PROJECT REPORT

Isolation and biochemical characterization of nickel resistant bacteria from Ganga river basin from Cossiepur industrial belt .Kolkata

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Abstract

Heavy metal resistance is a global cause of concern particularly in developing countries where stricter environmental norms are not practiced. This leads to anthropogenic pollution of environment by metals such as Nickel, Cadmium, Lead etc. Our current project aims to isolate nickel resistant bacteria from Ganga river water from the Cossiepur industrial belt. The bacteria were subjected to selection in nickel supplemented media both solid and liquid for multiple generations and performing subsequent subcultures to yield pure colony of resistant bacteria. The pure colony was subjected to different biochemical tests (Biotyping) like IMViC test, growth in EMB, Endo agar, catalase, Oxidase, Protease,Dnase,Lipase etc. The pure culture was further subjected to Antibiotypic sensitivity assay to 9 different antibiotics (Antibiotyping) both in solid media to yield zone of inhibition as well as in liquid media to yield minimal inhibitory concentration (MIC) of the isolate. Finally the bacteria was subjected to 16S rDNA analysis to identify the genus and species of the isolate.

Introduction

Heavy metal contamination in soil results mainly from natural weathering processes and anthropogenic activity. Ross (1994) classified the anthropogenic sources of metal contamination to five main groups: (i) metalliferous mining and smelting (As, Cd, Pb and Hg), (ii) industry (As, Cd, Cr, Co, Cu, Hg, Ni and Zn), (iii) atmospheric deposition (As, Cd, Cr, Cu, Pb, Hg and U), (iv) agriculture (As, Cd, Cu, Pb, Se, U and Zn), and (v) waste disposal (As, Cd, Cr, Cu, Pb, Hg and Zn).

Nickel (Ni) is the 24th most abundant element in the Earth's crust, comprising about 3% of the composition of the earth. It is the 5th most abundant element by weight after iron, oxygen, magnesium and silicon. It is a member of the transition series and belongs to group VIII B of the periodic table along with iron, cobalt, palladium, platinum and five other elements. Nickel is a naturally occurring element that can exist in various mineral forms. As a member of the transition metal series, it is resistant to corrosion by air, water and alkali, but dissolves readily in dilute oxidizing acids.

PROJECT REPORT

Title of the project: Isolation and Purification of Lactic Acid Bacteria from Curd Samples

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Introduction: Lactic acid bacteria (LAB) are group of gram positive cocci or rod shaped catalase-negative non spore forming bacteria that are generally recognized as safe organism. Mankind has exploited these bacteria for thousands of years for the production of fermented food for its ability to cause desirable change in taste, flavor and texture of the food. Moreover, different antimicrobial agents like lactic acid, acetic acid, hydrogen peroxide, carbon dioxide, bacteriocin are widely helpful to inhibit food borne pathogen and spoilage microorganisms, thereby extending the shelf-life and enhancing the safety of food products. Bacteriocins of LAB are extracellular bactericidal proteins that are secreted by the cells. On the basis of the protein structure, bacteriocins constitute a heterogeneous group of small peptides or high molecular weight proteins or protein complexes. The inhibition spectrum of bacteriocins produced by LAB towards Gram-positive bacteria varies widely but is mostly confined to closely related species of the producing bacteria.

In recent years, much attention is being given to a large variety of bacteriocinogenic LAB from different sources, because bacteriocins are considered to be safe in the form of food biopreservatives.

Objective: Isolation and purification of lactic acid bacteria from curd samples.

Materials and method:

a. **Sample:** curd samples procured from sweet shop near Belurmath, Howrah

b. **Chemicals:** MRS agar supplemented with 0.5% (w/v) $CaCO_3$ and 0.05% (w/v) L-cysteine-hydrochloride, MRS broth

c. **Labwares/Instruments:** Petridish, test tube, desiccator, incubator, autoclave etc.

Sampling: A total of twenty curd samples were collected from sweet shop near Belurmath at Howrah

Isolation of lactic acid bacteria: Loop full of curd sample was transferred to a test tube containing10 ml of MRS broth and incubated at 30°C for 48 hours. Those tubes that showed turbidity (positive result) were selected for isolation of LAB. Loop full of inoculum from positive tubes was transferred to MRS agar plate supplemented with 0.5% (w/v) $CaCO_3$ and 0.05% (w/v) L-cysteine-hydrochloride by dilution streak method. The plates were then kept in a closed desiccator with a moistened blotting paper and burning candle (candle jar method) and incubated at 30°C for 48-72 hours. Upon production of acid by LAB, $CaCO_3$ will be converted to CO_2 which will diffuse to form transparent zone around the acid forming colony. L-cysteine removes oxygen from the environment, transforming itself to cystine to make the environment anaerobic. All

Extraction of Biodiesel from Algae

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The rapidly diminishing petrochemical resources and environmental pollution are two critical challenges, which need to be addressed by the society. The use of petroleum-based fuels has become more restricted due to the declining supply of fossil oils and the demand for the reduction of greenhouse gas emissions that cause global warming. Renewable, carbon-neutral, and economically viable alternatives to fossil fuels are urgently needed in order to avert the impending oil crisis and the dramatic consequences of climate change (Chisti, 2007). Owing to the limited availability and associated environmental problems with fossil fuel utilization, both social and industrial researchers have started looking for renewable energy alternatives that can partially replace fossil fuel resources for establishing a more sustainable society and promoting economic recovery in the world.

The renewable energy based biofuel viz biodiesel and bioethanol are viewed as future substitute fuels for diesel and gasoline respectively. Sugar based fuel alcohol production is not feasible unless methods are developed to convert lingo-cellulosic biomass into ethanol and there is no competition with food supplies.The production of biodiesel has recently received much attention worldwide because of the world energy crisis.The biodiesel from edible oils is also not a sustainable option due to heavy competition with seed plants and accordingly, non-edible oils like Jatropha, Pongamia, Castor, Neemoils etc. are accorded top priority for biodiesel production in India. Apart from these non-edible oil resources, microalgae is also becoming the focus as future source of biodiesel as these are found exceedingly rich in oil that can be converted to biodiesel using existing technology.

Microalgae strains with high lipid contents are essential for feasible biodiesel production. A significant number of algae strainshave been shown to contain lipid content of about 20–50% by weight of algae biomass (Chisti, 2007). Different algae species vary in their lipid productivity depending on their photosynthetic system and adaptation to the culture media.

Depending on the specific algae species and their cultivation conditions, however, microalgal lipid production may range widely from 2 to 75% (Mata et al., 2010). In some extreme cases, it can reach 70%–90% of dry weight (Chisti, 2007). In the present study, maximum lipid content of 32.5%, 28% was obtained in USB16 (*Chlorella vulgaris*) and USB15-14 (*Neocystis brevis*) whereas the lowest lipid content of 16.5% was observed in HBB10(*Scendesmus pectinatus*). Lipid productivity can be dramatically increased by external application of stress factors and is considered a survival strategy for microalgae under adverse conditions. Most notably these include nutrient