SCREENING OF CELLULOLYTIC BACTERIA FROM SOIL

Project submitted to Department of Microbiology

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Accredited by NAAC' A 'grade in third cycle
Sri y.n college , autonomous, narsapur,
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(2019-2020)

Certificate

This is to certify that the minor research project entitled "SCREENING OF CELLULOLYTIC BACTERIA FROM SOIL" being submitted by R.V.L.S.Padmavathi, G.Sushma devi, P.Divya sree, CH.Anusha, MD.Nasreen, CH.Sai Krishna of III BSC C.B.M, during the academic year 2019-2020 under my guidance to the coordinator RUSA.

Signature of the Head of the Department

Signature of RUSA coordinator

Signature of the principal

DECLARATION

I hereby declare that the project work entitled “SCREENING OF CELLULOLYTIC BACTERIA FROM SOIL **”** submitted to RUSA 2.0 scheme, under the guidance of **Smt.Sk. Khareemunisa,** Lecturer in Microbiology, Department of Microbiology(UG), Sri Y.N. college (Autonomous), Narsapur.

SIGNATURE OF PROJECT STUDENTS

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ACKNOWLEDGEMENT

First and foremost, we praise and thank the almighty for his blessings and also for giving strength and support throughout the project work.

We wish to express our deep sense of gratitude to **Dr.KVRL VENKATESWARALU**, Principal, Sri Y.N College (Autonomous), Narsapur for giving us an opportunity to work in one of the esteemed departments in this institution.

We express our gratitude to **Smt.Sk.Khareemunisa**, Lecturer in Microbiology, Department of Microbiology, Sri Y.N College (Autonomous), Narsapur.West Godavari District, A.P., for her able guidance and constant encouragement in planning the project, valuable suggestions and regular follow up throughout the period of the study.

We would like to thank all our classmates and friends for their constant support and encouragement during our studies and in the preparation of this project.

CONTENTS

ABBREVIATIONS

mg – milligrams

ml-milliliters

Lts-Liters

M- Molar

% - percentage

µg-micro grams

µl - microlitres

Gms - grams

˚C- degree centigrade

CMC - Carboxy Methyl Cellulose

Abstract

In the present study bacteria were isolated from compost or decomposing leaves to determine their ability to degrade most complex biological molecule called cellulose in *in vitro* conditions. Bacteria having such ability are having industrial importance and are the need of the hour to convert the cellulosic biomass into useful products such as glucose, bio-ethanol.

- Cellulose a polymer of glucose residues connected by β-1-4 linkages being the primary structural material of plant cell wall.
- It is most abundant carbohydrate in nature.
- Cellulase is the enzyme that hydrolyze the β-1-4 glycosidic bonds in polymer to release glucose units.
- Cellulase can be used in washing powders, extraction of fruit and vegetable juices and starch processing.
- Cellulase can be produced by number of microorganisms, they are either cell bound or extra cellular.
- Due to the vast usefulness of cellulase, this project aims to isolate cellulase from cellulase producing bacteria.
- Various techniques such as chromatography and electrophoresis have also revealed that that the complex enzyme contains three different components.
  (i) endo-β-gluconase (E.C 3.2.1.4)
  (ii) exo-β-gluconase(E.C.3.2.1.9.1)
  (iii) β-gluosidase (E.C.3.2.1.21)

INTRODUCTION

SCREENING OF CELLULOLYTIC BACTERIA FROM SOIL

Objective: The aim of the present study is to demonstrate the isolation, identification and screening of bacteria with cellulase activity from soil samples.

Introduction to cellulose:

cellulose is the most abundant biological compound on terrestrial and aquatic ecosystem and is the main component of plant biomass. It is the dominant waste material from agricultural industry in the form of stalks, stems, husks, there has been great interest in utilizing cellulose as an energy resource and feed. The cellulose is composed of D-glucose units linked together to form linear chain via β-1, 4- glycosidic linkages.

Cellulose is also the most common organic compound on earth. It is well known that plants are the most common source of renewable carbon and energy on the earth. Cellulose is basically the structural component of the primary cell wall of green plants, many forms of algae and the Oomycetes. Cellulose is the major component of plant biomass. Plants produce 4x10 tons of cellulose annually.

It is also considered as one of the most important sources of carbon on this planet and its annual biosynthesis by both land plants and marine occurs at a rate of 0.85x10 tons per annum.

There are two types of hydrogen bonds in cellulose molecules: those that form between the C_3OH group and the Oxygen in the Pyranose ring within the same molecule and those that form between the C_6OH group of one molecule and the Oxygen of the glucosidic bond of another molecule. Ordinarily the beta -1-4 glycosidic bonds themselves are not too difficult to break. However, because of these hydrogen bonds, cellulose can form very tightly packed crystallites. These crystals are sometimes so tight that neither water nor enzyme can penetrate them; only exo gluconase, a sub group of cellulose that attacks the terminal glucosidic bonds, effective in degrading it. The inability of water to penetrate cellulose also explains why crystalline cellulose is insoluble. On the other hand, amorphous cellulose allows the penetration of endo gluconase, another subgroup of cellulose that catalyzes the hydrolysis of internal bonds. The natural

consequence of this difference in the crystalline structure is that the hydrolysis rate is much faster for amorphous cellulose than crystalline cellulose. The process of breaking the glucosidic bonds that hold the glucose basic units together to form a large cellulose molecule is called hydrolysis, because a water molecule must be supplied to render each broken bond inactive. In addition to crystallinity, the chemical compounds surrounding the cellulose in plants, e.g. lignin, also limit the diffusion of the enzyme into the reaction sites and play an important role in determining the rate of hydrolysis.

Cellulose degradation and its subsequent utilizations are important for global carbon sources. The value of cellulose as a renewable source of energy has made cellulose hydrolysis the subject of intense research and industrial interest. There has been much research aimed at obtaining new microorganisms producing cellulose enzymes with higher specific activities and greater efficiency.

Cellulose degrading enzyme:

Cellulolytic enzymes play an important role in natural biodegradation processes in which plant lignocellulosic materials are efficiently degraded by cellulolytic fungi, bacteria, *actinomycetes* and protozoa. In industry, these enzymes have found novel applications in the production of fermentable sugars, ethanol, organic acids , detergents and other chemicals. Cellulases provide a key opportunity for achieving tremendous benefits of biomass utilization (Wen *et al* ., 2005).

The conversion of cellulose into glucose is now known to consist of two steps in the enzyme system. In the first step, β -1,4 glucanase breaks the glucosidic linkage to form cellobiose, which is a glucose dimer with a β-1, 4 bond as opposed to maltose, a counterpart with an α-1, 4 bond. Subsequently, this β -1, 4glucosidic linkage is broken by β- glucosidase:

β-1,4glucanase β- glucosidase

Cellulose----------→Cellobiose----------→Glucose

The kinetics of cellulose hydrolysis has been widely studied, and michaelis- menten types of rate expressions with substrate or product inhibition terms have been proposed to describe the observed reaction kinetics.

Cellulose degrading microorganisms:

Cellulolytic enzymes are synthesized by a number of microorganisms. Fungi and bacteria are the main natural agents of cellulose degradation (Lederberg, 1992). The cellulose utilizing population includes aerobic, anerobic, mesophilic bacteria, filamentous fungi, thermophillic and alkaliphilic bacteria, actinomycetes and certain protozoa (Alexander , 1961). However , fungi are well known agents of decomposition of organic matter, in general and of cellulosic substrate in particular (Lynd et al ., 2002).

Microorganisms bring about most of the cellulose degradation occurring in nature. They meet this challenge with the aid of a multi-enzyme system (Aubert et al., 1987). Aerobic bacteria produced numerous extra – cellular enzymes with binding molecules for different cellulose conformations, while anerobic bacteria possess a unique extra cellular multi enzyme complex, called cellulase. However the main cellulose utilizing species are the aerobic and anaerobic hemophilic bacteria, filamentous fungi, basidiomycetes thermophilic bacteria and actinomycetes (Wright, 2003). At the first step, the microorganisms responsible for cellulose decomposition bring about an enzymatic hydrolysis of the complex polymer, that is , the enzymes system which involves a group of different enzymes, is collectively known as cellulose.

Many efficient cellulase activities are observed in fungi but there is an increasing interest in cellulase production by bacteria because bacteria have high growth rate as compared to fungi and has good potential to be used in cellulase production. The search for a novel and improved bacterial strain, having hyper cellulose productivity with more activity and high stability against temperature, pH and under non-septic conditions might make the process more economical.

The cellulase was first discovered in 1983 from the anaerobic, thermophillic spore forming *Clostridium thermocellum*. The production of cellulose generally depends on variety of growth parameters which includes inoculum size, pH value, temperature, presence of inducers, medium additives aeration, growth and time (Immanuel et al., 2006) and also the cellulose activity is appear to be depend on the presence of various metal ions as activators and inhibitors.

Cellulose is commonly degraded by cellulase. Cellulolytic enzyme system consists of three major components such as endoglucanases and β- glycosidase. Cellulases have a potential applications in biotechnology and in industry such as , starch processing, alcoholic beverage, malting and brewing, clarification of juice, pulp bleaching, textile industry and animal feed.

Certain cellulose producing bacteria also inhibit the other factors which are responsible for decomposition of organic matter and composting (Shankar et al., 2011). Beyond free bacterial cellulases, is the opportunity for whole cells in bacterial co-culture and strains with multiple exploitable characteristics to reduce the time and cost of current bio-conversion processes. It is also noticeable- as the final product of cellulose degradation by cellulose enzyme is glucose which is a soluble sugar . so, isolation and characterization of cellulose producing bacteria will continue to be an important aspect of biofuel research, biodegradation and bioremediation.

LITERATURE REVIEW

CELLULOSE:

Anselmepayen (1795-1871) coined the term cellulose and introduced it to scientific literature in 1839 after isolating a fibrous substance mostly found in wood, cotton and other plants (Payen,1838). Higher plant tissues such as trees, cotton, flax, cereal straw represent the main sources of cellulose i.e. it makes up 35-50% of dry plant weight (Lynd et al.,1999). Algae such as valoniaventricosa and microdicyan are representatives of lower plants that synthesize cellulose (Boisset, et al., 1999: Fierobe, et al., 2002). In addition to plants , non- photosynthetic organisms such as bacteria i.e. aerobic *Acetobacter xylinum*, marine invertebrates from the ascite family i.e. tunicates, fungi, slime moulds and amoebae also produce cellulose (Tommeet al., 1995; Lynd et al, 2002).

Kushwaha et al (2012), conducted another study where soil samples were obtained (10gm)from hardoi district , uttar Pradesh in india. Bacterial colonies were grown over CMC –Agar medium. Maximal cellulose production was obtained after 48hrs of incubation at 45^{o}C in medium containing 1.5% carboxymethyl cellulose (CMC) as substrate. The optimum pH for the enzyme was found to be ranging between 6.5 and 7.5 at which it was found to be most stable. Bacteriological studies indicated bacillus substilis to be the most frequent cellulytic bacteria to be found in the agricultural fields. The purpose of the current investigation was to screen bacillus species isolated from soil in order to study its suitability with regard to waste treatment in agricultural fields (bioremediation).

Balamurugan et al (2011) performed experiment where cellulose degrading bacteria of tea garden soil were isolated, screened in vitro and its characterization, in relation to cellulose activity, was studied. Among the 25 isolates, the five strains showed higher enzyme activity when compared to other strains. Cellulose activity was expressed at a higher level by strain CDB12 when blotting paper was used as a cellulose source on comparison with the other two substrate sources incorporated with minimal salt medium and followed by CDB13 and CDB21 in blotting paper . maximum growth of cellulose degradation bacteria (CDB) was recorded at 30 oC and pH 7.0. Among the carbon sources tested , maximum growth was observed in glucose amended mineral

salts medium followed by fructose and maltose . ammonium sulphate , ammonium nitrate and potassium nitrate were good nitrogen sources better survival of CDB isolates.

Mechanism of cellulose hydrolysis:

A cellulose enzymes system comprises of three classes of enzymes; endoglucanases (EC3.2.1.4), exoglucanases (EC3.2.1.91) and B glucosidase (EC3.2.1.21). Exoglucanases are further grouped in to glucano hydrolases (cellodextrinases) and cellobiohydrolases. These categories are based on their structural properities and mode of action (Henrissat et al., 1998; Henrissat & Davies, 2000). Endoglucanases randomly cleave at the internal sites of cellulose to yield oligosaccharides of various lengths. Exoglucanases on the other hand act on the reducing or non-reducing end of cellulose to liberate glucose , cellobiose or cello oligosaccharides, which are finally hydrolysed to glucose by β- glucosidases (Sukumaran et al., 2005).

This enzyme system exhibits synergy, a phenomenon in which the collective enzyme activity is higher than the sum of activities of individual enzymes. Four forms of synergy have been reported . Exo-exo synergy between endoglucanases and exoglucanases; Exo-β- glucosidase synergy between the catalytic domain and the CBMs (Din et al., 1994; Driskill et al.,1999).

Cellulolytic anaerobes have an extra cytoplasmic cellodextrinase for hydrolyzing cellodextrins and intracellular cellodextrin and cellobiose phosphorylases (CdP and CbP). These phosphorylases catalyse Pi mediated phosphorylation of cellodextrins and cellobiose respectively to yield glucose 1 mono phosphate (G-1-P) which is converted to Glucose 6 Phosphate (G-6-P), the entry point to Embden-Meyerhoffpathway (Lynd et al., 2002). Other bacteria produce intracellular β- glucosidases which cleave cellobiose and cellodextrins to produce glucose which is assimilated by the microbes (Karmakar & Ray, 2011). Simultaneous presence of extracellular cellodextrinases, intracellular CbP and CdP activities, and intracellular β glucosidases in cellulolytic microorganisms suggest that metabolism of cellobiose and cellodextrins probably occurs through several pathways. (i) Extracellular hydrolysis of the substrates i.e. cellobiose and

cellodextrins and subsequent uptake and metabolism. (ii) Direct uptake followed by intracellular phosphorolytic cleavage and subsequent catabolism. (iii) Direct uptake by the organism followed by hydrolytic cleavage and metabolism (Lynd et al., 2002). Cellulosic substrates occurring in nature contain hemi cellulose and lignin which impedes the access of cellulase components to β (1-4) glucosidic linkages thus other hydrolytic enzymatic activities distinct to those of cellulases are required. Enzymatic cleavage of the β 1 - 4-glucosidic linkages in cellulose proceeds through an acid hydrolysis mechanism, using a proton donor and nucleophile or base (Lynd et al., 2002).

The three types of reactions catalyzed by cellulases: (1) Breakage of the non covalent interactions present in the amorphous structure of cellulose by endoglucanase (2) Hydrolysis of chain ends to break the polymer into smaller sugars by exoglucanase (3) Hydrolysis of disaccharides and tetrasaccharides into glucose by β-glucosidase (Adapted from Karmakar & Ray, 2011).

Cellulase enzyme systems

Cellulose utilization takes place in both aerobic and anaerobic microorganisms. Members of the genus Cellulomonasare the sole facultatively anaerobic degraders reported so far (Bagnara, et al 1985; Bagnaraet al., 1987; Clemmer& Tseng, 1986; Dermoun&Belaich, 1988). Cellulase enzyme systems are generally classified into two; complexed (Shohamet al.,1999; Schwarz, 2001) and non complexed (Stutzenberger, 1990; Teeri, 1997). This classification is dependent on whether the microorganism is aerobic or anaerobic (Lynd et al., 2002).

Non complexed systems :

In non complexed cellulase systems components are free and mostly secreted thus can be recovered from the culture supernatant. These are normally found in aerobic cellulose degraders i.e. both fungi and bacteria (Rapp & Beerman, 1991). Cellulases from aerobic fungi are by far the most studied group (Lynd et al., 2002). Representatives in this category include Trichoderma reesei previously Trichoderma viride. T. reesei produces cellobiohydrolases CBHI and CBHII, eight endoglucanases EGI-VIII and seven β- glucosidases BGI-VII (Pakula&Penttila, 2005). The cellulase enzyme system from Humicolainsolens is homologous to that of T. reesei with at least

seven cellulases i.e. Two cellobiohydrolases CBHI and CBHII and five endoglucanases EGI, EGII, EGIII, EGV and EGVI (Schülein, 1997).

Most aerobic bacteria species are found in soil. They fall in genera that are known for non growth associated metabolism (secondary metabolism) that include formation of dormant states (Bacillus, Miromonospora and Thermobifida and production of secondary metabolites such as antibiotics (Bacillus and Micromonospora(Lynd et al., 2002). Most aerobic bacteria adhere to cellulose but the physical contact is not necessary for cellulose hydrolysis (Kauri & Kushner, 1985).

Complexed system :

Anaerobic cellulose degraders degrade cellulose via a complexed system; a cellulosome (Schwarz, 2001). Cellulosomes are protuberances on bacterial cell wall that harbor enzyme complexes. These enzyme complexes are firmly bound on to the cell wall but flexible enough to bind cellulose. Cellulosomes from different Clostridia (*Clostridium thermocellum, Clostridium cellulolyticum*, *Clostridium cellulovorans*, and *Clostridium josui*) and Rumino coccus species in the rumen have been studied. Cellulosome enzyme sub units are not any different from free cellulases. Both have catalytic domains from the same glycosyl hydrolase families. The major difference between these two enzyme types is that all cellulosomal enzymes have a dockerin domain which mediates the integration of the enzyme into the cellulosome complex. Free cellulases however lack a docker in domain but have a catalytic binding module that helps binding of a given catalytic domain to the substrate (Bayer et al.,1994; Tomme et al., 1995b; Be´guin&Lemaire, 1996).

Taxonomic diversity of cellulolytic microorganisms

Ability to degrade cellulose is widely distributed in several fungal and bacterial genera. In addition to these two, the domain eubacteria has a considerable distribution of cellulolytic capability. Members in the aerobic order Actinomyctes and anaerobic order Clostridiales. Fungi are the main agents of decomposition of organic matter in general and especially cellulosic substrates (Lynd et al., 2002; Montegut et al., 1991) and it‘s no surprise that cellulolytic capability is distributed across the entire kingdom from the advanced Basidiomycetes to the primitive Chytridomycetes (Lynd et al., 2002). Chytridomycetes are known to degrade cellulose

in gastrointestinal tracts of ruminant animals (Orpin, 1977).

Cellulolytic capability is however not exclusive to microorganisms. Species such as termites and cray fish produce their own cellulases that are different from those produced by their indigenous micro flora (Orpin, 1977). There is a broad distribution of cellulolytic capability and it‘s possible that a primordial ancestor acquired it early in the evolutionary development. This however may not be the case because cellulose biosynthesis capability evolved much later with the development of land plants, algae amongst others (Lynd et al., 2002)

.1: Fungi and bacteria with cellulolytic capability (Adapted from Kuhad et al., 2011).

Fungi

Soft rot fungi

Aspergillus niger, A. nidulans, A. oryzae, A. terreus; Fusarium solani, F.oxyspourm; Humicola insolens, H.grisea; Melanocarpus albomyces; Penicillium brasilianum, P.occitanis, P.decumbans, P. janthinellum ; Trichoderma reesei, T. harzianum, T. longibrachiatum, T.atroviride; Chaetomium cellulyticum, C. thermophilum; Thermoascus aurantiacus; Mucorcircinelloides; Paelomyces inflatus, P. echinolatum.

White rot fungi

Phanerochaete chrysosporium; Sporotrichum thermophile; Trametes versicolor; Agaricus arvensis; Pleurotus ostreatus; Phlebia gigantea.

Brown rot fungi

Coniophora puteana; Lanzites trabeum; Poria placenta, Tyromyces palustris; Fomitopsis sp

Bacteria

Aerobic bacteria

Acinetobacter junii, A. amitratus; Acidothermus cellulolyticus; Anoxybacillus sp; Bacillus subtilis, B.pumilus, B. licheniformis, B. amyloliquefaciens, B. circulans, B. flexus; Bacteroides sp; Cellulomonas biazotea; cellvibrio gilvus; Eubacterium cellulosolvens, Geobacillus sp; Microbispora bispora; Paenibacillus curdlanolyticus; Pseudomonas cellulosae; Salinivibrio sp; Rhodothermus marinus.

Anaerobic bacteria

Acetovibrio cellulolyticus; Butyrivibrio fibrisolvens; Clostridium thermocellum; C. llulolyticum; C. acetobutylium; C. papyrosolvens; Fibrobacter succinogenes; Ruminoccus albus

MATERIALS AND METHODS

The soil samples were collected from different areas such as Botanical garden soil from Sri Y.N College, Narsapur. Playground soil and then Ten fold serial dilutions of each soil sample were prepared in sterilized distilled water and 0.1 ml of that diluted sample was spread plated on Carboxy methyl Cellulose medium.

Isolation of cellulolytic bacteria

Cellulolytic bacteria were isolated from soil by using serial dilutions and spread plate technique. The medium used for isolation of cellulolytic bacteria contains 1.0 % peptone, 1.0 % carboxy methyl cellulose (CMC), 0.2 % K_2HPO_4, 1 % agar, 0.03 % $MgSO_4.7H_2O$, 0.25%$(NH_4)_2SO_4$ and 0.2 % Gelatin at pH 7 for 48 hours of incubation at 30˚C. Bacterial colonies were purified by repeated streaking. The purified colonies were preserved at 4°C for further identification and screening for cellulase production.

Screening of cellulolytic bacteria

Pure cultures of bacterial isolates were individually transferred on to CMC agar plates. After incubation for 48 hours, CMC agar plates were flooded with 1 % Congo Red and allowed to stand for 15 min at room temperature. 1M NaCl was thoroughly used for counter staining the plates. Clear zones were appeared around growing bacterial colonies indicating cellulose hydrolysis. The bacterial colonies having the largest clear zone were selected for identification and cellulase production.

Identification of cellulolytic bacteria

Identification of cellulolytic bacteria was carried out, which was based on morphological and biochemical tests.

Morphological characterization

Gram stain test was carried out on the bacterial isolates as described in the manual of veterinary laboratory techniques in Kenya (1981). A bacterial smear from a pure culture was prepared and fixed on a clean glass slide. The slide was flooded with Crystal violet for 1 minute, and rinsed with running tap water. The slide was then flooded with Gram‘s Iodine for 1 minute, and again rinsed with running tap water. This was followed by decolourisation with 95% ethanol and with tap water. The slide was counter stained with Safranin for 1 minute, rinsed with running tap water and allowed to air dry. The dry slide was covered with immersion oil and viewed under a microscope.

Biochemical characterization

1) Oxidase test :

A small piece of filter paper was soaked in 1% Kovac‘s oxidase reagent and dried.

With the help of a loop a well-isolated colony from a fresh (18- to 24-hour culture) bacterial plate was taken and rubbed onto treated filter paper and observed for color changes.

Microorganisms are considered as oxidase positive when the color changes to dark purple within 5 to 10 seconds. Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds. Microorganisms are oxidase negative if the color does not change or it takes longer than 2 minutes.

2) Catalase test:

The enzyme catalase converts hydrogen peroxide into water and oxygen, thus helping an organism to cope with toxic O* species. The catalase test is used to detect an organism‘s ability to produce catalase. With the help of a loop a small lump of bacterial colony from a fresh culture was taken and introduced into few ml of hydrogen peroxide in a test tube or into a drop of hydrogen peroxide on a glass slide and observed for the formation of bubbles due to release of oxygen. The organism which reacts is considered as catalase positive.

3) Indole test

Indole test is used to determine the ability of an organism to spilt amino acid tryptophan to form the compound indole.

Method:

a. Inoculate the tryptophan broth with broth culture or emulsify isolated colony of the test organism in tryptophan broth.

b. Incubate at 37°C for 24-28 hours in ambient air.

c. Add 0.5 ml of Kovac‘s reagent to the broth culture.

Expected results:

Positive: Pink colored rink after addition of appropriate reagent

Negative: No color change even after the addition of appropriate reagent.

3) MR-VP test

Methyl red test and Voges-Proskauer test both are done in methyl red–Voges-Proskauer (MR VP) broth, but the reagents that we add differs in terms of reaction.

Methyl Red (MR) Test:

 Positive methyl red test is indicated by the development of red color after the addition of methyl red reagent.

 A negative methyl red test is indicated by no color change after the addition of methyl red

Voges-Proskauer (VP) test:

1. Negative test is indicated by lack of color change after the addition of Barritt‘s A and Barritt‘s B reagents.
2. A positive Voges-Proskauer test is indicated by the development of red-brown color after the addition of Barritt‘s A and Barritt‘s B reagents.

5) Citrate Utilization Test:

Citrate utilization test is performed on Simmons citrate agar:

A. Negative citrate utilization test is indicated by the lack of growth and color change in the tube

B. A positive citrate result as indicated by growth and a blue color change.

RESULTS

Sampling:

The study was conducted on two soil samples one from Botanical garden area

& another from play ground.

Isolation of soil bacteria

Figure (4) showed Three isolates from soil samples collected from two different areas exhibited cellulose hydrolysis , which were cultured in agar medium containing CMC as the sole carbon source. They are named as CP-I,CP-II and CP-III (CP = Cellulase Producer) respectively.

Gram's Staining:

CP-I Gram Negative Bacilli

CP-II Gram Positive Bacilli

CP-III Gram Positive Bacilli

Cellulase Producer	Indole	Methyl Red	Voges Proskauer	Citrate	Catalase
CP-I	-Ve	-Ve	+Ve	+Ve	+Ve
CP-II	-Ve	-Ve	-Ve	+Ve	+Ve
CP-III	-Ve	-Ve	+Ve	+Ve	+Ve

CELLULASE HYDROLYSIS

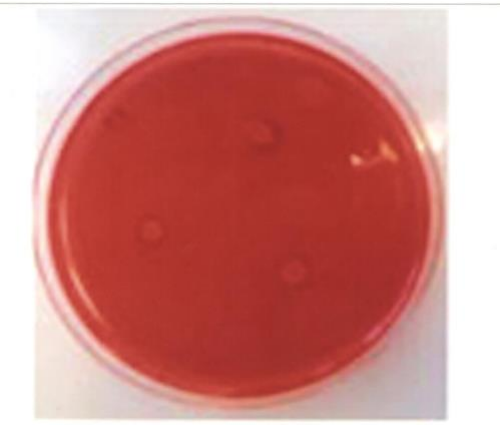

Fig 01 Negative Control

Fig 02 CP-I

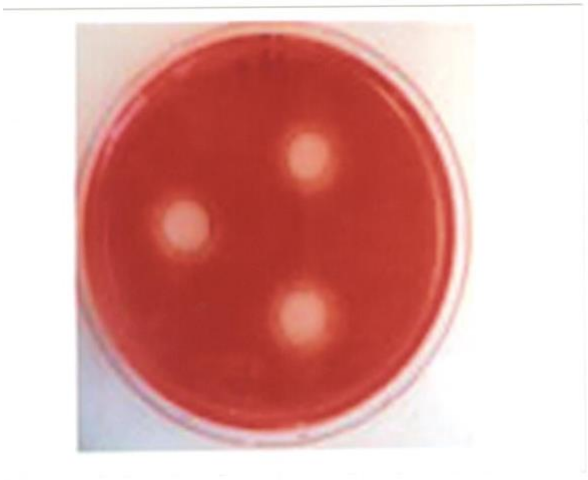

Fig 03 CP-II

Fig 04 CP-III

DISSCUSSION

A total of 24 bacterial colonies were obtained on CMC agar plates, when performed Congo red test only three samples exhibited hydrolytic reaction with clear zones of cellulose hydrolysis ranging from 2to 5mm diameter.

The preserved samples were again tested individually after naming as Cellulase Producers I to III. The soil sample harbours mostly Gram Positive bacteria. Bacterial samples when stained showed two isolates as Gram Positive and One Isolate as Gram Negative Bacteria. Their Biochemical and staining test results suggest that they are bacilli of both Gram Positive and Gram Negative nature.

CONCLUSSION

Application of these three isolates in industries may have several advantages such as high growth rate and ability to secrete proteins extracellularly; features of the *Bacillu*s species. More studies are however needed before industrial application of this isolates. These include enzyme activity assays of the purified specific cellulases for comparison with the results in this study and with those that have been purified. These studies would shed more light on whether to use the whole organism in the industry or harvest the enzymes and carry out downstream processes or purify the gene to know whether the gene is to be added to the genetic pool for protein engineering and directed evolutionary studies to come up with super enzymes. Similar studies should be extended to other environments in the country.

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