Vidhyayana - ISSN 2454-8596

An International Multidisciplinary Peer-Reviewed E-Journal

www.vidhyayanaejournal.org

Indexed in: Crossref, ROAD & Google Scholar

6

OPTIMIZING MICROBIAL ENZYME PRODUCTION FROM AGRICULTURAL WASTE

Ankita H. Parmar

Research Scholar, Microbiology, Surendrangar University, Wadhwan

Supervisor

Dr. Atul Makasana

Associate Professor- Microbiology, Surendranagar University

Abstract

The present study identified the pectinase-producing bacterium isolated from the contaminated broth as *Bacillus* sp. on 16S rDNA sequence analysis. The bacterium illustrated water-like droplets on the colony grown on the Sabouraud dextrose agar plate. It also exhibited multi-enzymes activities, such as pectinase, polygalacturonase, xylanase, and cellulase by using various agro-wastes as low-cost substrates. The orange peel was observed to be the best substrate among the agro-wastes used for maximum multi-enzymes (pectinase, polygalacturonase, xylanase, and cellulase). However, the bacterium demonstrated its capability to produce different enzymes according to the different substrates/agro-wastes used. The Plackett–Burman design was used to determine the essential influencing factors, while the Box Behnken design response surface methodology was for optimizing cultural conditions. At their optimal conditions (40°C incubation temperature, 24 h of incubation period, 1% w/v orange peel, and 2% v/v inoculum volume), the bacterium exhibited the maximum pectinase (9.49 ± 1.25 U/ml) and xylanase (16.27 ± 0.52 U/ml) activities.

Furthermore, the study explored the ability of the bacterium to produce bacterial lipids and observed about 25% bacterial lipid content on a dry weight basis. Therefore, the bacterium is a good candidate for producing important multi-enzymes and subsequent agro-waste degradation controlling the environment and facilitating waste management. Also, the bacterium can be a potential feedstock in producing renewable biofuel.

Keywords: Bacillus sp., multi-enzymes, agro-wastes, optimization, lipid content

Introduction

Agro-wastes are low-cost, renewable, and sustainable resources for industrially important enzyme production. Agricultural waste is usually produced during processing, pre-harvesting, post-harvesting, marketing procedures, and household activities. The loss of agricultural products during these processes may decrease the products and increase production costs. Additionally, the agro-wastes pollute the environment and create disposal problems. Population growth and waste generation are directly proportional and it is expected the waste generation per year will increase by 83% by 2050.[1] Dumping or landfill is the common method of waste disposal. However, it is not safe. The air near or surrounding the area of the dumping site may have air pollution due to the gasses produced and the suspended particles in the air. The leachate from landfill may contain toxic byproducts and may contaminate water bodies or soil which finally may affect living things including human and aquatic life. At the same time, there is a continuous rise in demand for enzymes, and the pure carbon and nitrogen source used in enzyme production is expensive. Therefore, these agro-wastes can be used as low-cost substrates to produce polysaccharides hydrolyzing enzymes as they contain different polysaccharides such as cellulose, xylose, and pectin in different compositions.

Besides, the multi-enzymes biocatalyst technology and production of enzyme cocktails from a single organism are gaining interest. The most common and commercially important enzymes are cellulase, pectinase, and xylanase, used for broad biotechnological applications. Pectinases and xylanases have been used in wastewater treatment, brewing technology, animal feed preparation, textile, pulp and paper industries, and food processing industries. Cellulolytic enzymes and other enzymes apply to cell wall disruption, juice extraction, and lipid extraction. In addition, the multi-enzymes help protects the environment by degrading

various plant and agro-wastes. Furthermore, enzymes production depends on the microorganisms exploited, and there are only a few studies for multi-enzymes productions from a single bacterium. Producing multi-enzymes by a single bacterium using different agro-wastes is cost-effective and time-efficient because using a single pure carbohydrate as substrate can only induce a single specific enzyme which is expensive and time-consuming.

Furthermore, the uninterrupted increase in the world's population is diminishing fossil fuel reserves, so there is a need to explore alternative energy sources. In this aspect, some oleaginous microorganisms are natural oil producers. They accumulate about 20% w/v of lipid on a dry weight basis and are the most promising feedstock for lipid and oleochemical production. Therefore, this study focuses on the isolation and identification of the bacterium that increased the pectinase activity significantly. Also, the current study exploits the bacterium for multi-enzymes production by utilizing different agro-wastes and optimization of the fermentation conditions. Furthermore, the bacterium was exploited to know its capability of producing lipids due to the unique colony of the bacterium with water-like droplets on Sabouraud dextrose agar plate.

Materials and Methods

Isolation and identification of bacteria

The bacterium was isolated from the contaminated broth. A loopful of the contaminated broth was streaked on Sabouraud dextrose agar (SDA) and nutrient agar (NA) plates and incubated at room temperature and 35°C, respectively, for 2–3 days. After the growth of colonies, a single colony was sub-cultured many times on SDA and NA plates to get the pure isolated colonies. Once the pure isolated colonies were observed on the agar plate, genomic DNA was extracted following the SDS-CTAB/NaCl method.

The 16S rDNA genes of the isolate were amplified by Taq DNA polymerase with universal primer sets: 16S rDNA forward primer 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse primer 5′-GGTTACCTTGTTACGACTT-3. The amplification systems for the 16S PCR reaction mixture contained 2 × *Taq* PCR master mix of 12 µl (10 × Taq DNA polymerase buffer, 10 mM dNTPs, 25 mM of $MgCl_2$, 1 U of *Taq* DNA polymerase), 3 µl of 10 µM forward and reverse primers, 2 µl of the genomic DNA template, and 4 µl of distilled water,

Vidhyayana - ISSN 2454-8596

An International Multidisciplinary Peer-Reviewed E-Journal

www.vidhyayanaejournal.org

Indexed in: Crossref, ROAD & Google Scholar

making a total volume of 24 µl. Furthermore, the PCR reaction conditions used were denaturation at 94°C for 5 min and the cycle starting at 94°C for 30 s, followed by annealing at 54°C for 30 s, extending at 72°C for 1.5 min for 33 cycles, and finally extending at 72°C for 10 min. Then the PCR products were determined by 1% (w/v) agarose gel electrophoresis. The 16S rDNA target fragments from the gel were cut, DNA was extracted using a gel extraction minipreps kit (Bio Basic), and the extracted DNA was sent for DNA sequencing.

Gene sequencing and phylogenetic analysis

The 16S rDNA sequences of the isolate provided by the sequencing company were compared with the known sequences found in the National Center for Biotechnology Information (NCBI) database using the basic local alignment search tool (BLASTn). The isolate was identified based on the percentage similarity with the known species sequences in the database. All the sequences were collected and parallelized using the Clustalw module in BioEdit v. 7.0.9.0 with default settings. Phylogenetic analysis was performed using a Neighbor-Joining (NJ) tree with 1,000 bootstraps using MEGA 7.

Screening tests

Different screening tests for pectinase, cellulase, xylanase, and amylase were performed by culturing the bacterium on agar plates containing pectin, cellulose, xylan, and starch, respectively. After the growth of the bacterium, potassium iodide solution for pectinase, Congo red for cellulase and xylanase, and iodine solution for amylase screening test were flooded. The clear halo zone around the colonies indicated the presence of respective enzymes.

Pectinase activity

The speck of pure isolated colony, which showed the clear pectinolytic zone on screening pectin agar plate, was subjected to prepare seed culture. Then, 1% v/v seed culture was inoculated into a flask with 50 ml of yeast extract pectin media (YEP) composed of 0.3% yeast extract, 1% pectin, 0.2% KH_2PO_4, and 0.2% K_2HPO_4 in distilled water. The inoculated flasks were incubated at 35°C for 4 days at 200 rpm. Samples from inoculated flasks were collected at regular intervals of 24 h, and enzyme activities were assayed and compared. Pectinase activity was calculated by measuring the reducing sugar content released from the

Vidhyayana - ISSN 2454-8596

An International Multidisciplinary Peer-Reviewed E-Journal

www.vidhyayanaejournal.org

Indexed in: Crossref, ROAD & Google Scholar

substrate following the 3,5-dinitrosalicylic acid (DNS) method, as mentioned in a previous study. In brief, 10 µl of crude enzyme extract was added to 20 µl of 1% citrus pectin solution as a substrate solution in the wells of a microplate, incubated in a 50°C water bath for 10 min, cooled, and 60 µl of DNS reagent was added. Then the microplate was covered and heated in boiling water for 5 min, followed by cooling down to room temperature. To the mixture, 200 µl of distilled water was added, and the absorbance was recorded at 540 nm to calculate the amount of reducing sugar released. Each enzymatic activity was expressed as the amount of enzyme that releases 1 µmol of galacturonic acid in 1 min under the mentioned conditions.

Effect of incubation period, temperature, pH, inoculum volume, and pectin concentration in pectinase production

The bacterium was cultured in YEP, pectinase production media, for different incubation periods (24–120 h). At different incubation periods, the cultured broth was aseptically taken in a sterile Eppendorf tube and centrifuged. The cell-free supernatant was used for enzyme activity assay. The fermentation condition for the bacteria regarding incubation temperatures and pH was studied by culturing at different incubation temperatures (30°C, 35°C, 40°C, and 45°C) and pH (5, 6, 7, 8, 9, and 10). Similarly, the different inoculum volume (0.5, 1, 2, 3, and 4% v/v), and different pectin concentrations (0.5, 1, 1.5, and 2% w/v) were added to the media, to study the enzyme activity for 96 h.

Agro-waste preparation and multi-enzyme production

Different agro-wastes which are easily and locally available were selected. Orange peel, banana peel, pomegranate peel, and pumpkin pulp+seeds were from the waste of those fruits and vegetables bought from the market. Barley straw and maple leaf were commercially available. Canola straw was accumulated from a local farm, and brewer's spent grains from a local brewing company (Sleeping Giant Brewing Co., Thunder Bay, Ontario, Canada). All those agro-wastes were dried, ground in a coffee grinder, and washed with hot water several times to remove contaminant and simple sugar. The presence of reducing sugar was determined by DNS method. Once the samples were free of reducing sugar, they were dried on a hot air oven at 50°C for 48 h (till constant weight). The dried agro-waste powders were kept in airtight containers for further use.

For multi-enzymes production using agro-wastes, the bacterium was cultured on the media containing 1% w/v agro-wastes as the carbon source instead of 1% w/v pectin in the YEP pectinase production media. Polysaccharides such as pectinase, polygalacturonase (PGase), xylanase, and cellulase activities were determined every 24 h. The citrus pectin, polygalacturonic acid, beechwood xylan, and carboxymethyl cellulose (CMC) were used as the respective substrates.

Optimization of cultural conditions for the maximum enzyme production

Orange peel was illustrated as the best substrate among the agro-wastes used in this study, so optimization of enzyme production was performed by using orange peel. Plackett–Burman design included seven different factors at two levels; incubation temperature, pH, incubation period, $MgSO_4$, NaCl, $FeSO_4$, and $(NH_4)_2SO_4$ to screen the main influencing factor, including 15 experimental runs with three central points. The high level (+1) indicates the maximum concentration, and the low level (−1) indicates the minimum concentration of the variables (Table 1). The Box–Behnken design (BBD) response surface methodology was used to optimize the cultural conditions for maximum enzyme production using the most significant factors from the Plackett–Burman design. Here, enzyme activity was considered response variable, whereas incubation period (h), orange peel concentration (% w/v), and inoculum volume (% v/v) were three independent variables. The BBD used all the factors at three levels assigned as −1, 0, and +1 for the lowest, central, and highest value (Table 2). Both Plackett–Burman and BBD experimental designs were generated by using the Minitab 16 software.

Table 1

Experimental design and enzymes activities (U/mL) using Plackett–Burman factorial design.

Run	Temperature (°C)	pH	Time (h)	$MgSO_4$ (%w/v)	NaCl (% w/v)	Fe_2SO_4 (%w/v)	$(NH_4)_2SO_4$ (%w/v)	Pectinase (U/ml)	PGase (U/ml)	Xylanase (U/ml)	Cellulase (U/ml)
1	40	6	96	0.02	0.1	0.01	0.5	6.54	4.98	3.39	3.10
2	40	9	12	0.05	0.1	0.01	0.1	2.25	3.72	1.46	0.00
3	30	9	96	0.02	0.5	0.01	0.1	5.01	5.25	5.84	0.00
4	40	6	96	0.05	0.1	0.05	0.1	5.91	8.01	6.26	7.26
5	40	9	12	0.05	0.5	0.01	0.5	1.99	3.60	0.71	0.00
6	40	9	96	0.02	0.5	0.05	0.1	3.73	1.85	0.00	0.00
7	30	9	96	0.05	0.1	0.05	0.5	6.60	5.60	8.25	6.96
8	30	6	96	0.05	0.5	0.01	0.5	5.69	5.49	7.25	4.15
9	30	6	12	0.05	0.5	0.05	0.1	2.39	3.00	1.83	0.00
10	40	6	12	0.02	0.5	0.05	0.5	2.03	2.75	1.19	0.00
11	30	9	12	0.02	0.1	0.05	0.5	2.34	2.50	2.60	0.00
12	30	6	12	0.02	0.1	0.01	0.1	1.51	1.89	0.71	0.00
13	35	7	54	0.035	0.3	0.03	0.3	7.42	7.13	8.13	6.00

Vidhyayana - ISSN 2454-8596
An International Multidisciplinary Peer-Reviewed E-Journal
www.vidhyayanaejournal.org
Indexed in: Crossref, ROAD & Google Scholar

Table 2

Box–Behnken Design for enzymes production by using orange peel as the substrate [−1, 0, and +1 are the codes for the variables].

Run	Time (h)	Orange peel (% w/v)	Inoculum volume (% v/v)	Pectinase (U/ml)		PGase (U/ml)	Xylanase (U/ml)		Cellulase (U/ml)
				Observed	Predicted	Observed	Observed	Predicted	Observed
1	12 (−1)	0.5 (−1)	2 (0)	1.71	2.60	1.25	0.96	1.27	0.00
2	36 (+1)	0.5 (−1)	2 (0)	7.41	8.33	5.65	8.59	8.41	0.00
3	12 (−1)	1.5 (+1)	2 (0)	4.58	3.66	7.98	1.03	1.21	13.68
4	36 (+ 1)	1.5 (+1)	2 (0)	6.53	5.64	6.12	7.09	6.77	17.22
5	12 (−1)	1 (0)	1 (−1)	2.34	2.21	0.16	2.67	2.52	0.00
6	36 (+1)	1 (0)	1 (−1)	5.52	5.37	5.87	8.68	9.02	8.8
7	12 (−1)	1 (0)	3 (+1)	1.01	1.16	1.48	0.00	0.00	0.00
8	36	1 (0)	3 (+1)	5.57	5.70	7.27	5.72	5.87	13.69

Run	Time (h)	Orange peel (% w/v)	Inoculum volume (% v/v)	Pectinase (U/ml)		PGase (U/ml)	Xylanase (U/ml)		Cellulase (U/ml)
				Observed	Predicted	Observed	Observed	Predicted	Observed
	(+1)								
9	24 (0)	0.5 (−1)	1 (−1)	5.24	4.47	8.24	6.57	6.41	7.04
10	24 (0)	1.5 (+1)	1 (−1)	4.49	5.54	4.62	8.23	8.20	17.34
11	24 (0)	0.5 (−1)	3 (+1)	7.04	5.99	7.43	6.03	6.05	7.51
12	24 (0)	1.5 (+1)	3 (+1)	2.54	3.30	0.11	2.38	2.54	0.00
13	24 (0)	1 (0)	2 (0)	10.62	9.49	13.83	16.79	16.27	19.54
14	24 (0)	1 (0)	2 (0)	8.15	9.49	10.66	15.75	16.27	17.90

Determination of lipid content

The bacterium was cultured in yeast extract peptone (YEP') culture media (consisting of 0.25, 0.25, 0.15, 2% w/v of yeast extract, peptone, $MgSO_4$, dextrose, respectively) and Mineral salt medium (MSM) culture media (consisting of 0.9, 0.15, 0.02, 0.01, 0.00012, 0.002, and 0.005% w/v of Na_2HPO_4, KH_2PO_4, $MgSO_4$, $(NH_4)_2SO_4$, ferric citrate, $CaCl_2$, and $NaHCO_3$, respectively). Every 24 h, lipid content was determined by Bligh and Dyer with

little modification. In brief, the biomass was first harvested by centrifugation at 10,000 g for 10 min, and 1 gm of the pellet was sonicated and then homogenized with a 20 ml mixture containing chloroform, methanol, and water (1:2:0.8 ratio), followed by 20 min of shaking in an orbital shaker at ambient room temperature. The homogenate was centrifuged at low speed (2,000 g) to separate two phases. The upper phase was siphoned off, and the lower chloroform phase containing lipids was evaporated. Finally, the lipids extracted were quantified by weighing, and lipid content (%) was calculated as,

Lipid content (%)=Weight of lipidpermLDryweight of bacteriapermL×100

Furthermore, lipid content was determined at two different media (YEP' and MSM), different incubation periods (24 to 96 h), temperature (30°C–45°C) and pH values (6–9) to optimize the lipid content from the bacterium.

Discussion

The production of industrially important, novel, and robust enzymes for biotechnological applications is increasing. Therefore, there is a need to isolate and identify the bacteria from natural diversities and optimize the cultural conditions for maximum enzyme production. In our current study, we isolated the bacterium and screened for enzymes. The presence of a relative enzyme hydrolyzes the substrate around the colony, and a clear hydrolysis zone around the colony is observed. However, the substrate not hydrolyzed by relative enzymes forms a complex and does not show a clear zone. On this basis, the bacterium was pectinase, amylase, cellulase, and xylanase producer.

The contaminant of the broth was identified as bacterium. Only 16S rDNA got amplified and showed the band at 1,500 bp region. Further, the bacterium was identified as BACILLUS sp. because the phylogenetic tree illustrated the bacterium closely related to the BACILLUS sp. (BACILLUS MEGATERIUM, BACILLUS SIAMENSIS, BACILLUS AMYLOLIQUEFACIENS, and BACILLUS VELEZENSIS). Furthermore, the uncorrected genetic divergence helped to confirm the bacterium as BACILLUS sp.

Vidhyayana - ISSN 2454-8596

An International Multidisciplinary Peer-Reviewed E-Journal

www.vidhyayanaejournal.org

Indexed in: Crossref, ROAD & Google Scholar

Bacillus sp. are the dominant bacteria. Our study too revealed the irregular colony of *Bacillus* sp. Additionally, we observed clear water-like droplets on the colony which is unique character. Each bacterium has specific optimal conditions for the maximum enzyme activity. For instance, the study illustrated the optimal conditions by *Bacillus* sp. for the maximum xylanase activity were 50°C, 72 h, pH 7.0 ± 0.2, and 1% xylan concentration. Another study recorded the maximum pectinase activity by *Aspergillus* sp. at 35°C, pH 6.5, 1% citrus pectin and after 192 h of incubation period. Similarly, *Streptomyces thermocoprophilus* demonstrated maximum cellulase and xylanase activity at 40°C, pH 6.5, 120 h, and 1% alkaline peroxide pretreated empty fruit bunch. And our study observed the highest pectinase activity by *Bacillus* sp. in 24 h, 40°C, 1% v/v inoculum volume, alkaline pH (7 and 9), and pectin 2% w/v. The variation in the optimal conditions for enzyme activities may be due to the differences in the microorganisms used. The optimal conditions affect the growth and metabolic rate of the organisms exploited, and the maximum activity is illustrated at the most favorable conditions depending on the organisms.

The cheapest and readily available agro-industrial wastes can be used for industrially important enzyme production. The various agro-wastes, when used as the carbon source for enzyme production, the bacterium demonstrated the highest enzyme activities from orange peel. This may be because the bacterium must have found all the favorable conditions from orange peel only to demonstrate all the studied enzyme activities. Also, the orange peel may have a lower lignin concentration, making it inaccessible to pectin, hemicellulose and cellulose for the bacterium. The bacterium exhibited xylanase and pectinase activity from canola straw, maple leaf, brewer’s spent grains, banana peel, and pomegranate peel. Further, the bacterium showed only pectinase activity from barley straw and pumpkin pulp+seeds. Thus, this study depicts the bacterium’s ability to use orange peel more effectively and efficiently than other agro-wastes and demonstrates orange peel as the best substrate for *Bacillus* sp. Similarly, another study demonstrated that maximum pectinase and cellulase activity by *Mucor circinelloides* and *M. hiemalis* using tangerine peel due to the low concentration of lignin, tissue structure flexibility, and easy access to pectin, cellulose and hemicellulose. Another study reported that banana peel with high pectin and starch content induced most of the target enzymes by *Aspergillus niger*. In contrast, cellulose-rich sugarcane

bagasse induced beta-glucanase and xylanase. At the same time, starch-rich cassava pulp induced amylase and other enzymes but was comparatively lower than banana peel. Such variation might be due to the various chemical compositions and carbon sources of agro-wastes which induce the target enzyme production by a microorganism differently.

The different concentrations (0.5, 1, 1.5, and 2% w/v) of agro-wastes were studied for enzyme activities. The enzyme activities were increased with an increase in the concentration of almost all agro-wastes used (orange peel, barley straw, pumpkin pulp + seeds, banana peel, barley spent grains, canola straw, and maple leaf). In contrast, a high concentration of pomegranate peel decreased the pectinase and PGase activities. This result relates that the bacterium's ability to illustrate enzyme activity is directly proportional to agro-waste concentrations except for pomegranate peel. The higher the concentration of agro-wastes, the higher the chance of exposure to relative carbon source (pectin for pectinase, and hemicellulose for xylanase) present in that agro-waste.

Plackett–Burman design is a powerful and unique design to screen, identify and evaluate important variables that affect the response of the experimental tests. Thus, the present study used the Plackett–Burman design as an initial statistical screening of seven cultural components (variables) for enzyme activities (response). Our study revealed incubation period as an influencing factor for pectinase, PGase, xylanase and cellulase activity from this design, with the p-value < 0.05. However, the cellulase activity was only affected by all six factors and was not significantly influential for other enzyme activities. Likewise, a study used the Plackett–Burman design and reported the pectinase activity by *Bacillus sonoresis* was strongly affected by the pectin mass fraction, pH, and $MgSO_4$ among eight different parameters studied.

Therefore, BBD was again used for optimizing the cultural condition considering incubation period, orange peel concentration and inoculum volume as the independent variables. Generally, the p-value less than 0.05 ensures the terms are statistically significant for each coefficient. The relationship between parameters was found to be significant for pectinase and xylanase but not for PGase and cellulase activities. Further, the statistical significance was checked by F-test to evaluate the coefficient of determination (R^2). The lesser R^2 value is

Vidhyayana - ISSN 2454-8596

An International Multidisciplinary Peer-Reviewed E-Journal

www.vidhyayanaejournal.org

Indexed in: Crossref, ROAD & Google Scholar

not good, so the model was not too good for PGase and cellulase activity. However, the model was perfect for pectinase and xylanase activities.

Due to the depletion of fossil fuel reserves, different alternate sources are being explored. Microorganisms have potential as an alternative for lipid production as they can accumulate the oil/lipid in them. The present study explored the capacity of bacterium to produce lipids because it depicted the water like droplets on the SDA media. The study illustrated that the lipid content was higher in YEP' media, possibly due to the higher cell growth, and the media favored the cell growth. In contrast, MSM inhibited the cell growth resulting in a decrease in lipid content. Our study illustrated that the lipid content continuously increased until 48 h of incubation period and decreased onward, which may be related to cell growth. Similarly, the incubation temperature of 35°C and pH 8 favored cell growth and resulted in higher lipid content at the respective temperature and pH. However, the bacterial lipid accumulation, lipid composition and even the cell membrane compositions of the same species vary with the environmental conditions of exposure. A study demonstrated that the lipid production was in the range of 25.5%–52.9% of cell dry weight by *Fusarium oxysporum* using the synthetic media containing different sugars (glucose, fructose, and sucrose) alone and mixture of them. Whereas *Bacillus cereus* accumulated lipid 5%–19% on a dry weight basis using palm oil mill wastewater. Another study reported that *Lipomyces starkey* could potentially be used as a lipid source and accumulate lipid (12%–29.5%) when cultured in oil mill wastewater-containing media. In addition, a study illustrated the microbial lipid from the yeast *Cryptococcus* sp. using corncob hydrolysate as a raw material. Therefore, microorganisms can be a potential source of lipid production by bioremediating the wastes. Further, studies relating to the isolation and identification of high lipid-containing microorganisms, the optimization of the culturing condition, compositional analysis, and more are recommended for maximum microbial lipid production.

Conclusion

The pectinase-producing bacterium isolated from the contaminated broth was identified as *Bacillus* sp. from 16S rDNA sequence analysis. The bacterium produced different polysaccharides degrading enzymes, such as pectinase, polygalacturonase, xylanase, and

cellulase. However, the different enzyme activities vary with agro-wastes used as low-cost substrates. The response surface methodology illustrated *Bacillus* sp. to exhibit maximum pectinase at 40°C, 29 h of incubation period, 1% w/v orange peel concentration, and 2% v/v inoculum volume. In contrast, the optimal conditions for xylanase activity were 40°C, 27 h, 1% w/v orange peel concentration, and 2% v/v inoculum volume. Furthermore, the bacterium has the potential to produce bacterial lipids. Therefore, the bacterium is a good candidate for producing biotechnologically important multi-enzymes and agro-waste degradation. Since the bacterium illustrates lipid content, it can be a potential feedstock in producing renewable biofuels and environmental resilience.

Reference

- Chantorn, S., Aekkawatchai, N., Chunya, P., Oontawee, S., Khumphai, P., & Charoenrat, T. (2021). Lignocellulolytic bacteria isolated from organic rice field soils for enzyme production using agricultural wastes: Screening, medium optimization, and co-culture. Biocatalysis and Agricultural Biotechnology, 33, 101988.
- Falah, F., Vasiee, A., Tabatabaei-Yazdi, F., Moradi, S., & Sabahi, S. (2022). Optimization of γ-aminobutyric acid (GABA) production by Lactobacillus spp. from agro-food waste. Biomass Conversion and Biorefinery, 1-13.
- Marimuthu, M., Sorimuthu, A., & Muruganantham, S. (2019). Production and Optimization of Xylanase Enzyme from Bacillus subtilis using Agricultural Wastes by Solid State Fermentation. International Journal of Pharmaceutical Investigation, 9(4).
- Mishra, S. K., Kumar, S., Kumar, S., & Singh, R. K. (2016). Optimization of process parameters for-amylase production using Artificial Neural Network (ANN) on agricultural wastes. Current Trends in Biotechnology and Pharmacy, 10(3), 248-260.
- Niyonzima, F. N., Veena, S. M., & More, S. S. (2020). Industrial production and optimization of microbial enzymes. Microbial enzymes: roles and applications in industries, 115-135.
- Salihu, A., Abbas, O., Sallau, A. B., & Alam, M. Z. (2015). Agricultural residues for cellulolytic enzyme production by Aspergillus niger: effects of pretreatment. 3 Biotech, 5, 1101-1106.
- Sethi, S., & Gupta, S. (2014). Optimization of cultural parameters for cellulase enzyme production from fungi. BioLife, 2, 989-996.
- Tepe, O., & Dursun, A. Y. (2014). Exo-pectinase production by Bacillus pumilus using different agricultural wastes and optimizing of medium components using response surface methodology. Environmental Science and Pollution Research, 21, 9911-9920.
- Zheng, Y., Zhang, Q., Zhang, Z., Jing, Y., Hu, J., He, C., & Lu, C. (2022). A review on biological recycling in agricultural waste-based biohydrogen production: Recent developments. Bioresource Technology, 347, 126595.