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Isolation and identification of ampicillin degrading bacterial strain from soil sample

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Abstract-The pharmaceutical compounds cause problems mainly in the terrestrial and aquatic environment. It is due to the accumulation of these compounds. The pharmaceutical compounds are converting into nontoxic compounds after biodegradation by microorganisms. Ampicillin is a semisynthetic antibiotic and belongs to β-lactam group of antibiotic. The ampicillin was degraded by bacterial isolates AMP6 and AMP8. The initial concentration was 50ppm of ampicillin for isolation of bacterial isolates. The isolates were identified as Ochrobacterum intermedium and Bacillus oceanisediminis respectively by using 16s RNA sequencing. The concentration ranging from 50 ppm to 500ppm of ampicillin was considered for its biodegradation. After 6 days of incubation at 400ppm the Ochrobacterum intermedium degraded 12.97±0.11% and the degradation by Bacillus oceanisediminis was noticed as 11.98±0.15 %.

Key words- Ampicillin, Ochrobacterum intermedium, Bacillus oceanisediminis, Biodegradation.

I. INTRODUCTION

Ampicillin is a semi-synthetic antibiotic belonging to the β-lactam group, which consist of β-lactam ring, It shows broad spectrum antibacterial activity against Gram positive bacterial and some Gram negative bacterial groups. The IUPAC name of ampicillin is (2*S*,5*R*,6*R*)-6-([(2*R*)-2-amino-2 phenylacetyl] amino)-3,3-dimethyl-7-oxo-4 thia-1 azabicyclo [3.2.0] heptane-2- carboxylic acid.

After metabolism of ampicillin antibiotic compounds, in the different systems of animal and plant body, enter in the soil and aquatic ecosystem. The unused ampicillin also enters in different ecosystems. These, after accumulation in the soil as well as in aquatic environment, cause great threat to the environmental health. Biodegradation is performed by the natural microbial communities in which the complex organic molecules are fragmented. Maki et al.,(2006) noticed that the ampicillin, doxycillin, oxytetracycline, thiamphenicol, and josamycin antibiotics were microbial degraded in the marine sediments. Emolla et al., (2009) measured the biodegradation of ampicillin, amoxicillin and cloxacillin in the municipal waste water treatment plant. Zhang et al., (2010) studied the degradation of 11 antibiotics (ampicillin, cephalexin, sulfamethoxazole, sulfadiazine, norfloxacin, ciprofloxacin, ofloxacin, tetracycline, roxithromycin, anhydro-erythromycin and trimethoprim) in fresh water and saline sewage sludge. Al-Gheethi et al., (2014) isolated Bacillus subtilis 1556WTNC from treated sewage effluents for biodegradation of B-lactams antibiotics the penicillin (amoxicillin and ampicillin) in treated sewage effluents. Otto et al., (2015) performed the experiments for the bio-degradation of B-lactam antibiotic with the help of Staphylococcus aureus in waste water effluents by advanced oxidation process. The present has been focused on the biodegradation of ampicillin was studied up to 21 days. 11 isolates were observed but only two isolates, the *Ochrobacterum intermedium, Bacillus* oceanisediminis, were capable of degrading at higher rate.

II. MATERIAL AND METHOD

A. Sample collection

Samples collections were categorized in following methods.

B. Culture enrichment

Antibiotic was buried in soil at 10cm deep for approximately two months and required humidity for better growth was maintained in this duration. After this the soil samples were collected for microbial culture of the degrading microbes and this culture was used as enrichment of the inoculum for further studies.

C. Antibiotics samples

Ampicillin (500mg) manufactured by Cadila pharmaceuticals Ltd. Dholka, Ahmedabad, India

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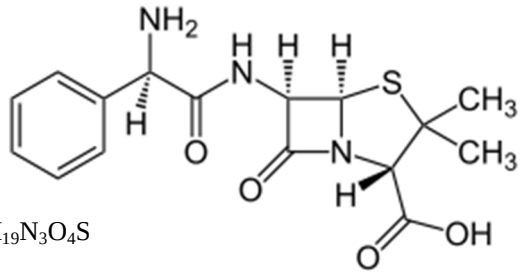
Structure of Ampicillin



Chemical fomula: $C_{16}H_{19}N_3O_4S$

Molecular weight: 349.41g/mol

IUPAC name: (2*S*,5*R*,6*R*)-6-([(2*R*)-2-amino-2 phenylacetyl] amino)-3,3-dimethyl- 7-oxo-4 thia-1 azabicyclo [3.2.0] heptane-2-carboxylic acid.

D. Isolation of antibiotic degrading microbes

The antibiotic degrading microbes in the soil sample were isolated by applying shaker flask method. In the 250ml of Erlenmeyer flask 100ml sterile Muller Hinton broth medium and 5gram of soil sample was added. In this flask, antibiotic solution of the ampicillin was added, so as to maintain the final concentration of 50 ppm of antibiotic. Then it was incubated at 37℃ for 15 days in shaker incubator controlled at 150 rpm and at 7pH. This broth was transferred on freshly prepared Muller Hinton Agar medium containing 50ppm concentration of antibiotics by spread plate method and was incubated at 37℃ for 24 hrs. and pH was adjusted at 7. Antibiotics degradation capacity was determined by the method of Maki et al., (2006) and Al-Gheethi et al., (2014). In broth the mixed colony developed.

E. Pure colony selection

After incubation of mixed culture from the broth obtained as discuss above, the respective bacteria colony was separated individually by streaking bacterial culture on Mueller-Hinton agar medium containing 50ppm concentration of antibiotics. This process was repeated until purity of isolated bacterial colony was conformed. The pure bacterial strains were stocked in nutrient broth with 15% glycerol at -20℃ (Maki et al., 2006).

F. Identification of the Ampicillin degrading bacterial isolates

Identification of ampicillin degrading bacterial isolates was performed according to their morphological, biochemical and molecular characteristics as detailed below.

G. Morphological identification

The morphological identification of screened bacteria was performed with the help of Gram staining techniques, observed under light microscope by 100X oil immersion lance.

H. Biochemical identification

These three isolates were selected for further biochemical identification.

I. Catalase test

The catalase test used to detect the character of bacterial species on the basis of production ability of catalase enzyme. One drop of test culture was put on microscopic slide then few drops of 3% hydrogen peroxide were added. If gas bubbles appeared test was positive (Cheesbrough et al., 1985)

J. Oxidase test

Filter paper soaked in oxidase reagent the P-aminodimethy laniline then test **culture was** smear on it. Change the medium color purple to blue. The test was positive (Cheesbrough et al., 1985).

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K. Urease test

Urease test is implemented for the determination of the production of urease enzyme by microorganism. The bacterial culture was incubated it at 35±2℃ for 24 hours, after incubation medium color changes from yellow to bright pink. The test was positive (Cheesbrough et al., 1985).

L. Hydrolysis of starch

This test is performed to detect the utilization of starch by the bacterial culture, which form the clear zone around the colony. After incubation of bacterial colony, the Lugols Iodine solutions was flooded on the plates and kept for 15-30 minutes. The clear zone observed around the colony confirms the positive test (Echford et al., 1927).

M. Hydrogen sulfide production test

The test was used for the production of the H_2S by microorganisms, which imparts the black colour in the plates. The H_2S production (black color formation in the medium along the line of stab inoculation) is the positive test of H_2S production (Aneja, 2003).

N. Indole test

In this test, the test culture was inoculated in tryptone broth and incubated at 35℃ for 48 hrs. After incubation few drops of Kovac's reagent were added. The appearance of pink color in broth is the positive to this test (Fellstrom et al., 1999).

O. Methyl Red and Voges-Proskaur test

The methyl red (MR) and the Voges-Proskaur (V-P) tests were used to differentiate two major types of facultative anaerobic enteric bacteria which produce a large amounts of acid and the neutral product acetoin as an end product. For the MR test the methyl red reagent was added to the culture and incubated at room temperature. It turns to red color is positive test. For the VP test alpha-naphthal and potassium hydroxide reagent was added and it was also incubated at room temperature, it turns to red color is positive test (Cheesbrough et al., 1985).

P. Liquefaction of gelatin Test

The bacteria produce a photolytic enzyme gelatinase, which hydrolyzed the gelatin. In this test nutrient gelatin in a deep tube was taken and inoculated the test culture in the tube. This was incubated at 35±2℃ for 24 hours then keep it at 4℃ for 30 minutes. Tubes with culture remained liquefied indicated the positive test (Blazevic and Ederer, 1975).

Q. Time study for the growth of antibiotics degrading bacteria

This study was executed for the maximum growth of ampicillin antibiotic degrading bacteria at different durations in Muller-Hinton medium containing 50ppm concentration of antibiotic. The pure bacterial isolate (from stocked culture) was streaked on petriplates containing Muller-Hinton agar medium. These petriplates were incubated at 37℃ for 24-48 hrs. After incubation one loop full of the pure colony, thus obtained, was inoculated in 5ml of Muller Hinton broth medium and incubated it at 37℃ for 24 hrs. After incubation, 1ml bacterial culture was inoculated in 100ml Muller-Hinton broth medium containing 50ppm concentrations of ampicillin antibiotic and incubated in shaker incubator (150rpm) at 37℃ for 24 hrs. and pH was adjusted at 7. This process allowed for each bacterial isolates. For this study the control was served without inoculum for every experiment. The determination of bacterial growth through UV/Vis (Ultra violet/visible) spectrophotometer at 600nm after inoculation in Muller-Hinton broth medium at 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 21 respectively .

R. Determination of antibiotics utilization by bacteria

In this experiment determined the utilization of ampicillin antibiotic as carbon and energy source. The pure bacterial isolate (from stock culture) was streaked on MH agar medium and incubated it at 37℃ for 24-48 hrs. After incubation, a pure bacterial isolate was inoculated in 10 ml MH broth medium tubes and incubated it at 37℃ for 24 hrs. After incubation, 1ml culture was inoculated in 100ml Muller-Hinton broth medium containing 50ppm concentrations of ampicillin and incubated it at 37℃ and pH 7 for 24 hrs in shaker incubator (150 rpm). After incubation, 5ml of culture was transferred in 95 ml of Muller-Hinton broth medium containing 50ppm concentration of ampicillin antibiotic and incubated it at 37℃ in shaker incubator (150ppm) pH was adjusted at 7. This

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procedure applied for each isolate. In the control flask, culture was not inoculated. After incubation, bacterial growth was observed by spectrophotometer at 600nm of OD (optical density) in MH broth.

S. Screening and selection of potential antibiotics degrading bacteria

In this experiment the screening and then the potentiality for ampicillin antibiotic degradation was determined. The pure bacterial isolate (from stock culture) was streaked on MH agar medium and incubated it at 37℃ for 24-48 hrs. and 7 pH was maintained. After incubation, a pure bacterial isolate was inoculated in 10 ml MH broth medium tubes and incubated it at 37℃ for 24 hrs. After this incubation, 1ml culture was inoculated in 100ml Muller-Hinton broth medium containing 50ppm concentrations of ampicillin and incubated it at 37℃ at pH 7 for 24 hrs in shaker incubator (150 rpm). After the 24hrs. of incubation, 5ml of culture was transferred to 95 ml of Muller-Hinton broth medium containing different concentrations of ampicillin antibiotics 50ppm, 100ppm, 200ppm, 300ppm, 400ppm and 500ppm respectively. These flasks were incubated at 37℃ at 150rpm. Same procedure was applied for each bacterial isolates. In control flask, the culture was not inoculated. Bacterial growth was observed at 0, 1, 2, 3, 4, 5, 6, 7, 14, and 21 days by UV/Vis spectrophotometer at 600nm.

T. Quantitative analysis of degraded erythromycin by UV/Vis spectrophotometer

In this study the quantity of antibiotics remaining after degradation by bacterial isolates, with the help of UV/Vis spectrophotometer was detected. After selection of the isolates, the further study was performed with the help of isolates, which were able to grow in higher concentrations of AMP antibiotics. The pure bacterial isolate (from stock culture) was streaked on MH agar medium and incubated it at 37℃ for 24-48 hrs. After incubation, a pure bacterial isolate was inoculated in 10 ml MH broth medium tubes and incubated it at 37℃ for 24 hrs. After incubation, 1ml culture was inoculated in 100ml Muller-Hinton broth medium containing 50ppm concentrations of AMP antibiotic and incubated it at 37℃ at pH 7 for 24 hrs in shaker incubator (150 rpm). After incubation, 5ml of culture was transferred in 95 ml of Muller-Hinton broth medium containing the concentration of AMP antibiotic individually, at which the maximum growth of isolates was noticed and incubated at 37℃ in shaker incubator (150ppm) at 7 pH. This procedure was applied for each isolate. In the control flask culture was not inoculated, it was run parallel. After incubation the 15ml of culture was taken in test tube and filtered through 0.45µm pore size syringe filter paper. Samples were analyzed by UV/Vis spectrophotometer and OD values were compared with standard curve (prepared by standard concentration for each antibiotic).

III. RESULT AND DISCUSSION

A. Isolation of ampicillin degrading bacteria

In the present research work eleven bacterial colonies were isolated from enriched soil sample with ampicillin antibiotic by enrichment method with the help of shaker incubator at the 50ppm concentrations of ampicillin antibiotic. For the purification of all bacterial colonies were collected on Mueller-Hinton agar medium containing 50ppm concentration of ampicillin antibiotic. Bacterial colonies were able to grow on Mueller-Hinton agar and Mueller-Hinton broth medium containing 50ppm concentration of ampicillin antibiotic. These bacterial cultures were purified on Mueller-Hinton agar medium containing 50ppm concentration of ERY, then given different code name of isolates as AMP 1, AMP 2, AMP 3, AMP 4, AMP 5, AMP 6, AMP 7, AMP 8, AMP 9, AMP 10 and AMP 11. The pure bacterial strains were stocked in nutrient broth with 15% glycerol at -20℃. Out of eleven bacterial isolates two bacterial isolates showed degradation of ampicillin antibiotics.

B. Identification of the ampicillin degrading bacterial isolates

1) *Morphological identification:* All two bacterial isolates such as AMP6 and AMP8 were subjected to Gram staining and the morphology of the respective bacterial isolate was visualized by 100X oil emersion lens under light microscope. Isolates were Gram negative and rod shaped and Gram positive rod shaped respectively (Fig. 1). The AMP6 and AMP8 showed motility, Catalase, Oxidase positive and MR positive. The VP and urease test were negative for AMP8 and positive for AMP6. Indole, starch hydrolysis and Gelatin liquefaction test negative for AMP6 and positive for AMP8. Both isolates were observed negative H_2S production test.

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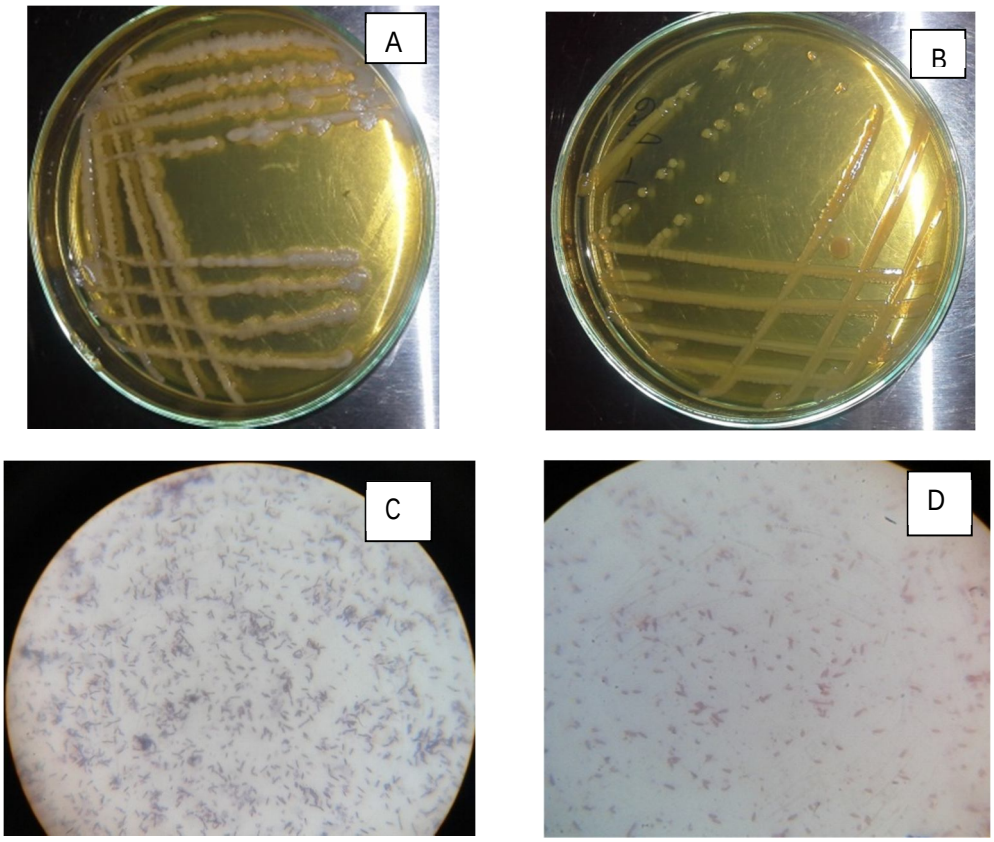

Fig. 1: The different bacterial isolates on Nutrient agar medium in Fig A- AMP6= white circular colony at 37^0C and Fig. B- AMP8= Creamish round small colony at 37^0C. Gram staining image of the isolates under 100X oil immersion. Fig. C- ERY6 and AMP6; Gram negative, rod shaped. Fig. D- ERY11, AMP8 and OFL9; Gram positive, rod shaped.

2) Biochemical identification of isolates:

Table 1: Biochemical identification of two isolates AMP6 and AMP8.

S. No.	Tests	AMP6	AMP8
1.	Gram's staining	_	+
2.	Cell shape	Rod shaped	Rod shaped
3.	Motility	+	+
4.	Catalase	+	+
5.	Oxidase	+	+
6.	Methyl-Red	+	+
7.	Voges-Proskaur	+	_
8.	H_2S production	_	_
9.	Indole test	_	+
10.	Hydrolysis of starch	_	+
11.	Urease test	+	_
13.	Gelatin liquefaction	_	+

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3) Molecular identification: On the basis of morphological and biochemical characteristics the bacterial isolates AMP6 as a member of the genus Ochrobacterum and AMP8 as a member of the genus Bacillus. This was further confirmed by assembled 16S rRNA sequences analysis and identified as *Ochrobacterum intermedium* (AMP6) and *Bacillus oceanisediminis* (AMP8). Complete sequencing and phylogenetic tree of AMP6 and AMP8 are given below.

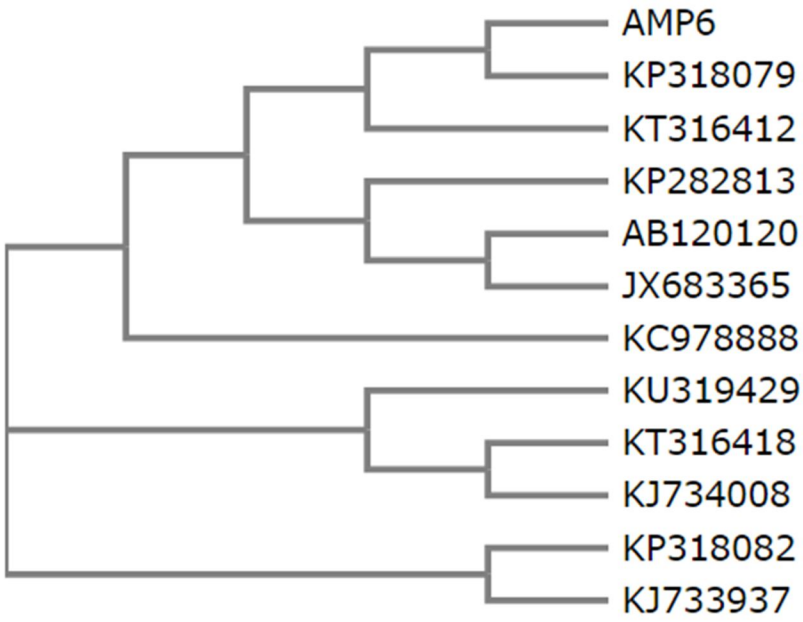

Fig. 2: Phylogenetic tree of AMP6 were conducted by MEGA4 software.

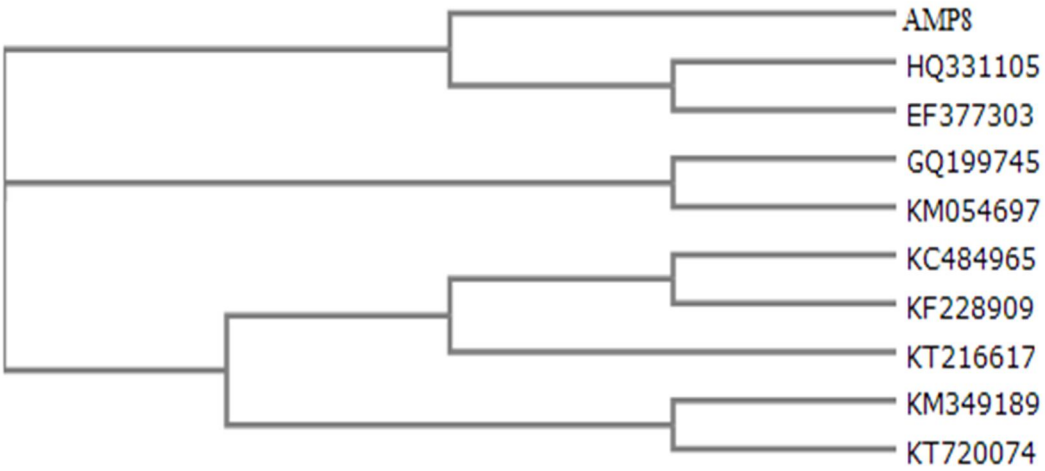

Fig. 3: Phylogenetic tree of AMP8 were conducted by MEGA4 software.

C. Time study for the growth of ampicillin degrading bacteria

In this experiment, eleven ampicillin degrading bacterial colonies were observed. For this study, the inoculum was prepared after purification. In the experimental section the inoculum preparation was explained. When these isolates inoculated in Muller-Hinton broth medium containing 50ppm AMP, the maximum growth of bacterial culture was recorded after 6 day of incubation. Corrected OD_{600nm} of eleven AMP degrading isolates is given in Table (2). The growth of the AMP degrading bacterial isolates was observed by using spectrophotometer at OD_{600nm} (Fig. 4). Don't observe any growth in uninoculated control sample.

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Volume 4 Issue X, October 2016
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Table 2: Corrected OD_{600} of AMP degrading bacteria in Mueller-Hinton broth medium containing 50ppm AMP at different time interval (in days), incubated at 37℃, pH 7.0 and 150rpm at different time interval.

AMP degrading bacteria	0 days	1 days	2 days	3 days	4 days	5 days	6 days	7 days	14 days	20 days
Control	0	0	0	0	0	0	0	0	0	0
AMP 1	0.004	0.196	0.226	0.386	0.454	0.499	0.521	0.525	0.530	0.536
AMP 2	0.003	0.262	0.346	0.421	0.562	0.582	0.599	0.602	0.604	0.609
AMP 3	0.002	0.192	0.286	0.349	0.398	0.485	0.551	0.555	0.560	0.562
AMP 4	0.001	0.281	0.396	0.426	0.448	0.526	0.681	0.685	0.690	0.693
AMP 5	0.005	0.254	0.348	0.398	0.486	0.510	0.596	0.598	0.600	0.604
AMP 6	0.004	0.145	0.430	0.602	0.786	0.845	0.889	0.892	0.895	0.898
AMP 7	0.003	0.118	0.295	0.364	0.495	0.508	0.592	0.594	0.597	0.599
AMP 8	0.003	0.210	0.530	0.614	0.690	0.824	0.950	0.955	0.960	0.963
AMP 9	0.002	0.208	0.365	0.489	0.546	0.598	0.600	0.603	0.608	0.612
AMP 10	0.001	0.182	0.265	0.414	0.518	0.584	0.661	0.665	0.668	0.672
AMP 11	0.002	0.192	0.346	0.484	0.546	0.618	0.678	0.680	0.683	0.686

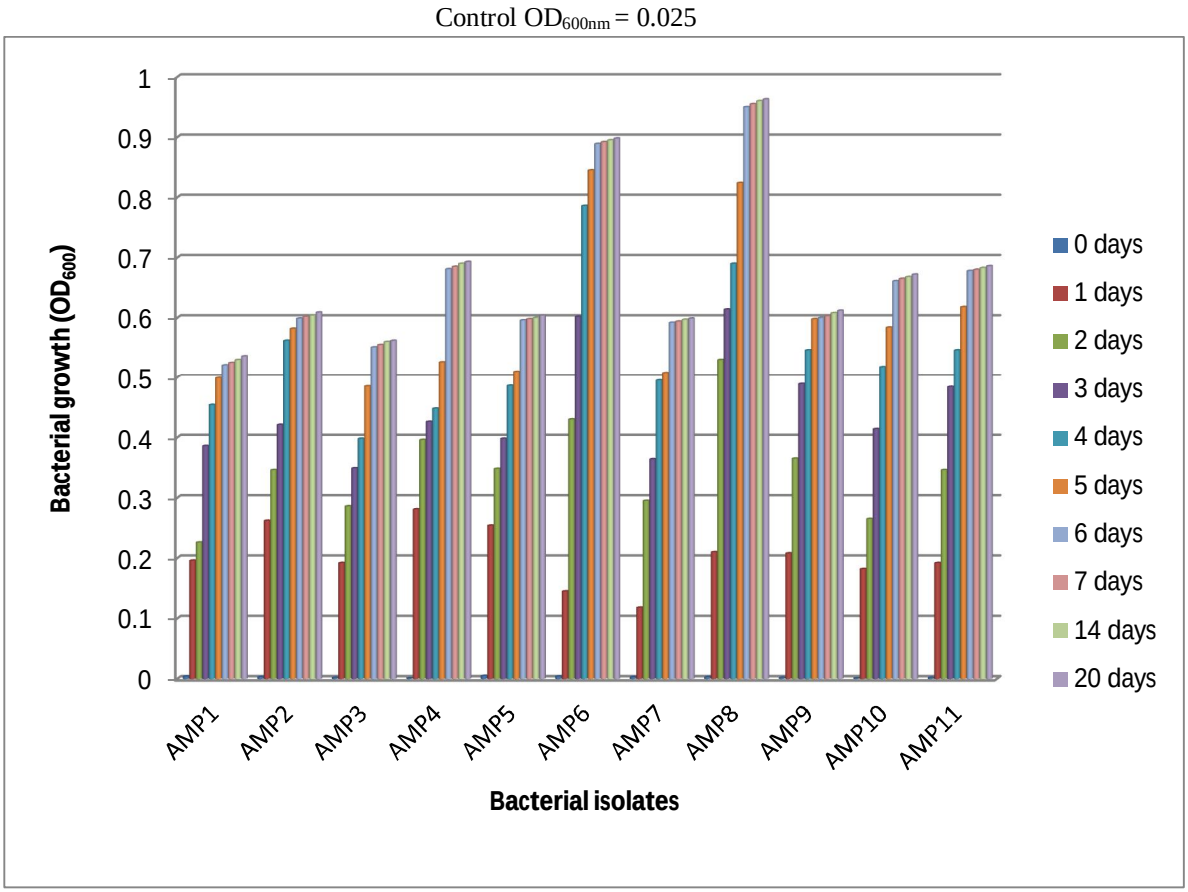

Fig. 4: The growth of AMP degrading bacteria in Mueller-Hinton broth medium containing 50ppm AMP, at different time intervals (in days), incubated at 37℃, pH 7.0 and 150rpm.

D. Isolates utilizing the ampicillin antibiotic as a carbon and energy source

In this studies, the bacterial utilization of ampicillin as energy and carbon source for their metabolic activities, was recorded.

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Eleven bacterial isolates, which were observed, were separately inoculated in Muller-Hinton broth medium containing 50ppm AMP and incubated at 37°C, 150 rpm and at pH 7.0. After 6 days of incubation turbidity appeared in flasks containing 50ppm AMP but in control flask no growth were observed in which AMP (as energy and carbon source) was not added (Table 3, Fig. 5). The bacterial isolate Labrysportucolensis F11 was capable to use the fluoroquinolones antibiotics ciprofloxacin, ofloxacin and norfloxacin as carbon and energy source was reported by Amorim et al., (2014).

Table 3: Normal OD_{600} of AMP degrading bacteria in Muller-Hinton broth medium in the absence and presence of 50ppm AMP at different time interval (in days), incubated at 37℃, pH 7.0 and 150rpm after 6 days of incubation.

AMP degrading bacteria	Without AMP	With AMP
AMP 1	0.053	0.542
AMP 2	0.042	0..610
AMP 3	0.035	0.572
AMP 4	0.046	0.690
AMP 5	0.051	0.600
AMP 6	0.049	0.874
AMP 7	0.038	0.598
AMP 8	0.048	0.626
AMP 9	0.052	0.963
AMP 10	0.043	0.677
AMP 11	0.044	0.682

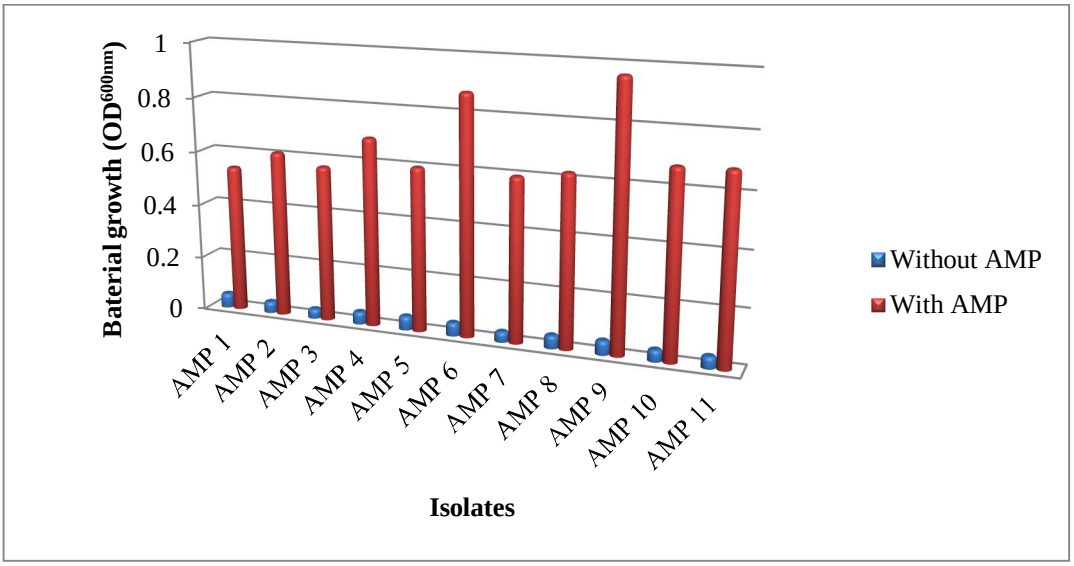

Fig. 5: Growth of AMP degrading bacteria in Muller-Hinton broth medium in the absence and presence of 50ppm AMP at different time interval (in days), incubated at 37℃, pH 7.0 and 150rpm after 6 days of incubation.

E. Screening and selection of ampicillin degrading bacteria

Out of eleven bacterial isolates obtained after inoculation in the MH medium, two bacterial isolates AMP 6 and AMP8 were selected on the basis of their growth at higher concentration, which was 400ppm of AMP. Above this concentration of AMP, the negative bacterial growth was noticed. In the present work, the higher growth was observed after 6 days of incubation. The corrected OD_{600nm} of fifteen isolates with different concentrations of AMP ranging 50ppm to 500ppm (Table 4 and Fig. 6).

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)

Table 4: Corrected OD_{600} of AMP degrading bacteria in Muller-Hinton broth medium with different concentration of AMP (50ppm to 500ppm) after 6 days incubation at 37℃, pH 7.0 and 150rpm.

AMP degrading bacteria	50ppm	100ppm	200ppm	300ppm	400ppm	500ppm
control	0	0	0	0	0	0
AMP 1	0.526	0.643	0.698	0.761	0.796	0.529
AMP 2	0.610	0.661	0.790	0.840	0.889	0.614
AMP 3	0.540	0.596	0.648	0.672	0.767	0.626
AMP 4	0.630	0.662	0.697	0.768	0.794	0.682
AMP 5	0.552	0.621	0.684	0.790	0.872	0.750
AMP 6	0.889	0.983	0.996	0.985	1.521	1.346
AMP 7	0.548	0.591	0.662	0.896	1.021	0.981
AMP 8	0.950	0.984	0.998	1.002	1.342	1.049
AMP 9	0.610	0.644	0.775	0.840	0.898	0.628
AMP 10	0.590	0.676	0.722	0.762	0.842	0.636
AMP 11	0.642	0.769	0.779	0.820	0.867	0.626

Control OD_{600nm} -- 50ppm = 0.030, 100ppm =0.075, 200ppm = 0.086, 300ppm = 0.119, 400ppm = 0.166, and 500ppm = 0.223

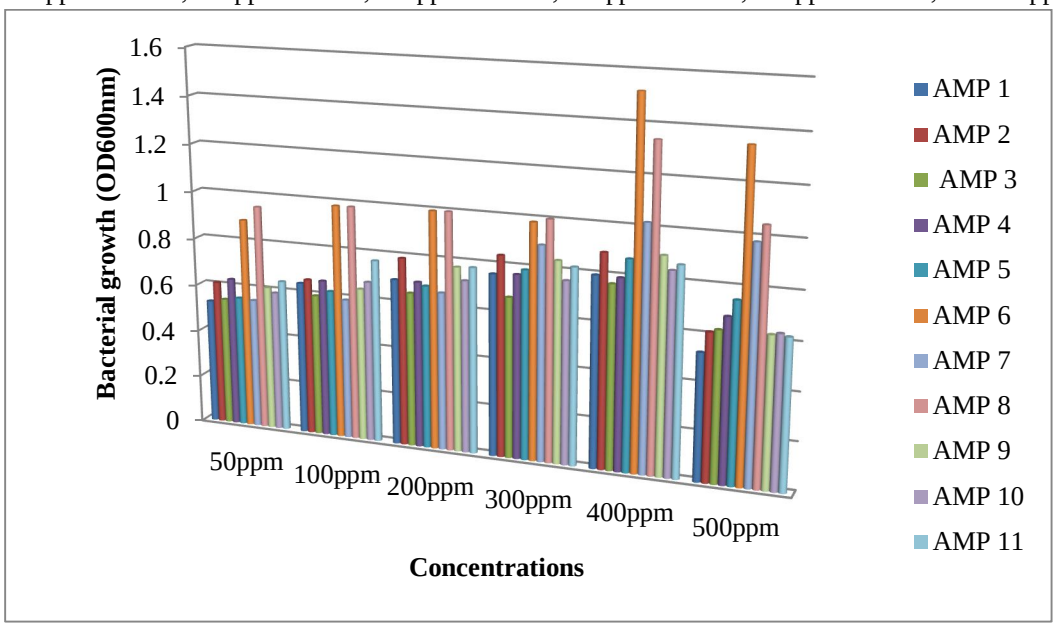

Fig. 6: Growth of AMP degrading bacteria in Mueller-Hinton broth medium at different concentrations (50ppm to 500ppm) at 37℃, pH 7.0 and 150rpm.

F. Quantitative analysis of degraded antibiotics by UV/Vis spectrophotometer

For the preparation of calibration curve of ampicillin, five concentrations were used (Table 5 and Fig. 7). The calculation of the concentrations of ampicillin, the regression equation from the calibration curve was used. The calculation was done by putting value of absorbance (Y) in the regression equation. The λ_{max} of ampicillin was 373_{nm}. The regression equations of ampicillin was Y=0.0275X-0.132. The concentrations values in percentage AMP after biodegradation, was noticed for different isolates. The isolates AMP6 and AMP8 showed degradation rate 12.97±0.11 and 11.98±0.15 (Fig. 8).

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Table 5: standard solution for ampicillin and their absorbance value at 373_{nm}.

Concentrations (πg/ml)	absorbance
18	0.398
36	0.797
54	1.402
72	1.803
91	2.399

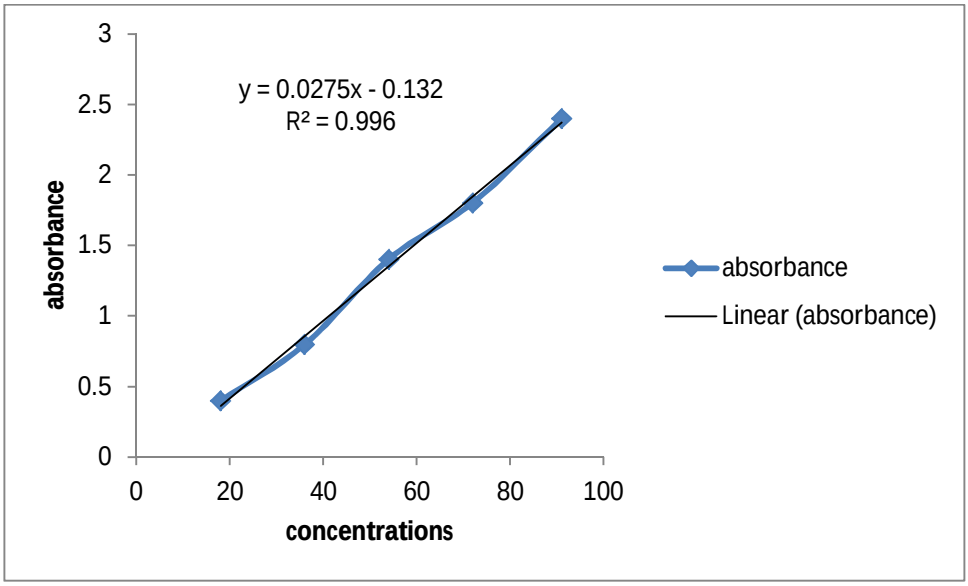

Fig. 7: Calibration curve for ampicillin.

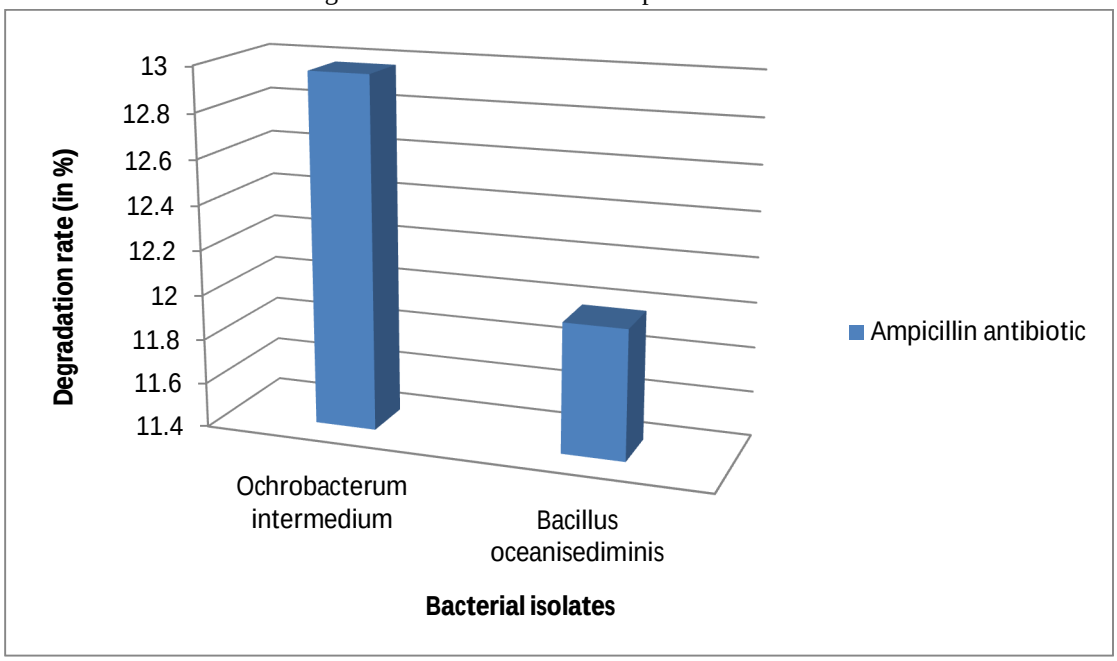

Fig. 8: The biodegradation rate of ampicillin by *Ochrobacterum intermedium* and *Bacillus oceanisediminis*.

IV. DISCUSSION

10 bacterial strains were isolated from marine sediments of Uranouchi Bay for the degradation of ampicillin at final concentration 50ppm, belonging to different bacterial groups, FCB group (RA1 and RA6), alpha-proteobacteria subdivision (RA2, RA5, RA8,

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RA9 and RA10) and gamma proteobacteria subdivision (RA3, RA4 and RA7) by Maki et al., (2006). Al-Gheethi et al., (2014) isolated 68 bacterial strains from 21 treated sewage effluent sample collected from three STPs from Penang, Malaysia. These were capable of the degrading the ampicillin, amoxicillin, cefuroxime, cephalexin and ciprofloxacin antibiotics. They further noticed that the most potent bacteria for enzymatic degradation was Bacillus subtilis 1556 WTNC for the ampicillin, amoxicillin, cefuroxime, cephalexin and ciprofloxacin antibiotics. Wang et al., (2015) studied the penicillin G degradation by Klebsiella pneumoniae Z-1.

Al-Ahmad et al., (1999) reported the higher bio- degradation of penicillin G by Pseudomonas putida after 28 to 40 days of incubation. Mitchell et al., (2013) focused the studied on the degradation of ampicillin, cefoxitin and cefalotin and recorded the higher rate after 5.3 to 27 days incubation. Al-Gheethi et al., (2014) noticed the maximum degradation of β-lactam antibiotics (amoxicillin, ampicillin, cephalexin and cefuroxime) by Bacillus subtilis 1956WTNC after 12 days of incubation. Lin et al., (2015) obtained the higher degradation rate of cephalexin by two Pseudomonas strains after 24 hrs. of incubation.

Maki et al. (2006) studied the biodegradation of ampicillin by ten AMP-resistant bacterial isolates .They further noticed that that the ampicillin residue was14 to 92% in Mueller-Hinton medium after 21 days of incubation. The degradation of β-lactam antibiotics by Bacillus subtilis 1556WTNC was studied by the Al-Gheethi et al. (2014). They noticed that the higher degradation rate of ampicillin, amoxicillin, cefuroxime and cephalexin was 15.56% at 0.8mg/ml, 25.03% at 1 mg/ml, 10.62% at 1mg/ml and 22.59 at 1 mg/ml respectively. Al-Gheethi et al. (2015) studied the degradation of cephalexin in pharmaceutical effluent. They recorded that the maximum degradation was 21% by Bacillus subtilis. Lin et al., (2015) demonstrated the degradation of cefalexin with the help of two strains CE21 and CE22 of Pseudomonas species. They observed the degradation rate as 46.7% and 90% after 24 hours of incubation respectively.

In present study the author isolated eleven isolates, out of eleven isolates only two AMP 6 and AMP 8 showed the higher degradation of ampicillin. The AMP 6 and AMP 8 identified as *Ochrobacterum intermedium* and *Bacillus oceanisediminis* respectively. These isolates were degraded the ampicillin after 6 days of incubation at 37^{o}C and 7 pH. The degradation rate of these antibiotics was 12.97±0.11 and 11.98±0.15 respectively.

V. CONCLUSION

The studies on the biological degradation of antibiotics will increase our knowledge for the remediation of ampicillin by microbes. The rate of biodegradation of ampicillin was 12.97±0.11 and 11.98±0.15 by *Ochrobacterum intermedium* and *Bacillus oceanisediminis* at 37^{o}C and 7 pH after 6 days of incubation respectively. The concentration of ampicillin was 400ppm in the culture medium. thus it is concluded that the biodegradation of Ampicillin antibiotic present in the soil, garbage sewage etc. can be possible in the field conditions also.

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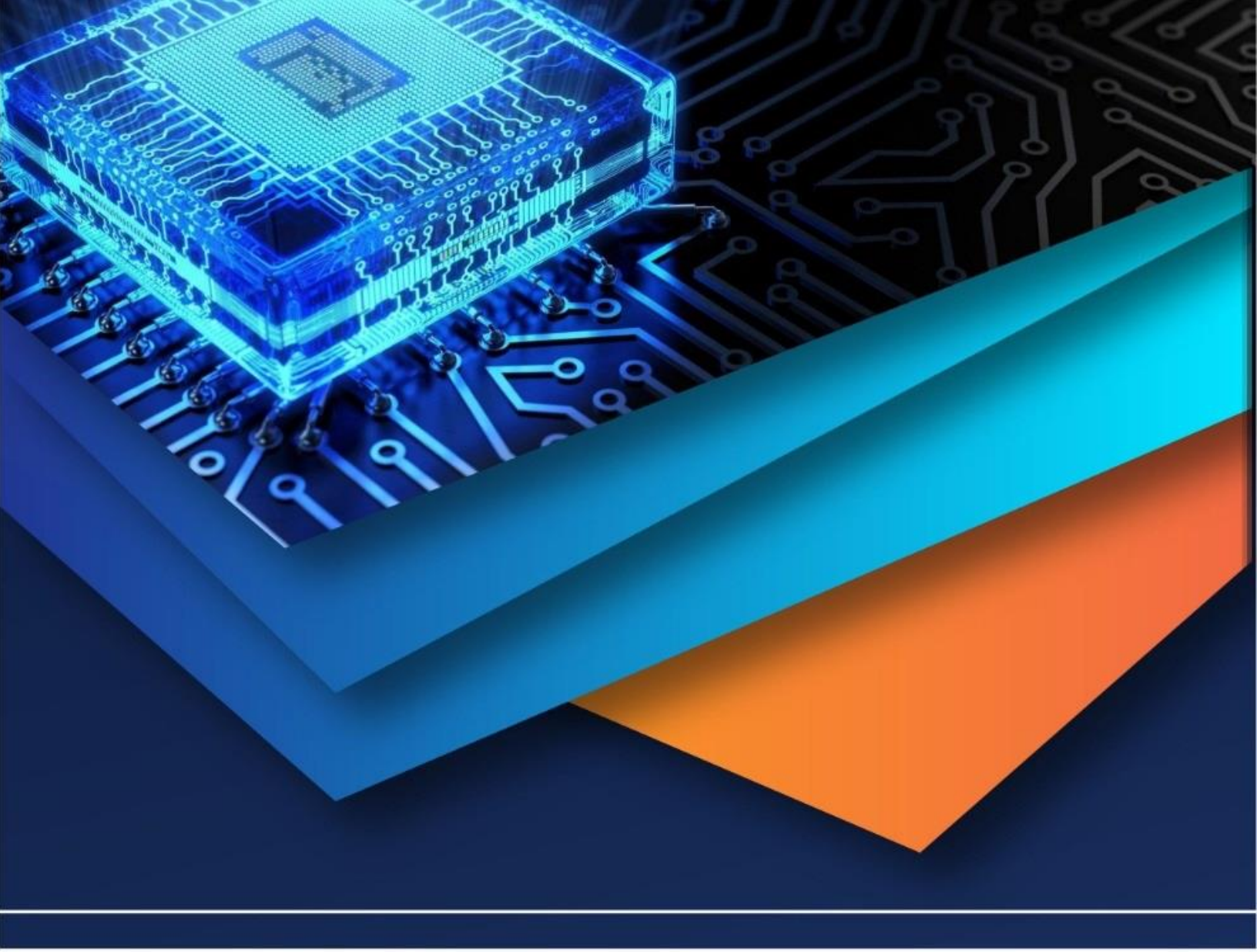

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