Optimization of Biocatalytic Conversion of Rice Husk to Single Cell Protein Using Bacterial Fermentation

ABSTRACT

The increasing global demand for protein has prompted a search for sustainable and cost-effective alternatives to traditional animal and dairy sources. Single-cell protein (SCP) produced from microorganism's offers a promising solution due to its high protein content and rapid growth rates. This study addresses the challenge of utilizing rice husk, an abundant yet underutilized agricultural waste in Nigeria, as a substrate for SCP production. This study also optimizedthe production of SCPusing *Bacillus sp. AT-b3* and *Bacillus sp. CMF 12*as model organisms.Molecular identification through 16S rRNA sequencing confirmed Bacillus sp. AT-b3 and *Bacillus sp. CMF 12* as the most potent isolates for SCP production. Optimization studies were conducted using the One-Factor-at-a-Time (OFAT) method to determine the ideal fermentation conditions. The results showed that the optimal temperature for SCP production was 40°C, with *Bacillus sp. AT-b3* demonstrating superior production efficiency. pH optimization revealed that neutral pH (pH 7) was ideal for maximizing SCP production, with *Bacillus spp. AT-b3* outperforming *Bacillus sp. CMF 12* at this pH. Substrate concentration studies indicated that 2.0% was optimal for SCP production, and incubation time optimization indicated 48 hours as the optimal period for maximum yield.Amino acid profiling of the SCP produced showed significant variations between the two isolates. *Bacillus sp. CMF 12* was richer in essential amino acids like Arginine and Methionine, while *Bacillus sp. AT-b3* had a higher Glycine content at ($p<0.05$). The findings of this study suggest that both strains have potential applications in nutritional supplements, with *Bacillus sp. AT-b3* being particularly suited for industrial-scale SCP production.This study concludes that *Bacillus sp. AT-b3* is an efficient SCP producer under optimal conditions of neutral pH, moderate temperature, and appropriate substrate concentration. Further research is recommended to explore pilot-scale production, alternative substrates, and comprehensive safety assessments.

Keywords: Optimization, Single Cell Protein, Bacterial Isolations, Biocatalytic Conversion, Rice Husk.

1. INTRODUCTION

The increasing global demand for protein has prompted a search for sustainable and cost-effective alternatives to traditional animal and dairy sources. Single cell protein refers to the protein obtainedfrom microbial cell mass, it develop when microbes ferments waste materials(including wood, straw, canary and food processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta) [1]. These microbes can be bacteria, yeast, fungi or microalgae [2]. The production of SCP from non-waste sources achieved industrial-scale production in the 1970s but was not economically competitive with other protein supplements. Recently, interest in SCP has been renewed, partly because of the identification of new, less expensive production processes, but largely due to the realization that SCP production has vast potential environmental benefits over traditional protein supplements in animal feed [3]. Microorganisms have capability to utilize agricultural waste to produce biomass, rich inprotein and amino acids. SCP production is a good way to overcome environmental pollution by utilizing waste materials. It is an efficient tool to transform agricultural waste such as rice hulls, rice straw, starchy residues and manure as a substrate into useful products [4].Several microorganisms have been used in SCP production using variety of substrates.

The goal of this research is to investigate the bioconversion of rice husk to single cell protein using bacteria isolated from rice processing site and optimization of the SCP produced by these bacterial strains. The finding of this study will contribute to the development of strategies for the effective utilization of rice husk and production of valuable biomass (i.e. SCP).

2. MATERIALS AND METHODS

2.1 Isolation of and Characterization of the Bacterial Strains

TheIsolation and Molecular Characterization of Industrially Significant Bacteria Obtained from Rice-Husks Dumping Sites capable of cellulose utilization was done as reported by [5].

2.2 Molecular Identification

2.2.1 Identification of Bacterial Strain Using 16S rRNA Gene Sequencing

2.2.2 DNA Extraction

The presumptive isolates were sub-cultured on nutrient agar and incubated for 24 hours at 37 ^{0}C. [6]. Part of the bacterial colony was picked with a sterile wire loop and suspended in 200 µl of TE buffer in order to prevent the DNA from degradation, at pH 8 containing RNase (50 ng /ml), then 400 ml of lysis buffer was added followed by mixing well and incubation for 15 min at 37^{0}C with intermittent shaking for every 5 min. Immediately chloroform and isoamyl-alcohol in the ratio (24:1) was taken and mixed by inversion. Tubes was centrifuged at 10000 rpm for 5 min, supernatant was transferred carefully to another micro-centrifuge tube. To the supernatant, 0.1 vol 3 M sodium acetate (pH = 5.2) and 0.6 vol isopropanol was added, mixed well by inversion and kept in the ice for 10 min followed by centrifugation at 1000 rpm for 10 min. The pellet was washed with 70% ethanol with gentle shaking and centrifuged at 10000 rpm for 3 min. Supernatant was removed and pellet was air dried. Extracted DNA was visualized in 0.8% agarose gel electrophoresis and images were documented [7].

2.2.3 Amplification of the Bacteria 16S rRNA gene

The reaction master-mix for each sample was prepared using 16S ribosomal RNA gene specific universal primers 27F 50-AGA GTT TGA TCC TGG CTC AG-30 and 1492R 50-GGT TAC CTT GTT ACG ACT T-30 (Sigma), the 16S rRNA gene was amplified with 2.5 µl of PCR master mix (Biolabs), 5.5 µl of Nuclease free water (Biolabs©), 5µl of DNA template and 1µL of each 16S forward primer and reverse primer 926R. The amplification was carried out in an Applied biosystem 9700 thermo-cycler using the following protocol:

Initial denaturation at 95 ^{0}C for 5 minutes, followed by a 36 cycles of denaturation at 94 ^{0}C for 1 minute, annealing at 55 ^{0}C for 1 minute, extension at 72 ^{0}C 1 minute 30 seconds and final extension 72 ^{0}C for 10 minutes.

2.2.4 Agarose Gel Electrophoresis

The electrophoresis was done using 1.5% agarose gel and stained with ethidium bromide DNA Gel stain (Sigma Aldrich Missouri USA) and 1X TBE buffer according to standard (85V, 3.00A, 300W) for 45 minutes. The cast and comb was set, the gel was poured and allowed to solidify. Positive bands were checked on the gel under UV-light using Bio Rad gel imager. Amplicons of the positive samples were sent to Inqaba Biotechnical Industries Ibadan, which was further transported to the industry's main branch in Pretoria, South Africa for sequencing.

2.2.5 Sequencing

PCR products was purified using a Monarch PCR and DNA clean up kit (Biolabs) following the instructions of the manufacturer.DNA (10–100 ng) was sequenced in only forward directionwith a Big-Dyeterminator version 3.1 cycle sequencing kit (AppliedBiosystems). Sequence studio genetic analyzer, (AppliedBiosystems) using the PCR primers (IDT) as sequencingprimers.

2.2.6 Bioinformatics Analysis

Basic Local Alignment Search Tool (BLAST) was used to determine sequence identities and query cover. The obtained sense and anti-sense sequences was submitted for quality evaluation using Phil's Read Editor (Phred) online application [8]. The sequences were assembled together with the Cap-Conting application in Bio-edit 7.0.9.0 software. Multiple Sequence Comparison with Log Expectation (MUSCLE) was used to align the sequences with the reference sequences downloaded from National Center for Biotechnology Information (NCBI) database as recommended by [9] using MEGA 7 software [10]. The software was used to construct a nucleotide Phylogenetic tree (Neighbor- joining, 1,000 bootstrap replications).

16S rRNA gene sequence from all relevant reference strains available in GenBank (NCBI) was used for comparison. The tree was used to determine the genotype of the sequenced 16S rRNA strains. The evolutionary distances were computed using the Maximum Composite Likelihood method [11]. All positions containing gaps and missing data were eliminated so that the total positions in the final dataset will be indicated.

2.2.7 Phylogenetic Analysis

The phylogenetic tree of the BLAST sequences and the evolutionary history were inferred using the Neighbor-Joining method [12]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [13]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using

the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site'' [10-11].

The Neighbor-Joining method was chosen for constructing the phylogenetic trees because it is fast, computationally efficient, and easy to implement. It works well with large datasets and is particularly useful for preliminary analyses and when resources are limited and it generates trees quickly by using a distance-based approach, making it ideal for a broad view of evolutionary relationships without requiring complex statistical models

2.3 Optimization of Conditions for Single Cell Protein Production

2.3.1 Preparation of the Rice Husk

Rice husk was obtained from Kofar-Kade rice processing company Sokoto. The sample was washed with cleaned water, dried, pulverized with pestle and mortar, and then sieved to tiny particles.

2.3.2 Pretreatment of the Rice Husk

At least 50g of the pulverized rice husk was mixed with 200mlof 2% NaOH and shacked until it homogenized, the solution was covered with cotton wool and aluminum foil and then allowed to suck for 24hrs, the mixture was filtered using Whatman filter paper No1. Washed with distilled water and dried [14].

2.3.3 Single cell protein production

Single cell protein production was carried out using submerged fermentation in a regulatory incubator shaker at 150 rpm. Minimal salt media was prepared in 100ml conical flasks and 1.0 ml of 0.5% Mac-Fernand standard of the bacterial isolates was used as the inoculums.

2.3.4 Determination ofOptimum Temperature for Maximum SCP Production

Optimum temperature for SCP production was determined in an incubator shaker at 30, 35, 40, 45 and 50 °C according to [15]. 50 ml of minimal salt media (MSM) was added in 100ml conical flasks in triplicate, autoclaved and inoculated with 1.0 ml of freshly prepared culture of the bacterial isolates; the pH was set at 7.5 and 150rpm in an incubator shaker.

2.3.5 Determination of Optimum pH for Maximum SCP Production

The experiment consists of five conical flasks of 100 ml capacity, in each of the flasks, 50ml of minimal salt media was added and the pH was adjusted accordingly with pH meter using 1.0M NaOH and 1.0 M NaCl. The fermentation was done in a shaker incubator at 150 rpm for a period of 48 hours to evaluate the optimum pH [15].

2.3.6 Determination ofOptimum Carbon Source Concentration for Maximum SCP Production

Different concentrations of rice husk 0.5, 1.0, 1.5, 2.0and 2.5% (w/v). 0.5% (w/v) was prepared by measuring 0.5 grams of rice husk and placed in a conical flask containing 100 ml of minimal salt media. The same procedure was involved in preparing other concentrations. The pH was adjusted accordingly and incubated at 150 rpm for 48 hrs.

2.3.7 Determination of optimum incubation time for maximum SCP production

Incubation time on SCP production was studied by setting the cultivation at different period of time for 24, 48, 72 and 96 hours. The best range of incubation period was identified [16].

2.4 Determination of Biomass

The biomass protein was determined in terms of dry weight in mg/g. An aliquot of the cultured bacteria was centrifuged at 6,000 rpm for 10 min, and the pellet was washed with distilled water

and transferred to a fresh centrifuge tube and left for an hour to settle the cell mass. The cell mass that was left in the tube was dried at 80 ^{0}C overnight and weighed for the dry mass of the bacterial culture at room temperature with a sensitive balance.

2.5 Estimation of Total Protein by Lowry Method.

Principle

Under alkaline condition, divalent copper ion forms a complex with peptide bonds in which it is reduced to a monovalent ion. Monovalent copper ion and the radical groups of tyrosine, tryptophan, and cysteine react with Folin reagent to produce an unstable product that becomes reduced to molybdenum/tungsten blue.

Method

The biomass obtained after each optimization was reconstituted in 2.0ml of distilled water, vortexed and centrifuged at 6000 rpm for 10minutes.Then 50.0µl of the supernatant was taken using micropipette into a test tube. 0.5ml of 1:2 dilution of Follin's reagent was added rapidly into a test tube containing the supernatant and mixed thoroughly. After keeping for 30 minutes at room temperature the optical density was measured using spectrophotometer at 660 nm. Protein concentration was estimated using the following formula:

Concentration of Protein = Optical Density (OD**)** Test / Bovine Serum Albumin (BSA) × concentration of the standard.

2.6 Total Protein Determination:

A small amount (115mg) of ground sample was weighed, wrapped in Whatman filter paper (No.1) and put in the Kjeldahl digestion flask. Concentrated sulphuric acid (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and

selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Six pieces of anti-bumping granules were added.

The flask was then put in Kjeldahl digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10ml) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70mL of distillate was collected.

The distillate was then titrated with standardize 0.01 N HCl to grey coloured end point.

$$\% \text{ Nitrogen} = \frac{\text{Va} \times \text{N} \times 0.014 \times \text{DF}}{\text{Weight of sample X ml of digested}} \times 100$$

Eq. 3.5

Where, Va = Volume of acid (T.V)

N = Normality of HCl used for the titration

DF = Dilution factor for the sample after digestion

0.014 = Milli- equivalent weight of nitrogen

CF = Conversion factor (6.16)

2.7 Amino Acids Profile Analysis

The Amino acid profiling was done using Model 120A PTH amino acid Analyzer(HPLC) which automatically analyzesphenyl-thiohydantoin (PTH) amino acids derived from Edman degradation of proteins and peptides.

Procedure

The amino acids profile in the sample was determined using methods described by Peace and [17].0.2g of the digested sample was weighed into glass ampoule. 7 ml of 6N HCl was added and oxygen was expelled by passing nitrogen into the ampoule (To avoid possible oxidation of some amino acids during hydrolysis such as methionine and cystine). The glass ampoule was then sealed and put in an oven preset at $105^{o}C \pm 5^{o}C$ for 22 hours. The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5 ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer. Then 60 µl was loaded into the Applied Biosystems PTH Amino Acid Analyzer.

The amino acids value was calculated automatically by an integrator attached to the analyzer which measures peaks of absorbance.

Statistical Analysis

The data were presented as mean ± SEM. A t-test was conducted to compare differences between groups, with statistical significance defined at a $p<0.05$, while GraphpadInstat software (version 3.0) was used for the t-test. Kolmogorov-Smirnov and Anderson-Darling tests were performed using stats model libraries to confirm the data meets the assumptions required for t-test.

3. RESULTS

3.1 Molecular Identification

Thepolymerase chain reaction (PCR) amplification of the 16S ribosomal RNA gene produced amplicons of approximately 400bpon agarose gel electrophoresis.The sequences identified by

NCBI BLAST indicate that the two isolates belonged to genera *Bacillussp.* with variation at species level. Their percentage hit similarity is above 90% as shown in Table 1.

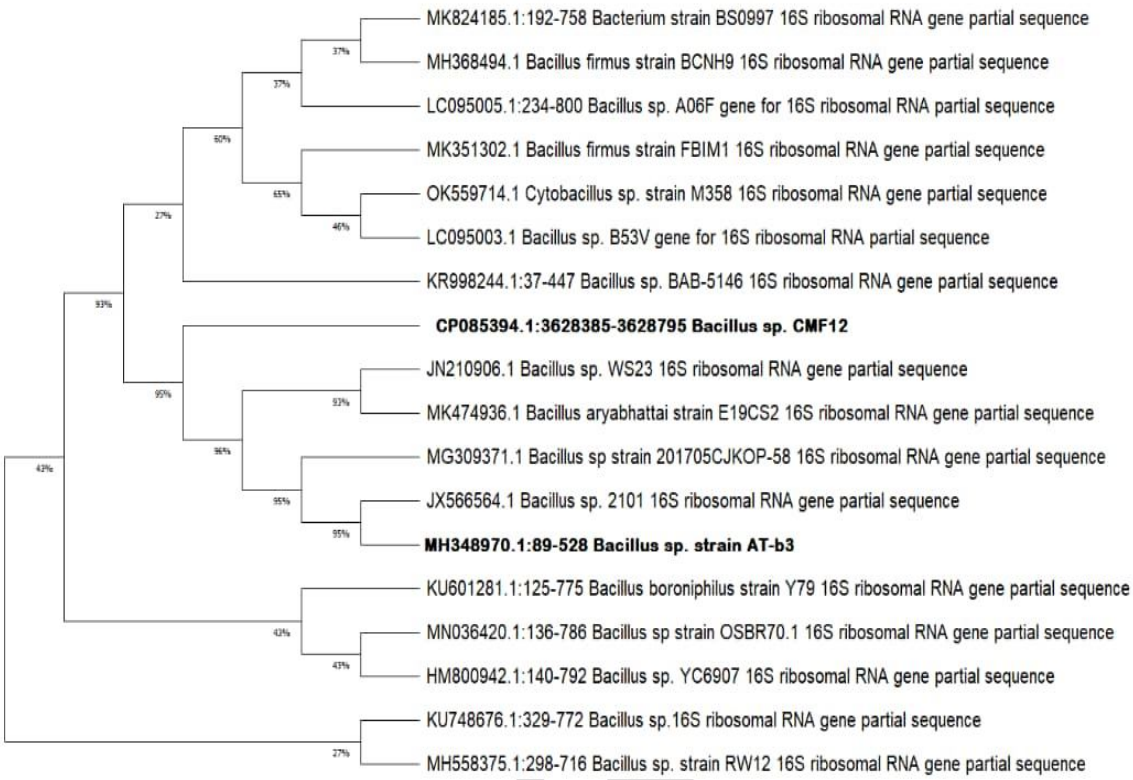
3.1.1 Phylogenetic Analysis of the Bacterial Isolates

Picture 1 shows the phylogenetic tree indicating the evolutionary relationship among the identified species and other species based on similarities and differences in their evolutionary genetic characteristics as compared with their related species from the database of the GenBank NCBI.

Molecular characterization of the most potent bacterial isolates revealed the two isolates as *Bacillus sp. AT-b3* and *Bacillus sp. CMF 12*. The occurrence of all these isolates in rice husk contaminated soil could be attributed to the abundance of microorganisms found in the soil and their ability to utilize or degrade the carbon source present in that soil.

Table 1. Molecular identification of Single cell production bacteria isolated from soil of rice husk dumping site at Kalambaina Area of Sokoto state.

Isolates	Organism	Strain code	Percentage Identity (%)	NCBI Accession Number
KLB 2B	*Bacillus sp.*	Strain Atb3	91.46	MH348970
KLB 2E	*Bacillus sp.*	Strain CMF 12	99.73	CP085392



Picture 1: Phylogenetic Tree for *Bacillus spp. AT-b3* and*Bacillus sp.CMF 12*based on 16S rRNA Sequence using Neighbor Joining Method.

3.2 Optimization of Fermentation Conditions for Maximum Single Cell Protein Production

One Factor at a Time (OFAT)method was employed in determining the effect of different conditions (temperature, pH, carbon source concentration and incubation time) on *Bacillus sp.* (Figure 1, 2,.3 and.4)

3.2.1 Effect of Temperature on SCP Production

Figure1 shows the effect of temperature range of 30 to 50 ^{0}C on single cell production. The result indicates that for *Bacillus sp. AT-b3*,the highest %SCP was observed at 45°Cwhereas the maximum %SCP concentration was at 40°C for *Bacillus sp.CMF12.*

3.2.2 Effect of pH on Single Cell Protein Production.

Figure2shows theeffect of pH range of 5 to 9 on SCP production.The result shows thatpH significantlyaffects the %SCP concentration.The highest %SCP for both *Bacillus sp. CMF 12*and *Bacillus sp.AT-b3* was observed at pH 7.

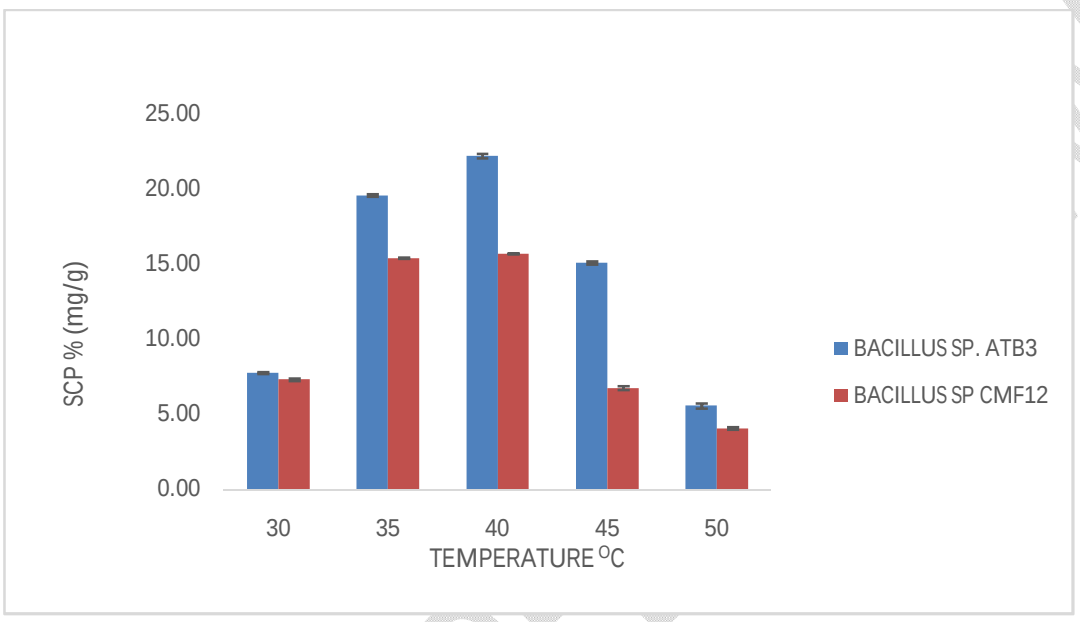


Figure1: Effect of Temperature on SCP Production

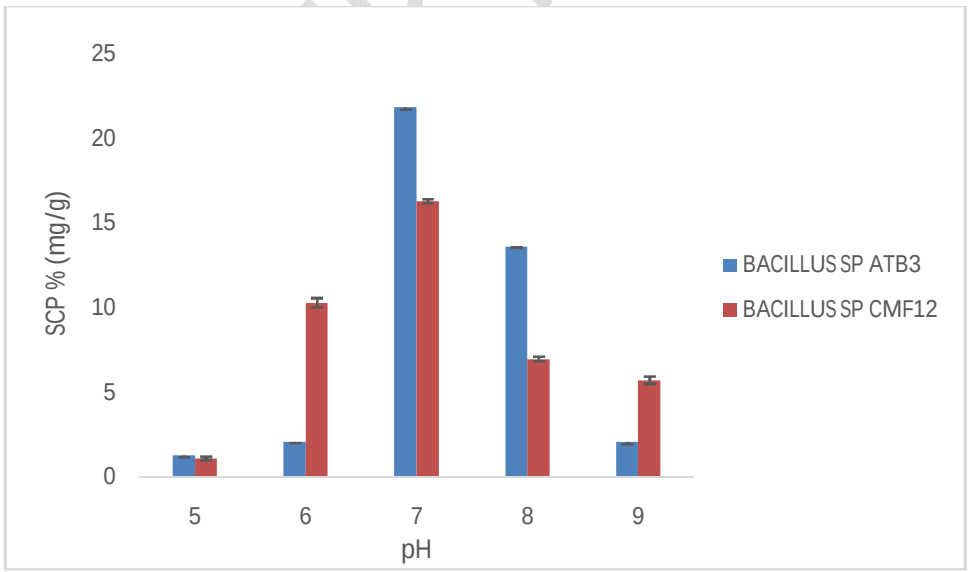


Figure2 Effect of pH on Single Cell Protein Production.

3.2.3 Effect of Carbon Source Concentration on SCP Production.

Figure3 shows the effect of carbon source concentration of 0.5% to 2.5% onSCP production. The result shows that the optimum carbon source concentration for *Bacillus sp.AT-b3* and *Bacillus sp. CMF 12* was observed at 2%.

3.2.4 Effect of Incubation Time on SCP Production.

Figure 4 shows the effect of incubation period of 12 to 96 hours on SCP production for *Bacillus sp.AT-b3* and *Bacillus sp.CMF 12.* From the result, the optimal incubation time for both the two isolates was observed after 48 hours.

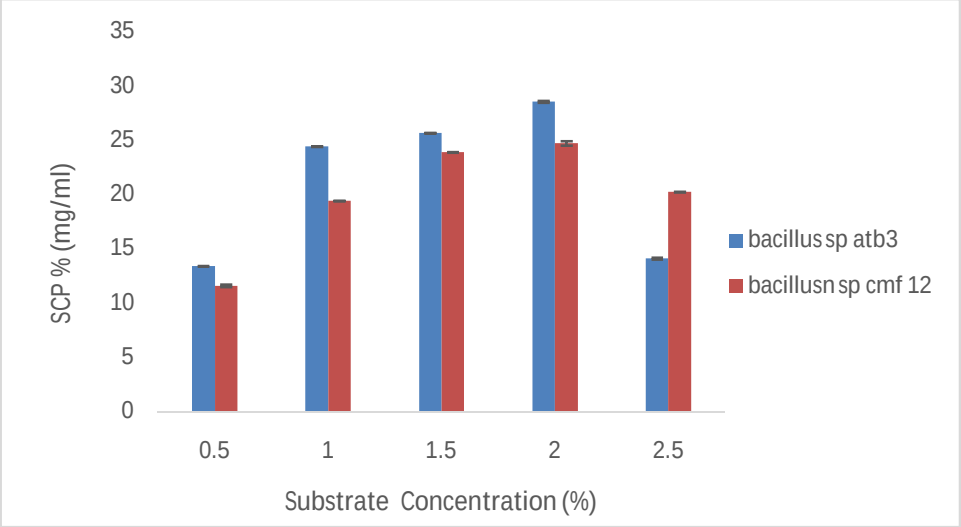


Figure 3: Effect of Carbon Source concentration on SCP Production.

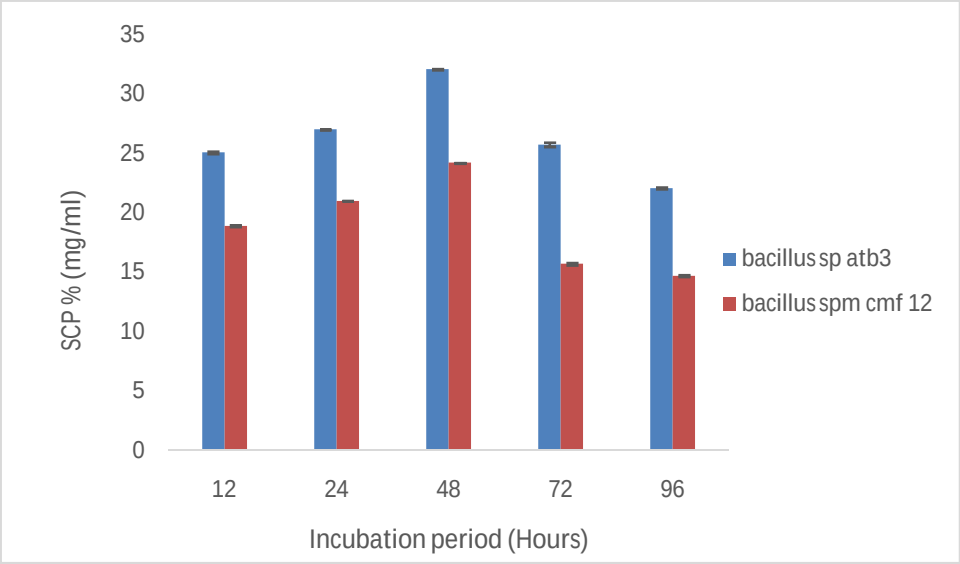


Figure4: Effect of incubation time on SCP production.

3.3 Amino Acid Profile of the Single Cell Protein

The amino acid profile of the selected bacterial isolates is presented in Table 2. This study revealed the presence of essential amino acids; Arginine, valine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and tryptophan. The non-essential amino acids detected are; proline, cysteine, alanine, glycine, glutamate, aspartate, serine and tyrosine were detected.

Table 2. Amino acid profile of the single cell protein produced from rice husk using *Bacillus sp. AT-b3*and*Bacillus sp. CMF 12*

Amino Acid	*Bacillus sp. AT-b3* (g/100g biomass)	*Bacillus sp. CMF 12* (g/100g biomass)
Essential		
Arginine	6.24±0.119[b]	8.40±0.302[a]
Valine	6.43±0.123[b]	8.07±0.098[a]
Histidine	2.09±0.115[a]	2.64±0.105[a]
Isoleucine	4.44±0.440[b]	5.30±0.266[a]
Leucine	6.95±0.134[a]	7.87±0.061[a]
Lysine	4.07±0.115[a]	3.39±0.156[a]
Methionine	2.33±0.180[b]	4.46±0.247[a]
Phenylalanine	4.79±0.210[b]	5.31±0.242[a]
Threonine	4.22±0.283[a]	4.58±0.290[a]
Tryptophan	1.80±0.020[b]	2.37±0.235[a]
Non-essential		
Proline	4.02±0.113[b]	7.35±0.172[a]
Cysteine	2.15±0.090[b]	3.71±0.191[a]
Alanine	5.57±0.240[a]	5.45±0.315[a]
Glycine	7.61±0.312[a]	5.57±0.331[b]
Glutamic acid	6.17±0.050[b]	19.03±0.349[a]
Aspartic acid	7.48±0.127[b]	8.7±0.332[a]
Serine	4.58±0.261[a]	5.54±0.115[b]
Tyrosine	3.52±0.230[b]	4.25±0.109[a]

Values are mean ± SD of three (3) replicates. The mean bearing the same superscriptare not significantly differentat $p<0.05$

4. DISCUSSION

Temperature optimization indicates that 40 °C is the optimal temperature for % SCP production for both *Bacillus sp. AT-b3* and *Bacillus sp. CMF 12*. However, *Bacillus sp. AT-b3* has higher production efficiency at this temperature, making it potentially more suitable for industrial applications where SCP production is the goal. This isolate shows both higher overall production and a wider range of high efficiency temperatures.

Temperature is an important factor that affects the growth of microorganisms and was reported by [8], while using whey as a sample, that thermal stress at higher temperatures led to decreased protein synthesis in *Bacillus subtilis* due to enzyme denaturation and impaired metabolic functions.According to their studies, the optimum temperature for SCP production for *Bacillus subtilis* was reported at 37˚C.

The effect of pH on SCP production indicates that there is a significant drop in %SCP production at pH levels lower than 7 and higher than 7. The production is minimal at pH 5, indicating that acidic conditions are less favourable for *Bacillus sp. AT-b3*.For *Bacillus sp CMF 12*, the % SCP production also peaks at pH 7, although the peak is *lower compared to Bacillus sp. AT-b3.Similar to Bacillus sp. AT-b3, Bacillus sp. CMF 12* exhibits a drop in % SCP production at pH levels outside the optimal range (5 and 9), with the lowest production at pH 5.*Bacillus sp. AT-b3* consistently shows higher % SCP production than *Bacillus sp. CMF 12* at pH 7 and 8.At pH 6, *Bacillus sp. CMF 12* outperforms *Bacillus sp. AT-b*3, indicating that *Bacillus sp. CMF 12* may be more tolerant to slightly acidic conditions.Both isolates show minimal % SCP production at pH 5 and 9, indicating that extreme acidic or alkaline conditions are unfavourable for SCP production in both Bacillus species.The graph indicates that pH 7 is the optimal pH for

maximizing % SCP production for both *Bacillus sp. AT-b3* and *Bacillus sp. CMF 12*, with *Bacillus sp. AT-b3* showing superior performance at this pH. *Bacillus sp. CMF 12*, however, performs better at pH 6, suggesting some tolerance to slightly acidic conditions. Maintaining a neutral pH is essential for effective SCP production; with *Bacillus sp. AT-b3* being the more efficient producer in neutral conditions. At neutral pH, enzymes involved in metabolic pathways function optimally, leading to enhanced biomass and protein production.

The effect of substrate concentration on % SCP production indicates that the % SCP production by *Bacillus sp. AT-b3* increases as the substrate concentration rises from 0.5% to 1.5%, peaking at around 30% SCP at 1.5% substrate concentration. Beyond 1.5%, the % SCP production begins to decrease, showing a decline at 2% and a significant drop at 2.5% substrate concentration. The highest efficiency in % SCP production is achieved at a substrate concentration of 2.0%, indicating this as the optimal concentration for *Bacillus sp. AT-b3 and Bacillus sp. AT-b3*which generally produces a higher % SCP across all substrate concentrations compared to *Bacillus sp. CMF 12*, except at 2.5%, where *Bacillus sp. CMF 12* outperforms *Bacillus sp. AT*-b3.The decline in % SCP production at substrate concentrations above 1.5% suggests that higher concentrations might lead to substrate inhibition or other metabolic limitations.This finding aligns with previous research by [19], which demonstrated that an optimal substrate concentration is critical for maximizing microbial growth and protein production, and that excessive substrate can lead to inhibitory effects. The significant effect of substrate concentration on both biomass and SCP production can be attributed to the balance between nutrient availability and metabolic capacity. At optimal concentrations, the bacteria can efficiently utilize the substrate for growth and protein synthesis. However, beyond the optimal point, excess substrate may cause metabolic imbalances or toxic by-product accumulation, leading to reduced biomass and protein production.

For incubation time optimization, the highest % SCP production for Bacillus species was observed after 48 hours. *Bacillus sp AT-b3* SCP production increases steadily, peaking at 48 hours with a yield of around 32%. After 48 hours, there is a decline in SCP production, which continues to decrease through 72 and 96 hours.

Therefore, for both isolates, the incubation period should be set to around 48 hours to maximize SCP yield before the onset of any decline in production. *Bacillus sp.AT-b3* is the better performer in terms of SCP yield across the incubation periods tested, making it potentially more suitable for industrial applications where higher yields are desired.

After obtaining the SCP, amino acid profile was performed, variations in concentration of essential amino acids such as Arginine, Valine, Histidine, and Methionine was observed. *Bacillus sp.CMF12* shows higher concentrations of Arginine and Methionine compared to *Bacillus sp. AT-b3.* There is also significant variation in non-essential amino acids. *Bacillus sp. AT-b3* has a high Glycine content whereas *Bacillus sp. CMF 12* is richer in Glutamic acid.The significant differences observed in the amino acid profiles of *Bacillus sp. AT-b3* and *Bacillus sp.CMF12* highlights the diversity within Bacillus species in terms of their nutritional content. These findings are consistent with [20-22], explained that different *Bacillus* strains can exhibit unique amino acid profiles, which can be optimized for specific applications in food and feed industries. The high content of essential amino acids such as Leucine and Isoleucine in *Bacillus sp. CMF 12* suggests its potential utility in nutritional supplements.

5. CONCLUSION

This research demonstrated the potential of Bacillus sp. for the production of Single Cell Protein (SCP) using rice husk as a substrate. The study isolated and characterized various Bacillus

strains, particularly *Bacillus sp. AT-b*3 and *Bacillus sp. CMF 1*2, which showed significant capabilities in SCP production. The optimal conditions for maximizing SCP yield were identified at a pH of 7, a temperature of 40 °C, carbon source concentration of 2%, and an incubation period of 48 hours at 150 rpm. The amino acid profile analysis revealed that *Bacillus sp. CMF* 12 had higher concentrations of essential amino acids compared to *Bacillus sp. AT-b*3, making it a superior candidate for SCP production. The essential amino acids detected included arginine, valine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and tryptophan. Non-essential amino acids such as proline, cysteine, alanine, glycine, glutamic acid, aspartic acid, serine, and tyrosine were also present in significant amounts. This research reportedfor the first time, that single cell production using *Bacillus sp.* can be improved by utilizing rice husk.

References

1. Anbuselvi A, Mahalanobis S, Jha M. Optimization of single-cell protein using green gram husk and Bengal gram husk using yeast. International Journal of Pharmaceutical Sciences Review and Research. 2014; 28:188-190.

2. Sharif M, Zafar MH, Aqib AI, Saeed M. Single-cell protein: Sources, mechanism of production, nutritional value and its uses in aquaculture nutrition, Aquaculture. 2021; 531(5):735885.
3. Matassa S, Nico B, Pikaar L, Verstraete W. Microbial protein: future sustainable food supply route with low environmental footprint, Microbial Biotechnology. 2016; 9(5):568-575.
4. Zhou M, Chen P, Guo S, Shen Y, Yan P, Yang X. 2019. Recycling of orange waste for single cell protein production and the synergistic and antagonistic effects on production quality. Journal of Cleaner Production. 2019; 213:384-392.
5. Abubakar AL, Musa SB, Maiyaki ZA, Lawal A, Abubakar GA. Isolation and Molecular Characterization of Industrially Significant Bacteria Obtained from Rice-Husks Dumping Sites, Asian Journal of Biotechnology and Bioresource Technology. 2024; 10(4):37-47.
6. Dashti AA, Jadion MM, Abdulsamad MA, and Dashti HM. 2009. Heat treatment of bacteria: A simple method of DNA extraction for molecular techniques. Kuwait Medical Journal, 41(2):117-122.
7. Balakrishnan K, Krishnaa, D, Balakrishnan, G, Manickam M, Abdulkader AM, Dharumadurai D. 2022. Association of Bacterial Communities with Psychedelic Mushroom and Soil as Revealed in 16S rRNA Gene Sequencing. Applied Biochemistry and Biotechnology, 196(5):2566-2590.
8. Togawa RC, Brigido MM. Sequencing analysis of next generation sequencing data. BriefingsinBioinformatics. 2012; 13(4):503-515.
9. Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD. Multiple sequence alignment with the CLUSTAL series of programs. Nucleic Acids Research,2003; 31(13):3497-3500.
10. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution. 2016; 33:1870- 1874.
11. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences, (USA). 2004; 101:11030-11035.
12. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 1987; 4:406-425.

13. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 1985; 39:783-791.

14. Nikzad M, Movagharnejad K, Talebnia F, Aghaiy Z, Mighani M. Modeling of alkali pretreatment of rice husk using response surface methodology and artificial neural network, Chemical Engineering Communications. 2015; 202(6):728-738.
15. Imran M, Anwar Z, Irshad M, Javid A, Hussaini H, and Ali S. Optimization of cellulose from a novel strain *Aspergillus tubingenesis* IMMIS 2 through response surface methodology, Biocatalysis and Agricultural Technology. 2017; 12:191-198.
16. Khokhar I, Haider M.S., Mushtaq, S. and Mukhtar, I. Isolation and screening of highly cellulolytic filamentous fungi. Journal of Applied Science Environment Management. 2012; 16(3): 223-226.
17. Gilani GS, Cockell KA, and Sepehr E. Effects of antinutritional factors on protein digestibility and amino acid available in food, JAOAC Int. 2005; 88(3): 967-987.
18. Gomashe AV, Pounikar MA, Gulhane P. Liquid whey: A potential substrate for single cell protein production from *Bacillus subtilis*. International Journal of Life Sciences, 2014; 2(2):119-123.
19. Zhang Z, Cai W. Enzymatic hydrolysis of sugarcane bagasse using dilute acid and hydrothermal pretreatment. *Applied Biochemistry and Biotechnology*, 2008;145:123-133.
20. Singh B, Sharma S, Kanwar SS. Single cell protein production by *Aspergillus terreus* and its evaluation. Biotechnology Research International,2012, 1-7.
21. Kaur, K., Sangwan, S. and Kaur, H. Biosurfactant production by yeast isolated from hydrocarbon polluted environment, Environmental Monitory Assessment. 2016; 189(12):603.
22. Li, J.L. and Chen, B.H. Surfactant-mediated biodegradation of polycyclic aromatic Hydrocarbons. Materials. 2018; 2:76–94.