Mol2Net-04, **2018**, BIOCHEMPHYS-01 (pages 1- x, *type of paper, doi: xxx-xxxx*
http://sciforum.net/conference/mol2net-4

SciForum
Mol2Net-04

Isolation of *Erwinia amylovora* Bacteriophage from Tunisian Soil and Pear Trees Plant Tissues

Asmahen Akremi *[1], Mouna Jlidi[1], Adel Haj Brahim[1], Manel Ben Ali[1,2], Lobna Daoud[1], Houda Hmani[1], Samir Bejar[1], Naser Aliye Feto[3], Mamdouh Ben Ali[1,2]

[1] Laboratory of Microbial Biotechnology and Engineering Enzymes (LBMIE), Center of Biotechnology of Sfax (CBS), University of Sfax, Road of Sidi Mansour km 6, PO Box 1177 Sfax 3018, Tunisia; mamdouh.benali@cbs.rnrt.tn (M.B.A)

[2] Astrum Biotech, Business incubator, Center of Biotechnology of Sfax (CBS), University of Sfax, Road of Sidi Mansour km 6, PO Box 1177 Sfax 3018, Tunisia.

[3] OMICS Research Group & Facility: Department of Biotechnology, Faculty of Applied & Computer Sciences, Vanderbijlpark Campus, Private Bag, X021 - Vanderbijlpark - 1911 - Andries Potgieter Blvd - South Africa.

***** Author to whom correspondence should be addressed; E-Mail: asmahen.akremi@gmail.com (A.A); Tel.: +216-50493606.

Received: / Accepted: / Published:

Abstract: Attributed to a Gram-negative bacterium, identified as *Erwinia amylovora*, the fire blight disease, recently detected in Tunisia, has become a real threat to our economy and our arboriculture. There is no effective curative treatment to eliminate the installed bacterium. It is therefore essential to prevent attacks and to limit the spread of the bacteria if it is already present. To do so we have chosen the biocontrol by bacteriophages.

In this work we have isolated new strains of *Erwinia amylovora* (10 strains) from plant tissues where fire blight symptoms are persistent. These strains have been the subject of a molecular study.

The isolated phages (30 isolates) were studied for their infectivity on *Erwinia amylovora* isolates and showed a surprising effect. Our phage isolates formed plaque of different sizes, with a diameter ranging between 0.8 and 7 mm on the soft agar layer containing the test bacterium. The phages thermostability showed that all these viruses resist at 80 ° C for 45 min.

Keywords: *Erwinia amylovora*, bacteriophage, soil, plant tissues, thermostability.

Mol2Net-04, **2018**, BIOCHEMPHYS-01 (pages 1- x, *type of paper, doi: xxx-xxxx*
http://sciforum.net/conference/mol2net-4

1. Introduction

Fire blight, a destructive disease caused by *Erwinia amylovora*, threatens several varieties of apple and pear trees (1). The bacterium can contaminate all aerial parts of plants (flowers, leaves, shoots, trunks, neck and rootstock), it is considered the most difficult bacteria to control because it can exist in two forms endophyte and epiphyte (2) and causes a severe infection that can cause total death of the plant in a single season. The disease was first discovered in the United States of America in 1794 (1) and was distributed to approximately 40 countries in North America, Europe, the Middle East and New Zealand. Similar symptoms of fire blight were observed in the spring of 2012 and 2013 for the first time in the Morneg area of Tunisia (3). The disease is caused by the production of exopolysaccharides (EPS) produced by *Erwinia amylovora* in the host plant. Control of the disease depends on the limitation of primary blossom infection in the spring and rapidly removing infected tissue.

The disease control potential of *E. amylovora* bacteriophages was first demonstrated in Britich Columbia by Erskine (4). Specific phages for this bacterium were abundant in orchards with symptoms of the disease. Studies have shown that individual phage isolates have broad host ranges within isolates *Erwinia amylovora*. Co-culture of the bacterium with phages resulted in a reduction of the bacterial population. This has led researchers to suggest that phages may be useful for the control of fire blight (5).

A number of different *Erwinia amylovora*-phages have been isolated, characterized and tested for their biocontrol efficacy worldwide (4-5). Moreover complete genomes of some of these phages have become available (6-10).

2. Results and Discussion

In Tunisia, symptoms similar to those of fire blight were observed on pear trees (Pyrus communis cv. Alexandrine, Williams) in the spring of 2012 and 2013 from the flowering stage. The symptoms of the disease appeared in 2012 in the region of Mornag and the following year they spread to the regions of Manouba and Tebourba. Percentages of orchard areas of infected plants varied around 100% (3, 11).

In order to combat this scourge, we started a research and isolation campaign of *Erwinia* strains in order to identify and characterize them in order to propose treatment solutions. 10 strains of *Erwinia amylovora* from different regions with symptoms of the disease in question were isolated and purified.

Phage isolation: A total 30 phages of *Erwinia amylovora* were isolated on LV medium. These bacteriophages are isolated from the plant tissues of pear and soil samples from the Manouba and Tebourba regions. Our phage isolates formed plaques of different sizes, with a diameter of 0.8–7 mm on the soft agar layer containing the test bacterium (Figure 1). On the different phages isolated from the plant tissue of pear and soil, we found that the diameter of the soil phage is larger and clear on the box than the phages isolated from the plant tissue. This is explained by the fact that soil phages are more stabilized by colloids loaded with humus and are well protected against desiccation and the effect of UV, which is consistent with the findings of Gill et al (5). In addition, Ritchie et al (12) have shown that plant tissue bacteriophages are unable to reside permanently in the same tissue after a long period of time since they could not isolate bacteriophage in the tissues on wintery months.

The phages thermostability test has been shown that all these viruses resist at 80 ° C for 45 min and some of them even withstand a heat treatment of the order of 100 ° C.

DNA extraction: The purpose of DNA extraction from the different purified phages is to sequence the genome. It was performed according to a protocol described above. The different DNA samples are migrated on a 0.8% agarose gel with a size marker that is DNA λ (42 kb) and after visualization sub-UV; we obtain low intensity bands and small. The sizes of the genomes studied vary between 40kb and 80kb.

Figure 1. Bacteriophage drop test on *Erwinia amylovora* strain

3. Materials and Methods

Bacterial strains: Strains of *Erwinia amylovora* were isolated from plant tissues of different station of Tunisia. The bacteria were grown in specific Levan (LV) medium (yeast extract 2 g; bactopeptone 5 g; NaCl 5 g; sucrose,50 g; pH 7.0–7.2) at 28°C while 24 to 72 hours. Liquid culture was carried out with Luria-Bertani (LB) added 1 % sucrose and incubated at 28°C in an orbital shaker. Molecular analysis was performed using tow primer that will be used for the amplification of the 16s RNA are RD1 and FD1. These primers allow the amplification of a 1500 bp fragment. PCR products were separated by electrophoresis on a 1% agarose gel, stained with ethidium bromide and photographed under UV light. A 1 kb DNA ladder (Invitrogen) was used as size marker.

Phage isolation: Collections samples were made on May 2017 from sites in the Mannouba region and Tebourba, Tunisia. Phages were isolated from aerial parts and soil of pear trees exhibiting fire blight symptoms. Only one bacterial host strain was used in the initial isolation and enrichment process in LB with 1% sucrose. Phage enrichment and isolation have been made according to procedures of Gill et al. (5), modified by Born et al. (6). Phage detection, purification and titre assessment were conducted with spot tests and Adams' double agar overlay method (7). Bacteriophage isolates were distributed on LBA soft layers supplemented with 1% (w/v) sucrose and containing *Erwinia amylovora* according to Adams' double agar overlay method (7). After incubation for 24 hours at 28 °C phage isolates were visually characterized based on plaque size and thickness of halos around the plaques.

The thermostability of the phages was observed at different temperatures of 50 ° C to 100 ° C as a function of time.

Analysis of phage DNA: For phage propagation and extraction of nucleic acid from phage particles, the method described by Su et al. (8) was followed and modified by Vinod et al. (9).

4. Conclusions

This work is part of the national program for combating the fire blight disease.

Attributed to a Gram-negative bacterium, identified as *Erwinia amylovora*, fire blight disease, recently detected in Tunisia, has become a real threat to our arboriculture and our economy.

There is no effective curative treatment to eliminate the bacterium installed. It is therefore essential to prevent attacks and to limit the spread of the bacteria if it is already present. To be done we chose the fight by the bacteriophages. This struggle is the first in Tunisia.

We have been able to isolate new strains of *Erwinia amylovorane* (10 strains) from plant tissues where fire blight symptoms are persistent.

Moreover we were able to isolate, for the first time, phages from the soil of different Tunisian territories. We also isolated the phages from plant tissues, which is also a first.

Isolated phages (30 isolates) were studied for their infectivity on *Erwinia amylovora* isolates and showed a surprising effect.

Acknowledgments

Conflicts of Interest

State any potential conflicts of interest here or "The authors declare no conflict of interest".

References and Notes

1. Roberts, R.G.; Hale, C.N.; Van Der, Z. T.; Miller, C.E.; Redlin, S.C. The potential for spread of *Erwinia amylovora* and fire blight via commercial apple fruit; a critical review and risk assessment. *Crop Protection* **1998**, *1*, 19–28.
2. Thomson, S. Epidemiology of fire blight. In *The Disease and its Causative Agent, Erwinia amylovora,* 2nd ed.; Vanneste, J.; HortResearch: Hamilton, New Zealand, 2000; Volume 3, pp. 370-382.
3. Rhouma, A.; Helali, F.; Chettaoui, M.; Hajjouji, R.; Hajlaoui, M. R. First Report of Fire Blight Caused by *Erwinia amylovora* on Pear in Tunisia. *Plant Disease* **2014**, *98(1),*158-158.
4. Erskine, J.M. Characteristics of *Erwinia amylovora* bacteriophages and its possible role in the epidemiology of fire blight. *Canadian Journal Microbiology* **1973**, *19*, 837–845.
5. Gill,J.J.; Svircev,A.M.; Smith, R.; Castle A.J. Bacteriophages of *Erwinia amylovora*. *Applied and Environmental Microbiology*, **2003**, 4, 2133–2138.
6. Born, Y.; Fieseler, L.; Marazzi, J.; Lurz, R.; Duffy, B.; Loessner, M.J. Novel Virulent and Broad-Host-Range *Erwinia amylovora* Bacteriophages Reveal a High Degree of Mosaicism and a Relationship to *Enterobacteriaceae* Phages. *Applied and Environmental Microbiology*, **2011**, 5945–5954.
7. Adams, M. H. (1959). *Bacteriophages*. New York: Interscience Publishers.
8. Su, M.T.; Venkatesh, T.V.; Bodmer, R. Large and small-scale preparation of bacteriophage λ lysate and DNA. *Bio-Technology*, **1998**, 25, 44–46.
9. Vinod, M.G.; Shivu, M.M.; Umesha, K.R.; Rajeeva, B.C.; Krohne, G.; Karunasagar, I.; Karunasagar, I. Isolation of Vibrio harveyi bacteriophage with a potential for biocontrol of luminous vibriosis in hatchery environments. *Aquaculture*, **2006,** 255, 117–124.
10. Yagubi, A. I., Castle, A. J., Kropinski, A. M., Banks, T. W., & Svircev, A. M. Complete genome sequence of *Erwinia amylovora* bacteriophage vB_EamM_Ea35-70. *Genome Announcements*, **2014**, 2, 413–414.
11. Gaaliche, B. ; Chehimi, S. Health status of the pear tree following the establishment of Fire blight in Northern Tunisia. *International Journal of Fruit Science*, **2018**,18,85-98.
12. Ritchie, D.F.; and Klos, E.J. Some properties of Erwinia amylovora bacteriophages. *Phytopathology*, **1979**, 69, 1078-1083.