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Editorial

The news describes the current situation well, but it may be overly optimistic. The plastic bag problem looms larger every day as they litter our environment and kill land animals and endangered marine life. Plastic is prevalent in our lives. It fills our fridges storing food in individual containers. It frames our glowing computer screens and nestles our DVDs. It totes our groceries around when we lack a free hand. Unfortunately, plastic has a darker side. Plastic is made from fossil fuel sources such as natural gas and petroleum. After researching the problem for about four years, Ashwath founded EnviGreen – a company that produces 100% organic, biodegradable, and eco-friendly bags.

This issue also focus on the current research that a chemical inhibitor that limits the growth of lung tumor cells.

The review that bacteria produce variety of biopolymers having different chemical properties via utilization of simple to complex substances. Naturally occurring EPS possess unique combination of environment friendly and functional properties. They are renewable, biodegradable and nontoxic. The study is conducted to produce cellulase from tilapia fish gut bacteria, using Plackett Burman design and carboxymethyl cellulose (CMC) as substrate. With the help of Plackett-Burman design, maximum cellulolytic activities was observed in high levels of CMC, Urea, Peptone, Calcium chloride, magnesium sulphate and Trace elements and Low levels of Ammonium sulphate, and Di Potassium Hydrogen phosphate, at 37 °C and pH 7.0 which is found to be optimized levels of components.

Stay tuned to 'QUEST' until the next installment get glued to this edition and dive in the pond of knowledge. We hope you enjoy reading the same as we enjoyed making it.

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Index

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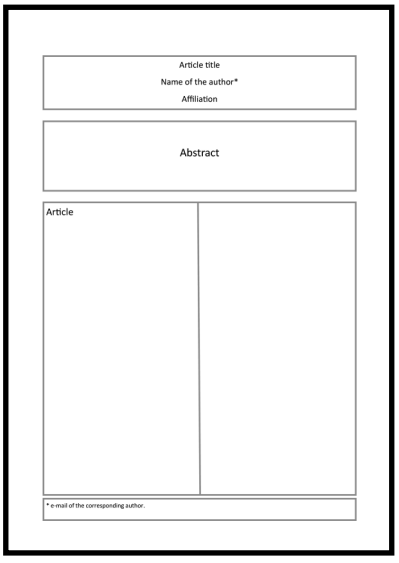

Plastic Bags That Are Actually Made of Potato & Tapioca – and Can Become Animal Food on Disposal

The partial or total ban on plastic bags in several Indian cities is often lauded as a welcome move by most people. But the inconvenience arising due to it was this problem faced by ordinary citizens that disturbed Ashwath Hegde, a Mangalore-born but now Qatar-based NRI entrepreneur.

After researching the problem for about four years, Ashwath founded EnviGreen – a company that produces 100% organic, biodegradable, and eco-friendly bags. According to the Minister of State for Environment, Forest and Climate Change, 15,000 tonnes of plastic waste is generated in India every day, out of which 9,000 tonnes is collected and processed, while 6,000 tonnes is not collected.

They look like plastic bags but are made of materials like natural starch and vegetable oil derivatives. If placed in a glass of water at normal temperature, an EnviGreen bag dissolves in a day. And when placed in a glass of boiling water, it dissolved in just 15 seconds! These bags take less than 180 days to biodegrade naturally once discarded. So users can throw them away without worrying about harming the environment. The bags are even edible and will cause no harm to animals if ingested.

The manufacturing process for these bags is entirely different from that of plastic, cloth, or paper bags. The company uses 12 ingredients, including potato, tapioca, corn, natural starch, vegetable oil, banana, and flower oil.

All raw materials are first converted into liquid form and then taken through a six-step procedure before the end product is ready. There is no use of any chemicals at all. Even the paint used for printing on the bags is natural and organic. The cost of one EnviGreen bag is about 35% more than that of a plastic bag, but 500% less than that of a cloth bag. To give you a rough idea, an EnviGreen bag measuring 13 inches by 16 inches costs Rs. 3, while a plastic bag with the same dimensions will cost Rs. 2.

The sheet neither melts nor sticks to the hot iron surface. The bags also don't melt, drip, or release any toxic fumes when burnt, unlike conventional plastic bags. They have been tested by the Central Institute of Plastic Engineering and Sriram Institute for Industrial Research as well.

Currently, the company has not started full-fledged sales in India, but the bags are already available in Qatar and Abu Dhabi.

Just the city of Bangalore consumes over 30,000 metric tonnes of plastic bags every month.

Once available for use, EnviGreen bags will not just help ease this problem but also help many consumers struggling to find a balance between their concern for the environment and ways of making things more comfortable in their daily lives .

- Contributed by
Janushi Patel
IGBT 6th sem

Skin patch to treat peanut allergy shows benefit in children

A wearable patch that delivers small amounts of peanut protein through the skin shows promise for treating children and young adults with peanut allergy, with greater benefits for younger children, according to one-year results from an ongoing clinical trial. The treatment, called epicutaneous immunotherapy or EPIT, was safe and well-tolerated, and nearly all participants used the skin patch daily as directed. The ongoing trial is sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, and conducted by the NIAID-funded Consortium of Food Allergy Research (CoFAR), which is led by Hugh Sampson, M.D., of Icahn School of Medicine at Mount Sinai in New York. Stacie Jones, M.D., of the University of Arkansas for Medical Sciences and Arkansas Children's Hospital, chairs the study. One-year outcomes are published online on October 26 in the *Journal of Allergy and Clinical Immunology*.

CoFAR researchers at five study sites randomly assigned 74 peanut-allergic volunteers aged 4 to 25 years to treatment with either a high-dose (250 micrograms peanut protein), low-dose (100 micrograms peanut protein), or placebo patch.

The investigators assessed peanut allergy at the beginning of the study with a supervised, oral food challenge with peanut-containing food. The patches were developed and provided by the biopharmaceutical company DBV Technologies under the trade name Via skin.

Each day, study participants applied a new patch to their arm or between their shoulder blades.

After one year, researchers assessed each participant's ability to consume at least 10 times more peanut protein than he or she was able to consume before starting EPIT. The low-dose and high-dose regimens offered similar benefits, with 46 percent of the low-dose group and 48 percent of the high-dose group achieving treatment success, compared with 12 percent of the placebo group. In addition, the peanut patches induced immune responses similar to those seen with other investigational forms of immunotherapy for food allergy. Investigators observed greater treatment effects among children aged 4 to 11 years, with significantly less effect in participants aged 12 years and older.

-Contributed by
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Chemical inhibitor may provide lung cancer treatment

A chemical inhibitor that limits the growth of lung tumor cells has been identified by a team of researchers. The inhibitor works by partially disrupting glycosylation, the addition of sugar chains to proteins.

Glycosylation is an essential process for all cells, but researchers have long thought that there is no way to disrupt glycosylation in cancer cells without disrupting the same process in other cells and thereby causing serious harm, according to Joseph Contessa, a senior author of the study. The inhibitor identified in the study -- called NGI-1 -- affects tumor cells most severely, while leaving other cells relatively unharmed.

NGI-1 disrupts the growth of cancerous cells by blocking the glycosylation of oligosaccharyltransferase, an enzyme known as OST that transfers sugar molecules called oligosaccharides to receptor proteins, according to the study. OST plays an important role in the spread of cancer, as it is used to glycosylate receptor proteins on which tumor cells depend for growth.

-Contributed by

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Bacterial Exopolysaccharides

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Abstract

Microorganisms secrete high molecular weight compound in the surrounding, known as Exopolysaccharides (EPS). EPS are mainly present in the form of capsule, sheath, and slime. EPS are generally closely associaated with bacterial outer cell surface and detached during growth period into growth medium. The exopolysaccharides are mainly comprised of polysaccharides but they may also contain non-sugar components such as proteins and nucleic acids.

Introduction

The history of bacterial EPS began during the mid-19th century with the discovery of an EPS from wine, which was later identified as dextran. The prokaryote responsible for its production was recognized as *Leuconostoc mesenteriodes*. Over the course of time, other EPS were discovered including cellulose, xanthan, alginate etc.

Bacterial EPS are generally synthesized and secreted into their exterior environment by bacteria of all taxa. EPS is usually of high molecular weight ranging from 10 to 1000 kDa. It may either be homopolymeric or heteropolymeric in composition. Bacteria produce variety of biopolymers having different chemical properties via utilization of simple to complex substances. Naturally occurring EPS possess unique combination of environment friendly and functional properties. They are renewable, biodegradable and non-toxic. Bacterial EPS may be ionic or nonionic and are water soluble[1].

Capsular EPS are formed primarily during the log phase of bacterial growth and are present close to the cell. The capsular material plays an important role in sludge flocculation. On the other hand, slime EPS are formed during the stationary phase and are not in bound form. The capsular EPS on the other hand, are held to the cell wall either by linkage between the carboxyl group of EPS and hydroxyl group of lipolysaccharides[2]. These polymeric substances mainly comprise of EPS (40-95%), protein (1-60%), nucleic acids (1-10%) and lipids (1-40%)[3,4]. Structure and composition of EPS vary with number of factors such as environment factors, carbon sources, pH, nitrogen content in medium etc. Studies have found that EPS composition is determined by different carbon substrate utilized[4]. EPS yield is also known to vary based on the nitrogen substrate utilization[5]. Studies have found that optimal pH for EPS production may differ from the optimal pH for bacterial growth[6, 7]. Studies have also found that EPS production is often favored by sub-optimal growth temperature.

EPS are either intracellular or extracellular with respect to their cellular location. The intracellular biopolymers are very few and have very limited use. However, the variety of extracellular biopolymers is huge[8]. They may be categorized into 4 major classes:

1. Polysaccharides
2. Inorganic polyanhydrides (such as polyphosphates)
3. Polyesters
4. Polyamides[9]

Polysaccharide components of the extracellular biopolymers are most abundant and their cellular location relative to the cells are as bellow;

a. At the cellwall - they provide structural and protective purposes and are found as constituents in teichoic acids.

b. Outside the cell - they might take the appearance of a covalently bound cohesive layer; termed capsule.

c. Completely secreted into the environment as slime.

Structure of EPS

Microbial EPSs are mainly categorized into 3 types:

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1. Homopolysaccharide,
2. Heteropolysaccharide[10]
3. Polysaccharides with irregular structure.

Homopolysaccharide consists of only one type of monosaccharide units that can be further categorized as a). Linear i.e. bacterial cellulose (polyglucose) or b). Branched polymers i.e. levans (polyfructoses) and dextrans (polyglucose), while Heteropolysaccharide is made up of repeating units, varying in size from disaccharides to heptasaccharides. Polysaccharides with irregular structure have no regular structure. Homopolysaccharide are further clustered into 4 groups based on linkage bonds and nature of monomeric units; which are α-D-glucans, β-D-glucans, fructans and polygalactan[11]. The composition of heteropolysaccharides includes the repeating units of D-galactose, D-glucose, L-rhamnose and in some cases, N-acetylgalactosamine (Gal NAc), N-acetyl glucosamine (Glc NAc), or glucuronic acid (GlcA). Sometimes non carbohydrate components such as glycerol, acetyl and phosphate are also present[12].

Bonds present between monomeric units are 1, 4-β - or 1, 3-β -linkages and 1, 2-α- or 1, 6-α linkages at the backbone of the polymers[13]. The former is characterized by strong rigidity while the later is more flexible. The differences between homopolysaccharide and heteropolysaccharide are not only observed in the chemical nature and linkage bonds, but also in synthetic enzymes and site of synthesis. The repeating units of heteropolysaccharide are produced intracellularly and then translocated across the membrane via carrier lipids for subsequent polymerization extracellularly. Homopolysaccharide synthesis requires specific substrate such as sucrose. The quality and quantity of EPSs produced varies from species to species of bacterial genera. The physicochemical factors such as incubation time, temperature, pH and media composition (carbon, nitrogen and cation sources) play important role in the synthesis of these compounds.

Bacterial polysaccharide allows for classification based on chemical structure, functionality, molecular weight and bound linkages. Following the chemical composition, EPS may be looked at based on monomeric composition and as such homopolysaccharides and hetropolysaccharides are the main group recognized[12]. Homopolysaccharides contain only one type of repeating unit of monosaccharide. EPSs have been similarly categorized on functionality and seven categories were proposed. They are constructive or structural, surface-active, redox-active, sportive- active, informative, and nutritive. Biomolecules classified as structural EPS include neutral polysaccharide because they serve architectural purpose in the matrix facilitating water retention and cell protection. Surface-active EPS having molecules with amphiphilic behavior; may be involved in biofilms formation and sometimes possess antibacterial and antifungal activities. Sportive EPS are made up of charged polymer whose function is sorption to other charged molecules involved in cell surface interaction[14]. EPSs are produced by variety of bacteria including rhizobiaceae and are able to secrete polysaccharide sugar polymer that form an adherent cohesive layer of cell surface and are arranged as capsular polysaccharide.

Monosaccharide composition is backbone of bacterial EPS and has been classified into hetropolysaccharides and homopolysaccharides[15]. They generally consist of monosaccharide and non-carbohydrate substituent such as acetate, pryuvate, succinate and phosphate.

EPS can be hetropolymer or homopolymer in natural environment. EPS contain non-sugar components like uronic acid, methyl esters, sulphates, pryuvate, protein, nucleic acid and lipids. EPS are long chain polysaccharide consisting of branched, repeating units of sugar derivative and non-carbohydrate components[2]. EPS categorized is very multifaceted and in some cases categorization factors are reapplied as additional construct distinctions between groups. It has been seen in case of homopolysaccharide of being further separated into groups and it has been seen in case of homopolysaccharide been further added into four group thus; α-D- glucan β-D –glucans, fructans, and polygalactan, this grouping is based on linkage of different bonds and nature of monomeric units .

On the other hand, the composition of hetropoly-

saccharide includes the repeating units of D-glucose. D-galactose, L-rhamnose and in some instances, N-acetyl glucosamine (GlcNAc), N-acetyl galactosamine (GalNAc) or glucuronic acid (GlcA). Non-carbohydrate substituent such as phosphate, acetyl and glycerol are sometimes present[4]. Bonds between monomeric units at the backbone of the polymer are 1,4β or 1,3β - linkage and 1.2α- or 1, 6-α- linkage. The former is characterized by strong rigidity. The distinction between homopolysaccharide and hetropolysaccharide is not only limited to chemical nature and linkage bound but in synthetic enzyme and site of synthesis also. The repeating units of hetropolysaccharide are formed in intracellular band, in isoprenoid glycosyl, as carrier lipids and are involved in translocation.

EPS has rapidly emerged as new and industrially important source of polymeric material, which are gradually becoming economically competitive[16]. Interest in exploitation of microorganism for production of valuable polysaccharide has greatly increased in recent years, since these biopolymer produced by a variety of microorganism are chemically well defined and have attracted worldwide attention due to their novel and unique physical properties.

EPS have divalent metal cations that go along with ionic bridges linking adjacent polysaccharide chains[4]. The existence of side-linkages and organic molecules influence the overall charge, stability binding capacity, rheology and solubility of the polymer.

Biosynthesis pathway of exoploysaccharide[17]

The biochemical pathway leading of EPS synthesis is well understood and can be divided into four distinct steps[2]:-

1. Monosaccharide activation by formation of sugar nucleotide.
2. Repeating unit assembly via addition of sugar to isoprenoid.
3. Polymerization of repeating units.
4. Polysaccharide excretion by cytoplasm membrane and cell wall.

Enzymes involved in the synthesis of EPS are divided into four groups by Sutherland et al[2] which broadly corresponds to the four steps of EPS synthesis. Firstly, initial substrate metabolism enzymes which perform synthesis and convert substrate into sugar nucleotide. Secondly, transferase enzyme that builds monosaccharide unit. Thirdly, enzyme which synthesizes the EPS molecules. Microbial biosynthetic pathway of alginate[18] is given in Fig. 1 below.

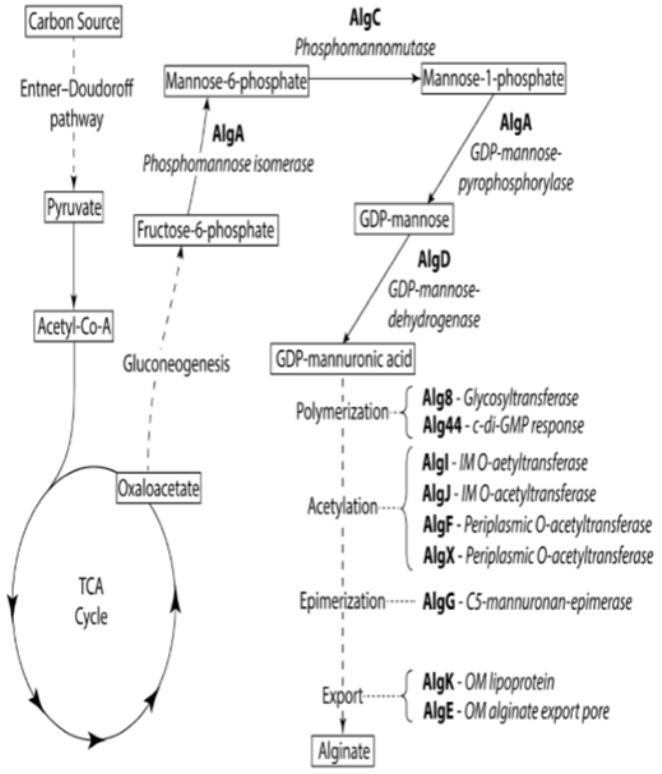

Fig.1 Microbial biosynthetic pathway of alginate[18]

Applications and Properties of EPS

EPSs have been a material of choice for a half a century because of their technical veracity, performance and cost effectiveness. They are widely used in everyday application because of their light weight, strength, durability, thermal insulation and shock absorption distinctiveness that provide economic, high performance product. The benefits of these properties are explained below: EPS can be used as bioadhesive, bioflocculant, biosorbent, gelling agent, probiotic, stabilizers, and thickeners, making them suitable for numerous commercial applications in the bionanotechnology, food, pharmaceutical, petroleum, civil construction, and environment sector.

i)Light weight - EPS is an extremely lightweight material since it is comprised of 98% air. This characteristic makes it perfect for utilization in packaging as it does not extensively add to the weight of the total product thereby reducing transportation costs.

Energy consumption for transport fuel is therefore reduced and vehicle emission minimized which contributes to lower global warming.

ii) Durability – The exceptional durability of EPS makes it an effective and reliable protective packaging material for a wide range of goods.

iii) Thermal efficiency – The superior thermal efficiency of EPS makes it ideal for packaging any product that is sensitive to temperature change. Product enclosed in EPS container can be maintained for long period at temperatures above or below ambient condition and can be protected from sudden temperature change that can occur in the transport through different climate zones.

iv)Shock Absorption – EPS exhibit excellent shock absorbing characteristics making it the first choice for packaging of a wide range of product including appliance, electronics product, computer and chemical.

v)Versatility – EPS products can be manufactured in almost any shape or size, or it can be easily cut and shaped when required. EPS is produced in a wide range of densities providing a varying range of physical properties. These are matched to the various applications where the material is used to optimize its performance.

vi) Characteristics of EPS: EPSs can be processed over a wide range of molding condition because of their strength, short cycle time and low stream consumption. This characteristic makes them suitable for block case molding

vii) EPS also has anti-static effects for products with physical characteristics similar to the EPS. Low residual styrene monomer content corresponding with national food standard.

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"Isolation of bacteria producing cellulase from tilapia fish gut and media optimization for Celluase Production using Plackett Burman design"

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Abstract:

Cellulase production from bacteria can be an advantage as the enzyme production rate is normally higher due to the higher bacterial growth rate as compared to fungi. Screening of bacteria, optimisation of fermentation conditions and selection of substrates are important for the successful production of cellulase. This study is conducted to produce cellulase from tilapia fish gut bacteria, using Plackett Burman design and carboxymethyl cellulose (CMC) as substrate. The highest cellulase producing ability of isolate from this study was found to be 0.323 IU/ml with 24 hours of incubation. With the help of Plackett-Burman design, maximum cellulolytic activities was observed in high levels of CMC, Urea, Peptone, Calcium chloride, magnesium sulphate and Trace elements and Low levels of Ammonium sulphate, and Di Potassium Hydrogen phosphate, at 37 °C and pH 7.0 which is found to be optimized levels of components.

Introduction:

Cellulases are a group of hydrolytic enzymes capable of hydrolyzing the most abundant organic polymer i.e. cellulose to smaller sugar components including glucose subunits. Cellulases are inducible enzyme complex involving synergistic action of endoglucanase, exoglucanase and cellobiase[1]. Cellulases gained a huge economic importance due to their wide range of application in industries like starch processing, animal food production, grain alcohol fermentation, malting and brewing, extraction of fruits and vegetable juices, pulp and paper industry and textiles industry[2].

The high cost of cellulase production owing to use of pure chemicals in production coupled with low enzyme activities limits its industrial use[3]. Major impediments to exploiting the commercial potential of cellulases are the yield stability and the cost of cellulase production. Research should therefore be aimed at taking advantage of the commercial potential of existing and new cellulases in nature. Cellulase enzymes account for approximately 20 percent of the world enzymes market and are mostly produced by *Aspergillus, Trichoderma* and *Penicillium* species[4]. Cellulase is widely produced by submerged fermentation, but the relatively high cost of production and recovery has hindered its industrial application. Cellulase of *Paenibacillus* spp was found to have maximum CMC activity at 60°C, pH 6.5. Due to the promising thermostability and slight acidic tolerance of this enzyme, it has good potential for industrial use in the hydrolysis of soluble cellulose as well as activity on microcrystalline sources of cellulose[2]. *Paenibacillus campinasensis* BLII is a thermophilic spore-forming bacterium which was found to grow between 25 and 60°C over a wide range of pH. Optimal growth is around neutral pH at 55^{0}C. The physiological properties of this strain and the vast number of free glycosyl hydrolases produced, give this strain potential for use in bio-refining industry[5]. *Bacillus subtilis* offers a potentially more valuable thermostable enzyme for the bio-refining industry due to extreme heat tolerance. Cultivation of thermophiles offers several advantages such as reducing the risk of contamination and viscosity thus making mixing easier and this leads to a high degree of substrate solubility while reducing the cost of cooling[6]. *Bacillus agaradhaerens* was shown to have increased optimal thermostability from 50 – 60°C and optimal pH range of 7 – 9.4[7]. *Cellulomonas flavigena* enzyme was found to have optimum cellulase and xylanase activities at pH 6 with an optimum temperature at 50°C[8]. Zaldivar et al. (2001)[1] observed that cellulase production by *Trichoderma aureoviridae* is best if pH doesn't fall below 3.5 and at an optimum temperature of 28^{0}C. Pei-Jun et al. (2004)[9] reported a pH of 6.5 for optimum cellulase production by

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Trichoderma koningii. Kalra and Banta (2008)[3] reported pH and temperature of 5 and 30^{0}C respectively as optimal for cellulase yield. Carbon source, cellulose quality, pH value, temperature, presence of inducers, medium additives, aeration and growth time have been reported to be important parameters in optimizing cellulase production[10].

As a result of the high cost of cellulase production with its low enzyme activities owing to the use of chemicals in its industrial production, efforts were needed to economize cellulase production through media (cultural) optimization and use of supplements and/or additives[3]. Researches have been carried out and are still ongoing all aimed at isolating potential cellulase producing microorganisms from diverse habitats[11]. This study was therefore aimed at investigating the cultural conditions necessary for optimal cellulase yield by cellulolytic bacterial organisms from gastrointestinal tract of *Tilapia* fish.

Materials & Methods

Sample-collection:

Carnivore, column feeder tilapia were sampled from Anand fish market for study. The average weight and length are studied & presented in Table 1.

Table 1 : Morphological characteristics of Tilapia

Fish species	Tilapia
Body weight (gm)	6.96
Total length(cm)	15.5
Gut Weight (gm)	5.59

Homogenate preparation

The gut of healthy fish was dissected out in cold and aseptic condition in phosphate buffer. Then it is rinsed in N-Saline and intestine was mechanically homogenized in sterile phosphate buffer (0.2 M) to give 10^{-1} diluted sample. Homogenates thus obtained were centrifuged for 15 min at 6000 rpm and supernatant was stored in refrigerator at 4ºC for further processing.

Isolation of cellulase producing microorganism on Nutrient agar Media:

Isolation of cellulase producing bacteria by spread plate technique is carried out by serially diluting the homogenates obtained in sterilized phosphate buffer using Normal saline as diluents.

0.1ml of aliquots from each dilution is used to spread inoculated onto Nutrient agar plates. (10^{-1} to 10^{-6}). Plates were incubated for 24 hours 37^{o}C and after incubation the isolates obtained were screened for cellulase production.

Screening of isolates obtained on Nutrient agar plates for cellulase activity:

A plate assay method with 1% (w⁄v) CMC in Nutrient Agar medium with 1.5 % (w/v) agar was used for screening of cellulase positive bacterial isolates. The plates were incubated at 37 ºC for 24 h and the cellulase activity was indicated as clear orange halos around the inoculated wells after staining with 1 % Congo red solution for 30 min and washing several times with 1 M NaCl[12]. The magnitude of the activity was calculated by measuring the diameter of the zones.

Fermentation process of Cellulase production

To 100 ml of the optimized culture medium, a loopful of cell culture from respective species was inoculated under controlled conditions. Then it was kept in incubator at 37°C for a 3days and after every 24 hours of incubation 5ml of broth from the flask was withdrawn aseptically and centrifuged at 5000rpm at 4^{0}C and the supernatant used for cellulase activity estimation. The composition of fermentation medium used for cellulase production at high level (+) contained (g / 100ml): Carboxymethyl cellulose,2.5; Urea,0.05; Proteose Peptone, 0.5; $(NH_4)_2SO_4$,0.5; KH_2PO_4, 0.5; $CaCl_2$, 0.5; $MgSO_4$, 0.05; $FeSO_4$, 0.5mg; $MnSO_4$, 0.16mg; $ZnSO_4$,0.14mg; $CoCl_2$, 0.2mg; pH – 7.0.

A ten times lower concentration of each component is maintained at low level (-) for cellulase production

Fermentation media optimization for Cellulase production using Plackett Burman Design:

Plackett-Burman experimental design assumes that there are no interactions between the different variables in the range under consideration. A linear approach is considered to be sufficient for screening. Plackett-Burman experimental design is a fractional factorial design and the main effects of such a design may be simply calculated as the difference between the average of measurements made at the high level (+) of the factor and the average of measurements at

the low level (−). To determine the variables that significantly affect cellulase activity, Plackett-Burman design is used. Eight variables (Table 2) are screened in 12 experimental runs (Table 3) and insignificant ones are eliminated in order to obtain a
smaller, manageable set of factors. The low level (−) and high level (+) of each factor are listed in (Table 2). The statistical software package Design-Expert software (version 6.1.5, Stat-Ease, Inc., Minneapolis, USA) is used for analyzing the experimental data. Once the critical factors are identified through the screening, the central composite design can be used to obtain a quadratic model.

The Plackett-Burman design was used for screening of the factors (media components) that significantly influenced on cellulase production. The design considers the main effect of the variables but not their interaction effects. It can represent by the first order polynomial equation:

$$Y = ßo + \sum^{n}_{i=1} ß_i X_i \quad \ldots\ldots\ldots\ldots (1)$$

Where Y represents the response, ßo is the model coefficient, bi is the linear coefficient, Xi is the variables, and n is the number of the parameters (variables).

Each variable was represented in the two levels, i.e. high (+), and low (-). The effect of each variable was determined by the following equation:

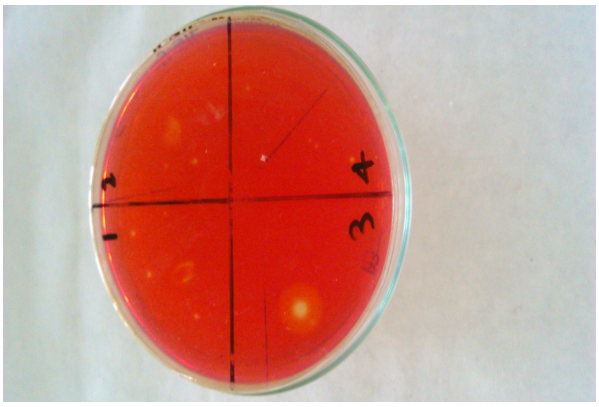
$$E_{(Xi)} = \frac{\sum Mi_{+} - \sum Mi_{-}}{N} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where $E_{(xi)}$ represents is the response value effect of the tested variable; $\sum_{Mi-}$ is the summation of the response value at low level, and N is the number of experiments.
Table 4 represented the selected variables to be evaluated at High and low levels, Table 5 showed the design matrix; eight assigned variables were screened in the 12 experimental runs. The cellulase production was carried out in the triplicate. The fraction significant at 95% level (p-value < 0.05) was considered reliable.

Enzyme Assay
Cellulase activity measured as CMC hydrolysing activity was assayed according to the method recommended by Ghose (1987)[13] and expressed as international units (IU).
One international unit of cellulase activity is the amount of enzyme that forms 1 μmol glucose (reducing sugars as glucose) per minute during the hydrolysis reaction. Reducing sugar was determined by the dinitro salicylic acid (DNS) method.

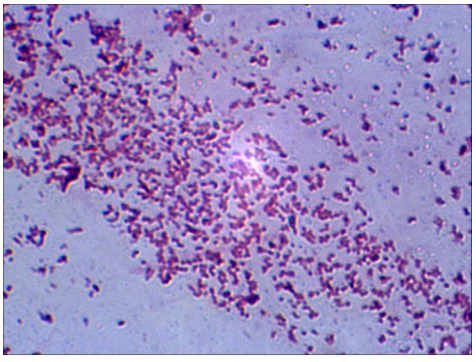
Result and Discussion

Eight isolates of bacterial organisms were obtained from the fish gut, Isolate no.3 was shown to produce cellulase enzyme in the primary screening carried out on nutrient agar medium supplemented with 1% CMC as seen in figure 2. The colony characteristics and Gram staining are tabulated in the Table 6 and figure 3 respectively.



Zone of CMC hydrolysis

Figure 2 : Screening of Cellulase producing Bacteria from Fish Gut



Gram positive cocci

Figure 3 : Gram staining of Cellulase producing bacteria.

Table 4 : Experimental range and levels of independent variables in the Plackett-Burman experiment

Variable	**Level (g%)**				
Variable	H (+)	L (-)	Trace elements		
Substrate CMC	2.5	0.25		mg %	Stock Solution
Urea	0.05	0.005	$FeSO_4.7H_20$	0.5	50mg%
Peptone	0.5	0.05	$MnSO_4.4H_20$	0.16	16mg%
$(NH_4)_2\ SO_4$	0.5	0.05	$ZnSO_4.7H_20$	0.14	14mg%
K_2HPO_4	0.5	0.05	$CoCl_2.6H_20$	0.2	20mg%
$MgSO_4\ 7H_2O$	0.05	0.01			
$CaCl_2H_2O$	0.5	0.05			
Trace elements	1ml	0.1ml			

Table 5 : The Plackett-Burman design for 11 parameters

Trial	**Substrate CMC**	**Urea**	**Peptone**	**$(NH_4)_2\ SO_4$**	**K_2HPO_4**	**$MgSO_4.7H_2O$**	**$CaCl_2.2H_2O$**	Trace elements	D1*	D2*	D3*	**Response (Cellulase IU/ml)**
1	+	+	-	+	+	+	-	-	-	+	-	0.101
2	-	+	+	-	+	+	+	-	-	-	+	0.156
3	+	-	+	+	-	+	+	+	-	-	-	0.161
4	-	+	-	+	+	-	+	+	+	-	-	0.084
5	-	-	+	-	+	+	-	+	+	+	-	0.130
6	-	-	-	+	-	+	+	-	+	+	+	0.085
7	+	-	-	-	+	-	+	+	-	+	+	0.264
8	+	+	-	-	-	+	-	+	+	-	+	0.323
9	+	+	+	-	-	-	+	-	+	+	-	0.293
10	-	+	+	+	-	-	-	+	-	+	+	0.083
11	+	-	+	+	+	-	-	-	+	-	+	0.071
12	-	-	-	-	-	-	-	-	-	-	-	0.034

* Dummy variables

Table 6 : Colony characteristics of well isolated colony of cellulase producer

Size	Medium
Shape	Round
Margin	Regular
Elevation	Convex
Texture	Mucoid
Opacity	Opaque
Pigmentation	Yellowish Orange
GramNature	Nagative

Bacteria present an attractive potential for the exploitation of cellulases due to their rapid growth rate, enzyme complexity and extreme habitat variability[14] . The high cost of cellulase production (due to use of pure chemical in production) coupled with low enzyme activities, limits its industrial use. Therefore, efforts are needed to economize cellulase production by media optimization[3] and hence, isolation and characterization of cellulase producing bacteria will continue to be an important aspect of biofuel research[7].

Sample used in this study included Tilapia fish gut, obtained from the Fish vendor. According to Doi (2008)[15], cellulase producing bacteria have been isolated from a wide variety of sources such as composting heaps, decaying plant material from forestry or agricultural wastes, the faeces of ruminants (cows etc) soil and organic matter. Identified cellulase producing bacterium in this study includes Gram positive cocci. In a similar study, Sangbrita et al. (2006)[16] isolated Bacillus species from the intestine of tilapia and Chinese grass carp. Weng-Jing et al. (2005)[17] isolated 15 mesophilic bacteria of which Bacillus species predominated.

In order to evaluate the accuracy of the applied Plackett-Burman statistical design, a verification experiment was applied to compare between the predicted optimum levels of independent variables, anti optimum levels and the basal condition settings. The highest cellulase producing ability of isolate from this study was found to be 0.323 IU/ml with 24 hours of incubation (Table 3). This was low when compared with the report of Wen-Jing et al. (2005)[17] who isolated Bacillus cereus with maximum cellulolytic activity of 7.9 – 28.0 IU/ml. Maximum cellulolytic activities of 67.02 IU/ml and 35.8IU/ml as reported by Sangbriba et al. (2006)[16] for Bacillus circulans and Bacillus megaterium respectively is also far higher than those obtained in present study. A possible explanation for the low cellulase yield in this study could be due to inadequate cultural conditions for isolated Gram positive cocci. According to Johnson et al. (1981)[18], Clostridium thermocellum is noted for producing a cellulase complex known as cellulosome which has a very high cellulolytic activity. Cellulosome eliminates the wasteful expenditure of microorganisms thus continuously producing copious amounts of free cellulases[19].

A high accumulation of sugar is a clear manifestation of high enzyme production and activity[20]. Glucose is one of the main products of cellulose breakdown. Glucose can be fermented to form ethanol which can be used as fuel. Ethanol can be dehydrated to produce ethylene – a major compound used in the chemical industries. Glucose (sugar) also has uses in the pharmaceutical, food and bioscience based industries[21]. Findings in this study did not exactly agree with those of other authors as cited. Most of the studies cited were carried out in other parts of the world especially in the temperate regions. Cellulolytic organisms by virtue of habitat or source were location and diet specific. Hence findings were peculiar to our environment. Though the total cellulase yield of 0.323 IU/ml as optimized by Plackett Burman design (Table 3) is much lower than commercial cellulase, it can be used in its crude and partially purified forms but at high ratios, to saccharify pretreated wastes first to sugars, then ethanol, ethylene, methane and biogas. By performing Plackett-Burman design, Isolate attained maximum cellulolytic activities in high levels of CMC, Urea, Peptone, Calcium chloride, magnesium sulphate and Trace elements and Low levels of Ammonium sulphate, and Di Potassium Hydrogen phosphate, at 37 $^{\circ}$C and pH 7.0 which is found to be optimized levels of components. The significant components for maximum cellulase production by isolated bacteria were found to be Carboxymethyl cellulose, Urea, ammonium sulphate, calcium chloride and trace elements (Fe, Mn,

Table 7 : Analysis of Plackett- Burman Design for Cellulase production

	Sub-strate CMC	Urea	Peptone	$(NH_4)_2SO_4$	K_2HPO_4	$MgSO_4.7H_2O$	$CaCl_2.2H_2O$	Trace ele-ments	D1	D2	D3
Σ H	1.22	1.04	0.89	0.59	0.81	0.96	1.04	1.05	0.99	0.96	0.98
Σ L	0.57	0.74	0.89	1.20	0.98	0.83	0.74	0.74	0.79	0.83	0.80
Difference	0.63	0.29	0.004	-0.61	-0.17	0.13	0.30	0.31	0.18	0.13	0.18
Effect	0.11	0.05	0.00	-0.10	-0.03	0.02	0.05	0.05	0.03	0.02	0.03
Mean Square	0.0114	0.0024	0.0000	0.0105	0.0008	0.0004	0.0026	0.0026	0.0010	0.0004	0.0009
Mean Square for Error	0.001										
t	14.70	3.17	0.00	13.58	1.09	0.57	3.30	3.34	1.26	0.58	1.16
p value	0.001	0.050	1.00	0.001	0.357	0.607	0.046	0.044	0.298	0.60	0.33
95% CI	**99.93***	**95.00***	0.039	**99.91***	64.33	39.32	**95.43***	**95.57***	70.23	39.81	67.06

*** Statistically significant at 95% of probability level.**

Zn and Co) as observed in table 5. The problem of high cost of cellulase production owing to low enzyme activities of organisms used industrially for its production can be solved by meticulously observing the optimal cultural growth conditions reported in this study.

Conclusion

In the present investigation, Tilapia fish species which was used for Cellulase producing bacteria isolation and isolate exhibited considerable cellulolytic activity. Effect of various substrates on cellulase enzyme production was studied by Plackett Burman design. The statistical design of experiment offers efficient methodology to identify the significant variables and to optimize the factors with minimum number of experiments for cellulose production by isolate from fish gut. Further optimization of cellulase production by central composite design would give the interactions of various substrates for better cellulase production as well the results would be further helpful for large scale production of cellulase under optimized environmental conditions. Further more this work can be extended in aspect of identification of isolate and large scale production, purification and application of cellulase enzyme.

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