Industrially Beneficial Microbes from Kitchen Waste: A Green Approach to Produce Cellulase Enzyme and Optimization of Culture Condition

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A Thesis report submitted to the Mathematics and Natural Sciences in partial fulfillment
of the requirements for the degree of
Bachelor of Science in Biotechnology

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The report does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The report does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Abstract

Bacterial cellulases nowadays have been gaining considerable attention due to its enormous use in biotechnology. The study here was pursued with the aim to isolate cellulolytic bacterial strains from kitchen wastes i.e. papaya peel, banana peel and cucumber peel. Here the kitchen wastes were chosen as sources because these were easily accessible and cost-effective. And also cellulases extracted from these sources could be an effective alternative to the conventionally used hazardous chemicals. Based on DNS Assay, the isolates with maximum cellulolytic activity were selected for optimization of culture condition using two physical parameters; pH and temperature. Among the selected isolates, *Bacillus subtilis* (0.5 U/ml) and *Bacillus cereus* (0.42 U/ml) were found to be the best cellulase producers at pH 5.0 and temperature 37^{o} C. These obtained cellulase producing bacteria could be used in industrial scale if further studied.

Keywords: Kitchen waste, Cellulose-degrading bacteria, Carboxymethylcellulose,

Enzymatic Activity, Biochemical Test

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List of Acronyms

PP	Papaya Peel
BP	Banana Peel
CP	Cucumber Peel
CMC	Carboxymethylcellulose
CD	Catalytic Domain
CBD	Cellulose Binding Domain
DNS	3,5-Dinitrosalicylic Acid
MR	Methyl Red
VP	Voges Proskauer
TSI	Triple Sugar Iron

Chapter 1

1. Introduction

Cellulose, a homopolymeric chain of D-glucose units connected via β-1, 4 glycosidic linkages, is the principle structural component of cell wall in most terrestrial plants (Sethi et al., 2013). Besides being most abundant organic biopolymer, cellulose is also called the largest reservoir of renewable carbon source on earth (Parkhey et al., 2017). It is also a biodegradable compound which has no taste and odor as well. However, inside the dynamic network of plant cell wall, cellulose is found as long-thread like crystalline structure called cellulose microfibrils, which are about 2-20 nm in diameter and 100-40,000nm in length. It is also a primary product of photosynthesis in terrestrial environment (Sethi et al., 2013). According to different estimations, each year approximately 100 billion tons of cellulose are produced in the biosphere. Hence, cellulose is considered as the most dominating renewable bioresource produced in the biosphere having the significant potential of being converted into numerous valued added bioproducts (Shah & Mishra, 2020). Cellulose also comprises biggest part of agricultural wastes such as crop residues, sawdust from timber, rice straw, wood products, animal excretes etc. And agricultural waste somehow can cause harm to our surrounding environment. Therefore, bioconversion of these cellulosic wastes into valuable products not only protects the environment but also helps to meet up the increasing demand.

Both chemical and biological means can be utilized to hydrolyze this biopolymer cellulose into monomeric glucose units. However, the microcrystal structure of cellulose sometimes hinders effective utilization and bioconversion (Premalatha et al., 2015). But chemical methods that involve use of acid, alkali, heat etc can serve good results. Although these chemical hydrolysis methods have been considered as viable options for a longer time, have already posed several harm to our environment. On the other hand, cellulolysis -the biological conversion of cellulosic biomass offers both environmental friendly and cheaper approach. The biological conversation of cellulosic biomass has the potential to develop sustainable bioprocess and products. One of the key steps of the global carbon cycle is the Enzymatic Hydrolysis of Cellulose by microorganism (Wilson, 2011).

Enzymes of the cellulase system control the biological process of cellulolysis. Three types of soluble extracellular enzymes comprise the cellulase enzyme system and these are 1, 4-β-exoglucanase, 1, 4-β-exoglucanase, and β-1,4-glucosidase. Here the 1, 4-β-endoglucanase makes the random cleavage of β-1, 4-glycosidic bonds along with a cellulose chain. Next 1,4-β-exoglucanase starts its action by staring to cleave the nonreducing end of a cellulose chain

and splits of the elementary fibrils from the crystalline cellulose. And, lastly β-1, 4-glucosidase produces glucose by hydrolyzing cellobiose and water-soluble cellodextrin. The complete conversion of cellulose to glucose through enzymatic hydrolysis is only possible with the synergy of above-mentioned three enzymes (Shewale, 1982).

Cellulose can be degraded by a large number of microorganism but few of them can completely hydrolyze crystalline cellulose by producing sufficient amount of free enzymes. Cellulase is either cell bound or extracellular (C. Koomnok, 2008). In the media, cellulases are extracellularly synthesized and excreted, so it is needed to estimate the relative capability of the cellulose-producing strains to produce all three enzymes and parameters of the production media affecting the synthesis (Sadhu et al., 2013).

Most of the studies on Cellulase production have been conducted on Fungi. The main Feed enzyme preparation is also produced by the Fungi. Recently, attention is put on cellulose producing bacteria due to their strong adaptability and many other advantages. Bacteria has higher growth rate than the fungi which allows recombinant production of enzymes in high amount, produces more complex glycoside hydrolases ensuring synergy. The extreme high natural diversity of bacteria makes the bacteria able to produce thermostable, alkali stable enzymes. So, the cellulolytic strains obtained from the harsh environment can be predicted to be stable in the bioconversion processes in the industry which would raise the rate of fermentation, enzymatic hydrolysis and product recovery (Sadhu et al., 2013).

The demand of cellulase as an industrial enzyme is 8% worldwide (Bon, 2007). Cellulose enzymes are very useful in many industrial application such as for denim finishing and cotton washing in Textile Industry, color care in Laundry Detergent, for mashing in food industry, for drainage improvement and modification of fiber in pulp industry (Cherry & Fidantsef, 2003). Moreover, the addition of cellulase in animal feed reduces the viscosity of chime, eliminates some anti-nutritional factors and improves the digestibility of the nutrients (Bhat, 2000). Animal diet costs can also be reduced by adding cellulose and cellulase to the diet of monogastric animals which will also improve intestinal health and reduce constipation occurrence (Bimrew, 2014) . To some up, cellulose enzyme has the potential to be the most stimulating technology in the future as the demand of very active, better stable and highly specific cellulases is increasing rapidly day by day in different industrial applications.

This study selected kitchen wastes i.e. Papaya Peels (PP), Banana Peels (BP) and Cucumber Peels (CP), as these could be potential sources for obtaining cellulolytic bacterial strains. Among all other available kitchen wastes, papaya peels were chosen due to their wide availability as edible fruit or vegetable, cultivable in all tropical countries and each year papaya wastes amount to more than one thousand (Rachtanapun, 2009). Then banana peels were selected as it was considered the most widely consumed fruit and ranked fourth after rice, wheat and corn as staple food (Phillips et al., 2021). Lastly, cucumber peels were selected because it was considered to contain greater cellulose content; 18.22% and it was another widely used vegetable (Mitra & Prasanna, 2020). This research was conducted to isolate the cellulose-degrading bacteria from kitchen wastes sample and optimize two environmental parameters-pH and temperature to improve the cellulase production by the isolated bacterial strains.

1.1 Literature Review:

1.1.1 Cellulose

Cellulose is a polysaccharide composed of a linear chain of glucose monomers (from a few hundred to more than ten thousand) linked via β-1,4-glycosidic bonds. In cellulose, the smallest repeating unit is cellobiose which ultimately can be converted into glucose. And, then the chains are packed into microfibrils where intramolecular hydrogen bonds along with intermolecular van der Waals forces hold them together. Then these microfibrils together form the cellulose matrix (Moo-Young, 2019).

There are four types of cellulose polymorphs: Cellulose I, Cellulose II, Cellulose III, and Cellulose IV. Cellulose I is the naturally occurring cellulose where in the parallel sheets where there is no intersheet hydrogen bonding (Thomas, 2017).

In the Crystalline structure of cellulose, the cellulose hydrogen Atoms are all situated in the axial position and the hydrogen groups are present in the equatorial position. The crystallization of cellulose occurs due to the hydrogen bonding of the equatorial hydroxyl groups with their nearest neighbours. In the figure given below, the Monoclinic crystalline unit cell for cellulose I is showed. In the "a" direction, medium-strength hydrogen bonds run (15 kcal $mol^{-1)}$). Weak Van der Waals forces hold the structure in the "C" direction (8 kcal $mol^{-1)}$. And in the "b"

direction the covalent bonds run which provides strength to the cellulose (50 kcal $mol^{-1)}$. Every molecule of cellulose run in similar direction; from non-reducing ends to reducing ends and so cellulose I is parallel (Caballero et al., 2003).

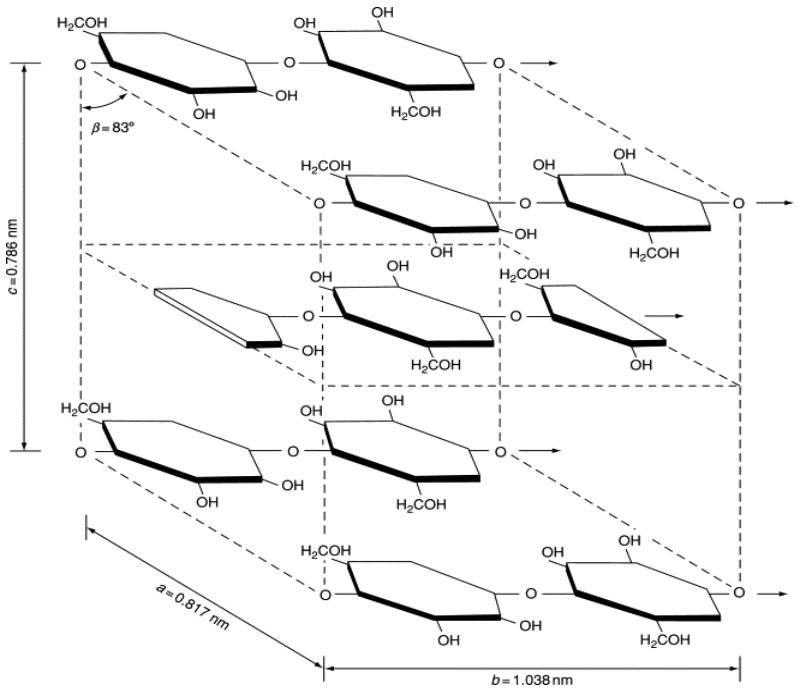


Figure1.1: Parallel Cellulose 1 Unit cell

Illustration Courtesy: Macrae R (1993). [Parallel cellulose I unit cell] https://www.sciencedirect.com/referencework/9780122270550/encyclopedia-of-food-sciences-and-nutrition#book-info

Enzyme cellulose synthase, known as membrane protein synthesizes cellulose. It possesses direct polymerization of the substrate UDP-glucose into cellulose product. In the globular motif of the cellulose synthase, the conserved residues are located and the identification of the Cellulose synthase's conserved residues from different organisms could be possible through the analysis of the predicted protein sequences (Inder, 2001).

Cellulose is one of the most widely found biopolymers in the world. From bacteria to terrestrial plants, many living organisms synthesize cellulose. Cellulose is the major component of plants' cell walls. High contents of cellulose are found in stalks, stems and other woody parts of the plants. It is also present in the cell walls of algae and fungi and occasionally found in bacteria. (De Leeuw and Largeau, 1993; Peberdy, 1990).

There are many applications of cellulose. Cellulose is the main constituent of the paper and pulp industry. It is also important in the textile industry where cotton linen and other plant fibres are used. Both of the pharmaceutical industry and food industry use microcrystalline cellulose as filler. The conversion of cellulose is also done to achieve various purposes such as cellophane from cellulose is used in photographic applications and nitrocellulose from cellulose is used in the household application and as gunpowder. Nowadays, Nanocrystalline cellulose or, Nanocellulose(which is prepared through the acid treatment of plant/bacterial cellulose) is getting attention rapidly for its various types of uses (*Green Chemistry*, 2018).

1.1.2 Background Information about Cellulase:

Enzymes are mainly proteins that function as catalysts in accelerating a chemical reaction. Almost all living beings can produce enzymes. And the enzymes coming from biological sources are termed as biocatalyst. Due to the hazardous effects of several chemical catalysts, recently the attention has been driven towards biological catalysts. However, in order to meet up the large scale demand, cost-effective substrates for these biocatalysts are necessary. Hence researchers have diverted their attention on identifying such enzymes that could use available plant polysaccharides as their substrate. In order to hydrolysis of cellulose, utilizing cellulase enzyme from the bacterial origin is one such suitable option that meets both criteria of cost-effective substrate and biocatalyst. Here, the term cellulase refers to the group of enzymes which assist in catalyzing the breakdown of cellulose into smaller oligosaccharides, cellobiose and glucose as end products. The digestible end products released by this enzyme can be used as a nutrient source for microorganisms itself, plants and animals (Ejaz et al., 2021). Therefore, cellulase can be considered an important enzyme of nature. However, the enzyme cellulase falls into the common group of glycosyl hydrolase, which is responsible for hydrolyzing glycosidic bonds between two carbohydrates or between a carbohydrate and a non-carbohydrate molecule. And based on amino acid sequence similarities and crystal structure, glycosyl hydrolases including cellulase have been classified into 115 families, out of which 13 different families contain cellulase.

1.1.3 Structure of Cellulase:

Cellulase is mainly composed of two binding sites: the active site and the substrate binding site. The active site is usually contained within the Catalytic Domain(CD) and the substrate binding site is a part of functionally different and separately folded Carbohydrate/Cellulose

Binding Domain(CBD) (Lakhundi et al., 2015).These two domains are held together by a peptide linker sequence, rich in proline and threonine.

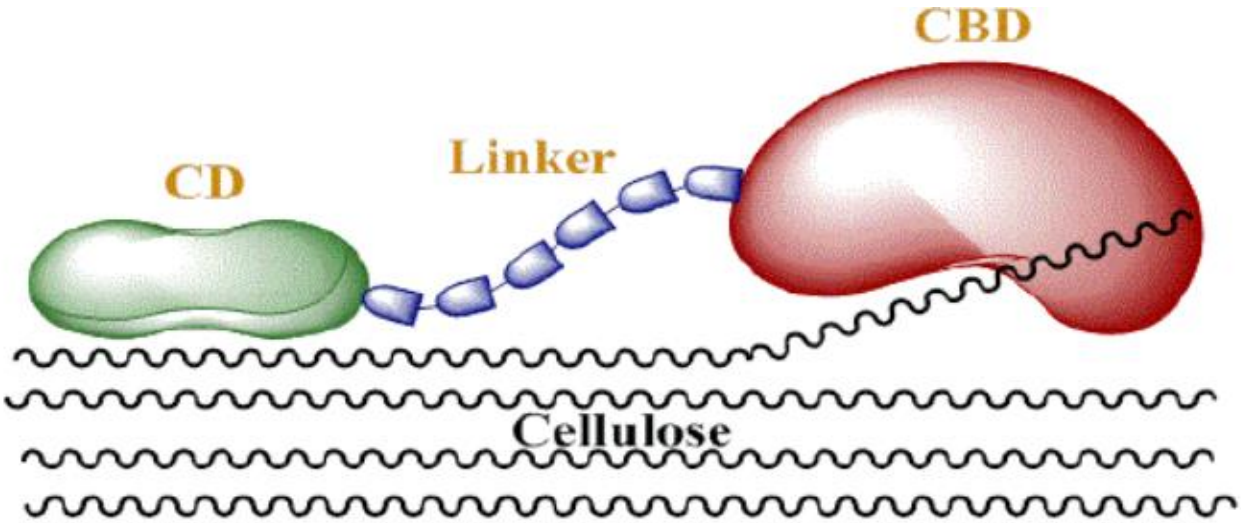


Figure 1.2: Two Domains of Cellulase Enzyme

Illustration Courtesy: Sajith (2016). [General structure of cellulase consisting of catalytic domain (CD) and carbohydrate binding domain (CBD) joined via a linker peptide] https://www.longdom.org/open-access/an-overview-on-fungal-cellulases-with-an-industrial-perspective-35246.html

i. **Cellulose Binding Domain(CBD):** Cellulose Binding Domain can be described as an adjoining amino acid sequence within any carbohydrate active enzyme, which has the ability to bind to cellulose substrate. It is usually composed of 30-200 amino acids. The CBD of a cellulase enzyme may exist as single, double or triple domain and can be located on both C-terminus or N-terminus and sometimes in the center of the protein (Lakhundi et al., 2015) . This CBD is considered to function by bringing the enzyme into closer and prolonged contact with its recalcitrant substrate. As the CBD binds to cellulose, cellulase enzyme can constantly bind to cellulose and continue its catalytic action by moving to next cleavage site following the movement of CBD on cellulose surface(Tanimura et al., 2013). However, not all cellulases contain CBD, rather have only CD in their protein chain and those are known as single-domain proteins. In this case, cellulase without CBD gets detached from its substrate after one cleavage reaction and then keeps searching for

next cleavage site for next catalytic reaction. Therefore, cellulases with a CBD are able to hydrolyze more efficiently that those with CBD (Tanimura et al., 2013).

ii. **Catalytic Domain(CD):** Catalytic Domain is considered as the longest domain within cellulase enzyme. This CD is mainly responsible for showing specificity towards a certain substrate. *Trichoderma reesei* cellobiohydrolase II (CBH II) was the first enzyme whose CD was structurally defined with the help of X-ray crystallography (Ohmiya et al., 1997). And CD of this enzyme was found to be a large α/β protein with 5 α-helices and 7 β-strands (Lakhundi et al., 2015). The active site here is an enclosed tunnel and was found near the C-terminal end of β-barrel through which cellulose chain can be threaded.

1.1.4 Mode of Action of Cellulase:

Due to the recalcitrant and resistant structure of cellulose, it becomes practically impossible for one single enzyme to clasp cellulose into its substrate site and hydrolyze it. Therefore, cellulose needs to be hydrolyzed by a multiple number of synergistically active enzymes functioning with each other to bring complete hydrolysis (Lakhundi et al., 2015). However, to completely hydrolyze polymeric substrate cellulose into its monomeric unit, at least three enzymes must work simultaneously.

Based on the cleavage site on cellulose substrate, cellulase can be classified into three different enzymes such as (1) β -1,4-endoglucanase, (2) β-1,4-exoglucanase and (3) β – glucosidase. With the help of the cooperation of these three enzymes, cellulases can disrupt the structure of cellulose at the solid-liquid interface, thereby making each fibre available for the hydrolysis process (Lakhundi et al., 2015). The mode of action of each enzyme to function cellulolysis is depicted below-

i. **β -1,4-endoglucanase Activity:** Cellulolysis is started with the activity of this enzyme. β -1,4-endoglucanase refers to those endocellulases which are able to cleave at the non-crystalline surface of cellulose molecule. It basically makes random cut within the amorphous region of cellulose and thus provides reducing and non-reducing ends of cello-oligosaccharides (Behera et al., 2017). By making random cuts it produces new chain lengths, which in turn makes hydrolytic process easier for next enzymes. As the

enzyme β -1,4-endoglucanase is able to bind to the interior of long cellulose fibers, therefore it is considered have open active site (Lakhundi et al., 2015). Furthermore, it's activity is assumed to be highest near soluble cellulose forms or amorphous regions (Jayasekara & Ratnayake, 2019).

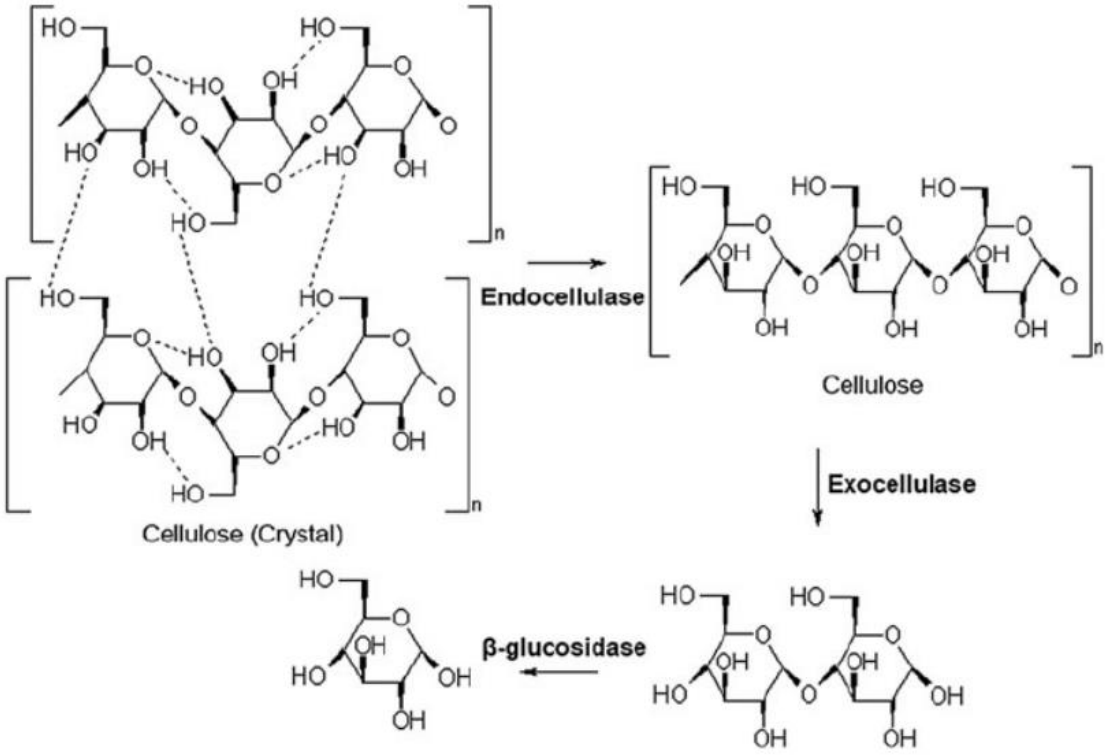


Figure 1.3: Enzymatic Hydrolysis of Cellulose

Illustration Courtesy: V (2019). [Enzymatic hydrolysis of cellulose]

https://www.researchgate.net/publication/328732983_Role_of_Cellulases_in_Food_Feed_and_Beverage_Industries_Enzymes_in_Industrial_Food_Processing

ii. **β-1,4-exoglucanase Activity:** Unlike the previous enzyme, β-1,4-exoglucanase has its active site inside a tunnel. Therefore, it is thought to be consistent with its natural progressive movement and sequential release of cellobiose from an end of a cellulose chain (Lakhundi et al., 2015). After β -1,4-endoglucanase cleaves into amorphous region and creates site for β-1,4-exoglucanase to proceed, it then starts working from reducing and/or non-reducing end of new cellulose chain (created by β -1,4-endoglucanase).

Which generates more shortened cellulose chains such as cellodextrins and cellobiose etc, where cellobiose is the major end product.

iii. **β – glucosidase Activity:** Finally, β – glucosidase comes into action and starts releasing glucose non-reducing ends of cello-oligosaccharides and convert cellobiose into glucose. Thus with cooperation of these enzymes, insoluble cellulose fiber is hydrolyzed into soluble glucose unit.

1.2 Cellulase Producing Bacterial Species Found in Different Studies:

1.2.1 Common Cellulase Producing Bacteria:

Bacillus spp, Acetivibrio Cellulyticas, Clostridium spp., Thermobispora bispara, Thermobifida fusca, Thermonospora spp., Streptomyces spp., Ruminococcus albus and *Cellumonas spp.,*- are found to be the most common cellulolytic bacteria (Sadhu & Maiti, 2013).

1.2.2 Findings of Recent Studies:

Some other bacterial species with cellulolytic activity were found in different recent studies.

- Due to having high amount of dietary fiber in the feed of Min pigs, the Min pig were considered to be a potential source of cellulase producing bacteria. From the feces of Min pig, 10 cellulase producing strains were isolated and among them *Bacillus velezensis* was reported to have the highest Enzymatic activity (Li et al., 2020).
- A study conducted on the gut of the *Holotrichia parallela* larvae where it was found for the first time that *Siphonobacter aquaeclarae, Paracoccus sulfuroxidans, Ochrobactrum cytisi, Cellulosimicrobium funkei, Ensifer adhaerens, Ochrobactrum haematophilum, Devosia riboflavina, Kaistia adipata, Labrys neptuniae, Shinella zoogloeoides, Citrobacter freundii and Pseudomonas nitroreducens*- these species isolated from the gut of the Holotrichia parallela larvae showed cellulolytic activity.
- From the surface seashore water, a novel marine bacterium with cellulolytic activity was isolated and characterized which was designated as *Oricola cellulosilytica* and affiliated to the family Phyllobacteriacea. Further research is here needed to know if these strains produce fascinating cellulases.
- A study was conducted on the intestinal tract of the sugarcane borer found *Klebsiella pneumoniae, Klebsiella* sp., and *Bacillus* sp. *K. pneumonia* to be the main

cellulase producing organism isolated from the intestinal tract of the sugarcane borer (Barbosa et al., 2020).

- Another study was conducted on the Sugar Industry waste (Molasses) which are cellulosic in nature and so are potential to be the source of cellulase producing bacteria. *Paenibacillus sp., Aeromonas sp., and Bacillus sp.* were isolated from the sugar industry waste and among them *Paenibacillus sp.* was found to have the highest cellulase production (Islam, 2019).

1.2.3 Thermostable Cellulase-Producing Bacteria

In recent times, studies on producing thermostable cellulases are also getting priorities so many studies are conducted on cellulase-producing thermo/alkaliphiles. Studies on improving the cellulase production condition are also carried out by optimizing the nutritional and environmental parameters of the production condition. Engineering of new recombinants which can produce a higher amount of cellulases is also attempted. Many thermophilic and alkaliphilic cellulose-producer bacteria already have been isolated from different types of environments such as –

- From a marine plant- *Ulva lactuca* ,a bacterial strain *Bacillus flexus NT* was isolated and this strain was reported to produce an extracellular alkali-halotolerant cellulose (Trivedi et al., 2011).
- From the 1.5 km deep surface of Homestake gold mine in Lead, South Dakota, USA , weathered soil-like sample was collected which contained mesophilic (37^{o} C) and thermophilic (60^{o} C) cellulolytic bacterial diversity. The cellulase producing mesophilic and thermophilic pure culture obtained from the enrichment culture of this sample belong to the genera *Brevibacillus, Paenibacillus, Bacillus*, and *Geobacillus* and some selected cultures were furthered studied (Rastogi et al., 2009).

1.2.4 Some Related Research Works Conducted in Bangladesh:

I. In Bangladesh, rice production comprises a large amount of straw waste. The components of these straw wastes include mainly cellulosic materials, therefore become resistant to microbial decomposition unlike other straw wastes coming from

protein-rich grains such as wheat and barley. Farmers have been using a traditional on-site burning method to manage waste. However, incomplete combustion of these straws causes severe damage to the environment such as disturbing air quality and soil nutrients etc. Focusing on these drawbacks, the university of Chittagong has conducted research by trying to isolate actinomycetes from rice straws of local paddy fields in Hathazari using a Starch Casein Agar medium. And among numerous numbers of isolates *Thermomonospora viridis* was found to be the best cellulose degrader (Sadida & Manchur, 2021).

II. Sugar mills also play a big role in impacting the environment by producing wastewater, emissions and solid wastes etc. Compared to other industrial wastes, sugar industry wastes mostly includes cellulose and the microorganisms present there can utilize these cellulosic materials as a nutritional requirement. Therefore, a research group utilized the sugar industry molasses from the Katakhali region of Rajshahi city to isolate several cellulose-degrading bacteria such as *Paenibacillus* sp. *Bacillus* sp. and *Aeromonas* sp. This research also included the optimization of cultural conditions for these identified bacterial samples (Islam & Roy, 2018).

1.3 Biotechnological Application:

Bacterial cellulases have many potential applications in many industries. Among all other enzymes, it has a very high demand in many fields of the industry. It also has applications in medical field and agricultural field. Some of the latest advances of the application of cellulase in different fields are mentioned below:

1.3.1 Industrial Application:

Industry always demands a cost-effective way of enzyme production and maintenance. And for industrial applications, enzymes are needed which are highly stable and can tolerate extreme pHs, and temperatures. So, ensuring the improved quality of cellulase in a cost-effective production way is the prime condition for the cellulase to be utilized in industrial applications. And, studies are conducted on how to improve the quality of cellulase cost-effectively.

Textile Industry:

For the biopolishing of fabrics and producing stonewashed looks in denims, the cellulase are applied in the textile Industry. The cellulases applied to be in the textile industry should have compatibility with the other components of the formulation, and tolerance to extreme temperature and pH (Adrio & Demain, 2014a). *Pseudomonas, Sphingomonas, Thermobifida and Actinomycetes* from *Streptomyces* genera are used for the degradation and decolourisation of the textile dye (McMullan et al., 2001). For the enzymatic processing of the denim fibres, alkaline/neutral conditions are preferred and endoglucanase of *Thermomonospora sp.*, which is an alkaline-stable enzyme, was reported to be used (Anish et al., 2007).

Detergent Industry:

To improve the brightness and softness of fabrics made of cotton, cellulase is used in household laundry detergent. As for other industries, the cellulases used in this industry should be tolerant to extreme pH and temperature and should be produced cost-effectively. Cellulase combined with other enzymes is added to detergents(where all other components of the detergent is present) for catalyzing the hydrolyzing of the chemical bonds in the water's presence under a thermophilic and/or alkalophilic environment (Adrio & Demain, 2014). The thermophilic bacterium *Bacillus* sp. SMIA-2 was reported to produce a cellulase which was very stable and active at the extreme environment with the presence of different commercial detergent brand (Yu et al., 2015).

Food Processing Industry:

With the combination of pectinases and hemicellulases, cellulases are frequently used in food processing, for example- to produce fruit juice, vegetable juices, degradation of plant cell walls and carotenoids for the wine and beer industry. *Bacillus* and *Paenibacillus* were reported to be the cellulase producers used in the fruit juice production industry (Singh, 2015). Indirectly, cellulases are used to produce colouring substances by being combined with pectinases to degrade the cell walls of oranges, carrots and sweet potatoes to extract carotenoids (Çinar, 2005). For producing sugar by degrading the peels of the grapes, cellulases are used in various industrial applications (Wilkins et al., 2007). In the extraction of the phenolic compound from grape pomace, cellulase participates (Meyer et al., 1998).

Animal Feed Processing Industry:

The combined use of cellulase with hemicellulase and pectinase is also applied in Animal Feed Processing Industry. To increase the digestibility of cereal-based food, cellulases are used in animal feed (Dhiman et al., 2002) . For monogastric animal feed application, cellulases from *Bacillus subtilis* can be applied, in soya grain hull degradation to enhance its nutritional value of it (Wongputtisin et al., 2014). More studies should be conducted for the discovery of new microbial cellulases that is adaptable to different processes of this industry. Here, the application of the metagenomic tool will be a very great help for the search.

Paper and Pulp Industries:

Cellulase has the potential to be applied in the paper and pulp industry based on it being capable of de-inking papers, but the application of cellulase in this field is yet not a reality. For having the probability of fibre degradation and loss of viscosity, cellulase should not be present in the preparation of pulp and paper, but for de-inking, specifically de-inking office waste paper (Viesturs et al., 1999), paper recycling (Bajpai, 1999), fibre modification and drainage improvement (Eriksson, 1990), the uses of cellulases were mentioned in many patents and studies. In this field of industry, only in one study bacterial cellulase CelB isolated from *Paenibacillus sp*. BP-23 was mentioned to be used for the improvement of paper properties by improving the drainage process (García et al., 2002).

Biorefinery and Biofuels:

Plant biomass degradation requires 3 steps in total: physicochemical pretreatments, ii. Enzymatic hydrolysis and iii. Fermentation. Cellulases are such enzymes that could efficiently hydrolyze lignocellulosic biomass and can reduce about 40% cost of bioprocessing by eliminating pretreatments. (Lynd et al., 2008). And a significant reduction in production cost is necessary to ensure the viability of cellulase production on a commercial scale. These biological enzymes are also compatible with bioreactor and processing environments(Menendez et al., 2015). *Trichoderma reseei* has been considered as the main industrial source of cellulase for a long time , where it has efficiently depolymerized plant biomass into simple sugars (Adrio & Demain, 2014). However, an extremely thermophilic bacterium *Caldicelluloseruptor bescii*, can produce cellulase with higher enzymatic activity

than *Trichoderma reseei* (Yang et al., 2010). Nowadays thermophilic bacteria are gaining popularity as efficient cellulase sources.

1.3.2 Medical Applications:

Bacterial cellulases have both direct and indirect role in medicinal sector.

Indirect Applications: Chitosan Obtention

Chitosan is a semi-crystalline derivative form of chitin, which can be obtained using enzymatic hydrolysis. And chitin is a polysaccharide composed of randomly distributed β-linked D-glucosamine and N-acetyl-D-glucosamine. Cellulase enzymes of fungal origin can degrade chitosan in combination with chitinase and lysozyme. The derived chitosan from chitin has multiple medical applications such as bone rebuilding, artificial skin production, anti-bacterial, and anti-cancer agent preparation (Zhang et al., 2010). With the help of the combined effect of unspecific cellulases regular-sized chitosan can be converted into low molecular weight chitosan, which can be easily used in medical sectors. A report says cellulase-treated chitosan contains anti-tumoral capacity (Qin, 2004).

Direct Applications: Phytobezoar Degradation and Anti-biofilm Agents

Phytobezoar refers to the concretion in the gastrointestinal tract composed of indigestible plant-originated swallowed substances. Such medical conditions can be treated with cellulase, diet coke or a combination of both without the help of any surgical intervention. Although, the use of bacterial cellulase, in this case, no evidence has been found, however, fungal cellulase has been used in some cases(Menendez et al., 2015). Another application of cellulase talks about disrupting cell walls and biofilm structures of pathogenic organisms. Biofilm refers to the assimilation of bacterial communities in an extracellular matrix, which is tough to be degraded by multiple drugs. Indeed, a major component in biofilm structure is cellulose. However, bacterial cellulases can be directly used as anti-biofilm agents in several medical conditions such as cystic fibrosis, nosocomial infections etc (Menendez et al., 2015).

1.3.3 Agricultural Applications:

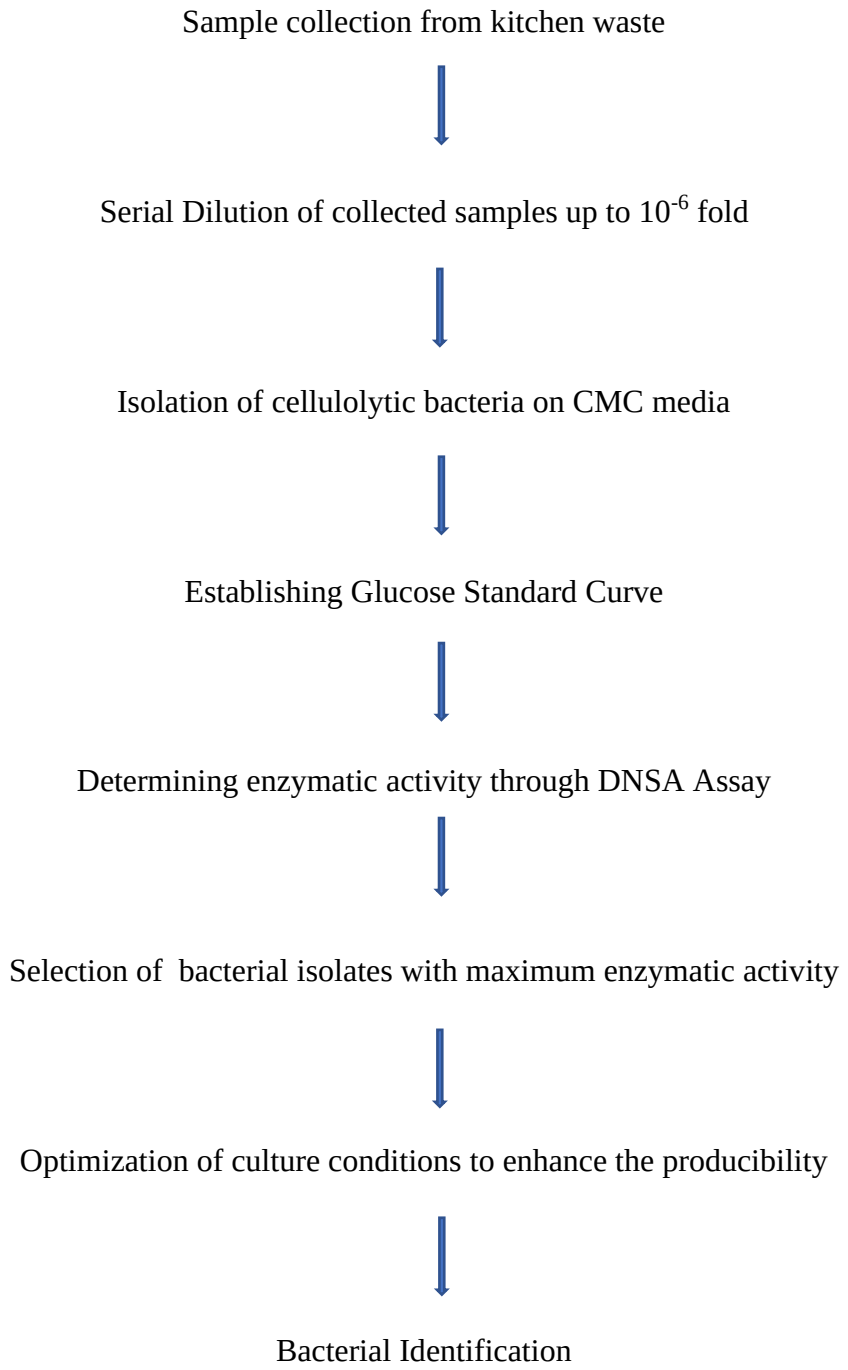
Plant Growth-Promoting Rhizobacteria (PGPRs) provide some beneficial mechanisms to agriculture such as reducing chemical fertilization treatment, increasing plant growth and development etc. It also provides some indirect benefits such as inhibiting pathogen growth by

antibiotic production, lytic enzyme production etc (Glick, 2012). Bacteria such kinds of PGPR mechanisms are known as biocontrol agents. And several biocontrol agents can produce lytic enzymes such as chitinase, protease, and catalase, which can degrade cell walls of many pathogenic fungi (El-Tarabily et al., 1996). Bacterial cellulase can function synergistically with antibacterial compounds from other bacteria, which can function against fungal diseases. One such example can be *Paenibacillus ehimensis KWN38* generates antifungal compounds in combination with cellulase and *ß-glucanases*, which safeguards crops from infection of oomycetes (Sopheareth et al., 2013).

Chapter 2

2. Materials and Methods:

2.1 Experimental Work Flow:



2.2 Sample Collection from Kitchen Waste:

Samples were collected from three different kitchen wastes, i.e. Papaya peels, Cucumber peels, and Banana peels etc. All of these samples were decomposed for 24 to 48 hours in order to ensure proper bacterial growth. 5 to 6 grams of each sample was measured and then soaked into 100ml saline in conical flasks and vortexed for 10 to 15 seconds.

2.3 Serial Dilution of Collected Samples:

Each of the samples was diluted for a ten-fold dilution up-to dilution factor of 10^{-6}. Where 1 ml of a sample was taken and then sequentially transferred into series of 6 test tubes containing 9mL solution of a sterile diluent (0.9% NaCl).

2.4 Screening and Isolation of Cellulase Producing Bacteria:

Carboxymethyl Cellulose (CMC) agar media was prepared to specifically isolate the cellulase producing bacteria with following ingredients(g/L) 1.36 g KH_2PO_4, 0.20 g $MgSO_4.7H_2O$, 2.00 g NaCl, 1.00 g $(NH_4)_2SO_4$, 0.01 g $FeSO_4.7H_2O$, 3.00 g CMC, 1.00 g Yeast Extract, 15.00 g Agar powder (Kunasundari et al., 2016). Then, 50 microliter sample from each dilution tube was taken by pipette and spread on prepared CMC plates. The plates were then incubated for 24 hours at 37^o C. After incubation period, colonies were selected from the plates on the basis of their morphological characteristics. Here 16 isolates from Papaya peels, 8 isolates from Banana peels and 10 isolates from Cucumber peels were selected for next steps.

Sample Name	Isolate Number	Selected Isolate Number
Papaya Peels (PP)	PP1-PP16	16
Banana Peels (BP)	BP1-BP8	8
Cucumber Peels (CP)	CP1-CP7	7
	Total	31

Table 2.1: Total Number of Initially Selected Isolates

2.5 Maintenance of the Selected Isolates:

The isolates selected on the basis of morphological characteristics (from previous step) were inoculated in nutrient agar medium and then incubated overnight at 37^{o} C.

2.6 Establishing Glucose Standard Curve:

Glucose concentration ranging from 0.2 to 1.4 micromole/ml were prepared. For that, 100 ml stock solution of 1.4 micromole/ml glucose was prepared initially. Then following the formula of $C_1V_1 = C_2V_2$ other concentrations, i.e., 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 micromole/ml were prepared in different test tubes using the stock solution. Next, 1ml solution from each test tube was taken and transferred into seven discrete test tubes labelled with respective concentrations. At the same time, in another test tube 1ml distilled water instead of glucose was taken as control. After that, 1ml of 0.05M citrate buffer (pH 5.0) was added in all of the test tubes. Afterwards, 3mL of 3,5-Dinitrosalicylic acid (DNS) was also added in each test tube. Finally, all test tubes were placed in water bath at 100^{o} C for 10 minutes (Miller, 1959). After allowing the test tubes to cool in room temperature, absorbance was measured at 540 nm in a spectrophotometer for each of the sample. Then using reading of the absorbance, a standard glucose curve was plotted keeping 'Glucose Concentration' in 'Y' axis and 'Absorbance' in 'X' axis.

2.7 Enzyme Assay:

Inoculation in Nutrient Agar Medium:

At first, 2 to 3 scoops of inoculum was taken from the maintenance media plates and inoculated again in Nutrient Agar media and then incubated for 24 hours at 37^{o} C.

Inoculation in Enzyme Production Medium:

Here 2 to 3 scoops inoculum was taken from Nutrient Agar plates of previous step and inoculated in 100ml conical flasks containing 10 ml of Luria Bertani Broth (Enzyme Production media) and kept in shaker incubator for 24 hours at 37^{o} C.

Preparation of Crude Enzyme:

After completing fermentation period, 1.5 ml culture from each conical flask was taken into 1.5 ml sized Eppendorf and then centrifuged at 3500 rpm for 10 minutes maintaining 4^{o} C temperature. After centrifugation, the obtained supernatant layer was served as crude enzyme.

2.8 Reducing Sugar Estimation by Dinitrosalicylic Acid (DNSA) Method:

3,5-Dinitrosalycylic Acid method was used to measure the amount of reducing sugar released in the hydrolysis of cellulose.

At first, for each sample, 1ml 0.05 M Citrate Buffer (pH 5.0) was taken in a test tube followed by the addition of 1ml substrate solution (1% CMC dissolved in 0.05 M Citrate Buffer (pH 5.0)). Then 1ml of the prepared crude enzyme was also added. In another test tube, 1mL distilled water instead of the crude enzyme was taken as Blank. After that, the test tubes were placed in a water bath for 30 minutes at 50^{o} C. The test tubes were then allowed to cool at room temperature and to stop the reaction 2ml DNS was added and kept for 10 minutes. Next, the test tubes were placed in a water bath for 10 minutes at 100^{o} C. After allowing the test tubes to cool in room temperature, absorbance was measured at 540 nm of wavelength for each of the samples (Miller, 1959).

Here, the amount of enzyme that could release 1 micromole of glucose by hydrolyzing CMC(Carboxymethylcellulose) within 1 minute of reaction, was defined as one unit of Endo-β-1,4-glucanase activity (Islam, 2018).

2.9 Optimization of Culture Condition for Maximum Cellulase Production:

Selection of bacterial isolates with the highest enzymatic activity:

The bacterial isolates with the highest enzymatic activity were selected-

Sample Name	No of the selected isolates
Papaya Peels (PP)	5
Banana Peels (BP)	3
Cucumber Peels (CP)	4
Total	12

Table 2.2: Number of Selected Isolates with Highest Enzymatic Activity

Determining the Effect of pH and Temperature of Culture Media:

For these selected isolates, two different parameters (pH and temperature) of culture media were set separately at different values to observe their effect on bacterial growth.

Effect of pH:

The pH of the culture media was set at 5.0 and 8.5, and adjusted using NaOH pellets and 37% HCL. At the time of changing pH, the standard temperature of bacterial growth; 37° C was maintained.

Effect of temperature:

The temperature of the shaker incubator was set at 30° C and 42° C. Here the pH of culture media was unchanged.

2.10 Bacterial Identification:

Morphological and microscopic identification of bacterial samples relies on several types of identification test. Here, each identification test required fresh and young culture. To obtain fresh and young culture, each time NA plates were streaked maintaining sterile environment for each bacterial sample and incubated for 24 hours at 37° C.

2.10.1 Gram Staining

After preparing bacterial smear, Crystal violet was used as a primary stain. Next, Gram's Iodine was added as a mordant to fix the dye. Then, decolourizer Ethanol was used to remove the primary strain if not retained by the bacterial cell wall. Finally, a counterstain- Safranin was added. If the Bacterial strain was Gram Positive, it would be stained as purple or, if Gram Negative, it would be stained as pink under microscope.

2.10.2 Biochemical Testing:

Total 9 biochemical tests were conducted. And for each test, 12 test tubes were taken for 12 bacterial samples and 1 extra test tube was kept as negative control.

Methyl Red Test:

For each sample, 7 ml of prepared MR-VP broth was taken to a test tube and was sterilized under autoclave. After allowing to cool in room temperature, one loopful of fresh bacterial culture was inoculated into these test tubes in a sterile environment and incubated for 24 to 48 hours at 37° C. After the incubation period, 5 drops of Methyl Red indicator were added to each test tubes and observed for any color change.

Voges-Proskauer Test:

For each sample, 7 ml of prepared MR-VP broth was taken to a test tube and was sterilized under autoclave. After allowing to cool in room temperature, one loopful of fresh bacterial culture was inoculated into these test tubes in a sterile environment and incubated for 24 to 48 hours at 37º C. After the incubation period, Barritt Reagent A (5% alpha-naphthylamine) and Barritt Reagent B (40% KOH) were added for 12 drops and 4 drops respectively to each test tube and waited for 30-45 minutes to observe for any change.

Citrate Utilization Test:

2.5 ml of prepared Simmons citrate agar was taken to vials for each sample and sterilized under autoclave. Then the autoclaved vials were kept in slanted position and allowed to be solidified in room temperature. Next one loopful of fresh bacterial culture was inoculated into these vials maintaining sterile environment and incubated for 24-48 hours at 37º C to observe the change in color.

Indole Test:

7 ml of prepared tryptophan broth was taken to the test tubes for each sample and sterilized under autoclave. After allowing to cool in room temperature, one loopful of fresh bacterial culture was inoculated into these test tubes and incubated for 24-48 hours at 37º C in order to observe for any color change.

Triple Sugar Iron test:

7 ml of prepared Triple Sugar Iron media was taken to test tubes for each sample and sterilized under autoclave. After that, the autoclaved test tubes were placed in slanted position and allowed to be solidified under room temperature. Next, one loopful of fresh bacterial culture was inoculated into them and incubated for 24-48 hours at 37º C in order to observe for any color change, gas formation and black precipitation.

Oxidase test:

After the oxidase reagent was prepared, a filter paper was soaked with 1 drop of it. Then using a sterile toothpick, small amount of fresh bacterial culture was picked and streaked on the soaked filter paper and waited for about 30 seconds to observe any color change.

Catalase test:

After 3% H_2O_2 reagent was prepared, one drop of it was placed on glass slides for each sample with a sterile dropper. Then using a sterile tooth pick, small amount of fresh bacterial culture was taken and mixed with H_2O_2 and then observed for any gas formation or no gas formation.

MIU (Motility-Indole-Urea) Test:

At first, MIU media base was prepared and sent to sterilization under autoclave. After sterilization is completed, the autoclaved media was allowed to cool in room temperature and previously prepared 40% urea solution was aseptically added into MIU base using syringe filter. Next, 6 ml of the MIU media (containing 40% urea solution) was transferred into all test tubes using a sterilized pipette while maintaining sterile condition. The test tubes were then kept in fridge until the media was solidified enough to be inoculated. Finally, one loopful of bacterial sample was very carefully stabbed for one time into those test tubes and kept for incubation at 37^{o} C for 24-48 hours until any observable result is found.

Nitrate Reduction Test:

7 ml of prepared Nitrate broth was taken to test tubes for each sample and sterilized under autoclave. After allowing the test tubes to cool in room temperature, one loopful of fresh bacterial culture was inoculated into these test tubes and incubated for 24-48 hours at 37^{o} C. Following the incubation period, 5 drops of Reagent A (Sulphanilic Acid) was added and then 5 drops of reagent B (α-naphthylamine) was added to each test tubes. After that, if no appearance of red color was observed in any test tubes, a small amount of Zinc powder was added to only those test tubes.

Chapter 3

3. Result:

3.1 Glucose Standard Curve Establishment:

A Standard Glucose curve was established using 0.2 to 1.4 micromole/ml concentration of glucose.

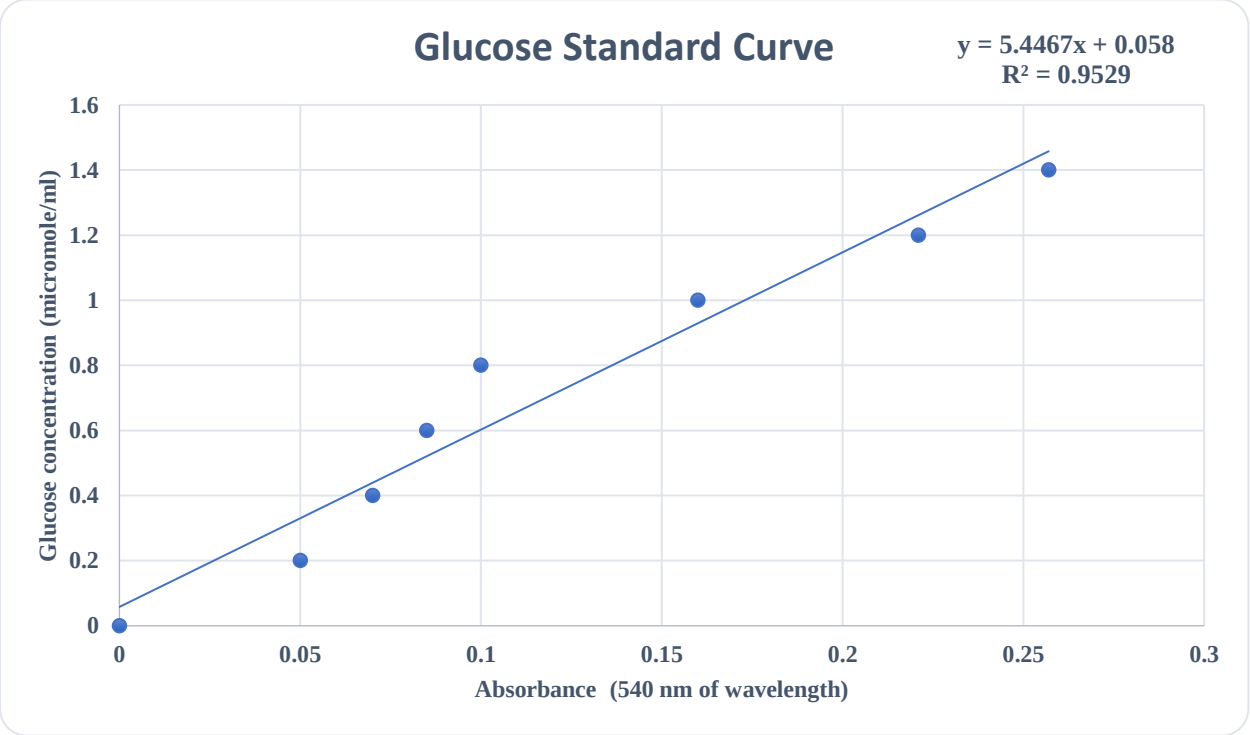


Figure 3.1: Glucose Calibration Curve

In the illustrated graph, the 'X' axis represented Absorbance (at 540 nm wavelength) and 'Y' axis represented glucose concentration. Here the established Glucose standard curve represented the relationship between Glucose and its corresponding Absorbance measured at 540 nm of wavelength. Here the correlation coefficient, R^2 =0.9529 indicated that 95.29% of data could be fitted by this regression model.

3.2 Cellulolytic Activity Measurement of Bacterial isolates:

In order to measure cellulolytic activity of the bacterial isolates from peels of papaya, banana and cucumber, 3,5-Dinitrosalycylic Acid method was used to measure the amount of reducing sugar released in the hydrolysis of cellulose.

Results of Enzymatic Assay for Isolates from Papaya Peel:

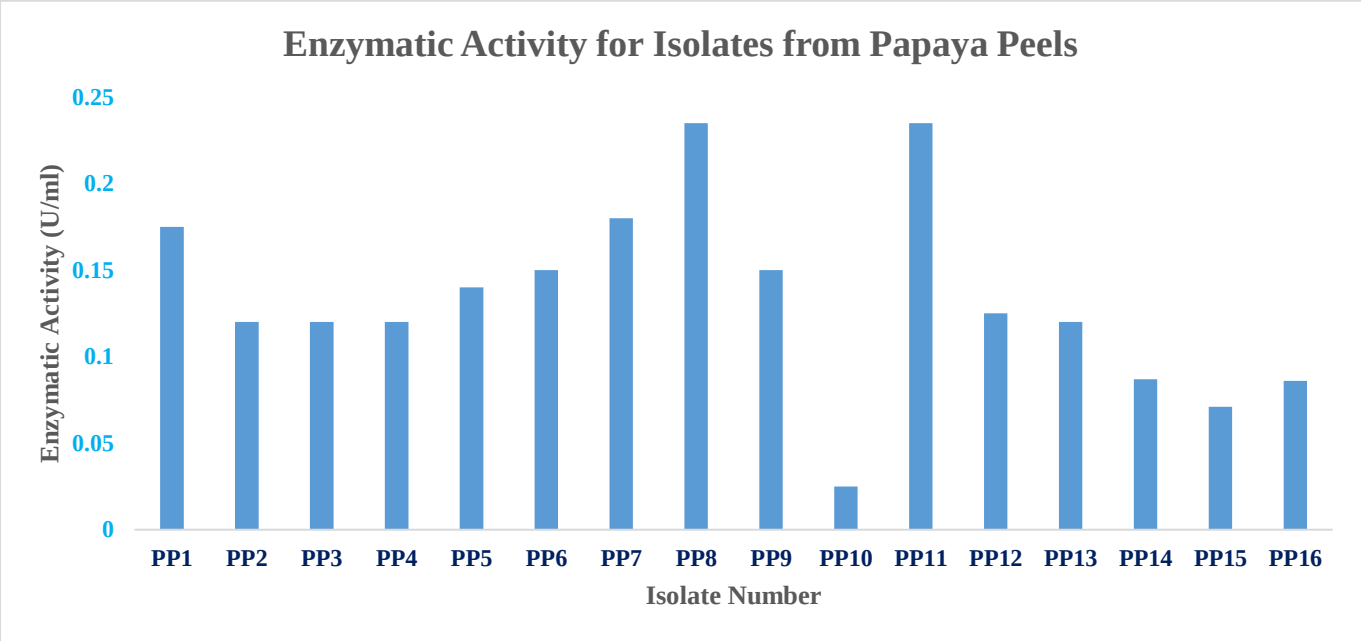


Figure 3.2: Enzymatic Activity for Isolates from Papaya Peels at Normal Condition

According to the illustrated graph, enzymatic activity was ranged from 0.025-0.235 U/ml (obtained from the Absorbance measured at 540 nm of Wavelength using Glucose Standard Curve). Where the lowest activity was observed in isolate number PP10 and the highest activity was observed in isolate numbers PP8 and PP11.

Results of Enzymatic Assay for Isolates from Banana Peel:

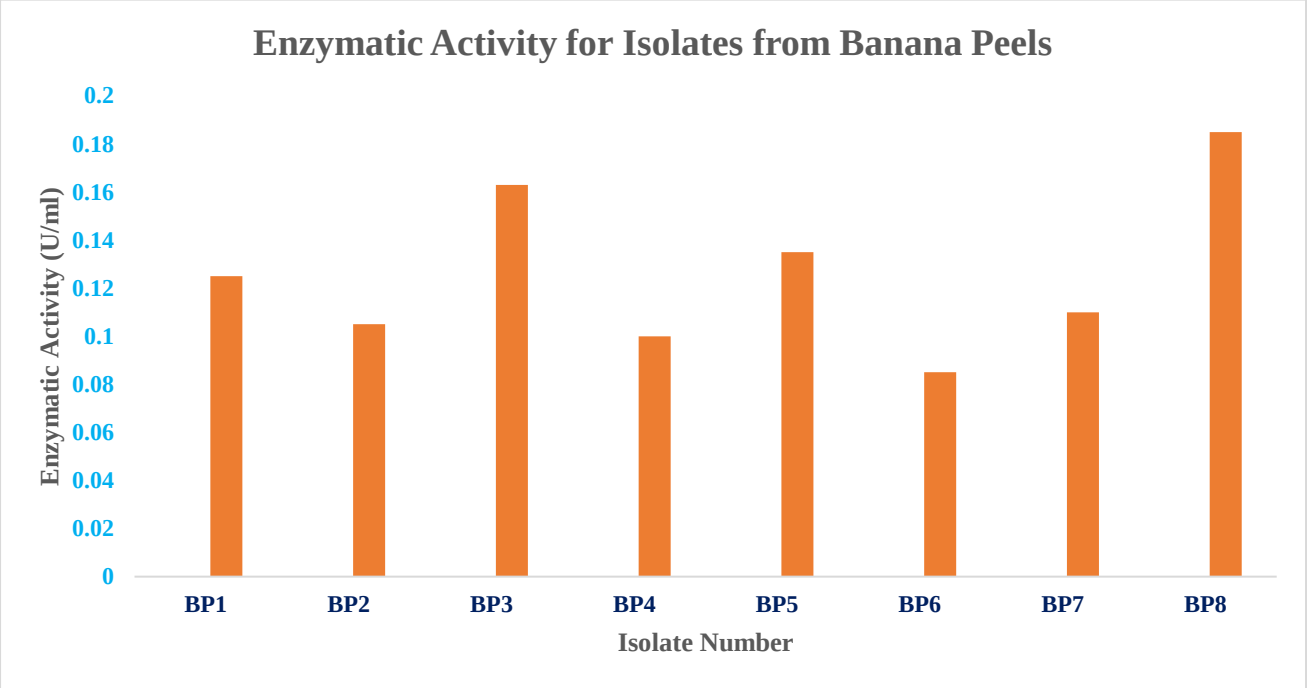


Figure 3.3: Enzymatic Activity for Isolates from Banana Peels at Normal Condition

According to the illustrated graph, enzymatic activity was ranged from 0.085-0.185 U/ml (obtained from the Absorbance measured at 540 nm of wavelength using Glucose Standard Curve). Where lowest activity was observed in isolate number BP4 and highest activity was observed in isolate number BP8.

Results of Enzymatic Assay for Isolates from Cucumber Peel:

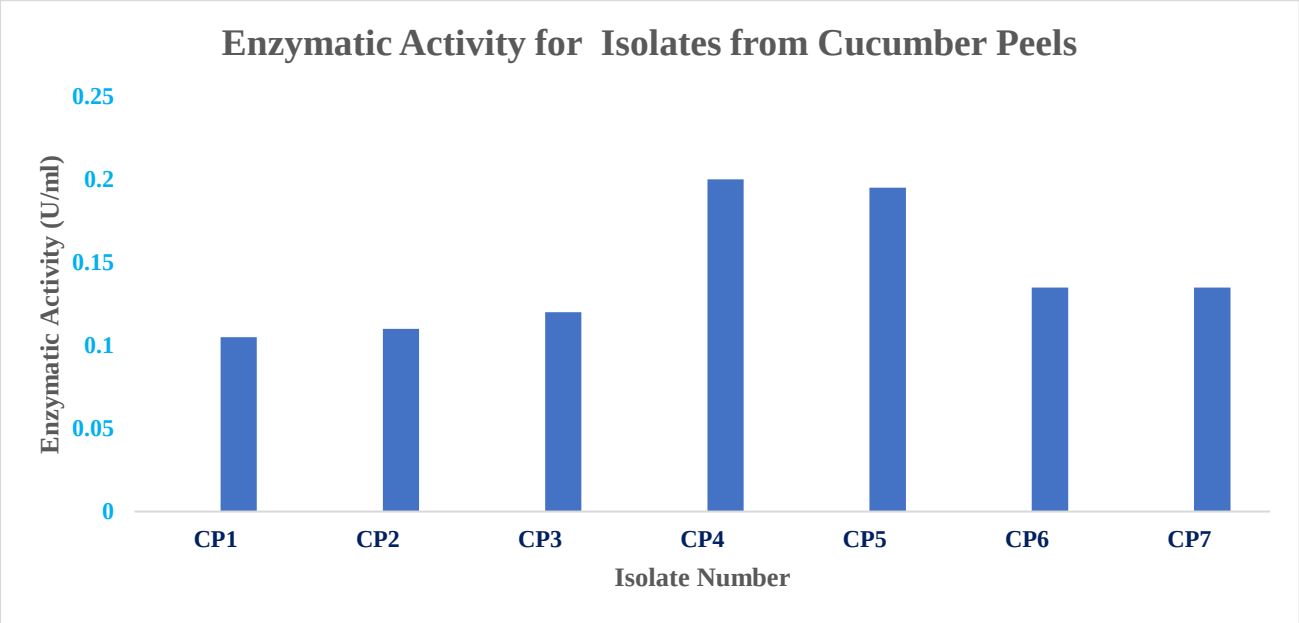


Figure 3.4: Enzymatic Activity for Isolates from Cucumber Peels at Normal Condition

According to the illustrated graph, enzymatic activity was ranged from 0.105-0.2 U/ml (obtained from the Absorbance measured at 540 nm of wavelength using Glucose Standard Curve). Where lowest activity was observed in isolate number CP1 and highest activity was observed in isolate number CP4.

3.3 Optimization of Culture Condition for Maximum Cellulase Production:

From the previous step, total 12 isolates with highest enzymatic activity were selected for optimization purpose i.e. from Papaya peels isolate number PP1, PP5, PP6, PP8, PP11, from Banana peel isolate number BP3, BP5, BP8 and from Cucumber peels isolate number CP4, CP5, CP6, CP7. Here, two parameters (pH and temperature) were focused on to know the optimal condition.

3.3.1 Optimization of pH of Culture Media:

Optimization of Culture Media at pH 5.0:

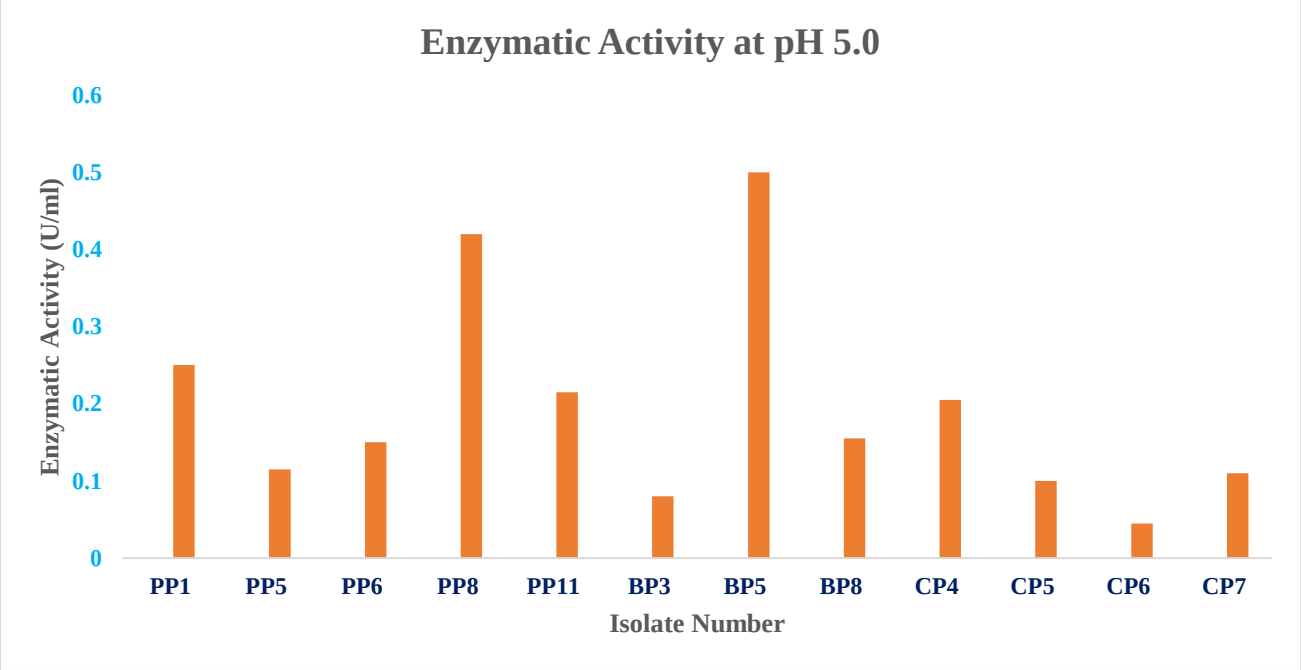


Figure 3.5: Enzymatic activity of 12 Selected Bacterial Isolates at pH 5.0.

Here, isolate number BP5 from Banana peels showed highest cellulolytic activity,0.5 U/ml and isolate number CP6 from Cucumber peel showed lowest cellulolytic activity, 0.045 U/ml (obtained from the Absorbance measured at 540 nm of wavelength using Glucose Standard Curve).

Optimization of Culture Media at pH 8.5:

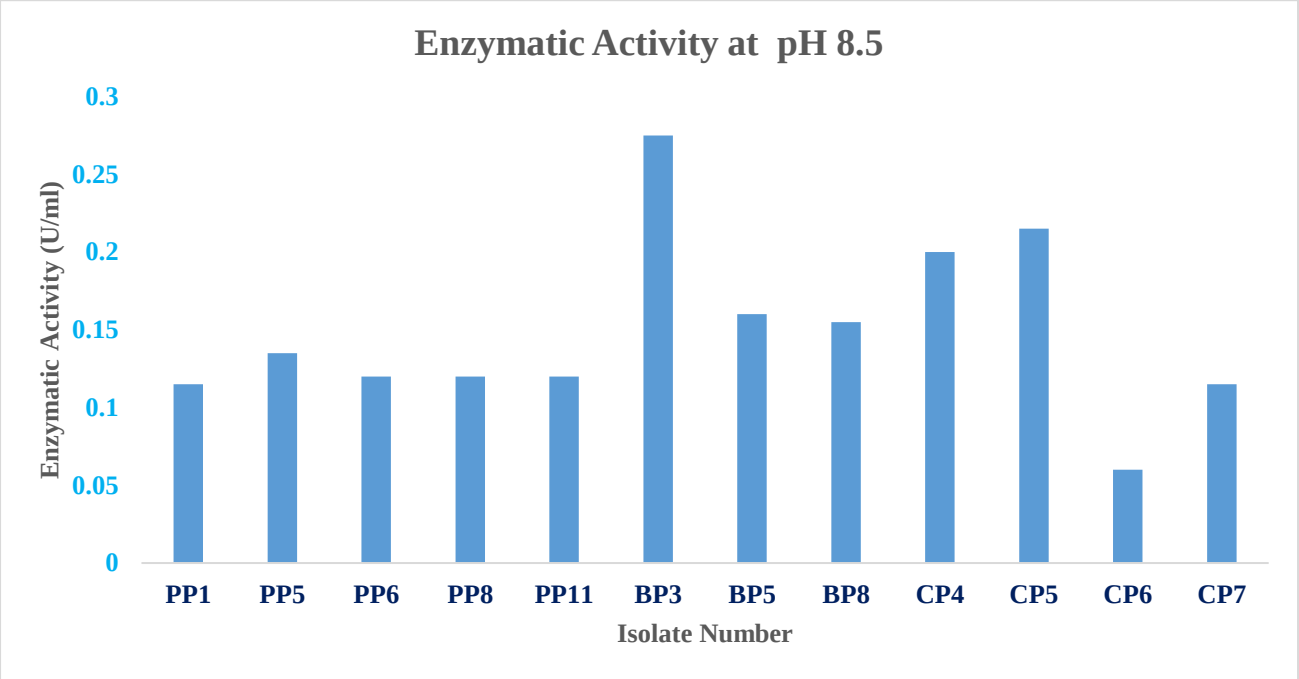


Figure 3.6: **Enzymatic Activity of 12 Selected Bacterial Isolates at pH 8.5**

Here isolate number BP3 from Banana peel showed highest cellulolytic activity, 0.275 U/ml and isolate number CP6 from Cucumber peel showed lowest activity, 0.06 U/ml (obtained from the Absorbance measured at 540 nm of wavelength using Glucose Standard Curve).

Comparison of Cellulolytic Activity at pH 5.0, pH 8.5 and pH 7.0 :

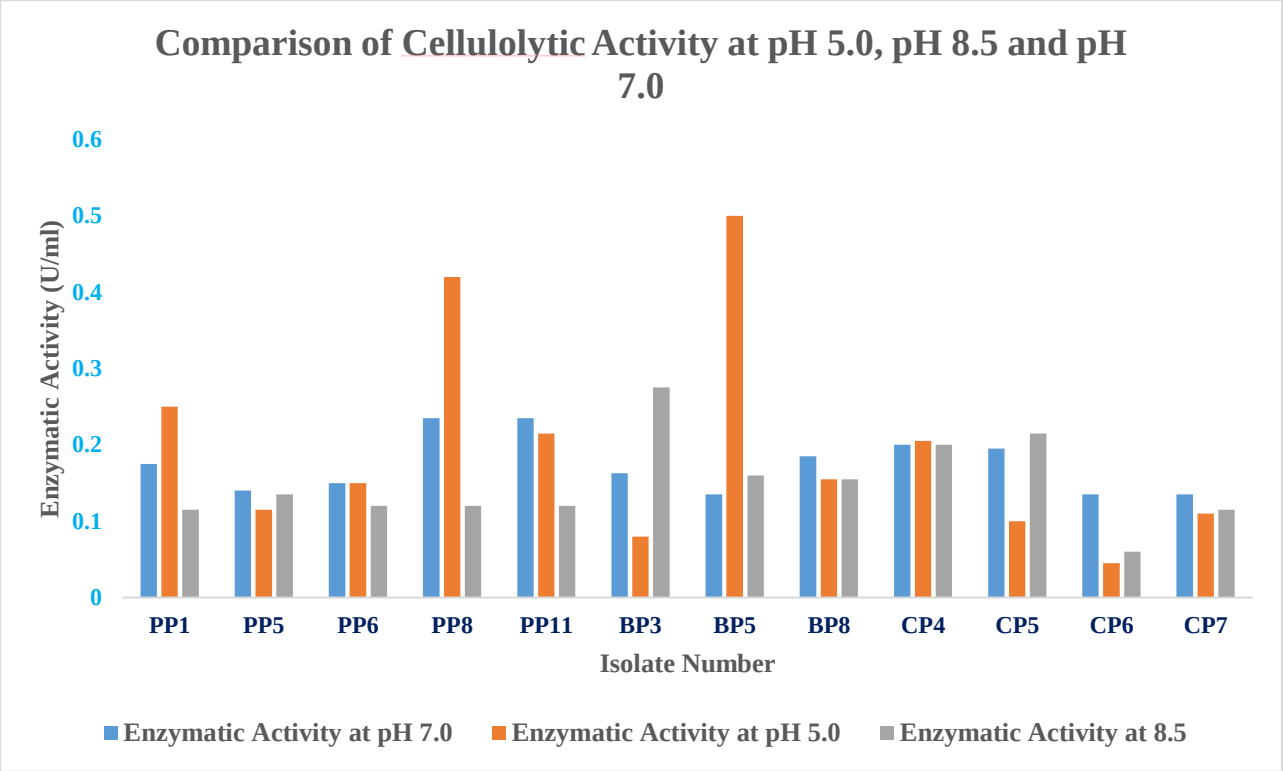


Figure 3.7: Comparison of Cellulolytic Activity for 12 Selected Bacterial Isolates at pH 5.0, pH 8.5 and pH 7.0

The graph illustrated above represented a comparison of cellulolytic activity among pH 5.0, pH 8.5 and pH 7.0, obtained from the Absorbance measured at 540 nm of wavelength using Glucose Standard Curve. Here, the highest enzymatic activity was mostly observed at pH 5.0. However, the enzymatic activity of CP6 from Cucumber peels was an exception here. And enzymatic activity for each of the bacterial isolates was found comparatively stable at pH 7.0.

3.3.2 Optimization of Temperature of Culture Media:

Optimization of Culture Media at 30º C:

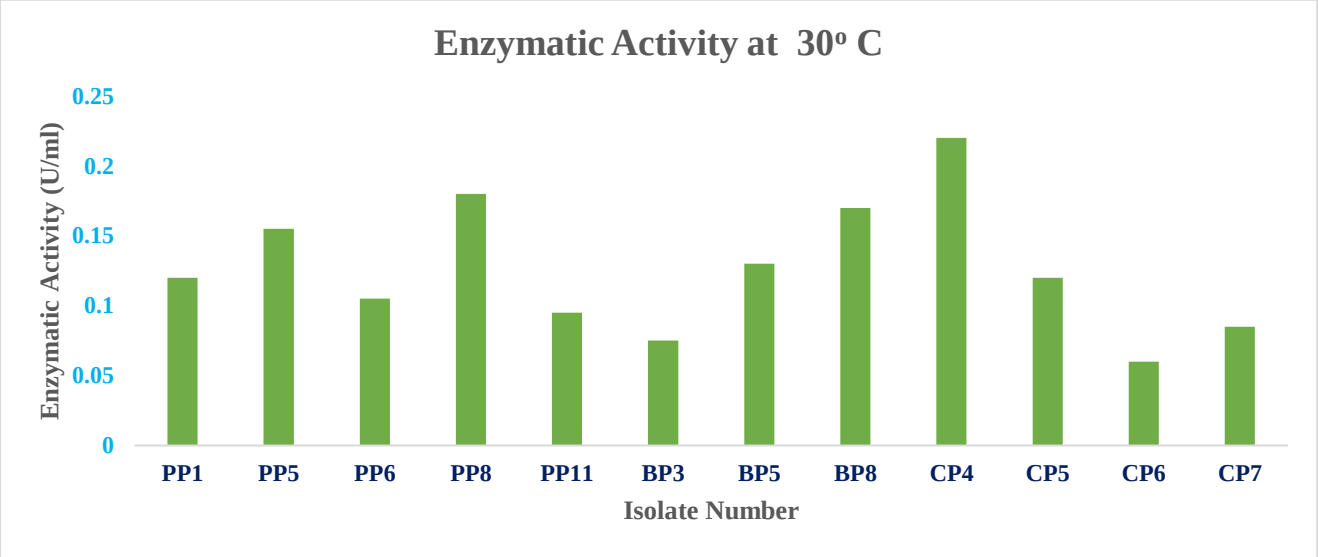


Figure 3.8: Enzymatic Activity of 12 Selected Bacterial Isolates at 30º C

Here**,** the isolate number CP4 from Cucumber peel showed highest cellulolytic activity,0.22 U/ml and isolate number CP6 from Cucumber peels showed lowest cellulolytic activity, 0.06 U/ml (obtained from the Absorbance measured at 540 nm of wavelength using Glucose Standard Curve).

Optimization of Culture Media at 42º C:

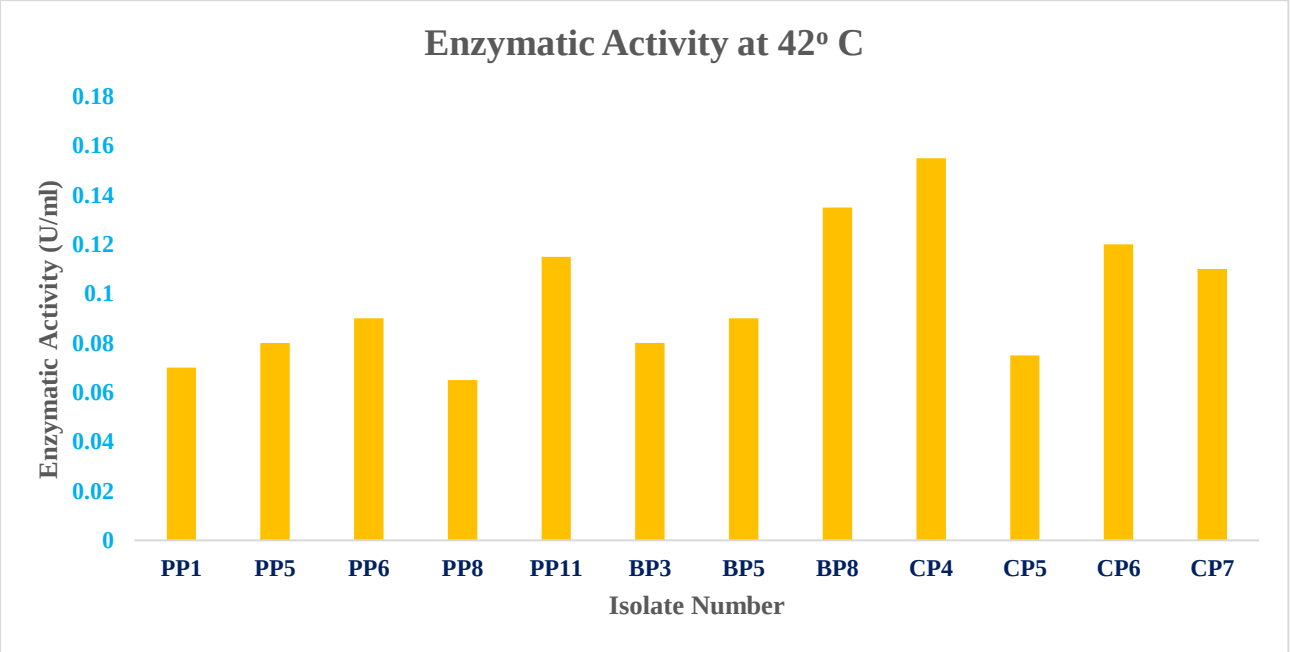


Figure 3.9: Enzymatic Activity of 12 Selected Bacterial Isolates at 42º C

Here also the isolate number CP4 from Cucumber peel showed highest cellulolytic activity, 0.155 U/ml, and isolate number PP8 from papaya peels showed lowest cellulolytic activity, 0.065 U/ml (obtained from the Absorbance measured at 540 nm of wavelength using Glucose Standard Curve).

Comparison of Cellulolytic Activity for 12 Selected Isolates at 30° C, 42° C and 37° C:

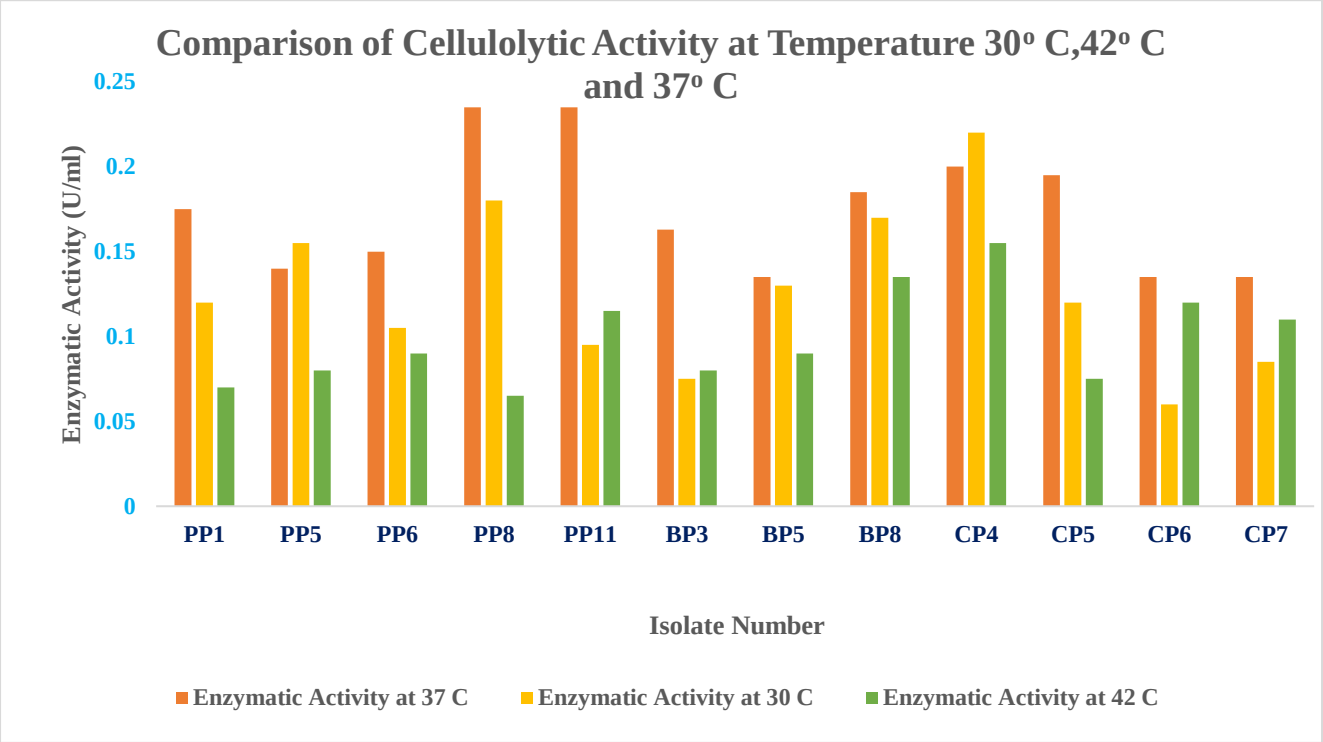


Figure 3.10: Comparison of Cellulolytic Activity for 12 Selected Bacterial Isolates at 30° C, 42° C and 37° C.

The graph illustrated above represented comparison of cellulolytic activity at 30° C, 42° C and 37° C, obtained from the Absorbance measured at 540 nm of wavelength using Glucose Standard Curve. Here, the highest enzymatic activity was mostly observed at 37° C. Also, enzymatic activity for each of the bacterial isolates was found comparatively stable at this temperature. Overall, most of the isolates showed comparatively lower enzymatic activity 42° C.

3.4 Result of Bacterial Identification:

Based on the morphological, microscopic and different biochemical activities conducted for selected 12 bacteria with highest Enzymatic Activity in the normal production condition, the samples were identified to be-

Isolate Number	Identifies as
PP1	*Enterobacter cloace*
PP5	*Proteus mirabilis*
PP6	*E.coli*
PP8	*Bacillus cereus*
PP11	*Bacillus cereus*
BP3	*Burkholderia spp.*
BP5	*Bacillus subtilis*
BP8	*Bacillus subtilis*
CP4	*Paenibacillus spp.*
CP5	*Streptomyces spp*
CP6	*Paenibacillus septentrionalis*
CP7	*Bacillus acidiproducens*

Table 3.1: Identification of Selected 12 Isolates

Chapter 4

4. Discussion:

The conducted research was aimed at isolating and optimizing cellulase-producing bacterial strains from some easily accessible kitchen wastes i.e. papaya peels, banana peels and cucumber peels. Till now several studies have been performed on isolating cellulose degrading bacteria from different wastes such as paper cup wastes((Karthika et al., 2020) sugar industry wastes (Islam, 2019), agricultural wastes (Waghmare et al., 2018) etc. However, one evidence was found where cellulolytic bacteria have been isolated from kitchen wastes (Mahmood et al. 2014). In our research, *Bacillus subtilis*, *Bacillus cereus* and *Burkholderia spp* showed maximun enzymatic activity. Similarly in previous studies these three isolates i.e. *Bacillus subtilis* (Kim et al., 2012), *Bacillus cereus* (Chaudhary et al., 2017) *Burkholderia spp* (Beladhadi et al., 2022) were also found to show cellulose degrading characteristics. Nontheless, in our research, *Paenibacillus septentrionalis* was found to have cellulolytic potentiality, whereas no previous study showed such evidence.

The DNSA (3, 5-Dinitrosalicylic acid) reagent in this enzyme assay played it's role by estimating the concentration of reducing sugar in enzymatic hydrolases. Here it detected the presence of free carbonyl(C=O) groups in reducing sugar. Since the reducing sugar here was glucose, DNSA at first reacted with the aldehyde function group in glucose and converted into 3- amino5-nitrosalicylic acid (ANSA). Under alkaline condition the 3- amino5-nitrosalicylic acid (ANSA) formed reddish-brown colored complex, which had maximum absorbance at 540nm.

After changing two parameters of culture condition- pH and Temperature, change of the enzymatic activity was observed for the samples and every sample showed different response towards the changed parameter. The notion of optimization was to get the highest yield. The isolated sample **BP5** showed the highest Enzymatic activity among all the samples (0.5 U/ml) while the production media condition was pH 5.0 and 37^{o} C (Figure 3.5). This **BP5** was identified as *Bacillus subtilis.* A similar study revealed that a strain *Bacillus subtilis* from cow dung showed maximum cellulolytic activity at acidic pH 5.0 (Malik & Javed, 2021). And, at the same parameter of the production condition, the second Enzymatic activity was observed on **PP8** 0.42 U/ml (Figure 3.5). The **PP8** was identified as *Bacillus cereus.* However, some

previous studies revealed that strains from *Bacillus cereus* from paper sludge showed their best cellulolytic activity at basic pH 8.0 (Kumar et al,.2012). Lastly, another isolate **BP3** was found to be the third highest in enzymatic activity 0.275 U/ml at different parameter, which is pH 8.5 (figure 3.5). And the **BP3** was identified as *Burkholderia spp.* Whereas, a previous study conducted on this *Burkholderia spp* from rice straw revealed that the optimal condition to produce maximum titer of cellulase was pH 7.0 and 40^{o} C (Beladhadi et al., 2022b).

This conducted research revealed that the isolates were affected differently with the change of pH and temperatures. However, in the case of changing pH, most of the isolates showed maximum cellulolytic activity at pH 5.0(Figure 3.7). On the other hand, while changing temperature, most of the isolates were highest at 37 ^{O} C.

Here, in this study, only two parameters; pH and temperature were optimized. However, some more physical parameters such as carbon source, nitrogen source, incubation time etc could also have been used to optimize the culture condition. Furthermore, to observe the effect of pH and temperature on culture condition, total three values for each parameter were taken. The effect could have been more precisely determined if more than three values for each parameter were taken. Bacterial Identification could have been more accurate if 16s rRNA sequencing could be conducted here. Lastly, strain improvement could have been obtained through mutagenesis that would bring improvement in cellulolytic activity of the bacterial isolates.

Chapter 5

5. Conclusion:

In this conducted study, bacterial isolates were chosen based on maximum cellulase yield in comparison to others. The bacterial species were identified via morphological characterization and biochemical identification. Identifying bacterial species would help to increase enzyme production by modifying culture conditions according to need of that particular organism. Furthermore, this identification would also help in analyzing cellulase structure of that particular organism, by which cellulolytic activity can be improved by any of two major strategies: rational design and directed evolution. Since cellulase are gaining considerable attention nowadays, this protocol of isolating cellulase producing bacteria from easily accessible kitchen wastes would be a stirring alterative to many hazardous components used for industrial purposes. It is optimistic that, if the identified strains are studied more, it would be possible to design such protocol where cellulases can be obtained from kitchen wastes at industrial scale for detergent industry, textile industry, agricultural industry, paper and pulp industry etc.

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