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BACTERIAL DECOMPOSITION OF SPIRULINA UNDER CONTROLLED INCUBATION PERIOD

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ABSTRACT

The molecular diversity of commercially available brands of "Spirulina" supplements and the occurrence of other cyanobacterial and heterotrophic bacterial microorganisms in these products. Since there are no reports of bacteria that causing decaying of spirulina shown elsewhere, a microbiological examination of spirulina degradation was performed. The isolated bacteria from the collected samples were screened for the production of enzymes in agar plate assay. Bacteria content comparing the natural as well as spirulina powder has been examined and found that both are predominated by proteobacterial group and found that many of the bacteria are absent in dry commercial powder.

Keywords: Spirulina, Enzyme activity, Proteobacteria, Lipolytic bacteria, Amylolytic bacteria, Cellulolytic bacteria.

1. INTRODUCTION

Multicellular, filamentous blue-green algae known as spirulina are becoming more and more popular in the health food sector and as a protein and vitamin addition to aquaculture diets. It is utilized as a protein supplement and a health food in many Asian countries, and it has grown significantly in popularity in the human health food sector. According to the International Association of Applied Microbiology, spirulina was designated as a "wonderful future food source" in 1967 (1). Spirulina's nutritional composition was examined, and the results revealed that it has an extraordinarily high protein content—between 60 and 70 percent of its dry weight—as well as excellent protein quality (a balanced essential amino acid content). The two genera Spirulina and Arthrospira, into which these microorganisms were initially divided in 1989, are still recognized today (2). The microalga being discussed is from the genus Arthrospira, although for a while it will probably be referred to as spirulina.

Depending on the source, spirulina has a very high protein content of between 55 and 70 percent by dry weight (3). As opposed to normal proteins like those from meat, eggs, or milk, it is a complete protein that contains all essential amino acids. Despite having less quantities of methionine, cystine, and lysine, it is still superior than all conventional plant proteins like those from beans. The polyunsaturated fatty acids (PUFAs) in spirulina account for 1.5–2.0% of the overall lipid content, which is 5–6%. Spirulina is particularly high in -linolenic acid (which makes up 36% of all PUFAs), and it also contains α-linolenic acid (ALA), linoleic acid (36% of all PUFAs), stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA). Vitamins B1 (thiamine), B2, riboflavin, B3, nicotinamide, B6, pyridoxine, B9 (folic acid), B12 (cyanocobalamin), C, D, and E is all present in spirulina. Along with calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, and zinc, spirulina is a rich source of potassium. Spirulina has a unique benefit in that its lack of cellulose in the cell walls makes it easily digestible. Commercial spirulina powder is a low-fat, low-calorie, cholesterol-free source of protein since it contains 60% protein, 20% carbohydrates, 5% lipids, 7% minerals, and 3-6% moisture.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 13.Iss 03, 2024

The nutraceutical properties of these microalgae have been attributed to their chemical composition, which is rich in proteins (60-70%) (4), polyunsaturated fatty acids (5), phenolic compounds (6) and phycocyanin (7).The microalgae biomass available for sale in pharmacies has been used primarily as a dietary supplement; however, to extend the use of these bio-compounds as nutraceuticals due to their antioxidant potential, knowledge about the care necessary for the maintenance of this potential is necessary. Chemical reactions, such as hydrolysis and oxidation, may occur due to environmental factors (temperature, humidity, light, atmospheric gases, pH and microbial contamination), potentially directly interfering with the stability of a compound (8). During the degradation, the increased temperature could provide the activation energy required to break the chemical bonds in the present study, the bacteria content of decomposed Spirulina powder as well as spirulina species were isolated with the aim of evaluating their hydrolytic capacities for potential applications in biotechnology. The isolated strains were examined for the production of five different hydrolytic enzymes such as protease, lipase, pectinase, cellulase and amylase. These results can contribute to determine the best storage conditions, especially by pharmacies that sell the microalgae as capsules.

There are reports on the molecular diversity of commercially available brands of “Spirulina” supplements and the occurrence of other cyanobacterial and heterotrophic bacterial microorganisms in these products (9). Since there are no reports of bacteria that causing decaying of spirulina shown elsewhere, a microbiological examination of spirulina degradation was performed here. The complicated and still debated taxonomy of Arthrospira and its relationship with Spirulina raises serious concerns regarding the “identity” of traditionally edible cyanobacteria and as a consequence, regarding their nutritional quality (e.g., unlike Spirulina, Arthrospira contains the unsaturated fatty acid γ-linolenic acid) and the safety (e.g., unlike Spirulina, Arthrospira is known to be potential toxin producer) of their products (10; 11; 12; 13).

2. MATERIALS AND METHODS

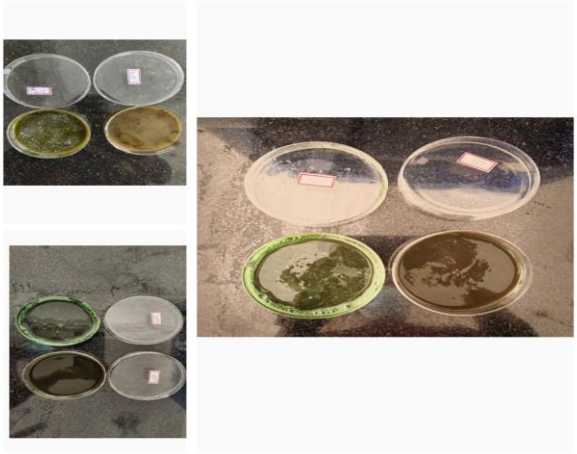
2.1. Sample

Raw Spirulina was collected from non-specific source in fresh water lake as blue green mat or clumps which are grown in cultivable large size. Both samples were separated into three incubation temperature of 18^0C, 27^0C and 37^0C, then they were subjected to analysis giving one week period interval, respectively.

Figure. 1. Spirulina (marketed by Ladumor pharma, Batch No: F-ZSP06)

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 13.Iss 03, 2024

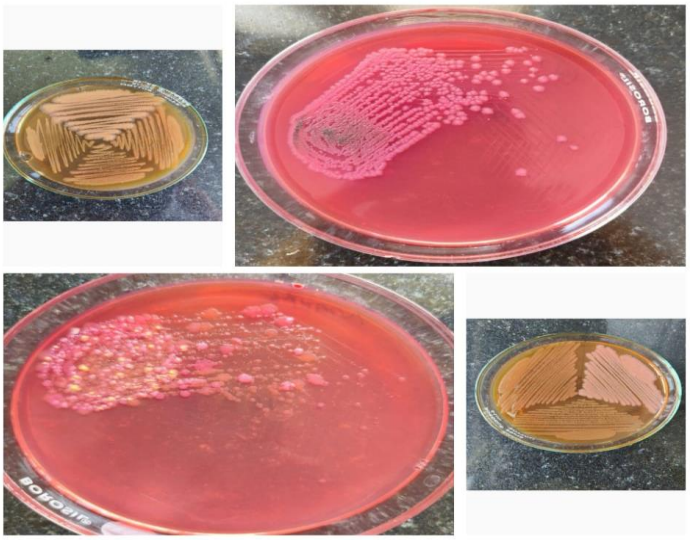
Figure. 2. Samples (spirulina and raw spirulina) were separated into three incubation temperature of 18^0C, 27^0C and 37^0C.



2.2. Bacteria Isolation and Identification

The collected spirulina and spirulina powder were sampled for microbiological examination and evaluate the results from 0^{th} day and continued until it degraded completely. Both samples were inoculated on Mac Conkey's and Blood agar and incubated at 37°C for 24 hrs. The isolates were identified with standard microbiological procedures. The VITEK 2 automated system (Biomerieux, France; with the GN and AST-N405 cards) was also used for identification of isolates. The decaying bacteria as well as its enzymatic properties were determined and as shown below.

Figure.3. Bacteria Isolation on Mac Conkey's agar



2.3. Enzymatic hydrolysis determination of bacteria

The isolated bacteria were screened for the production of protease, lipase, pectinase, cellulase and amylase enzymes in agar plate assay. Activated culture of each isolate was streaked on agar media containing suitable substrate specific for each of the enzyme activities. For example, gelatin, Tween-80, pectin, carboxymethylcellulose (CMC) and starch were used for the detection of proteolytic, lipolytic, pectinolytic, cellulolytic and amylolytic activities, respectively. After incubation at 30◦C for 48 h, the culture-media were treated with specific staining solutions as described below. Formation of zones of clear halo surrounding the colonies indicated presence of the respective enzymes. For the detection of proteolytic activity, the isolates were inoculated onto gelatin-agar media (10 g/L gelatin, 5 g/L tryptone,

1 g/L glucose, 2.5 g/L yeast extract, 20 g/L agar; pH 7) and incubated at 30◦C for 48 h followed by staining the media with mercuric chloride solution (150 g/L HgCl2 in 20% v/v HCl). Development of transparent circles around the colonies indicated a positive reaction (14). Similarly, for lipolytic activity, the isolates were inoculated on Tween 80-agar media (15 mL/L Tween 80, 5 g/L tryptone, 2.5 g/L yeast extract, 5 g/L NaCl, 20 g/L agar; pH 7) and incubated at 30◦C for 48 h. The appearance of clear halos after staining with methyl red solution (0.2 g/L methyl red in 95% ethanol) indicated the presence of lipolytic activity (15). For pectinolytic activity, isolates grown on pectin-agar media (5 g/L pectin, 5 g/L tryptone, 2.5 g/L yeast extract, 5 g/L NaCl, 15 g/L agar; pH 7) were flooded with potassium iodide solution (20 g/L potassium iodide and 10 g/L iodine) and examined for the appearance of clear zones to confirm pectinase production (16). For the determination of cellulolytic activity, the isolates were inoculated onto CMC-agar plates (10 g/L CMC, 2 g/L tryptone, 4 g/L KH2PO4, 4 g/L Na2HPO4, 0.2 g/L MgSO4.7H2O, 0.001 g/L CaCl2, 0.001 g/L FeSO4.7H2O, 20 g/L agar; pH 7). After incubation at 30◦C for 48 h, the plates were first stained with Congo red solution (2 g/L) for 10 min and then destained with 1 M NaCl for 15 min; halo zones surrounding the colonies indicated cellulase production (17). For amylolytic activity, bacteria grown on starch-agar media (10 g/L soluble starch, 5 g/L tryptone, 3 g/L yeast extract, 20 g/L agar; pH 7) were flooded with potassium iodide solution; transparent zones surrounding the colonies indicated amylase production (18).

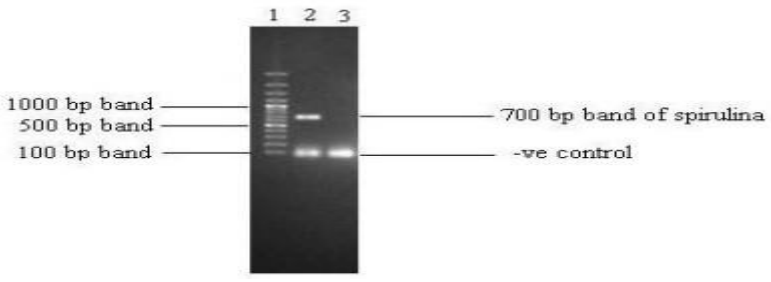
Measurement of Enzyme-Production

Amount of enzyme-production by the isolates was determined by agar diffusion method. The isolates were grown on media The Spirulina and spirulina powder were separated into three samples containing specific substrates for the respective enzymes as performed in the screening experiment described above and diameter of the zones of clearance and that of the colonies were measured. Amount of the enzyme produced was then calculated, and expressed as enzyme intensity (EI) where EI = (colony diameter + halo zone diameter)/colony diameter (19; 20). Each experiment was performed in triplicate and averaged.

Measurement of Nutrient Content

Nutrient content was performed using AOAC (Association of Official Analytical Chemists, Rockville, MD, USA) methods. The protein content of pasta was calculated by determining nitrogen using micro Kjeldahl method (34) with fully automatic digester and distillation unit (Velp Scientifica, Usmate, Italy) and multiplying by factor of 6.25 [35]. The crude lipids content was estimated by using chloroform: methanol extraction method [36]. Derivatization of total lipids as fatty acid methyl esters (FAME) was performed according to Ichihara and Fukubayashi [37] the fatty acids profile for each of the pasta samples was determined by Gas Chromatography (Shimadzu GC-2014, Serial no.—C121652, Kyoto, Japan) on Restek Stabilwax column (30 m, 0.25 mm ID) using flame ionization detector (FID, Shimadzu, Kyoto, Japan). The carbohydrates content was determined by Anthrone method [38]. Calorific value of pasta was calculated based on the composition, using the Atwater conversion factors of 4 kcal/100 g for protein and carbohydrates, and 9 kcal/100 g for lipids [39].

Figure. 4. Molecular characterization of Spirulina



ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 13.Iss 03, 2024

For molecular characterization PCR of 16s rDNA was done after isolating the DNA from Spirulina. DNA of Spirulina was extracted as followed by (41). PCR was done by using the cyclic conditions 94°C for 5 min, 94°C for 1 min, 60°C for 1 min, 72°C for 1 min and final extension at 72°C for 10 min (40). The following specific oligonucleotide primers were used for amplification of Spirulina 16s rDNA (CYA781-F):5´- CGGACGGGTGAGTAACGCGTGA-3´ (CYA781-R): 5´-GACTACTGGGGTATCTAATCCCATT-3´

3. RESULTS AND DISCUSSION

The observations of spirulina dry powder as well as spirulina natural source at different incubation period comparing with the onset of decaying as represented in day are shown in the Table 1, 2 and 3. The dominant species of bacteria were shown to have proteinase activity. It was followed by lipase activity. Amylase and Cellulase activity were rarely obtained. The details of enzyme activity were shown in Table 4.

Table 1. Bacterial decaying series pattern observed at 18°C

Days of decaying	Observed bacteria	
	Spirullina (Natural)	Spirulina powder (Commercial)
7 th Day	*Spingomonas paucimobilis* *Citrobacter farmer*	*Spingomonas paucimobilis*
14 th Day	*Ochrobactrum anthropi*	No growth
21 st Day	*E.coli*	No growth
28 th Day	*No growth*	No growth
35 th Day	*E.coli*	No growth
42 nd Day	*Aeromonas salmonicida* *Achromobacter xylosoxidans* *Enterococcus casseliflavus*	No growth

Table 2. Bacterial decaying series pattern observed at 25°C

Days of decaying	Observed bacteria	
	Spirullina (Natural)	Spirulina powder (Commercial)
7 th Day	*Acinetobacter baumanii complex* *Enterobacter clocae complex*	No growth
14 th Day	*Brevundimonas diminuta/vesicularis,* *Brevundimonas diminuta/vesicularis*	No growth
21 st Day	*Stenotrophomonas maltophila*	No growth
28 th Day	*Stenotrophomonas maltophila*	No growth
35 th Day	*Stenotrophomonas maltophila*	No growth
42 nd Day	*Achromobacter denitrificans*	*Stenotrophomonas maltophilia*

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 13.Iss 03, 2024

Table 3. Bacterial decaying series pattern observed at 37°C

Days of decaying	Observed bacteria	
	Spirullina (Natural)	Spirulina powder (Commercial)
7 th Day	*E.coli*	*Spingomonas paucimobilis*
14 th Day	*Aeromonas salmonicida, Escherichia hermannii*	*Spingomonas paucimobilis*
21 st Day	*Spingomonas paucimobilis*	*Spingomonas paucimobilis*
28 th Day	*Spingomonas paucimobilis*	*Spingomonas paucimobilis*
35 th Day	*Spingomonas paucimobilis*	*Spingomonas paucimobilis*
42 nd Day	*Spingomonas paucimobilis*	*Spingomonas paucimobilis*

Characteristics of Bacteria

Spingomonas paucimobilis is a polymorphic gram-negative rod and is strictly aerobic, weakly oxidase positive, and catalase positive and produce a yellow pigment. *Citrobacter farmeri* form small, circular, convex dark pink colonies on MacConkey agar. They are Gram-negative, nonsporing, straight rods; catalase-positive and oxidase-negative. *Acinetobacter baumanii* complex is a domed, mucoid, and nonpigmented. They may be presumptively identified as aerobic, gram-negative, catalase-positive, oxidase-negative, nonmotile, nonfermenting coccobacilli. *Enterobacter clocae complex* is a Gram-negative, facultatively-anaerobic, rod-shaped bacterium. They are greyish to white-colored large, circular, and convex colonies. *E.coli* is a gram negative, rod shaped, non-spore forming, motile with peritrichous flagella. Colonies are rough, flat, and irregular and circular.

Ochrobactrum anthropic is an aerobic, oxidase-positive, urease-positive, Gram-negative, motile, non-lactose-fermenting bacillus. *Brevundimonas diminuta/vesicularis* are non-lactose-fermenting environmental Gram-negative bacilli and produce yellow-pigmented colonies. *Aeromonas salmonicida* is a Gram-negative, facultatively anaerobic, nonmotile bacterium. It is rod-shaped. The bacterium readily ferments and oxidizes glucose, and is catalase- and cytochrome oxidase-positive. *Escherichia hermannii* is a Gram-negative bacillus, facultative anaerobe and produce yellow pigment. *Stenotrophomonas maltophilia* is a motile, aerobic, glucose non-fermenting, gram-negative bacterium. They are frequently pigmented colonies (yellow or yellowish orange) and a negative oxidase reaction. *Achromobacter denitrificans is a* Gram-negative, oxidase- and catalase-positive and motile bacterium

Achromobacter xylosoxidans is an aerobic, motile, oxidase and catalase positive, non-fermenting, gram negative bacillus. *Enterococcus casseliflavus is a* Gram-*positive spherical or ovoid cell arranged in pairs or chains motile, produce yellow colonies.*

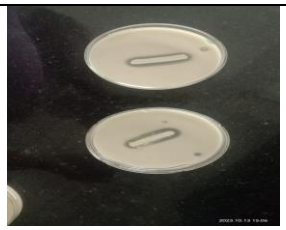
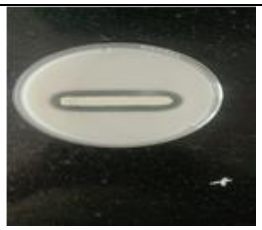
Table 4. Enzyme characteristics of bacterial isolates

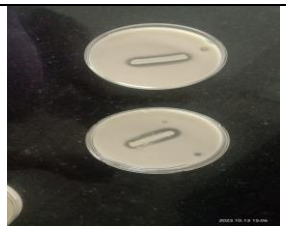
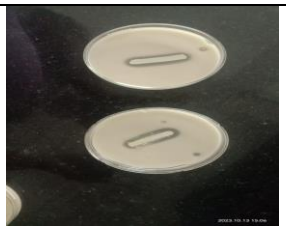
Sp.	*Protease*	*Lipase*	*Amylase*	*Cellulase*
Spingomonas paucimobilis	+	+	-	-
Citrobacter farmer	+	-	+	-
Acinetobacter baumanii complex	+	+	-	-
Enterobacter clocae complex	+	+	+	-

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 13.Iss 03, 2024

E.coli	-	-	+	+
Ochrobactrum anthropic	+	+	-	+
Brevundimonas diminuta/vesicularis	+	+	+	+
Aeromonas salmonicida	+	-	-	-
Escherichia hermannii	+	+	+	-
Stenotrophomonas maltophilia	+	+	+	-
Achromobacter denitrificans	+	-	-	-
Achromobacter xylosoxidans	+	-	-	-
Enterococcus casseliflavus	+	+	+	+

Figure.5. production of extracellular hydrolytic enzymes by isolated bacteria

Proteolytic activity of the isolated bacteria			
			
Spingomonas paucimobilis	*Citrobacter farmer and Acinetobacter baumanii* complex	*Enterobacter clocae complex*	*Ochrobactrum anthropic*

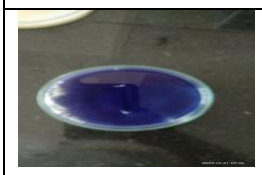
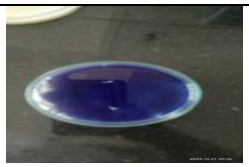
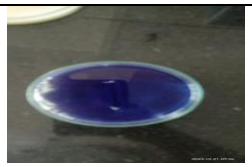
Proteolytic activity of the isolated bacteria			
			
Brevundimonas diminuta/vesicularis and Aeromonas	*Escherichia hermannii* and *Stenotrophomonas*	*Achromobacter denitrificans and Achromobacter*	*Enterococcus casseliflavus*

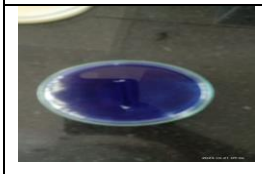
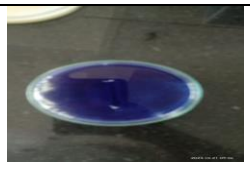
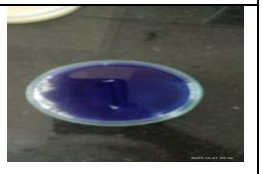
ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 13.Iss 03, 2024

salmonicida	*maltophilia*	*xylosoxidans*	

Lipase activity of the isolated bacteria			
			
Spingomonas paucimobilis	Acinetobacter baumanii complex	Enterobacter clocae complex	Ochrobactrum anthropic

Lipolytic activity of the isolated bacteria			
			
Brevundimonas diminuta/vesicularis	Escherichia hermannii	Stenotrophomonas maltophilia	*Enterococcus casseliflavus*

Amylolytic activity of the isolated bacteria			
			
Citrobacter farmer	Enterobacter clocae complex	E.coli	*Brevundimonas diminuta/vesicularis*

Amylolytic activity of the isolated bacteria		
		

ISSN PRINT 2319 1775 Online 2320 7876
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Escherichia hermannii	Stenotrophomonas maltophilia	*Enterococcus casseliflavus*

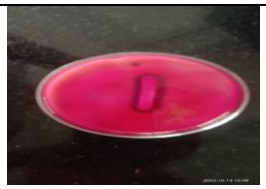
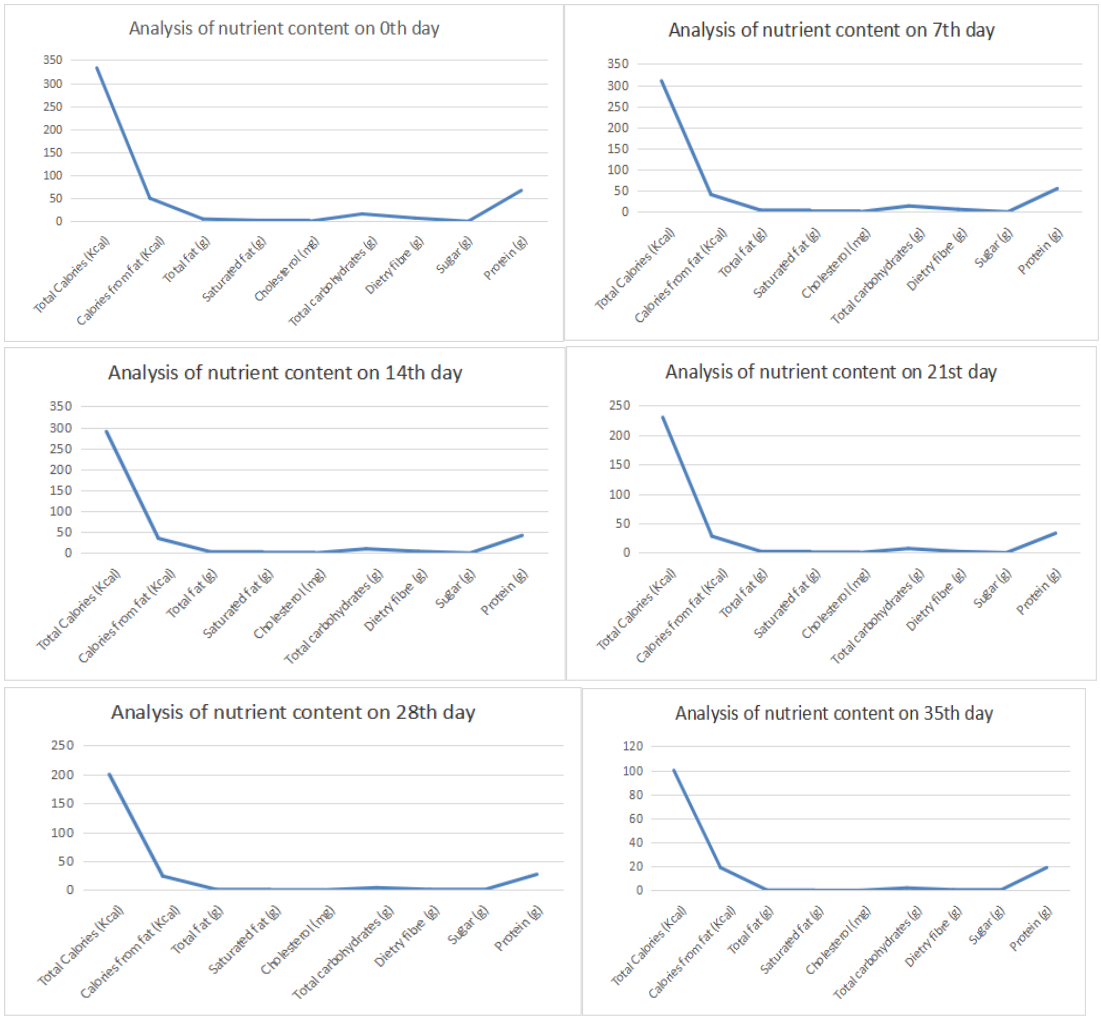
Cellulolytic activity of the isolated bacteria			
			
E.coli	Ochrobactrum anthropic	Brevundimonas diminuta/vesicularis	*Enterococcus casseliflavus*

Figure 6. Nutrient Content of Spirulina Powder on day progression at 37^0C



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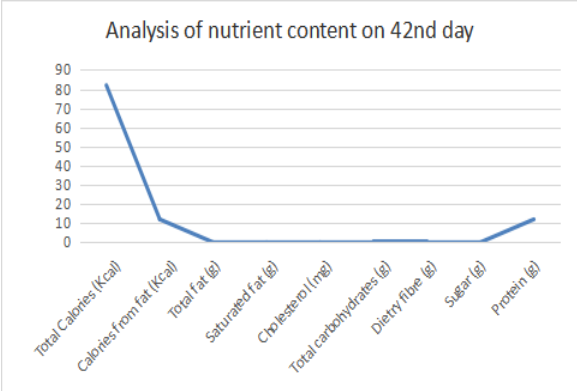
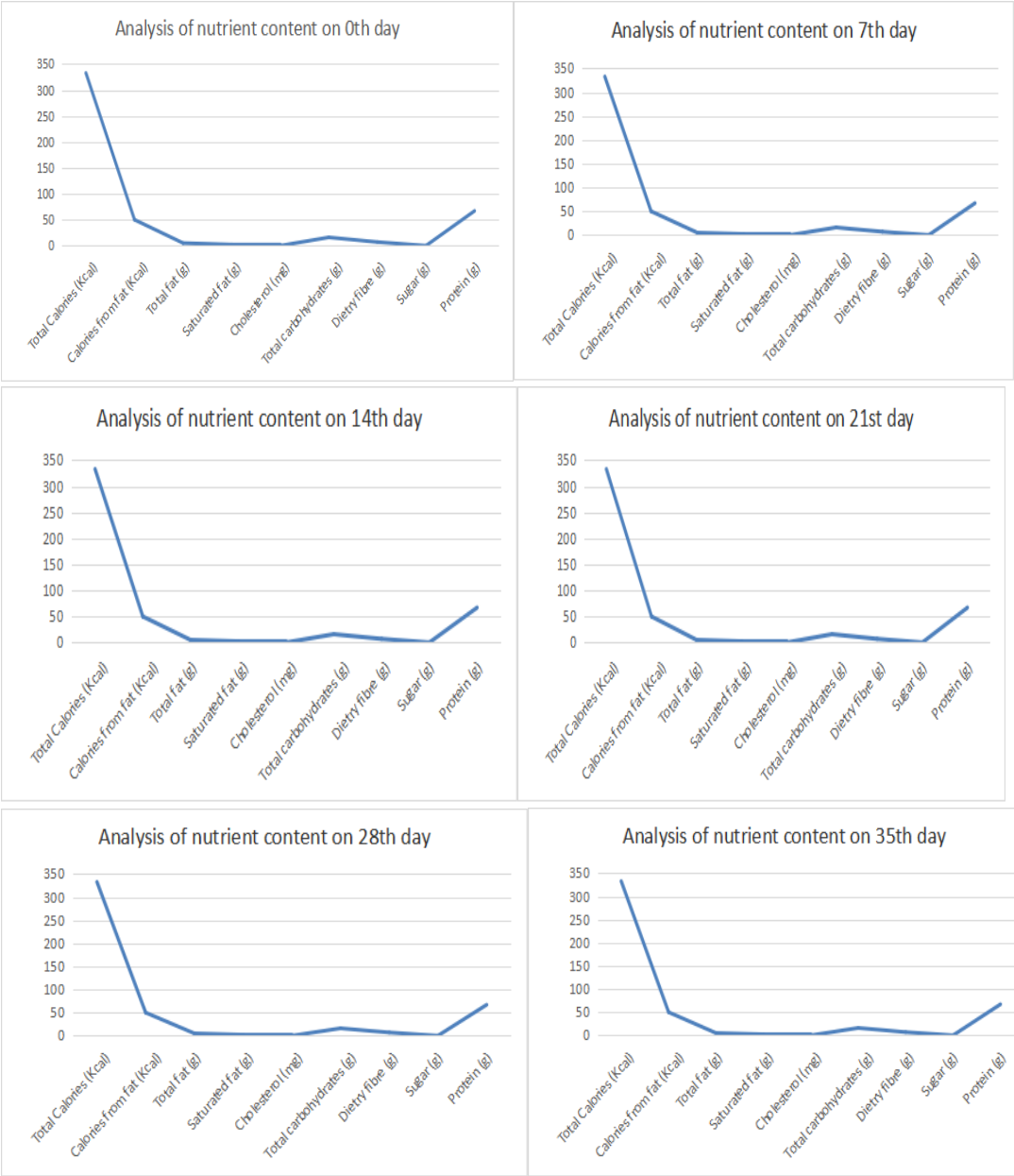


Fig 7. Nutrient Content of Spirulina Powder on day progression at 25^0C



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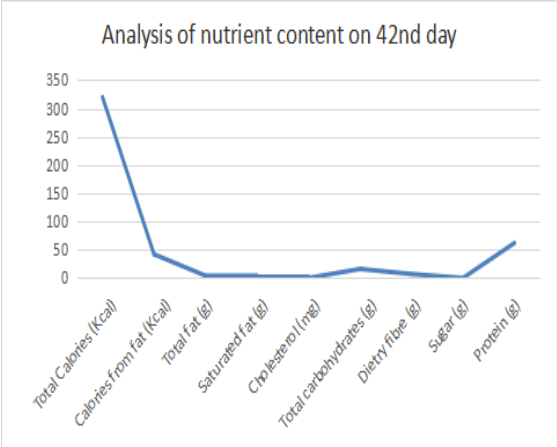
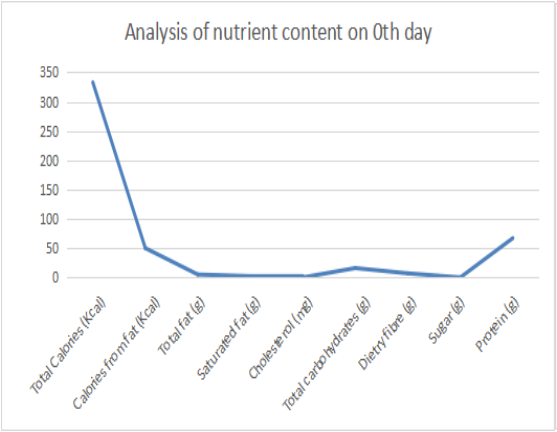
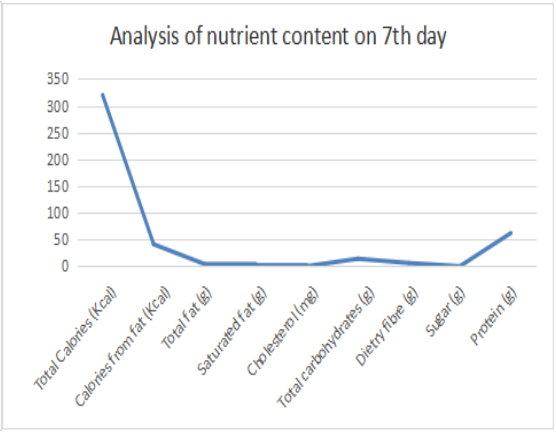
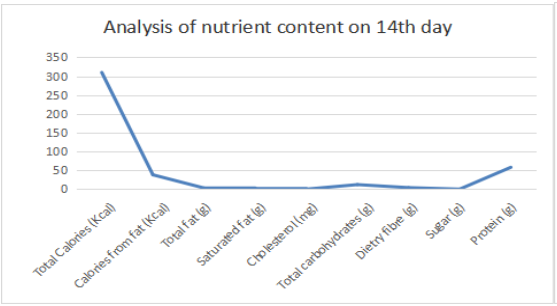
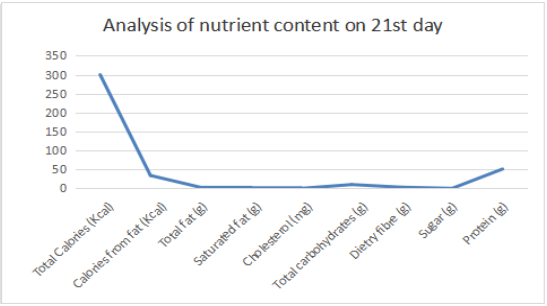


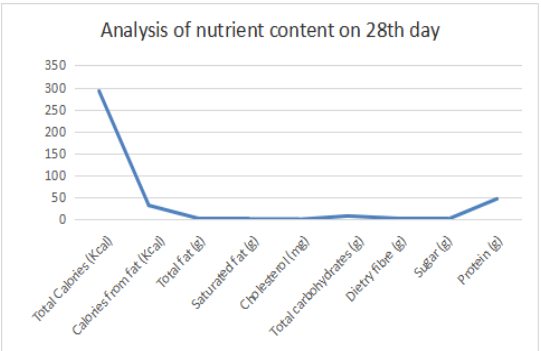
Fig 8. Nutrient Content of Spirulina Powder on day progression at 18^0C

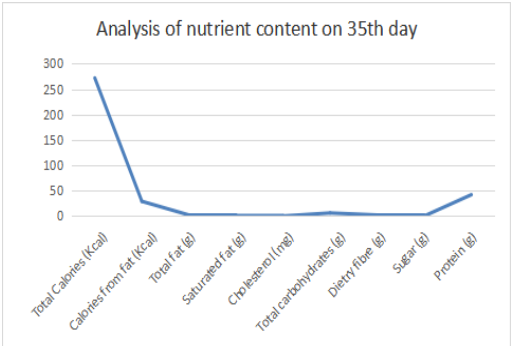




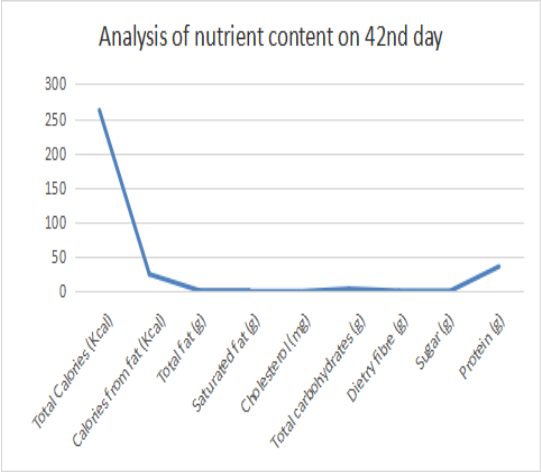








ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 13.Iss 03, 2024



During the incubation period at 18^0C

Spirulina contains unusually high amounts of protein, between 55 and 70 percent by dry weight, depending upon the source (21). It is a complete protein, containing all essential amino acids, though with reduced amounts of methionine, cystine, and lysine, as compared to standard proteins such as that from meat, eggs, or milk; it is, however, superior to all standard plant protein, such as that from legumes. Therefore, many of the bacteria obtained have proteinase activity.

From the Table 1. it has been showed that only *Spingomonas paucimobilis* has been isolated form the spirulina powder. No further bacterial growth was observed. Therefore, enzyme catalyse degradation was absent as there is no any presence of bacteria has determined in the sample. The degradation of protein and lipids was only predominant at 7 days incubation. While in spirulina natural source, it has been showed both *Spingomonas paucimobilis* and *Citrobacter farmeri* on the 7th day which has got proteinase, lipase and amylase activity. The essential lipids (unsaturated fatty acids) in spirulina are about 1.3–15 percent of total lipid (6–6.5 percent), mainly constituting γ-linolenic acid (30–35 percent of total lipid) (22; 23). Some researchers found that polyunsaturated fatty acids (PUFAs) could represent 25 to 60 percent of total fatty acids in spirulina. Therefore, on day 14 another bacterium *Ochrobactrum anthropic* was detected which had got in addition to proteinase and lipase the degradation of cellulase was there. Then *E.coli* was observed which shown that the degradation of amylase and cellulase has been determined in the sample on day 21. On day 28th no bacterial growth was shown which may be due to completion of degradation and decaying bacterial started with the onset of *E.coli* in the next week. *Aeromonas salmonicida, Achromobacter xylosoxidans, Enterococcus casseliflavus* was observed at 42nd day progression which are decaying bacteria has got proteinase activity in the first two and all enzyme activity in the last, respectively. The decaying phenomenon was only shown at this incubation temperature, since the presence of *E.coli* was observed before and after 28th day, respectively. The enzyme activity of the bacterial isolates was shown in Table 4. The evaluation of the chemical kinetics and the reaction orders can determine the knowledge of the degradation profile or concentration-time profile of a given substance (Jimenez-Kairuz, 2004). The kinetics of thermal degradation was determined using the Arrhenius method, even considering the Spirulina powder a complex material. Others authors used this model in pharmaceutical formulations (considered complex) (25; 26; 27). As for thermal stability, the kinetics degradations in all temperatures were of the zero order, for which the degradation rate is independent of the concentration of the reactants; i.e., the reaction rate of degradation is constant. From Fig 7. It has been showed that the nutrient is loosed as the day progression. The bacterial action is acting slowly on the nutrient content in this incubation period.

During the incubation period at 25^0C

Spirulina has high quality protein content (59–65 percent), which is more than other commonly used plant sources such as dry soybeans (35 percent), peanuts (25 percent) or grains (8–10 percent). A special value of spirulina is that it is readily digested due to the absence of cellulose in its cell walls (as

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 13.Iss 03, 2024

it is the case for eukaryotic green microalgae such as Chlorella, Ankistrodesmus, Selenastrum, Scenedesmus): after 18 hours more than 85 percent of its protein is digested and assimilated (28). It has been found the storage of spirulina powder was effective at this temperature, since there is no growth of bacteria was obtained until 35th day. *Stenotrophomonas maltophilia* was obtained after that which has got proteinase, lipase and amylase activity. The thermal stability evaluation showed that the degradation of S. platensis powder was higher at 50°C, and after 63 days. At 25°C, the S. platensis powder demonstrated the highest stability, followed by 40ºC (*29*). *Acinetobacter baumanii complex* and *Enterobacter clocae complex* was observed in the natural spirulina in the first week sampling. There is proteinase as well as lipase activity for *Acinetobacter baumanii complex*, while the other bacteria *Enterobacter clocae complex* has got proteinase, lipase and amylase activity. *Brevundimonas diminuta/vesicularis* was shown predominantly in the next week analysis which shown wide range of enzyme activity. The activity include proteinase, lipase, amylase and cellulase, digestion phenomenon. The essential lipids (unsaturated fatty acids) in spirulina are about 1.3–15 percent of total lipid (6–6.5 percent), mainly constituting γ-linolenic acid (30–35 percent of total lipid) (30; 31). Some researchers found that polyunsaturated fatty acids (PUFAs) could represent 25 to 60 percent of total fatty acids in spirulina. *Stenotrophomonas maltophilia* was observed in the next week onwards until 35th day observation, which has got proteinase, lipase and amylase activity. It may be due to more nitrogenous compound at this incubation period, there was production of *Achromobacter denitrificans* on the 42nd day. These bacteria have got degradation of high nitrogen containing proteins.Storage shelf life of spirulina powder was found to be effective at this incubation period. From Fig 6. It has been showed that nutrient loss is shown at the 42nd day since the bacterial presence was observed at this incubation period only. This result suggest that nutrient component of spirulina powder can be effectively preserved at this incubation period.

During the incubation period at 37^{0}C

On a dry-weight basis, spirulina is 60–77% protein, 9–15% lipids, and 10–19% carbohydrates, with variation depending on spirulina species and growing conditions (e.g., pond versus lab-grown). The protein content is of relatively high quality for a plant-based protein, having a biological value of 75% and a digestibility of 83%. From the Table 1. it has been showed that only *Spingomonas paucimobilis* has been isolated form the spirulina powder. No other bacterial growth was observed throughout the day progresses. Therefore, enzyme catalyse degradation was proteinase which has been suspected periodically on day progression. At this incubation time only proteinase activity was reported. Almost same observation was there in spirulina natural sample also, since there is only one bacterium was observed from 21st day onwards. Studies indicate that in addition to its 50% to 70% protein content in dry matter, it has essential amino acids, essential lipids, unsaturated fatty acids, important vitamins and minerals of high nutritional value. These has been utilized in the day prior to the observation made on 21st day. *Escherichia hermannii* and *E.coli* which has shown lipase, amylase and cellulase activity in addition to proteinase activity. This activity was observed in the starting day examination for spirulina natural source with presence of these bacteria. There was also shown the presence of *Aeromonas salmonicida* which has got high proteinase activity. Since Arthrospira has an optimum growth temperature in the range of 35–38°C, large-scale cultivation is mainly located in tropical, sub-tropical and warm temperate climate zones. (32). There is risk in contamination of the spirulina by fungi, bacteria and protozoa are very common in this condition also. (33). From fig 5. It has been shown that nutrient loss is predominantly higher in this incubation period. Since there is presence of bacteria was observed at 7th day onwards. The storage of spirulina powder is not recommended at this incubation temperature.

In the present study confirmation of Spirulina was done by polymerase chain reaction using primer pair CYA106 F and CYA781 R which was specific for the amplification of 16S rDNA gene segment from the genus Spirulina. Similar study was conducted by Nubel et al. (40); Boutte et al. (42) their results were also similar to the present work, as they used same set of primers and similar amplification conditions.

4. CONCLUSION

Bacteria content comparing the natural as well as spirulina powder has been examined and found that both are predominated by proteobacter group and found that many of the bacteria are absent in dry commercial powder. But the progress of bacteria is different at different incubation period. The dominant species of bacteria were shown to have proteinase activity. It was followed by lipase activity. Amylase and Cellulase activity were rarely obtained. *Spingomonas paucimobilis* was the predominat bacteria obtained which show that spirulina enzyme activity is due to the presence of its high protein substrate.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY

Data will be available on request

Statement of Previous Publication

A preprint has previously been published Jithu et al., https://www.researchsquare.com/article/rs-3890178/v1

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