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# From Waste to Protein: Optimizing Rice Husk Utilization for Sustainable Single Cell Protein Production

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## 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. Through optimization of the production of SCP using *Bacillus sp. AT-b3* and *Bacillus sp. CMF 12* as model organisms. Molecular identification

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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, food security and sustainability has prompted a search for sustainable and cost-effective alternatives to traditional animal and dairy sources (Jones AW, et al. 2020). It has been projected a global population increase 9.3 billion by 2050, pressing need arises to address the challenge posed by rising food demands (Junaid et al., 2020). Single cell protein (SCP) refers to the protein obtained from microbial cell mass, it develop when microbes degrade and utilize waste materials (including wood, straw, canary and food processing wastes, residues from alcohol production, hydrocarbons, or human and animal excreta) (Anbuselvi et al., 2014). These microbes can be bacteria, yeast, fungi or microalgae (Sharif et al., 2021). The production of SCP from non-waste sources achieved industrial-scale production in the 1970s but was not economically competitive with other protein supplements. This emerged as a promising solution to address the growing global demand for protein sustainability (Kieliszek et al., 2017), by allowing low-cost production with minimized demand for resources. 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 (Matassa et al., 2016).

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 (Zhou et al., 2019). 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

The Isolation and molecular characterization of industrially significant bacteria obtained from Rice-Husks dumping sites capable of cellulose utilization was done and reported by Abubakar et al. (2024).

### 2.2 Molecular Identification

#### 2.2.1 DNA extraction

The presumptive isolates were sub-cultured on nutrient agar and incubated for 24 hours at 37 °C (Dashti et al., 2009). 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, 0.1ml 3M sodium acetate (pH = 5.2) and 0.6ml 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 (Balakrishnan et al., 2022).

### 2.2.2 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 °C for 5 mins, followed by a 36 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C 1 min 30 secs and final extension 72 °C for 10 mins.

### 2.2.3 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.

### 2.2.4 Sequencing

PCR products were 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 direction with a Big-Dyeterminator version 3.1 cycle sequencing kit (AppliedBiosystems). Sequence studio genetic analyzer, (AppliedBiosystems) using the PCR primers (IDT) as sequencing primers.

### 2.2.5 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 (Togawa et al., 2012). 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 (Chenna et al., 2003) using MEGA 7 software (Kumar et al., 2016). 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 (Tamura et al., 2004). All positions containing gaps and missing data were eliminated so that the total positions in the final dataset will be indicated.

### 2.2.6 Phylogenetic analysis

The phylogenetic tree of the BLAST sequences and the evolutionary history were inferred using the Neighbor-Joining method (Saitou et al., 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). 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 (Kumar et al., 2016; Tamura et al., 2004).

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 Single Cell Protein Production

### 2.3.1 Preparation of the rice husk

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

**Pretreatment of the Rice Husk:** At least 50g of the pulverized rice husk was mixed with 200ml of 2% NaOH and shacked until it homogenized, the solution was covered with cotton wool and aluminum foil and then allowed to ferment for 24 hrs, and the mixture was filtered using Whatman filter paper (Nikzad et al., 2015).

### 2.3.2 Optimization of conditions for 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.

**Determination of Optimum 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 (Imran et al., 2017). 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 using an incubator shaker.

**Determination of Optimum pH for Maximum SCP Production:** The experiment consists of five (5) 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 using incubator shaker at 150 rpm for a period of 48 hrs to evaluate the optimum pH ((Imran et al., 2017).

**Determination of Optimum 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 0.5g 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 150rpm for 48 hrs.

**Determination of Optimum Incubation Time for maximum SCP production:** Incubation time for SCP production was studied by setting the cultivation at different period of time for 24, 48, 72 and 96 hrs. The best range of incubation period was identified according to (Khokhar et al., 2012).

## 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 pellets were washed with distilled water and then transferred to a fresh centrifuge tube and left for an hour to settle the cell mass. The cell mass left in the tubes were dried at 80 °C overnight and weighed for the dry mass of the bacterial culture at room temperature with a sensitive balance.

## 2.5 Determination of Total Protein by Lowry Method

Total protein was determined using Lowry method (Lowry et al., 1951) as modified by Gomashe *et al.* (2014). Lysis buffer was used to disrupt the cells after reconstitution in distilled water, the cells were centrifuged and Folilins's reagent was added. Total protein was determined spectrophotometrically at 660nm.

## 2.6 Amino Acids Profile Analysis

The amino acids profile of the sample was determined using methods described by Paece and Gilani, (2005); (Benitez, 1989). The amino acid analysis was performed using Model 120A PTH amino acid Analyzer (HPLC) automatically analyzes phenylthiohydantoin (PTH) amino acids derived from Edman degradation of proteins and peptides. The biomass samples were dried to constant weight, defatted, hydrolyzed,

evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer. An integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids.

### 2.7 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 Graphpad Instat 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 AND DISCUSSION

### 3.1 Molecular Identification

The polymerase chain reaction (PCR) amplification of the 16S ribosomal RNA gene produced amplicons of approximately 400bp on Agarose gel electrophoresis as shown in Plate 1. The sequences identified by NCBI BLAST indicate that the two isolates belonged to genera *Bacillus sp. AT-b3* and *Bacillus CMF 12* with variation at species level. Their percentage hit similarities were above 90% as shown in Table 1.

#### 3.1.1 Phylogenetic analysis of the bacterial isolates

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 in (Fig. 1).

### 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.* (Figs. 2, 3, 4 and 5).

The effect of temperature range of 30 to 50 °C on single cell production in (Fig. 2) indicates that for *Bacillus sp. AT-b3,* the highest %SCP was observed at 45 °C whereas the highest %SCP concentration was at 40 $^0$C for *Bacillus sp. CMF 12.* The pH range investigated (5 to 9) on SCP production in (Fig. 3) shows that pH significantly affects the %SCP concentration. The highest %SCP for both *Bacillus sp. CMF 12* and *Bacillus sp. AT-b3* was observed at pH 7. The effect of carbon source concentration of 0.5% to 2.5% on SCP production in Fig. 4 shows that the optimum carbon source concentration for *Bacillus sp. AT-b3* and *Bacillus sp. CMF 12* was observed at 2%. The incubation period from 12 to 96 hours on SCP production for *Bacillus sp. AT-b3* and *Bacillus sp. CMF 12* in (Fig. 5) indicated that the optimal incubation time for both the two isolates was observed after 48 hours.

### 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.

## 4. DISCUSSION

The Molecular characterization of the two (2) most potent bacterial isolates revealed the isolates as *Bacillus sp. AT-b3* and *Bacillus sp. CMF 12*. The occurrence of 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 which enables them strive nutritionally as well as providing them with shelter.

**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 *AT-b3* | 91.46 | MH348970 |
| KLB 2E | *Bacillus sp.* | Strain *CMF 12* | 99.73 | CP085392 |

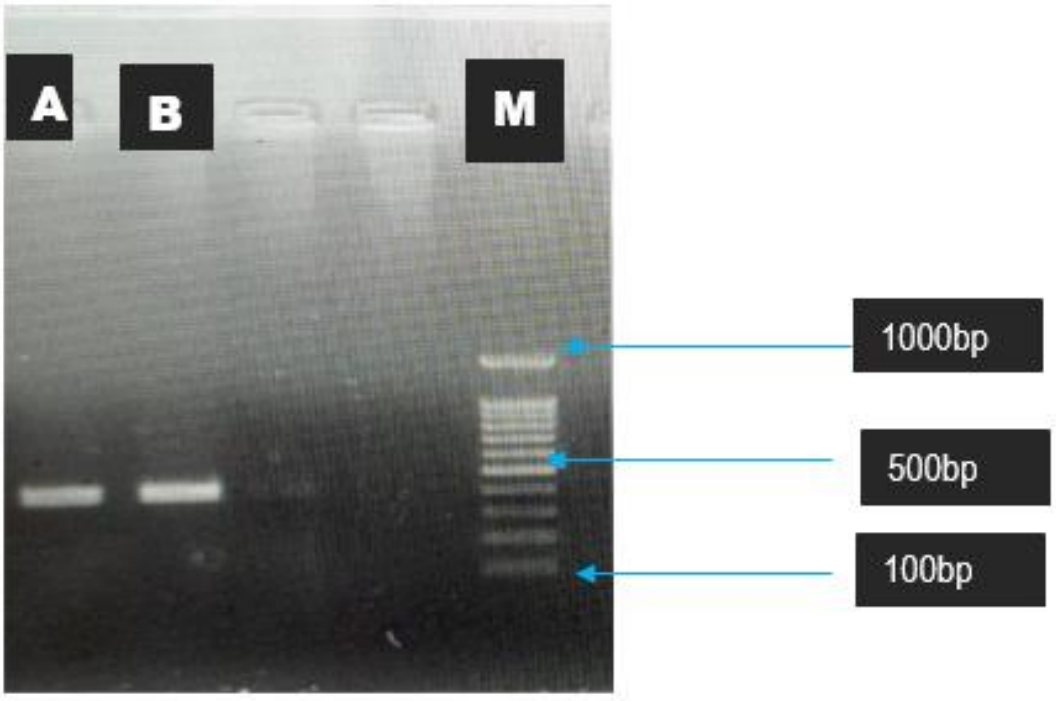


**Plate 1. Gel image of the isolates on Agarose gel electrophoresis from Abubakar et al. (2024)**
*A= Bacillus sp. CMF 12*
*B= Bacillus sp. AT-b3*
*M=DNA ladder*

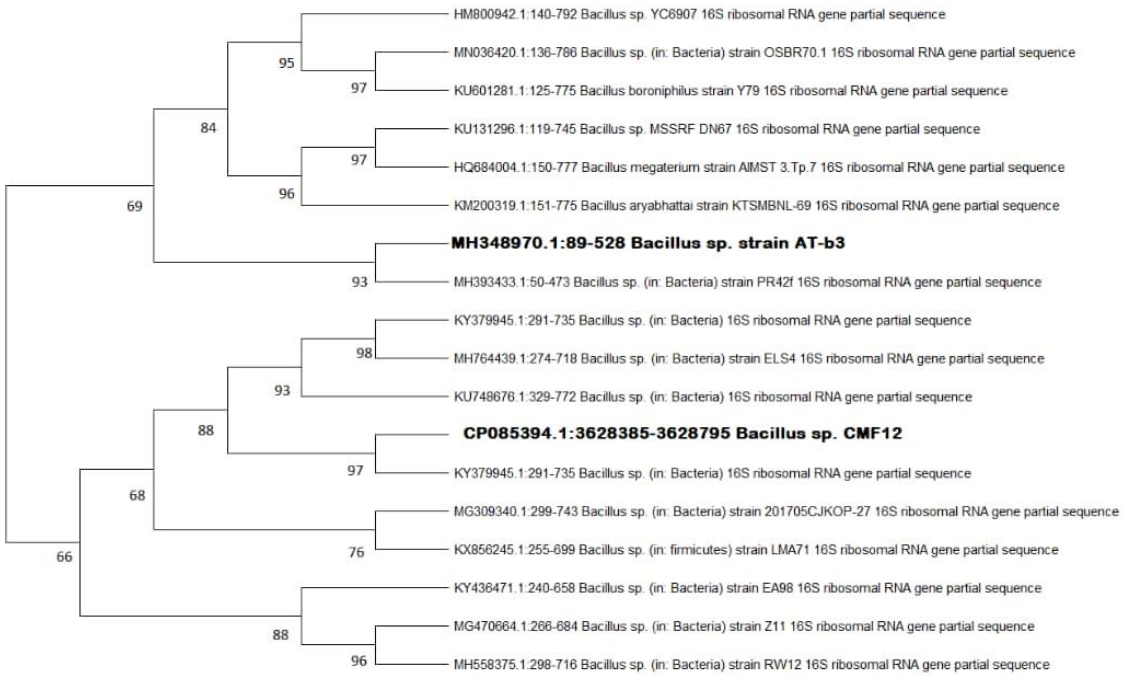


**Fig. 1. The Phylogenetic Tree for *Bacillus sp. AT-b3* and *Bacillus sp. CMF 12* based on 16S rRNA Sequence using Neighbor Joining Method**

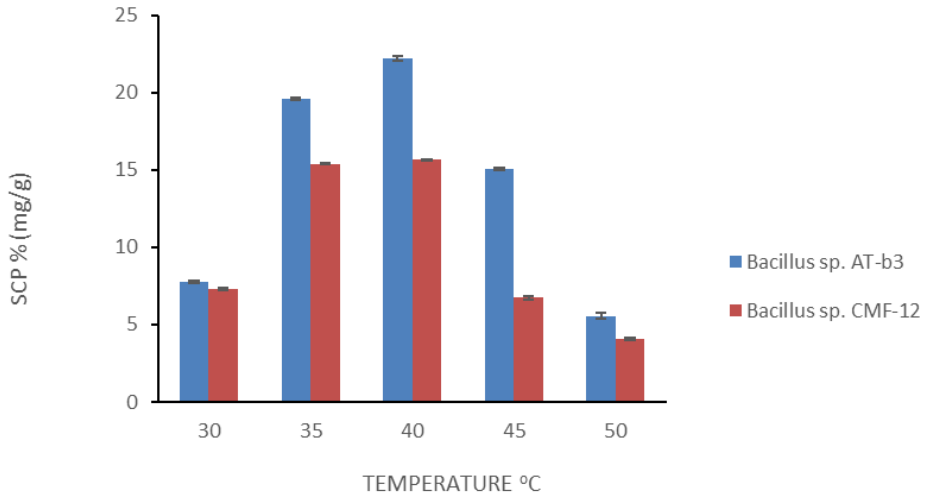


**Fig. 2. Effect of different temperature on SCP production using *Bacillus sp. AT-b3* and *Bacillus sp. CMF 12* obtained from rice husk dumping sites**

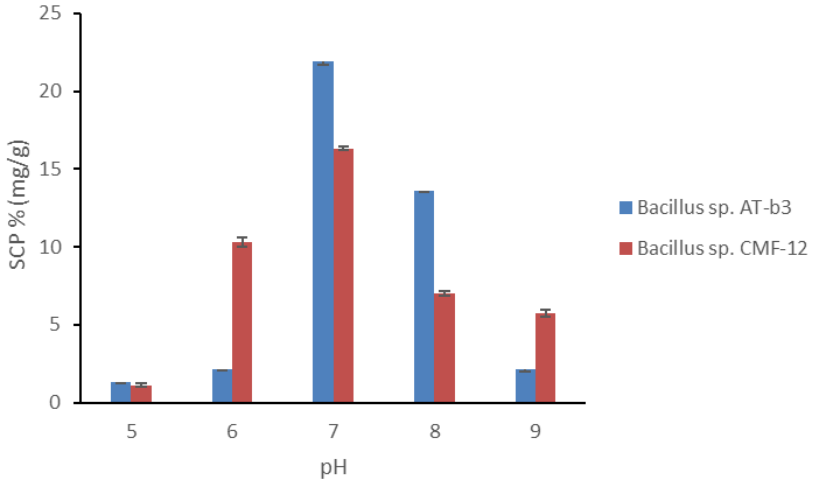


**Fig. 3. Effect of pH on Single Cell Protein Production using *Bacillus sp. AT-b3* and *Bacillus sp. CMF 12* obtained from rice husk dumping sites**

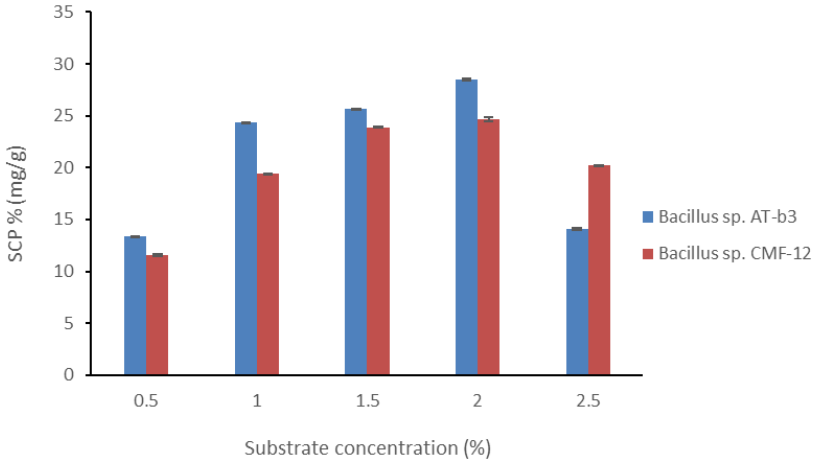


**Fig. 4. Effect of Carbon Source Concentration on SCP Production using *Bacillus sp. AT-b3* and *Bacillus sp. CMF 12* obtained from rice husk dumping sites**

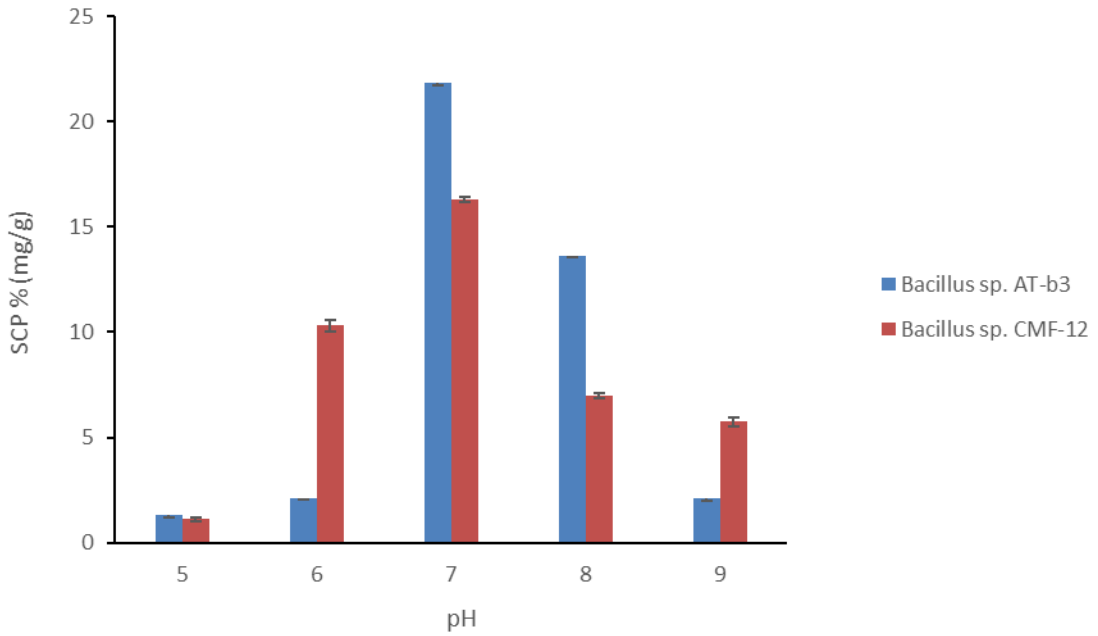


**Fig. 5. Effect of incubation time on SCP production using *Bacillus sp. AT-b3* and *Bacillus sp. CMF 12* obtained from rice husk dumping sites**

**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 superscript are not significantly different at p<0.05*

The optimal temperature for %SCP production for both *Bacillus sp. AT-b3* and *Bacillus sp. CMF 12* was observed at 40 °C. 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. These isolates show higher overall production with a wider range of high efficiency at this temperature.

Temperature is an important factor that affects the growth of microorganisms and was reported by (Gomashe et al., 2014), 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 indicated that there was 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 reached its peak at pH 7, although the peak is *lower compared to Bacillus sp. AT-b3.* Similarly to *Bacillus sp. AT-b3, Bacillus sp. CMF 12* exhibits a significant drop in %SCP production at pH levels lower or higher than the optimum pH (i.e 7), with the lowest production recorded at pH 5. *Bacillus sp. AT-b3* consistently shows higher %SCP production than *Bacillus sp. CMF 12* at pH 7 and 8. But 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%, with the highest peak 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 optimum substrate concentration for *Bacillus sp. AT-b3*. *Bacillus sp. AT-b3* was generally observed to 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 (Zhang and Cai, 2008), 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, the highest % SCP productions for the *Bacillus species* were observed after 48 hours. *Bacillus sp AT-b3* SCP production increases steadily, the peak was observed at 48 hrs with a yield of around 32%. After 48 hrs, there was a decline in SCP production, which continues to decrease through 72 and 96 hrs.

Therefore, for both isolates, the incubation period should be set to 48 hrs in other to maximize SCP yield before the onset of any decline in production. *Bacillus sp. AT-b3* was better in terms of SCP yield across the incubation periods tested, making it potentially 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. CMF 12* 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* was richer in Glutamic acid. The significant differences observed in the amino acid profiles of *Bacillus sp. AT-b3* and *Bacillus*

*sp.CMF 12* highlights the diversity within *Bacillus species* in terms of their nutritional content. These findings are consistent with (Singh et al., 2012; Kaur et al., 2016; Li and Chen, 2018), were they 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 possible exploitation for nutritional supplements.

## 5. CONCLUSION

The study isolated and characterized various *Bacillus strains*, particularly *Bacillus sp. AT-b*3 and *Bacillus sp. CMF 12*, which showed significant capabilities in SCP production. The optimal conditions for maximizing SCP yield were identified at a pH of 7, an optimum temperature of 40 °C, carbon source concentration of 2%, and an incubation period of 48 hours at 150 rpm. *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. This research reported for the potential of *Bacillus strains* and *Bacillus sp. AT-b*3 for single cell production using rice husk as substrate.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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