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Analysis of Isolated Bacterial Bioremediation and biochemical degradation studies of Seafood Plant Effluent

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Abstract:
Veraval, Gujrat fish landing stations are being examined to assess the prevalence and antibiotic resistance of E. coli bacteria, respectively (E. coli). Samples were analyzed at two locations in Veraval, Gujrat. Fish landings, seafood processing plants, and local fish markets are all included in the sampling area (fish samples). Total bacterial counts were determined in 48 swabbed samples collected from two distinct fish processing facilities. Pre- and post-packaging samples from fish markets and seafood processing industries provided the majority of the E. coli strains and Ballicius sp strains used in this study Ampicillin resistance was at least 60%, and chloramphenicol resistance was at least 3% for E. coli and Chloramphenicol(C) resistance was 61% and Erythromycin (E) resistance was 2% for ballicius sp in this study.

Keywords: *ampicillin-resistant, chloramphenicol, E. coli., bioremediation, effluent treatment, eutrophic componenet degradation*

1. Introduction

Fish is an essential part of human nutrition, providing 60 percent of the world's protein needs. Fish provides 30% of the annual protein needs of 60% of developing countries. About 16 percent of the animal protein consumed by the world's population comes from this high-quality protein source. Fish accounts for 17% of the continent's protein intake, making it one of the most affordable and readily available. Fish is an excellent source of protein and nutrients because of its digestibility and nutritional value [1]. There is a large range of bacterial pathogens that can infect fish, the majority of which can cause disease and are therefore called saprophytic by some.

According Fresh fish muscle's microbial richness is highly dependent on the fishing areas and the surrounding environment. There are a number of examples of non-indigenous organisms that contaminate the fish or the environment, including Escherichia coli and Clostridium botulinum. Gram-negative, rod-shaped bacteria, E. coli among warm-blooded creatures, it is most typically found in the lower intestine [2]. A wide variety of E. coli serotypes can cause serious food poisoning in their hosts and lead to the recall of products as a result of contaminated food being sold. Examples of naturally occurring bacterial diseases found in fish habitats are Vibrio and Aeromonas species.

Escherichia coli strains, both normal and atypical, were found in fish sold at retail in Gujrat, India, by Thampuran et al. (2005). Poor water quality, overfishing, and other stressors, such as hormonal imbalances in fish and nutritional deficiencies, can all lead to pathogenic bacteria in fish, but this is not always the case. Streptococcus species, Vibrio species, Aeromonas species, Salmonella species and others are among the pathogenic and potentially harmful bacteria associated with fish and shellfish. Veraval area having a large seafood processing industry makes this research essential for local biodivercity.

Sewage discharges from outfalls, combined sewer overflow, and rainwater discharges can cause water contamination even in clam farming locations. Energetic Escherichia coli enteropathogens can cause human gastroenteritis, which is widespread in coastal areas and frequently found in seafood that has been contaminated by the bacteria. The microorganisms that might cause many infectious diseases in humans can be concentrated by bivalves as filter-feeding organisms. Furthermore, oysters can be eaten raw or barely cooked, which makes them a possible source of pathogenic E. coli infection [3]. Gram-negative, anaerobic bacteria are most common in the gastrointestinal tracts of humans and other endothermic animals? Even while most of these commensal E. coli strains are innocuous, there are a number of pathogenic variants that can infect people. As a biomarker of fecal contamination, E. coli has also been recommended as an indicator of antibiotic resistance in food, marine, and freshwater ecosystems.

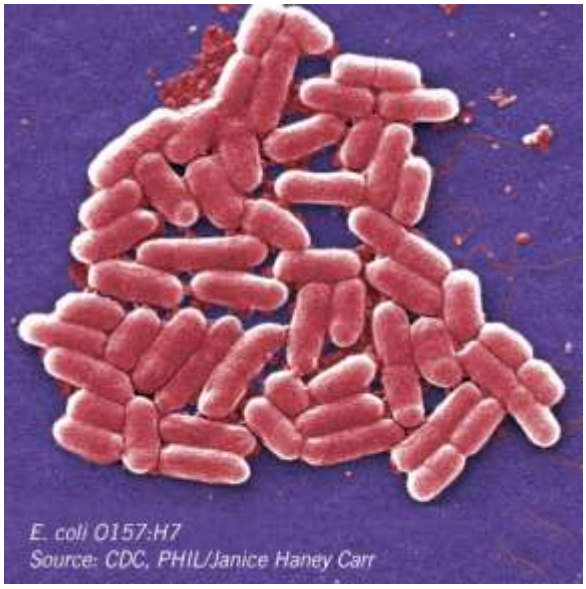

Fig. 1: E, Coli Strain Structure

People should be worried about eating seafood since it may contain harmful strains of E. coli. As a result, regulatory limitations and monitoring programs based on E. coli counts, bivalve fecal coliform levels, or bivalve growing area fecal coliform levels have been implemented in nations like Brazil, the United States, and the European Union. Many outbreaks of foodborne illness have been linked to E. coli that produce shiga toxin, particularly E. coli O157:H7. Non-O157 isolates from serogroups O26, O45, OC9 and OC91 have also been shown to cause major human illnesses. However fresh research suggests that these other serogroups may potentially be responsible for human illness.

For E. coli strains, serotypes are a useful tool, but they don't completely characterize a strain. Pulsed-field gel electrophoresis (PFGE) and other genotyping methods have been used to identify and link E. coli strains (PFGE). As a result of its high level of discrimination, PFGE is widely regarded as the gold standard technology for the development of a reproducible DNA fingerprinting method.

For the identification of bacteria, antimicrobial resistance (AMR) might be used. An increase in AMR E. coli is blamed on widespread use of antimicrobials by both humans and nature. As bivalve mollusks can accumulate AMR bacteria, it has been suggested that bivalves could be used to monitor environmental pollution by the developing problem of AMR bacteria.

The phrase "wastewater" refers to water that has been contaminated by human activity. More than 99.9% of all wastewater is water and the rest solids; it comes from a wide range of sources, including home, commercial, agricultural, and stormwater runoff. The chemical components and flow conditions of wastewater are utilized in the design of each wastewater treatment facility to identify the characteristics of the wastewater [4]. When it rains, storm water will flow into the sewer system,

resulting in a higher volume of wastewater. The organic and inorganic constituents of wastewater are used as a chemical quality indicator. The following parameters are commonly taken into account when determining the chemical characteristics of wastewater: In addition to BOD and COD, other parameters to consider include total solids and volatile solids as well as nitrogen, phosphorus, pH, and alkalinity [11].

A. Chemical oxygen demand (COD)

To put it another way, a higher concentration of COD in wastewater usually means a higher level of contamination within. COD levels in industrial wastewater are frequently higher than in municipal or domestic wastewater. If the BOD/COD ratio is greater than 0.5, biological treatment of the wastewater is an option. The amount of oxygen necessary to chemically stabilize carbonaceous organic stuff is what's used to calculate it. This apparatus can be used to measure wastewater components such as organic matter, nitrite, sulfide, and ferrous salts.

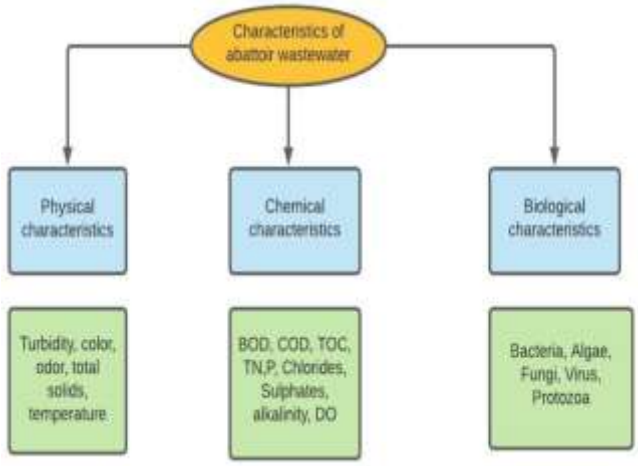

Fig. 2: characteristics of wastewater

B. Biochemical oxygen demand (BOD)

Under aerobic conditions, bacteria need this much oxygen to decompose organic matter. BOD, like COD, is a measure of contamination because it affects the quantity of dissolved oxygen that aquatic species require and, if it is less than 6 mg/L, may cause death. This ranges from 100 to 200 mg/L to 200 to 300 mg/L to 300 to 560 mg/L for home wastewater with small industrial waste in it, depending on the strength of the wastewater. This means that the presence of low dissolved oxygen in wastewater indicates the presence of high BOD levels in the water. Water's organic biodegradable content increases in proportion to the BOD content. Due to an increase in biodegradable organic pollutants, microbial fuel cell technology, or microalgae, can lower wastewater's BOD level [5]. According to Zhang et al., MFC can remove up to 98.6% of BOD, while Marassi et al. found that the tubular MFC can remove up to 96% of BOD. By producing O2 during photosynthesis, microalgae have been shown to successfully reduce the BOD level of wastewater, with 87 percent removal efficiency.

C. Total nitrogen and phosphorus

Nitrogen and ammonia are plant nutrients that can be found in wastewater from fertilizer producing organizations, agricultural industries and businesses that use corrosion inhibitors. Among the nitrogen compounds present in waste water are organic and inorganic nitrates and nitrites, ammonium, the amino acids, and organic nitrogen composites. There is a greater whole that each of these nitrogen atoms is a small piece of. This mineral is found in the form of orthophosphates, condensed and organic phosphates in all aquatic environments. If wastewater is discharged without treatment, eutrophication, which can lead to the extinction of aquatic habitats, can occur [6]. Microalgae treatment processes have been

shown to remove between 87 and 93 percent of nitrogen and phosphorus from wastewater, according to industry reports.

D. Metals

Wastewater from the manufacturing, mining and textile industries tends to include a lot of metals. There are several typical pollutants in industrial wastewaters that are made up of metals including arsenic and iron as well as chromium and lead, copper, tin, sodium, potassium, mercury, aluminum, and nickel. Heavy metals are frequently found in the wastewater produced by various industries, including iron and steel, mining, microelectronics, and textiles. In addition to increasing treatment costs, metals in waste water are known to cause a wide range of environmental issues, including plant deformation, algal blooms, the death of aquatic organisms and the development of debris and sediment. Carcinogenicity, recurrent asthma, skin problems, depression, damage to internal organs, coughing, and disorders of the neurological system are just some of the human health implications.

E. Viruses and bacteria

Human pathogenic viruses are frequently found in wastewater, and as a result, previously unrecognized occurrences of these pathogens are now classified as wastewater pollutants. Environments where wastewater is discharged are frequently linked to waterborne epidemics of both bacterial and viral diseases, according to the CDC. Gastroenteritis, hepatitis, and respiratory tract infections are just a few of the illnesses that enteric viruses have been linked to [7]. These pathogens are abundant in wastewater, which has been extensively studied for their prevalence and amount in untreated and treated whey as well as in wastewater itself, including polyomaviruses (PyVs), bacteriophages, fecal coliforms (FCEs), E. coli and others. Human excrement brought a number of viruses to sewer systems, including SARS-CoV-2, which has been linked to the COVID-19 pandemic. Current studies are looking into the long-term effects of SARS-CoV-2 on aquatic environments.

F. Pharmaceutical compounds

The long-term effects of these chemicals on human and aquatic ecosystems remain unknown, making them a developing contaminant. Anti-convulsants, anti-cancer medicines and beta-blockers are just a few of the pharmaceutical substances that have lately been detected in wastewater. Lipid-regulators and antidepressants are also found in wastewater. This is due to the fact that medications administered to humans are excreted either in their original form or after being digested. The effluent from a wastewater treatment facility contains some pharmaceutical chemicals despite the fact that most of these compounds are biodegradable. Sewage treatment facility effluent contained over 2 g/L of tetracycline and ibuprofen, as well as contrast media, caffeine, and codeine [8]. Antibiotics were found at quantities less than 0.05 ng L1 in another effluent by Clara et al. Antibiotics, Bisphenol A, and analgesics, anti-inflammatory medications, and beta-blockers had a clearance rate of roughly 50% and 30%–40%, respectively, according to studies.

G. Water quality

Due to increasing stocking density, reticulated systems for intensive culture produce large volumes of particulate organic and soluble inorganic excretory waste. Overfeeding and a high nutrition food composition are the primary causes of this waste, which has an impact on the fish's ability to survive, grow, and reproduce. In addition to endangering the ecosystem, nitrous and phosphorus waste can weaken fish immune systems, making them more vulnerable to parasite and disease infection.

Fish excrete ammonia by bronchial diffusion, which is a key metabolic waste product. Mineralization of sediment and bacterial ammoniafication both contribute to the production of nitrates. Fish are particularly vulnerable to ammonia and nitrite, which are both oxidized to nitrate through the nitrification process. Denitrification can also lead to the production of nitrite. Higher amounts of nitrate are hazardous to fish, causing gill hyperplasia and necrosis, as well as histological evidence for kidney

and liver damage and a decrease in growth rate [9]. Ammonia-induced epithelial lifting on gill filaments results in respiratory impairment and death when exposed to high ammonia concentrations.

No imbalance in nature means nitrite is an important intermediate in the processes of nitric and denitrification, ammonia decomposition and the uptake of nitrate. It is possible to derive all three nitrogen gases (nitrous and nitric) from nitrate by denitrification. In the end, it's a waste product. At values of 0.15 mg/l and above, fish die from brown blood disease because the conversion of haemoglobin to methaemoglobin in blood restricts oxygen delivery and causes fish to perish. Nitrite concentrations are also known to alter weight gain, specific growth rate, and the efficiency with which food is converted into energy.

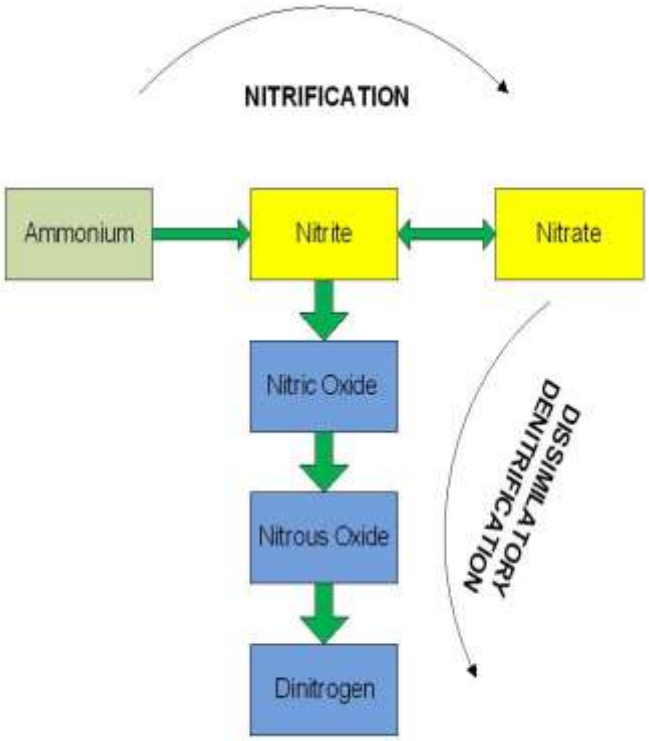

Fig. 3: Nitrification and denitrification cycle

Adding phosphorus to fish meals boosts weight gain and feed conversion ratio, making it a crucial component. In many cases, the lack of an acidic stomach in some species and the presence of phytic acid in vegetable protein prevent the phosphate in vegetable protein from being fully utilized. As a result of phosphorus excretion in the feces, water quality deteriorates, increasing algal blooms and eutrophication.

2. Materials and methods

1. Sample collection and E. coli & Bacillius sp isolation

Samples collected from Veraval, Gujrat in Tamil Nadu, India, totalling 48 in all. Fishery landing facilities, seafood processing plants (packing and ice-packed fishes), and local fish markets are included in the sampling region (fish samples). A 10 cm2 sample of each type of fish, ice, and serving utensil was taken and suspended in pH-8.0 peptone water before being sent to the laboratory within 1-2 hours. EMB agar (Himedia, Mumbai) and Zobell marine agar (Himedia, Mumbai) were used to incubate the samples for 24 to 48 hours. On 12 EMB agar plates, the isolate was subcultured even though nearly pure culture growth had been achieved from a fish on the media.

Antimicrobial substances produced by Bacillus bacteria and their capacity to sporulate make them ideal for use as probiotics in humans and animals because of the wide range of different habitats where they can thrive. After being isolated from the intestines of an L. calbasu, the FS1, FC3, and FC6 bacteria

thrived for 14 hours at 37 °C in a nourishing broth under constant shaking. Methods for studying fish illnesses included agar well diffusion and cross-stripping.

2. Sequencing and phylogenetic analysis

In order to purify the PCR products, a Genei purification kit was employed (Genei, Bangalore). The amplified 16S rDNA genes' substantially complete sequences were extracted using automated sequencers (1 510 bp). Clustal X mega software was used to edit the sequences, and an NCBI BLAST search was conducted to discover the amplified sequence's nearest neighbor. Homology searches were conducted using the sequencing data. In order to create phylogenetic trees, the neighbor-joining method was used.

3. Results

One E. coli was isolated and identified from the seven samples. The isolated E. coli was kept at 4°C on nutrient agar slants with 1 percent of the nutrient solution. The appearance of pink color colonies on MaC Conkey agar (0.5 percent bile salts) verified the presence of E. coli after isolation and basic confirmation. E. coli was initially found in the intestines of wild fish, according to Hanson et al. E. coli was found to be present in the fish's intestines. Researchers are trying to isolate E. coli from various fish components, including the intestines and the heads and tails. An incubation period of 24 hours in nutritional broth was used to extract the pathogen from the fish (E. coli). In this investigation, we isolated and identified E. coli strains from incubated broth and subcultured them in E. coli isolation media appropriate to the study. E. coli was isolated using MaC Conkey agar, a particular medium.

Fig. 4: Fish pathogen isolation

Table 1: Tests biochemistry

Name of the tests	Result
VP	-ve
Indole	+ve
Citrate	-ve
MR	+ve

A. Ecoli isolated strains

Swab samples collected from two separate fish handling regions were tested for the total bacteria count and E. coli count. All of the peptone water swabbed samples were taken to the lab and tested for total bacterial count and E. coli on plates. It was found that every sample taken from a landing center or fish market had E. coli in it even if none of the other tests were negative. From Veraval, Gujrat, 80 E. coli strains were identified based on their colony shape and size. Before and after the packaging process, E. coli germs were found in fish markets and seafood processing factories, where the vast majority of

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these outbreaks began. Antimicrobial resistance and susceptibility to commercially available 10 antibiotics were evaluated for each of the 80 strains (Figure 5).

Table 2: Percentage of E. coli strains resistant to 10 antibiotics

Antibiotics	Isolated E. coli Resistance (%)
ciprofloxacin	35
amoxicillin	39
ceftriaxone	10
cephalothin	31
chloramphenicol	3
ampicillin	60
nalidixic acid	24
tetracycline	27
rifampicin	7
streptomycin	5

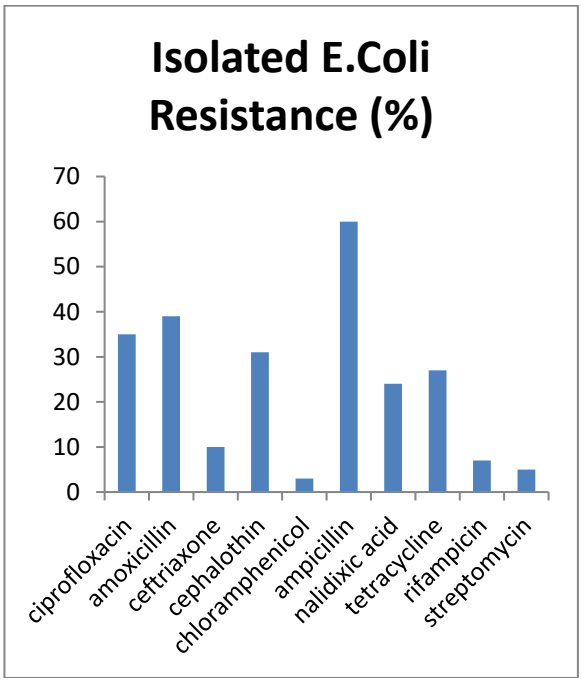

Fig. 5: Antibiotic-resistant E. coli strains

As a result, at least 60 percent of the strains were ampicillin-resistant, and at least 3 percent of the bacteria were chloramphenicol-resistant. Antibiotic resistance was found in over 20% of the isolates. Antibiotic-resistant E. coli strains CE2, CE3, CE6, CE12, CE19, CE21, PE4, PE6, PE7, PE10, PE14, and PE18 were identified as 12 distinct strains. With a 25 mm zone of inhibition, E. coli CE21 was determined to be the most resistant to ampicillin of the six tested antibiotics. As a result, molecular approaches such as 16S rDNA sequencing were used to locate the E. coli CE21. Genomic DNA from E. coli CE21 was extracted and amplified using a universal primer in a PCR reaction. Using the sequence from the amplified product and the one from GenBank, NCBI, the researchers sequenced the 1 510 base pair DNA fragment they had obtained. Bioserve Pvt Ltd in Hyderabad, India performed partial 16S rDNA sequencing. The phylogenetic tree was generated by comparing the sequencing data to E. coli.

B. Ballicius sp isolated strains

Polythene bags filled with oxygenated water were used to ship live Indian big carp samples, L. calbasu, to the lab from several fish handling facilities in Tamil Nadu, India. Table 3 summarizes the findings of an antibiotic sensitivity test performed on the chosen bacterial strains. Chloramphenicol, ciprofloxacin, erythromycin, and vancomycin were all found to be extremely effective against the three selected isolates. All antibiotics were shown to be ineffective against strains FS1 and FC6. Ampicillin, amoxicillin, and Ciprofloxacin were all found to be ineffective against the strain FC3.

Table 3: bacterial strains' antibiotic sensitivity tests

Antibiotics	FS1	FC3	FC6	Isolated Ballicius sp Overall Resistance (%)
Erythromycin (E)	+	+	+	2
Ampicillin (A/S)	+	–	+	19
Vancomycin (VA)	+	+	+	21
Chloramphenicol(C)	+	+	+	61
Ciprofloxacin (CIP)	+	–	+	52
Amoxicillin (AMS)	+	–	+	38
Penicillin (P)	+	+	+	7
Gentamicin (G)	+	+	+	46

–, resistant; +, susceptible.

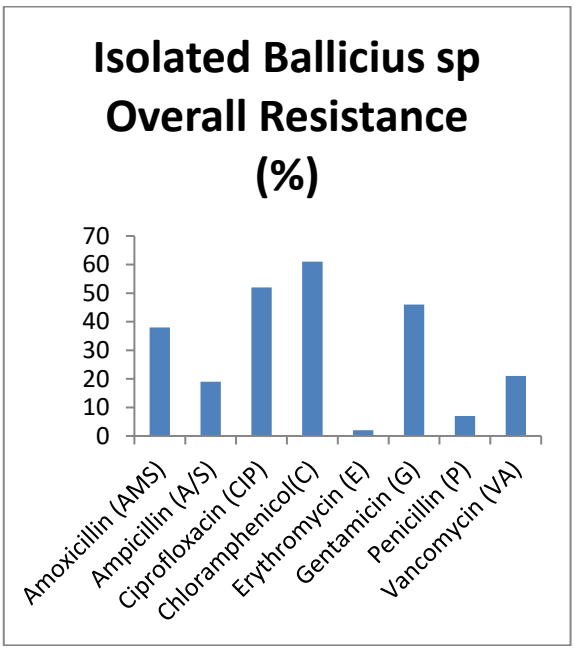

Fig. 6: Antibiotic-resistant Bacillius sp strains

C. Bio Analysis of Bacterial Strains

A total of 12 strains were chosen from a pool of 48 for bioremediation of pond water according to their favorable morphological and biochemical properties. Effluent from a seafood processing company was flocculated and 10 percent individual bacterial culture was added to aid in the degradation of the waste. Ten days of degradation investigations were conducted, during which time ammonia (NH3-N), nitrate (NO3-N), and nitrite (NO2-N) concentrations were measured. For Bio analysis we consider the

individual isolates of different bacterial strains and we tested the physical and chemical analysis of those compounds for 5th day and 10th day and they are tabulated below

Table 4: Changes in COD (mg/l) during bioremediation studies with individual isolates

Isolate	1 day	5day	Change in %	10th day	Change in %
BS3	3.23	2.95	8.7	2.45	24.15
BS7	2.85	2.12	25.61	1.98	30.53
BS12	3.94	3.05	22.6	2.96	24.87
BS21	3.69	2.97	19.5	2.68	27.37
BS26	2.46	1.49	39.4	0.65	73.58
BS28	2.18	1.56	28.4	0.98	55.05
BS29	3.56	2.98	16.29	2.21	37.92
BS31	3.85	3.15	18.18	2.56	33.51
BS34	2.44	1.98	18.85	1.35	44.67
BS39	2.89	2.02	30.1	1.18	59.17
BS42	3.64	2.97	18.4	2.31	36.54
BS47	2.75	2.15	21.8	1.62	41.09

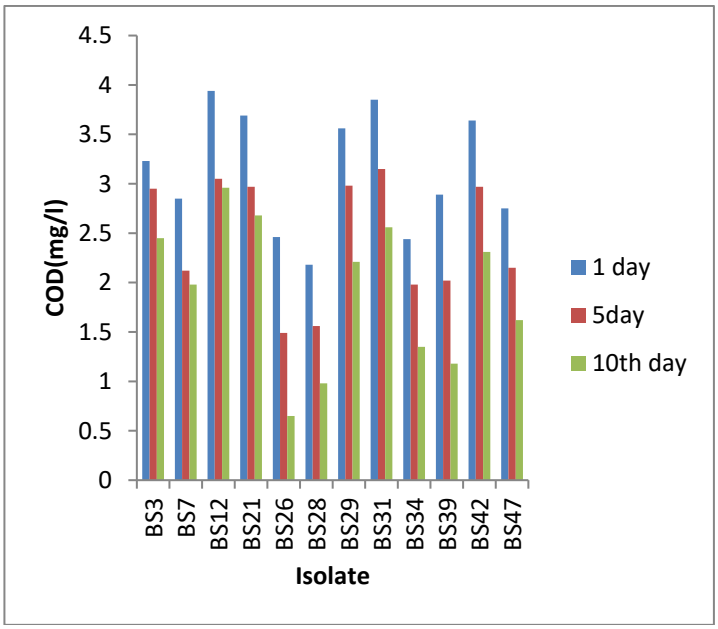

Fig. 7: Changes in COD (mg/l) during bioremediation studies with individual isolates

Table 5: Bioremediation investigations employing individual isolates BOD (mg/l)

Isolate	Initial BOD	5th day BOD	Change in BOD %	10th day BOD	Change in BOD %
BS3	4.29	3.51	18.18	2.97	30.769
BS7	4.29	2.49	41.96	1.98	53.846
BS12	4.29	3.26	24.01	2.95	31.235
BS21	4.29	2.97	30.77	2.16	49.650
BS26	4.29	1.56	63.64	1.04	75.758
BS28	4.29	0.94	78.09	0.56	86.946
BS29	4.29	2.66	38.00	2.13	50.350
BS31	4.29	3.16	26.34	2.95	31.235
BS34	4.29	0.45	89.51	0.23	94.639

BS39	4.29	3.1	27.74	2.89	32.634
BS42	4.29	2.32	45.92	1.98	53.846
BS47	4.29	1.98	53.85	1.64	61.772

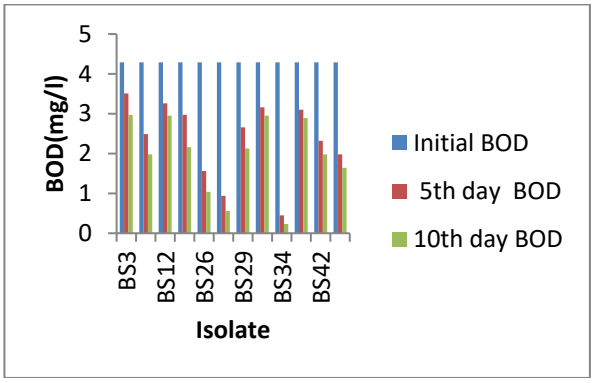

Fig. 8: Bioremediation investigations employing individual isolates BOD (mg/l)

Table 6: Changes in Ammonia NH3-N (mg/l) during bioremediation employing specific isolates

Isolate	1 day	5th day	Change in %	10th day	Change in %
BS3	59.8	61.3	-2.51	63.1	-5.52
BS7	31.5	26.9	14.60	25.8	18.10
BS12	2.89	3.15	-9.00	3.24	-12.11
BS21	63.8	66.2	-3.76	68.1	-6.74
BS26	42.8	44.8	-4.67	45.6	-6.54
BS28	7.5	9.6	-28.00	10.4	-38.67
BS29	36.8	41.8	-13.59	43.2	-17.39
BS31	4.9	2.8	42.86	2.1	57.14
BS34	56.6	61.3	-8.30	64.4	-13.78
BS39	48.9	44.6	8.79	41.5	15.13
BS42	9.5	7.9	16.84	7.1	25.26
BS47	10.6	11.6	-9.43	13.1	-23.58

Table 7: Changes in nitrate (NO3-N) (mg/l) during bioremediation trials using individual isolates

Isolate	1 day	5th day	Change in %	10th day	Change in %
BS3	0.001	0.001	0	0.001	0
BS7	0.007	0.005	28.57	0.004	42.86
BS12	0.005	0.003	40	0.0025	50
BS21	0.004	0.005	-25	0.0056	-40
BS26	0.007	0.006	14.29	0.0055	21.43
BS28	0.009	0.0095	-5.56	0.01	-11.11
BS29	0.006	0.007	-16.67	0.008	-33.33
BS31	0.005	0.0045	10	0.0039	22
BS34	0.044	0.041	6.82	0.038	13.64
BS39	0.038	0.056	-47.37	0.061	-60.53
BS42	0.015	0.018	-20	0.021	-40
BS47	0.009	0.01	-11.11	0.014	-55.56

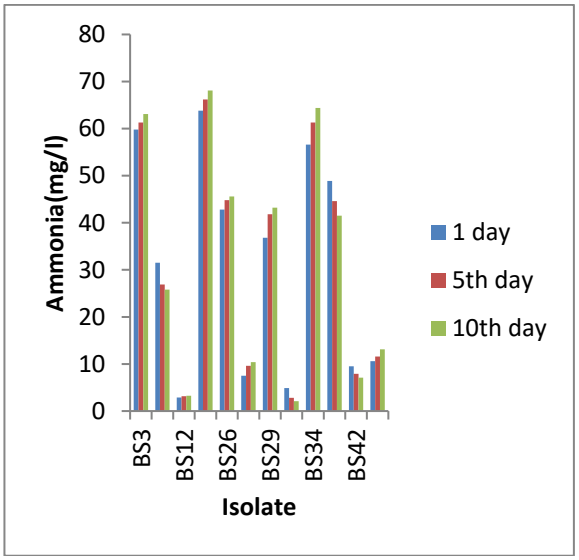

Fig. 9: Changes in Ammonia NH3-N (mg/l) during bioremediation employing specific isolates

Table 8: Changes in nitrite (mg/l) during bioremediation trials using individual isolates

Isolate	1 day	5th day	Change in %	10th day	Change in %
BS3	0.002	0.0018	10	0.0015	25
BS7	0.006	0.006	0	0.006	0
BS12	0.003	0.0025	16.67	0.0022	26.67
BS21	0.007	0.006	14.29	0.0056	20
BS26	0.008	0.009	-12.5	0.0035	56.25
BS28	0.01	0.008	20	0.007	30
BS29	0.005	0.007	-40	0.0075	-50
BS31	0.004	0.003	25	0.0026	35
BS34	0.042	0.039	7.14	0.037	11.90
BS39	0.029	0.016	44.83	0.015	48.28
BS42	0.018	0.019	-5.56	0.02	-11.11
BS47	0.012	0.01	16.67	0.009	25

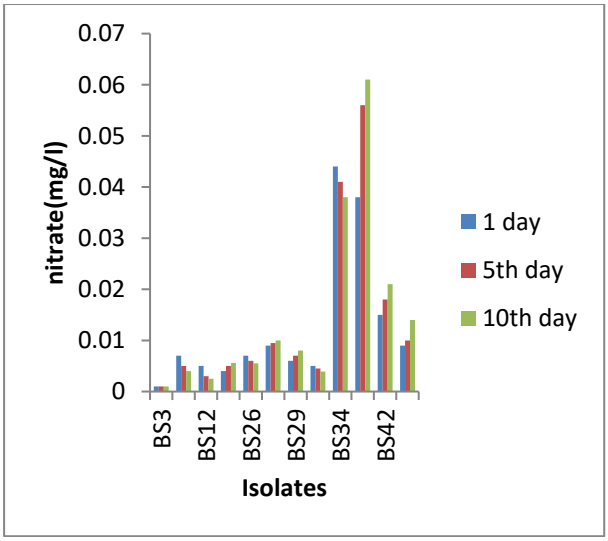

Fig. 10: Changes in nitrate (NO3-N) (mg/l) during bioremediation trials using individual isolates

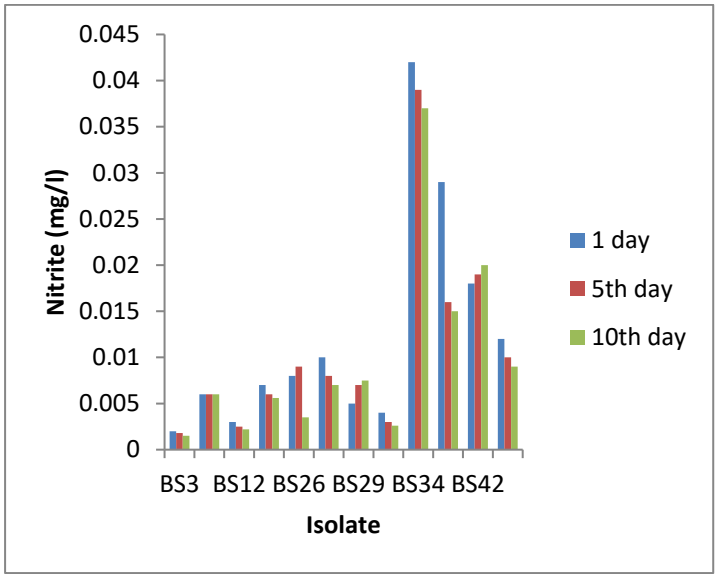

Fig. 11: Changes in nitrite (mg/l) during bioremediation trials using individual isolates

4. Conclusions

As a final step, E. coli and bacterial isolates were tested for MDR characteristics. Many tests were performed to ensure that the bacterium was in fact E. coli. Water temperature, salt content, distance from polluted areas (human and animal feces), naturally occurring bacterial populations in the water, and the amount of food eaten by fish can all affect the microbiology of seafood. India and other countries currently use E. coli testing in seafood processing industries to determine contamination levels. Mead et al. estimate that around 73,000 cases of human illness and 61 deaths are linked to this organism in the United States each year. All warm-blooded animals have E. coli in their digestive systems. The E. coli count in fish handling at local markets is higher than in other sampling locations because of poor sanitation control in the markets. When compared to fish sold in local markets, fish from the landing center had a lower E. coli count. E. coli can't survive in the ocean for long, thus it's unlikely to be found in fish caught off the coast. 80 E. coli strains were identified and described from all of the sampling locations.

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