ROLE OF CYANOBACTERIAL STRAINS ON TRITICUM AESTIVUM GROWTH UNDER CHROMIUM STRESSES IN LABORATORY

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ABSTRACT: Cyanobacterial strains isolated from different places near Lahore, Pakistan. They were proved to be efficiently promoting the growth of economically valuable crop Triticum aestivum, even under the stress of metal contaminant chromium in laboratory conditions, where petri plates were used. Seedling length of Triticum aestivum was severely affected by the application of chromate salt especially with the Cr (VI). About 18% and 61% reduction in seedling length respectively under 300 µg mL\(^{-1}\) of CrCl\(_3\), and \(\text{K}_2\text{CrO}_4\) was observed. Hexavalent chromium had drastic effect on root numbers while trivalent chromium had no effect. Both trivalent (10.4%) and hexavalent (97.2%) chromium salts caused reduction in the leaf area. Fresh weight of seedlings was adversely affected with the application of chromium salts. With trivalent salt 15.1% and with hexavalent 59.3% reduction was observed. With chromium salts dry weight accumulation increased and under hexavalent salt dry weight accumulation was almost double than that of control. Protein content of seedlings increased with chromium salts. Cyanobacterial inoculations caused significant stimulation in auxin content in seedlings when compared to non-inoculated control. CrCl\(_3\) (300 µg mL\(^{-1}\)) and \(\text{K}_2\text{CrO}_4\) (300 µg mL\(^{-1}\)) treatments, stimulated more synthesis of auxin content in seedlings 142.8% and 285.7% increases respectively. About 45% and 230% increases in acid phosphatase activity, over control, were observed under 300 µg mL\(^{-1}\) for each CrCl\(_3\) and \(\text{K}_2\text{CrO}_4\), respectively. Seedlings grown under 300 µg mL\(^{-1}\) \(\text{K}_2\text{CrO}_4\) uptake more chromium content as compared to the seedlings which were grown under 300 µg mL\(^{-1}\) of CrCl\(_3\).

Key word: Cyanobacteria, Triticum aestivum, chromium stress, plant growth promotion

INTRODUCTION:

Environmental conditions are under the constant influence of human activities that are causing harmful effects on all life forms on earth including plant. Chromium is naturally present in earth crust but industries are the main source of increasing its amount in soil, water and air above the appointed level which is approximately 1-300 mg/kg in soil and 9.7 µg/L in fresh water [1]. Untreated Waste material from the various industries in which chromium is being used for the various purposes like Electroplating, leather tanning, dyeing and painting when exposed to environment, pollute the soil and water reservoir [2].

When plants are grown in Cr contaminated soil or irrigation of crops is done with Cr contaminated water, the plant start to accumulate the chromium within the root cells by up taking via transpiration molecules that are usually use for essential metal ion e.g.; Fe, S, and P [3]. When Cr accumulates in roots it affects the cell elongation and division which ultimately retards the root length and growth as well as reduce the ability of seedlings to absorb water necessary for their germination [4, 5]. Due to this less amount of water, nutrients are not certainly transported to stem which results in the short stature of plant. Cr transportation to the stem and leaves cause the direct drastic effect on cells metabolism which also cause the shortening of plant height. It is also reported that Cr produces reactive oxygen species that cause the oxidative damage to cell organelles such as mitochondria and chloroplast which in result reduce the plant growth [6]. These ROS also damage the cell membrane transport mechanism for inorganic molecule and H\(^+\)ATPase which increases the uptake of Cr(VI) in the cell [7]. It also causes competition of Cr with Mn, Fe, Zn that are essential for growth and other enzyme activity [8]. Cr also affect the nitrate reduction property of plant by minimizing NR enzyme activity [9].

For the safe, unharmed and undamaged removal of metals from soil, Bioremediation is the effective way. For this purpose microbes are playing leading role. Microbes that are present in rhizosphere may play an important role in plant growth promotion either by competing with pathogen, by providing essential molecules or by reducing toxic metals. Among microbes, cyanobacteria belongs to very important photosynthetic organisms that diversify the universe from its most reducing state to the most oxygenic state and made life possible on earth by producing oxygen [10]. Current study indicates that not only bacteria are associated with plant growth promotion but cyanobacteria also play many important roles by reducing toxic metals. One way to positively affect nutrient content and soil structure is to add cyanobacteria [11-13]. Cyanobacteria can be incorporated into soil as organic matter and also as a source of enzymes as they produce acid and alkaline extracellular phosphatases that are active in solution or located in the periplasmatic space of the cell wall. The effects of *N. muscorum* mass on soil physical, chemical and biological properties indicate the possible benefits of cyanobacteria as soil inoculants [12]. Cyanobacteria are also known for fixing nitrogen and are also reported to establish association with plants and stimulate plant growth [14]. These properties of cyanobacteria enhance the plant growth by ensuring nutrient as
well as water availability to the plants. This study utilizes the Cr resistance property of cyanobacteria and effect of them on plants that are grown in Cr stress which can hinder the plant growth in the absence of cyanobacteria. Different growth parameters of *Triticum aestivum* var. Uqab-2000 are observed in the presence and absence of inoculum while Cr stress (trivalent (CrCl₃) and hexavalent (K₂Cr₂O₇) salts (300 µg mL⁻¹) are provided in both cases. In laboratory conditions inoculated un inoculated seeds were grown in petriplates for 10 days by providing Cr stress in its trivalent and hexavalent form in order to determine the difference in germination and growth pattern (biochemical analysis, chromium content of seedlings [15]).

**MATERIAL AND METHOD**

Cyanobacterial strains and inoculum preparation

Strains of chromium resistant cyanobacteria were previously isolated, characterized and molecularly identified from local environment which were used in this study [16-18]. For preparation of Cyanobacterial suspensions, these strains were grown in liquid cultures; cells were then harvested, washed and resuspended in sterilized glass-distilled water. To ensure the equal cyanobacterial growth (cell population of cyanobacterial strains in the suspension for different experiments), chlorophyll-a concentration was measured.

**Experimental plant and seed inoculation**

Certified seeds of *Triticum aestivum* var. Uqab-2000 were procured from the National Agriculture Research Centre - NARC, Islamabad, Pakistan. Healthy seeds were surface sterilized with 0.1% HgCl₂ for 5 minutes, with continuous shaking followed by repeated washing with sterilized glass-distilled water, so that all traces of HgCl₂ were removed from the seeds. Surface sterilized seeds were dipped in cyanobacterial suspension for Fifteen minutes.

**Seed germination and growth**

Sterilized petriplates having filter paper were taken and 10 ml of 300 µg mL⁻¹ of each trivalent (CrCl₃) and hexavalent (K₂Cr₂O₇) chromium treatment was supplied to respective petriplates. Previously sterilized and inoculated seeds were uniformly spread on filter paper and All the plates were kept in dark at 22 ± 2°C for three days. Germination was noticed daily.

**Addition of nutrient supplement**

After germination, 10 mL of [19] nutrient solutions, containing the respective chromium salt concentration, were added to supplement the nutrient requirements of the seedlings and plates were shifted to light (195µE m² s⁻¹ for 16 hours at 22 ± 2°C). Seedlings were grown for 10 days. Daily observations were made and general appearance of seedlings was noticed. The experiments were repeated four times and data was analyzed statistically [20].

**Harvesting and growth parameters**

Seedlings were removed from petriplates and excess of moisture was removed by gently pressing the seedlings between blotting papers and growth measurements such as percentage germination seedling length, no of roots, leaf area, fresh and, dry weight g⁻¹ were noted.

**Determination of biochemical parameters**

For the estimation and extraction of Auxin produced by seedlings (µg g⁻¹ fresh) [21] method was followed. Optical densities of the material was taken at 535nm on spectrophotometer and amount of auxin in the extract was measured and calculated as µg gm⁻¹ fresh weight of cyanobacteria/ plants by using standard curve. Extraction of Soluble protein content of seedlings (µg g⁻¹ fresh) was done by following [22], whereas for soluble protein analysis method of [23] was adopted and for Acid phosphatase activity (unit g⁻¹ fresh) [24] were followed. Optical density of the material was taken at 750 nm on spectrophotometer. Amount of soluble proteins was calculated using standard curve.

Chromium content in different part of plant was measured by digesting them according to the protocol followed by [25]. Cr content for both forms was analyzed with the help of UV-Vis spectrophotometer [26].

**RESULTS:**

**Seed Germination**

Germination of *Triticum aestivum* was hindered with CrCl₃ (11.3 %) and K₂Cr₂O₇ (44.3%) but cyanobacterial inoculation lessen this adverse effect when compared with respective non-inoculated control and 3.5 to 33% increases in this parameter were observed with different strains (Table-1). Strains *Gloeocapsopsis* sp and *Cyanoascrina* sp caused maximum 13.9% stimulation whereas with *Oscillatoria “S.A”* the least i.e. 4.6%, increase in seed germination was observed (Table-1).

**Seedling Length (cm)**

Seedling length of *Triticum aestivum* was severely affected by the application of chromate salt especially with the Cr (VI). About 18 and 61% reduction in seedling length respectively under 300 µg mL⁻¹ of CrCl₃ and K₂Cr₂O₇ was observed. All cyanobacterial inoculations stimulated seedlings length as compared to non-inoculated control. *Gloeocapsopsis* sp caused maximum stimulation by increasing 39% and 51% seedling length under CrCl₃ and K₂Cr₂O₇ stresses respectively (Table-1).

**Number of Roots**

Hexavalent chromium had drastic effect on root numbers while trivalent chromium had no effect on this parameter. Inoculation of cyanobacterial strains had no effect on number of roots and they remained same as that of control in CrCl₃ treatment, whereas under K₂Cr₂O₇ stress inoculation of strain *Gloeocapsopsis* sp caused increase (10%) in number of roots over respective non-inoculated control (Table-1). Inoculation of cyanobacterial strains *Arthospira* sp, *Oscillatoria “O”*, *Oscillatoria “F.W”* and *Cyanoascrina* sp caused increases (20%) in root numbers over non-inoculated control. Changes in the texture of roots as compared to control were also observed. Roots were stunted and become brownish black. Appearance of roots was much improved by the application of cyanobacterial strains.

**Leaf Area (cm²)**

Both trivalent (10.4%) and hexavalent (97.2%) chromium salts caused reduction in the leaf area. The mean leaf area of
the inoculated seedlings was greater than those of control plants in chromium free treatment (Table-2). Under 300 µg mL⁻¹ CrCl₃ treatment, strains *Cyanosarcina* sp and *Gloeocapsosis* sp exerted a substantial promoting effects on the leaf area of the *Triticum aestivum* (almost 14 and 12%, respectively), over control. In case of hexavalent chromium treatment, seedlings inoculated with strain *Gloeocapsosis* sp enhanced the leaf area up to 21.4% as compared to non-inoculated control.

**Fresh Weight of Seedlings**

Fresh weight of seedlings was adversely affected with the application of chromium salts. With trivalent salt 15.1% and with hexavalent 59.3% reduction was observed. All cyanobacterial inoculations caused increases (3 to 36%) in fresh weight when compared to non-inoculated respective control. At 300 µg mL⁻¹ of CrCl₃, cyanobacterial strains stimulated 18 to 44% increases over respective control in the fresh weight of seedlings. Inoculation of *Gloeocapsosis* strain caused maximum increase in this parameter in the presence of 300 µg mL⁻¹ of K₂CrO₄, all strains also enhance the fresh weight of seedlings and 11 to 54% increases relative to respective non-inoculated control were observed with different strains (Table-2).

**Dry Weight g⁻¹ Fresh Weight**

With chromium salts dry weight accumulation increased and under hexavalent salt dry weight accumulation was almost double than that of control. The dry weight of *Triticum aestivum* seedlings was increased with inoculations of 5 strains, *Oscillatoria* “F.W”(7%), *Anabaena* sp (7%), *Gloeocapsosis* sp (14%), *Cyanosarcina* sp (14%) and *Synechocystis* “MK(S)”(7%) over non inoculated chromium free control, while with other strains no change in this parameter was observed. Under chromium stresses all cyanobacterial inoculations caused decreases in the dry weight of seedlings relative to non-inoculated respective treatments. The decrease with cyanobacterial strains was more pronounced with hexavalent chromium, where 16 to 27% reductions in dry weight were observed (Table-2).

**Protein Content**

Protein content of *Triticum aestivum* seedlings increased with chromium salts. However, inoculation of strains *Gloeocapsosis* sp (17%), *Cyanosarcina* sp (13%) and *Anabaena* sp (13%) significantly increased the protein content of seedlings when compared with that of control. At 300 µg mL⁻¹ CrCl₃, *Oscillatoria* “S.A” strain caused relatively low increase (0.2%) whereas strain *Gloeocapsosis* sp enhanced maximally (14%) over respective control (Table-3). Under K₂CrO₄ stress, inoculation with *Anabaena* treatment increased protein content up to 12.9% relative to respective non inoculated (Table-3).

**Auxin Content (µg g⁻¹ fresh weight)**

Cyanobacterial inoculations caused significant stimulation in auxin content in wheat seedlings when compared to non-inoculated control. CrCl₃ (300 µg mL⁻¹) and K₂CrO₄ (300 µg mL⁻¹) treatments, stimulated more synthesis of auxin content in *Triticum aestivum* seedlings 142.8% and 285.7% increases respectively (Table-3). *Anabaena* sp inoculated seedlings in chromium free treatment showed 114 % increase in auxin content. Generally at 300 µg mL⁻¹ CrCl₃, all cyanobacterial inoculations caused an increment in auxin content when compared to their respective control. At 300 µg mL⁻¹ K₂CrO₄, High increases were observed with inoculations of strains *Anabaena* sp, *Arthrospira* sp, *Cyanosarcina* sp and *Gloeocapsosis* sp where 22.2%, 18.5%, 18.5% and 14.5% increases respectively, over non-inoculated respective treatment were observed (Table-3).

**Acid Phosphatase (unit g⁻¹ fresh weight)**

About 45% and 230% increases in acid phosphatase activity, over control, were observed under 300 µg/mL⁻¹ of CrCl₃ and K₂CrO₄ respectively (Table-3). Under CrCl₃ stress cyanobacterial strains enhanced 2.8-24.7% activity of this enzyme over that of control. Under K₂CrO₄ stress, stimulation in the enzyme activity was more pronounced with inoculations of strains *Gloeocapsosis* sp, *Cyanosarcina* sp and *Arthrospira* sp, which caused 18.9%, 13 % and 12.6% increases respectively, in acid phosphatase activity of wheat seedlings over their respective un-inoculated treatment.

**Chromium Content (mg Cr g⁻¹ d.w.**

Seedlings which were grown under 300 µg mL⁻¹ K₂CrO₄ uptake more chromium content as compared to the seedlings which were grown under 300 µg mL⁻¹ of CrCl₃ (Table-3). In general there was a reduction in chromium uptake with the cyanobacterial inoculation. In case of 300 µg mL⁻¹ K₂CrO₄ treatment, except strain *Cyanosarcina* sp where some increase in chromium content of wheat seedlings was observed, all other cyanobacterial strains manifested a reduction or no change in the chromium content of seedlings when compared to respective non-inoculated control treatment (Table-3).

**DISCUSSION:**

In the present study ten filamentous chromium resistant cyanobacterial strains that exhibited greater reduction potential were used for inoculating seeds of *Triticum aestivum* var. Uqab-2000, which were subsequently germinated and grown under different chromium salts (300 µg mL⁻¹ of CrCl₃ and K₂CrO₄) in both laboratory and pots condition. The effects of hexavalent salt were more severe than that of trivalent salt. Hexavalent chromium is highly soluble and easily available to seeds, hence is different valence state of chromium when compared with control. Without cyanobacterial inoculation, about 11 and 44.3% more toxic. Higher uptake of metallic salt disturbs nuclear division and hinders cytokinesis, which causes adverse effects on seed germination [27]. Cyanobacterial strains not only enhanced the plant growth but also provided resistance to crop against reduction in seed germination was observed in *T. aestivum* under 300 µg mL⁻¹ of CrCl₃ and K₂CrO₄, respectively. In fact these cyanobacterial strains gave some alleviation to plants under Cr (VI) stress by decreasing the availability of toxic Cr (VI) to plants. One possible explanation for stimulation in germination and growth parameters might be that cyanobacteria take up chromium in
### Table 1: Effect of Inoculation of Chromium Resistant Cyanobacterial Strains on Seed Germination, Seedling Length and Number of Roots of *Triticum aestivum* var. Uqab-2000 Grown at 0 and 300 μg mL$^{-1}$ of CrCl$_3$ and K$_2$CrO$_4$ Concentrations (Means of Four Replicates)

<table>
<thead>
<tr>
<th>Strains</th>
<th>% Germination</th>
<th>Seedling length (cm)</th>
<th>No. of roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.8±0.5</td>
<td>4.3±0.1</td>
<td>0.1±0.0</td>
</tr>
<tr>
<td>Arthrospira sp</td>
<td>5.5±0.6</td>
<td>4.9±0.1</td>
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<tr>
<td>Lyngbya sp</td>
<td>4.8±0.5</td>
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</tr>
<tr>
<td>Oscillatoria &quot;G&quot;</td>
<td>4.9±0.5</td>
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</tr>
<tr>
<td>Oscillatoria &quot;B1&quot;</td>
<td>5.0±0.6</td>
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<tr>
<td>Oscillatoria &quot;B3&quot;</td>
<td>5.0±0.6</td>
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<tr>
<td>Synechocystis &quot;AH-Z-MX&quot;</td>
<td>5.0±0.6</td>
<td>4.5±0.1</td>
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</tr>
<tr>
<td>Synechocystis &quot;AH-Z-FPA&quot;</td>
<td>5.0±0.6</td>
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</tr>
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</table>

### Table 2: Effect of Inoculation of Chromium Resistant Cyanobacterial Strains on Leaf Area cm$^2$, Fresh Weight and Dry Weight g$^{-1}$ Fresh Weight of *Triticum aestivum* var. Uqab-2000 Seedlings Grown at 0 and 300 μg mL$^{-1}$ of CrCl$_3$ and K$_2$CrO$_4$

<table>
<thead>
<tr>
<th>Strains</th>
<th>Leaf area cm$^2$</th>
<th>Fresh wt (g)</th>
<th>Dry wt / Fresh wt (g)</th>
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<tr>
<td>Control</td>
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### Table 3: Effect of Inoculation of Chromium Resistant Cyanobacterial Strains on Protein Content, Auxin Content, Acid Phosphatase and Chromium Content of *Triticum aestivum* var. Uqab-2000 Seedlings Grown at 0 and 300 μg mL$^{-1}$ of CrCl$_3$ and K$_2$CrO$_4$ Concentrations (Mean of Four Replicates)

<table>
<thead>
<tr>
<th>Strains</th>
<th>Protein content (mg g$^{-1}$ fresh weight)</th>
<th>Auxin content (μg g$^{-1}$)</th>
<th>Acid phosphatase (μg g$^{-1}$ fresh weight)</th>
<th>Chromium content (mg g$^{-1}$)</th>
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their cells and may be capable of reducing the availability to seeds. Thus cyanobacteria by lessening the harmful effects of chromium are provoking germination and subsequently stimulating seedling growth. Under chromium stress, different growth parameters of crop *T. aestivum* were also severely affected. Actually under hexavalent chromium stress different vital functions such as electron transport chain and photosynthesis [28, 29] severely affected, which ultimately lead to poor growth of seedlings in comparison to respective control plants. Majority of cyanobacterial inoculations caused an increment in the seedling length both under chromate stress and unstressed medium. In *T. aestivum*, strains Anabaena sp and *Arthrospira* sp posed relatively more stimulatory effects both in chromium stressed and unstressed conditions. It may be due to the fact that growth stimulatory cyanobacteria released some chemotaxis to root exudates, metabolism which helps for the suppression of competitor microorganism and most importantly the ability to bind with plant root surface [12, 30-32] which helps plant for better growth. Chromium stress also caused reduction in the number of leaves. In *Triticum aestivum* 300 µg g⁻¹ of K₂CrO₄ caused much reduction in the number of leaves as compared to chromium free control. Cyanobacterial inoculation caused increment in the number of leaves not only in chromium free but also in the presence of trivalent and hexavalent chromium in *T. aestivum*. A major effect of chromium stress on roots was shortening and thickening, which may be due to decrease in the rate of cell elongation and growth. This morphological adaptation of roots is a typical change under stress environment [33]. Plant growth promoting microorganisms / rhizobacteria in addition to stimulating plant growth (length and weight parameters) and nutrient uptake, also enhance number and length of root hairs [34].

In the presence of chromate, weight parameters of *T. aestivum* were severely affected while cyanobacterial strains caused increase in fresh weight of this crop both under normal and chromium stress conditions, when compared with non-inoculated respective control. The response of wheat seedlings upon *Azospirillum* inoculation, particularly the stimulation of root differentiation, in addition to the promotion and elongation of the primary root has been attributed to compounds presenting auxin activity, such as 1AA and nitrate [35, 36]. The reduction in fresh weight of *Triticum aestivum* seedling under hexavalent chromium stress might be due to reduced uptake of water and probably cell expansion as indicated by stunted growth was also inhibited. Heavy metal toxicity hampered cell division and decrease turgor pressure of plant cell [37]. With application of 300µg mL⁻¹ of K₂CrO₄, dry weight and dry mass accumulation increased significantly. Enhanced dry weight could be attributed to the uptake and increase level of organic salts in cytoplasm [38]. In the presence of hexavalent chromium stress, inoculation with all strains caused reduction in dry weight of *Triticum aestivum* seedlings. The decrease in dry weight and dry mass accumulation in inoculated seedlings might be due to the alleviation of stressed conditions by the cyanobacterial strains. Several others workers also reported increased fresh weight, plant height and leaf size of plants with microbial inoculation [39].

Cyanobacterial strains also showed production of plant growth regulators such as auxin, gibberellins, ethylene or abscisic acid [40-43]. In the present study amount of auxin content increased in *Triticum aestivum*, seedling under chromium stress by the help of cyanobacterial inoculations that exhibited some increment in auxin content both under chromium stress and normal conditions. According to researchers [44], cyanobacterial strains stimulated plant growth by synthesizing and liberating growth hormones. Chromium stress also caused some increment in soluble protein content in the seedlings as compared to respective chromium free control. Majority of cyanobacterial inoculations enhanced the protein content of seedlings relative to respective non-inoculated treatment. In view of the above results it can be believed that along with many other mechanisms involved for the growth promotion of seedling of this important cash crops, it is apparent that strains supported the plant growth by lessening the availability of toxic Cr (VI), which was reduced by the cyanobacterial strains into a less soluble Cr (III).

Chromium content of *Triticum aestivum* seedlings increased in case of hexavalent chromium stress as compared to trivalent chromium. In the present study under 300 µg mL⁻¹ of Cr (VI), majority of cyanobacterial inoculation resulted a decrease in chromate uptake in *Triticum aestivum* seedlings as compared to their respective non-inoculated control. In fact majority of the cyanobacterial strains were also capable of reducing more toxic and mobile Cr(VI) in to Cr(III). Hence majority of the strains reduced this Cr(VI) in the vicinity of root, thus limiting the entry of chromium into the plant roots. In the present study majority of cyanobacterial inoculations caused an increment in the acid phosphatase activity as compared to their respective non-inoculated control. The activity of acid phosphatase is related with metal accumulation by the cell [45]. In *Triticum aestivum* The enhanced activity was much higher with inoculations of *Arthrospira* sp., as compared to other strains, which are also showing good chromium uptake, chromium reduction and auxin production.

These findings urge us for the safe and long term use of cyanobacteria in fields to enhance the growth of plant by acting as bioremediation and biofertilitation.

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