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Isolation and Screening of Zinc and Potassium Solubilizing Microorganisms from Different Rhizospheric Soil

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Abstract: ***Zinc is considered as a crucial trace micronutrient for proper growth, reproduction and driving of many metabolic reactions in all crops. Potassium is classified as a macronutrient associated with movement of water and nutrients as well as enzyme activation. The present study was aimed for isolation and screening of zinc and potassium solubilizing microorganisms from different rhizospheric soil of rice. Eight different isolates- ZSB1, ZSB2, ZSB3, ZSB4, KSB1, KSB2, KSB3 and KSB4 were obtained and were screened for their Zinc and Potassium solubilizing activities on TRIS minimal agar medium and Aleksandrov's medium, respectively. Upon characterization, ZSB1, ZSB4 and KSB3 were observed as motile Gram-negative organisms, ZSB2, KSB2 and KSB4 as motile Gram-positive organisms while ZSB3 and KSB1 had mycelial growth with some conidial spore. All isolates gave positive results for starch and lipid hydrolysis. In gelatin hydrolysis, only ZSB3, ZSB4, KSB1 and KSB2 gave positive results. The main purpose to obtain these zinc and potassium solubilizing microorganisms was for their use in plant growth promotion as biofertilizers to increase soil fertility and crop yield.***
Keywords: Zinc, Potassium, Zinc and Potassium Solubilizing activity, Biofertilizers.

I. INTRODUCTION

Zinc (Zn) is a trace element which is required in critical concentrations for crops and its inadequacy can lead to physiological stress causing dysfunctional in enzymatic functions and metabolic activities. It becomes available to the rhizosphere by the action of both mass flow as well as diffusion. Diffusion is a likely to be the foremost mechanism within the rhizosphere for the supply of zinc to plants as its concentration in soil solutions is generally too low [2]. Potassium (K) is considered to play an active role in growth, metabolism and plant development and is the most essential plant nutrient. Potassium solubilizing bacteria (KSB) are effective in releasing K from inorganic and insoluble pools of total soil K through solubilization [3].

Plant growth promoting rhizobacteria (PGPR) promote plant growth either by solubilizing and aiding in nutrient acquisition or by liberating phytohormones or biocontrol agents for protection against various plant pathogens [8]. Various PGPR have been found to be effective zinc and potassium solubilizers and colonize the rhizosphere for improving plant growth and development.

II. METHODOLOGY

A. Sample Collection

Rhizospheres of rice (Oryza sativa) from different region were collected from the depth of 9 cm from Rohina, Jujwa and Dungri regions of Valsad District, from South Gujarat, India. Soil samples were packed properly in polythene bags, labelled and further processed.

B. Analysis of Physiochemical Properties of Rhizospheric Soil Samples

Soil samples collected from rhizosphere of rice of different region were analysed for different physiochemical properties such as- colour, odour, temperature, pH, texture, as well as type of fertilizer used.

C. Isolation and Screening of Zinc and Potassium Solubilizing Microorganisms

Suspension of soil samples was made with distilled water and the soil particles were allowed to settle down, then supernatant was used for streaked plate method. Isolation was carried out on sterile TRIS minimal medium with 0.1% Zn oxide for Zinc Solubilizing Microorganisms, plates were incubated at 28°C in incubator [15]. For Potassium Solubilizing Microorganisms, isolation was carried out on sterile Tryptic Soya Agar Medium [12], plates were incubated at 28°C in incubator. The growth was observed after 2-3 days [12,15]. The screening was done by spot assay method and zone formation was observed surrounding Zinc and Potassium solubilizing microorganisms.

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D. Screening of Collected Isolates for their Zinc Solubilizing Activity

The isolates were placed on sterile TRIS minimal medium supplemented with 0.1% Zn oxide [6]. The test organisms were placed on these media and were incubated at 28°C for 3-4 days. The diameters of the colony and clearing zones around the colonies were measured with the help of zone meter [15].

E. Screening of Collected Isolates for their Potassium Solubilizing Activity

Potassium solubilizing isolates were placed on sterile Aleksandrov's medium. The test organism on the medium was incubated at 28°C for 3-4 days. Zone was observed around the colonies and the diameter of the colony and clearing zones were measured by using zone meter [15].

F. Characterization of Isolates

All the isolates were studied for their different morphologic, biochemical and enzymatic characteristics.

G. Morphological Characterization

Morphological characterization was done by performing Gram`s staining and Motility [4].

H. Biochemical Characterization

1) *Indole production Test:* In 1% sterile peptone broth, bacterial isolates were inoculated and incubated for 24 hours. After incubation, Kovac`s reagent was added in the broth tubes and pink ring formation in the upper layer was observed.
2) *Methyl red (M-R) Test:* In sterile Glucose phosphate broth, bacterial isolates were inoculated and incubated for 24 hours. After incubation, methyl red indicator was added and red colour formation in the broth was observed.
3) *Voges Proskauer (V-P) Test:* In sterile Glucose phosphate broth, bacterial isolates were inoculated and incubated for 24 hours. After incubation, few drops of reagent A (α-naphthol solution) and reagent B (40% KOH) were added in same order and tube was kept in slanting position to increase the aeration. Development of red color was observed within 15 min.
4) *Citrate Utilization Test:* Isolates were streaked on sterile Simmon's citrate agar slant and incubated at 37°C for 24 hours. Change in colour was observed after incubation.
5) *Urea Hydrolysis Test:* Isolates were inoculated in sterile Stuart's urea broth and incubated at 37°C for 24 hours. The change in color was observed in broth after incubation.
6) *Ammonia Production:* In 4% sterile peptone broth, bacterial isolates were inoculated and incubated for 24 hours. Nessler`s reagent was added in broth and formation of yellow to brown precipitation was observed.
7) *Nitrate Reduction Test:* Isolates were inoculated in sterile peptone nitrate broth (PNB) and incubated at 37°C for 24 hours. After incubation, reagent A (α-naphthylamine reagent) and reagent B (sulphanilic acid reagent) were added in this order and development of red color within 30 secs was observed after adding test reagent.

I. Tests for Enzymatic Activities

Various enzymatic properties of the obtained ZSMs and KSMS were studied including starch hydrolysis, gelatine hydrolysis, and lipid hydrolysis test.

1) *Starch Hydrolysis Test:* The isolates were placed on sterile Starch nutrient agar plates and the plates were incubated at 25-28°C 24 hours. After incubation, starch agar plates were flooded with iodine solution [9].
2) *Gelatine Hydrolysis Test:* Isolates were placed on sterile Nutrient gelatine agar plate and then incubated at 25-28°C for 24 hours. After incubation, gelatine agar plates were flooded with Frazier`s reagent [9].
3) *Lipid Hydrolysis Test:* Isolates were placed on sterile Tributyrene agar plate and then incubated at 25- 28°C for 24 hours [13].

III. RESULTS AND DISCUSSION

A. Sample Collection

Rhizospheric soil of *Oryza sativa,* was collected from the depth of 9 cm from different regions of Valsad.

B. Analysis of Physiochemical Properties of Rhizospheric Soil Samples

Soil samples were collected from rhizosphere of rice from different regions and were analyzed for different physiochemical properties. Colour of the samples was brown with earthy smell with clayey texture. Temperature and pH of the samples was 27° C and 7, respectively while organic type of fertilizer was used.

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Fig. 1: Soil sample region and its pH

The current study was carried out at Department of Microbiology, Dolat Usha Institute of Applied Sciences, Valsad, Gujarat, India. In the present study, different types of rhizospheric organisms were isolated. Zinc solubilizing microorganisms were isolated on TRIS minimal agar medium and Potassium solubilizing microorganisms were isolated on Tryptic Soya agar medium (Fig. 2 & Fig. 3).



Fig. 2: Zinc and Potassium Solubilizing microorganisms

C. Screening of Zinc and Potassium Solubilizing Microorganism

In the present study, all isolates were screened and tested their solubilizing activity. Four isolates obtained that has ability to solubilize Zinc were coded as ZSB1, ZSB2, ZSB3 and ZSB4 and the other four isolates obtained that has ability to solubilize Potassium were coded as KSB1, KSB2, KSB3 and KSB4. ZSM`s gave clear zone around the colonies on TRIS minimal agar medium while KSM`s gave yellow colour zone on Aleksandrov`s agar medium as observed in Fig. 3 and Fig. 4, respectively.

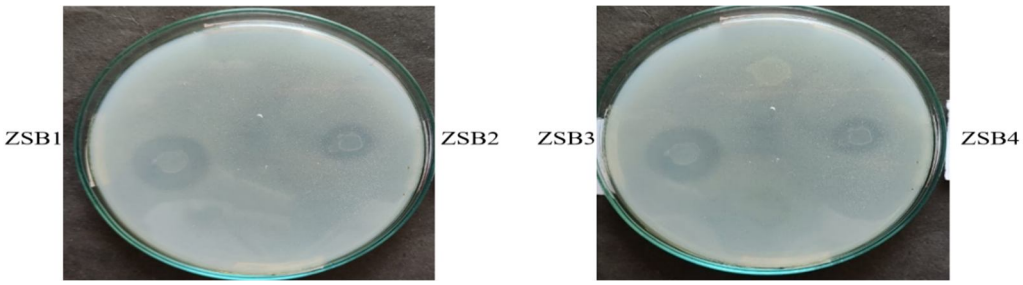


Fig. 3: Screening of ZSM on TRIS minimal agar medium

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Figure 4: Screening of KSM on Aleksandrov`s agar medium

Similar study was done by Saravanan *et al.,* in 2003 where they found *Bacillus* and *Pseudomonas* sp. has ability to solubilize Zinc. Fatharani and Rahayu in 2018 isolated seven bacteria which were able to solubilize Potassium.

TABLE I
Zinc and Potassium solubilizers

Isolates	Zinc solubilizers	Potassium solubilizers
ZSB1	+	+
ZSB2	+	-
ZSB3	+	+
ZSB4	+	-
KSB1	+	+
KSB2	-	+
KSB3	+	+
KSB4	-	+

In the current study, all obtained isolates were studied for their colonial characteristic on TRIS Minimal medium and Tryptic Soya agar medium for Zinc Solubilizing Microorganisms and Potassium Solubilizing Microorganisms and were reported with different size, shape, opacity, surface and many with each other (TABLE II & TABLE III).

TABLE II
Characterization of ZSMs

Isolates	Colony Characteristics on TRIS Minimal Medium (ZSM)
ZSB1	Large, irregular, erose, flat, rough, opaque, bluish green pigmentation.
ZSB2	Large, round, entire, convex, smooth, opaque, greyish white colony.
ZSB3	Large, myceloid, filamentous, raised, opaque, brownish pigmentation.
ZSB4	Pinpoint, round, entire, convex, smooth, opaque, red pigmentation.

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TABLE III
Characteristics of KSM`s

Isolates	Colony Characteristics on Tryptic Soya agar (KSM)
KSB1	Large, myceloid, filamentous, raised, opaque, brownish pigmentation.
KSB2	Large, round, smooth, entire, convex, opaque, no pigmentation.
KSB3	Large, irregular, erose, flat, rough, opaque, bluish green pigmentation.
KSB4	Large, round, undulate, convex, smooth, opaque, no pigmentation.

D. Results of Morphological and Biochemical characterization

In current study, all isolates were morphologically characterized by performing Gram staining, Motility and Fungal mounting. By performing the test, it was found that from ZSM`s, two isolates were Gram negative (ZSB1 and ZSB4), one was Gram positive (ZSB2) and one was fungi (ZSB3) and from KSM`s, one isolate was Gram negative (KSB3), two Gram positive isolates (KSB2 and KSB4) and one fungus was found (KSB1). All bacterial isolates were found motile. The ZSB3 and KSB1 showed fungal growth, which was confirmed by their fungal mounting.

TABLE IV
Results of Biochemical characterization

Tests	Isolates							
	ZSB1	ZSB2	ZSB3	ZSB4	KSB1	KSB2	KSB3	KSB4
Indole test	-	-	-	-	-	-	-	-
M-R test	-	+	-	+	-	-	-	-
V-P test	-	-	-	-	-	-	-	-
Citrate utilization	-	-	-	-	-	-	-	-
Urea hydrolysis	+	+	+	+	+	+	+	+
Nitrate reduction	-	+	+	+	-	-	+	+
Ammonia production	+	+	+	+	+	+	+	+

In present study, all isolates gave positive results for Urea hydrolysis and ammonia production and negative for Indole, Voges Proskauer and Citrate utilization test. In methyl red test, ZSB2 and ZSB4 gave positive results and rest all isolates gave negative results. In nitrate reduction test, ZSB2, ZSB3, ZSB4, KSB3 and KSB4 gave positive results and others gave negative results (TABLE IV).

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TABLE V
Results of Enzymatic activities

Isolates	Test		
	Starch hydrolysis	**Gelatin hydrolysis**	**Lipid hydrolysis**
ZSB1	+	-	+
ZSB2	+	-	+
ZSB3	+	+	+
ZSB4	+	+	+
KSB1	+	+	+
KSB2	+	+	+
KSB3	+	-	+
KSB4	+	-	+

In our current study as indicated in TABLE Vand Fig. 5, Fig. 6, Fig. 8, and Fig. 9, it was found that all isolates were able to hydrolyze Starch and Lipid. While only four isolates were able to hydrolyze Gelatin (Fig. 7) (ZSB3, ZSB4, KSB1 and KSB4).

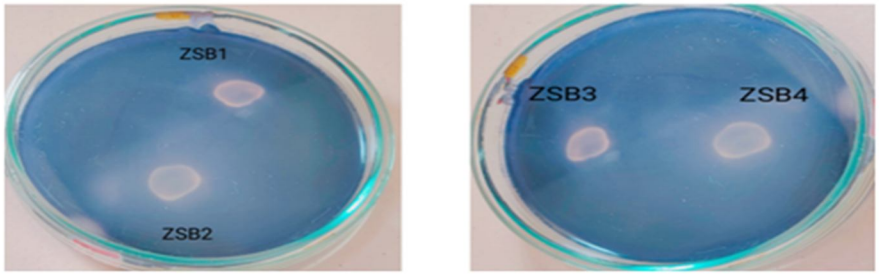


Fig. 5: Starch hydrolysis test results obtained from ZSM



Fig. 6: Starch hydrolysis results obtained from KSM

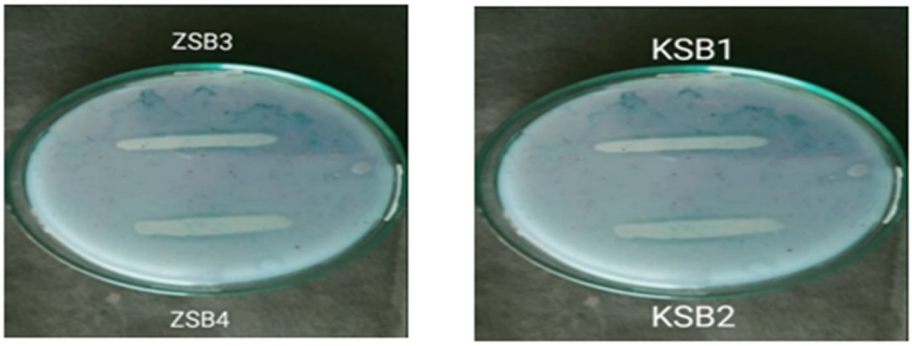


Fig. 7: Gelatin hydrolysis test results obtained from ZSM and KSM

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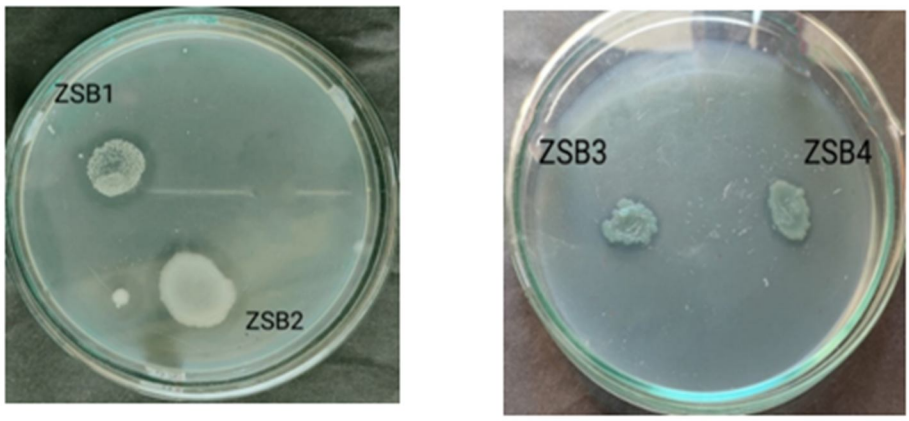


Fig. 8: Lipid hydrolysis test results obtained from ZSM

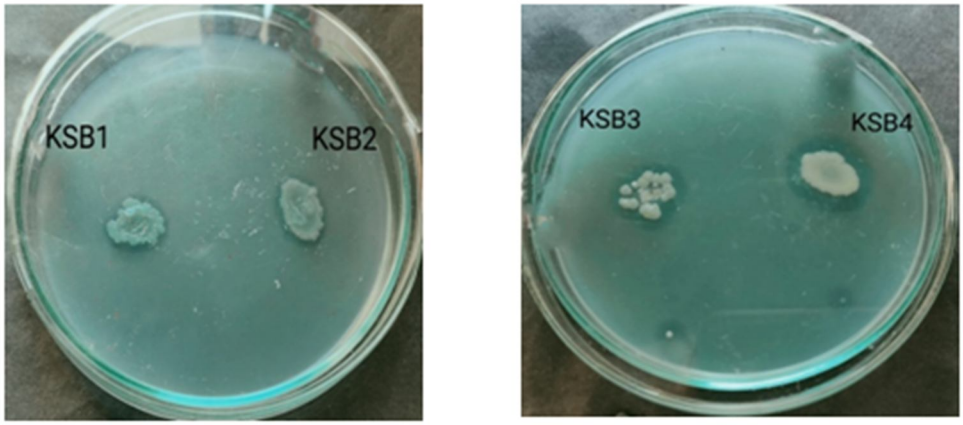


Fig. 9: Lipid hydrolysis test results obtained from KSM

In 2012, Akhter *et al.*, reported in their studies that they obtained 15 isolates which were able to produce amylase enzyme. Same as this in our study also all isolates were found that had ability to hydrolyze starch. In 2015, similar study was also done by Shree *et al.*, which showed that their all four isolates were able to hydrolyze gelatin. In our study, out of eight isolates four isolates were able to hydrolyze gelatin (ZSB3, ZSB4, KSB1 and KSB4).

IV. CONCLUSIONS

In the current study, zinc and potassium solubilizing microorganisms were isolated from rhizospheric soil of rice (*Oryza sativa*). Four isolates were found which have capability of solubilizing zinc and four isolates were found that can be solubilize potassium. Among these eight isolates, four isolates were able to solubilized both zinc as well as potassium. 6 isolates were identified as bacteria and two were fungi. Screening was done in which zinc solubilizing microorganisms gave clear zone around colonies and potassium solubilizing microorganisms gave yellow color zones. The isolates were characterized morphologically and biochemically and their enzyme activity also checked. The isolates coded as ZSB3, ZSB4, KSB1 and KSB2 were able to hydrolysed starch, gelatine and lipid. The main purpose to obtain these zinc and potassium solubilizing microorganisms was for their use in plant growth promotion as biofertilizers to increase soil fertility and crop yield.

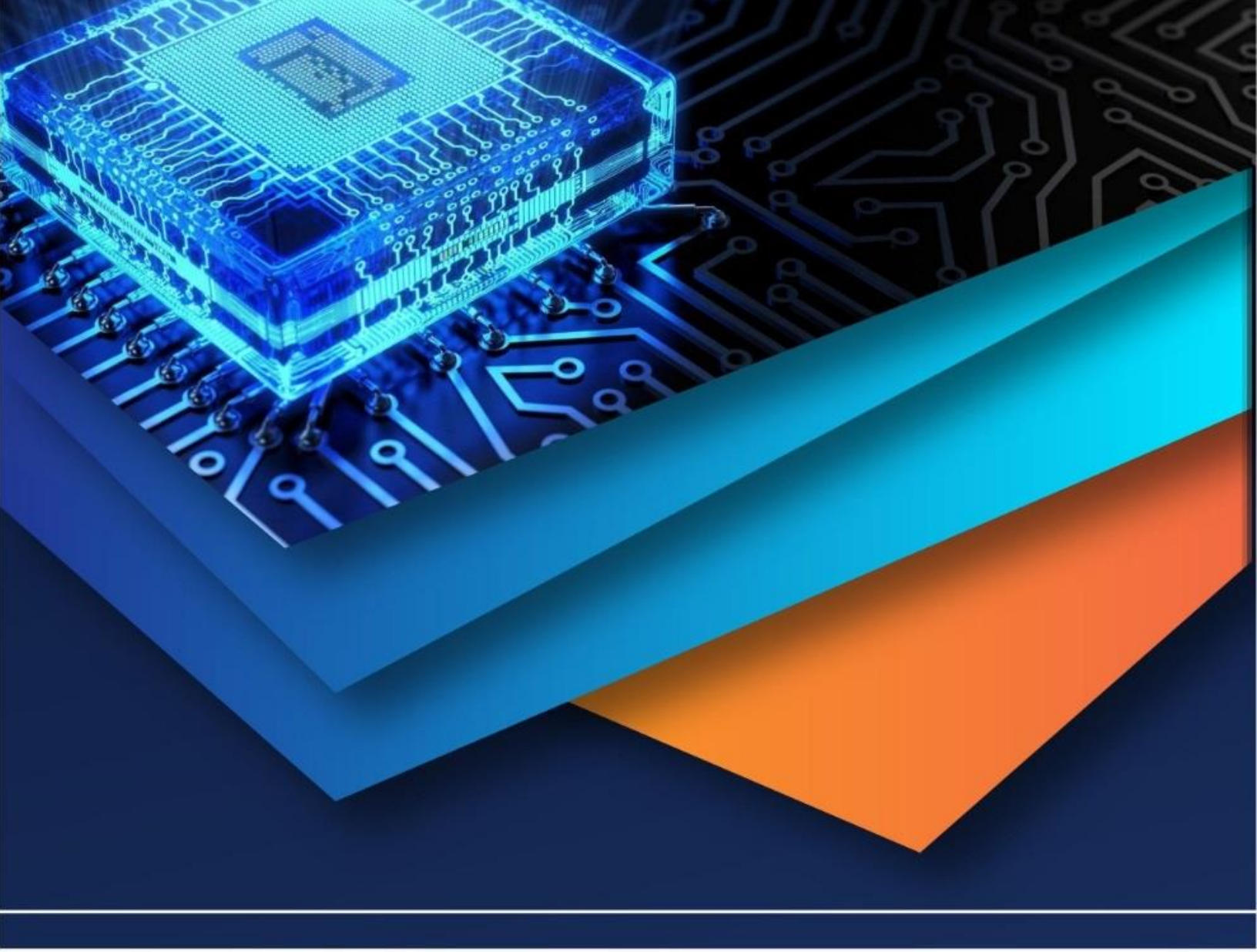
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