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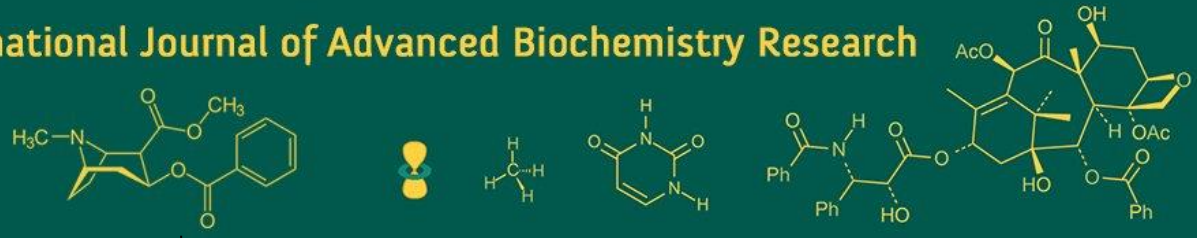

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Selection and characterization of effective *Azospirillum* isolates from rice field of Chhattisgarh plain

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Abstract
Twenty-two soil samples were collected from farmer's field of Bilaspur district for isolation & enumeration of *Azospirillum* bacteria. Out of these 13.64% soil samples lacked *Azospirillum*. Based on a population threshold of ≥2 × 10^4 g^{-1} of soil, 9 samples were selected for further physiological characteristics and performance assessments. These 9 local isolates were compared with a state check under both laboratory and pot conditions. The selected *Azospirillum* isolates, including the state check, were tested for different salt concentration, temperature, and pH tolerance under *in vitro* conditions. All isolates tolerated up to 3% salt concentration. The optimal pH range was 7-8, with 8 out of 10 isolates (excluding Azs-02 and Azs-03) tolerating pH 5-8, but none could withstand pH 4. In terms of temperature, all isolates grew between 20 °C and 40 °C, while only Azs-05 and the state check could tolerate 50 °C. Pot experiments evaluating Biological Nitrogen Fixation (BNF) parameters—such as plant height, grain per panicle, yield, and nitrogen uptake—identified local isolate Azs-13 as the most efficient among all isolates, including the state check. Thus, Azs-13 is suggested as the most potent nitrogen-fixing strain for rice cultivation in the Chhattisgarh plains.

Keywords: Soil samples, *Azospirillum* bacteria, isolation and enumeration, pH tolerance

Introduction
Rice (*Oryza sativa* L.) is a staple food for over 60% of the global population, second only to wheat (Fukagawa & Ziska, 2019) [8]. Asia contributes about 90% of global rice production and consumption (Tomar *et al*., 2018) [16]. Rice fulfils 43% of the calorie requirements for more than 70% of both the Indian and global population, making its production crucial for global food security (Anonymous, 2009) [1].

India's total rice production in 2021-22 was estimated at 127.93 million tonnes, surpassing the previous five-year average of 116.44 million tonnes by 11.49 million tonnes, indicating significant growth (Anonymous, 2022a) [2]. In Chhattisgarh, the rice cultivation area in 2021-22 was 3,899.34 hectares, with a productivity of 3,212 kg per hectare (Anonymous, 2022b) [3].

The excessive use of chemical fertilizers to improve soil fertility and crop productivity disrupts biogeochemical cycles (Perrott *et al*., 1992) [11] and leads to nutrient leaching, particularly nitrogen, causing environmental degradation (Gyaneshwar *et al*., 2002) [9].

In view of the rising costs and limited availability of nitrogenous fertilizers, alternative methods for supplying essential nutrients are necessary. In this regard, biofertilizers offer marginal farmers an effective strategy to enhance productivity.

Inoculation of biological nitrogen fixing bacteria like *Azospirillum* is a sustainable alternative for agro-ecosystems. Similar to that of other crop beneficial microbes, *Azospirillum* inoculation improve plant growth and soil fertility as well. *Azospirillum* inoculant is cost-effective and ecofriendly compared to the chemical N fertilizers.

Azospirillum is one, which colonizes on root surface and helps to fix atmospheric nitrogen. This bacterium was first time isolated and described as *Spirillum lipoferum* in 1925 by Beijerink. In 1978, Tarrand renamed this organism as *Azospirillum*. It is a heterotrophic, micro-aerophilic, gram negative, spiral shaped, N-fixing bacterium. *Azospirillum* being associative as well as free living N fixers, it is exclusively recommended for paddy, maize, bajra, ragi etc. as an inoculant.

Materials and Methods

The study was carried out at the Instructional Farm of Barrister Thakur Chhedilal College of Agriculture and Research Station, Bilaspur (C.G.), with the objective of selecting effective *Azospirillum* isolates. The experiment followed a Completely Randomized Design (CRD) with three replications and twelve treatments to evaluate the performance of selected indigenous *Azospirillum* inoculants for key Biological Nitrogen Fixation (BNF) parameters, including plant height, grains per panicle, yield, and nitrogen uptake. Additionally, the study involved the characterization of *Azospirillum* isolates under varying pH levels (4-8), temperature conditions (10-50 °C), and salt tolerance tests using sodium chloride concentrations of 1%, 2%, and 3% (w/v).

Microbial Analysis

The dilution plate method was used to estimate the microbial population in relation to the *Azospirillum* count of the soil (Subba Rao, 1988) [14].

Isolation of crop beneficial bacteria

Soil samples were collected from a depth of 6 inches (15 cm) below the surface. Approximately 200 grams of soil was placed in a polythene bag to retain moisture. These samples were appropriately labeled, sealed, and stored in a refrigerator for the isolation of *Azospirillum* One gram of rhizosphere soil was suspended in 9 ml of sterile water, and serial dilutions of the suspension were prepared using sterile water blanks. The suitable dilutions were then plated on malate medium, which contained NH4Cl (ammonium chloride) as a nutrient source to support the initial growth of bacteria.

Physiological tests

Growth at different temperature

The growth of different isolates was assessed at various temperatures. The plates containing different isolates were streaked and incubated at 10, 20, 30, 40, and 50 °C. After four days of incubation, growth observations were recorded (Tensingh Baliah and Rajalakshmi, 2015) [5].

Growth at different pH levels

The pH tolerance of all *Azospirillum* isolates was evaluated using NFB medium adjusted to different pH levels. The pH was set to 4.0, 5.0, and 6.0 using 1N HCl, and to 8.0 using 1N NaOH. The plates containing various isolates were streaked and incubated at 28±2 °C. After a four-day incubation period, growth was visually observed (Usha and Kanimozhi, 2011) [17].

Growth at different levels of NaCl concentration

Laboratory studies were conducted to assess the tolerance of *Azospirillum* to different NaCl concentrations. NFB media plates were prepared with NaCl concentrations of 1%, 2%, and 3%. The isolates were then streaked onto the plates and incubated at 28±2 °C for four days. Growth was visually observed (Tensingh Baliah and Rajalakshmi, 2015) [5].

Results & Discussion

The experiments were carried out in both laboratory & pot conditions to evaluate the efficiency of various native *Azospirillum* isolates from Bilaspur district on rice crops. The result of different characteristics are presented in Table 1.1.

Study of different characteristics of efficient *Azospirillum* isolates pH tolerance test

All isolates exhibited growth across a pH range of 5 to 8, except for isolates Azs-02 and Azs-03, which showed no growth at pH 5. The optimal pH for *Azospirillum* isolate growth was between 7 and 8, while none of the isolates could tolerate pH 4. These variations are primarily attributed to the nature of the strains and the nature of the soil conditions. (Baldani *et al*., 1990) [4]. Similar views were also expressed by Yogaraj *et al* (2021) [18] & Shankar (2014) [13].

Temperature tolerance test

All isolates exhibited excellent growth at 30 °C. However, they were able to grow within a temperature range of 20 °C to 40 °C. Isolates Azs-05 and Azs-State check showed poor growth even at 50 °C, while no growth was observed at 10 °C. The results are in agreement with findings of El-Akhdar *et al*. (2019) [7].

Salt tolerance test

All isolates exhibited good growth at the minimum concentration of 1% NaCl, but their growth gradually declined as the concentration increased. However, all isolates, including the state check, were able to grow at all three salt concentrations (1%, 2%, and 3%). The findings were confirmed by Yogaraj *et al*. (2021) [18] & Shankar (2014) [13].

Table 1: Characteristisation of efficient *Azospirillum* isolates

Isolate no.	pH					Temperature					Salt concentration		
	4	5	6	7	8	10 °C	20 °C	30 °C	40 °C	50 °C	1%	2%	3%
Azs-State check	-	+	+	+	+	-	+	+	+	+	+	+	+
Azs-01	-	+	+	+	+	-	+	+	+	-	+	+	+
Azs-02	-	-	+	+	+	-	+	+	+	-	+	+	+
Azs-03	-	-	+	+	+	-	+	+	+	-	+	+	+
Azs-04	-	+	+	+	+	-	+	+	+	-	+	+	+
Azs-05	-	+	+	+	+	-	+	+	+	+	+	+	+
Azs-09	-	+	+	+	+	-	+	+	+	-	+	+	+
Azs-12	-	+	+	+	+	-	+	+	+	-	+	+	+
Azs-13	-	+	+	+	+	-	+	+	+	-	+	+	+
Azs-17	-	+	+	+	+	-	+	+	+	-	+	+	+

Pot experiments

The findings from the pot experiment are presented in table 1.2.

Plant height

The maximum plant height (105.77 cm) was observed in the plants of RDF (100:60:40 kg NPK ha^{-1}) which was at par with plants raised from seed inoculated with Azs-13 (104.55 cm), Azs-State check (103.35 cm), Azs-02 (102.59 cm), Azs-01 (102.18 cm) & minimum plant height (82.54 cm) was recorded in plants of control pots.

No. of grains panicle-1

No. of grains panicle-1 were recorded maximum (157.43) with the application of RDF: 100:60:40 kg NPK ha^{-1} which was at par with the application of Azs-State check (154.00), Azs-13 (151.89), Azs-02 **(**151.38), Azs-01 **(**150.38) & least no. of grains panicle-1 (126.16) were observed on control pots.

Zaw Lwin Oo (2010) [19] also reported that inoculation with specific strains of *Azospirillum* sp. significantly increased the panicle length of rice, ranging from 3.95% to 7.01%. Additionally, the total number of seeds per panicle showed a significant difference compared to the control.

Yield (g/plant)

Grain and straw yields of rice grown under pot conditions significantly increased with *Azospirillum* inoculation compared to the control. The highest straw yield among the isolates, including the state check, was recorded in plants inoculated with isolate Azs-13 (12.40 g/plant), which was statistically at par with Azs-State check (12.33 g/plant), Azs-02 (12.12 g/plant), and Azs-01 (11.85 g/plant). However, the highest straw yield (12.42 g/plant) was observed under RDF: 100:60:40 kg NPK ha^{-1}, while the lowest (6.06 g/plant) was recorded in control pots. Among the isolates, including the state check, the highest grain yield was recorded in plants inoculated with isolate Azs-13 (9.33 g/plant), which was statistically at par with Azs-State check (9.26 g/plant), Azs-02 (9.17 g/plant), and Azs-01 (9.14 g/plant). However, the maximum grain yield (9.36 g/plant) was observed under RDF: 100:60:40 kg NPK ha^{-1}, while the lowest (4.67 g/plant) was obtained from control pots. Rodrigues *et al.,* (2008) [12] reported that inoculation of rice plants with *Azospirillum* has been found to cause significant increases in growth and yield which is equivalent to the application of 15-20 kg N/ha.

N-accumulation study

Bio-priming with the native *Azospirillum* isolate, including the state check, showed non-significant effects on the nitrogen content in both grain and straw.

N uptake by straw was obtained highest in isolate Azs-13 (53.80 mg/plant) which was at par with state check (52.39 mg/plant), Azs-01 (50.24 mg/plant), Azs-02 (48.28 mg/plant). However highest (54.16 mg/plant) N-uptake by straw was found in plants raised with RDF: 100:60:40 kg NPK ha^{-1} and minimum (21.28 mg/plant) N-uptake was found on plants of control pots.

While amongst isolates including state check, N uptake by grain was obtained highest in isolate Azs-13 (113.82 mg/plant) which was at par with state check (112.04 mg/plant), Azs-02 (110.95 mg/plant) & Azs-01 (108.76 mg/plant). However highest N-uptake (115.13 mg/plant) by grain was found in plants raised with RDF: 100:60:40 kg NPK ha^{-1} and minimum (52.77 mg/plant) N-uptake was found plants of control pots. Similar views were also expressed by Kumar and Shrivastav (1994) [10].

Table 2: Effect of bio-priming with native *Azospirillum* isolate on various growth parameters of rice

Treatments	Plant height (cm)	Grains panicle-1	Yield (g/plant)		N-content (%)		N-uptake (mg/plant)	
			Grain yield	Straw yield	Grain	Straw	Grain	Straw
T_1-Control	82.54	126.16	4.67	6.06	1.13	0.35	52.77	21.28
T_2-RDF : 100:60:40 kg NPK ha^{-1}	105.77	157.43	9.36	12.42	1.23	0.44	115.13	54.16
T_3-Azs-State check	103.35	154.00	9.26	12.33	1.21	0.42	112.04	52.39
T_4-Azs-01	102.18	150.38	9.14	11.85	1.19	0.42	108.76	50.24
T_5-Azs-02	102.59	151.38	9.17	12.12	1.21	0.40	110.95	48.28
T_6-Azs-03	91.50	139.90	8.05	10.47	1.14	0.37	91.77	39.11
T_7-Azs-04	94.31	142.15	8.17	10.66	1.19	0.37	97.22	39.23
T_8-Azs-05	91.90	137.41	8.05	10.56	1.15	0.37	92.57	39.45
T_9-Azs-09	89.46	139.72	7.92	10.45	1.14	0.35	90.28	36.40
T_{10}-Azs-12	94.35	136.24	8.07	10.47	1.18	0.36	95.22	38.04
T_{11}-Azs-13	104.55	151.89	9.33	12.40	1.22	0.43	113.82	53.80
T_{12}-Azs-17	94.77	141.13	8.12	10.69	1.15	0.37	93.38	39.92
Sem (±)	2.09	2.78	0.33	0.39	0.05	0.02	3.07	2.19
CD (5%) =	6.11	8.11	0.95	1.13	NS	NS	8.96	6.38

Note:-75% N & full dose of P & K fertilizer had been given as per recommended dose from treatment T_3 to T_{12}.

Conclusion

The study demonstrated that native *Azospirillum* isolates from Bilaspur district exhibit promising potential in enhancing rice crop growth and yield. The isolates were tolerant to a wide pH and temperature range, with optimal growth observed at pH 7-8 and temperatures between 20 °C and 40 °C. Salt tolerance tests showed growth at various NaCl concentrations, with all isolates showing some level of resilience. Pot experiments confirmed that *Azospirillum* inoculation significantly increased plant height, grain number, and yield compared to the control. The highest grain yield was achieved with isolate Azs-13, comparable to the recommended dose of fertilizers.

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