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Biological Activity of Native Bacterial Isolates Against Aphids in Beans and Peas Field Production

Vinelina P. Yankova[1*], Dima M. Markova[1], Mladen K. Naydenov[2]

1 - Maritsa Vegetable Crops Research Institute, Department of Technologies in vegetable crops production, 32 Brezovsko shosse St., 4003 Plovdiv, BULGARIA
2 - Agricultural University, Faculty of Plant Protection and Agroecology, Department of Microbiology and Environmental Biotechnologies, 12 Mendeleev Boul., 4000 Plovdiv, BULGARIA
[*]Corresponding author: vinelina@abv.bg

Abstract. Aphids (Hemiptera: Aphididae) are a major group of pests in legume crops. They cause direct damage to the host plants by sucking of plant sap. In addition to direct damage from their feeding, aphids also act as vectors for carrier of several viruses. The main method to control these pests is still the use of chemical insecticides. This practice hides risks due to harmful environmental effects, residual quantities in production, the emergence of resistance populations, destruction of beneficial species and many more. In recent years, there has been a growing interest in the discovery and development of new bioinsecticides as environmental friendly pest control tools to be integrated in combination or rotation with chemical pesticides in pest management programmes. Since the bacteria are relatively poorly studied as biocontrol agents for aphids control, the aim of this study was to evaluate the aphicidal effect of native bacterial isolates. Strains of *Bacillus amyloliquefaciens*, *Paenibacillus polymyxa* and *Providencia rettgeri* were used in experiments, conducted in the period 2019-2020. *Bacillus amyloliquefaciens* A1, *Paenibacillus polymyxa* AB3 and *Providencia rettgeri* K10 were tested against black bean aphid (*Aphis fabae* Scop.) and pea aphid (*Acyrthosiphon pisum* Harris) in the field growing of peas and beans. Among the studied microorganisms, *B. amyloliquefaciens* A1 and *P. rettgeri* K10 showed good aphicidal activity (E≥75%) against black bean aphid (*A. fabae*) and pea aphid (*A. pisum*) in the growing of beans and peas in the field.

Key words: bean, pea, aphids, biocontrol, *Bacillus amyloliquefaciens* A1, *Paenibacillus polymyxa* AB3, *Providencia rettgeri* K10.

Introduction

Of the food legume crops grown in our country, bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.) are the most preferred food for consumption due to their high nutritional value and taste qualities. Their proteins in a nutritional value are close to that of meat, fish and other animal products. Beans and peas also have important agro-technical importance, as they enrich the soil with nitrogen and release the areas early. They are an excellent precursor in crop rotation for many crops. The production of beans and peas is limited by certain environmental stress factors, particularly biotic and abiotic stresses. Among the biotic factors, the attack by pests causes a significant reduction in the yield of legumes.

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Aphids (Hemiptera: Aphididae) are a major group of pests that have a high degree of reproduction. They can cause direct damage to the host plants by sucking plant sap. In addition to direct damage from their feeding, aphids are vectors of various disease causing viruses (Shannag, 2007).

The black bean aphid, *Aphis fabae* Scopoli, is a polyphagous pest that attacks more than 200 legumes and can damage all the parts of the plant (Purhematy et al., 2013). In peas, pea aphid, *Acyrthosiphon pisum* Harris is considered a serious pest as it reduces both the mass and caloric value of young pea plants by 64% and 113%, respectively, depending on the number of aphids (Melesse & Singh, 2012). All stages of plant development are sensitive to direct damage to aphid feeding, but young pods are most susceptible. Feeding damage to flowers and pods is highly correlated with aphid density and show significant reductions in yield. Peas must be protected from aphids for a 2 to 3week period beginning when pods start to form (Maiteki & Lamb, 1985).

The main method of aphid control is still the use of chemical insecticides. This practice hides risks due to harmful environmental effects, residual quantities in production, the emergence of resistance in populations, destruction of beneficial species and many more.

In recent years, there has been a growing interest in the discovery and development of new bioinsecticides as environmental friendly pest control tools to be integrated in combination or rotation with chemicals in pest management programmes.

According to Gonzalez et al. (2016) the most effective control of aphids in integrated and biological production systems is the combination of microbiological plant protection products with bioagents.

Microorganisms namely bacteria are widespread in the environment and have developed various interactions with insects, including symbiosis. While many bacterial species inhabit the insect bodies, establishing different levels of relationships, a limited number of them behave as insectopathogens. The latter have developed a number of strategies for penetrating the host, overcoming its immune barriers and ultimately the ability to infect and kill it. The mechanisms leading to these interactions are thought to be of ancient origin and have evolved through a long process of coevolutionary development (Vilcinskas, 2010).

Such is the case with protein toxins produced by *Bacillus thuringiensis* Berliner (De Maagd et al., 2003). These toxins are usually very specific to a limited number of hosts, while in other cases the bacteria produce metabolites that show a wider insecticidal spectrum.

Entomopathogenic bacteria are traditionally well represented by members of the *Bacillaceae* family, such as *Bacillus* spp. and *Paenibacillus* spp.

Important information about the understanding of the mechanisms involved in the interactions between different pathogens and hosts is obtained as a result of modern research. However, many aspects have not yet been discovered and after several decades of microbial pest control dominated by *B. thuringiensis*, new bacterial species with innovative modes of action have been discovered and formulated as new biopesticide products (Ruiu et al., 2013).

Bacillus amyloliquefaciens commonly found in the soil. There is very little research on its properties as a biological agent to pest control. At this stage, *B. amyloliquefaciens* was studied for mosquito control (Geetha et al., 2010, 2011), tomato leaf miner (*Tuta absoluta* Meyrick) (Saoussen et al., 2015) and citrus mealybug (*Planococcus citri* R.) (Mohamedova et al., 2017). Experiments were performed to determine aphicidal effect of the strains *B. amyloliquefaciens* against *Myzus persicae* Sulzer. Received results propose that *B. amyloliquefaciens*, could function as a novel eco-friendly biopesticide for the control of *M. persicae* (López-Isasmendi et al., 2019).

Experiments with the bacterial isolates *Bacillus amyloliquefaciens* A1, *Paenibacillus polymyxa* AB3 and *Providencia rettgeri* K10, derived from soil samples were performed. They have been screened for their effectiveness in controlling citrus mealybug (*Planococcus citri* Risso). The studied bacterial strains show approximately the same effectiveness. It was found that the mortality rate of larvae from the first age caused by *B. amyloliquefaciens* A1, *P. rettgeri* K10 and *P. polymyxa* AB3 reached 84.29%, 82.62% and 90.37%, respectively (Mohamedova et al., 2017).

To date, several observations have been made on the effect of entomopathogenic bacteria on *Psedococcidae* species, as a result of extracellular production of enzymes or lipopolysaccharides, which destroys hemocytes and internal insect organs after the bacteria enter the hemocoele (*Pseudomonas aeruginosa*, *Serratia marcescens* and *Providencia rettgeri*) (Lysenko, 1985) or because of its ability to produce toxins that destroy the epithelial cells covering the intestines of insects (*Bacillus thuringiensis*) (Van-Rie et al., 1990).

Many authors have concluded that the mechanism of action of entomopathogenic bacteria is based on substances with contact action - enzymes and other metabolic products.

The aim of the study was to determine the biological activity of three native bacterial isolates *Bacillus amyloliquefaciens* A1, *Paenibacillus polymyxa* AB3 and *Providencia rettgeri* K10 against aphids in beans and peas grown in open field conditions.

Material and Methods

Microorganisms

The present study was performed with *Bacillus amyloliquefaciens* A1, *Paenibacilluspolymyxa* AB3 and *Providencia rettgeri* K10 from the collection of the Laboratory of Microbiological Technologies, Agricultural University, Plovdiv. The tested bacterial strains were isolated from the different natural soils. The bacterial isolates were maintained for long-term storage at -20°C storage temperatures in sterile glycerol. For the performed tests, the bacteria were activated by repeated sub-culturing on Bacto nutrient agar (Difco laboratories, Detroit, USA) at 28°C.

Cultivation

For biological tests, the microorganisms were cultured in a liquid medium. For this purpose, 500 ml flasks, containing 200 ml of sterile Triptic soy broth (TSB) (Biolife, Milano, Italy) were inoculated with 1 ml of bacterial suspension obtained by washing colonies of solid medium with sterile water. Inoculum cell density was adjusted to 10^9 CFU ml^{-1} before inoculation as determined by optical density. Cultivation was performed on a rotary shaker at 195 min^{-1} at 28°C for 48 h. Bacterial suspensions (10^7 CFU ml^{-1}) of each isolate were used as inocula for the treatment of experimental variants.

Entomological tests for aphids (Homoptera: Aphididae)

In aphid-infested plants are sprayed with the appropriate dose of test microorganisms. The number of live individuals before spraying and in intervals after 1, 3, 5, 7, 10 and 14 days was reported. Test pests: black bean aphid (*Aphis fabae* Scop.) and pea aphid (*Acyrthosiphon pisum* Harris). Test microorganisms: *B. amyloliquefaciens* A1, *P. polymyxa* AB3 and *P. rettgeri* K10. Total area of the experimental plot was 100 m^2 of bean variety Evros and 100 m^2 pea variety Skinado.

The effectiveness (%) was calculated by the formula of Henderson-Tilton (1955):

$$E\% = \left(1 - \frac{TaxCb}{TbxCa}\right).100,$$

where:

Ta - number of live aphids in the variant after treatment;

Tb - number of live aphids in the variant before treatment;

Ca - number of live aphids in the control after treatment;

Cb - number of live aphids in the control before treatment

A comparative analysis was made using Duncan’s multiple range test (1955).

Results and Discussion

Aphids are pests that have a high degree of reproduction. Successful control with them is associated with problems, as in populations often developed resistance to the used chemical plant protection products. In recent years, efforts have focused on exploring the possibilities of biological control - the use of phytopesticides, microbial products and products of mineral origin.

The biological activity of three microorganisms against black bean aphid (*Aphis fabae* Scop.) in bean variety Evros grown in the field was studied. Good effectiveness of *Bacillus amyloliquefaciens* A1 (E = 77.09% on the 5th day after treatment) and *Providencia rettgeri* K10 (E = 78.02% on the 7th day after treatment) was found. This trend was observed in both experimental years, as reported effectiveness in *Bacillus amyloliquefaciens* A1 was 73.42% (2019) and 80.76% (2020) on the 5th day after treatment, respectively. In *Providencia rettgeri* K10, the effectiveness was 76.55% (2019) and 79.49% (2020) on the 7th day after treatment. *Paenibacillus polymyxa* AB3 showed unsatisfactory biological activity (E <60%) against this pest (Table 1 and Fig. 1). The aphid density changes and it is significantly lower in the variants by the end of the experiment compared to the control. During the period 5 - 7 days after treatment in the variants *Providencia rettgeri* K10 and *Bacillus amyloliquefaciens* A1, the average number of aphids is less than 10 per plant (Fig. 2).

In pea variety Skinado, experiments to determine the biological activity of the same microorganisms included in the studies in bean variety Evros were made to control the pea aphid (*Acyrthosiphon pisum* Harris). The same trend for good biological activity of *Bacillus amyloliquefaciens* A1 (E = 75.25% on the 7th day after treatment) and *Providencia rettgeri* (E = 77.10% on the 7th day after treatment) was observed (Table 2).

Good effectiveness of the studied microorganisms during the experimental years was found in *Bacillus amyloliquefaciens* A1 74.52% (2019) on the 7th day after treatment and 78.74% (2020) on the 5th day after treatment, in *Providencia rettgeri* 76.03% and 78.17% on the 7th day after treatment, respectively. *Paenibacillus polymyxa* AB 3 showed unsatisfactory biological activity (E <60%) against this pest (Table 2 and Fig. 3).

Table 1. Biological activities of the studied microorganisms against black bean aphid (*Aphis fabae* Scop.) in bean variety Evros. *Legend:* a, b, c … – Duncan's multiple range test ($p < 0.05$).

Variants	Days after treatment	Effectiveness 2019	Effectiveness 2020
Providencia rettgeri K10	1	40.24 b	43.00 c
Paenibacillus polymyxa AB3		28.22 b	31.55 n.s.
Bacillus amyloliquefaciens A1		33.00 b	43.29 b
Providencia rettgeri K10	3	50.70 b	61.68 b
Paenibacillus polymyxa AB3		39.82 ab	48.93 n.s.
Bacillus amyloliquefaciens A1		46.43 b	66.01 ab
Providencia rettgeri K10	5	57.23 b	67.32 ab
Paenibacillus polymyxa AB3		48.43 ab	58.26 n.s.
Bacillus amyloliquefaciens A1		**73.42** a	**80.76** a
Providencia rettgeri K10	7	**76.55** a	**79.49** a
Paenibacillus polymyxa AB3		58.70 a	49.58 n.s.
Bacillus amyloliquefaciens A1		49.92 b	68.91 ab
Providencia rettgeri K10	10	50.16 b	65.30 ab
Paenibacillus polymyxa AB3		49.67 ab	49.33 n.s.
Bacillus amyloliquefaciens A1		39.94 b	58.28 ab
Providencia rettgeri K10	14	40.54 b	54.81 bc
Paenibacillus polymyxa AB3		42.23 ab	46.47 n.s.
Bacillus amyloliquefaciens A1		33.49 b	53.90 ab

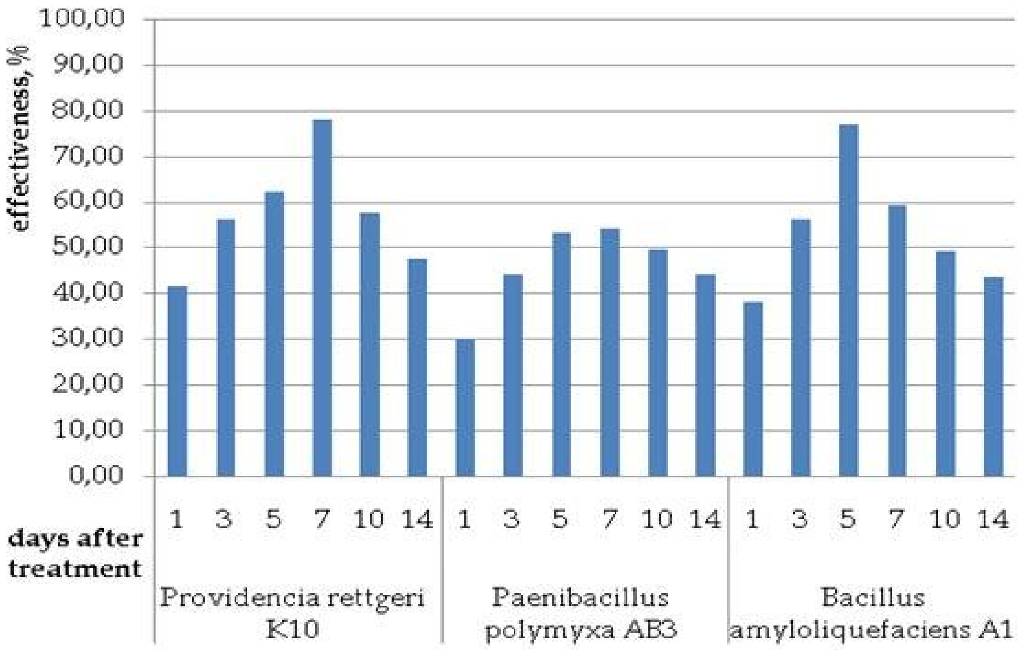

Fig. 1. Average effectiveness of the studied microorganisms against black bean aphid (*Aphis fabae* Scop.) in bean variety Evros.

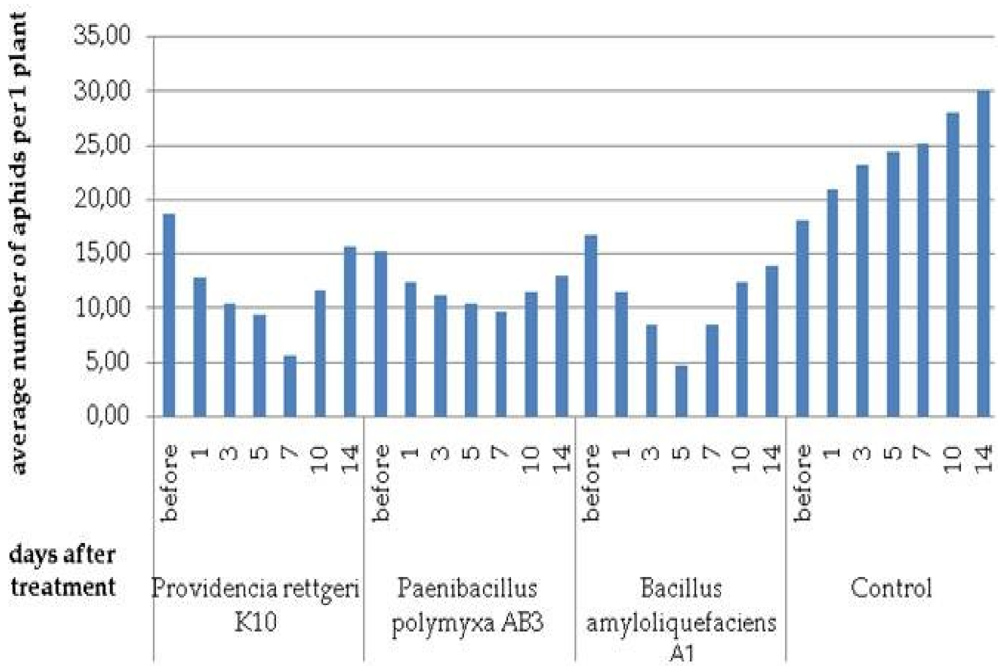

Fig. 2. Average population density of black bean aphid (*Aphis fabae* Scop.) in bean variety Evros.

Table 2. Biological activities of the studied microorganisms against pea aphid (*Acyrthosiphon pisum* Harris) in pea variety Skinado. Legend: a, b, c … – Duncan's multiple range test ($p < 0.05$).

Variants	Days after treatment	Effectiveness	
		2019	**2020**
Providencia rettgeri K10	1	34.84 c	47.89 b
Paenibacillus polymyxa AB3		25.33 d	45.56 n.s.
Bacillus amyloliquefaciens A1		29.67 b	46.21 c
Providencia rettgeri K10	3	48.96 b	66.57 a
Paenibacillus polymyxa AB3		32.47 cd	47.48 n.s.
Bacillus amyloliquefaciens A1		38.56 b	67.35 ab
Providencia rettgeri K10	5	57.08 b	70.99 a
Paenibacillus polymyxa AB3		41.48 bc	55.83 n.s.
Bacillus amyloliquefaciens A1		49.52 b	**78.74** a
Providencia rettgeri K10	7	**76.03** a	**78.17** a
Paenibacillus polymyxa AB3		52.46 ab	59.51 n.s.
Bacillus amyloliquefaciens A1		**74.52** a	75.98 ab
Providencia rettgeri K10	10	51.28 b	66.88 a
Paenibacillus polymyxa AB3		55.91 a	58.69 n.s.
Bacillus amyloliquefaciens A1		45.90 b	65.24 ab
Providencia rettgeri K10	14	46.46 b	61.62 ab
Paenibacillus polymyxa AB3		47.48 ab	49.13 n.s.
Bacillus amyloliquefaciens A1		41.00 b	61.40 b

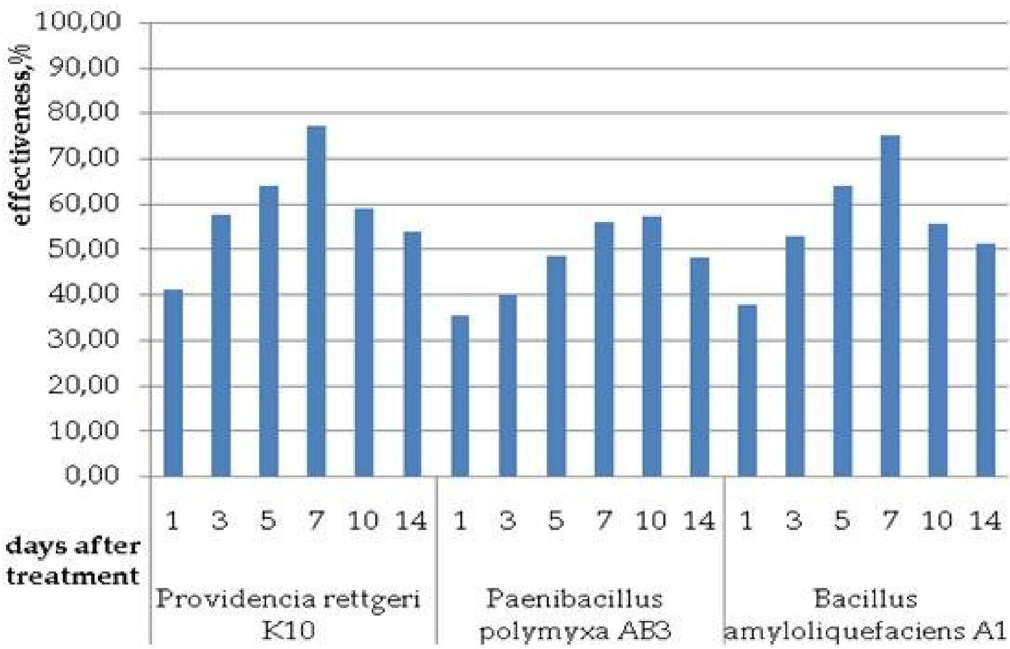

Fig. 3. Average effectiveness of the studied microorganisms against pea aphid (*Acyrthosiphon pisum* Harris) in pea variety Skinado.

The aphid density changes and it is significantly lower in the variants by the end of the experiment compared to the control. In the variants *Providencia rettgeri* K10 and *Bacillus amyloliquefaciens*, the density was lowest on day 7 after treatment, with an average number of aphids per stem 6.25 and 8.63, respectively (Fig. 4).

In the two crops included in the study, beans and peas, there was a decrease in the effectiveness of the microorganisms against aphids after the 7th day of treatment, which should be included in the treatment and re-introduction scheme after this period.

Of the studied microorganisms those that showed good biological activity against aphids in legume crops could be included in subsequent studies as effective bioagents to control these pests.

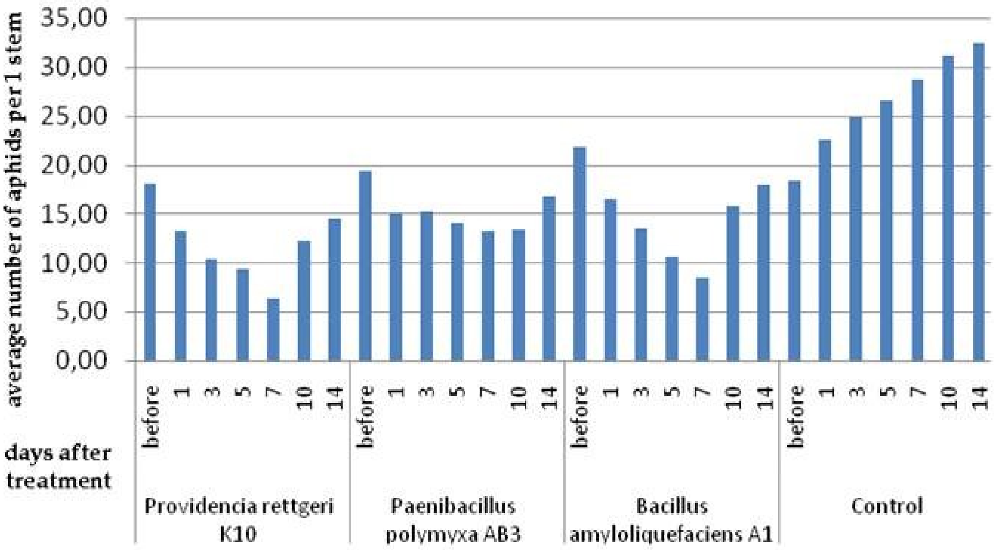

Fig. 4. Average population density of pea aphid (*Acyrthosiphon pisum* Harris) in pea variety Skinado.

Conclusions

Of the studied microorganisms, *Bacillus amyloliquefaciens* A1 and *Providencia rettgeri* K10 showed good aphicidal activity (E≥75%) against black bean aphid (*Aphis fabae* Scop.) and pea aphid (*Acyrthosiphon pisum* Harris) in growing of beans and peas in the field.

Due to the shown best effectiveness of the tested microorganisms *Bacillus amyloliquefaciens* A1 and *Providencia rettgeri* K10 on the fifth to seventh day after treatment, it can be included in the scheme and re-introduction after this period.

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