Isolation and Characterization of Naga king chilli rhizobacteria

Abstract

Amongst the many landraces of chilli that are cultivated in the North east region of India, the Naga king chilli (*Capsicum chinense* Jacq.) is one of the best known worldwide and is widely grown in the state of Nagaland.27 rhizospheric bacteria of Naga king chilli was isolated for their characterization and results suggested that the rhizosphere of Naga king chilli is dominated by smooth, round, either orange or milky white, smooth surface, convex with entire margin and translucent bacteria. The rhizosphere was also observed to be dominated by Gram negative bacteria (21 isolates) while, all the isolates were observed to give positive reaction catalase test and gelatine hydrolysis activity with 18 isolates being able to hydrolyse starch.

Keywords: Characterization, Naga King chilli, Rhizobacteria, Isolation

Introduction

The Northeast region of India is recognized as hot-spot for chilli diversity (Mathur *et al*., 2000) and amongst the many landraces of chilli that are cultivated in this region, the Naga King Chilli (*Capsicum chinense* Jacq.) is the best known worldwide. Naga king chilli is a self-pollinated, semi perennial plant if grown under favourable condition (Borgohain and Devi, 2007). Chilli fruits constitute large amounts of beneficial compounds including antioxidants, carbohydrates, minerals, phytochemicals, proteins, amino acids, and vitamins (Olatunji and Afolayan, 2018). The narrow zone of soil directly surrounding the root system is referred to as rhizosphere (Walker *et al.*, 2003). This zone is rich in nutrients when compared with the bulk soil due to the accumulation of a variety of plant exudates, such as amino acids and sugars, providing a rich source of energy and nutrients for bacterial growth (Gray and Smith, 2005). The rhizosphere is populated by a diverse range of microorganisms and the bacteria colonizing this habitat are called rhizobacteria (Schroth and Hancock, 1982). Studies have suggested that these rhizobacteria increases the absorption capacity of nutrients and protection

against plant pathogens thus increasing plant growth and production (Pérez-García *et al*., 2023). Previous studies showed that treatment of chilli seeds with rhizobacteria before sowing increased field germinability and seedling height (Yanti *et al*., 2020). The present investigation was carried out with an aim to understand the native rhizobacterial population of Naga king chilli and characterize it for their better understanding.

Materials and method

A field survey was undertaken for the collection of rhizobacteria from the farmer's field of Peren district, Nagaland. Where, the soil samples from the rhizospheric zone of healthy Naga king chilli plants at flowering stage were collected to be used for the isolation of rhizobacteria.

Isolation and maintenance of bacteria

Soil dilution plate technique described by Waksman, 1927 was followed for the isolation of rhizobacteria. For which, the soil adhering to the roots from the plant sample were collected to get 0.5g of the soil and then 4.5 ml of sterilized distilled water and mixed vigorously at 140 rpm for 2 minutes. Then the suspension was diluted serially to 10^{-8} and 0.1 ml of the last 3 serial dilution were spread with a glass spreader on nutrient agar and King's B medium (Peptone-20g; Glycerol-15ml; K_2HPO_4 -1.5g; $MgSO_4.7H_2O$-1.5g; Agar-agar-20g; Distilled water-1000ml) plates and incubated for 48h at 26±1°C. To obtain pure colonies, colonies with distinct morphological appearances were chosen from the countable plates and re-streaked on a fresh Petri plate (Patel *et al*., 2014).

Characterization of rhizobacteria

Morphology of bacterial colonies were characterized based on various traits such as size, shape, elevation, surface, optical properties, margin, and pigmentation on bacterial cultures previously grown for 48 h on nutrient agar medium and incubated at 28°C.

A series of biochemical tests were conducted using the criteria of Bergey's Manual of Systemic Bacteriology (1994).

Catalase test

To detect the production of catalase a loop full of 24-48 hours old test bacterium was smeared on the slide and then covered it with a few drops of 3% hydrogen peroxide. The smear was observed for bubble production. (Koche and Gade, 2013)

KOH test

A loopful of bacteria was mixed in a drop 3% aqueous KOH solutions for few seconds. The inoculating loop was raised a few centimetres from the microslide and the formation of a mucoid thread was recorded. Gram positive bacterium do not produce strands even on repeated strokes of the inoculating loop while gram negative bacterium do.

Starch hydrolysis

Starch hydrolysis was evaluated using nutrient agar amended with 0.2% starch. After incubating the test bacterium on the medium for 7 days, the agar plates were then flooded with Lugol's iodine and allowed to act for few minutes. The cultures showing a clear zone was considered to be positive reaction.

Gelatine liquefaction test

Stab method described by Koche and Gade. (2013) was followed for gelatine liquefaction test (Peptone-10g; Beef extract-5g; Gelatin-20g and Distilled water - 1000ml). Inoculation was done by stabbing a straight inoculating needle charged with 48 hours old growth of the test bacterium. The tubes were incubated at 20^{o}C and observation were recorded for liquefaction of gel column.

Result and discussions

Morphological characters of the rhizobacterial isolates are presented in Table 1. The isolates were observed to be predominantly smooth, round, either orange or milky white in colour, smooth surface, convex with entire margin and translucent. Result on biochemical characterization are shown in Table 2 and depicted in Fig 1. It was Observed that out of the 27 isolated rhizobacteria six were found to be Gram positive and 21 Gram negative, 19 isolates were recorded to utilize starch whereas, only six

isolates were found to be negative for KOH test. All the isolates were also observed to liquify gelatine and gave positive reaction for catalase test.

The findings of the present investigation were found to be in contrary to the findings of Banerjee *et al.* (2011) who reported that the bacterial population in chilli rhizosphere was dominated by gram positive bacteria with white, irregular, opaque colonies as the Naga king chilli rhizosphere was found to be dominated by Gram negative, orange coloured, opaque colonies. This difference in observations may be due to the fact that different ecological and environmental conditions favour the growth, development and establishment of different bacterial population and hence reflected in the population of the rhizobacteria. The results on catalase productions were found to be in conformity with the findings of Patel and Desai (2015) who observed that all rhizobacterial isolates were positive for catalase production and hence, are aerobic in nature.

Conclusion

From this study we can conclude that the rhizosphere of Naga king chilli is inhabited by various bacteria; predominantly by Gram negative, small, round, orange, smooth, convex, entire and opaque with mostly being able to hydrolyse starch and all the isolates being able to liquify gelatine.

Table 1: Morphological characterization of rhizobacteria

Isolates	Size	Shape	Colour	Surface	Elevation	Margin	Opacity
T_1	Small	Round	White	Smooth	Flat	Erose	Translucent
T_2	Small	Round	White	Smooth	Convex	Entire	Translucent
T_3	Small	Round	Creamy white	Smooth	Convex	Entire	Opaque
T_4	Small	Irregular	Light yellow	Smooth	Raised	Undulated	Translucent
T_5	Small	Round	Orange	Smooth	Convex	Entire	Opaque
T_6	Small	Round	Light orange	Smooth	Convex	Entire	Opaque
T_7	Small	Irregular	Orange	Corrugated	Raised	Undulated	Opaque

T_8	Small	Round	Reddish	Smooth	Convex	Entire	Opaque
T_9	Small	Round	Yellow	Smooth	Pulvinate	Entire	Opaque
T_{10}	Small	Round	Yellow	Smooth	Convex	Entire	Translucent
T_{11}	Small	Round	Pale yellow	Smooth	Convex	Entire	Opaque
T_{12}	Small	Round	Greenish yellow	Smooth	Convex	Entire	Opaque
T_{13}	Medium	Round	White	Smooth	Convex	Entire	Opaque
T_{14}	Small	round	Yellow	Smooth	Raised	Entire	Opaque
T_{15}	Small	Round	Milky white	Smooth	Convex	Entire	Translucent
T_{16}	Medium	Round	Orange	Smooth	Convex	Entire	Opaque
T_{17}	Medium	Round	Dull white	Smooth	Convex	Entire	Translucent
T_{18}	Small	Round	Orange	Corrugated	Raised	Undulated	Opaque
T_{19}	Small	Round	Light orange	Smooth	Flat	Entire	Translucent
T_{20}	Small	Round	Pale white	Smooth	Convex	Entire	Translucent
T_{21}	Small	Round	Orange	Smooth	Convex	Entire	Opaque
T_{22}	Small	Round	Yellow	Smooth	Flat	Entire	Opaque
T_{23}	Small	Round	Orange	Smooth	Convex	Entire	Opaque
T_{24}	Small	round	Pale white	Smooth	Flat	Entire	Translucent
T_{25}	Small	Round	Creamy white	Smooth	Convex	Entire	Opaque
T_{26}	Medium	Round	Dull white	Smooth	Convex	Entire	Opaque
T_{27}	Small	Round	Pale yellow	Smooth	Convex	Entire	Opaque

Table 2. Biochemical characterization of the rhizobacteria

Isolates	Gram staining	Starch Hydrolysis	KOH test	Gelatine liquification	Catalase test
T_1	-	-	+	+	+
T_2	-	-	+	+	+
T_3	-	+	+	+	+
T_4	-	+	+	+	+
T_5	+	-	-	+	+
T_6	-	+	+	+	+
T_7	-	+	+	+	+
T_8	-	+	+	+	+
T_9	-	+	+	+	+
T_{10}	-	+	+	+	+
T_{11}	-	-	+	+	+
T_{12}	-	-	+	+	+
T_{13}	-	+	+	+	+
T_{14}	-	+	+	+	+
T_{15}	+	+	-	+	+
T_{16}	-	-	+	+	+
T_{17}	+	+	-	+	+
T_{18}	-	+	+	+	+
T_{19}	-	-	+	+	+
T_{20}	+	+	-	+	+
T_{21}	-	-	+	+	+
T_{22}	+	+	-	+	+
T_{23}	-	+	+	+	+
T_{24}	-	+	+	+	+
T_{25}	+	+	-	+	+
T_{26}	-	+	+	+	+
T_{27}	-	+	+	+	+

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

References

Banerjee, S., Datta, M., Palit, R., Sengupta, C., Pandit, M. K. (2011). Plant growth promoting rhizobacteria enhance growth and yield of chilli (*Capsicum annuum* L.) under field conditions. *Australian Journal of Crop Science*. **5**(5):531-536.

Borgohain, R., Devi, J. (2007). The hottest chilli: A new horizon in agri-entrepreneurship. *Science Tech Entrepreneur.* http://www.assamagribusiness.nic.in.

Gray, E. J., Smith, D. L. (2005). Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signalling processes. *Soil Biology and Biochemistry*. **37**: 395–412.

Koche, M. D., Gade, R. M. (2013). Effect of sodium chloride, streptomycin sulphate and different agro-chemical on growth of *Pseudomonas fluorescence*. Journal of *Mycopathilogical Research*. **51**(2): 295-299.

Mathur, R., Dangi, R. S., Dass, S. C., Malhotra, R. C. (2000). The hottest chilli variety in India. *Current Science.* **79**: 287–288.

Olatunji, T. L., Afolayan, A. J. (2018). The suitability of chili pepper (*Capsicum annuum* L.) for alleviating human micronutrient dietary deficiencies: A review. *Food Science & Nutrition.* **6**:2239-2251.

Patel, P. V., Desai, P. B. (2015). Isolation of Rhizobacteria from Paddy Field and Their Traits for Plant Growth Promotion. *Research Journal of Recent Sciences.* **4**: 34-41.

Patel, M., Sheth, U., Hajela, P., Joshi, B., Animasaun, D. A. (2014). Isolation and diversity analysis of rhizobacteria from sugarcane and its biocontrol potential against *Rhizoctonia solani* a common plant pathogen. *International Journal of Current Microbiological Applied Science*. **3**(12): 69-76.

Pérez-García, L. A., Sáenz-Mata, J., Fortis-Hernández, M., Navarro-Muñoz, C. E., Palacio-Rodríguez, R., Preciado-Rangel, P. (2023). Plant-Growth-Promoting Rhizobacteria Improve Germination and Bioactive Compounds in Cucumber Seedlings. *Agronomy*. **13**(315):1-10.

Schroth, M. N., Hancock, J. G. (1982). Disease-suppressive soil and root-colonizing bacteria. *Science.* **216**:1376-81.

Walker, T. S, Bais, H. P., Grotewold, E., Vivanco, J. M. (2003). Root exudation and rhizosphere biology. *Plant Physiology*. **132**: 44–51.

Yanti, Y., Astuti, F. F., Nasution, C. R., Lubis, C. C., Nasution, A. S. (2016). Isolation and screening of indigenous rhizobacteria in West Sumatera to increase growth rate of

Chilli pepper seedling (*Capsicum annuum* L.). *Indian Phytopathology.* 69 (4):516-520

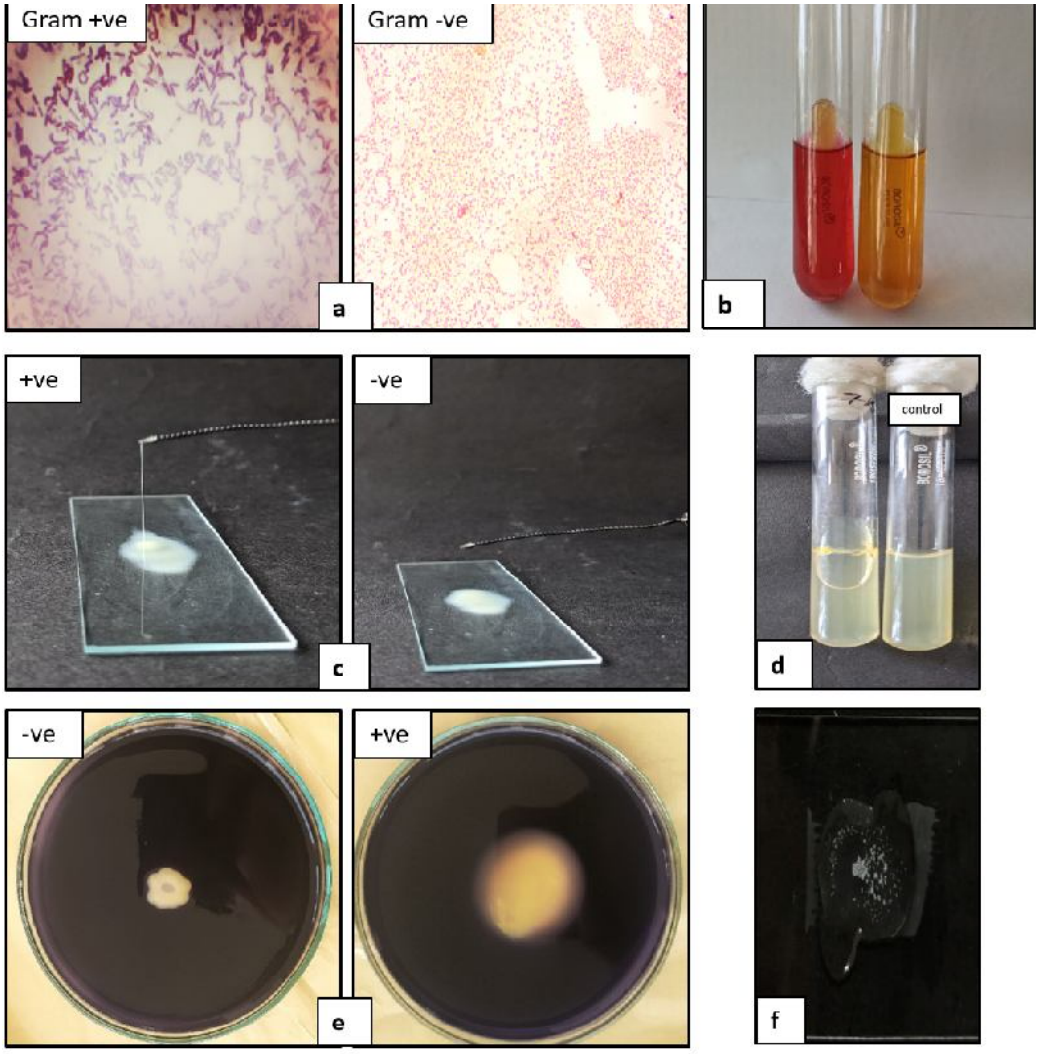

Fig 1. Biochemical test of the rhizobacteria (a. Gram reaction, b. Lactose utilization test, c. KOH test, d. Gelatine liquification test, e. Starch Hydrolysis test, f. Catalase test)