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A comparative primary structure analysis of phosphofructokinase from different plant pathogenic bacteria

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Abstract

The tremendous increase in modern sequencing techniques has provided us with ample scope to carry out conclusive analysis of the different facets of polymeric macromolecules such as nucleic acids and proteins. In this study, a detailed amino acid sequence analysis of the enzyme phosphofructokinase from five different plant pathogenic bacteria was carried out to find out the compositional variability of this important enzyme both at the inter as well as intraspecific level. The plants pathogens that have been considered in this study includes species of *Dickeya, Erwinia, Pectobacterium, Pseudomonas* and *Xanthomonas*. All these organisms have been included in the list of top ten plant pathogenic bacteria in molecular plant pathology based on scientific and/or economic importance. The frequency of the different amino acids at the first 30 positions starting from the N-terminal end of the PFK structure was also computed in course of this analysis. Relative amino acid usage frequency profile of PFK sequences from *Dickeya, Erwinia* and *Pectobacterium* were found to be quite similar. Compared to *Dickeya, Erwinia* and *Pectobacterium*, the genus *Pseudomonas* exhibited comparatively higher frequency of neutral amino acid residues in their PFK structure whereas the amino acid compositional profile of PFK sequences from *Xanthomonas* was found to be quite distinct from the other four plant pathogenic genera considered in this study. The spatial amino acid configuration profile at the N-terminal end of the PFK sequences was also found to be quite similar in the three Enterobacteriaceae genera in comparison to *Pseudomonas* and *Xanthomonas*.

Keywords: Plant pathogenic bacteria, phosphofructokinase, glycolysis, amino acid usage, isoelectric point, GRAVY, compositional variability.

Introduction

Glucose metabolism is known to play a significant role in establishment of pathogenesis. The metabolic needs of several pathogens are met by up regulation of host cell glycolysis, whereas in some cases, down regulation of cellular glycolysis has also been seen (Rojas, Senthil-Kumar, Tzin, & Mysore, 2014). Glycolysis is a linear sugar oxidation pathway through which glucose is metabolized into pyruvate in the cytoplasm yielding two molecules of ATP per mole of glucose. Regulation of glycolytic enzymes can also occur by different mechanisms such as controlling their transcription and mRNA stability or through allosteric activation. One of the key regulating enzymes of the glycolytic pathway is phosphofructokinase (PFK), which is regulated by several kinases that are upregulated during infection (Ghukasyan & Heikal, 2014). PFK (EC number 2.7.1.11) is known as a rate-limiting enzyme of glycolysis and has even been detected in organisms like phytoplasma which lack many vital biosynthetic and catabolic genes related to metabolic pathways such as hexose monophosphate shunt, fatty acid and amino acid biosynthesis, TCA cycle and others as a result of reductive evolution (Oshima et al., 2007).

The tremendous increase in modern sequencing techniques has provided us with ample data to carry out satisfactory and conclusive analysis of the different facets of polymeric macromolecules such as nucleic acids and proteins. In this study, a detailed sequence analysis of the amino acid sequence of the PFK enzyme from different plant pathogenic bacteria was carried out to find out the compositional variability of this important enzyme both at the inter as well as intraspecific level. The plants pathogens that have been considered in this study includes species of *Dickeya, Erwinia, Pectobacterium, Pseudomonas* and *Xanthomonas*. All these organisms have been included in the list of top ten plant pathogenic bacteria in molecular plant pathology based on scientific and/or economic importance. The bacterium, making the resilient appearance on scientific and economic grounds is *Pseudomonas syringae* (Mansfield et al., 2012). *P. syringae* is a prolific plant pathogen which exists as over 50 different pathovars, many of which show a high degree of host specificity. Another important plant pathogenic genus is *Xanthomonas* which is a large genus of gram negative bacteria causing disease in hundreds of plant hosts, including many economically important crops and display a high degree of host plant as well as tissue specificity (Ryan et al., 2011). Members of this genus cause disease on at least 124 monocot species and 268 dicot species, including fruit and nut trees, Solanaceous and Brassicaceous plants, and cereals (Jackson, 2009). *Dickeya, Erwinia* and *Pectobacterium* represents three prominent genera of the family Enterobacteriaceae and are very potent plant pathogens. *Dickeya* consists mainly of pathogens from herbaceous plants whereas, *Erwinia* infects many woody plants. *Pectobacterium* used to be a member of the genus *Erwinia*, which was later split into three genera— *Erwinia, Pectobacterium*, and *Brenneria* (Toth, Bell, Holeva, & Birch, 2003).

Materials and Methods

In order to conduct this study, amino acid sequences of the PFK enzyme from the selected plant pathogenic genera of *Dickeya, Erwinia, Pectobacterium, Pseudomonas* and *Xanthomonas* were obtained from GenBank (Benson et al., 2013). The amino acid frequency of the different amino acids constituting the PFK protein primary sequence was computed using our own script developed in Python. In this analysis the amino acid composition profile of the entire 98 amino acid sequences of PFK was computed. In addition to this, the frequency of the different amino acids (up to thirty residues) present at the N-terminal end of the PFK sequences was also computed. The grand average of hydropathy (GRAVY) score was calculated using GRAVY CALCULATOR hosted at http://www.gravy-calculator.de The grand average of hydropathicity or GRAVY (Kyte & Doolittle, 1982) of the linear polypeptide sequence is calculated as the sum of hydropathy values of all amino acids, divided by the number of residues in the sequence. Increasing positive score indicates greater hydrophobicity. The calculation is based on the Kyte-Doolittle scale (Kyte & Doolittle, 1982). It is a simple method for displaying the hydropathic character of a protein.

Results and Discussion

The amino acid sequences of the PFK enzyme from the five genera belonging to the class Gammaproteobacteria were thoroughly analyzed in terms of their amino acid composition. In total 100 PFK sequences were analyzed and initial observation suggested that the average molecular weight of the PFK sequences is 33275 Da. *Xanthomonas hyacinthi* (31653 Da) and *X. sacchari* (31933 Da) demonstrated the lowest molecular weights, whereas, *Erwinia mallotivora* (35001 Da) and *E. tracheiphila* (35027 Da) were found to have the highest molecular weights. All the 100 PFK sequences that were analyzed in course of this study were found to be composed of 317 to 320 amino acids. A graph showing the fluctuations in the molecular weights of the plant pathogenic PFK sequences is given in Figure 1.

A comprehensive intra-generic comparison of the PFK amino acid sequences was carried out in the five plant pathogenic genera.

i) *Dickeya* — This genus is a member of the family Enterobacteriaceae and consists mainly of pathogens from herbaceous plants. The different species belonging to this genera whose PFK amino acid sequences were analyzed includes *D. chrysanthemi, D. dadantii, D. paradisiaca* and *D. solani*. PFK sequences belonging to different pathovars of *Dickeya* were found to display fluctuations in the protein length from 312 to 320 amino acid residues. Relative amino acid usage analysis of the PFK sequences from *Dickeya* species show that the neutral amino acid residues are used in higher proportions followed by the hydrophobic, polar, aliphatic and the aromatic residues in decreasing frequency. The frequency of the neutral side chain containing residues like A, G, H, P, S, T and Y were in between 35 to 40 percent. The frequency of the aliphatic residues I, L and V appeared constant at about 25%. Fluctuations were observed in the distribution of the hydrophobic (C, F, I, L, M, V,

and W) and polar residues (D, E, R, K, Q and N). The distribution of the aromatic residues F, H, W and Y was found to hover in the order of about 8%.

The GRAVY score of the PFK sequences from this genus was found to range between -0.054 and 0.071.

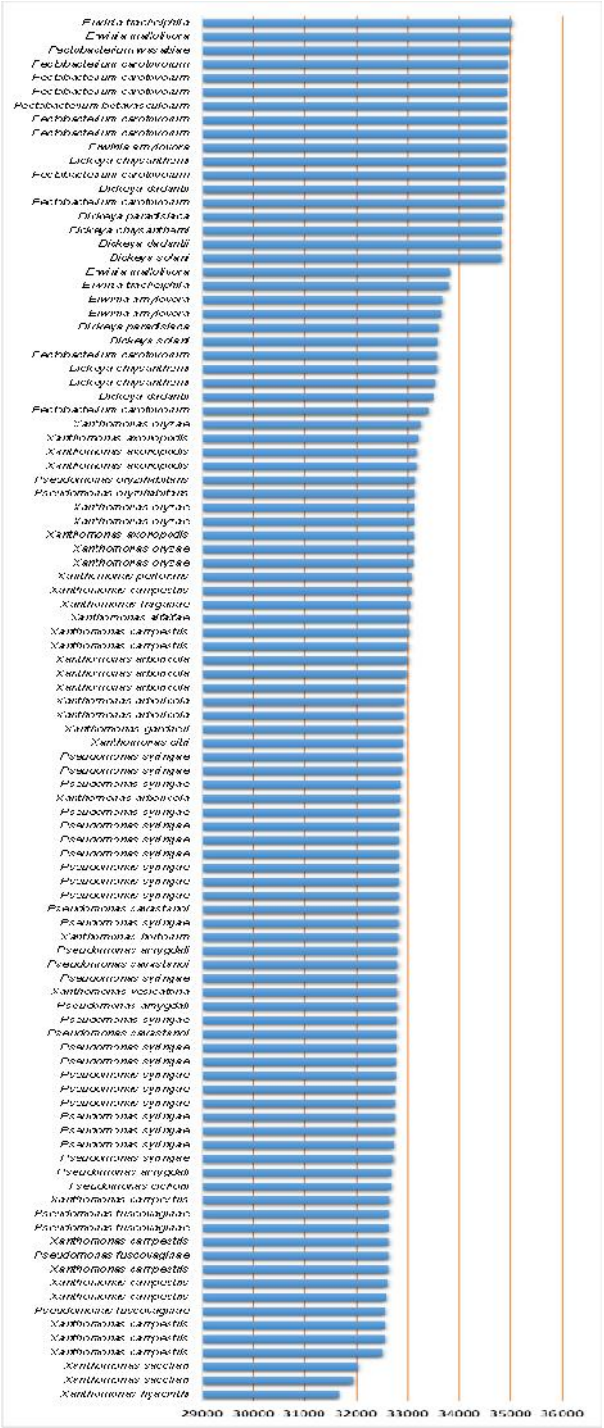

Figure 1: A horizontal bar graph showing the fluctuation in molecular weight (Da) in the 100 PFK amino acid sequences from plant pathogenic bacteria.

ii) *Erwinia* — The genus *Erwinia* also belongs to the family Entreobacteriaceae and contains mostly plant pathogenic species causing diseases in Rosaceae plants such as apples and pears. *E. tracheiphila* is known to cause wilt of cucurbits. PFK amino acid sequences of *Erwinia* analyzed in this study included sequences from species like *E. amylovora*, *E. mallotivora* and *E. tracheiphila*. Similar to *Dickeya*, PFK sequences belonging to different strains of *Erwinia* were found to display fluctuations in the protein length from 312 to 320 amino acid residues. The relative amino acid usage profile of the PFK sequences from *Erwinia* displayed features quite similar to *Dickeya*. The GRAVY score of the PFK sequences from this genus was found to range between -0.0612 and 0.116.

iii) *Pectobacterium* — Similar to *Dickeya* and *Erwinia*, this genus too belongs to the family Enterobacteriaceae. *Pectobacterium* used to be a member of the genus *Erwinia*, which was later split into three genera namely, *Erwinia*, *Pectobacterium*, and *Brenneria* (Toth et al., 2003). Eleven PFK amino acid sequences were analyzed from this genera which included the species *P. betavasculorum*, *P. carotovorum* and *P. wasabiae*. PFK sequences belonging to different strains of *Pectobacterium* were found to display fluctuations in the protein length ranging from 312 to 320 amino acid residues. Relative amino acid usage frequency profile of *Pectobacterium* PFK sequences were found to be quite in line with that of *Dickeya* and *Erwinia* but the frequency of polar and hydrophobic residues appeared much steadier except two sequences from *P. carotovorum* where the relative frequencies of the polar residues were lower than their average and the frequency of the hydrophobic residues were greater than their average frequencies. The GRAVY score of the PFK sequences from this genus was found to range between -0.104 and 0.080.

iv) *Pseudomonas* — In total 35 PFK sequences belonging to six different species of *Pseudomonas* were obtained from the sequence database and analyzed. These species include *P. amygdali*, *P. cichorii*, *P. fuscovaginae*, *P. savastanoi* and *P. syringae*. *Pseudomonas* represents the type genera of the family Pseudomonadaceae which belongs to the class Gammaproteobacteria. Among all the *Pseudomonas* species, *P. syringae* is the most prolific plant pathogen existing as more than 50 different pathovars, many of which exhibit a high degree of host specificity. In comparison to the three genera belonging to the Enterobacteriaceae, the relative amino acid usage profile of the genus *Pseudomonas* is much more complex. Compared to *Dickeya*, *Erwinia* and *Pectobacterium*, the genus *Pseudomonas* exhibited comparatively higher frequency of neutral amino acid residues in their PFK structure which ranges from 40% to 50%. The frequency of the aromatic residues F, H, W and Y was also found to much lower (about 5%) in comparison to the Enterobacteriaceae genera. A careful examination of the amino acid compositional profile of PFK sequences from different species of *Pseudomonas* showed that *P. fuscovaginae* has lower frequency of hydrophobic residues (about 30%). The polar residues and the hydrophobic amino acid residues were found to be utilized nearly in equal frequencies in the PFK sequences of the different *Pseudomonas* species. Different strains of the species *P. syringae* demonstrated variability with respect to their hydrophobic, neutral, polar, aromatic and aliphatic amino acid residues. The GRAVY score of the PFK sequences from this genus was found to vary between 0.0501 and 0.248.

v) *Xanthomonas* — This genus belongs to the family Xanthomonadaceae in the gamma subdivision of the Proteobacteria, and consists of 27 plant-associated species causing significant diseases of crops and ornamentals (Jackson, 2009) such as bacterial spots and blights of leaves, stems, and fruits (Boch & Bonas, 2010). About 36 PFK amino acid sequences from 14 different *Xanthomonas* species were analyzed in course of this study. These species include *X. alfalfa*, *X. arboricola*, *X. axonopodis*, *X. campestris*, *X. citri*, *X. fragariae*, *X. gardneri*, *X. hortorum*, *X. hyacinthi*, *X. oryzae*, *X. perforans*, *X. sacchari* and *X. vesicatoria*. The PFK protein length was found to range between 317 to 319 amino acid residues. The genus *Xanthomonas* thus, demonstrates the lowest range in PFK protein length fluctuation compared to the other genera. The amino acid compositional profile of PFK sequences from *Xanthomonas* was found to be quite distinct from the other four genera considered in this study. The frequency of the aromatic amino acid residues was found to hover consistently near the 5% range. The neutral amino acid residues were found to constitute a larger proportion of the PFK amino acid sequences. *X. hyacinthi* demonstrated the greatest frequency of neutral side chain containing amino acid residues. The frequency of the hydrophobic residues was found to consistent at the 30% level in all the PFK sequences from the different *Xanthomonas* species. Fluctuation was observed in the frequency of the polar residues, particularly, in the case of *X. hyacinthi*. This PFK of *X. hyacinthi* demonstrated the lowest frequency of polar side chain containing residues

which is about twenty percent. The GRAVY score of the PFK sequences from this genus was found to vary between 0.0509 and 0.445.

A comparative depiction of the different classes of amino acid residues constituting the PFK enzyme amino acid sequences in the different species of the five plant pathogenic bacteria is given in Figure 2.

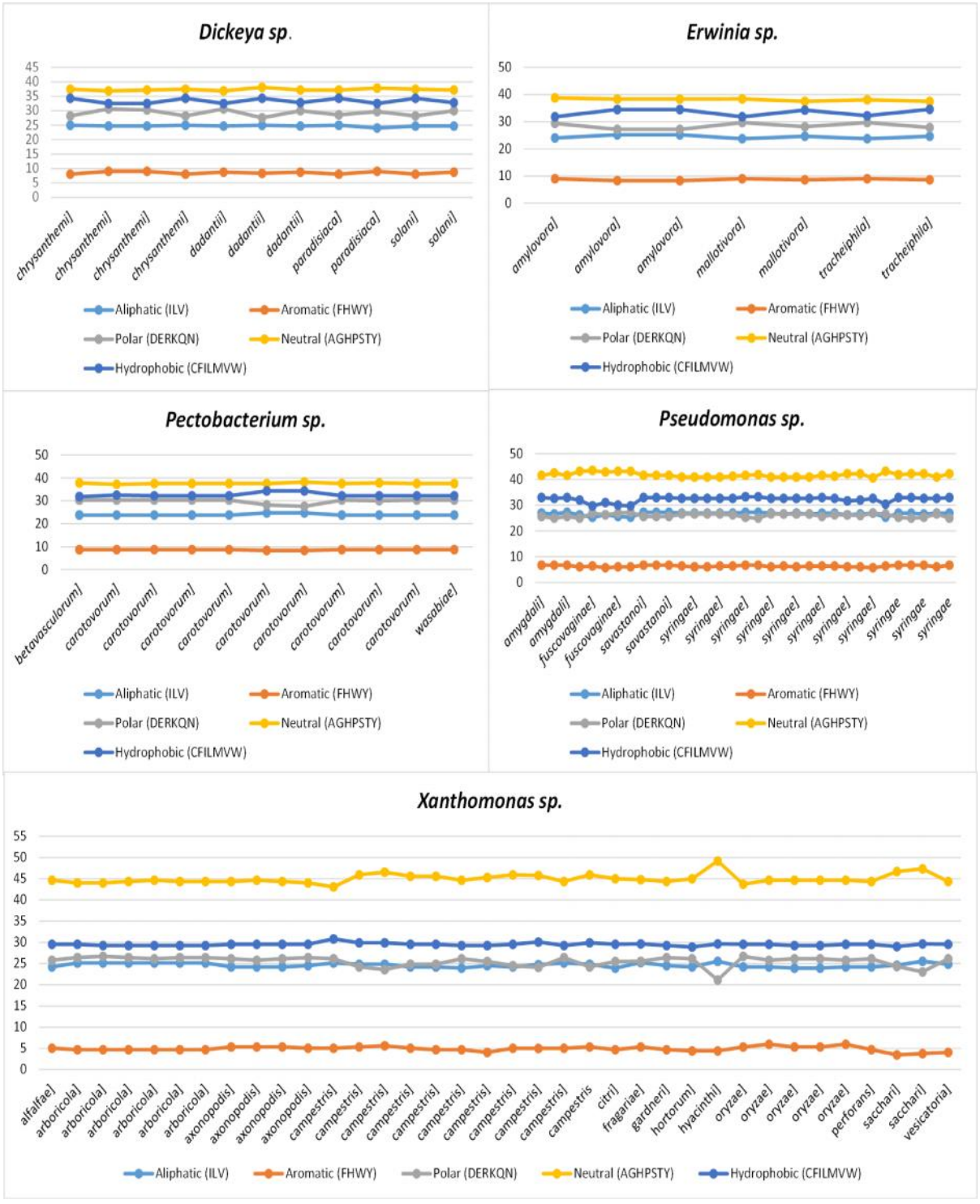

Figure 2: A comparative depiction of the different classes of amino acid residues constituting the PFK enzyme amino acid sequences in the different species of the five plant pathogenic bacteria.

Position effect on amino acid composition of PFK

In this study an attempt was also made to capture the frequency of amino acid usage with respect to the position of the amino acids on the PFK primary structure. For this study, the frequency of the different amino acids at the first 30 positions starting from the N-terminal end of the PFK structure was computed. A comparative genera wide study of the five plant pathogenic bacteria genera revealed the following:

i) *Dickeya* — The PFK sequences obtained from the members of this genera demonstrated a constant frequency of aspartic acid (3.33%), serine (3.33) % and valine (10%) at the first 30 positions of the N-terminal end. Amino acids such as phenylalanine, glutamine and tryptophan are totally absent at the first 30 positions of the N-termini of the PFK sequences.

ii) *Erwinia* — The PFK sequences from this genus demonstrated the same spatial composition signature of amino acids as was evident in *Dickeya*. In addition to that, cysteine residues were also found to be completely lacking at the first 30 positions of the N-terminal end.

iii) *Pectobacterium* — The PFK sequences from *Pectobacterium* demonstrated a profile quite similar to both *Dickeya* and *Erwinia* except for two sequences belonging to *P. carotovorum* (NCBI gene ids: gi|746310841|ref|WP_039357678.1 and gi|746445646|ref|WP_039485593.1). The amino acid compositional profile at the N-terminal end of PFK within this genus was found to be highly conserved barring the two aforementioned sequences. The frequency of only serine and threonine residues were found to fluctuate between 6.66% and 3.33% at the N-terminal end of the PFK sequences from different *Pectobacterium* species.

iv) *Pseudomonas* — In the case of *Pseudomonas*, a total avoidance of five different amino acids at the first 30 positions from the N-terminal end of PFK was observed. These residues include cysteine, phenylalanine, histidine, tryptophan and tyrosine. Leucine appeared to be the most preferred residue at the N-terminal end of PFK with a frequency of about 24%. Apart from *P. fuscovaginae*, the ratio of the different amino acids were found to be constant in all the PFK sequences from the other *Pseudomonas* species. In contrast to the other three genera of the family Enterobacteriaceae mentioned above, the PFK sequences from *Pseudomonas* was found to possess a relatively higher frequency of glutamine residues (6.67% - 10%) at the N-terminal end.

v) *Xanthomonas* — Similar to *Pseudomonas*, a total avoidance of five different amino acids at the first 30 positions from the N-terminal end of PFK was observed in *Xanthomonas*. These residues included cysteine, phenylalanine, lysine, tryptophan and tyrosine. In *Xanthomonas* too, leucine appeared to be the most preferred residue at the N-terminal end of PFK with a frequency of about 13.33% which is comparatively lower than *Pseudomonas*. Most of the PFK sequences from *Xanthomonas* was found to possess the highest frequency of glutamine residues (10% - 13%) at the N-terminal end which is the highest among all the studied genera.

Conclusion

Relative amino acid usage frequency profile of PFK sequences from *Dickeya, Erwinia* and *Pectobacterium* were found to be quite similar. Compared to *Dickeya, Erwinia* and *Pectobacterium*, the genus *Pseudomonas* exhibited comparatively higher frequency of neutral amino acid residues in their PFK structure whereas the amino acid compositional profile of PFK sequences from *Xanthomonas* was found to be quite distinct from the other four plant pathogenic genera considered in this study. The spatial amino acid configuration profile at the N-terminal end of the PFK sequences was also found to be quite similar in the three Enterobacteriaceae genera in comparison to *Pseudomonas* and *Xanthomonas*.

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