapaya. The authors isolated *Lactobacillus plantarum, Lactobacillus paracasei* and *Lactobacillus pentosus* strains with strong antifungal capacity from two fermented beverages(*Tepache and Tejuino*). All the strains inhibited spore germination by at least 60% and mycelial growth by 100%.

Ferments containing lactic acid bacteria have been used for decades in agricultural systems to improve soils, control disease and promote plant growth, however, the functional roles of lactic acid bacteria in the phytomicrobiome have yet to be discovered [20]. The ease of culturing wild lactobacilli without the use of laboratory equipment or microbiological expertise, paired with its ability to preserve, and even improve, the nutritional quality and flavor of foods, have contributed to the widespread use of these microbes by farmers and the general public [21,22].

2. CONCLUSIONS

Based on the above, taking these evidences into account it was shown that lactic acid bacteria isolated from diverse sources could improve agricultural plant production.

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