Section A-Research paper
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BACILLUS SUBTILIS CAN IMPROVE WATER QUALITY

Bhavna Mehla , Dr.Garima Sharma
Apex University ,Jaipur

ABSTRACT
The use of subtilis in the improvement of water might provide significant economic benefits. Bacillus subtilis, Bacillus pumilus, and Bacillus licheniformis may all improve water quality when exposed to it. Recent research examined the effectiveness of four bacterial isolates: Bacillus subtilis, Bacillus pumilus, Pseudomonas pseudoalcaligenes, and Brevibacterium halotolerant. When applied to water, probiotics improve the quality of the water supply by decreasing harmful ammonia levels and raising nitrite and nitrate levels. Additionally, it inhibits the development of several pathogenic microorganisms in water. Water microorganisms' diversity might be affected by bacterial complexes. B. subtilis was found to have the highest total metal content of all of the isolated bacteria. Variations in immobilising activity were shown to be very sensitive to water temperature, humidity, and pH. Prior to treatment, the effluent was analysed for Biochemical Oxygen Demand (BOD), Dissolved Solids (DS), Colour, Intensity, and Heavy Metals. After determining which Bacillus subtilis strains had the greatest potential for degradation, those bacteria were transferred to an air-sparged bioreactor. In this investigation, we used bacterial isolates that can survive in the presence of metals, including Pseudomonas aeruginosa KP717554, Alcaligenes feacalis KP717561, and Bacillus subtilis KP717559. In order to determine the frequency of faecal coliforms (FC) and faecal streptococci (FS), membrane filtration techniques were used. Bacillus subtilis spores and MS2 phages were tested for their susceptibility to chlorine disinfection, and the role that water quality (pH, temperature, turbidity, and natural organic matter) played in the disinfection process was also investigated. Systems based on Biofloc Technology (BFT) improve water quality with the help of beneficial bacteria. Prior to treatment, dissolved organic matter, biochemical oxygen demand, nitrates, phosphates, and other metals were all reduced by at least 10%.

Keywords: BOD, Dissolved Solids (DS), Probiotic treatment, Heavy Metals, Bacillus Subtilis

1. INTRODUCTION

By converting ammonia to nitrite and nitrate, probiotic therapy improves water quality. It also acts as a barrier against certain harmful microorganisms in the water. Water management is an important aquaculture strategy for disease prevention. Water management is an important aquaculture method for preventing disease outbreaks in a large-scale fish production system (Cha et al., 2013). The pH-temperature relationship was also quite significant. After controlling for NOM disinfectant intake, the presence of natural organic matter had no effect on MS2 phage or B. suhtilis spore inactivation (Barbeau and colleagues, 2004). Bacillus subtilis is a probiotic species that is commonly utilised in aquaculture for water quality management, growth promotion, and immune enhancement. Guo and colleagues (2016). Bacillus subtilis spores are inactivated by ozone at a particular microorganism inactivation threshold that is independent of ozone dosage and water type. Bacillus subtilis is a probiotic that is commonly utilised in aquaculture. Microorganisms have a crucial role in maintaining water quality, waste management, increasing production, and nutrient cycling. Bacillus species are frequent probiotics found in aquatic animals. (Nayak, 2021). In general, litter breakdown rates were faster after Bacillus subtilis addition treatment, while litter bag mesh diameters were comparable. In terms of bacterial effect on water parameters, there were no significant variations between treatments in ammonianitrogen (NH3-N), total phosphorus (TP), dissolved oxygen (DO), and chemical oxygen demand (COD). Litter quality and water factors influenced the rate of decomposition. Litter C/P and N/P ratios were excellent indicators of litter decomposition. (Zhai and colleagues, 2020). Metal-tolerant, plant growth-promoting bacteria might be employed to create bio-inoculants to improve phytoextraction efficiency. Three Bacillus strains were tested for their ability to detoxify heavy metals such as lead, chromium, and copper. Surface waterways, such as lakes and rivers, that are utilised

for drinking water supply, agricultural irrigation, fisheries, and energy generation, are under danger from home, industrial, and agricultural pollution caused by the world's fast rising population.

1.1. Bacillus Subtilis

This research looked at how adding Bacillus subtilis SC02 to a grass carp (Ctenopharyngodon idellus) culture affected the water quality, microbial diversity, and structure of the pond. The treatment group had a greater Shannon diversity index (Shannon) and species richness estimate (Chao), according to pyrosequencing. Differences in microbial communities were seen between the control and treatment groups at the genus level. We draw the conclusion that alterations in the microbial community diversity in grass carp cultures may be the ultimate cause of the improvement in water quality brought about by the addition of the SC02 strain in water. (Zhang et al., 2013)

When added to grass carp production water, Bacillus subtilis SC02 and Pseudomonas stutzeri F1M considerably reduce hazardous nitrogen compounds. It's possible that the BC group didn't find a biomarker on day 15 since they didn't start using the BC water addition until day 14. These bacterial complexes, if they join forces, may alter the make-up of the microorganisms living in water. Based on previous research (Zhang et al., 2019)

Nile tilapia, Oreochromis niloticus, were fed Bacillus subtilis under a biofloc system at concentrations of 0, 1, 2, 3, and 4 grammes (1.19 108 CFU/g) per kilogramme of baseline food. The levels of tannic acid nitrogen, nitric oxide, and nitric oxide were all drastically cut down. Fish given probiotics grew more quickly and had higher enzyme activity. Parameters that were altered by LPS were recovered after treatment with a probiotic. subtilis increased tilapia survival rates, protected them against LPS-induced damage, and boosted their immunity under the biofloc system. To wit: (Mohammadi et al., 2020)

The larval survival rate of Litopenaeus vannamei (white shrimp) was increased by administering the probiotic Bacillus subtilis IS02. The supplement was administered to the water at a concentration of 108 CFU mL-1 once every three days during the 21-day breeding period. When the probiotic was applied, the larvae developed faster and had a higher survival rate. (Mirbakhsh, et al., 2021)

The potential probiotic effects of Bacillus subtilis—a Gram-positive, aerobic, endospore-forming bacterium—were studied in the Indian main carp, Labeo rohita. Both the experimental and control groups received an intraperitoneal feeding of a virulent strain of Aeromonas hydrophila. In the treatment group, as opposed to the control group, significantly more people survived after being challenged with A. hydrophila. Increased enzyme activity was seen after the challenge (Po0.05). The findings provide strong evidence that B. subtilis has economic potential for application in aquaculture. Kumar et al. (2006)

1.2. Survival Of B. Subtilis

The fish used in this research were grouper, Epinephelus coioides, and the goal was to see whether the probiotic Bacillus subtilis E20 might help them develop faster, respond better to illnesses, and be less susceptible to them. Feeding fish probiotic B.subtilis at concentrations of 0 (control), 104, 106, and 108 colony-forming units (cfu) g/f) g1 for 28 days led to increased growth and improved feed conversion ratios (PWG and FE, respectively). The BPHYF/BPHYR primer pair was used to analyse B.Subtilis survival in the distal part of the gut. Grouper, Erichthys coioides, had B. subtilis E20 enter its digestive tract, which led to an increase in cellular and humoral innate immune responses. Fish fed for 28 days were challenged with Streptococcus sp. and an iridovirus, and their relative survival rates were 22.8, 40.9, and 45.5 days, and 21.7, 30.4, and 52.2 days, respectively. The 108 cfu g1 group that was fed for 28 days had the greatest outcomes. B.subtilis in the diet is a great way to boost E.coioides's development and immunological resistance. Liu et al. (2012)

1.3. Inoculation With Bacillus Subtilis

By biosorption, bioaccumulation, and conversion to more stable forms, inoculation with Bacillus subtilis DBM dramatically reduced the bioavailability of Pb in soil. There is a need for B. subtilis DBM to aid in phytostabilization of soil polluted with several heavy metals. X-ray diffraction examination revealed that $Pb_5(PO_4)_3OH$, $Pb_5(PO_4)_3Cl$, and $Pb_{10}(PO_4)_6(OH)_2$ precipitated out on the cell surface during the biosorption process. Several researchers (Bai et al., 2014)

1.4. Dried Bacillus Subtilis

The researchers looked at how a dried Bacillus subtilis culture affected the quality of eggs laid by layers. Results revealed that supplementing diets with 500 mg of B.subtilis culture/kg increased egg production, feed intake, and feed conversion. In the first treatment group, the layers received a placebo diet. The remaining groups were

given either 4 mg of colistin sulphate or 20 mg of zinc bacitracin per kilogramme of body weight. The findings show that dietary supplementation with DBSC at 30.0 106 cfu/g has positive benefits.(Xu et al., 2006)

1.5. Bacillus Subtilis-Fermented Rice Bran (FRB)

The purpose of this research was to examine the impact of feeding Bacillus subtilis-fermented rice bran (FRB) to white leg shrimp (Litopenaeus vannamei) during their rearing at varying salinities on a variety of water quality metrics, water microbial counts, growth performance, body composition, antioxidants/oxidants, and immune biomarkers. In a study using 27 1-m3 fibreglass tanks, young shrimp (0.51 0.11 g) were split into triplicates and placed in each tank. In both the 100 CD+FRB and FRB groups, half of the water in each tank was swapped out every day for fresh water with the same salinity level. Shrimp thrived in tanks with a constant salinity of 35 g/L and no water exchange when fed solely FRB. Antioxidants and oxidants, as well as immunological biomarkers, of white leg shrimp were dramatically modified by water salinity and feeding type. By eliminating the need for additional feed and the associated costs, the use of FRB in shrimp culture at 35 g/L salinity with 0% water exchange should support much superior development and production of the shrimp. According to the research (Abdel-Tawwab, et al., 2022),

Protease-producing Bacillus subtilis E20 was isolated from natto (fermented soybeans) and then introduced into shrimp grow-out culture. There was no mortality among shrimp fed diets containing 0 (control), 106, 107, or 108 colony forming units (CFU) kg1 of B.subtilis E20. In comparison to the control group, shrimp demonstrated superior growth performance and productivity. This is due to the fact that the novel bacteria enhanced protease activity in the digestive system. Methods and Results The natto-isolated protease-producing bacterium, designated E20, was determined to be B. Based on previous research (Liu et al., 2009)

1.6. Water Supplemented

Shrimp in the Mekong Delta, Vietnam, grew faster and had a higher survival rate when their water was fed with B. subtilis CM3.1. The ultimate weight, weight growth, and daily weight gain of the shrimp were not different ($p>0.05$) between the control and treatment groups. When comparing a control group to those given T3, no statistically significant difference was seen in either growth rate or survival.(Truong et al., 2021)

1.7. Degrade The Polysaccharides

When B. subtilis is introduced to a clogging material, it may breakdown the polysaccharides within, therefore increasing the porosity of the substrate. Column 1 (with addition) had a sixfold increase, or 57 times, the hydraulic 18 conductivity of the control column (Column 2). The richness and variety of the microbial communities inside the substrate have risen following its inclusion. Findings suggest that bio-clogging in CWs may be mitigated by adding B.subtilis without posing any health risks.(Ping et al., 2021)

1.8. Bacteria Bacillus Subtilis

To that end, we're doing this research to see what happens when the beneficial bacteria Bacillus subtilis is introduced into carp diet. The density of the bacteria in the medium is 108 CFU/mL, and the technique employed is the exploration approach with three treatments (A, B, and C). Treatment A exhibited the greatest increase in gene expression, with increases in white blood cell count of 29% and red blood cell density of 21%, respectively, and in liver and kidney samples of 19.76% and 16.04%. As reported by (Andieny et al., 2021)

Diets containing probiotic Bacillus subtilis and Bacillus licheniformis at concentrations of 1 9 104 and 1 9 108 CFU/g were given to Litopenaeus vannamei postlarvae for 60 days. At the conclusion of the feeding experiment, many aspects of the animals' growth performance, including their body composition, blood biochemical parameters, and hemocytes count, were assessed. When compared to the control group, shrimp given diets supplemented with the probiotics demonstrated greater gains in body weight, total length, specific growth rate, FCR, and overall survival. according to (Sadat Hoseini Madani et al., 2018)

2. Biosurfactants

Some of the most promising commercial uses of biosurfactants include surfactin. The strain of Bacillus subtilis ATCC 21332 was grown on medium with varying doses of Mn2C. Surfactin yield rose from 0.001 to 0.1 mmol/L with addition of mn2C, with maximum production at 1500 mg/L, or 6.2-fold that of yield obtained in conditions without it. When the ammonium nitrate concentration rose over 0.05 mmol/l, nitrate consumption increased and the nitrogen use pattern shifted to favour nitrate. In contrast, M2C boosted glutamate synthase activity, which improved nitrogen uptake and transformation and increased surfactin synthesis by freeing up more amino acids.(Huang et al., 2015)

2.1. Quality Of The Rearing Water

Olive flounder's growth performance, innate immunity, and disease resistance, as well as the quality of the rearing water, are all improved by exposure to Bacillus subtilis, Bacillus pumilus, and Bacillus licheniformis. In the first experiment, a control meal consisting of a basic preparation and no additional nutrients was made, and three experimental diets were made by adding 0.5% of one of three different Bacillus spp.

Fish given diets containing probiotics saw considerable increases in phagocytic activity. In a 5-day fast in a static water system, fish were given 104 CFU ml-1 of one of two probiotics every 24 hours. Ammonia levels and fish mortality both dropped dramatically throughout the study. The diet should include B. subtilis at a 0.5% concentration for olive flounder breeding water.(Cha et al., 2013)

2.2. Probiotic Species In Aquaculture For Water Quality Control

The purpose of this research is to identify new strains of B.subtilis from fish that have the potential to be employed as probiotics. Both GC-21 and GC-22 are able to withstand antibiotics, suppress pathogens, sporulate, form biofilms, produce cellulase, adhere to epithelial cells and mucus in the gut, and induce anti-inflammatory cytokines.(Guo et al., 2016)

2.3. Impact Of Disinfection Efficacy In Natural Waters

Four untreated surface waters and one ultra-pure buffered water were put through disinfection tests to determine the influence of disinfection effectiveness on natural waters. These fluids were laced with Bacillus subtilis spores, and disinfection tests using free chlorine and chlorine dioxide were performed at 22 1C. As long as disinfectant degradation was taken into account, free chlorine's inactivation kinetics of native spores were not statistically different (at p 14 0:05). The sporicidal efficiency of chlorine was unaffected by passing untreated water through a 0.45 mm filter.(Barbeau et al., 2005)

2.4. Ozonation Process

It will be helpful for treatment facilities that utilise ozonation to disinfect drinking water and want to increase the efficacy of that procedure. The optimal location for the ozone disinfection process may be determined by calculating the dosage of ozone needed for each scenario, which corresponds to the application of ozone at different places throughout the various unit processes.(Choi et al., 2007)

2.5. Isolates

Selected and tested in the presence of Aer. hydrophila in vitro and in an initial in-vivo experiment with C. carpio, isolates B001, B002, and B003 were ultimately chosen for further study. It was shown that the concentrations of waste ions were reduced and that the development of pathogens was stifled. The isolates were categorised as Bacillus subtilis, Bacillus cereus, and Bacillus licheniformis according to their 16S rRNA sequence homology.

Bacillus spp. hydrophila, and a reduction in ammonium, nitrate, and phosphate ion concentrations by the mixed culture of the three chosen isolates during the course of the 80-day experiment. An essential factor in the prospective use of the isolates is that the therapy had no detrimental effects on oxygen sufficiency, growth, or health of the test specimens. Further studies have shown that good bacteria may benefit animal health in a variety of ways, including via increased efficiency in waste ion elimination and decreased prevalence of harmful bacteria (Larmoyeux and Piper 1973; Liao and Mayo 1974). Although testing the potential biological agents in vivo may have produced a more complicated ecological system due to their interaction with the wider bio-community, the results were consistent with those shown in in vitro experiments. Data from (Lalloo et al., 2007)

When given to fish, the probiotic strains B. cereus var. toyoi and B. subtilis C-3102 colonised the intestines without altering the fishes' development rate, proximate composition, or water quality parameters ($P > 0.05$). The haematological profile, including levels of haemoglobin, hematocrit, glucose, and neutrophils, is affected by the use of probiotic supplements. Wild et al. (2014) found that including probiotics into Nile tilapia production systems led to a decrease in nutrient input to the ponds, which contributed to the system's environmental sustainability. According to a recent study (Garcia-Marengoni et al., 2017),

Four different bacterial isolates—Bacillus subtilis, Bacillus pumilus, Pseudomonas pseudoalcaligenes, and Brevibacterium halotolerans—were put through their paces. Similar 16S rRNA gene sequences were found in bacterial isolates S3, S28, S22, and S29. Most Cr (5 g kg-1) was stored in S. The PGPR strain Kluyvera ascorbata SUD165 protects plants against Ni, Pb, and Zn toxicity after being inoculated into the seeds of Indian

mustard and canola (Brassica campestris). This strain generates siderophores and includes the enzyme 1-aminocyclopropyl-1-carboxylate (ACC) deaminase. According to research (Wang et al., 2014),

2.6. Bacillus Subtilis Isolate

Prawns were fed a probiotic strain of Bacillus subtilis that had been isolated from the digestive tracts of young M. rosenbergii. When compared to the control group, prawns that were given their meals using the soaking technique grew at a faster rate (mean weight gain = 2.09) and a larger size (328.84%) after 60 days. Weight increase, feed intake, and FCR all varied significantly (P 0.05) between the soaking technique, other treatment, and control groups. When comparing the water quality and biochemical composition of the treated and control groups, there was no statistically significant difference (P > 0.05).

The number of Bacillus spp. in the prawn stomach and faeces may be decreased after a bathing procedure, leading to slower development. Compared to mixing and spraying, soaking significantly increased the prawns' rate of growth and production. Prawns in the control group had a considerably larger number of Gram-negative bacteria than those in the treatment groups. Results from using probiotic bacteria as a growth stimulant might vary depending on the specifics of the culture. According to a study (Keysami et al., 2012),

2.7. Aquaculture effluent

When it comes to keeping aquaculture water quality high while keeping costs low, bacillus species have shown themselves time and time again to be beneficial. The chemical oxygen demand and biological oxygen demand, as well as the concentrations of nitrates, nitrites, and ammonia, are all known to rise in aquaculture effluent. Heavy metals and dangerous bacteria found in water are two additional sources of pollution that should cause alarm due to their potential to enter the food chain. Since the capacity of microbes to modify water quality is tied to their genetic composition, it is also advised that we improve our genetic knowledge of Bacillus and create new genetic tools. It is also suggested that new genetic techniques be developed and a deeper genomic knowledge of Bacillus be attained.

Genetic makeup is correlated with a microbe's bioremediation potential. Alkalinity, pH, COD, DO, BOD, TDS, phosphates, nitrogenous species, hardness, transparency, heavy metals, and oil leakage are just some of the water quality metrics that Bacillus may affect. It also shows that probiotic Bacillus treatment of aquaculture waters has promise for further usage. To paraphrase (Hlordzi et al., 2020)

2.8. Rice-Crayfish Coculture System (RCCS)

Commercial use of Bacillus subtilis to alter soil and water physicochemical properties are common in the rice-crayfish coculture system (RCCS). However, the effects of this modulation on Cd bioavailability and accumulation need to be more carefully evaluated in light of the increasing cadmium (Cd) contamination in RCCS and its possible consequences for food safety. Inoculating the soil with varying concentrations of soil Cd led to a 40- to 128-fold increase in the number of B. subtilis in the intestines of crayfish. Cd bioavailability was poor because to a decrease in exchangeable Cd (EXC-Cd). (Mo et al. 2022)

Symphytum officinale planted in smelter-contaminated soil raised soil pH by 0.11 unit (T3) and electrical conductivity by 754 mS cm 1 (T2). The soil bacterial population, enzymatic activity, and HM bioavailability were all affected by Bacillus subtilis and saponin. The levels of zinc in the plant's leaves and roots dropped by 12.75 and 27.32%, respectively, during T1. Due to their accumulation in dead cells and cell vacuoles, pyrrolizidine alkaloids remained undetectable in the shoots while increasing by 40% in the roots. The entry of HMs and pyrrolizidine alkaloids into the human food chain is a real concern if S. Officinale is cultivated on polluted ground. The microbial community structure of amended soils changed across treatments. Roots and shoots both saw a reduction in their Zn concentrations as a result of B. subtilis, whereas Cd concentrations rose and then fell. (Li et al. 2021)

3. Biofertilization

Nitrogen losses due to excessive fertiliser usage in agriculture are one of the 15 major causes of non-point pollution. Nitrogen loss from runoff and leaching was drastically cut down on, and NO3-N buildup in the soil was slowed down, thanks to the use of biofertilizer. As a result, the populations of two key groups of bacteria involved in the breakdown of organic matter in soil—Bacteroidetes 28 and Chloroflexi—increased. In addition to a 5% improvement in crop productivity, this 21 technique also improved nitrogen usage efficiency by 11.2%. By reducing the number of amoA-producing bacteria, application of biofertilizer 493 delayed the nitrification process.

Parallelly, it resulted in an increase of 495 narG, nirS, and other denitrifying functional genes. The combination of nirK and nosZ. Because of this, less NO3—N was allowed to build up in the soil, 496 therefore lowering the amount of N lost to drainage and leaching. Up to 11.2 percentage points were gained in both yield and nitrogen usage efficiency (Sun et al., 2020)

3.1. Preventing Pathogens Development

Around 95% of all microorganisms in nature have a net positive impact, such as enhancing food digestion and absorption, blocking the growth of pathogens, or bettering environmental factors. Growing human populations and careless antibiotic usage have put significant strain on farming, cattle, and fisheries, not to mention the natural world. This stress has led to the breakdown of environmental factors and the emergence of pathogenic strains resistant to the vast majority of drugs. One such multipurpose probiotic bacterium that may address these issues is probiotic bacteria. According to research (Olmos et al., 2020)

3.2. Effect Of Hot Water

The purpose of this research was to compare the effectiveness of using hot water (HW), antagonists, and sodium bicarbonate (SBC) alone or in combination to manage Monilinia spp. when storing stone fruits after harvest. Three different types of peaches and nectarines were intentionally infected with M, and their response to various combinations of these treatments was assessed. laxa. Significant differences between individual or mixed treatments were mitigated when fruit was incubated for 21 days at 0°C followed by 5 days at 20°C (Casals et al., 2010)

3.3. Larval Survival

Macrobrachium rosenbergii (de Man) larvae fed both Artemia salina nauplii treated with Bacillus subtilis and untreated Artemia salina. There were considerably more Gram-negative bacteria in the untreated group compared to the treated group. The B. group had a considerably higher larval survival rate after 40 days (P 0.05). Keysami, et al. (2007)

Bacillus subtilis was studied for its effects on Litopenaeus vannamei development and viability after being isolated from the digestive system of Macrobrachium rosenbergii. There were 16 treatment and control aquaria, each with 4 duplicates. The B. subtillis treatment group had a considerably higher survival rate (75.524.62%) and yield (190.0013.13 g) than the other treated and control groups (P0.05). There was a significant decrease in the abundance of Vibrio spp. in both the raising water and the digestive system of the fish over the cultural period. According to research (Far et al., n.d.)

4. Probiotic

Ammonia, nitrite, and nitrate ion levels were all significantly lowered by both probiotic groups in both in vitro and in vivo settings. Survival rates in the BM8 group were significantly different from those in the control group. The results suggest that the immune response had a role in mediating shrimp resistance to illness. The BM5 and BM8 groups had substantially higher mean values for body mass at the end of the study, weight gain, specific growth rate (SGR), food conversion ratio (FCR), and digestive enzymatic activity compared to the untreated control group. according to (Zokaeifar et al., 2014)

Feed is the primary factor in the high overall cost of production in shrimp and fish aquaculture. The use of probiotic microorganisms has recently come to the forefront as a viable option with far-reaching implications. FDA has only officially approved a few number of probiotic strains, and B. subtilis is one of those strains (FDA). The most popular genera are Lactobacillus and Bifidobacterium, and they are mostly employed to prevent the growth of infections in the digestive systems of people and animals. Over the last half-century, the global market for probiotics supplements has steadily expanded. (Olmos, 2014)

Feeding a baseline diet supplemented with 0 g/kg (CT) and BS to fish (53.01 1.0 g) resulted in no growth or disease. All probiotic BS supplemented groups improved their weight gain, specific growth rate, and feed conversion ration at trial's conclusion, although the BS10 group fared the best. All treated groups showed increased catalse activity in the serum, and the B10 group also showed increased myeloperoxidase activity (P > 0.05). Fish in the control (CT), BS3, BS5, BS7, and BS10 groups had 80%, 47.5%, 42.8%, 30%, and 20% cumulative mortality after a Streptococcus agalactiae exposure, respectively. Farms raising tilapia may want to think about using probiotic BS to boost their fish's health and development. Serum and skin mucus from the BS10 group had the greatest levels of lysozyme, protease, antiprotease, superoxide dismutase activity, and immunoglobulin M (P 0.05). The results of this study were published in 2018 (Abarike et al.).

4.1. Marine Aquatic Probiotics

In China, only a handful of marine aquatic probiotics have been approved for use. It exhibited promising properties as probiotics, including the capacity to sporulate and withstand high temperatures. Many studies have shown that probiotics may boost immunity, particularly the capacity of the blood serum of aquatic animals to kill bacteria. Isolation and characterization of Bacillus spp. subtilis 7k, a process-enduring, growth-stimulating, immunity-enhancing, and health-promoting probiotic for use in grouper culture, were the primary aims of this research.

As a possible probiotic, B. subtilis 7k isolated from grouper GIT displays desirable resistance and sporulation. After 8 weeks of food administration, the grouper given probiotics had considerably greater serum bactericidal activity against V. alginolyticus than the control fish. Zhou et al. (2019).

4.2. Dietary Supplementation Of Probiotics

It is becoming more accepted that supplementing animals' diets with probiotics is a viable scientific alternative to antibiotics for improving animal health and production in aquaculture. Bacillus subtilis strains are seen as promising probiotic options in the aquaculture sector. The purpose of this research was to compare the impact of the NZ86 and O14VRQ strains on many aspects of Nile tilapia's innate immunity (Oreochromis niloticus). According to a recent study (Galagarza et al., 2018)

4.3. Prebiotics And Synbiotics

We supplemented the diets of Pengze crucian carp (Carassius auratus var. Pengze) with prebiotics and probiotics for 70 days to see how it would affect their growth rates. Pengze fish fillet quality, immunological responses, and antioxidant status were greatly enhanced by treatment with beta-glucan and B. subtilis. Results indicated that B. (Cao et al., 2019)

This bacteria uses a wide range of complementary and antagonistic processes, including competitive exclusion and immunological stimulation. The commercial potential for Bacillus species in general, and B. subtilis in particular, is unquestionably rising, and this is true for a variety of reasons. The evidence supporting their use as probiotics and synbiotics to improve host nutrition, immunity, and disease resistance is overwhelming. The actual mode(s) of action of the strains utilised need to be uncovered, and this can only be done with further scientific validations. Most research supports the idea that this bacteria may be beneficial to aquatic creatures' health. (Nayak, 2021)

4.4. The Cellulase – Synthesizing Probiotic Bacteria

Leaf meal from inoculated Leucaena leucocephala was fed to rohu, Labeo rohita, fingerlings, and fish to boost their growth rates. To create the diets, either raw or cooked (inoculated with fish intestinal bacteria). Incorporating more Leucaena leaf meal resulted in higher a-amylase activity. For 15 days at 37 1C, Leucaena leaf meal was inoculated with Circobacter circulans (isolated from Oreochromis mossambicus), which has extracellular cellulolytic and amylolytic activity. Including more raw leaf meal in one's diet resulted in a rise in cellulase activity.

This shows that the fish were able to successfully break down the starch in the treated leaf meal diets. Before the meal was formulated, the cellulase-synthesizing probiotic bacteria had already broken down the Leucaena leaf into cellulose, hemicellulose, and crude carbohydrates. Fish given diets with increasing amounts of raw leaf meal exhibited cellulase activity, suggesting that the bacteria were actively digesting the cellulose in the diet. This indicates that bacteria are actively breaking down dietary cellulose. To wit: (Bairagi et al., 2004)

4.5. Shrimp Larval Development

When the probiotic was added to the larval rearing water at a concentration of 109 cfu L1, the growth of the shrimp larvae was greatly sped up. Treatment with 109 cff laboratory water resulted in a considerably greater larval survival rate compared to the control. Serine proteinase and glutathione peroxidase gene expressions were not found to be significantly different. Scientists believe that the probiotic B subtilis E20 might be useful in the rearing of shrimp larvae. "(Liu et al., 2010)"

4.6. Relative Percentage Survival (RPS)

Isolated from the tropical estuary environments of Cochin, Bacillus subtilis MBTDCMFRI Ba37 is a strain of bacterium known for its antibacterial properties. Aquaculture pathogens including Vibrio and Aeromonas were killed, and it also showed exoenzymatic activities and tolerance for a range of physiological circumstances. Efficacy on growth, immunological parameters, and antioxidant enzyme activities was measured throughout the

course of a 21-day feeding study in juvenile Etroplus suratensis (E.suratensis). B.subtilis Ba37-fed fish had a greater relative percentage survival (RPS) than the control group after 15 days of a challenge test. A study by Nair and colleagues (2021)

4.7. The Role Of Water Quality

The effectiveness of chlorine in killing Bacillus suhtilis spores and MS2 phages was studied, and the influence of water quality (pH, temperature, turbidity, and natural organic matter) was determined. The effectiveness of chlorine was found to be most greatly explained by temperature and pH. With the consumption of disinfectant by NOM taken into consideration, the presence of NOM either had no effect on spore inactivation of B. subtiiis or greatly enhanced it. Until a concentration of 5 mg/L, kaolin particles offered hardly little defence.

When compared to hypochlorite ions (OCP), hypochlorous acid's (HOCL) effectiveness was less affected by changes in water temperature. When compared to the other three factors affecting water quality, inorganic turbidity (produced by adding kaolin) had a little influence. There needs to be further investigation on the effects of pH and temperature on chlorine's effectiveness. This has been shown to be true (Barbeau et al., 2004).

5. Biofloc Technology (BFT)

In a biofloc technology (BFT) system, using probiotics may enhance water quality, which in turn boosts fish productivity. The condition factor of fish raised in BFT + BS was greater, and they grew and gained weight more rapidly. A culture solution containing 4 108 cells per mL was added to the water supply once a week. Even in the fish of the system to which it was introduced, the bacteria Bacillus subtilis is often detected in the tambaqui stomach. (dos Santos et al., 2021).

5.1. Acute Hepatopancreatic Necrosis Disease (AHPND)

The Pacific white shrimp business is negatively affected by Acute Hepatopancreatic Necrosis Illness (AHPND), a severe disease caused by several Vibrio spp. Water-soluble and feed applications of the viable probiotic Bacillus subtilis AQAHBS001 were tested in vitro for their ability to suppress several VPAHPND strains. The addition of 1 103-1105 CFU/mL of this probiotic to the water successfully decreased total ammonia, but it did not enhance shrimp development or resistance to VBAHPND. In addition to boosting the expression of the genes for prophenoloxidase, lysozyme, and anti-lipopolysaccharide factor, this probiotic also greatly increased immune responses through phagocytic activity and clearance efficiency. according to (Kewcharoen and Srisapoome, 2019)

5.2. The Control Group (CON)

Six weeks were spent raising broiler chicks, during which time they were randomly assigned to one of four treatments. The CON group of birds were given meals free of BS fmbJ and antibiotics. At 42 days, the BS-2 group had gained more weight than the CON group overall (P 0.05). Broiler hens' response to antibiotics resulted in improved growth rates, antioxidant capacities, and meat quality. Drip loss of breast meat after one day of storage was higher when broiler birds were fed more probiotics, for reasons that were not entirely understood (Park and Kim, 2015). Nearly 85% of customers regarded colour a main issue when purchasing meat, despite the fact that the link between softness and meat colour was still contentious (Huff-Lonergan et al., 2002). (Bai et al. 2017)

5.3. Microbial Biochar Formulations (MBFs)

Agricultural waste biochar was loaded with Bacillus subtilis SL-13 to produce microbial biochar formulations (MBFs). Common agricultural fertilisers and pesticides may have serious side effects; MBFs can help mitigate these risks. Soil amended with biochar was shown to be more productive for plant growth than untreated soil. According to a recent study (Tao et al., 2019),

6. Heavy Metals

Bacillus subtilis B38 is a mutant strain created by irradiation and selection in a high cadmium environment. Initially in the biosorption process, metal bound quickly to both living and nonliving B38. With variations in solution pH, temperature, biomass dosage, and ionic strength, the maximal adsorptive amount of the heavy metals on B38 also varied. The bacteria preferred the cationic heavy metals over the anionic ones. The exponential equation adequately represented the biosorption kinetics of metal binding by L B38 biomass.

The kinetic model for the biosorption of heavy metals onto NL B38 was consistent with the experimental data, in contrast to the pseudo-second-order model. Initial exposure of both biosorbents to Cd(II), Hg(II), and Pb(III) ions resulted in rapid metal binding on the cell walls. The results revealed that in the monocomponent systems,

the metals with the highest absorption capability had the most inhibitory influence on the bioremediation of other metal ions. According to research (Wang, Sun, 2013)

Slow growth and limited bioavailability of heavy metals significantly reduce the effectiveness of this green technology, despite the fact that phyto-extraction is a developing cost-effective alternative for the rehabilitation of heavy metal-contaminated soils. Pseudomonas aeruginosa KP717554, Alcaligenes feacalis KP717561, and Bacillus subtilis KP717559, all of which are metal-tolerant bacterial isolates, were employed. For instance, (Ndeddy Aka and Babalola, 2016)

6.1. Heavy Metal Contaminated Water

Due to the hazardous effects of these metals on living creatures, the cleanup of heavy metal polluted soils has become an urgent concern. This study examined the impact of Pseudomonas fluorescens RB4 and Bacillus subtilis on the development and phytoremediation capability of Catharanthus roseus in soils polluted with copper and lead. Water extractable Pb and Cu levels were found to be considerably higher in Pb- and Cu-accumulating bacterial strains. According to research (Khan et al., 2017)

Table I. Sediment isolates' minimum inhibitory concentrations (MIC) for heavy metals

Isolates	Heavy metals (mM)				
	Hg	Cr	Cu	Zn	Ni
B. cereus BF1	0.01	3.0	6.0	6.0	4.0
B. thuringiensis BF2	0.01	3.0	6.0	6.0	4.0
B. atrophaeus BF3	0.01	3.0	4.0	2.0	4.0
B. aguimaris BF4	0.01	2.0	4.0	2.0	2.0
B. thuringiensis BF5	0.01	3.0	6.0	6.0	4.0
B. aguimaris BF6	0.01	2.0	4.0	6.0	4.0
B. cereus BF7	0.01	3.0	4.0	6.0	4.0
B. subtilis BF8	0.01	3.0	6.0	1.0	4.0
B. simplex BF9	0.01	2.0	6.0	0.6	2.0
B. amyloliquefaciens BF10	0.01	2.0	2.0	1.0	2.0
B. drentensis BF11	0.01	2.0	4.0	2.0	2.0
B. aguimaris BF12	0.01	3.0	6.0	6.0	4.0
B. fusiformis BF13	0.01	2.0	4.0	2.0	2.0
B. subtilis BF14	0.01	3.0	6.0	6.0	4.0
B. cereus BF15	0.01	3.0	6.0	4.0	4.0
Arthrobacter nitroguajacolicus BF16	0.01	3.0	6.0	6.0	4.0
B. pumilus BF17	0.01	3.0	4.0	4.0	2.0

The lake's high metal concentration may be traced back to the mafic and ultramafic rocks that are exposed in the Büyük Menderes Rivers' drainage region. The agricultural region around the lake is a likely source of heavy metals due to the usage of pesticides, with human sources also contributing.

Bacillus subtilis' impact on Ipomoea pes-caprae has been examined at 25, 50, and 100 ppm nickel. pes-caprae increased in prevalence in B-infected plants. The impact of inoculating Bacillus subtilis on I. pes-ability caprae's to accumulate nickel in the presence of nickel-contaminated soil was evaluated. Nickel in the soil had a major impact on the development of the plants, particularly their stature and the depth of their roots. This may be because of PGPR's capacity to store heavy metals and boost phytoextraction activities in plants, as well as its mechanism that may influence plant development. To wit: (Acinas et al., n.d.)

To address heavy metal pollution, the biosorption method may be used instead of the less effective chemical precipitation and ultrafiltration. Atomic absorption spectrometry (AAS) and inductively coupled plasma optical emission spectroscopy were used to evaluate the biosorption capability of each isolate (ICP-OES). the bacteria Bacillus subtilis NSPA13 (85%). according to (Syed, Chinthala, 2015)

6.2. Physico-Chemical Properties Of Water

Lake Bafa is the biggest coastal lake in Turkey, thus scientists decided to look into its chemistry and physics. Membranefiltration methods were used to ascertain the prevalence of faecal coliforms (FC) and faecal streptococci (FS). According to the results of this preliminary research, public health officials should keep a close eye on the lake because of its importance to agriculture, fishing, and pleasure. Kacar et al. (2014)

Table II. Water physico-chemical parameter values

Physico-chemical parameters			
Temperature	**Salinity**	**pH**	**DO**
28.1	14.2	8.30	6,68
27.9	14.2	8.25	5,15
28.7	14.1	8.32	7,05
29.2	14.1	8.39	7,55
29.3	14.2	8.44	7,2
28.6	14.3	8.22	6,05
29.6	14.3	8.37	7,46
28.5	14.1	8.35	7,59
28.5	14.0	8.32	7,17
28.7	14.1	8.36	7,4
32.0	14.2	8.40	6,49
28.5	14.7	8.55	9,33
28.6	14.1	8.72	11,2
28.7	14.0	8.38	8,81
26.5	14.3	8.21	11,29
26.8	16.3	7.99	3,65
29.7	17.4	9.08	6,89

(Table.II) displays the minimum inhibitory doses of several heavy metals. Isolates of B. cereus BF1, B. subtilis BF14, and A. nitroguajacolicus BF16 fared best when exposed to a battery of metals.

Isolates found in sediment have greater resistance to Cu, Zn, and Ni. All of the isolates had the highest concentration of mercury among the hazardous metals tested. Highly resistant strains were Bacillus aguimaris BF 12, Bacillus subtilis BF14, and Acinetobacter nitroguajacolicus BF16.

There is a synergistic impact between the bioaugmentation of a UV-mutated microbe, Bacillus subtilis 38, and the amendment of a bio-fertilizer, NovoGro, for the immobilisation of cadmium (Cd) in soil. This research suggests a method that might be used for in-situ cleanup of Cd-polluted soils at a reasonable cost. Temperature, soil moisture, and pH all played major roles in determining the intensity of the immobilisation effect. It would seem that the optimal circumstances for microbial activity coincided with the peak immobilisation on Cd. The combination of B. subtilis 38 and NovoGro (SNB therapy) was more effective than either SB or SN treatment alone. According to research (Jiang et al., 2009)

6.3. The Physicochemical Characterization

Effluent from International Textile Industry (Nig.) LTD at the Odongunyan Industrial Estate in Ikorodu, Lagos was analysed physicochemically. Effluent from International Textile Industry (Nig.) LTD at Odongunyan Industrial Estate, Ikorodu, Lagos, was analysed physicochemically. Biochemical Oxygen Demand (BOD), Dissolved Solids (DS), Color, Intensity, and Heavy Metals were all measured in the effluent before it was treated. Degradation-capable strains of Pseudomonas aeruginosa and Bacillus subtilis were immobilised on agar-agar and then transferred to a bioreactor with an air sparger. The study showed that COD, BOD, nitrates, sulphates, phosphates, and heavy metal biosorption were all reduced by over a hundred percent. According to research (Aajao et al., 2011),

6.3.1. Lead(Pb) Toxicity

Mung bean's growth capacity and resistance to Pb toxicity might be enhanced by inoculating the plant with Pb tolerant Bacillus subtilis PbRB3, which in turn would speed up Pb rhizoaccumulation from metal polluted environments. Pb-toxicity may have many negative impacts on plant development, but those effects can be mitigated by inoculating the plant with beneficial bacteria that are resistant to PB. Based on the results of this research, a chosen group of bacteria may make a good inoculant for removing lead. Changes in root Pb content after inoculation were similar for the Pb250 and Pb500 treatments. Superoxide dismutase activity was much greater in both Pb toxic and non-treatments compared to the inoculation control group. Based on previous research (Arif et al., 2019)

6.3.2. Adsorption Of Multiple Heavy Metals

Bacillus subtilis is an effective biosorbent for the simultaneous removal of several metal ions (cadmium, chromium, mercury, and lead). SNB treatment resulted in a 55.4%-97% reduction in edible plant parts in the examined plants (lettuce, radish, and soybean). The growth of both local and foreign microorganisms may be stimulated by NovoGro. Bioremediation of heavy metal-contaminated soil using B38 in combination with NG.

Heavy metal absorption by edible plant parts was drastically reduced when B38 and NG were introduced. This lessened the impact on soil bacteria and helped exotic B38 and native microbes flourish. Reference: (Dih et al., 2019)

6.3.3. Bioremediation Of Heavy Metals

One of the greatest health and environmental issues is the bioremediation of heavy metals in the ecosystem. Non-biodegradable heavy metals may be hazardous to microorganisms. Technologies based on microbial fuel cells, microbial gene transfer, and biofilms have emerged as serious rivals in this space in recent years. Using gene transfer inside biofilms to remove heavy metals is an area that requires further study. To wit: (Igiri et al., 2018)

6.4. Biopolymers

The biological properties of g-PGA produced by Bacillus subtilis (natto) in both small- and large-scale fermenter setups have been analysed. g polyglutamates are completely water soluble in their K+, Na+, NH4 +, Ca2+, and Mg2+ salt forms. They are biopolymers that serve a variety of purposes and are both safe for humans and the environment. In vivo feeding experiments were conducted using broilers and egg-layers to help describe some of the biological functions. Using 1H- and 13C-NMR spectroscopy and FT-IR spectroscopy, the structural properties of the salts of g-Polyglutamates (Na+,K+,NH4 +,Ca2+, and Mg2+) were determined. Significant roles in defining the biopolymers' chemical and biological usefulness are played by the conformational states and unique binding features of the a-carboxylate anionic groups. Calcium and magnesium compounds will be soluble in future biopolymers, and the resulting ionic complexes will be rather stable. Natural, protein-based biopolymers like G-PGA and its derivatives provide potential benefits to the economy and the environment. The unusual polyanionic properties and structural characteristics are important for bioflocculant functions, detoxification of heavy metallic ions, and possibly activities against pathogenic microbial activity and the growth of tumour cells, in addition to their high hydrophilicity and reactivity, which are important for many biological functions. Ho et al. (2006)

CONCLUSION

Both the experimental and control groups received an intraperitoneal feeding of a virulent strain of Aeromonas hydrophila. The findings provide strong evidence that B. subtilis has economic potential for application in aquaculture. By irradiating and selecting Bacillus subtilis (B38) in a high cadmium environment, we were able to isolate a strain with desirable properties. The results revealed that in the monocomponent systems, the metals with the highest absorption capability had the most inhibitory influence on the bioremediation of other metal ions. Slow growth and limited bioavailability of heavy metals significantly reduce the effectiveness of this green technology, despite the fact that phyto-extraction is a developing cost-effective alternative for the rehabilitation of heavy metal-contaminated water. There were three metal-tolerant bacterial isolates found in the mining waste: BCr3, BCd33, and BNi11. Isolates of B. cereus BF1, B. subtilis BF14, and A. nitroguajacolicus BF16 fared best when exposed to a battery of metals. All of the isolates had the highest concentration of mercury among the hazardous metals tested. The International Textile Industry (Nig. LTD) at Odongunyan Industrial Estate, Ikorodu, Lagos, had its effluent analysed for its physicochemical composition. COD, BOD, Nitrate, Phosphates, and other heavy metals were all reduced by % before treatment.

Conflicts Of Interest

There are no competing interests in investigating Bacillus subtilise potential usefulness in enhancing water quality. The study's overarching goal is to help scientists learn more about the positive effects of this bacterium. The research was done without bias, by all applicable procedures and principles. The writers have no financial or personal ties to the topic which may be seen as a conflict of interest. These results are based entirely on the data we gathered and examined.

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