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#### DAFTAR ISI

|                                                                                                                                                                                                                                                | Halaman |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| Farming system research on Madura Tobacco<br>SOENARDI, MUCHAMAD YUSRON, A.S. MURDIYATI and<br>MUKANI .....                                                                                                                                     | 99      |
| A study of optimum cropping pattern and irrigation system on cotton + soybean<br>cropping at lowland of rainfed area, South Sulawesi<br>J. LIMBONGAN, J. WIROATMODJO, I. GONARSYAH, HASNAM D, MURDIYARSO,<br>and H.M.H. BINTORO DJOEFRIE ..... | 109     |
| Cotton and sesame intercropping on dry land<br>SYAFRUDIN KADIR and PETER TANDISAU .....                                                                                                                                                        | 120     |
| Isolation of <i>Pseudomonas syzygii</i> bacterium from <i>Batrachomorphus cocles</i><br>a potential insect vector of xylem limited bacterium in clove<br>ADRIA and HERWITA IDRIS .....                                                         | 129     |
| The effect of the ash from oilpalm fruit bunch and micro foliar fertilizer<br>on the growth of vanilla stem cutting<br>ROSIHAN ROSMAN, SUDIRMAN YAHYA and M. IDRUS MARPAUNG .....                                                              | 136     |
| Development of cotton pest <i>Helicoverpa armigera</i> Hubner on several alternate hosts<br>ELNA KARMAWATI and AGUS KARDINAN .....                                                                                                             | 145     |
| Patchouli oil products as insects repellent<br>TRI L. MARDININGSIH, TRIANTORO, S.L. TOBING and S. RUSLI .....                                                                                                                                  | 152     |



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# ISOLATION OF *Pseudomonas syzygii* BACTERIUM FROM *Batrachomorphus cocles* A POTENTIAL INSECT VECTOR OF XYLEM LIMITED BACTERIUM IN CLOVE

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## ABSTRACT

*Batrachomorphus cocles* is one the insects found on clove trees. It was assumed to be a potential insect vector of *Pseudomonas syzygii* causing xylem limited bacterium (local term : BPKC) disease in clove trees. This insect has a life pattern similar to *Hindola fulva*, which is known as the insect vector of *P. syzygii*. To find out the role of *B. cocles* on clove trees, isolation of *P. syzygii* bacterium from *B. cocles* was carried out at the Solok Sub. Research Institute for Spices and Medicinal Crops from September 1991 to October 1992. Insect samples for isolation material were collected eight times, each time on 10 diseased clove trees and 10 healthy clove trees. For each collection 10 nymphs and 10 imagoes were caught. The isolated bacteria were tested using antiserum plant serological test of agglutination method and Koch postulate test on three-year old clove plants. The results of the serological tests showed that the nymphs and imagoes collected from diseased trees as well as imagoes collected from healthy trees were positively infected with *P. syzygii* bacteria with a percentage of 1.67-2.78 %, 0.16-1.32 %, and 0.03-0.08 % respectively and with bacterium population of 2.09-3.21, 0.47-1.36, and 0.14-0.28 CFU per insect respectively. The results of Koch postulate test indicated that the isolated bacteria of the imagoes collected from diseased trees had the highest pathogenicity (19.17%) and the earliest incubation period (182 days). The isolated bacteria of the imagoes collected from healthy trees had the lowest pathogenicity (9.17%) and latest incubation period (194 days). Microscopic examination showed that more vessels were plugged by bacteria in the plants which were inoculated with isolated bacteria of imagoes collected from diseased trees, 4.12 vessels of roots and 1.54 vessels of stem. While the plants which were inoculated with isolated bacteria of imagoes collected from healthy trees showed the lowest amount of plugged vessels, 2.88 vessels of root and 0.75 vessels of stems.

Key words : Clove, *Syzygium aromaticum*, insect vector, *Pseudomonas syzygii*, *Batrachomorphus cocles*

## RINGKASAN

**Isolasi bakteri *Pseudomonas syzygii* dari tubuh *Batrachomorphus cocles* serangga berpotensi vektor penyakit BPKC**

Pada tanaman cengkeh ditemukan beberapa jenis serangga, diantaranya *Batrachomorphus cocles* yang diduga

berpotensi sebagai vektor penyakit bakteri pembuluh kayu cengkeh (BPKC). Serangga ini pola hidupnya mirip *Hindola* spp. yang telah diketahui berperan sebagai vektor. Untuk mengetahui peranan *B. cocles* pada tanaman cengkeh, dilakukan penelitian bakteri *Pseudomonas syzygii* dari serangga *B. cocles* di Sub Balitro Solok dari bulan September 1991 sampai Oktober 1992. Koleksi serangga contoh untuk bahan isolasi bakteri dilaksanakan delapan kali masing-masing pada 10 tanaman cengkeh sakit dan sehat. Dari tiap-tiap tanaman ditangkap 10 ekor nimfa dan imago. Bakteri hasil isolasi diuji memakai antiserum tes serologi tanaman dengan metode aglutinasi serta uji postulat koch secara buatan pada tanaman bibit umur tiga tahun. Dari uji serologi terhadap isolat diketahui bahwa imago dan nimfa yang dikoleksi dari tanaman sakit serta imago dari tanaman sehat, positif terinfeksi bakteri *P. syzygii* dengan persentase masing-masing antara 1.67-2.78 %, 0.16-1.32 %, dan 0.03-0.08% serta kandungan bakteri antara 2.09-3.21, 0.47-1.36, dan 0.14-0.28 koloni tiap serangga. Dari uji postulat Koch secara visual diketahui, bahwa isolat bakteri dari imago asal tanaman sakit mempunyai tingkat infeksi paling tinggi terhadap tanaman (19.17%) dan gejala awal paling cepat (182 hari). Isolat bakteri dari imago asal tanaman sehat mempunyai tingkat infeksi paling rendah (9.17 %) dengan gejala awal paling lambat (194 hari). Secara mikroskopis terlihat penyumbatan xylem lebih banyak terdapat pada tanaman yang diinokulasi dengan isolat bakteri dari imago asal tanaman sakit dengan rata-rata 4.12 pembuluh dalam akar dan 1.54 pembuluh dalam batang. Sebaliknya bibit yang diinokulasi dengan isolat bakteri dari imago asal tanaman sehat penutupan xylemnya rata-rata 2.88 pembuluh dalam akar dan 0.75 pembuluh dalam batang.

Kata kunci : Cengkeh, *Syzygium aromaticum*, *Pseudomonas syzygii*, vektor, *Batrachomorphus cocles*

## INTRODUCTION

The infection of xylem limited bacterium disease in clove (local term BPKC) which is caused by *Pseudomonas syzygii* is still a problem in the clove plants in Indonesia, which is indicated from the increasing attacked area and number of infected plants. According to Asman (1988), BPKC distribution had already reached Central Java



Province, formerly it was only found in Sumatera and West Java. Others (ANON., 1991) reported that the clove trees in three districts of East Java Province were positively infected with BPKC disease. Moreover, according to BALFAS and ESTER (in ANON., 1994) the symptoms of the disease were also found in South Kalimantan.

According to BALFAS and GREEN (1988), and Asman (1988) *Hindola striata* and *Hindola fulva* (Homoptera : Machaerotidae) are two insect species known as a vector. Other insects which are potential to be the vectors are *Clovina sp.*, *Cofana sp.*, *Selenoce phalinii sp.*, *Botrogonia sp.*, *Gununga cara*, *Kolla spp.*, *Hishimonus sp.*, *Bharagonalia walshae*, and *Batracomorphus coclus*. The life pattern similar to of these insects that of *Hindola* spp., although they were grouped as phloem sucker (BALFAS *et al.*, 1988).

Based on the observations in Laing Experimental Garden and several clove plantations in Solok, *B. cocles* was always found on the clove trees, from egg stadium to nymph and imago stadium. This condition indicated that *B. cocles* was strongly associated with clove trees and was rarely found on other crops. This support the assumption that *B. cocles* functions as a vector. To prove this assumption, *P. syzygii* bacterium was isolated from *B. cocles* and the isolated bacteria were tested using antiserum plant serological test and Koch postulate test.

#### MATERIALS AND METHOD

The research was conducted in the Solok Sub Research Institute for Spices and Medicinal Crops from September 1991 to October 1992. The research used 10 healthy and 10 diseased clove trees for collecting the *B. cocles*. Observations were conducted every months from September 1991 to April 1992. In every collection 10 nymphs and 10 imagoes were caught from each clove tree. Each individu was isolated in the laboratorium. The insect was washed with alcohol (4%) for 1 minute, after that it was ground in a mortar untill soft, then mixed with 0.1 ml peri-

winkle wilt (PW) solution and stirred until it became a suspension. Further it was poured on to periwinkle wilt medium (PWM) and flattened with a spatulla. After it was kept in an incubator for 48 hours at 28°C, the growing bacterium colonies were observed and counted. Later on the bacterium colonies was processed into bacterium isolate for serological test and Koch postulate test.

The serological test used antiserum of agglutination slide test method, later on a glob formed on the part of the tested slide (P.2/2). The Koch postulate test was conducted through artificial inoculation in 8 steps, each step used 3-year old clove plants, 15 plants per isolate. Each plant was pricked with a pin containing bacterium isolate at its stem base until the pin penetrated the xylem. After the first symptom occured, the plant was dug up and preparates were made from diagonal cuts of the roots and stem using a sliding microtome, after that, the preparates were observed microscopically pically to identify the number of plugs in the vessels caused by the bacterium.

#### RESULT AND DISCUSSION

The results of the serological test showed that the nymphs and imagoes of *B. cocles* collected from diseased trees were positively infected with *P. syzygii*. While from the healthy tree insect collections, only the imagoes were positively infected. The presence of the bacterium in the nymphs and imagoes of *B. cocles* is due to that this insect has a similar feeding pattern to *Hindola* spp. which suck in the liquid of the young parts of the plant, so that the bacteria existing in the plant are also sucked. This case was made clearer by the isolation of the nymphs collected from healthy trees, all the nymphs were not infected by the bacterium. As to the infected imagoes collected from healthy trees, it was assumed that the imagoes did not grow and develop on the healthy trees, but they originated from diseased trees and moved to the healthy trees after they were infected by the bacterium.



The results indicated that *B. cocles* was infected by *P. syzygii* which lived in the xylem, although the insect belonged to the phloem suckers (BALFAS *et al.*, 1988). According to Chapman (1969) the phloem suckers insect has shorter stilet, however, on certain plants or on plant tissue where the border between xylem and phloem was very thin, the insect stilet could penetrate to the xylem. This case was found in the Leaf of Brinjal disease distribution with the same type of vector *Rishimonus* sp which belonged to the phloem sucker insect (HORSFALL and COWLING, 1978).

The percentage of infected imagoes collected from diseased trees was higher than that of nymphs and also was higher than the imagoes collected from healthy trees, i.e., 1.67-2.78 %, 0.16-1.32 % and 0.03-0.08 % (Table 1).

The infection of the bacterium on imagoes was high, because of difference in movements and relatively uneven bacterium distribution in the plant, so that on diseased plant there were still some healthy parts. In this case the imagoes

which could fly freely from one part to another has more chances to suck the liquids from parts of the plant containing bacterium compared to that of nymphs with movement limited to certain parts.

Other reasons were probably that the stilets of the nymphs were shorter than those of the imagoes, so that the stilets did not penetrate the xylem. This happened because the nymphs consisted of several instars which were still in metamorphic process so that their organs were not as perfect as the imagoes (CHAPMAN, 1969); ELZINGA, 1978). The possibility of bacterium infection on the nymphs was on the last instar nymph which already looked like an imago. This assumption could not be proven by this research, because the nymph collection was conducted as a whole without clasifying the instars.

The bacterium contents of the imagoes collected from diseased trees were higher than those of the nymphs or the imagoes collected from healthy trees, the contents, respectively ranged

Table 1. Percentage of *P. syzygii* infection on the nymphs and imagoes of *B. cocles*.

Tabel 1. Persentase bakteri *P. syzygii* terinfeksi oleh nimfa dan imago *B. cocles*

| Insects collection time<br>Waktu koleksi serangga |      | Collection source Sumber koleksi |                            |               |
|---------------------------------------------------|------|----------------------------------|----------------------------|---------------|
|                                                   |      | Healthy trees*) Pohon sehat      | Diseased trees Pohon sakit |               |
|                                                   |      | Imagoes Imago                    | Nymphs Nimfa               | Imagoes Imago |
|                                                   |      | (%)                              |                            |               |
| September                                         | 1991 | 0.03                             | 1.32                       | 2.78          |
| October                                           | 1991 | 0.07                             | 1.24                       | 2.21          |
| Nopember                                          | 1991 | 0.06                             | 1.21                       | 2.57          |
| December                                          | 1991 | 0.04                             | 0.16                       | 1.95          |
| January                                           | 1992 | 0.08                             | 0.57                       | 1.67          |
| February                                          | 1992 | 0.07                             | 1.30                       | 2.71          |
| March                                             | 1992 | 0.06                             | 1.28                       | 2.44          |
| April                                             | 1992 | 0.04                             | 0.69                       | 2.52          |
| Average Rata-rata                                 |      |                                  | 0.05                       | 0.972.36      |
| CV K.K. (%)                                       |      | 4.81                             | 11.13                      | 8.56          |

\*) No bacterial infection in the nymph collected from healthy trees

Tidak ada bakteri yang terinfeksi pada koleksi nimfa dari pohon sehat



between 2.09-3.21, 0.47-1.36, and 0.14-0.28 colonies per insect (Table 2). The bacterium contents differed because the bacterium characteristic was not persistent, it did not develop in its host (HORSFALL and COWLING, 1978). In relation to this characteristic, it was assumed that the bacteria existing in the nymphs and imagoes of *B. cocles* would decrease, even vanish in a certain period of time if the insect did not suck on diseased trees. This assumption can be seen from the imagoes collected from healthy trees where their bacterium contents were relatively low, because there was no bacterium source on healthy trees. So that the bacterium contents of the imagoes decreased in line with the characteristic of the bacterium, which was not persistent. On the other hand the imagoes have high activities which needs plenty of energy, so that the sucking intensity of the imagoes was more frequent compared to the nymphs. Therefore, eventually the imagoes would contain more bacteria, just like the imagoes collected from diseased trees.

Based on the differences in the bacterium contents, it could be said that *P. syzygii* in *B.*

*cocles* was not persistent, it could decrease or vanish in a certain period of time, as long as the insects did not suck diseased plants. However, as long as the insects were still infected they might function as a vector of xylem limited bacterium disease.

Koch postulate test visually indicated that all isolated bacteria from the imagoes and nymphs derived from diseased trees and from the imagoes derived from healthy trees attacked the clove plants. The isolated bacteria of the imagoes derived from diseased trees showed the highest rate of infection and the fastest in showing the symptom, with an average of 19.17 percent and 182 days. While the isolated bacteria of the imagoes from healthy trees showed the lowest rate of infection and the latest in showing the symptom, with an average of 9.17 percent and 194.13 days respectively (Table 3).

The difference in the rate of infection and the period of the first symptom occurred because of the difference in the bacterium virulence due to the physiological environment inside the insect body, particularly between the imagoes and the

Table 2. Average of *P. syzygii* contents in the nymphs and imagoes of *B. cocles*.

Tabel 2. Rataan kandungan bakteri *P. syzygii* pada nimfa dan imago *B. cocles*

| Insects collection time<br>Waktu koleksi serangga |      | Collection source Sumber koleksi |              |                            |
|---------------------------------------------------|------|----------------------------------|--------------|----------------------------|
|                                                   |      | Healthy trees *) Pohon sehat     |              | Diseased trees Pohon sakit |
|                                                   |      | Imagoes Imago                    | Nymphs Nimfa | Imagoes Imago              |
|                                                   |      | ..... (%) .....                  |              |                            |
| September                                         | 1991 | 0.21                             | 1.15         | 2.88                       |
| October                                           | 1991 | 0.14                             | 1.18         | 3.14                       |
| November                                          | 1991 | 0.28                             | 0.96         | 3.21                       |
| December                                          | 1991 | 0.21                             | 0.47         | 2.54                       |
| January                                           | 1992 | 0.18                             | 1.27         | 2.42                       |
| February                                          | 1992 | 0.27                             | 0.81         | 2.09                       |
| March                                             | 1992 | 0.22                             | 0.63         | 2.16                       |
| April                                             | 1992 | 0.28                             | 1.36         | 2.39                       |
| Average                                           |      | 0.22                             | 0.98         | 2.60                       |
| CV KK (%)                                         |      | 6.93                             | 10.76        | 9.35                       |

\*) No bacterial infection in the nymph collected from healthy trees

Tidak ada bakteri yang terinfeksi pada koleksi nimfa dari pohon sehat



Table 3. The average infection of bacterium on clove plants and duration of first symptom through Koch postulate test  
 Tabel 3. Rataan tanaman terinfeksi bakteri dan waktu gejala awal dalam uji postulat Koch

| Inoculation time<br>Waktu inokulasi | Inoculated with bacterium isolates from<br>Inokulasi dengan isolat bakteri dari |                                               |                                                    |                                               |                                                    |                                               |
|-------------------------------------|---------------------------------------------------------------------------------|-----------------------------------------------|----------------------------------------------------|-----------------------------------------------|----------------------------------------------------|-----------------------------------------------|
|                                     | Imagoes on diseased trees<br>Imago pada pohon sakit                             |                                               | Nymphs on diseased trees<br>Nimfa pada pohon sakit |                                               | Imagoes on healthy trees<br>Imago pada pohon sehat |                                               |
|                                     | Symptom<br>Gejala<br>(%)                                                        | First symptom<br>Gejala awal<br>(days) (hari) | Symptom<br>Gejala<br>(%)                           | First symptom<br>gejala awal<br>(days) (hari) | Symptom<br>Gejala<br>(%)                           | First symptom<br>gejala awal<br>(days) (hari) |
| <b>1991</b>                         |                                                                                 |                                               |                                                    |                                               |                                                    |                                               |
| September                           | 26.67                                                                           | 167.50                                        | 13.33                                              | 182.50                                        | 6.67                                               | 194.00                                        |
| October                             | 20.00                                                                           | 179.50                                        | 6.67                                               | 178.00                                        | 6.67                                               | 187.00                                        |
| November                            | 26.67                                                                           | 184.50                                        | 6.67                                               | 193.00                                        | 13.33                                              | 202.00                                        |
| December                            | 6.67                                                                            | 185.00                                        | 13.33                                              | 201.50                                        | 6.67                                               | 184.00                                        |
| <b>1992</b>                         |                                                                                 |                                               |                                                    |                                               |                                                    |                                               |
| January                             | 20.00                                                                           | 186.50                                        | 20.00                                              | 203.00                                        | 6.67                                               | 191.00                                        |
| February                            | 20.00                                                                           | 192.00                                        | 6.67                                               | 186.00                                        | 13.33                                              | 199.00                                        |
| March                               | 6.67                                                                            | 181.00                                        | 13.33                                              | 201.00                                        | 13.33                                              | 207.50                                        |
| April                               | 26.67                                                                           | 180.00                                        | 13.33                                              | 196.50                                        | 6.67                                               | 188.00                                        |
| Average Rata-rata                   | 19.17                                                                           | 182.00                                        | 11.67                                              | 192.69                                        | 9.17                                               | 194.00                                        |
| CV (%)                              | 35.32                                                                           | 0.15                                          | 36.59                                              | 0.18                                          | 36.64                                              | 0.15                                          |

nymphs. While between the imagoes, it probably happened because of the difference in the bacterium collection, for example in healthy tree imagoes the collection of the bacteria was longer because they had collected the bacteria from diseased trees before they moved to healthy trees. According to HORSFALL and COWLING (1978), the infection of the disease on the plant strongly depended on the level of the pathogen virulence, which was not constant every time because of condition changes inside or outside the host. Further, NURMANSYAH *et al.*, (1990) stated that elevation influenced the virulent level of *P. syzygii*, the higher the elevation the stronger the virulence, therefore the first symptom of BPKC was faster in the highland clove plantations (ASMAN, 1992).

From microscopic observation, it was seen that the highest number of plugged vessels occurred on the clove plants which were inoculated with bacteria isolated from imagoes collected

from of diseased trees imagoes, with an average of 4.17 vessels of the root and 1.54 vessels of stem. While the lowest number of plugged vessels occurred on the clove plants inoculated with bacteria isolated from imago collected from healthy tree imagoes, with an average of 2.88 vessels of root and 0.75 vessels of stem (Table 4). The difference was due to the characteristic of *P. syzygii* which was frequently found on roots whose environment condition was suitable for *P. syzygii* growth (NUR-MANSYAH *et al.*, 1990).

#### CONCLUSION AND SUGGESTION

The imagoes and the nymphs of *B. cocles* collected from diseased clove trees and the imagoes of *B. cocles* collected from healthy trees were positively infected with *P. syzygii* with a per-



Table 4. Numbers of blocked xylems in inoculated plants

Tabel 4. Jumlah penyumbatan xylem (pembuluh) pada tanaman inokulasi

| Inoculation time<br>Waktu inokulasi | Inoculated with bacterium isolates from <i>Inokulasi dengan bakteri dari</i> |                       |                                                            |                       |                                                             |                       |
|-------------------------------------|------------------------------------------------------------------------------|-----------------------|------------------------------------------------------------|-----------------------|-------------------------------------------------------------|-----------------------|
|                                     | Imagoes from diseased trees<br><i>Imago dari pohon sakit</i>                 |                       | Nymph from diseased trees<br><i>Nimfa dari pohon sakit</i> |                       | Imagoes from healthy trees<br><i>Imago dari pohon sehat</i> |                       |
|                                     | Root<br><i>Akar</i>                                                          | Stem<br><i>Batang</i> | Root<br><i>Akar</i>                                        | Stem<br><i>Batang</i> | Root<br><i>Akar</i>                                         | Stem<br><i>Batang</i> |
| <b>1991</b>                         |                                                                              |                       |                                                            |                       |                                                             |                       |
| September                           | 5.50                                                                         | 2.75                  | 3.50                                                       | 1.50                  | 3.00                                                        | 0.00                  |
| October                             | 4.33                                                                         | 1.67                  | 4.00                                                       | 1.00                  | 4.00                                                        | 0.00                  |
| November                            | 4.00                                                                         | 1.75                  | 4.00                                                       | 1.00                  | 2.50                                                        | 1.50                  |
| December                            | 3.00                                                                         | 0.00                  | 2.50                                                       | 0.50                  | 2.00                                                        | 1.00                  |
| <b>1992</b>                         |                                                                              |                       |                                                            |                       |                                                             |                       |
| January                             | 4.67                                                                         | 2.33                  | 3.33                                                       | 2.00                  | 2.00                                                        | 1.00                  |
| February                            | 3.67                                                                         | 1.33                  | 3.00                                                       | 0.00                  | 3.00                                                        | 1.00                  |
| March                               | 4.00                                                                         | 1.00                  | 2.00                                                       | 0.50                  | 0.50                                                        | 1.50                  |
| April                               | 3.75                                                                         | 1.50                  | 2.50                                                       | 1.00                  | 3.00                                                        | 0.00                  |
| Average <i>Rata-rata</i>            | 4.12                                                                         | 1.54                  | 3.04                                                       | 0.93                  | 2.88                                                        | 0.75                  |
| CV KK. (%)                          | 7.08                                                                         | 17.83                 | 9.45                                                       | 16.79                 | 9.18                                                        | 19.23                 |

centage of 1.67-2.78 %, 0.16-1.32 % and 0.03-0.08 % and a bacterium content of 2.09-3.21, 0.47-1.36, and 0.14-0.28 colonies per insect respectively.

The isolated bacteria of the imagoes collected from diseased trees had the highest infection rate on clove plants and the fastest symptom duration, with an average of 19.17 % and 182 days. While the isolated bacteria of the imagoes collected from healthy plants had the lowest infection rate and the slowest symptom duration, with an average of 9.17 % and 194 days.

Blocked xylems occurred more on the clove plants inoculated with bacteria isolated from the imagoes collected from diseased trees, with an average of 4.12 vessels of root and 1.54 vessels of stem. While on clove plants inoculated with bacteria isolated from imagoes collected from healthy trees, the blocked xylems was 2.88 vessels of root and 0.75 vessels of stem.

*Pseudomonas syzygii* in *B. cocles* (nymphs and imagoes) tended to be non-persistent so that it may disappear automatically. When the insects are still infected by the bacterium they still function as a vector of xylem limited bacterium disease.

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