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Isolation, Characterization and Activity of Amidase Producing Paenibacillus Polymexa from Semi Arid Soils

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Abstract: ***Soil samples from different locations were collected and the amidase producing organisms were isolated by enrichment culture method in soil extract medium using acetamide as sole source of C and N and incubating at 37°C. A total of 29 bacterial isolates were obtained. Eight strains showed amidase production. The highest amidase producing strain (HSS22) was revealed as Paenibacillus polymexa. Acetamide containing mineral salt media when supplemented with Sodium citrate for P. polymexa resulted in higher growth of bacteria but addition of NaCl did not show remarkable effect on bacterial growth, this indicated that amides could be efficiently utilized as N source. Isolate P. polymexa was found to show highest amidase activity (19.34 U/ml) among the nine amidase producing isolates. The pH tolerance, temperature and amidase production capabilities of P. Polymexa were compared. It was found that P. polymexawas able to grow at temperature of 37°C, the pH 8.0, whereas another six isolates showed amidase activity in the pH range of (6.0-9.0) and temperature (30°C) thus P. polymexawas selected for subsequent studies. The results for enzyme activity under the performance of different physiochemical conditions and purification as well as molecular weights are recorded and discussed.***

Keywords: Amidase, Soil Samples, Bacteria, Physiochemical condition, Purification.

I. INTRODUCTION

An amidase (EC 3.5.1.4), acylamidases, acylase (misleading), amidohydrolase (ambiguous), deaminase (ambiguous), fatty acylamidases, N-acetylaminohydrolase (ambiguous) are enzymes that catalyze the hydrolysis of an amide.

Amidase (Richards &Rolinson, 1961) act on the peptide linkage of the prosthetic group. Amidase belongs to the

Family of hydrolyses, those acting on carbon-nitrogen bonds other than peptide bonds, specifically in linear amides. These enzymes are involved in nitrogen metabolism in cells and are widely distributed in nature. They have been found in prokaryotic and eukaryotic cells. (Kotlova *et al.*, 1998) This enzyme utilizes two substrates, which are monocarboxylic acid amide and H_2O and result in the formation of monocarboxylate and NH_3.

In enzymology, an amidase (EC 3.5.1.4), acylamidase, acylase (misleading), amidohydrolase (ambiguous), deaminase (ambiguous), fatty acylamidase, N-acetylaminohydrolase (ambiguous) are enzyme that catalyze the hydrolysis of an amide.

A. Potential Applications Of Amidase

1) Food industry
2) Waste treatment
3) Production of acrylic acid
4) Biosynthesis of hydroxamic acids
5) Production of nicotinic acid
6) Peptide synthesis
7) Production of amino acids and their derivatives

II. MATERIALS AND METHOD

A. Collection Of Soil Sample

The soil samples for the isolation of amidase producing bacteria were collected from different fields viz., cultivated soil – Farm of Unjha district, Non- Cultivated soil – garden of Gandhinagar district, Arid soil – Santalpur and High saline soil – white desert, Kutch district. The pH of all sites was between 6.0 and 7.5. Samples were brought to the laboratory and were kept at 4°C in refrigerator till further processing.

B. Physico-Chemical Test Of Soil

1) Enrichment And Isolation
2) General media –nutrient broth/agar
3) Selective media- soil extract broth/agar

C. Characterization Of Isolates

1) Cultural Characters: - since isolate hss-22 exited highest amidase activity among all the isolates, therefore, this bacterium was selected for further characterization.

2) Colony Morphology: - bacterial strains were identified by gram's staining and various morphological characteristics like margine, pigment etc. all the results were observed and recorded according to the methods given by (goss, 1972) and (case and johanson, 1984).

3) Biochemical Characters:- various biochemical tests like tsi test, urea hydrolysis test, indole test, emb test, m.r. test, v.p. test, catalase test, starch utilization test, citrate test, h_2s test, gelatine liquidification test, lipid hydrolysis test, nitrate reduction test, dehydrogenase test, carbohydrate fermentation, were performed to identify the bacterial isolate hss-22.

4) 16s Rdna Sequence Determination:-the isolates were identified by morphological and biochemical tests and 16s rrna sequence analysis. 16s rrna gene sequencing of the isolated microorganisms were carried out at chromous biotech pvt. ltd., bangalore, india

D. Screening of Enzyme

1) Qualitative Assay: A qualitative estimation of amidase producing organism was done using a modified method. (Rahim *et al.*, 2003)

2) Quantitative assay:

a) Effect Of Different Growth Factors On Amidase Enzyme:-

i) EFFECT OF TEMPERATURE
ii) EFFECT OF PH
iii) EFFECT OF CARBON SOURCE
iv) EFFECT OF NITROGEN SOURCES
v) EFFECT OF INCUBATION TIME

b) Determination Of Pgp Characters:-

i) INDOLE ACETIC ACID ANALYSIS
ii) PHOSPHATE SOLUBILISATION
iii) PRODUCTION OF AMMONIA:-
iv) SIDEROPHORE PRODUCTION:
v) HYDROGEN CYANIDE PRODUCTION

c) *Purification Of Amidase:* Purification of amidase enzyme performed by SDS-PAGE.

III. RESULTS AND DISCUSSION

Table:- 1 - Physico-chemical analysis of soil samples

Chemical analysis							
Soil sample	pH	EC	C (%)	P (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)
Arid soil	6.8	0.5	0.12	0.10	70	4.5	4.4
Non- Cultivated soil	7.9	0.3	0.18	0.02	110	5.4	4.6
Cultivated soil	6.9	0.4	0.16	0.03	68	4.6	2.4
High saline soil	7.3	0.6	0.25	0.04	78	6.0	1.2

Physical analysis						
	Soil texture (%)					WHC (%)
Soil sample	Gravel	Coarse sand	Fine sand	Silt	Clay	
Arid soil	1.0	1.0	1.10	3.30	93.59	28.35

Non- Cultivated soil	0.09	0.06	0.65	7.54	91.66	24.54
Cultivated soil	0.74	1.08	9.46	58.0	30.68	31.68
High saline soil	22.18	4.77	7.27	35.25	28.53	26.71

A. Screening

1) A qualitative analysis of amidase producing organism was done using a modified method by Rahim *et. al.*, 2003.
2) The pH of the medium was adjusted to 6.6 ± 0.2.
3) The broth and plates were regularly examined for colour change from yellow to pink.
4) Among twenty nine isolates, eight isolates were give positive result in qualitative screening of Amidase.

B. Qualitative Screening

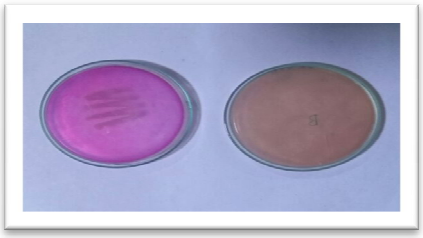


Fig: 1 Qualitative Screening of HSS22

Table:- 2 – Quantitative assay

Source of Soil	Bacterial isolates	Enzyme activity (U/ml)	Source of Soil	Bacterial isolates	Enzyme activity (U/ml)	Bacterial isolates	Enzyme activity (U/ml)	Bacterial isolates	Enzyme activity (U/ml)	Bacterial isolates	Enzyme activity (U/ml)
Arid Soil	HSS 1	---	Non Cultivated Soil	HSS 8	11.64	HSS 11	---	HSS 14	11.04	HSS 14	---
	HSS 2	12.21		HSS 9	---	HSS 12	---	HSS 15	---	HSS 15	---
	HSS 3	13.08		HSS 10	---	HSS 13	---	HSS 16	---	HSS 16	---
Saline Soil	HSS 4	---	Cultivated Soil	HSS 17	---	HSS 21	---	HSS 25	---	HSS 29	---
	HSS 5	---		HSS 18	16.92	HSS 22	19.32	HSS 26	---		
	HSS 6	---		HSS 19	---	HSS 23	---	HSS 27	---		
	HSS 7	---		HSS 20	12.6	HSS 24	12.9	HSS 28	---		

C. Characterization & Identification:

Table:- 3 – Morphological Characteristics

Organism	HSS 22
Size	Small
Shape	Irregular
Margin	Entire
Elevation	Mucoid
Texture	Smooth
Consistency	dry
Opacity	Translucent
Pigment	White shiny
Gram reaction	Gram positive

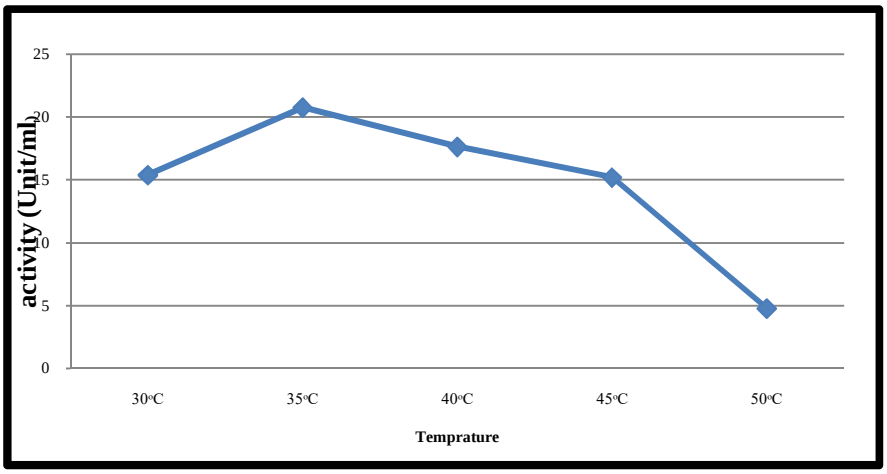
Table:- 4 - Biochemical Characterization

No.	Test		HSS 22
1	TSI Test		-
2	Urea Hydrolysis Test		-
3	Indole Test		-
4	EMB Test		Blue colour
5	M.R. Test		+
6	V.P. Test		-
7	Catalase Test		+
8	Starch Utilization Test		-
9	Citrate Test		+
10	H_2S Test		-
11	Gelatine liquidification Test		+
12	Lipid hydrolysis Test		+
13	Nitrate Reduction Test		-
14	Dehydrogenase Test		+
15	Carbohydrate Fermentation	Glucose	-
16		Lactose	-
17		Xylose	-
18		Mannose	-
19		Maltose	-

Table:- 5 - Identification of isolate by 16S rRNA:

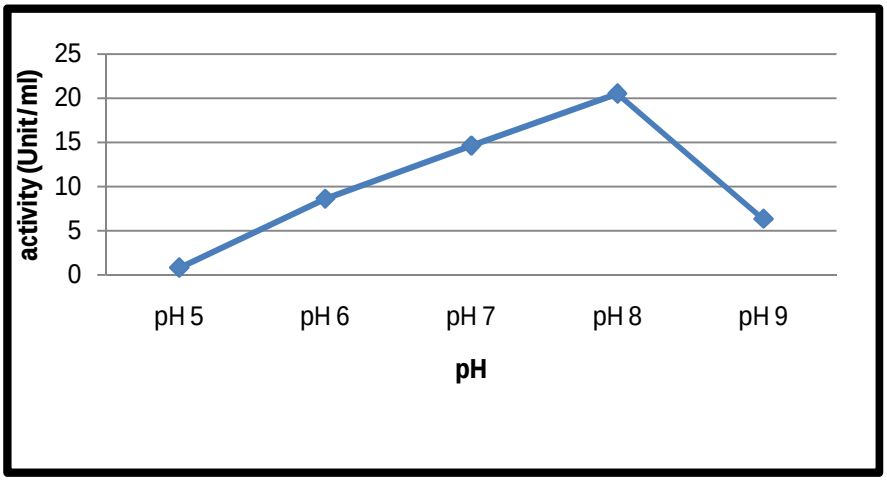
Sample Id	Sequence	Organism	Similarity	Accession No.
HSS 22	TTTGCCACTCCTGGAT…….....CTATTGCCCTTGTAGGACTG	*Paenibacillus polymexa*	100%	LN774600.1

D. Physio-chemical Characteristics



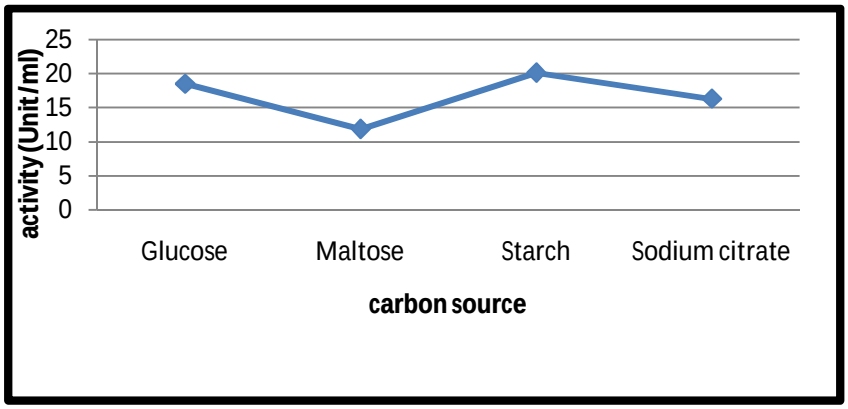
The results are shown in fig.2 P. polymexa showed highest activity at 35°C beyond these temperatures isolate recorded decrease in activity. Isolate recorded highest unit activity of 20.76 U/ml 35 °C

E. Effect of $_p$H



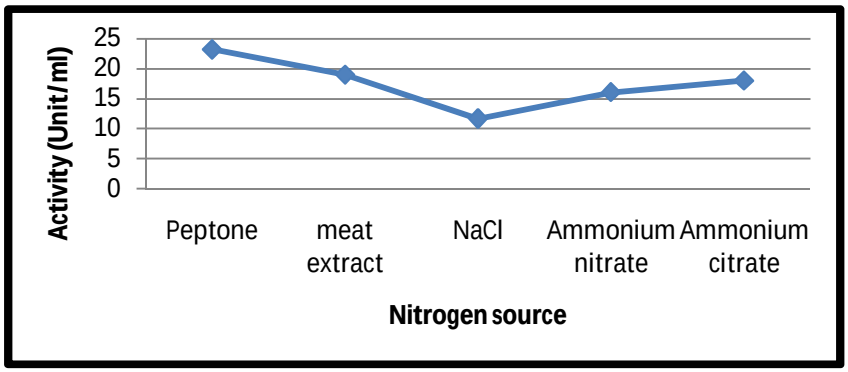
The results are shown in fig. 3. The highest activity of amidase from *P. polymexa* was at pH 8 and the activity was 20.52U/ml.

F. Effect Of Carbon Source



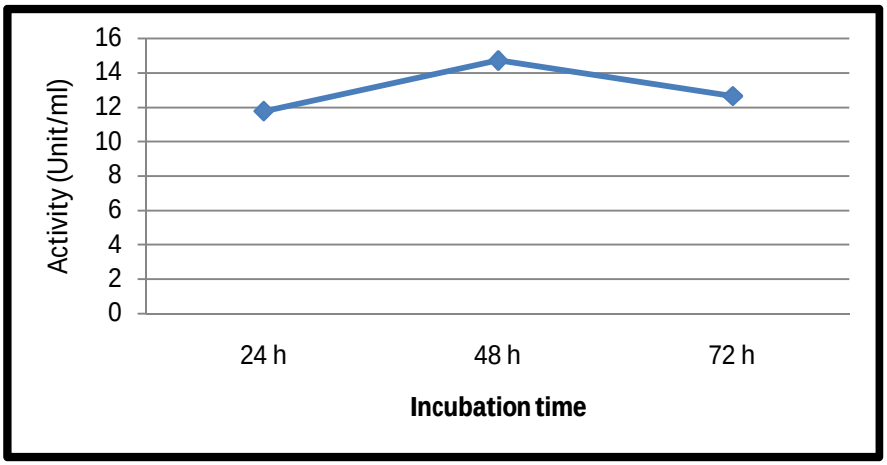
The results are shown in fig. 4. It is clear from that starch is better carbon source for *P. polymexa and the activity was 20.16.*

G. Effect of nitrogen source



The results are shown in fig. 5. The activity of amidase with different nitrogen source used for growth of organisms. The highest activity of amidase by *P. polymexa* showed high enzyme activity in peptone was used as nitrogen source and the activity was 23.24 U/ml.

H. Effect Of Incubation Time



The results are shown in fig. 6. The optimize activity of amidase on different incubation time. The highest activity of amidase producing organism P. polymexa was at 48h and the activity was 14.72 U/ml

I. Purification of enzyme

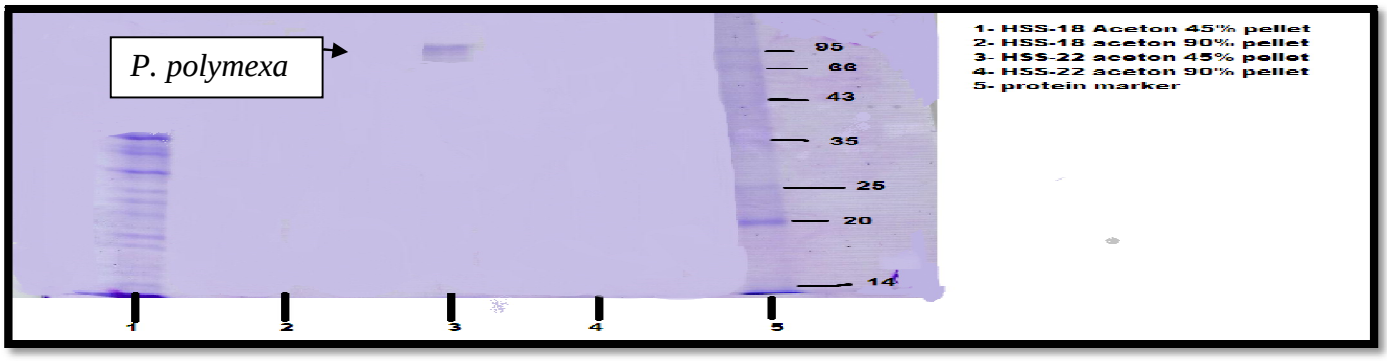


Fig. 7 result of purification

Amidase enzyme from *Paenibacillus spp.* was purified by SDS PAGE method.
The enzyme was corresponding with std. (range 10 to 110 kDa)
The molecular weight of amidase from *Paenibacillus* polymexa was found 95 kDa.

IV. CONCLUSION

From ths study was focused on amidase enzyme producing bacteria and its activity. The objectives set forth for the study were achieved and out some of the work is summarized below:

A. In this work total 29 bacterial strains were isolated from various types of soil collected from different locations of North Gujarat region. On the basis of qualitative as well as quantitative screening, one bacterial isolated was selected as potent amidase producer.

B. HSS 22 showed maximum enzyme activity 19.32 U/ml compared to other isolates.

C. HSS 22 was identified as *Paenibacillus polymexa* on the basis of morphological characteristics, biochemical tests and 16 S rRNA sequencing.

D. Effect of various physico chemical parameters was checked on the amidase activity of*P. polymexa* which showed maximum amidase activity at temperature 35^{o}C, pH - 8, starch as a sole source of carbon, peptone as a nitrogen source and 48 hrs incubation time.

E. The enzyme from *P. polymexa* by SDS PAGE method and the molecular weight were recorded as 95 kDa for *P. polymexa.*

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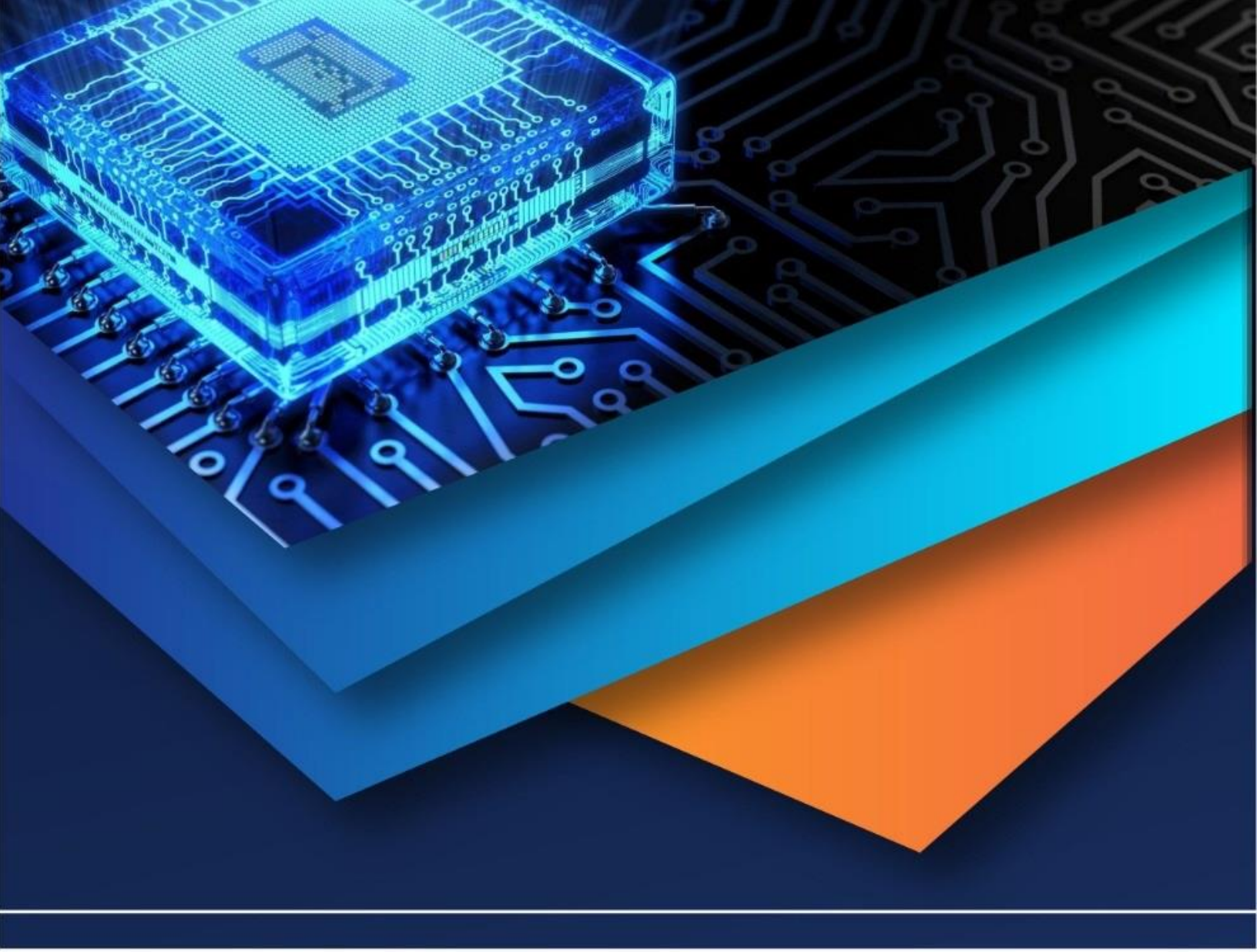

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