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Use of Beneficial Microbes (Probiotics) in Aquaculture

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Abstract: Aquaculture is an important aquatic food-producing sector to fulfill nutritional food demand of a continuously growing world population. However, diseases outbreak became a major issue in aquaculture which results in huge economic loss to the aquaculture sector. The use of expensive health care drugs for treatment have negative impacts on the aquaculture species and also on the environment. So there is a growing concern to find other safe, non-antibiotic based and eco-friendly alternative for the improvement of the health and treatment of the various diseases. The use of probiotics is a secure alternative approach for the control the infections, boost the immunity and treatment of diseases. The benefits of probiotics include improvement of improved digestion, stimulation of growth, boosting immune response and recuperate the soil and water quality. Probiotics supplements use via in water, soil and feed in the shrimp and fish farming to fight against various pathogens and improve the overall health as they show antibacterial, antifungal and anti-viral properties use of probiotics in aquaculture has become a recent trend.
Keywords: Aquaculture, Beneficial microbes, Prebiotics, Probiotics

I. INTRODUCTION

Aquaculture is an important and rapidly growing sector as it plays an important role to achieve global protein food demand compared to capture fisheries and terrestrial farmed meat. The role of aquaculture to improve the socio-economic status of any region is highly appreciable because it is not only limited to the source of essential nutrients but it also generates various employment opportunities [1]. India ranks second in the world after China in fish production through aquaculture with a contribution of 6.3% of the global aqua production, which is very less as compared to that of China (60.5%) [2]. Fishes are dominant in aqua products, and around 200 fish species are produced for their commercial value [3]. With the increasing intensification and commercialization of aquaculture production, diseases have become a hurdle in the fish farming industry [4]. During the last decades, antibiotics used as a traditional strategy for fish diseases management and also for the improvement of growth and efficiency of feed conversion. However, the development and spread of antimicrobial-resistant pathogens were well documented [5, 6]. In aquaculture, chemotherapeutic agents like antibiotics and chemicals are the classical cure for microbial infection. However, the extensive usage of these chemotherapeutic drugs leads to their accumulation in aquatic habitat and results in harmful consequences such as emergence of antibiotic-resistant bacteria, accumulation of antibiotic residues in the flesh, kill the beneficial microbes of the gastrointestinal tract and alterations in microbiota (effect on non-target microbes) of the aquatic environment[7,8]. There is a possibility of risk associated with the transmission of resistant bacteria from aquaculture environments to humans, and risk associated with the introduction in the human environment of nonpathogenic bacteria, containing antimicrobial resistance genes, and the subsequent transfer of such genes to human pathogens [9].
Considering these factors, there has been heightened research in developing new dietary supplementation strategies in which various health and growth-promoting compounds as probiotics, prebiotics, symbiotics, photobiotic and other functional dietary supplements have been evaluated[10]. In this context, the microbial intervention can play a vital role in aquaculture production, and effective probiotic treatments may provide broad-spectrum and greater nonspecific Disease protection [11, 12].
This review summarizes and evaluates about the probiotics, selection of probiotics, commonly used probiotic organism, their mode of action and safety regulation of probiotics in aquaculture.

A. Definition of Probiotics

The word “probiotic” was introduced by Parker, 1974[13]. According to his original definition, probiotics are “organisms and substances which contribute to intestinal microbial balance”. Fuller, 1989 [14] revised the definition as “live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”. Therefore probiotics called such as “favorable", "useful", “beneficial”, and “friendly” or “healthy” bacteria are also commonly used to describe probiotics. Although the application of probiotics in aquaculture seems to be relatively recent [15] the interest in such environment-friendly treatments is increasing rapidly [16] proposed to extend the definition of probiotics in aquaculture to microbial ‘‘water additives’’. A growing number of studies have dealt explicitly with probiotics, and it is now possible to survey its state of the art, from the empirical use to the scientific approach [17, 18]. This definition signifies that the living bacterial cells are an imperative part of

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potential probiotics and also clarifies the confusion created by the use of the term "substance". WHO [19] has termed probiotics as live microbes, which when administered insufficient amount, confer a health benefit to the host. Probiotics protect the host organism from pathogenic bacteria by liberating metabolites like bacteriocins and different organic acids. These metabolites hinder the adhesion of different pathogens and also inhibit them by limiting the available resources such as nutrients and space [20, 21].

Probiotics have the potential to improve the host's defenses, including the innate and acquired immunity system. This is important for the prevention and treatment of infectious diseases and also to cure inflammation in the digestive tract. Probiotics also have a direct influence on other microbes, either commensal or pathogenic *Vibrio* or other harmful bacterial species.

B. Selection of Probiotics

Selection of probiotic bacteria has usually been an empirical process based on scientific evidence. Many of the failures in probiotic research can be attributed to the selection of inappropriate non-useful microorganisms. Probiotics selection steps have been defined, but they need to be adapted for different species and environments. It is essential to understand the mechanism of probiotic action and to define selection criteria for important probiotics.

Methods of probiotics production and processing:

1) Method of administration of the probiotic.
2) The location in the body where the microorganisms are expected to be functional.
3) The probiotics should have a beneficial effect on the growth, development and protection of shrimp/ fish against various pathogenic bacteria.
4) The probiotic bacteria should not show any harmful effect on the shrimp/ fish.
5) The probiotics should not have the ability of drug resistance power, they should have the ability to keep up the hereditary traits.

a) Probiotics might be able to modulate the host's gut defenses including the innate as well as the acquired immune system and this mode of action is most likely important for the prevention and therapy of infectious diseases but also for the treatment of inflammation of the digestive tract or parts thereof.
b) Probiotics canals have a direct effect on other organisms, commensal and or pathogenic ones and this principle is in many cases is of great importance in the prevention, treatment, and restoration of the microbial equilibrium in the gut.
c) Finally, probiotic effects may be based on their function affecting microbial products, host products.

For the utilization of probiotics as an efficient feed, they should exhibit the following properties:

i) Acid and bile tolerance
ii) Resistance to gastric juices
iii) Adherence to digestive system surface
iv) Antagonism towards pathogens
v) Stimulation of the immunity
vi) Increase in the gut motility
vii) Survival in mucous
viii) Production of enzymes and vitamins

According to the application or by the function of the probiotics, probiotics are considered three types based on their modes of action are all likelihood associated with gut and/or gut microbiota. Therefore, it has become apparent that we are in fact dealing with another "organ", the so-called "macrobiotic canal" with the increased knowledge of the specific activity of the gut microbiota [22].

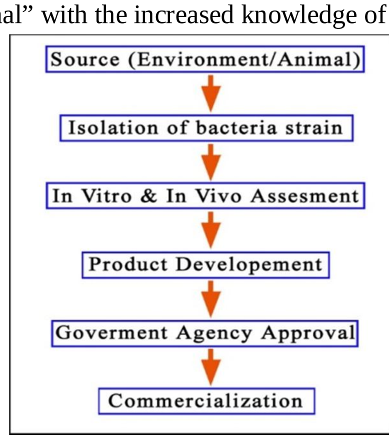

Fig. 1 Flow chart of the screening process for the selection of probiotic bacteria Modified (Balcazar *et al.*, 2006) [23].

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A probiotic agent with all these features has a considerable advantage over antibacterial supplements such as antibiotics currently in use. They do not induce resistance to antibiotics which will compromise therapy. They are not toxic and therefore will not produce undesirable side effect when being fed and in the case of food the animal will not produce toxic residues in the carcass. They may stimulate immunity whereas the immune status remains unaffected by antibiotics.

Table I. Probiotic species and their beneficial effects used in aquaculture.

S. No	Probiotic species	Beneficial effect	Reference
1	Lactobacillus acidophilus Streptococcus faecium	Best growth performance and feed efficiency.	[24]
2	Bacillus Subtilis Lactobacillus acidophilus	Enhanced the non-specific immune parameters and enhance the challenge against Edwardsiella tarda infection	[25]
3	Bacillus cereus Paenibacillus polymyxa	Improved resistance against pathogenic Vibrio spp.	[26]
4	Lactococcus lactis CLFP 101 Lactobacillus plantarum CLFP 238 Lactobacillus fermentum CLFP 242	Reduce the adhesion of pathogens i.e. Aeromonas salmonicida, Aeromonas hydrophila, Yersinia ruckeri and Vibrio anguillarum to intestinal mucus and shows antibacterial activity against these fish pathogens.	[27]
5	Lactobacillus plantarum Bacillus subtilis	Shows antagonistic activity against Aeromonas hydrophila.	[28,29]
6	Bacillus coagulans Bacillus mesentericus Bifidobacterium infantis	Probiotic bacteria significantly established in gut of P. conchonius and significant effects on the pathogenic gut inhabitants of the fish.	[30]
7	Nitrosomonas species Nitrobacter species	Improves water quality and lowers the pathogenic Pseudomonas species bacterial loads in fish.	[31]
8	Lactococcus lactis (D1813)	Exhibit highest amount of IFN-γ production and bactericidal activity. Inhibit the infection caused by Vibrio penaeicida.	[32]
9	Enterobacter sp. strain C6–6	Protects the fish against Flavobacterium psychrophilum infection, reduce the mortality and enhance the immunity of fish.	[33]
10	Bacillus subtilis	Increase in the growth, survival, improve food digestion, reduce the mortality caused by pathogenic bacteria Aeromonas hydrophila.	[34]
11	Bacillus cereus	Shows high growth performance like specific growth rate, body weight and also shows inhibition against the pathogenic strain Aeromonas hydrophila.	[35]
12	Bacillus firmus Bacillus aerophilus	Improves digestion and fight against the fish pathogens such as Providencia rettgeri and Aeromonas species.	[36]
13	Lactococcus lactic Lactobacillus plantarum	Show improve phagocytic activity of innate immune cells, skin mucus lysozyme activity and improves host innate immunity, weight gain and survival rate following Streptococcus iniae challenge.	[37]
14	Pediococcus acidilactici	Increase growth performance, health status and also modulate intestinal microbial community.	[38]
15	Bacillus subtilis Pediococcus acidilactici Enterococcus faecium Lactobacillus reuteri	Strains are more efficient in converting organic matter, adhere to the intestine, and enhance the growth and survival of L. rohita.	[39]
16	Saccharomyces cerevisiae Bacillus licheniformis	Increase the growth, immune response and disease resistance of juvenile tilapia against Streptococcus iniae.	[40]
17	Bacillus pumilus	Bacillus pumilus treated fish show maximum percentage of total erythrocyte count, haemoglobin concentration and haematocrit concentrations which improves survival and therefore establish better health conditions.	[41]
18	Bacillus mojavensis	Shows antagonism against Vibrio parahaemolyticus.	[42]
19	Lactobacillus gasseri TSU3 Lactobacillus gasseri TSU3	Capable of adhering to epithelial cells and mucosal surfaces and exhibit strong anti-bacterial activity against all pathogens including Aeromonas hydrophila.	[43]
20	Pseudomonas psychrotolerans Vibrio ichthyoenteri Labrenzia sp.	Enhance the immune defence of fish. Show antagonism against three fish pathogens: Vibrio anguillarum, Photobacterium damselae and Pseudomonas anguilliseptica.	[44]
21	Bacillus amyloliquefaciens (KF623290) Bacillus sonorensis (KF623291)	Shows antagonistic activity against Pseudomonas putida and Aeromonas salmonicida.	[45]

22	Lactobacillus plantarum	Stimulates growth rate, feed efficiency, and conferred the best performance and immune response of Nile tilapia challenged with Aeromonas hydrophila and Show inhibitory activity against pathogens including S. aureus, S. typhimurium, S. enteritidis, E. coli O157:H7, V. ichthyoenteri, S. iniae, and V. parahaemolyticus.	[46, 47]
23	Bacillus stratosphericus (KM277362) Bacillus aerophilus (KM277363) Bacillus licheniformis (KM277364) Solibacillus silvestris (KM277365)	Strains grow better in intestinal mucus and produce various cellular components which exhibit bactericidal activity against the fish pathogens.	[48]
24	Bacillus amyloliquefaciens	Improve the growth performance, enhance the immune parameters in turbot and also fight against V. anguillarum infection.	[49]
25	Kocuria sp. Rhodococcus sp.	Produce extracellular enzymes (secondary metabolites) which is inhibitory to Virbio anguillarum, V. ordalii, E. coli, Pseudomonas aeruginosa and Staphylococcus aureus.	[50]
26	Enterococcus hirae	Persist in simulated gastric conditions with the inhibition capability of various pathogens like Staphylococcus aureus (MTCC 3160), Escherichia coli (MTCC 40), Pseudomonas aeruginosa (MTCC 424) and Salmonella typhi (MTCC 3215).	[51]
27	Bacillus pumilus AQAHBS01	Improves immunity of Nile tilapia and enhance disease resistance against Streptococcus agalactiae.	[52]
29	Bacillus sp.	Shows antibacterial activity against four fish pathogens, Aeromonas salmonicida, A. hydrophila, A. sobria and Pseudomonas fluorescens.	[53]
28	Lactobacillus farraginis Pediococcus acidilactici	Produce antimicrobial compounds against fish pathogens, have good colonization capacity on gastrointestinal tract of salmon.	[54]

Different modes of action or properties are desire on the potential probiotic like antagonism to pathogens shown in Table. I. and Fig.2. Ability of cells to produce metabolites (like vitamins) and enzymes [55] colonization or adhesion properties [56] enhance the immune system [57].

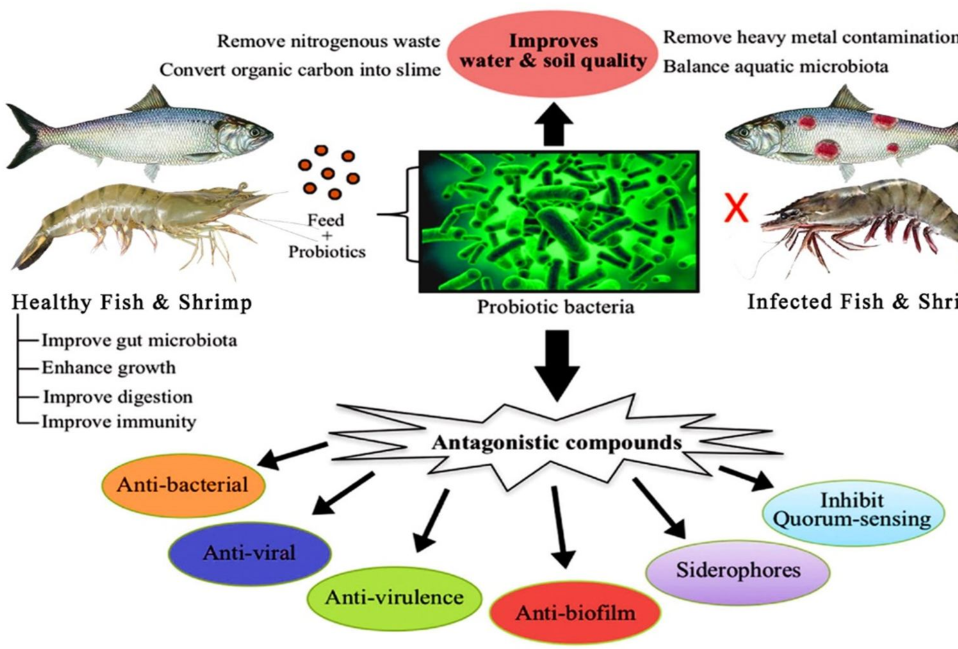

Fig. 2 Probiotics mode of action. Modified from Chauhan and Singh (2018) [58].

Competitive exclusion of harmful pathogenic bacteria Competitive exclusion is a phenomenon whereby an established microflora prevents or reduces the colonization of a competing bacterial challenge for the same location on the intestine. The aim of probiotic products designed under competitive exclusion is to obtain: stable, acceptable and controlled microbiota in cultures based on the following; competition for attachment sites on the mucosa, competition for nutrients and production of inhibitory substances by the microflora which prevents replication and/destroys the challenging bacteria and hence reduce colonization [59].

Different strategies are displayed in the adhesion of microorganisms to those suitable sites as passive forces, electrostatic interactions, hydrophobic, steric forces, lipoteichoic acids, adhesions and specific structures of adhesion. Adhesion and colonization of the mucosal surfaces are possible protective mechanisms against pathogens through competition for binding sites and nutrients [60, 61].

C. Probiotics As Water Quality Enhancers

Probiotics have proven their effectiveness in improving water quality in different approaches. They enhanced decomposition of organic matter, reduced nitrogen and phosphorus concentrations, and controlled ammonia, nitrite, and hydrogen sulfide [62, 63]. Probiotics reduced organic matter accumulation [64], mitigated nitrogen [65] and phosphate pollution in the sediments and enhanced environmental conditions for a prawn farm. Probiotics reduced metabolic wastes during transportation of cardinal tetra *(Paracheirodon aexlrodi).* Probiotics improved water quality by reducing the number of pathogenic bacteria [66].

D. Probiotics As A Survival And Growth Promoters

Applications of probiotics have improved aquatic animal growth rates, feed utility by influencing digestive enzyme processes, and survival rates. Bacterial strains promoted the growth of black tiger prawn nauplii, and giant freshwater prawn, *Macrobrachium rosenbergii, Pseudomonas aeruginosa* and *Ps. synxantha* improved the western king prawn growth [67]. *Haliotis asinine* fed a diet pudding probiotic *Vibrio* Alg3.1RfR-Abn1.2RfR-enriched protein exhibited an increased growth. In fact, probiotics improved the digestibility of feed due to enhancement of digestive enzymes

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E. Antagonistic Activity Of Probiotics

Antagonistic compounds are defined as chemical substances produced by microorganisms (in this case bacteria) that are anthropogenic (bactericidal) or inhibitory (bacteriostatic) toward other microorganisms. The presence of bacteria producing antibacterial compounds in the intestine of the host, on its surface, or in its culture water is thought to prevent the proliferation of pathogenic bacteria and even eliminate these. The structure of the antibacterial compound is often not elucidated and their mode of action has not been found. Furthermore, none of these reports demonstrate that the antibacterial compound is produced in vivo. This will be of significant importance if the production of these compounds and its mode of action are understood. If the production of the antibacterial compound is the only mode of action, it is possible that the pathogen eventually will develop resistance toward the compound. This will result in an ineffective treatment. The risk of the pathogen to develop resistance against the active compound has to be evaluated, to assure a stable effect of the probiotic bacterium [68].

F. Probiotics As Immune Response Enhancers

The immune systems of aquatic animals have two integral components: a) the innate, natural or nonspecific defense system formed by a series of cellular and humoral components, and b) the adaptive, acquired or specific immune system characterized by the humoral immune response through the production of antibodies and by the cellular immune response which is mediated by T-lymphocytes, capable of reacting specifically with antigens. The normal microbes in the GI ecosystem influences the innate immune system, which is of vital importance for the disease resistance of fish and is divided into physical barriers, humoral and cellular components. Innate humoral parameters include antimicrobial peptides, lysozyme, complement components, transferring, pentraxins, lectins, anti-proteases, and natural antibodies, whereas nonspecific cytotoxic cells and phagocytes constitute innate cellular immune effectors. Cytokines are an integral component of the adaptive and innate immune response, particularly IL-1b, interferon, tumor necrosis factor-a, transforming growth factor-b and several chemokines regulate innate immunity [69].

The nonspecific immune system stimulated by probiotics. It has been demonstrated that oral application of Clostridium butyricum bacteria to rainbow trout enhanced the resistance of fish to vibriosis, by increasing the phagocytic activity of leucocytes. These probiotics positively influenced the growth and survival of juveniles of white shrimp and presented a protective effect against the immune system, by increasing phagocytosis and antibacterial activity in the animal cells.

Although the exact mechanism by which these bacteria exerts its antiviral effects is not known, laboratory tests indicate that the inactivation of viruses can occur by chemical and biological substances, such as extracts from marine algae and extracellular agents of bacteria. It has been reported that strains of Pseudomonas sp., Vibrio sp., Aeromonas sp., and groups of coryne forms isolated from salmonid hatcheries, showed antiviral activity against infectious hematopoietic necrosis virus (IHNV) with more than 50% plaque reduction [70] studies reported that a marine bacterium, tentatively classified in the genus Moraxella, showed antiviral activity against poliovirus. Direkbusarakim et al, 1998. Isolated two strains of Vibrio spp. from a black tiger shrimp hatchery. These isolates displayed antiviral activities against IHNV and Oncorhynchus masou virus (OMV), with percentages of plaque reduction between 62 and 99%, respectively [71, 72].

G. Antibacterial Activity Of Probiotics

Many probiotics used in aquaculture are well-known for their antibacterial property against known pathogens. Lactococcus lactis RQ516 probiotic shows inhibitory action against Aeromonas hydrophila when given to Tilapia (Oreochromis niloticus) [73]. Lactic acid bacteria such as Lactobacillus acidophilus, Lactobacillus buchneri, Lactobacillus fermentum, Lactococcus lactis, and Streptococcus salivarius were isolated from Spanish mackerel (Scomberomorus commerson) intestine and were capable to inhibit the Listeria innocua growth [74]. Many Lactobacilli species isolated from the intestine of Anguilla species, Clarias orientalis, Labeo rohita, Oreochromis species and Puntius Carnatic showed significant antimicrobial activity against Aeromonas and Vibrio species [75].

H. Antiviral Activity Of Probiotics

In recent years, the antiviral activity of probiotics has gained attention [76] but the exact mechanism of action through which probiotic bacteria show antiviral effects is still unknown. However, the in-vitro analysis reveals that the inhibition of viruses can occur by secretion of extracellular enzymes produced by the bacteria. For example, Aeromonas species, Corynebacterium, Pseudomonas, and Vibrio species show the antiviral activity against the IHNV (Infectious hematopoietic necrosis virus) [77]. Feeding of probiotic strain Bacillus megaterium has increased the resistance against WSSV (white-spot syndrome virus) in the shrimp, Litopenaeus vannamei [78]. The previous studies have reported that probiotics strain Bacillus and Vibrio species are effective against WSSV and efficiently protect L.vannamei [79]. Application of Lactobacillus as probiotic, either as a single strain or as a mixture with Sporolac resulted in better resistance against the lymphocytic viral disease which is found in Paralichthys olivaceus (olive flounder) [80].

I. Antifungal Activity Of Probiotics

Only few studies have been reported about the antifungal activity of probiotics. Aeromonas strain A199 from Anguilla australis (eel) culture water, had high inhibitory property against Saprolegnia species [81]. In another study, Pseudomonas species M162, Pseudomonas species M174 and Janthinobacterium species M169 have increased the immunity against saprolegniasis in Oncorhynchus mykiss (rainbow trout) [82].

J. Probiotics Safety Regulation

The safety profile of a potential probiotic strain is of critical importance in the selection process. This testing should include the determination of strain resistance to a wide variety of common classes of antibiotics such as tetracycline, nitro furan metabolites, quinolones and macrolides and subsequent confirmation of non-transmission of drug resistance genes or virulence plasmids. Evaluation should also take the end-product formulation into consideration because this can induce adverse effects in some subjects or negate the positive effects altogether. A better understanding of the potential mechanisms whereby probiotic organisms might cause adverse effects will help to develop effective assays that predict which strains might not be suitable for use in probiotic products. Furthermore, modern molecular techniques should be applied to ensure that the species of probiotics used in aquaculture are correctly identified, for quality assurance as well as safety [83].

II. DISCUSSION

The use and application of probiotics in aquaculture shows promise, but needs considerable efforts of research. However, many probiotic products have been thoroughly researched and evidenced their efficacy a possible use on aquaculture. Useful bacterial preparations that are species-specific probiotics have become more widely available to the aquaculture community. These preparations show the specific beneficial effect as disease prevention and offer a natural element to obtain a stable healthy gut environment and immune system. The establishing of strong disease prevention and disease control program, including probiotic and good management practice, can be beneficial to raise aquatic organism production.

III. CONCLUSION

The use and application of probiotics in aquaculture shows promise, but needs considerable efforts of research. It is essential to understand the mechanisms of action in order to define selection criteria for selective probiotics. Therefore, more information on the host/microbe interactions in- vivo, in-vitro, and development of monitoring tools (e.g. microbiology, molecular biology) are still needed for better understanding of the composition and functions of the indigenous bacteria, as well as of microbial cultures of ‘‘probiotic sps.’’ The use of probiotics is an important management tool, but its efficiency depends on understanding the nature of competition between species or strains.

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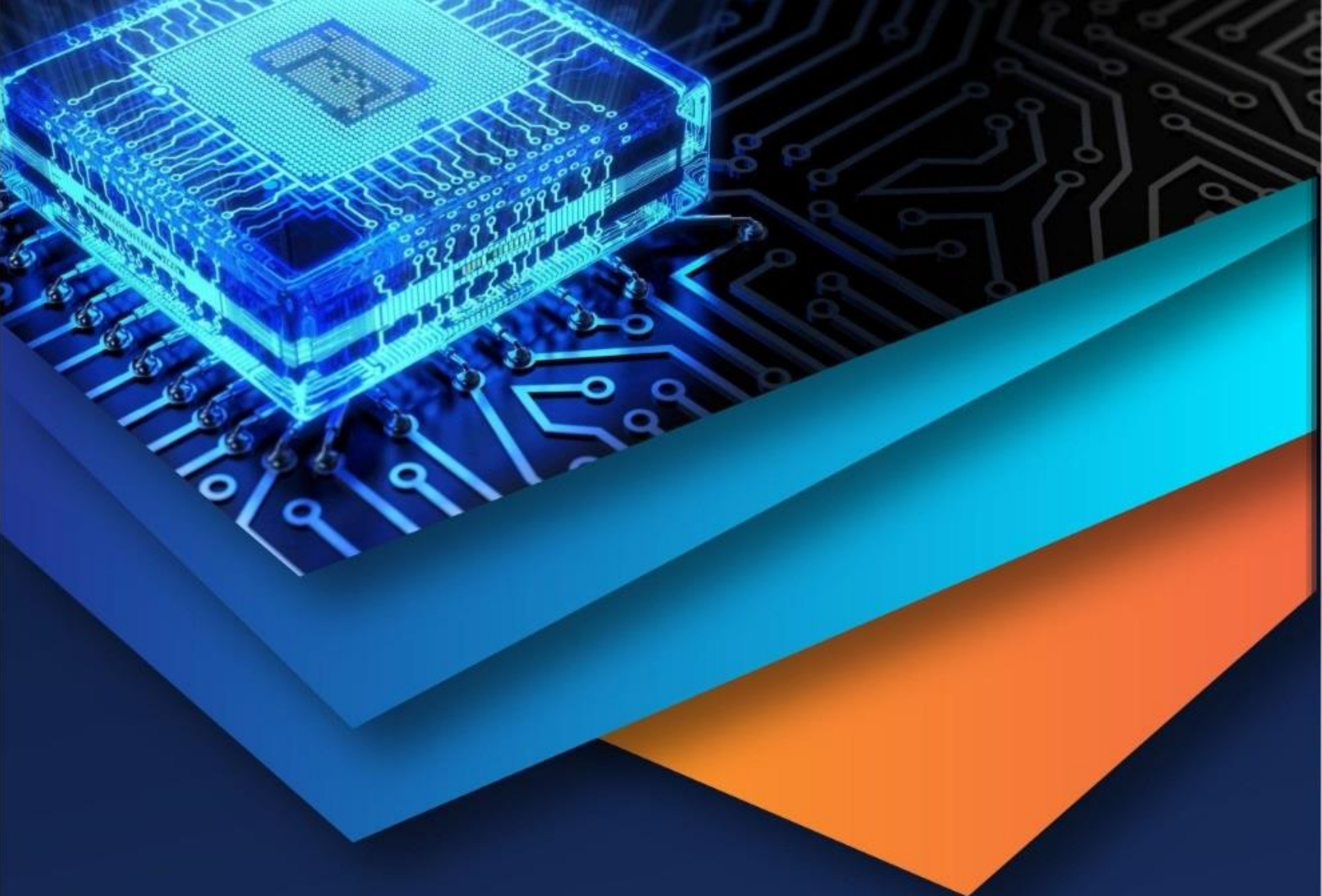

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