RESEARCH PAPER

Comparative Studies on Optimization of Cellulase Production by Bacterial Isolates S4 and S5 Recovered from Coir Industry Waste-Associated Soil Using Agro-Industrial Residues

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Abstract

Cellulases constitute an important group of extracellular hydrolytic enzymes that participate in the biological conversion of cellulose-rich biomass into soluble carbohydrate products. The increasing generation of lignocellulosic residues from agricultural and industrial activities has created a need for efficient biological approaches for their utilization, particularly processes that can simultaneously reduce waste accumulation and generate commercially valuable bioproducts. Microbial cellulases are particularly attractive in this context because their production can potentially be achieved using inexpensive renewable substrates. The present investigation was undertaken to comparatively evaluate the cellulase-producing characteristics of two bacterial isolates designated S4 and S5, which were obtained from coir industry waste-associated soil. The study focused on determining the influence of important physicochemical and nutritional variables on cellulase production and on identifying suitable agro-industrial residues that could serve as low-cost substrates for enzyme production. The isolates were evaluated under different incubation periods, initial pH values, temperatures, carbon sources, nitrogen sources, mineral supplements and agro-industrial substrates. The experimental observations demonstrated considerable differences in the physiological responses of the two isolates. S4 exhibited its maximum recorded response at 48 h of incubation, whereas S5 reached its highest response at 36 h. Both isolates showed their highest responses at pH 6 and 30 °C under the tested conditions. However, their nutritional requirements differed substantially. Glucose supported the highest response in S4, while maltose was the most favorable carbon source for S5. Ammonium sulfate produced the highest response among the nitrogen sources tested for both isolates. Mineral supplementation also produced isolate-specific responses, with $MgSO_4$ being most favorable for S4 and K_2HPO_4 producing the highest response for S5. Evaluation of agro-industrial residues revealed that rice bran was the most favorable substrate for S4, whereas wheat bran produced the highest response for S5. The results demonstrate that cellulase production by the selected bacterial isolates is strongly influenced by cultivation conditions and substrate composition. The differential responses of S4 and S5 indicate that strain-specific medium formulation may be advantageous for developing economical cellulase production systems based on agro-industrial residues. The findings provide a preliminary experimental basis for subsequent statistical optimization, enzyme characterization and validation studies using lignocellulosic biomass.

Keywords: cellulase; cellulolytic bacteria; coir industry waste; bacterial isolates; S4; S5; agro-industrial residues; enzyme production; rice bran; wheat bran

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1. Introduction

Cellulose is the predominant structural polysaccharide present in plant biomass and represents one of the most abundant renewable organic resources available on Earth. Because of its extensive availability in agricultural residues, forestry wastes and agro-industrial by-products, cellulose represents an important feedstock for the development of sustainable biotechnological processes. However, the highly organized structure of cellulose, together with its association with hemicellulose and lignin in lignocellulosic materials, makes its efficient conversion into soluble sugars a challenging process. Biological degradation through microbial enzymes provides an environmentally compatible alternative to

severe chemical processing and has consequently received considerable attention in industrial biotechnology.

Cellulases constitute a complex group of hydrolytic enzymes involved in the degradation of cellulose. The cellulolytic enzyme system generally functions through the coordinated action of different catalytic activities that act upon crystalline and amorphous regions of cellulose and ultimately generate soluble oligosaccharides and glucose. Because of their ability to modify cellulose-containing materials under relatively mild conditions, cellulases have found applications in several sectors, including pulp and paper processing, textile processing, food and feed industries, detergent formulations, biomass saccharification and renewable fuel production. The increasing demand for environmentally sustainable manufacturing processes has further stimulated interest in microbial cellulases as alternatives to conventional chemical treatments.

Microorganisms represent an important biological source of cellulases because they can produce extracellular enzymes while growing on cellulose-containing substrates. Bacteria are particularly attractive enzyme producers because many bacterial species possess comparatively rapid growth rates, diverse metabolic capabilities and the ability to adapt to nutrient-limited environments. The production characteristics of cellulolytic bacteria, however, vary substantially among strains. Consequently, isolation of microorganisms from environments naturally enriched in cellulose-containing material may provide an effective strategy for identifying organisms with useful cellulolytic properties.

The coir industry represents one such environment. Coir is a lignocellulosic material derived from coconut husk, and its processing generates substantial quantities of solid residues. Continuous accumulation of these residues may create disposal and environmental-management problems. At the same time, the cellulose-rich nature of coir-associated material provides a selective ecological environment in which microorganisms capable of utilizing complex plant polymers may become established. Consequently, coir industry waste-associated soils constitute a potentially valuable source for the recovery of microorganisms possessing extracellular hydrolytic enzymes.

Although the isolation of cellulolytic microorganisms represents an important first step, the presence of cellulolytic activity alone does not necessarily indicate suitability for industrial enzyme production. Microbial enzyme synthesis is controlled by several interacting physiological and nutritional factors. Incubation time can influence the relationship between microbial growth and extracellular enzyme secretion, whereas pH and temperature directly influence cellular metabolism and enzymatic activity. Similarly, the nature of the available carbon and nitrogen sources can influence both biomass development and the regulation of extracellular enzyme synthesis. Mineral ions may additionally affect microbial metabolism and enzyme-associated processes.

The economic feasibility of cellulase production is also strongly influenced by the cost of the fermentation substrate. Carboxymethyl cellulose is widely used as a laboratory substrate for screening and enzyme assays, but its use as a primary production substrate may not be economically attractive at industrial scale. For this reason, considerable attention has been directed toward the use of agricultural and agro-industrial residues as alternative fermentation substrates. Materials such as rice bran, wheat bran and oil cakes contain carbohydrates, proteins, minerals and other nutrients that may support microbial growth and enzyme biosynthesis. Their utilization could therefore provide a dual benefit by reducing substrate costs while simultaneously contributing to the valorization of agricultural residues.

The present study was designed to comparatively evaluate two cellulolytic bacterial isolates, designated S4 and S5, obtained from the coir industry waste-associated microbial collection. The investigation specifically examined the effects of incubation period, initial pH, temperature, carbon source, nitrogen source, mineral supplementation and agro-industrial substrate on cellulase production. Particular emphasis was placed on identifying differences between S4 and S5, since strain-specific physiological characteristics are important when designing an optimized fermentation process. The study further aimed to identify inexpensive agro-industrial residues that could serve as alternative substrates for cellulase production and thereby provide a basis for subsequent process development.

2. Materials and Methods

2.1 Source and selection of bacterial isolates

The bacterial isolates examined in the present study were obtained from coir industry waste-associated soil samples collected from locations in Kanniyakumari District, Tamil Nadu, India. The original experimental work involved isolation and preliminary screening of bacterial colonies for cellulolytic activity using a CMC-containing medium. Among the recovered bacterial collection, the isolates designated S4 and S5 were selected for comparative optimization studies. The experimental dataset contains comparative measurements for S1–S5, including the responses of S4 and S5 under different cultivation and nutritional conditions.

2.2 Isolation and preliminary screening of cellulolytic bacteria

Bacterial isolation was carried out using a conventional serial dilution and agar-plating procedure. The cellulolytic screening medium contained carboxymethyl cellulose as the principal cellulose-containing substrate together with peptone, K_2HPO_4, $MgSO_4 \cdot 7H_2O$, ammonium sulfate, gelatin and agar. Following inoculation, the plates were incubated under appropriate conditions to allow bacterial colonies to develop.

The cellulolytic potential of the recovered isolates was evaluated using Congo red staining. After incubation, the CMC agar plates were flooded with Congo red solution and subsequently treated with sodium chloride. The appearance of a distinct transparent or clear hydrolysis zone surrounding a bacterial colony was considered evidence of extracellular cellulose-degrading activity. This approach was used for the preliminary identification of bacterial isolates possessing cellulolytic potential. The original dataset records 25 screened isolates with hydrolysis zones ranging from 10 to 35 mm.

2.3 Characterization of selected isolates

The selected bacterial isolates were subjected to morphological and biochemical characterization. Colony characteristics were evaluated with respect to general morphology, while microscopic examination included Gram staining. Biochemical characterization included tests such as catalase, oxidase, urease, indole, methyl red, Voges–Proskauer, citrate utilization, starch hydrolysis, casein hydrolysis and carbohydrate fermentation. The previous experimental records document biochemical characterization of the selected isolates and provide the corresponding reaction profiles. Because the present S4–S5 manuscript is focused on comparative enzyme-production behaviour, the isolates are retained here primarily by their experimental designations rather than assigning an unsupported taxonomic identity to S5.

2.4 Preparation of cellulase-producing cultures

A CMC-based production medium was employed for the comparative evaluation of cellulase production. The production medium contained cellulose substrate together with appropriate mineral and nitrogen components. Actively growing bacterial cultures were used as inoculum, and the inoculated production cultures were incubated under shaking conditions. Following cultivation, the bacterial cultures were separated from the extracellular enzyme-containing liquid fraction by centrifugation. The resulting culture supernatant was subsequently used as the crude enzyme preparation for cellulase activity determination. The previously documented experimental protocol describes centrifugation of the culture broth and use of the resulting supernatant for enzyme analysis.

2.5 Determination of cellulase activity

Cellulase activity was determined by estimating the reducing sugars liberated from the cellulose-containing substrate using the dinitrosalicylic acid assay. The assay was based on the reaction of reducing sugars with DNS reagent, followed by spectrophotometric measurement against a glucose standard. The experimental records define one unit of cellulase activity as the quantity of enzyme required to release 1 µmol of glucose per minute under the specified assay conditions. The values presented in the following sections correspond to the experimental observations recorded for S4 and S5 in the supplied dataset.

2.6 Effect of incubation period

The influence of cultivation time on cellulase production was evaluated at 12, 24, 36, 48 and 72 h. The enzyme-production response was determined at each incubation interval while maintaining the other experimental variables under the respective conditions. The purpose of this experiment was to identify the period at which extracellular cellulase production reached its maximum recorded response for each isolate.

2.7 Effect of initial pH

The effect of initial medium pH on cellulase production was examined using pH values of 5, 6, 7, 8 and 9. The pH of the production medium was adjusted before inoculation, while other culture conditions were maintained as specified in the experimental design. This experiment was performed to determine the pH range that supported the highest cellulase-production response of each isolate.

2.8 Effect of temperature

The influence of incubation temperature was investigated at 20, 30, 40, 50 and 60 °C. The bacterial cultures were cultivated under the respective temperatures, and cellulase activity was subsequently determined from the culture supernatants. The temperature producing the highest experimental response was considered the most favorable condition within the tested range.

2.9 Effect of carbon source

The influence of carbon-source composition was examined using glucose, maltose, fructose, lactose and sucrose. Each carbon source was evaluated individually while maintaining the other components of the production system constant. The purpose of this experiment was to identify the carbon source that most effectively supported cellulase production by S4 and S5.

2.10 Effect of nitrogen source

The influence of nitrogen nutrition was evaluated using peptone, casein, yeast extract, beef

extract and ammonium sulphate. Each nitrogen source was examined independently, and the corresponding cellulase-production response was determined.

The comparative analysis was used to identify whether organic or inorganic nitrogen sources were more favorable for cellulase production by the two isolates.

2.11 Effect of mineral supplementation

The effects of NaCl, $MgSO_4$, $FeSO_4$ and K_2HPO_4 were examined as mineral supplements. Each component was evaluated separately under otherwise comparable production conditions. The response obtained for each mineral treatment was compared between S4 and S5 to determine the isolate-specific influence of mineral supplementation.

2.12 Evaluation of agro-industrial residues

The potential of selected agro-industrial residues as alternative substrates for cellulase production was evaluated using rice bran, wheat bran, castor oil cake, coconut oil cake and groundnut oil cake. These materials were selected because agricultural and agro-industrial residues can provide inexpensive sources of nutrients and structurally complex carbohydrates. The experimental response obtained with each substrate was compared between S4 and S5.

3. Results

3.1 Comparative screening of cellulolytic isolates

The preliminary screening experiment demonstrated considerable variation in cellulolytic activity among the bacterial isolates recovered from coir industry-associated soil. The measured hydrolysis zones ranged from 10 to 35 mm, indicating that the isolates differed substantially in their ability to hydrolyze the cellulose-containing screening substrate. The largest hydrolysis zone was recorded for S18 at 35 mm, followed by S5 at 33 mm, S7 and S22 at 32 mm, and S3 at 29 mm. The complete screening dataset included 25 coded isolates.

The wide variation in hydrolysis-zone diameter indicates that the microbial population associated with the coir industry waste environment was physiologically diverse with respect to cellulose degradation. However, qualitative plate hydrolysis should be regarded as a preliminary screening criterion rather than a direct quantitative measurement of extracellular enzyme productivity. Consequently, subsequent comparative optimization was undertaken to evaluate enzyme-production responses under submerged culture conditions.

3.2 Influence of incubation period on cellulase production

Incubation period exerted a pronounced influence on the cellulase-production response of both S4 and S5. As shown in Table 1, S4 exhibited a progressive increase in response from 2.1 at 12 h to 2.4 at 24 h and subsequently to 3.7 at 36 h. The response reached a maximum recorded value of 5.9 at 48 h before declining to 4.9 at 72 h. This pattern indicates that 48 h represented the most favorable incubation period among the conditions tested for S4. S5 exhibited a somewhat different temporal profile. The response increased from 2.9 at 12 h to 3.2 at 24 h and subsequently reached 5.2 at 36 h. Beyond this point, the response decreased to 4.8 at 48 h and 3.6 at 72 h. Therefore, the highest recorded response for S5 occurred at 36 h.

The difference between the two isolates is noteworthy because it indicates that the timing of maximum cellulase production was not identical even though both isolates were evaluated under the same general experimental framework. Such differences may be associated with strain-specific growth characteristics, differences in the timing of extracellular enzyme secretion and changes in nutrient availability during cultivation. The reduction observed following the respective optimum periods may additionally be associated with depletion of readily available nutrients or changes in the physicochemical environment of the culture.

3.3 Influence of initial pH

Initial medium pH markedly influenced cellulase production by both bacterial isolates. S4 showed an increase in response from 2.1 at pH 5 to 5.2 at pH 6. Thereafter, the response decreased to 3.7 at pH 7, 2.1 at pH 8 and 1.4 at pH 9. Thus, pH 6 produced the highest recorded response for S4. A similar pattern was observed for S5, although the magnitude of the decline under alkaline conditions was more pronounced. The response increased from 1.38 at pH 5 to 5.09 at pH 6 and subsequently decreased to 2.12 at pH 7. At pH 8, the response decreased sharply to 0.18, followed by a value of 1.20 at pH 9.

The common preference for pH 6 suggests that mildly acidic conditions were favorable for cellulase production by both isolates under the tested experimental conditions. The substantial reduction at alkaline pH values further indicates that maintenance of an appropriate initial pH is important for obtaining an effective cellulase-production response.

3.4 Influence of temperature

Temperature was another important variable affecting cellulase production. S4 exhibited a response of 2.1 at 20 °C, which increased substantially to 5.2 at 30 °C. At 40 °C, the response decreased to 3.7 and subsequently declined further to 2.1 and 1.4 at 50 and 60 °C, respectively. S5 demonstrated a closely related pattern. The response increased from 1.38 at 20 °C to 5.09 at 30 °C and subsequently declined to 2.12 at 40 °C. A particularly strong reduction was observed at 50 °C, where the response was 0.18, while a value of 1.20 was recorded at 60 °C. The highest response for both isolates was therefore obtained at 30 °C. The similarity

in temperature preference indicates that moderate-temperature cultivation may be appropriate for the production of cellulase by both isolates. From a process-development perspective, moderate cultivation temperatures may also be advantageous because they avoid the greater energy requirements associated with high-temperature fermentation.

3.5 Influence of carbon source

The two isolates exhibited distinct responses to the carbon sources evaluated. S4 produced its highest recorded response of 6.4 in the presence of glucose. Fructose and sucrose resulted in identical responses of 4.8, while maltose and lactose produced lower responses of 3.4 and 2.1, respectively. In contrast, S5 demonstrated its maximum response with maltose, reaching a value of 5.9. Lactose and fructose produced responses of 3.7 and 3.5, respectively, while sucrose and glucose resulted in lower responses of 2.9 and 2.1.

The contrasting carbon-source preferences provide clear evidence of nutritional differences between S4 and S5. Glucose was most favorable for S4, whereas maltose was most favorable for S5. This observation emphasizes the importance of evaluating carbon-source requirements independently for individual microbial strains rather than assuming that the same carbon source will maximize enzyme production across different isolates.

3.6 Influence of nitrogen source

Nitrogen nutrition produced a relatively consistent pattern between the two isolates. Among the five nitrogen sources tested, ammonium sulfate generated the highest response for both S4 and S5. S4 exhibited a value of 3.3 with ammonium sulfate, followed by peptone at 2.8. The responses associated with casein, yeast extract and beef extract were comparatively lower. S5 also demonstrated its highest response with ammonium sulfate at 2.9. Beef extract generated a response of 1.7, whereas casein, peptone and yeast extract resulted in values of 1.1, 0.8 and 0.3, respectively.

The consistent preference for ammonium sulfate suggests that an inorganic nitrogen source may be favorable for cellulase production by both isolates. However, the magnitude of the response differed between the strains, indicating that although their preferred nitrogen source was similar, their overall nitrogen utilization efficiency was not identical.

3.7 Influence of mineral supplementation

The responses to mineral supplementation differed between S4 and S5. S4 showed its highest response in the presence of $MgSO_4$, with a value of 1.3. $FeSO_4$ and K_2HPO_4 each produced a response of 1.2, while NaCl resulted in the lowest value of 0.4. S5 exhibited a different pattern. K_2HPO_4 produced the highest response at 1.7, followed by $MgSO_4$ at 1.1 and NaCl at 0.8. $FeSO_4$ generated the lowest response, with a value of 0.3.

The contrasting responses demonstrate that mineral requirements were isolate specific. Therefore, the indiscriminate addition of mineral salts to a production medium may not necessarily improve cellulase production. Instead, mineral supplementation should be evaluated according to the physiological requirements of the selected microorganism.

3.8 Influence of agro-industrial substrates

The utilization of agro-industrial residues produced substantial differences in cellulase-production responses between S4 and S5. In S4, rice bran generated the highest response, with a value of 6.4. Castor oil cake and groundnut oil cake produced intermediate responses of 4.8 each, whereas wheat bran and coconut oil cake resulted in lower responses of 3.4 and 2.1, respectively. S5 displayed a markedly different substrate preference. Wheat bran produced the highest response at 5.8, followed by coconut oil cake at 3.7 and castor oil cake at 3.5. Groundnut oil cake produced a response of 2.9, while rice bran generated the lowest response of 2.1 among the tested substrates. These findings demonstrate that the nature of the agro-industrial substrate strongly influenced cellulase production and that the most effective residue differed between the two isolates. Rice bran was particularly suitable for S4, whereas wheat bran was most favorable for S5.

Table 1. Effect of incubation period on cellulase production by S4 and S5

Incubation period (h)	S4	S5
12	2.1	2.9
24	2.4	3.2
36	3.7	5.2
48	5.9	4.8
72	4.9	3.6

Table 2. Effect of initial pH on cellulase production

Initial pH	S4	S5
5	2.1	1.38
6	5.2	5.09
7	3.7	2.12
8	2.1	0.18
9	1.4	1.20

Table 3. Effect of temperature on cellulase production

Temperature (°C)	S4	S5
20	2.1	1.38
30	5.2	5.09
40	3.7	2.12
50	2.1	0.18
60	1.4	1.20

Table 4. Effect of carbon source

Carbon source	S4	S5
Glucose	6.4	2.1
Maltose	3.4	5.9
Sucrose	4.8	3.5
Lactose	2.1	3.7
Sucrose	4.8	2.9

Table 5. Effect of nitrogen source on cellulase production

Nitrogen source	S4	S5
Peptone	2.8	0.8
Casein	1.3	1.1
Yeast extract	1.2	0.3
Beef extract	1.2	1.7
Ammonium sulfate	3.3	2.9

Table 6. Effect of mineral supplementation on cellulase production

Mineral supplement	S4	S5
NaCl	0.4	0.8
$MgSO_4$	1.3	1.1
$FeSO_4$	1.2	0.3
K_2HPO_4	1.2	1.7

Table 7. Effect of agro-industrial residues on cellulase production

Agro-industrial substrate	S4	S5
Rice bran	6.4	2.1
Wheat bran	3.4	5.8
Castor oil cake	4.8	3.5
Coconut oil cake	2.1	3.7
Groundnut oil cake	4.8	2.9

Table 8. Comparative optimum conditions identified from the present experimental dataset

Parameter	S4	S5
Optimum incubation period	48 h	36 h
Optimum pH	6	6
Optimum temperature	30 °C	30 °C
Preferred carbon source	Glucose	Maltose
Preferred nitrogen source	Ammonium sulfate	Ammonium sulfate
Preferred mineral supplement	$MgSO_4$	K_2HPO_4
Preferred agro-industrial substrate	Rice bran	Wheat bran
Highest recorded response in tested conditions	6.4	5.9

Table 9: BLAST analysis and molecular identification of cellulolytic isolates

Strain	Isolates	Max Score	Total Score	Query Cover	E-value	Max Identity	Accession No
EJ 4	*Bacillus pacificus* strain	2680	2680	98%	0.0	99.8%	PX116275.1
EJ 5	*Bacillus paramycoides* strain	2770	2770	99%	0.0	99.1%	PX116242.1

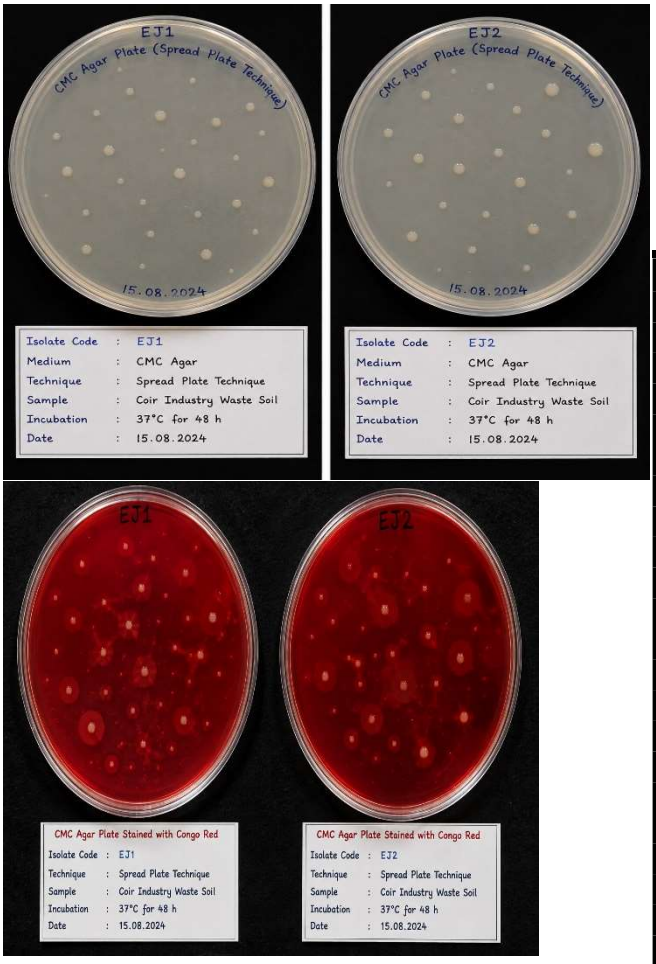

Fig: 1 Primary Isolation of Cellulolytic Bacteria
Fig: 2 Primary screening and isolation of cellulolytic bacterial isolates EJ1 and EJ2 on CMC Agar

on CMC agar using the spread plate technique and Congo red staining.

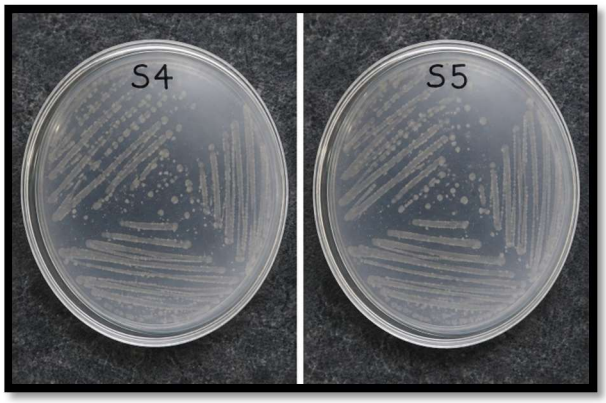

Fig 3: Cultural morphology of cellulolytic bacterial isolates

Fig: 4 Gram staining of Cellulolytic Bacterial isolates

Fig: 5 Phylogenetic tree of Bacterial strains based on 16S rRNA gene sequences

SR2589-S4-RSF1_B11.ab1
GATAACTCCGGGAAACCGGGGCTAATACCGG
ATAACATTTTGAACCGCATGGTTCGAAATTGA
AAGGCGGCTTCGGCTGT
CACTTATGGATGGACCCGCGTCGCATTAGCTA
GTTGGTGAGGTAACGGCTCACCAAGGCAACG
ATGCGTAGCCGACCTGA
GAGGGTGATCGGCCACACTGGGACTGAGACA
CGGCCCAGACTCCTACGGGAGGCAGCAGTAG
GGAATCTTCCGCAATGGA
CGAAAGTCTGACGGAGCAACGCCGCGTGAGT
GATGAAGGCTTTCGGGTCGTAAAACTCTGTTG
TTAGGGAAGAACAAGTG
CTAGTTGAATAAGCTGGCACCTTGACGGTACC
TAACCAGAAAGCCACGGCTAACTACGTGCCA
GCAGCCGCGGTAATACG
TAGGTGGCAAGCGTTATCCGGAATTATTGGGC
GTAAAGCGCGCGCAGGTGGTTTCTTAAGTCT
GATGTGAAAGCCCACGG
CTCAACCGTGGAGGGTCATTGGAAACTGGGA
GACTTGAGTGCAGAAGAGGAAAGTGGAATTC
CATGTGTAGCGGTGAAAT
GCGTAGAGATATGGAGGAACACCAGTGGCGA
AGGCGACTTTCTGGTCTGTAACTGACACTGA
GGCGCGAAAGCGTGGGAG

Figure 6: Isolate EJ4 16S rRNA gene sequence

>SR2589-S5-RSF1_C11.ab1
TGAGTTAGCGGCGGACGGGTGAGTAACACGT
GGGTAACCTGCCCATAAGACTGGGATAACTCC
GGGAAACCGGGGCTAAT
ACCGGATAACATTTTGAACCGCATGGTTCGAA
ATTGAAAGGCGGCTTCGGCTGTCACTTATGGA
TGGACCCGCGTCGCAT
TAGCTAGTTGGTGAGGTAACGGCTCACCAAG
GCAACGATGCGTAGCCGACCTGAGAGGGTGA
TCGGCCACACTGGGACTG
AGACACGGCCCAGACTCCTACGGGAGGCAGC
AGTAGGGAATCTTCCGCAATGGACGAAAGTC
TGACGGAGCAACGCCGCG
TGAGTGATGAAGGCTTTCGGGTCGTAAAACT
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TTGGGCGTAAAGCGCGCGCAGGTGGTTTCTT
AAGTCTGATGTGAAAGCCCACGGCTCAACCG
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TGGGAGACTTGAGTGCAGAAGAGGAAAGTG
GAATTCCATGTGTAGCGGTGAAATGCGTAGAG
ATATGGAGGAACACCAGT
GGCGAAGGCGACTTTCTGGTCTGTAACTGAC
ACTGAGGCGCGAAAGCGTGGGGAGCAAACA
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AGAGGGTTTCCGCCCTTTAGTGC

Figure 7: Isolate EJ5 16S rRNA gene sequence

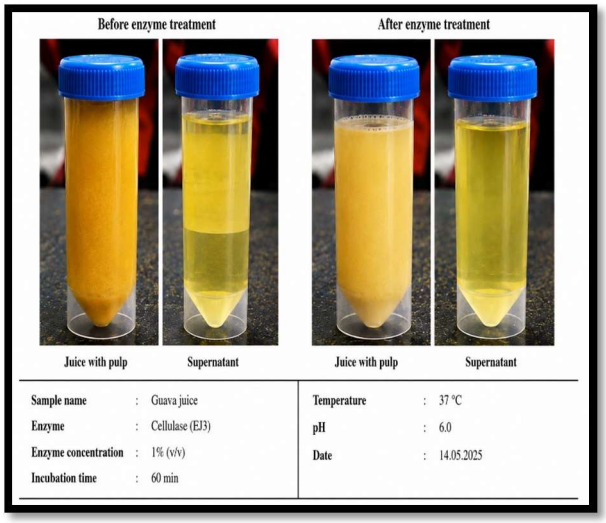

Fig: 8 Fruit Juice Clarification using cellulase enzyme

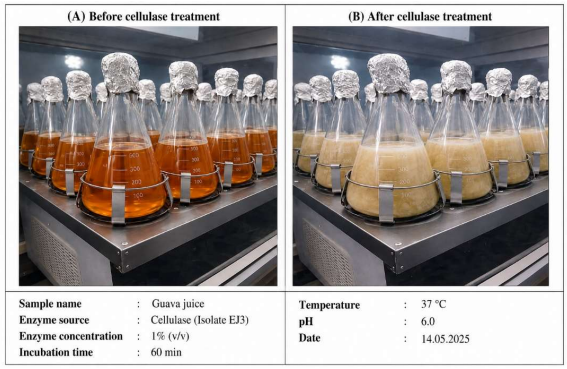

Fig: 9 Fermentation setup showing bioethanol production from crude cellulase enzyme

4. Discussion

The present investigation demonstrates that S4 and S5 possess distinct physiological characteristics with respect to cellulase production, despite their evaluation under a common experimental framework. The observed differences were particularly evident in their responses to incubation period, carbon source, mineral supplementation and agro-industrial substrates. These observations emphasize the importance of strain-specific optimization when developing microbial enzyme-production processes.

The effect of incubation period demonstrated that maximum cellulase production was not achieved simultaneously in the two isolates. S4 exhibited its highest recorded response at 48 h, whereas S5 reached its maximum at 36 h. Microbial enzyme production is closely associated with the physiological state of the organism, and extracellular enzyme synthesis may increase as cells enter particular stages of growth. Continued cultivation beyond the most productive period may not necessarily increase extracellular enzyme accumulation because nutrient availability may decline and metabolic conditions may change. The decline observed after 48 h in S4 and after 36 h in S5 is therefore consistent with the presence of an isolate-specific optimum cultivation period.

The response to pH was comparatively consistent, with both isolates producing their highest values at pH 6. This result suggests that mildly acidic conditions were favorable for cellulase production by the selected bacteria. The pronounced reduction in response at pH values above 7, particularly for S5, demonstrates that maintenance of an appropriate hydrogen-ion concentration is essential for maintaining the physiological conditions required for enzyme production. Changes in pH can influence nutrient availability, membrane-associated processes and the overall metabolic state of bacterial cells, thereby affecting extracellular enzyme secretion.

Temperature produced a similarly clear response. Both S4 and S5 demonstrated their highest recorded values at 30 °C, whereas increasing the cultivation temperature above this point resulted in a progressive reduction in the measured response. The common optimum indicates that both isolates are well suited to moderate-temperature fermentation under the conditions investigated. From an industrial perspective, the use of moderate cultivation temperatures may also contribute to process economy by avoiding the additional energy input required for high-temperature fermentation systems.

The most pronounced difference between S4 and S5 was observed in their carbon-source preferences. Glucose supported the highest response in S4, whereas maltose was most favorable for S5. This difference demonstrates that carbon metabolism is strongly strain dependent. Although carbon sources provide energy and cellular building blocks, their influence on enzyme production may extend beyond simple nutritional support. Different carbohydrates can influence metabolic regulation and consequently alter the synthesis and secretion of extracellular

hydrolytic enzymes. Therefore, the carbon source should be selected based on the physiological characteristics of the individual production strain.

The nitrogen-source experiment revealed greater similarity between the isolates. Ammonium sulfate generated the highest response for both S4 and S5. The favorable response to this inorganic nitrogen source suggests that readily assimilable nitrogen may support efficient cellulase production under the experimental conditions. However, nitrogen source alone does not determine enzyme productivity. The relative balance between carbon and nitrogen availability is also important, and future optimization should therefore investigate the interaction between carbon and nitrogen concentrations rather than evaluating them independently.

Mineral supplementation produced clearly isolate-specific responses. $MgSO_4$ was most favorable for S4, whereas K_2HPO_4 generated the highest response for S5. Such variation may reflect differences in cellular requirements for mineral nutrients and differences in the physiological response of the isolates to ionic composition. The comparatively low responses obtained with some mineral supplements further indicate that supplementation should be carefully optimized rather than applied universally.

The use of agro-industrial residues represents one of the most significant aspects of the present study. Rice bran supported the highest response of S4, while wheat bran was most favorable for S5. These findings indicate that agricultural residues can serve as alternative substrates for microbial cellulase production and may reduce dependence on commercially prepared cellulose-containing substrates. The different substrate preferences of S4 and S5 are particularly relevant to process development. A substrate that supports efficient enzyme production by one isolate may not necessarily produce the same response in another. The composition of agricultural residues is complex and includes varying proportions of carbohydrates, proteins, minerals and structural polymers. The relative availability of these components may influence both bacterial growth and enzyme biosynthesis. Consequently, detailed chemical characterization of the most favorable substrates would be an important component of subsequent process development.

For S4, the combination of 48 h incubation, pH 6, 30 °C and rice bran represents a promising preliminary production condition based on the present dataset. The response of 6.4 obtained with rice bran was comparable to the highest response observed among the tested carbon sources. For S5, the combination of 36 h incubation, pH 6, 30 °C and wheat bran appears promising, with wheat bran producing a response of 5.8. These findings suggest that low-cost substrate-based production may be feasible for both isolates, although additional replicated experiments are necessary to confirm these preliminary observations. An important implication of this study is that the optimization of microbial cellulase production should be approached as a strain-specific process. Although S4 and S5 shared certain favorable physicochemical conditions, including pH 6 and 30 °C, their nutritional preferences differed substantially. This finding supports the use of individualized medium formulations rather than a single universal formulation for different cellulolytic isolates.

The present results therefore provide a useful preliminary framework for further process optimization. The next stage should involve statistical experimental designs capable of evaluating interactions among the most influential parameters. Response surface methodology could be employed to determine whether simultaneous adjustment of incubation period, pH, temperature, substrate concentration and nitrogen level produces a higher cellulase yield than the individual optimization approach.

5. Comparative evaluation of S4 and S5

The comparative analysis indicates that S4 and S5 possess both common and distinct characteristics with respect to cellulase production.

The comparison reveals that both isolates responded favorably to similar physicochemical conditions, whereas their nutritional requirements were considerably different. This distinction is important because it demonstrates that optimization of cellulase production should consider both environmental and nutritional variables.

S4 appears particularly suited to a production system involving rice bran, whereas S5 demonstrated a stronger association with wheat bran. These findings provide an opportunity to develop separate low-cost fermentation strategies for the two isolates.

6. Proposed low-cost production strategy

Based on the experimental observations, a preliminary production strategy can be proposed for each isolate.

S4
S4 → pH 6 → 30 °C → 48 h → glucose → ammonium sulfate → $MgSO_4$ → rice bran
S5
S5 → pH 6 → 30 °C → 36 h → maltose → ammonium sulfate → K_2HPO_4 → wheat bran

These combinations represent preliminary experimental conditions identified from one-factor-at-a-time observations and should not yet be described as statistically validated optimum conditions. Confirmation using independent biological replicates, statistical analysis and validation experiments is

necessary before making definitive process-optimization claims.

7. Industrial and Biotechnological Significance

The utilization of agro-industrial residues for enzyme production provides an attractive approach for integrating waste management with bioprocess development. Rice bran and wheat bran are generated in substantial quantities from agricultural processing and are relatively inexpensive compared with purified laboratory-grade substrates. Their utilization as fermentation substrates could therefore contribute to reducing the cost of cellulase production. The present results further indicate that the two isolates may be developed through different substrate-based production strategies. S4 demonstrated a strong response with rice bran, whereas S5 responded particularly well to wheat bran. This substrate-specific behavior could potentially be exploited to design separate fermentation processes tailored to the physiological characteristics of each bacterial isolate.

However, industrial-scale application requires additional information concerning substrate composition, enzyme stability, specific activity, productivity per unit substrate and reproducibility between fermentation batches. The present results should therefore be considered an initial step toward developing a low-cost cellulase-production platform rather than evidence of immediate industrial scalability.

8. Limitations of the Study

Although the present dataset provides useful comparative information, several experimental parameters required for comprehensive statistical validation are not available in the supplied records. In particular, replicate values and standard deviations are not provided for the optimization experiments. Consequently, statistical significance and confidence intervals cannot be reliably calculated from the available data alone. The study also does not presently include detailed enzyme characterization such as specific activity, protein concentration, SDS-PAGE profiling, purification-fold analysis, kinetic parameters or enzyme stability over a range of pH and temperature conditions.

Similarly, the agro-industrial substrates were evaluated primarily on the basis of their ability to support cellulase production, while detailed physicochemical characterization of their cellulose, hemicellulose, lignin, protein and mineral contents was not included. These limitations provide clear directions for future work and should be addressed before making strong claims regarding industrial-scale application.

9. Future Research Directions

The present investigation provides a foundation for several subsequent studies. The most important next step would be to conduct replicated experiments under statistically designed conditions. Response surface methodology could be applied to identify interactions among the major factors controlling cellulase production.

For S4, rice bran should be investigated further as a potential low-cost production substrate, whereas wheat bran represents an appropriate candidate for further optimization with S5. Substrate concentration, moisture or medium composition, nitrogen supplementation and incubation conditions should be systematically evaluated.

The crude enzymes produced by the selected isolates should subsequently be characterized with respect to pH optimum, temperature optimum, pH stability, thermal stability, substrate specificity and storage stability. Determination of CMCase and FPase activities would provide a more comprehensive assessment of cellulolytic capability.

Protein profiling using SDS-PAGE would provide additional information regarding the extracellular enzyme profile. Furthermore, FTIR and SEM analysis of untreated and enzyme-treated lignocellulosic substrates could be used to investigate structural changes resulting from enzymatic treatment.

Finally, validation using actual coir-derived lignocellulosic biomass would be valuable for determining whether the laboratory cellulase-production results translate into effective biomass hydrolysis.

10. Conclusion

The present study demonstrated that bacterial isolates S4 and S5 obtained from a coir industry waste-associated environment possess distinct cellulase-production characteristics. Comparative evaluation of incubation period, pH, temperature, carbon source, nitrogen source, mineral supplementation and agro-industrial substrates revealed substantial differences in the physiological and nutritional requirements of the two isolates.

S4 exhibited its highest recorded response at 48 h, pH 6 and 30 °C, while glucose was the most favorable carbon source, ammonium sulfate was the most favorable nitrogen source, $MgSO_4$ produced the highest mineral response and rice bran was the most effective agro-industrial substrate. In contrast, S5 showed its highest response at 36 h, pH 6 and 30 °C, with maltose as the preferred carbon source, ammonium sulfate as the preferred nitrogen source, K_2HPO_4 as the most favorable mineral supplement and wheat bran as the most effective agro-industrial substrate.

The common preference for moderate temperature and slightly acidic pH, together with the different responses to nutritional variables, demonstrates that the two isolates possess both shared

and strain-specific characteristics. The ability of S4 and S5 to produce substantial cellulase responses using inexpensive agro-industrial residues is particularly relevant to the development of economically viable enzyme-production processes.

Overall, the findings establish S4 and S5 as promising candidates for further cellulase-production research. Nevertheless, the preliminary optimum conditions identified in this study require confirmation through replicated experiments, statistical optimization and independent validation. Further enzyme characterization and evaluation using actual lignocellulosic biomass will be necessary to establish their suitability for practical biotechnological applications.

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Author Contributions

Eglin Juno B K: Conceptualization, methodology, investigation, experimental work, data interpretation and manuscript preparation.

Dr. Helen Rathi: Scientific review, manuscript revision, and critical evaluation of the manuscript.

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Conflict of Interest

The authors declare that there is no conflict of interest associated with the present study.

Data Availability Statement

The experimental data underlying the findings of this study are derived from the authors' laboratory investigation. Additional information may be made available by the corresponding author subject to institutional and research-data requirements.

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