GENUS BACILLUS SPECIES PROMISING PROBIOTIC IN AQUACULTURE (FROM MOLLY FISH) FOR BIOREMEDIATION (GREEN SYNTHESIS AND SUSTAINABILITY)

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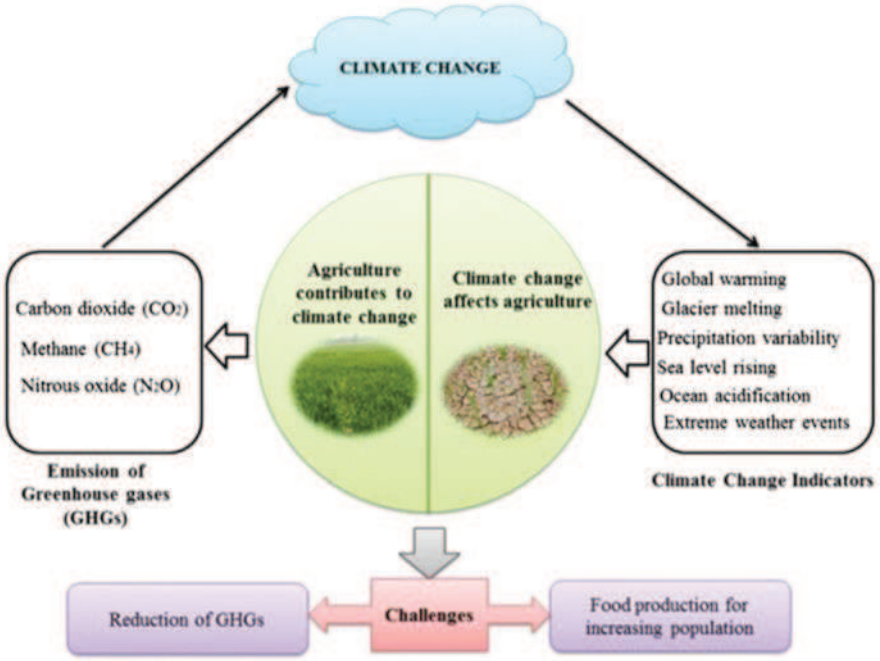
Abstract: Climate change is the biggest challenge planet Earth facing in this century. Organic aquaculture seems to play a promising role to mitigate clean and sustainable Environment. Organic aquaponics is gaining importance for its healthy method of growing fish and plants Like leafy vegetables, medicinal plants and even cereal crops for their mutual benefits. Aquaculture fisheries seem to have an important role in increasing food productivity in an eco-friendly manner for sustainability to cover-up land cultivation problems to some extent.

This study is basis for assessing the general health of fish, identification of dominant bacterial species present in its gut for future probiotic work for aquatic animals, animal husbandry and bioremediation. The present study has established evidence by experimental tank system in which required sustainable ecosystem is created with fish culture. In that it was observed for keratin and chitin solubilisation and the luxuriant plants growth. The suspected bacterium identification and characterisation, was carried out when its source soil from fish tank is enriched with marine soil (sand). The mixed sample was boiled (Vegetative forms get killed for viable spores) cooled and streaked on starch casein agar for culture isolation and characterisation of Actinomycetes for phyto pathogenic effect. It is identified with rRNA analysis and 16s sequence as Bacillus *cereus*. This establishes evidence that the fish gut bacteria played vital role for luxurious growth of plants by aquaculture fisheries.

Keywords: Climatic Change, Mitigation Measures, Aquaponics, *Bacillus Cereus*.

Introduction: Climate change is the biggest challenge planet Earth is facing this century. It takes place either due to natural phenomenon or human activities. The changes are attributable to human activities-mainly burning of fossil fuels. Continues to rise due to increased release of Green House Gases in the atmosphere. Some of the gases in the earth's atmosphere can trap heat and maintain the earth's temperature acting as thermal blanket. These gases are mainly carbon dioxide(CO_2),methane (CH_4) and nitrous oxide(N_2O).The concentration of the naturally present GHGs in the atmosphere is increasing and new gases are being added , which lead to more heat being retained in the atmosphere. The result is the warming of our atmosphere.This is known as Green House Effect, which is causing the global temperature to rise.

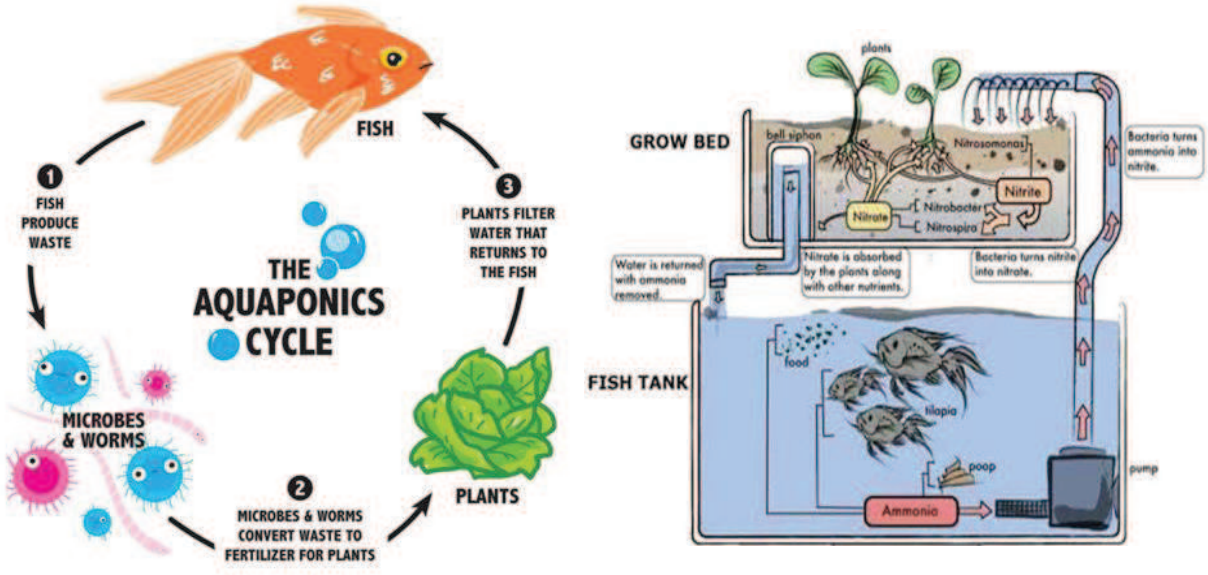
The earth's climate had been changing due to natural causes since ages. However the present change is man-made. Since industrial revolution the concentration of greenhouse gases in the atmosphere has increased due to various human activities such as burning of fossil fuel for energy generation ,industrial processes and transportation, deforestation and agriculture practices etc. Moreover, new gases like HFCs (hydro Flourocarbon),)PFCs (perforate chemicals) and SF_6 (Sulphur Hexachloride) used in refrigeration and air conditioning have been added. With urbanisation population growth and fancier lifestyles green house gas emissions have reached unprecedented levels.



The impacts of climate change are already visible. The average surface temperature increase is causing the polar caps to melt with subsequent increase in the sea level. However there are primary and direct impacts and their onset is slow. The immediate direct impacts are the increase in extreme weather conditions and irregularity in precipitation which has indirect impacts on health, agriculture and water resources. At other levels are the indirect impacts like migration, economic losses and increase in various conflicts. In other words impacts of climate change are cascading in nature -change as a trigger in one of the systems will alter and impact all the associated ecosystems and earth's elements as well as socio-economic systems.

Climatic change impacts in India, high health risk due to increase in heat waves, vector borne diseases and epidemics. Increase in the number of heat wave days from about 5 to between 30 to 40 every year. Change in the spatial and temporal pattern with increased frequency of vector borne diseases. Loss of ecosystem and biodiversity and increased frequency of forest fire is leading to ecological degradation, coastal inundation, sea water ingress and loss of life due to sea level rise. Loss due to extreme weather events, decreased agriculture production due to variability in precipitation and temperature rise- results into shrinkage in rice, wheat, maize production. Loss of soil fertility even with increased level of fertiliser application, besides more fertility in some cultivable, less chance of natural soil fertility build up by microbes due to continuous cultivation, frequent drought conditions and predictability of climatic weather pattern, decline in ground water etc, are threatening food production industry by soil based agriculture . Under such circumstances in near future it will be impossible to feed the entire population using open field of agriculture production only.[1]

Naturally soil less culture is becoming more relevant in the present scenario to cope with these challenges. Soil less culture has shown promising results all over the world due to poor waste management, river pollution and water management. Organic aquaculture plays an important role in increasing soil fertility, minimising input costs and producing an eco-friendly and safe products. Organic aquaculture is breeding of fish, shellfish , algae, seaweed and other organisms in all types of water environments is promising as mitigation measures to cope-up with impacts of climate change.

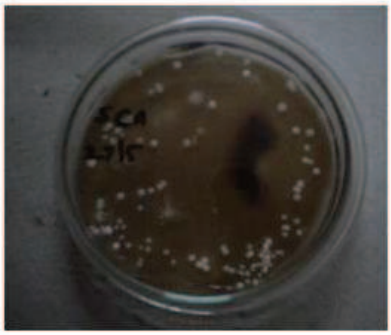


Materials and Method: Soil sample from sea shores to isolate Actinomycetes, Soil from fish tank, plants growing in the fish pond and mangrove for its soil (*Asparagus* racemosus root).

Isolation of Actinomycetes: 0.5 grams of soil sample (mixture of fish tank soil and sea shore sand was air dried then boiled) was suspended in 9.5 ml sterile water and was 10 fold diluted. 1ml of dilution was spread on various culture medias such as Bennet agar, L glycine glycerol agar,, L -arginine glycerol agar, Starch casein agar, humus agar with pH 7.8 and anti-fungal agents fluconazole &streptomycin. The plates were incubated at 30^{o} C for 2weeks.

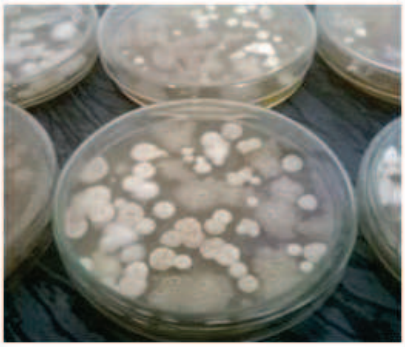
Observations: Colonies were opaque and variously pigmented. The colony surface was waxy white, chalky orange, red purple grey. Pink pigment is seen with smooth band granular margins. Colonies were velvety depending on the abundance of growth. Biochemical tests carried out include catalase, amylase, cellulase and lipase for the secondary screening.

Bacteria from fish gut for bioactive compounds released into Fish tank are showing keratin degradation and chitin degradation (bird feathers ,insects and worms) are seen growing on plate count agar with glucose at 37'c with pH 7.8 to +2, upon incubation produced isolated grey colonies . On Gram staining it is Gram -positive, rod shaped. Facultative anaerobic, motile, spore forming bacteria in soil has growth promoting factors (PGPR) seen by the growth of marine Actinomycetes . The sample gave well isolated chalk like colonies of marine Actinomycetes producing novel enzymes and antibiotics. Isolated colonies of *Bacillus* and Actinomycetes were-seen. Starch casein agar, cellulose casein agar, and arginine glycerol agar showed respective enzyme activity.

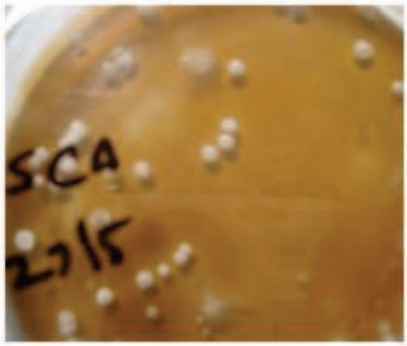


Amylase Test Positive

Caesin Hydrolysis



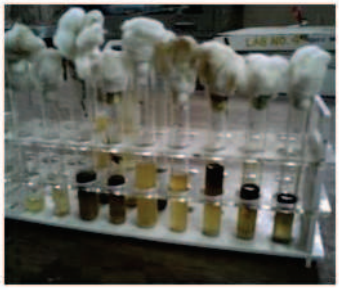
Isolated Colony
On Glyscerol Arginine Agar



Isolated Colonies On
Starch Agar

Amylase production was observed by iodine test and Di nitro salicylic method, casein degradation in starch casein agar, cellulase release in cellulose agar plate is observed. *Bacillus* species showed anti-fungal and antibacterial effect by its growth in chitin and keratin media for its proteases. [2]

Methyl Red Positive
Showing Glucose Fermentation.

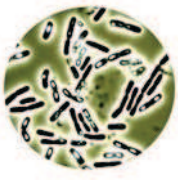


Vogus- Prosker Negetive In
Glucose Broth

Indole Test In Tryptone Broth.

This *Bacillus* species from fish gut was confirmed by its rRNA analysis and is identified by 16s sequence as *Bacillus cereus*.

Observation:



Micro scopic view *Bacillus cereus*

A: *Bacillus cereus* is Gram -positive rod shaped, facultatively anaerobic bacterium, soil saprophyte, spores are widely spread ,commonly found in soil and food. Some strains are harmful to humans and cause food borne illness while other strains can be beneficial as probiotics for animals.

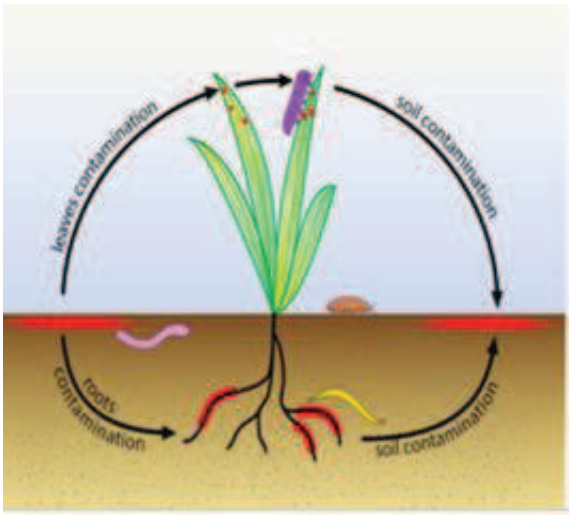
B: *Bacillus paramycoides* from rhizosphere of *Asparagus racemeouses Bacillus paramycoides* is a spore -forming bacterium of the genus Bacillus they are usually larger then 3micrometers, flat , relatively rough with silken threads around the colony .Fform chains of cells , gram positive, spores can form acid from glucose , and this form is non motile . , Vogue's -Proskaur test positive test . hydrolysis starch , cellulose and inulin which is reserve food in the root.
C.: Keratin degradation on keratin enriched agar.

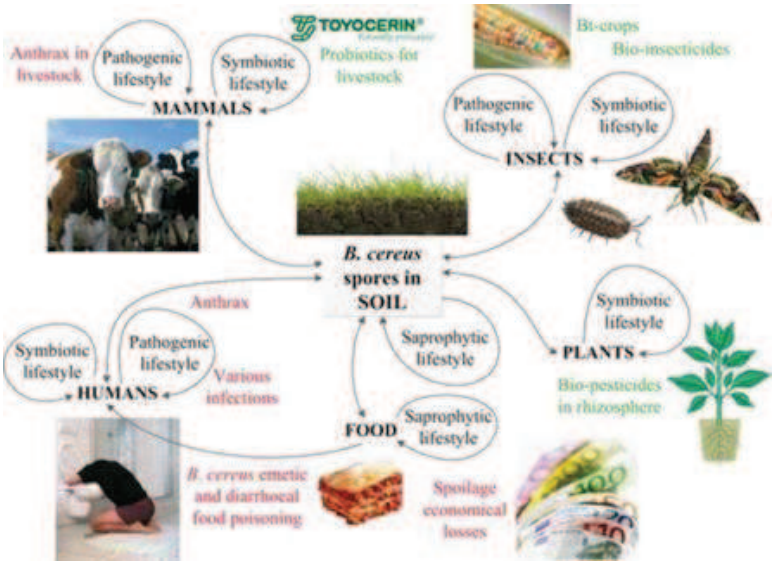
A .Isolated Colonies from Fish Gut.

B. Isolated Colonies from Asparagus Root.

C. Feather Media Showing Its Lysis

Life Cycle of *Becillus Cereus*





Field Setup:

Experimental Fish Tank

Live Fish in Fish Tank

Based on its reviews, it is Plant growth promoting bacteria (PGPR) as well as harmful bacteria found in soil and food . *Bacillus* sps is spore forming that is resistant to aggressive physical and chemical methods. Name *cereus* means waxy in latin. It normally grows at room in rice soups and milk leading to spoilage. [3,4] *Bacillus cereus* being source of nitrogen fertiliser and bio pesticide (with its

chitinase activity against pests and worms) proved by the growth of Actinomycetes on culture plates and plants in the tank. The fish tank shows luxuriant growth of plants such lotus, mint, *Alternantheria sessilis* and ornamental creeper as well as fish . Aquaculture is closed system for the continuous food production where in fish provided nitrogenous wastes to plants and in turn plants provide fresh oxygen by trapping solar energy which can also be supplied by LED lamps with solar battery for indoor plants.

Conclusion: The objective of the review paper is on *Bacillus cereus* as a probiotic in aquaculture. Bioactive compounds for bioremediation, efficacy in fish.

Scope: This case study has scope for food industry, Diary industry, Textile industry, Cosmetic industry, Leather industry, Probiotic in Fish & Prawn culture ,Agriculture for Bio-pesticide & Bio-fertilizer, Bioremediation of heavy metals and plastics.

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