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Delipidation of Chicken Feathers by Lipolytic *Bacillus* Species Isolated from River-Borne Sediments

Tariro Shiri, Nonso E. Nnolim * and Uchechukwu U. Nwodo

Patho-Biocatalysis Group, Department of Biochemistry and Microbiology, University of Fort Hare, Alice 5700, South Africa; 201926984@ufh.ac.za (T.S.); unwodo@ufh.ac.za (U.U.N.)
* Correspondence: nnnolim@ufh.ac.za; Tel.: +27-406022683

Abstract: Though the keratin content of chicken feathers is being explored for many potential uses, the crude lipid content of the biomass significantly hinders the valorization processes. Therefore, this study explored the potential of bacteria isolated from sediment for lipolytic properties. Sediment-associated strains were evaluated for lipolytic activity on tween 80–peptone agar. The best lipolytic bacterium was used to break down the lipid content of chicken feathers. The results showed that out of six bacterial strains with variable lipolytic activity, strain TTs1 showed the largest zone of precipitate around the colony, which is why it was selected and identified as *Bacillus* sp. TTs1. The maximum lipase production of 1530.5 U/mL by strain TTs1 was achieved at 96 h post-fermentation, with optimal process conditions of initial pH (10), incubation temperature (45 °C), agitation speed (140 rpm), inoculum size (2% v/v) and tween 80 (10% v/v). The total free fatty acid (0.58%) was liberated from chicken feathers at a concentration of 6% (w/v). Crude fat extraction from both untreated and TTs1-pretreated chicken feathers showed fat contents of 2.1 ± 0.42% and 0.92 ± 0.13%, respectively. The findings of this study highlight the biotechnological relevance of strain TTs1 in lipase production and the sustainable delipidation of lipid-rich bioresources.

Keywords: chicken feather; fatty acid; delipidation; biomass valorization; *Bacillus* sp.

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1. Introduction

Livestock production remains on a growth trajectory, providing the needed resources for the increasing global population. A significant proportion of the generated keratin-rich wastes accumulate in the ecosystem due to their durability and the lack of proper recycling technology used by farmers [1]. Keratinous biomass typically exists as α-keratin or β-keratin, depending on the structural architecture and molecular configuration [2]. Keratinous wastes are resistant to decomposition by environmental factors due to the existence of a high network of disulfide linkages, hydrogen, and hydrophobic interactions in the biopolymer [3]. Nonetheless, the biomass has been processed as feed and has also been utilized as feedstock in the biodigesters for green energy production [4,5]. The hydrolysates derived from keratinous biomass degradation are gaining traction for use in cash crop cultivation because of their sufficient nitrogen content and other crucial elements that support plant growth and development [6]. The hydrolysates' slow nitrogen releasing tendency makes them good candidates for use in soil fertility augmentation while promoting soil ecosystem and sustainable agroecology [7]. The European Union (EU) Parliament's regulation on the use of keratinous biomass as organic fertilizers and soil improvers states that such products should not pose public or animal health risks [8].

Avian feathers remain the topmost keratinous waste emanating from livestock production [4,9]. The pecking order of poultry production shows that the US, Brazil, China, and the EU are the lead producers globally. South Africa produces several million tons of chicken due to the imposition of anti-dumping duties on the poultry sector. This high-capacity poultry processing results in the generation of high amounts of keratinous residues

as by-products [10]. The EU regulations require that poultry farmers dispose of poultry carcasses without undue delay via incineration at a Department of Agriculture- or Environmental Protection Agency-approved facility [11]. Again, the regulations concerning good practice guidelines stipulate that poultry litter which does not contain dead birds or pose any health risks should be transported to farms, compost sites or biogas plants where it can be adequately exploited. The regulations also add that wastewater from poultry facilities should not be discharged on livestock grazing land or land adjacent to water courses as such practice could lead to serious health issues [12].

In 2021, a total of 1089 million broilers were processed for chicken in the Republic of South Africa, and this generated a huge amount of feather waste of about 98,000 tons, supposing a mature broiler weighed an average of 1.8 kg [13]. A small portion of widely generated feather waste is transformed into important products such as feed supplements and organic fertilizers [14]. The poultry trading projection predicts growth in developing countries because of the rising human population, which would result in higher keratinous waste generation [15]. In developing countries, the majority of the feather waste generated is landfilled or incinerated in an improper facility, creating variable forms of environmental pollution. The adverse impact of waste on the environment has spurred the National Waste Management Strategy (NWMS) of South Africa to encourage the diversion of 25% of recyclables from municipal landfills for re-use, recycling, and recovery [16]. However, the municipalities have struggled with the implementation of these measures owing to the lack of capacity or infrastructure. Keratin from different sources has been utilized to produce dietary protein, bio-adhesives, and nanocomposite materials, among other useful products [17]. Its extraction from cheap and readily available feather waste represents a sustainable development from the economic and ecological perspectives. A few selected patents related to innovative development from keratinous biomass are presented in Table S1.

Although the keratin content of chicken feathers is significantly high (about 85–90%), the biomass is equally reported to contain about 11% crude lipid [18]. The feather lipids provide a protective and waterproof layer on the feathers' surface and a physical barrier against microbial attack [18]. Consequently, the lipid moiety interferes with the bio-valorization of keratinous feathers by limiting the catalytic activity of keratinases [19]. Therefore, the crude lipids in chicken feathers are dealt with before the transformation process during the value-addition stage [20]. The conventional methods, such as ethanol soaking and high temperature drying, have been fundamentally used to remove excess lipids from keratinous biomass [19]. The relatively lipid-deficient keratin can then be valorized into value-added products through alkaline hydrolysis or by using oxidizing and reducing agents [21]. Considering the above methods' environmental concern and energy cost, the bio-treatment approach signifies sustainable development.

In view of the foregoing, up-regulation of the lipolytic esterase-encoding gene was reported during the growth of *Streptomyces* sp. SCUT-3 on chicken feather medium, suggesting the bacterium's involvement in lipid digestion [22]. Adelina and colleagues reported a decrease in the lipid content from 2.44% to 1.42% after chicken feather biotreatment to improve the nutritional quality for potential use in fish feeds [23]. Sediments comprise a large community of bacterial species with diverse metabolic activity crucial for the biogeochemical cycle within lotic ecosystems [24]. Therefore, this study assessed the lipolytic potential of some bacteria isolated from sediment samples from the Tyume River, South Africa. The most potent isolate was employed as a bio-treatment candidate for the digestion of chicken feathers' lipids in a submerged-state fermentation. Lipolytic enzyme production by the selected isolate was enhanced through optimization studies, and the crude lipid extraction from the chicken feathers was likewise evaluated.

2. Materials and Methods

2.1. Collection and Processing of Samples

The Tyume River sediments were collected in sterile sampling bags and placed in a cooler box with ice packs. The samples were transported to the Patho-Biocatalysis

Laboratory at the University of Fort Hare, South Africa for analysis. The Tyume River is in the Eastern Cape Province, South Africa, at these coordinates: 32°46′55″ S 26° 50′47″ E. The samples were further processed under aseptic conditions by air-drying for seven days in a lamina flow cabinet, properly crushing using a sterilized ceramic mortar and pestle, and being kept in sterile bottles at room temperature.

2.2. Bacterial Isolation

Starch casein agar with the following composition, starch, 10.0 g/L; casein, 1.0 g/L; seawater, 37.0 g/L; and agar, 15.0 g/L (HiMedia Laboratories Pvt. Ltd., Mumbai, India), was used as the growth medium for the bacterial isolation. Gram-negative bacteria and fungi were prevented from growing on the plates by the respective addition of nalidixic acid (50 mg/L) and nystatin (25 mg/L) (Merck, Darmstadt, Germany) to the isolation medium [25]. In 100 mL of sterile normal saline, 5 g of crushed samples was homogenized and serially diluted to about 10^{-5}. An aliquot (0.1 mL) was withdrawn from each dilution and evenly distributed onto the isolation agar plates [26]. The plates were inspected for bacterial growth after 7 days of incubation at 30 °C. Colonies with variable morphological characteristics were selected and purified by streaking them onto newly prepared media. The axenic bacterial cultures were stored on slants at 4 °C.

2.3. Evaluation of Potential Lipase Activity

Tween 80–peptone agar was used for the study of the lipolytic capacity of the strains, as previously reported [26,27]. The following composition was used to formulate the screening medium in g/L: peptone, 10.0 g (Merck, Modderfontein, South Africa); NaCl, 5.0 g; $MgSO_4 \cdot 7H_2O$, 2.0 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; tween 80, 10% (*v*/*v*) (Sigma-Aldrich, St. Louis, MO, USA); and bacteriological agar, 15.0 g (Merck (Pty) Ltd., Modderfontein, South Africa). Separately, tween 80 and basal salt medium were autoclaved at 121 °C and 15 psi for 15 min. The tween 80 was aseptically mixed with the basal media after their temperature had dropped to about 45 °C. The tween 80-peptone media was aseptically dispensed into Petri dishes and allowed to set. After that, the plates were inoculated with 10 µL of the axenic cultures and incubated for 48 h at 30 °C. The visible precipitate surrounding a colony caused by the deposition of crystals of the calcium salt generated by the fatty acid released indicated that the isolate possessed lipase activity [27]. Each plate was examined for precipitate formation, and the diameter of the zone of precipitation was recorded in millimeters (mm).

2.4. Enzyme Production and Assay for Lipase Activity

Enzyme production was carried out in Erlenmeyer flasks containing medium with a composition similar to that in Section 2.3., without the addition of bacteriological agar. The pH of the medium was adjusted to 7, and the flasks were autoclaved for 15 min at 121 °C. The flasks were placed in an incubator and incubated for 72 h at 30 °C and 120 rpm. After the incubation, the fermented medium was pipetted into microtubes and centrifuged at 15,000× *g* for 10 min and at 4 °C. The supernatant was transferred into a new tube, which served as the crude enzyme for the lipase activity assay.

The lipase activity was determined using titration, and the principle was based on olive oil hydrolysis [28]. In 20 mL test tubes, the assay was carried out with the combination of 3 mL olive oil, 2.5 mL deionized water, 1 mL of 200 mM Tris-HCl buffer (pH 7.5), and 1 mL crude lipase solution. The solution was properly vortexed until fully mixed prior to incubation at 37 °C for 30 min. The control experiment had a similar composition, without the active enzyme. Following the incubation period, 3 mL of 95% ethanol was added to the test and control samples. Four drops of thymolphthalein indicator (Merck (Pty) Ltd., Modderfontein, South Africa) were added to the test and control solutions in 250 mL Erlenmeyer flasks. These mixtures were titrated against 50 mM NaOH until a pale blue coloration appeared. One unit (U) of lipase is the amount of enzyme liberating one micromole of fatty acid per minute under the assay conditions described [29].

2.5. Lipolytic Bacteria Identification

The most efficient lipolytic bacterium, strain TTs1, was extracted for its genomic DNA using a Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Cat. No. D6005; Irvine, CA, USA) according to the manufacturer's guidelines. The 16S rRNA gene target region was amplified with OneTaq® 2X Master Mix (Promega, Madison, WI, USA) under standard thermal cycling conditions. The forward and reverse primers used for the polymerase chain reaction (PCR) were the universal oligonucleotide sequences 16S-27F 5′-AGAGTTTGATCMTGGCTCAG-3′ and 16S-1492R 5′-CGGTTACCTTGTTACGACTT-3′, respectively [30]. The amplicons were separated on an ethidium bromide-stained agarose gel (1% w/v), and the bands were extracted using a Zymoclean™ Gel DNA Recovery Kit (Zymo Research, Cat. No. D4001; Irvine, CA, USA). A ZR-96 DNA Sequencing Clean-Up Kit™ (Cat. No. D4050; Irvine, CA, USA) was used to purify the fragments following the manufacturer's instructions. After purification, an ABI 3500xL Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific, Johannesburg, South Africa) was used to analyze the fragments. The ab1 files generated by the ABI 3500xL analyzer were further analyzed using the CLC Bio Main Workbench v7.6. The assembled 16S rRNA gene sequence was BLASTed against the National Centre for Biotechnology Information (NCBI) database to identify the closely related taxa for strain TTs1 [31]. The closely related taxa revealed by the basic local alignment search tool hits were extracted from the NCBI and used to conduct the phylogenetic analysis. The neighbor-joining approach was used to infer the evolutionary relationship, and the analysis was carried out in MEGA-X [32]. The isolate was identified as *Bacillus* sp. TTs1, and the 16S rRNA gene sequence for strain TTs1 was deposited in GenBank with accession number MW556206.

2.6. Fermentation Condition Optimization to Enhance Lipase Production

Using a one-variable-at-a-time strategy, the influence of physicochemical variables on lipase production was studied. The initial medium pH (4–11), incubation temperature (25–50 °C), and agitation speed (100–180) were studied at intervals of 1 unit, 5 °C, and 20 rpm, respectively. Similarly, the influence of the inoculum size (2–10%) was determined at 1% unit intervals. The starter culture was standardized based on the 0.5 McFarland turbidity standard, which is comparable to the density of a bacterial suspension with 1.5×10^8 CFU/mL [33]. The inducers' effect (tween 20, avocado oil, tween 80, olive oil, sunflower oil, castor oil) on the extracellular lipase secretion by the isolate was determined at 10% (v/v). In each step, the incubation was performed for 48 h, and the crude enzyme was extracted via centrifugation. The lipase activity assay was performed using the previously described conditions.

The time course study was performed in 250 mL Erlenmeyer flasks containing the basal salt medium and at optimized fermentation conditions of pH 10, 45 °C, 140 rpm, 2% inoculum and 10% tween 80. At 24 h intervals, aliquots of the fermentation medium were removed and centrifuged at 15,000× g for 10 min and at 4 °C; thereafter, the cell-free filtrates served as crude enzymes and were used to determine the lipase activity under the previously specified conditions.

2.7. Influence of Chicken Feather Concentration on the Medium Free Fatty Acid

At different concentrations (2, 4, 6, and 8% (w/v)) of chicken feathers, the fermentation medium was formulated. The chicken feathers were collected from a local poultry farm. The flasks were autoclaved at 121 °C for 15 min and 15 psi. For the test samples, 2% (v/v) bacterial suspension was used to inoculate the flasks. The negative control flasks were prepared with a similar composition, except for the addition of the inoculum. After incubation at 45 °C for 96 h and 140 rpm, the fermented medium was filtered using Whatman™ filter paper 1 (Cat No 1001-150; GE Healthcare Ltd., Buckinghamshire, UK) with a pore size of 11 µ to separate the feather biomass from the liquid part. The filtrate was assessed for the presence of free fatty acid by titrating against 0.1 N NaOH, and phenolphthalein (Merck (Pty) Ltd.;

Modderfontein, South Africa) served as an indicator [34]. The fatty acid concentration (%) was computed using the formula stated below.

$$\text{Free fatty acid } (\%) = \frac{[(Y - Z) \times N_{\text{NaOH}} \times 28.2]}{W} \quad (1)$$

where Y represents the titrant volume, Z represents the blank volume, N is used to denote the normality factor (0.1), and W indicates the weight of the sample (g).

The above calculation assumes that oleic acid is the major component of the fatty acids released from the keratinous feathers [35].

2.8. Quantitation of the Crude Fat Content of Untreated and Biotreated Chicken Feathers

The crude fat content of the untreated and biotreated chicken feathers was determined using the Soxhlet extraction method [36]. In the case of the chicken feather (8 g) pretreatment with the lipolytic bacteria, fermentation was carried out for 4 days under optimal conditions. Thereafter, the chicken feather fermentation medium was filtered through Whatman™ filter paper (150 mm, Cat. No 1001-150; GE Healthcare Ltd., Buckinghamshire, UK), thoroughly washed with tap water, and dried to a constant weight at 60 °C. The control experiment (untreated) passed through the same treatment, except for inoculating the flasks with strain TTs1. The extraction of crude fat from the untreated and TTs1-pretreated chicken feathers (8 g) was carried out for 6 h at 45 °C. Petroleum ether (250 mL) (Merck (Pty) Ltd., Modderfontein, South Africa) was used per cycle, and the experiment was carried out in triplicate. A rotary evaporator (Apex Scientific, Bloemfontein, South Africa) was used to eliminate the extraction solvent from the samples at the end of the experiment. Subsequently, the extract was placed in an oven at 100 °C for 1 h to ensure complete vaporization of the solvent, and then it was stored in a desiccator before being weighed [37]. The crude fat concentration (%) of the chicken feathers was determined using the USDA Food Safety and Inspection Service's formula, which is given below [38].

$$\text{Crude fat} = \frac{(Y - Z)}{X} \times 100\% \quad (2)$$

where X represents the sample weight, Y represents weight of the flask and extract, and Z represents weight of the flask before extraction

2.9. Statistical Analysis

Unless otherwise stated, triplicate experiments were carried out in each case, and the data accrued were analyzed using an analysis of variance (ANOVA) in the Statistical Package for Social Sciences (SPSS) version 23. The significant differences between the means were compared at $p < 0.05$ using Duncan's multiple range test.

3. Results

Chicken feather waste recycling has been a challenge to poultry farmers due to the intractable nature of the biomass; hence, its valorization strategy remains a research hotspot. Sediments harbor a unique microbial community with genetic diversity that could be explored for various biotechnological processes. Avian feathers contain crude lipids that may hinder their bioconversion into useful products. Consequently, this study investigated the lipolytic potential of sediment-associated bacterial species. The isolate that showed promising lipase-producing capacity was employed for the digestion of the crude lipids in chicken feathers.

3.1. Lipolytic Bacterial Isolation and Identification

The sediment-associated bacterial strains were evaluated for lipase production. Out of twenty strains coded as ACT001–ACT020, six showed variable degrees of calcium salt precipitation around the colonies (Supplementary Table S1), with halo zones between 9 mm and 30 mm, which was an indication of lipase activity by the bacterial

strains. Among the six strains, strain TTs1, with the largest zone of crystalized calcium salt precipitate around the colony on tween 80–peptone agar (Figure 1A), was selected for further study.

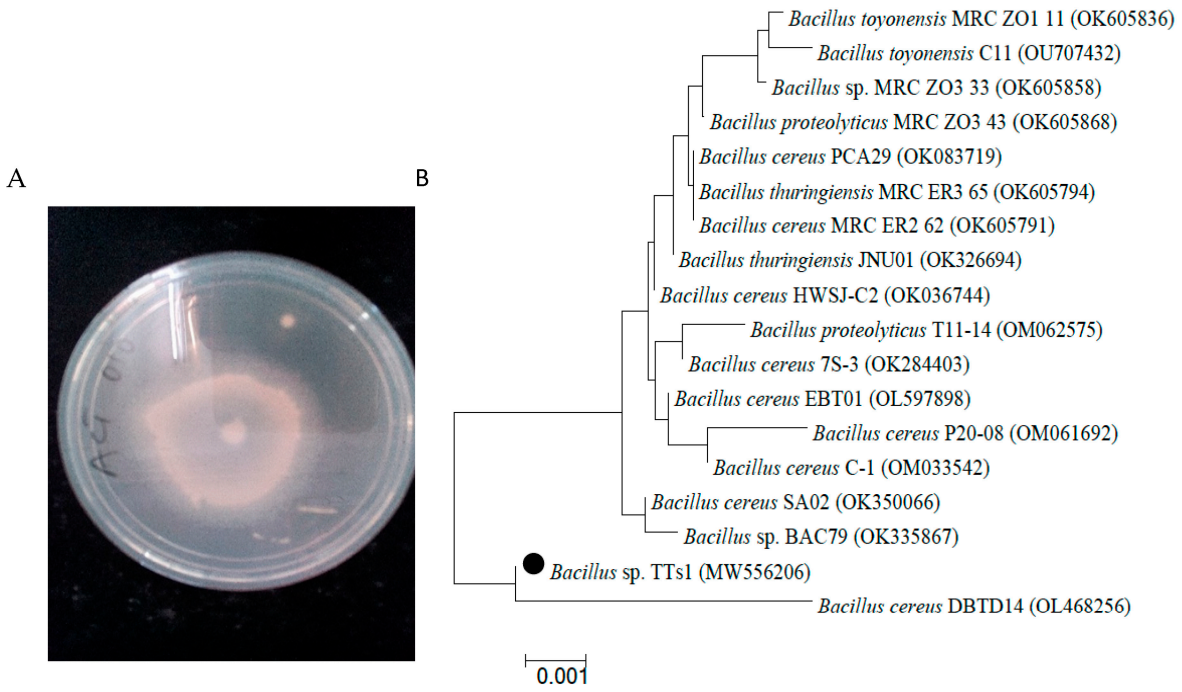

Figure 1. (**A**) Evaluation of lipolytic activity on tween 80–peptone media; a visible precipitate around the colony showed positive lipase activity by the bacterial isolate. (**B**) A phylogenetic tree showing the evolutionary relationship between the strain TTs1 (round black tip) utilized for the study and other closely related taxa. The GenBank accession numbers are presented in parentheses.

Based on 16S rRNA gene sequencing and analysis, strain TTs1 demonstrated a high percentage of sequence similarity with *Bacillus* spp., including *Bacillus* cereus DBTD14 (accession number OL468256); hence, it was identified as *Bacillus* sp. (Figure 1B). Accordingly, the 16S rRNA gene sequence of strain TTs1 was deposited in the NCBI GenBank under the accession number MW556206.

3.2. Optimization of the Fermentation Process Conditions

The optimization process indicated differential lipase activity at the various initial pH conditions tested. Lipase production by *Bacillus* sp. TTs1 increased from acidic to alkaline conditions, with an optimum performance at pH 10 (Figure 2A).

However, there was no significant difference between the lipase production at pH 9 (510.0 ± 42.43 U/mL) and pH 10 (577.65 ± 6.15 U/mL), with $p = 0.495$. Above pH 10, the lipase activity dropped significantly compared to the optimum, with $p < 0.000$. The study of the incubation temperature showed that strain TTs1 produced lipase at all the temperatures evaluated, with the highest lipase production (630.1 ± 13.72 U/mL) at 45 °C (Figure 2B). At 50 °C, the lipase production drastically decreased, with an enzyme titer of 326.25 ± 51.27 U/mL. Agitation of the fermentation medium promoted lipase production by strain TTs1, with the maximum activity (594.55 ± 0.78 U/mL) displayed at 140 rpm (Figure 2C). Beyond 140 rpm, the enzyme production by the isolate decreased with an increasing agitation speed.

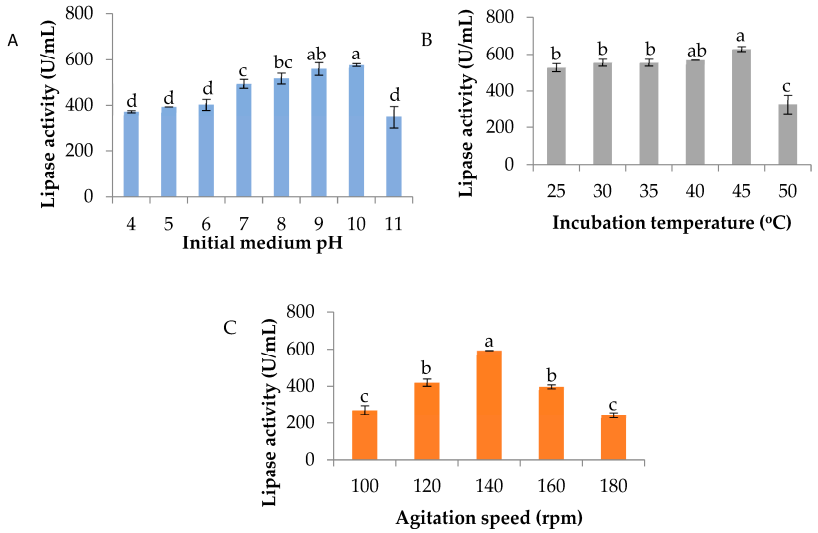

Figure 2. Effect of fermentation conditions: (**A**) initial medium pH, (**B**) incubation temperature, and (**C**) agitation speed on lipase production by *Bacillus* sp. TTs1. The bars represent the mean and standard deviation (error bar) of three independent experiments. The bars sharing the same letter(s) (a, b, c, d) are not statistically different.

In addition, the study of the starter culture concentration showed that 2% (*v*/*v*) inoculum was optimal for lipase production (575.15 ± 28.21 U/mL) by *Bacillus* sp. TTs1 (Figure 3A). An increase in the concentration of the starter culture beyond 2% resulted in a decrease in enzyme production, with the least lipase yield obtained at 10% inoculum (248.55 ± 19.73 U/mL). The inductive effect of different substrates was studied, and the results indicated that tween 80 optimally induced lipase production (510.45 ± 2.33 U/mL), followed by tween 20 (382.1 ± 12.58 U/mL), while sunflower oil had the least inductive effect, with lipase activity of 139.95 ± 9.41 U/mL (Figure 3B).

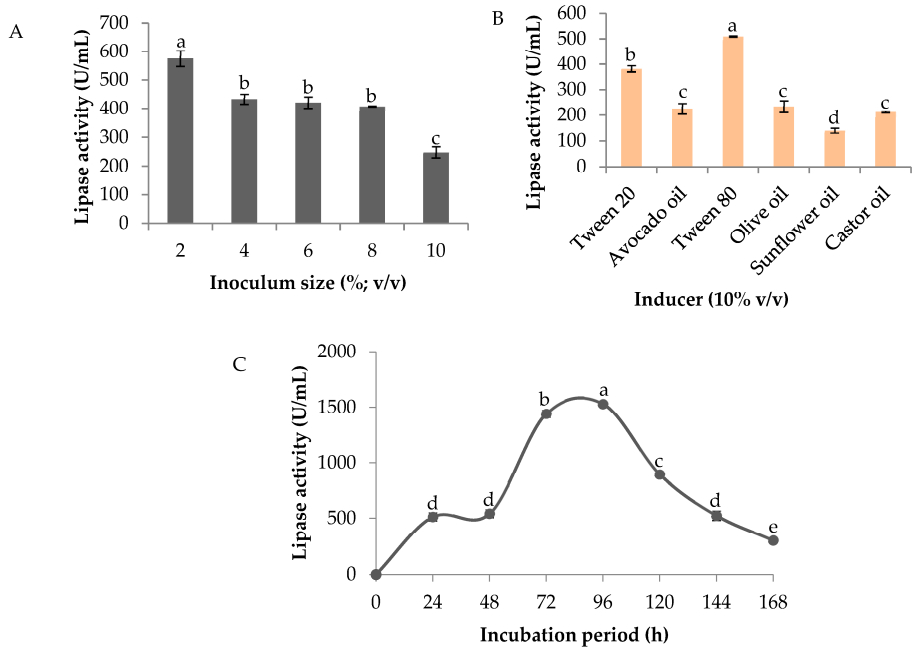

Figure 3. Effect of (**A**) inoculum size and (**B**) inducer on lipase production by *Bacillus* sp. TTs1. (**C**) Time course study of lipase production by *Bacillus* sp. TTs1. The bars or points represent the mean and standard deviation (error bar) of three independent experiments. The bars or points sharing the same letter(s) (a, b, c, d, e) are not statistically different.

The time course study of lipase production by *Bacillus* sp. TTs1 showed that extracellularly secreted lipase displayed an incremental titer with an increasing fermentation period, and subsequently, it peaked at 96 h, with an enzyme activity of 1530.5 U/mL (Figure 3C). Beyond 96 h, the lipase activity consistently declined with an increasing incubation time.

3.3. Influence of Chicken Feather Concentration on the Medium Free Fatty Acid

The free fatty acid released from the chicken feathers via the lipolytic activity of *Bacillus* sp. TTs1 increased with an increasing feather concentration. Free fatty acid of 0.26%, 0.43% and 0.58% were obtained at the respective chicken feather concentrations (*w*/*v*) of 2%, 4% and 6% (Figure 4). A further increase in the feather concentration to 8% (*w*/*v*) showed a comparable result (0.58%) to that obtained at 6% (*w*/*v*).

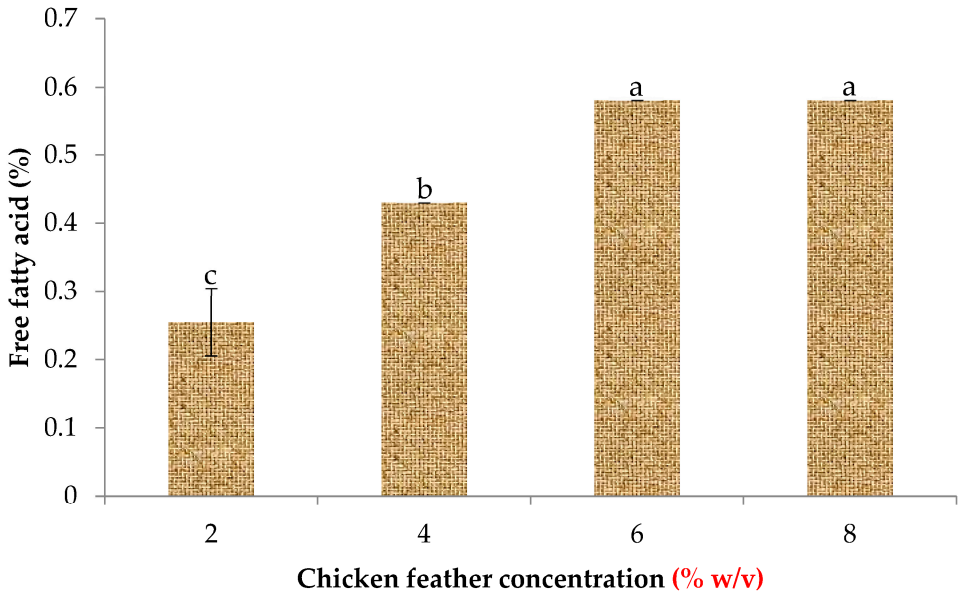

Figure 4. Effect of chicken feather concentration on free fatty acid liberation directed by *Bacillus* sp. TTs1. Each bar represents the mean and standard deviation (error bar) of three independent experiments. Bars sharing the same letter(s) (a, b, c) are not statistically different.

3.4. Quantitation of Crude Lipid Content of Untreated and TTs1-Pretreated Chicken Feathers

The crude lipids from both untreated and TTs1-pretreated chicken feathers was studied using Soxhlet extraction. The results showed that a crude lipid content of 2.1 ± 0.42% was extracted from the untreated chicken feathers, while 0.92 ± 0.13% was recovered from the TTs1-pretreated feathers (Table 1 and Figure S1).

Table 1. Crude fat determination from both untreated and TTs1-pretreated chicken feathers.

Feather Sample	Crude Fat (%)
Untreated	2.1 ± 0.42 [a]
TTs1-pretreated	0.92 ± 0.13 [b]

[a,b] The letters show a statistical difference between the two treatments.

4. Discussion

Chicken feathers, a by-product of the chicken processing industry, have a high crude protein content in the form of keratin, making them a good source of dietary protein. Efficient valorization of keratinous feathers has been hindered by the lipid component of the biomass. Hence, this study investigated a sustainable route for removing the feather lipids to generate lipid-deficient keratin. Consequently, bacterial stains from sediment samples were evaluated for lipolytic potential. Sediments have been described as important sources

of bacteria with great capacity to produce secondary metabolites [39,40]. The community of bacterial strains recovered from the sediment samples showed variable efficiencies in terms of the lipase activity, as indicated by formation of insoluble precipitate on tween 80–peptone agar. This observation highlighted their metabolic diversity and served as an index in the selection of competent strains [41].

The complexation of calcium salts with free fatty acids released via the action of extracellularly secreted lipase has been one of the efficient methods of assessing the lipolytic properties of bacteria on solid media [42]. Similarly, Lee et al. [41] reported visible precipitate formation on tween–agar medium by bacterial strains recovered from oil-contaminated soil samples. However, another study employed the phenol red method in the evaluation of bacterial lipolytic potential on solid medium [43]. Interestingly, the tween 80–peptone agar method has been preferred over the phenol red method, as the latter is highly dependent on the pH of the medium and any alteration in the pH could affect the process sensitivity [41,44]. Therefore, the selection of strain TTs1 was based on its promising lipolytic activity on tween 80–peptone agar.

Based on partial 16S rRNA gene sequencing and an NCBI database search, strain TTs1 was identified as *Bacillus* sp. TTs1. Even though reports on lipase production among different strains of *Bacillus* spp. abound [45–48], the genetic variation and geographical distribution of microbial species also influence the catalytic robustness of their secondary metabolites. Secondary metabolites are produced by microbes in response to their immediate need to either defend themselves against predation or utilize complex substrates for homeostasis.

Alkaline conditions enhanced lipase production by *Bacillus* sp. TTs1, and this result suggests that the biosynthetic pathways and/or extracellular transportation of the synthesized lipolytic enzyme were favored by the prevailing micro-environmental conditions. In agreement with the present finding, *Bacillus* sp. LBN 2 achieved maximum lipase production at pH 9.0 [49]. The maximum lipase secretion by *Bacillus* spp. in a submerged state fermentation has been extensively reported at a pH ranging from 7 to 10 [50]. However, extremophilic *Bacillus pumilus* and *Bacillus subtilis* from the oil extraction industry maximally produced lipase at pH 1.0 [51]. The incubation temperature influences enzyme production by regulating the microbial growth or biosynthetic pathways via transcription, translation, and enzyme secretion [49]. The strain's metabolic capacity also plays a crucial role in relation to the optimal conditions for microbial productivity. Hence, *B. pumilus* and *B. subtilis* isolated from a similar environment maximally secreted lipases at 50 °C and 35 °C, respectively [51].

Agitation enhances the aeration of the culture medium and promotes the homogeneity of medium nutrients. Adequate dissolved oxygen in an aerobic fermentation is crucial for optimal cell growth and enzyme production [52]. The nature of the nutrients in the fermentation medium significantly influences/regulates the microbial metabolism in relation to gene expression, enzyme biosynthesis and extracellular production [53]. An increased agitation speed beyond the optimum could create shear forces that negatively impact the cell biomass integrity, which therefore affects the enzyme productivity [54]. Likewise, other studies have shown that lipase production by *Bacillus licheniformis* [55] and *B. subtilis* [56] was optimum at 140 rpm and 150 rpm, respectively. The size of the starter culture influences enzyme production by bacterial strains; overpopulation of the culture medium at the inception of fermentation could be a limiting factor in terms of the optimal enzyme yield, as this might result in the quick depletion of nutrients and dissolved oxygen [47]. This might explain why the lipase activity of *Bacillus* sp. TTs1 decreased beyond a 2% inoculum size. *Bacillus aryabhattai* SE3-PB optimally secreted lipase when fermentation was initiated with an inoculum size of 2.2% (*v*/*v*) [57].

Various substrates have been utilized in the fermentation medium to induce extracellular lipase production, and the enzyme titer in response to the available substrate might be strain-specific. Consequently, *Bacillus methylotrophicus* [46], *B. subtilis* [58], *B. pumilus* [51], *Ps. gessardii* [59], and *B. subtilis* [47] maximally secreted lipolytic enzyme when the production media were constituted with tween 80, olive oil, palm oil, beef tallow, and waste cooking oil, respectively. Among the surfactants, tween 80, which showed the maximum inducible effect

in the present study, has been reported to be a potent lipase inducer compared to tween 20, sodium dodecyl sulfate and triton X-100 [60].

Lipase production by *Bacillus* sp. TTs1 reached the highest titer at 96 h, and the timeline for the maximum lipase production in a pilot-scale fermentor by bacterial strains might be attributed to the strain efficiency and media composition, as the induction mechanism by the inducer-substrate could differ significantly [61]. Similar to the present finding, *B. pumilus* achieved a maximum lipase titer of 1100 U/mL at 96 h [51]. However, Riaz and colleagues reported a maximum lipase activity of 5.12 U/mL after 48 h with *Bacillus* sp. FH5 [50]. Generally, the optimal lipase production timeline of *Bacillus* spp. has been extensively reported to be between 48 h and 96 h [28,47,62,63].

The increasing fatty acid liberation with the incremental chicken feather concentrations suggests an up-regulation of the lipase-encoding gene. Lipase production among prokaryotes is generally inducible in the presence of lipid-rich substrates that promote the biosynthesis and extracellular secretion of the lipolytic enzymes [64]. However, when the amount of chicken feathers exceeded the optimum, further increment showed no significant change in the fatty acid concentration. This assertion could explain why the free fatty acid concentration remained constant at 6% and 8% (w/v) chicken feathers.

The decrease in crude fat obtained from the biologically pretreated chicken feathers compared to the untreated feathers indicates that a significant amount of lipids has been hydrolyzed by the action of *Bacillus* sp. TTs1 lipase. The crude fat extracted from the untreated chicken feathers is in the same percentage range as that reported by Prajapati and colleagues, who recorded 2.30 $\pm$ 0.05% [20]. Lipids have a detrimental effect on chicken feather conversion into digestible proteins and other biomaterials such as nanofibers [19]. Lipid-deficient keratin is an ideal substrate for the value-addition stage as it permits a keratinolytic system or chemical agents to efficiently penetrate and dismember the polymer into desired products [65]. This finding of our study highlights the delipidation ability of *Bacillus* sp. TTs1 lipase and its relevance to sustainable and eco-friendly developments.

An approach spurring on a complete hydrolysis of readily available keratinous feathers into amino acid-rich protein hydrolysates augurs well for the agro-industrial sector. A sustainable method of digesting feather lipids provides a cheap protein substrate that could be harnessed as an alternative source of livestock feed protein while promoting eco-friendliness. The lipolytic potential of the studied bacterium warrants further research that would include the following. (1) Comparative study of protein hydrolysates recovered from the biodegradation of bio-delipidated and untreated chicken feathers. This objective would shed more light on the efficacy and relevance of chicken feather delipidation before enzymatic/chemical keratinolysis. (2) Another pertinent future study would focus on bacterial genomic profiling and the mining of relevant genes involved in lipolytic activity, and on the expression of such genes in competent industrial hosts. It is considered that the recombinant host would enhance lipolytic enzyme production in a short timeline of fermentation as against the wild bacterial strain.

5. Conclusions

In conclusion, bacterial strains with variable lipolytic activity were isolated from sediment samples. Remarkably, strain TTs1, which was identified as *Bacillus* sp. TTs1, displayed promising lipase activity, as indicated by the formation of a large halo zone precipitate of calcium salt crystals around the colony on tween 80–peptone agar. An alkaline condition (pH 10) and a moderate temperature (45 °C) optimally supported extracellular lipase production by strain TTs1. Tween 80 elicited the maximum positive effect in terms of inducing bacterial lipase among the different inducers that were evaluated. The crude lipid extracted from untreated chicken feathers differed from that obtained from TTs1-pretreated chicken feathers, and this variation suggests the lipolytic potential of *Bacillus* sp. TTs1. Therefore, the findings of this study highlight the biotechnological prospects of strain TTs1 in lipase production and the sustainable delipidation of keratinous biomass.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cleantechnol5040062/s1, Figure S1: A representative flow of crude fat extraction showing (A) raw chicken feather, (B) chicken feather post-extraction (C) crude fat at the bottom of the flask. Table S1: A few selected patents on innovative application of keratinous biomass. Table S2: Qualitative evaluation of sediment-associated bacteria for lipolytic potentials on tween 80 amended agar media.

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References

1. Laba, W.; Rodziewicz, A. Biodegradation of hard keratins by two *Bacillus* strains. *Jundishapur J. Microbiol.* **2014**, *7*, e8896. [CrossRef]
2. Chilakamarry, C.R.; Mahmood, S.; Saffe, S.N.B.M.; Arifin, M.A.B.; Gupta, A.; Sikkandar, M.Y.; Begum, S.S.; Narasaiah, B. Extraction and application of keratin from natural resources: A review. *3 Biotech* **2021**, *11*, 220. [CrossRef]
3. Kokwe, L.; Nnolim, N.E.; Ezeogu, L.I.; Sithole, B.; Nwodo, U.U. Thermoactive metallo-keratinase from *Bacillus* sp. NFH5: Characterization, structural elucidation, and potential application as detergent additive. *Heliyon* **2023**, *9*, e13635. [CrossRef] [PubMed]
4. Khumalo, M.; Tesfaye, T.; Sithole, B.B.; Ramjugernath, D. Possible beneficiation of waste chicken feathers via conversion into biomedical applications. *Int. J. Chem. Sci.* **2019**, *17*, 298. [CrossRef]
5. Nnolim, N.E.; Udenigwe, C.C.; Okoh, A.I.; Nwodo, U.U. Microbial keratinase: Next generation green catalyst and prospective applications. *Front. Microbiol.* **2020**, *11*, 580164. [CrossRef]
6. Bokveld, A.; Nnolim, N.E.; Nwodo, U.U. *Chryseobacterium aquifrigidense* FANN1 produced detergent-stable metallokeratinase and amino acids through the abasement of chicken feathers. *Front. Bioeng. Biotechnol.* **2021**, *9*, 720176. [CrossRef] [PubMed]
7. Gurav, R.G.; Jadhav, J.P. A novel source of biofertilizer from feather biomass for banana cultivation. *Environ. Sci. Pollut. Res.* **2013**, *20*, 4532–4539. [CrossRef]
8. European Union. Regulation 2019/1009, 2019. (EU) of the European Parliament and of the Council of 5 June 2019 Laying Down Rules on the Making Available on the Market of EU Fertilising Products and Amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and Repealing Regulation (EC) No 2003/2003. *Off. J. Eur. Union* **2019**, *170*, 1–114. Available online: http://data.europa.eu/eli/reg/2019/1009/oj (accessed on 23 June 2023).
9. Ningthoujam, D.S.; Tamereihao, K.; Mukhererjee, S.; Khunjamayum, R.; Devi, L.J.; Asem, R.S. Keratinaceous Wastes and Their Valorization through Keratinolytic Microorganisms. In *Keratin*; Blumenberg, M., Ed.; IntechOpen: London, UK, 2018. [CrossRef]
10. Kakonke, G.; Tesfaye, T.; Sithole, B.; Ntunka, M. A novel method for rapid extraction of biofibres from waste chicken feathers. *J. Solid Waste Technol. Manag.* **2021**, *47*, 31–45. [CrossRef]
11. European Union. Code of Good Practice for Poultry Farmers. Legal Obligations and Good Practice Guidelines for Poultry Farmers in Relation to Dead Birds and Poultry Litter. 2022. Available online: https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwifk7_csfKBAxXoMjQIHS9-C8oQFnoECBMQAQ&url=https%3A%2F%2Fassets.gov.ie%2F120140%2Fa247d156-9404-4c8a-aa99-605ffc3f66cf.pdf&usg=AOvVaw2DD4cfZraILeMsD-2ph0Qg&opi=89978449 (accessed on 23 June 2023).
12. Mozhiarasi, V.; Natarajan, T.S. Slaughterhouse and poultry wastes: Management practices, feedstocks for renewable energy production, and recovery of value added products. *Biomass Conv. Bioref.* **2022**. [CrossRef]
13. South African Poultry Association (SAPA). Broiler Production Annual Report 2021. Available online: https://www.sapoultry.co.za/wp-content/uploads/2022/06/SAPA-ANNUAL-REPORT-2021.pdf (accessed on 24 June 2023).
14. Chen, H.; Gao, S.; Li, Y.; Xu, H.J.; Li, W.; Wang, J.; Zhang, Y. Valorization of Livestock Keratin Waste: Application in Agricultural Fields. *Int. J. Environ. Res. Public Health* **2022**, *19*, 6681. [CrossRef]
15. Conway, A. World Poultry Production at Nearly 123 Million Tons in 2018. 2019. Available online: https://www.wattagnet.com/articles/38018-world-poultry-production-at-nearly-123-million-tons-in-2018 (accessed on 21 June 2022).

16. Godfrey, L.; Oelofse, S. Historical review of waste management and recycling in South Africa. *Resources* **2017**, *6*, 57. [CrossRef]
17. Fagbemi, O.D.; Sithole, B.; Tesfaye, T. Optimization of keratin protein extraction from waste chicken feathers using hybrid pretreatment techniques. *Sustain. Chem. Pharm.* **2020**, *17*, 100267. [CrossRef]
18. Kondamudi, N.; Strull, J.; Misra, M.; Mohapatra, S.K. A green process for producing biodiesel from feather meal. *J. Agric. Food Chem.* **2009**, *57*, 6163–6166. [CrossRef]
19. Khumalo, M.; Sithole, B.; Tesfaye, T. Valorization of waste chicken feathers: Optimization of keratin extraction from waste chicken feathers by sodium bisulphite, sodium dodecyl sulphate and urea. *J. Environ. Manag.* **2020**, *262*, 110329. [CrossRef] [PubMed]
20. Prajapati, S.; Koirala, S.; Anal, A.K. Bioutilization of chicken feather waste by newly isolated keratinolytic bacteria and conversion into protein hydrolysates with improved functionalities. *Appl. Biochem. Biotechnol.* **2021**, *193*, 2497–2515. [CrossRef]
21. Fagbemi, O.D.; Sithole, B. Evaluation of waste chicken feather protein hydrolysate as a bio-based binder for particleboard production. *Curr. Res. Green Sustain. Chem.* **2021**, *4*, 100168. [CrossRef]
22. Li, Z.W.; Liang, S.; Ke, Y.; Deng, J.J.; Zhang, M.S.; Lu, D.L.; Li, J.Z.; Luo, X.C. The feather degradation mechanisms of a new *Streptomyces* sp. isolate SCUT-3. *Commun. Biol.* **2020**, *3*, 191. [CrossRef]
23. Adelina, A.; Feliatra, F.; Siregar, Y.I.; Suharman, I.; Pamukas, N.A. Fermented chicken feathers using *Bacillus subtilis* to improve the quality of nutrition as a fish feed material. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *348*, 012008. [CrossRef]
24. Nealson, K.H. Sediment bacteria: Who's there, what are they doing, and what's new? *Annu. Rev. Earth Planet. Sci.* **1997**, *25*, 403–434. [CrossRef]
25. Shrivastava, P.; Yandigeri, M.S.; Kumar, R.; Arora, D.K. Isolation and characterization of *Streptomycetes* with plant growth promoting potential from Mangrove ecosystem. *Pol. J. Microbiol.* **2015**, *64*, 339–349. [CrossRef]
26. Praveen Kumar, P.; Preetam Raj, J.P.; Nimal Christhudas, I.V.S.; Sagaya Jansi, R.; Murugan, N.; Agastian, P.; Arunachalam, C.; Ali Alharbi, S. Screening of actinomycetes for enzyme and antimicrobial activities from the soil sediments of Northern Tamil Nadu, South India. *J. Biol. Act. Prod. Nat.* **2015**, *5*, 58–70. [CrossRef]
27. Gopinath, S.C.; Hilda, A.; Anbu, P. Extracellular enzymatic activity profiles in fungi isolated from oil-rich environments. *Mycoscience* **2005**, *46*, 119–126. [CrossRef]
28. Bharathi, D.; Rajalakshmi, G.; Komathi, S. Optimization and production of lipase enzyme from bacterial strains isolated from petrol spilled soil. *J. King Saud Univ. Sci.* **2019**, *31*, 898–901. [CrossRef]
29. Kanjanavas, P.; Khuchareontaworn, S.; Khawsak, P.; Pakpitcharoen, A.; Pothivejkul, K.; Santiwatanakul, S.; Matsui, K.; Kajiwara, T.; Chansiri, K. Purification and characterization of organic solvent and detergent tolerant lipase from thermotolerant *Bacillus* sp. RN2. *Int. J. Mol. Sci.* **2010**, *11*, 3783–3792. [CrossRef] [PubMed]
30. Turner, S.; Pryer, K.M.; Miao, V.P.; Palmer, J.D. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis 1. *J. Eukaryot. Microbiol.* **1999**, *46*, 327–338. [CrossRef] [PubMed]
31. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [CrossRef] [PubMed]
32. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef] [PubMed]
33. Kralik, P.; Beran, V.; Pavlik, I. Enumeration of *Mycobacterium avium* subsp. paratuberculosis by quantitative real-time PCR, culture on solid media and optical densitometry. *BMC Res. Notes* **2012**, *5*, 114. [CrossRef] [PubMed]
34. Vicentini-Polette, C.M.; Ramos, P.R.; Gonçalves, C.B.; De Oliveira, A.L. Determination of free fatty acids in crude vegetable oil samples obtained by high-pressure processes. *Food Chem. X* **2021**, *12*, 100166. [CrossRef]
35. Suresh, K.S.; Suresh, P.V.; Kudre, T.G. Prospective ecofuel feedstocks for sustainable production. In *Advances in Eco-Fuels for a Sustainable Environment*; Woodhead Publishing: Cambridge, UK, 2019; pp. 89–117.
36. Redfern, J.; Kinninmonth, M.; Burdass, D.; Verran, J. Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. *J. Microbiol. Biol. Educ.* **2014**, *15*, 45–46. [CrossRef]
37. Laroche, M.; Perreault, V.; Marciniak, A.; Gravel, A.; Chamberland, J.; Doyen, A. Comparison of conventional and sustainable lipid extraction methods for the production of oil and protein isolate from edible insect meal. *Foods* **2019**, *8*, 572. [CrossRef] [PubMed]
38. United States Department of Agriculture Food Safety and Inspection Service, Office of Public Health Science. 2009. Available online: https://www.fsis.usda.gov/wps/wcm/connect/dd881c92-c19b-4530-b6ee-931c368b8904/CLG_FAT_03.pdf?MOD=AJPERES (accessed on 6 March 2023).
39. Dias, A.C.; Andreote, F.D.; Dini-Andreote, F.; Lacava, P.T.; Sá, A.L.; Melo, I.S.; Azevedo, J.L.; Araújo, W.L. Diversity and biotechnological potential of culturable bacteria from Brazilian mangrove sediment. *World J. Microbiol. Biotechnol.* **2009**, *25*, 1305–1311. [CrossRef]
40. Jankoski, P.R.; Correa, A.P.F.; Brandelli, A.; Motta, A.S. Biological activity of bacteria isolated from wetland sediments collected from a conservation unit in the southern region of Brazil. *An. Acad. Bras. Ciênc.* **2021**, *93*, e20191269. [CrossRef] [PubMed]
41. Lee, L.P.; Karbul, H.M.; Citartan, M.; Gopinath, S.C.; Lakshmipriya, T.; Tang, T.H. Lipase-secreting *Bacillus* species in an oil-contaminated habitat: Promising strains to alleviate oil pollution. *BioMed Res. Int.* **2015**, *2015*, 820575. [CrossRef]
42. Lanka, S.; Latha, J.N.L. A short review on various screening methods to isolate potential lipase producers: Lipases-the present and future enzymes of biotech industry. *Int. J. Biol. Chem.* **2015**, *9*, 207–219. [CrossRef]
43. Kanchana, R.; Muraleedharan, U.D.; Raghukumar, S. Alkaline lipase activity from the marine protists, thraustochytrids. *World J. Microbiol. Biotechnol.* **2011**, *27*, 2125–2131. [CrossRef]

44. Pham, V.H.T.; Kim, J.; Chang, S.; Chung, W. Investigation of lipolytic-secreting bacteria from an artificially polluted soil using a modified culture method and optimization of their lipase production. *Microorganisms* **2021**, *9*, 2590. [CrossRef]
45. Ghori, M.I.; Iqbal, M.J.; Hameed, A. Characterization of a novel lipase from *Bacillus* sp. isolated from tannery wastes. *Braz. J. Microbiol.* **2011**, *42*, 22–29. [CrossRef] [PubMed]
46. Sharma, P.; Sharma, N.; Pathania, S.; Handa, S. Purification and characterization of lipase by *Bacillus methylotrophicus* PS3 under submerged fermentation and its application in detergent industry. *J. Genet. Eng. Biotechnol.* **2017**, *15*, 369–377. [CrossRef]
47. Suci, M.; Arbianti, R.; Hermansyah, H. Lipase production from *Bacillus subtilis* with submerged fermentation using waste cooking oil. *IOP Conf. Ser. Earth Environ. Sci.* **2018**, *105*, 012126. [CrossRef]
48. Shart, A.O.; Elkhalil, E.A. Biochemical Characterisation of Lipase Produced by *Bacillus* spp. Isolated from Soil and Oil Effluent. *Adv. Enzym. Res.* **2020**, *8*, 39–48. [CrossRef]
49. Bora, L.; Bora, M. Optimization of extracellular thermophilic highly alkaline lipase from thermophilic *Bacillus* sp. isolated from hotspring of Arunachal Pradesh, India. *Braz. J. Microbiol.* **2012**, *43*, 30–42. [CrossRef]
50. Riaz, M.; Shah, A.A.; Hameed, A.; Hasan, F. Characterization of lipase produced by *Bacillus* sp. FH5 in immobilized and free state. *Ann. Microbiol.* **2010**, *60*, 169–175. [CrossRef]
51. Saranya, P.; Kumari, H.S.; Jothieswari, M.; Rao, B.P.; Sekaran, G. Novel extremely acidic lipases produced from *Bacillus* species using oil substrates. *J. Ind. Microbiol. Biotechnol.* **2014**, *41*, 9–15. [CrossRef]
52. Lv, P.J.; Qiang, S.; Liu, L.; Hu, C.Y.; Meng, Y.H. Dissolved-oxygen feedback control fermentation for enhancing β-carotene in engineered *Yarrowia lipolytica*. *Sci. Rep.* **2020**, *10*, 17114. [CrossRef]
53. Singh, V.; Haque, S.; Niwas, R.; Srivastava, A.; Pasupuleti, M.; Tripathi, C. Strategies for fermentation medium optimization: An in-depth review. *Front. Microbiol.* **2017**, *7*, 2087. [CrossRef]
54. Potumarthi, R.; Subhakar, C.; Vanajakshi, J.; Jetty, A. Effect of aeration and agitation regimes on lipase production by newly isolated *Rhodotorula mucilaginosa*–MTCC 8737 in stirred tank reactor using molasses as sole production medium. *Appl. Biochem. Biotechnol.* **2008**, *151*, 700–710. [CrossRef] [PubMed]
55. Popoola1, B.M.; Olanbiwoninu, A.A. Characterisation of Some Selected Bacterial Strains from Vegetable Oil Contaminated Soil. *Microbiol. Res. J. Int.* **2021**, *31*, 22–37. [CrossRef]
56. Olusesan, A.T.; Azura, L.K.; Abubakar, F.; Mohamed, A.K.S.; Radu, S.; Manap, M.Y.A.; Saari, N. Enhancement of thermostable lipase production by a genotypically identified extremophilic *Bacillus subtilis* NS 8 in a continuous bioreactor. *J. Mol. Microbiol. Biotechnol.* **2011**, *20*, 105–115. [CrossRef]
57. Isiaka Adetunji, A.; Olufolahan Olaniran, A. Optimization of culture conditions for enhanced lipase production by an indigenous *Bacillus aryabhattai* SE3-PB using response surface methodology. *Biotechnol. Biotechnol. Equip.* **2018**, *32*, 1514–1526. [CrossRef]
58. Saraswat, R.; Bhushan, I.; Gupta, P.; Kumar, V.; Verma, V. Production and purification of an alkaline lipase from *Bacillus* sp. for enantioselective resolution of (±)-Ketoprofen butyl ester. *3 Biotech* **2018**, *8*, 491. [CrossRef] [PubMed]
59. Ramani, K.; Kennedy, L.J.; Ramakrishnan, M.; Sekaran, G. Purification, characterization and application of acidic lipase from *Pseudomonas gessardii* using beef tallow as a substrate for fats and oil hydrolysis. *Process Biochem.* **2010**, *45*, 1683–1691. [CrossRef]
60. Al Mohaini, M.; Farid, A.; Muzammal, M.; Ghazanfar, S.; Dadrasnia, A.; Alsalman, A.J.; Al Hawaj, M.A.; Alhashem, Y.N.; Ismail, S. Enhancing lipase production of *Bacillus salmalaya* strain 139si using different carbon sources and surfactants. *Appl. Microbiol.* **2022**, *2*, 237–247. [CrossRef]
61. Soleymani, S.; Alizadeh, H.; Mohammadian, H.; Rabbani, E.; Moazen, F.; Sadeghi, H.M.; Shariat, Z.S.; Etemadifar, Z.; Rabbani, M. Efficient media for high lipase production: One variable at a time approach. *Avicenna J. Med. Biotechnol.* **2017**, *9*, 82–86.
62. Hasan, F.; Shah, A.A.; Hameed, A. Influence of culture conditions on lipase production by *Bacillus* sp. FH5. *Ann. Microbiol.* **2006**, *56*, 247–252. [CrossRef]
63. Haniya, M.; Naaz, A.; Sakhawat, A.; Amir, S.; Zahid, H.; Syed, S.A. Optimized production of lipase from *Bacillus subtilis* PCSIRNL-39. *Afr. J. Biotechnol.* **2017**, *16*, 1106–1115. [CrossRef]
64. Adetunji, A.I.; Olaniran, A.O. Production strategies and biotechnological relevance of microbial lipases: A review. *Braz. J. Microbiol.* **2021**, *52*, 1257–1269. [CrossRef]
65. Tesfaye, T.; Sithole, B.; Ramjugernath, D. Valorisation of waste chicken feathers: Optimisation of decontamination and pre-treatment with bleaching agents using response surface methodology. *Sustain. Chem. Pharm.* **2018**, *8*, 21–37. [CrossRef]