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REVIEW ARTICLE **OPEN ACCESS**

A Review on the aspects of *Azosprillum*: Used as a tool in sustainable agriculture

Shagun Sharma, Adesh Kumar*, Jenita Nongthombam and KartikNakum
School of Agriculture, Lovely Professional University, Phagwara (Punjab), India-144411
E-mail: adesh.19078@lpu.co.in, phytopath06@gmail.com

ABSTRACT
Among growth promoting rhizobacteria, Azospirillum is known to be a very active nitrogen fixer under laboratory as well as under soil conditions providing fast growth, better health for the plants and higher yield. Azospirillum has potential use in agriculture as biofertilizer, as growth promoter, as a plant protector, as a nitrogen fixer, as a soil manipulator etc. in agriculture. Azospirillum reduces the use of chemical fertilizers by 50% and promotes plant growth by producing phytohormones viz auxins, gibberellins, cytokinins, ethylene, abscisic acid etc. As these bacteria promote plant growth they are also called as Plant Growth Promoting Rhizobacteria (PGPR). Plant growth promoting rhizobacteria are indigenous to soil and play a major role in nitrogen fixation. Azospirillum biofertilizer has zero to minimum residual effect on soil and also helps to suppress specific plant diseases, soil-borne diseases and parasites.
Keywords: Azospirillum, PGPR, Biofertilizer, Nitrogen Fixation

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INTRODUCTION

For the growth and development of plants, Nitrogen is required in bulky amounts as it is the crucial constituent of nucleic acids and proteins [11].In soils it is used for intensive cropping whereas soil fertility is maintained by addition of nitrogenous fertilizers [50]. *Azospirillum* spp. is among the most important bacteria involved in Nitrogen fixation in cereal crops [105]. It's a diazotrophic bacteria usually found in the rhizosphere region and inside the plant roots [92], gram negative, vibrio or spirillum shaped often with pointed ends [1], having flagella [113], 1.0µm in diameter and 2.1-3.8 µm in length, highly motile in nature [7] and a genus of family Rhodospirillaceae[125]. The genus *Azospirillum* is associated with α-subclass of proteobacteria[45] and includes a species formerly named *Spirillum lipoferum*[59] and after that *Azospirillum* name was given by Beijerinck, 1922 [93]. Beijerinck was first to detect the growth of spirillum-like bacteria in 1922 [93].

The major goal of this publication is to provide a quick overview of *Azosprillum* as a whole body that plays an important role in bioremediation and phytoremediation by primarily replacing synthetic fertilizers and pesticides (partly).

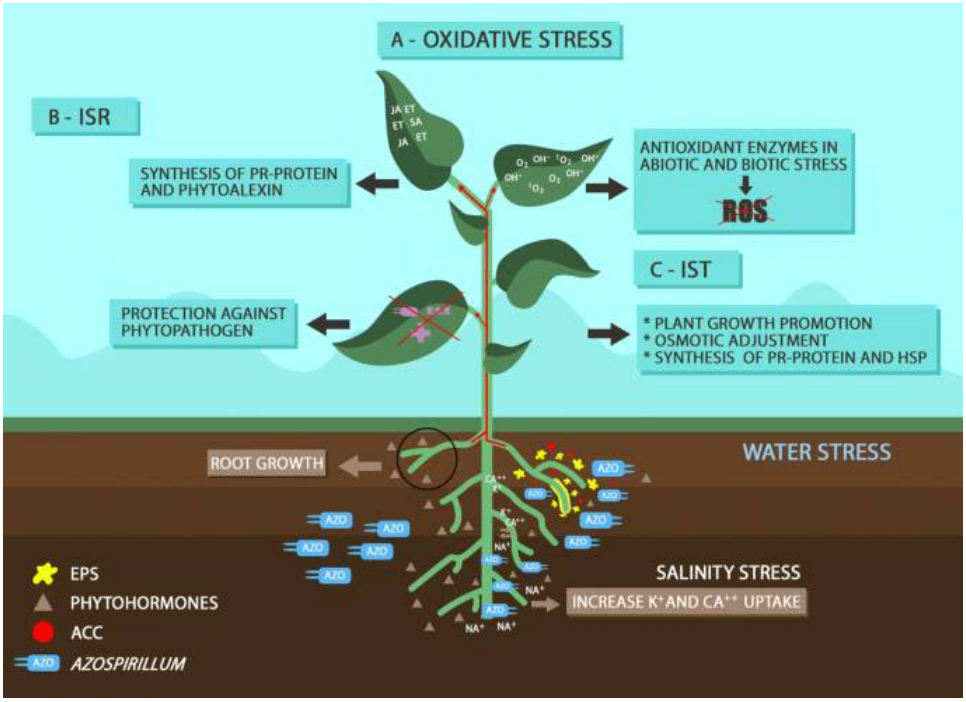

Fig. 1 Mechanism of biotic and abiotic stress induced by *Azospirillum*into plant system. Image source [38]

Azospirillum species grow well on organic acids like malate and succinate but vary in their ability to use dissimilar sugars and amino acids as carbon and energy sources [8] but *Azospirillum* spp. usually isolated with nitrogen-free culture media which can be inoculated with soil or roots [2]. With time and culture's age cells transform its size and shape, and produce cysts [11]. Mechanism of tolerance of biotic and abiotic stresses induced by *Azospirillum* in plants is shown in **Figure 1**, **Table 1.**

Table 1. Five species of *Azospirillum*

Azospirillumspp	**Source**
Azospirillum brasilense	[83]
Azospirillum lipoferum	[42]
Azospirillum amazonense	[96]
Azospirillum halopraeferens	[91]
Azospirillum irakense	[10]

Azospirillum brasilense

Azospirillum brasilense is well known to grow healthy in culture media on a variety of carbon sources and produces plenty phytohormones viz, indole 3-acetic acid (IAA), gibberellic acid (GA3), abscisic acid (ABA), ethylene, and growth regulators putrescine, spermine, spermidine, and cadaverine (CAD). *A. brasilense* is potentially capable of promoting direct plant growth or agronomic yield increase. Az39 and Cd are generally used as free living PGPR which are capable of affecting the yield of numerous plant species of agronomic interest. Az39 and Cd also showed differential potential to produce the five major phytohormones and CAD in chemically defined medium because of this *A. brasilense* is suggested for maize and wheat crop inoculation [83].

Azospirillum lipoferum

Azospirillum lipoferum strains are habitually obtained from rice fields. Two strains 4B and 4T are generally used for study which stands for *A. lipoferum* 4T and *A. lipoferum 4BpHT2. A. lipoferum*4Tis a non-motile species, whereas *A. lipoferum*4B is also a non-motile rifampicin resistant mutant of a motile spontaneous rifampicin resistant strain. The letter was derived from *A. lipoferum* 4B, the motile parental strain. The special character of these bacteria is that both strains produce dark brown color [42].

Azospirillum amazonense

Azospirillum amazonense species is very scarce in the rhizosphere. That's why it is difficult to isolate, however forty-two isolates are confirmed as *A. amazonense* by the fluorescent in situ hybridization (FISH) technique. All *A. amazonense* strains tested produce indoles, but only 10% of them showed high production, above 1.33 µM mg protein-1. The nitrogenase activity also was variable and only 9% of

isolates showed high nitrogenase activity and the majority (54%) exhibited a low potential. However, in the greenhouse experiment, inoculation of strains of *A. amazonense* increased grain dry matter accumulation (7 to 11.6%), the number of panicles (3 to 18.6%) and nitrogen accumulation at grain maturation (3.5 to 18.5%) [96].

Azospirillum halopraeferens

Azospirillum halopraeferens cells are gram negative, vibrioid to S shaped; only few helical cells occur in alkaline media. These gram negative cells are motile in liquid medium with a rapid corkscrew like motion by one polar flagellum. Fluorescent pigments are not produced. No growth on plates of congo red medium. Good growth on tryptic soy agar, forming cream-colored, circular, flat colonies with an entire margin. When cultivated on N-free semisolid medium at 30 or 35"C, young cultures which have just developed a fine pellicle contain cells of small cell width, averaging from 0.7 to 0.9 pm. When growing at 41°C in medium supplement with 0.25% NaCl at pH 7.2, the width of young cells is 1 to 1.2 pm [91].

Azospirillum irakense

Some isolates of *A. irakense* were obtained from surface-sterilized field-grown rice roots, indicating their ability to penetrate plant roots and suggesting the involvement of plant cell wall-degrading enzymes in this infection process. There are reports on the molecular characterization of a Pel (PelA) and the cloning of the corresponding structural gene from *AzospirillumirakenseKBC1*. *A. irakensePelA* defines a new class of Pel enzymes since it displays no homology to other known bacterial, plant or fungal pectinases [10].

In attachment of *Azospirillum* to roots various nutritional, physiological, environmental, and chemical factors matters [15]. As for many other rhizobacteria, *Azospirillum* colonizes the plant at the expense of seed and root exudates [35]. This involves microbial proliferation and microcolony or biofilm formation at the surface of seeds and roots [85]although still the particular type of mechanism by which *Azospirillum* attaches itself to roots still remains anonymous [123]. Recently it was speculated that agglutinins may be positioned in the fibrillar material, helping cells and Lectin to encourage binding, also been suggested as possible [31]. However, these theories have not been further explored [7]. It is also difficult to discover because in comparison to the *Rhizobium* legume symbiosis, *Azospirillum* grass relation does not produce visible structures on roots which point out successful infection [117].

Numerous major changes are seen in growth parameters of plants where inoculation is made with *Azospirillum*, viz. Nutrient uptake, tissue N content, increase in plant area, tiller number, leaf size, plant height, root length, volume [78, 62, 78, 32] in different cereals [40]. These organisms grow well under aerobic circumstances and on a variety of collective nitrogen sources, including nitrate, ammonia, or amino acids [102].

Azospirillum is extremely versatile, along with being a quality plant colonizer [101]. *Azospirillum* not only fixes atmospheric Nitrogen [14], but can also mineralize nutrients from the soil, sequester Iron (Fe), stay alive under critical environmental conditions [5], and support valuable mycorrhizal-plant associations [104]. In addition; *Azospirillum* can help plants minimize the negative effects of abiotic stresses [118].

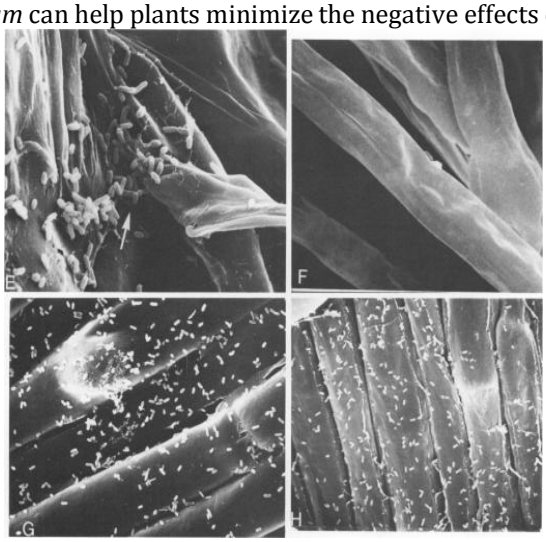

Fig. 2 (E) *Azospirillum brasilense* occupying void spaces (arrow) created by desquamation at lateral root emergence at x3000. (F) Lack of adherent bacteria on root hairs grown in Fahraeus medium supplemented with 5 mM KNO3. Compare with the adsorbed bacteria associated with the granular surfaces of root hairs grown in the nitrogen-free medium (A and B) x3000. (G) *A. brasilense* adsorbed to undifferentiated epidermal cells of roots grown in N-free Fahraeus medium. x3000. (H) Same as (G) **Image Source:** [117]

Azospirillum is the most studied Plant Growth Promoting Rhizobacteria (PGPR), apart from *Rhizobium*, and reached commercialization in numerous countries, including India, Italy, France, Argentina and Mexico [105]. Symbiotic or free-living rhizobacteria which are considered beneficial for plant growth and development have been termed as plant growth-promoting rhizobacteria (PGPR) [20]. The nomenclature "plant growth-promoting bacteria (PGPBs)" is increasingly used for bacteria [71] which able to promote plant growth by a variety of individual or combined mechanisms [38] Inoculation of soil with PGPB is used to enhance plant growth [98], yield [74], crop quality [37], germination pace [54], root growth [109], protein content [68], mineral content, stress tolerance of plants [53], and as biocontrol agents [61]. In inoculation with PGPR along with growth changes there are physiological and morphological changes also which are usually considered within a "black box" model [18].

AZOSPIRILLUMAS BIOFERTILIZER

Biofertilizers are commonly called microbial inoculants [90] of bacteria, algae and fungi [28]. The Biofertilizers like *Rhizobium*[70]*, Azotobacter*[81]*, Azospirillum*[106]*,* and Blue green algae [43] are known to be responsible for nitrogen fixation in grasses, cereal crops, vegetables and plantation crops [79]. Application of biofertilizers can not only cut down chemical fertilizer consumption [80] by 20 to 50 per cent but also can simultaneously boost the yield of crop by 10 to 20 per cent [50]. Biofertilizers, being less costly, ecofriendly and sustainable [17] are likely to assume greater implication as a complement or supplement to inorganic fertilizers [65]. It is important to build up integrated fertilization strategies for crop production that enhance the competitive ability of the crop, minimize weed competition [86], and reduce the risk of nonpoint source pollution from nitrogen [77].

The work on *Azospirillum* throughout the past twenty years showed increasing scientist interest because of the nitrogen fixing capability [89] and their association close to the roots of the forage and cereal crops [79]. Experiments have exposed that field application of *Azospirillum* provides an opportunity to decrease the need for chemical nitrogen fertilizer by 50% without unpleasant effect on crop yield and quality [111].This made many scientists to have an inquiry in the field crop like rice and sugarcane to associate the nitrogen effectiveness [79]. One major property of *Azospirillum* relies on the synthesis of phytohormones and other compounds, including auxins[107], cytokinins [27], gibberellins [19], abscisic acid [25], ethylene [95], and salicylic acid [38]. *Azospirillum* sp. increases the health of roots during the growth stage by competing with root pathogens and increasing the absorption of nutrients and water [77].

*Azospirillum*helps in increasing the organic matter content of the soil [5], with improving the exchange capacity of nutrients [105], increasing soil water retention and promoting soil aggregates [22]. Several strains of bio-fertilizers like *Azospirillum* etc. have been developed for cereals, pulses, vegetables, oils seeds, cotton and sugarcane [76] etc. to improve the growth and yield [88]. *Azospirillum* have shown a significant effect on maize, wheat, rice, sorghum and sugarcane production [66].*Azospirillum* biofertilizer contains 35% moisture and 63% lignite and 2% *Azospirillum* bacteria [60]. Use of lignite along with *Azospirillum* is accountable for increase in crop production at large scale [106]. In India *Azospirillum*is available in two forms one is in liquid form (Fig. 3) and other is in wettable powder (Fig. 4) as shown in images.

Fig 3. Liquid form of *Azospirillum* **Fig 4.** Powder form of *Azospirillum*

A suitable bio-fertilizer like *Azospirillum* can become a promising alternative for many problems like pH, acidity and alkalinity [67]. *Azospirillum* can be very useful in case of developing seeds because they utilize nitrogen from vegetative parts of seed, due to this nitrogen content decrease [46].

AZOSPIRILLUM AS GROWTH PROMOTER

Among the microorganisms that reside in the rhizosphere, a diverse group of bacterial species also recognized as PGPB [41] which are capable of promoting plant growth [63]. These bacteria can play a significant role in nutrients acquired by plants, acting as biofertilizers[52], phytostimulants[9] and biotic and abiotic stress controllers [126]. Numerous *Azospirillum* strains have been used as PGPR [85] and by using these strains inoculation is done in plants resulting in improved crop yield in several regions of the world [108]. Along with this *Azospirillum* spp. also diminishes the requirement for chemical fertilizers [48].

Azospirillum's flexible metabolism is well-matched to competition and to the harsh environment which exists in the rhizosphere and in soil [12]. It has been repeatedly shown that *Azospirillum* has the potential for agricultural development [49]. Extensive studies on *Azospirillum* genetics, biochemistry and physiology have been carried out making this genus one of the best characterized genera among associative PGPR [96]. In past years, the research is focusing on the phytohormone production by *Azospirillum*[64], as a most vital mechanism for plant growth promotion [110]. *Azospirillum*spp. produces several plant growth regulating substances such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), cytokinins (CK) [114] and several gibberellins (GA, i.e., GA1, GA3, GA9, GA19, and GA20) in chemically defined media [24], which are known to promote better growth [21].

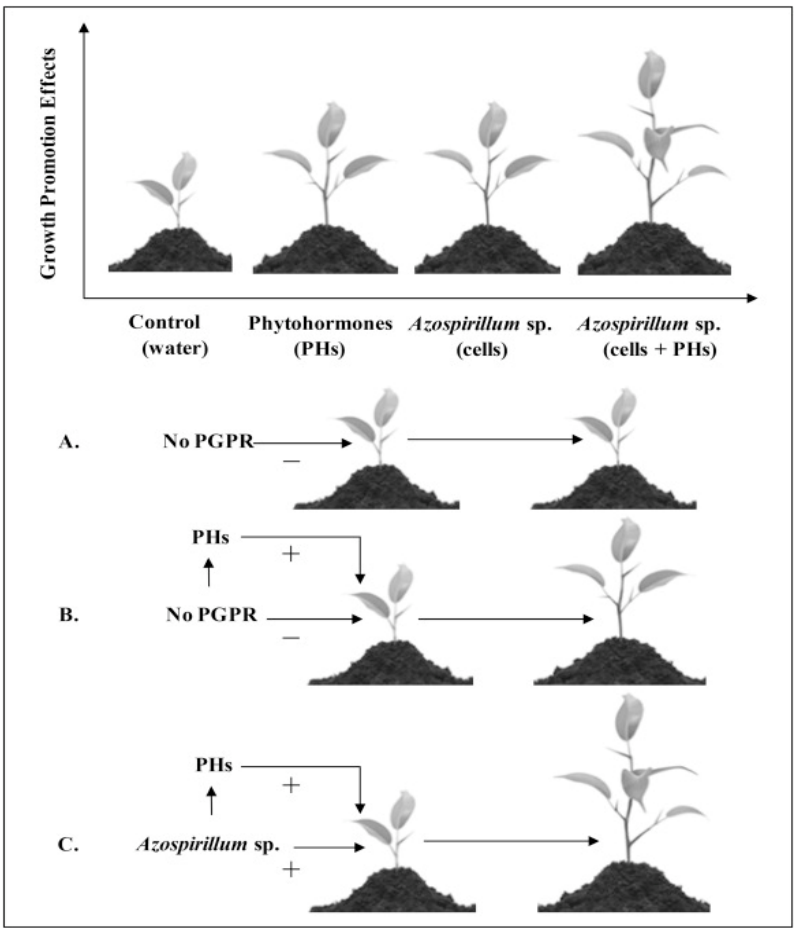

Fig. 5 Illustrative model to explain the positive short-term effects of seeds and seedlings inoculation with *Azospirillum* spp **Image Source:**[18]

A model to explain the positive effects of inoculation with *Azospirillum* spp. a phytohormones producer (PGPR) are summarized and illustrated in (Image 5) by [18]. *Azospirillum* species/strains are unfocused PGPB affecting the metabolism of plants in general and some strains have a broad host range [34]. This promotion ability has been studied mainly in terms of biological nitrogen fixation [83]. In another

experiment maize seeds were inoculated with *A. brasilense* and within 15 days the aerial sprouts size increased about 22 to 60% and the mature dry weight increased from 27 to 34% [116].

AZOSPIRILLUM AS PLANT PROTECTOR

Azospirillum helps to suppress plant diseases, soil-borne pathogens and specific pests [22]. Along with this *Azospirillum* stimulates plant natural defenses [39] by a mechanism called induced systemic resistance (ISR) [99]. Some plant pathogens, beneficial rhizospheric pathogens [87] and endophytic microbes [34] can activate plant resistance by ISR mechanism, which also has beneficial effects, on plant growth [69]

A recent study indicated that tomato plants inoculated with *Azospirillumspp* showed better disease resistance against a virulent bacterial pathogen ,*Pseudomonas syringaepv. tomato*[6, 124]. Another study done by [125] found that colonization of the bacterial endophyte *Azospirillum* sp. B510 [56] induced disease resistance in rice plants against rice blast disease and bacterial blight disease.

Along with plant protection *A. brasilense* BNM65 [26] also cause developmental changes on tomato plants that favored a better plant-water relationship adjustment [100]. The utilization of PGPR strains is use to combat plant diseases and reduce the use of pesticides [119] which is a promising approach for the sustainability of agro ecosystem [36]. Although *Azospirillum* is not known as a typical biocontrol agent, it has been reported that few strains are capable of stimulating plant growth also [94] ultimately by suppressing soil-borne pathogens [122] and other deleterious microorganisms in various systems.

AZOSPIRILLUM AS NITROGEN FIXER

Associative and symbiotic nitrogen-fixing bacteria are common valuable microorganisms of the monocot [115] and leguminous family including 80–90% of higher plants [13]. *Azospirillum* is an associative symbiotic nitrogen fixing bacteria which fixes nitrogen by the formation of Para nodules [23]. *Azospirillum* has higher nitrogen fixing potential in non-leguminous crops [78] in contrast to other nitrogen fixing bacteria [104],*Azospirillum* can exist freely or in associative symbiosis [33] with cereal crops and helps in entrapping atmospheric nitrogen [127]. The entrapped nitrogen is converted to Ammonia (NH_3), from unreactive nitrogen by an enzyme called Nitrogenase[47]. This process is termed as Biological Nitrogen Fixation (BNF) [120]. Nitrogenase is an oxygen sensitive enzyme [84], which is present inside the bacteria and the process of conversion is easily utilized by plants by following reaction; [104]

$$N_2 + 8H + 8_{e^-} + 16ATP \xrightarrow{\text{Nitrogenase}} 2NH_3 + H_2 + 16ADP + 16Pi$$

Transcription of the nitrogen fixation genes (nif genes) in Proteobacteria is activated by the NifA protein [2], whose nifA gene expression is under different, complex and species-specific regulatory mechanisms[51]. *Azospirillum* have complicated systems to control nitrogen fixation in response to environmental conditions [127]. More specifically, the *Azospirillum* perform nitrogen fixation only under microaerobic conditions and when fixed nitrogen is limiting [78].In an experiment by [112] shows the range of nitrogen fixing ability with *Azospirillum* sp. was between 3.3 to 9.3 mg N/g. Among them, the maximum nitrogen fixing ability (9.3 mg N/g) was recorded from *A. brasilense* and minimum (3.3 m N/g) was recorded in *A. zeae*. **Table 2.**

AZOSPIRILLUMAS SOIL MANIPULATOR

*Azospirillum*helps in increasing soil water withholding capacity [4], along with promoting soil aggregates, and buffering the soil adjacent to acidity, alkalinity, salinity, pesticide activity and lethal heavy metals [30, 72, 29]. *Azospirillum* and other organic fertilizers release nutrients little by little, which contributes to residual collection of organic nitrogen and phosphorus in the soil [44] besides reducing N leaching loss and P fixation. *Azospirillum* and other soil manipulator also provide micronutrients like copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn) to soil[73]. They boost root growth due to healthier soil structure [121]. *Azospirillum* improves plant traits which help in foster exploration of water in the soil [38]. They increase the organic matter content in the soil which ultimately enhance soil biological movement [33], and improve nutrient mobilization from organic and chemical sources with decomposition of toxic substances[22].

Table 2. Nitrogen fixed by different spp. of *Azospirillum* in mg/g

Identified Isolated	Mg N fixed/g
Azospirillum lipoferum	6.9
Azospirillum brasilense	9.3
Azospirillum dobereinerae	5.4
Azospirillum zeae	3.3

Table Source: [112]

CONCLUSION

*Azospirillum*is recognized as PGPR promotesplant growth by production of phytohormones, can reduce the use of chemicals, fertilizers by half without effecting quality and quantity of the plant and produce. Extensive studies on *Azospirillum* genetics, biochemistry and physiology have been carried out making this genus one of the best characterized genera among associative PGPRs. *Azosprillum* is much more than any other biofertilizers, *Azospirillum* species are known for fixing nitrogen in non-leguminous crops by formation of para nodules and the bacterium helps in suppressing both phytopathogens and soil borne pathogens which cause severe infection in plants, therefore, Azospirillum is very healthy approach used for sustainable agriculture with no or very less residual effect on environment and human health.

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