Journal of Advanced Zoology
*ISSN: 0253-7214*
*Volume* ***44*** *Issue* ***S-8 Year 2023*** *Page* ***133:140***

# Isolation and Characterization of Bacterial Lipids and its Application as Antioxidant Agent

**B. M. Govilkar[1], A. N. Vemula[1]***

[1]*Department of Microbiology, Changu Kana Thakur Arts, Commerce & Science College, New Panvel*

***Corresponding Author:** A. N. Vemula*
*Email: vemulaanvi@gmail.com*

***Abstract***

The study was aimed to isolate the oleaginous bacteria from various soil samples and characterize the bacterial lipids to study its suitable application. The study was carried out from June 2023 to October 2023 in the microbiology lab at Changu Kana Thakur Arts, Commerce & Science College, New Panvel (Autonomous). The soil samples were collected from the garden, dumping, and compost area at New Panvel. The bacterial isolation was carried out by simple streak plate method, followed by screening by Sudan black B staining method. Production of oleaginous bacteria was done, followed by extraction of lipids by Bligh and dyer method. The extracted lipids were characterized by TLC and FTIR. The antioxidant potential of extracted lipids was assessed by DPPH method. The different oleaginous bacteria were isolated from soil sample and screened by Sudan black B staining method. The results indicate phospholipids, TAG, esters of fatty acids were mainly present. The extracted lipid samples S1C2, S2C4, S2C2, S1C1, S3C4, S2C1, S3C2 have 83.30%, 80%, 36.60%, 23.30%, 26.60%, 16.6%, 6.6% antioxidant potential respectively.



***Keywords: Oleaginous bacteria, FTIR, Phospholipids, Antioxidant potential***

**Introduction**:

Single-cell microorganisms (SCM) are a newly emerging source of high-value lipids for a number of expanding applications that seek low-cost, high-quality substitutes. (**Bajwa et al.; AJB2T, 3(1): 1-12, 2018; Article no. AJB2T. 39260**) Oleaginous microorganisms are those that can accumulate lipids in amounts more than 20% and up to 70% of their dry weight. **(Tamene and Dawit, 2014)** Microbial lipid sources have several advantages over other sources, including better lipid productivity in terms of g/L/day, being unaffected by seasonal climate fluctuations, being low labour intensive, and being easily scaled-up**. (Alok Patel, Fabio Mikes and Leonidas Matsakas; 2018)** Lipids are a class of molecules that are soluble in organic solvents but insoluble in water. They include fat-soluble vitamins (e.g., A, D, E, and K), monoglycerides, diglycerides, triglycerides, hydrocarbon-like compounds (e.g., sterols, terpenes, waxes), and glycerophospholipids. **(A. Daniel Jones et al;2019)** Temperature, pH, substrate, C/N ratio, and oxygen pressure are all factors that influence the productivity of accumulating lipids. (**Bajwa et al.; AJB2T, 3(1): 1-12, 2018; Article no. AJB2T. 39260**). Microbial lipids can potentially be used to produce safe and clean biomaterials at low cost and with continuous availability. (**Bajwa et al.; AJB2T, 3(1): 1-12, 2018; Article no. AJB2T. 39260**). Antioxidants have ability to neutralize free radicals. They reduce the risk of various diseases. Many lipids have antioxidant

potential. The antioxidant potency is determined by DPPH assay. DPPH is a free radical neutralize by antioxidant, give colour changes from purple to colourless. The decrease in absorbance is proportional to antioxidant capacity. **(Durval, I. J. B., Ribeiro, B. G., Aguiar, J. S., Sarubbo, L. A., Rufino, R. D., & Converti, A, 2021)**

**Materials and Methods**:

1. **Collection and enrichment of soil samples:** Soil samples were collected from garden, dumping, compost and 1 gram of each sample was added into different sterile nutrient broth. Incubate for 24 hrs. at R.T. on shaking condition.
2. **Isolation of bacterial species:** After incubation period loopful inoculum was streak on sterile nutrient agar plate and kept it at R.T. for 24 hrs.
3. **Morphological characterization and screening of oleaginous bacteria:** Morphologically different colonies were selected and streak on Tryptone Yeast Extract agar plates. Gram nature of isolates was identified by Gram staining method. Sudan black B method was used for the screening of oleaginous bacteria.
4. **Production of lipids:** Sudan black B positive isolates were inoculated into 20 ml sterile Peptone yeast extract (PY) broth and kept it at R.T. for 24 hrs. in a shaking condition. Cell pellets were collected by centrifugation from 10 ml of incubated PY broth and inoculated into sterile mineral medium broth for the production of lipids.
5. **Extraction of lipids:** Cell pellets were collected from 24 hrs inoculated mineral medium and kept it for completely get dried for 2-3 days. All dried cell biomass was subjected to total lipid extraction by using Bligh and dyer method.
6. **Characterization of lipids by using TLC:** The extracted lipids were characterized by using TLC in which the sample spotted TLC plates were kept in solvent system n- Hexane: diethyl ether: acetic acid (90:10:2). After running the sample with solvent, kept it in iodine vapour.
7. **Characterization of lipids by using FTIR spectroscopy:** Secondary lipid characterization of the samples is done by using FTIR spectrophotometer (Model- Nicolateis 5) (make- Thermoscientific) at Quality solution lab, Navi Mumbai.
8. **Assessment of antioxidant potential of extracted lipids:** The antioxidant activity of extracted lipids was determined by DPPH assay. The standard with ascorbic acid concentrations (0 ug/ml, 5 ug/ml, 10 ug/ml, 15 ug/ml, 20 ug/ml) were used. The absorbance was determined at wavelength 517 nm.

**Result**:

**1. Enrichment and isolation of bacteria:** The collected soil samples were enriched by using Nutrient broth.12 isolated colonies were obtained after streaking loopful samples on Nutrient agar plates from enrichment broth.

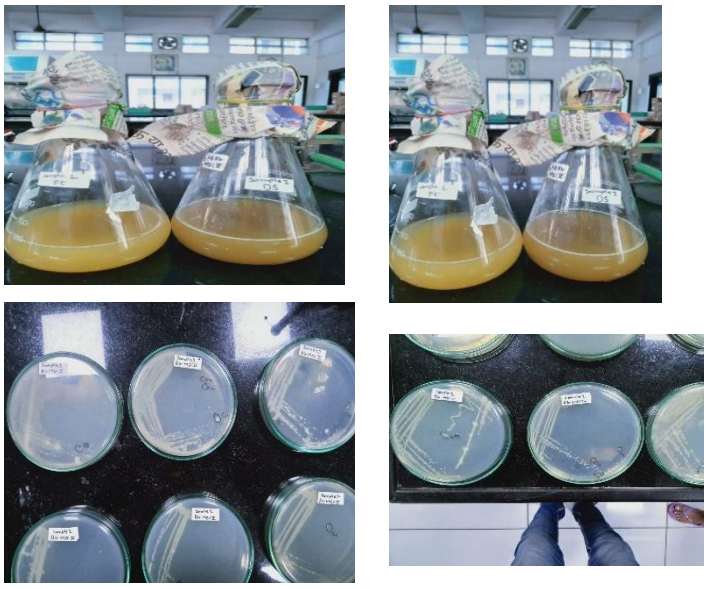


**Figure No. 1: Enrichment and isolation of bacteria**

**2. Morphological characterization and screening of oleaginous bacteria:**
Out of 12, 7 colonies were Sudan black B (SB) positive (black centred bacteria when observed under microscope).

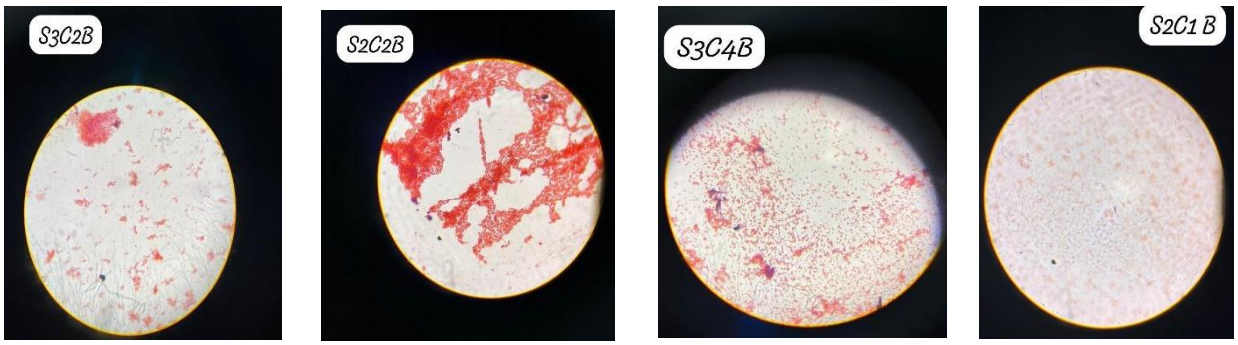



**Figure No. 2: Sudan black B staining**

**3. Production of lipids:** Cell pellets were collected by centrifugation of incubated mineral medium and dried it.

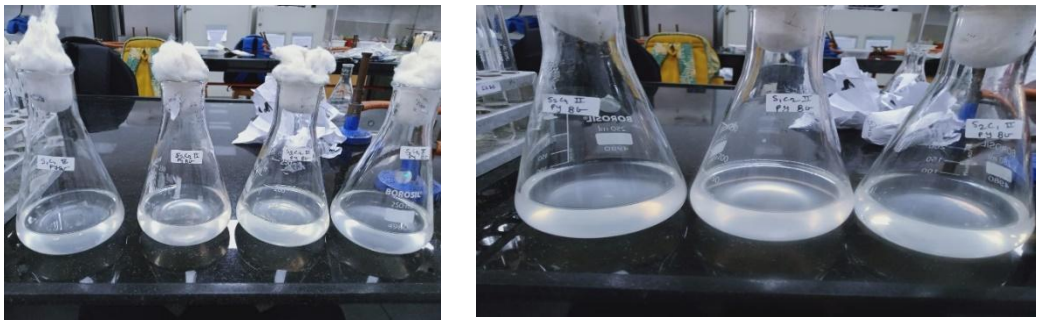


**Figure No. 3: Production of lipids**

**4. Extraction of lipids:** After performing Bligh and dyer the lipids were extracted and present at bottom chloroform phase.

**Figure No. 4: Dried cell biomass**

**Figure No. 5: Extracted lipids**

**5. Characterization of lipids by using TLC:** The Rf values 0.76, 0.15, 0.23, 0.30, 0.15, 0.39, 0.84 were obtained after performing the TLC. From these values they may be TAG, esters of fatty acids, monoacyl glycerol, phospholipids.

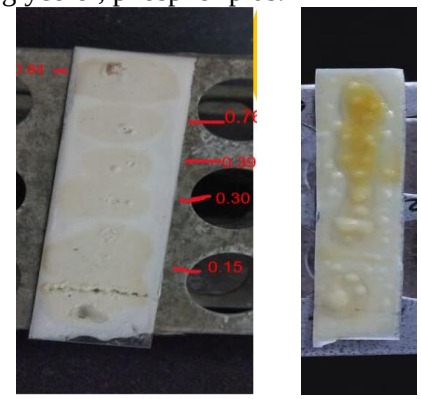


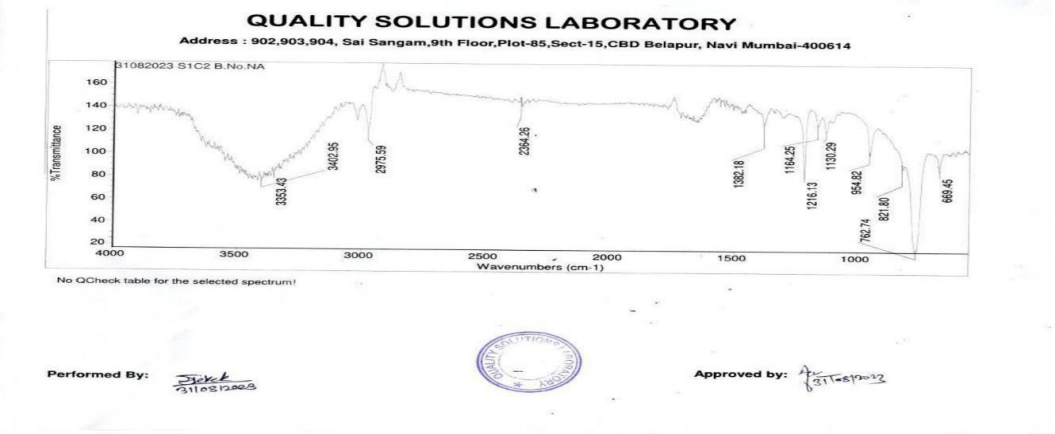

**Figure No. 6: Results of TLC**

**6. Characterization of lipids by using FTIR:**

Peaks are obtained after characterization of lipid samples by FTIR spectroscopy. Lipid content can be identified by peaks related to C-H stretching vibrations at 762.74, 2855-2954 cm-1. C-H bending at 821 cm-1, CH, C-O stretching at 1130, 1216.13, 1164.25 are present. N-H, O-H stretching at 2975,59, 3353.43, 3402.95cm-1. All these signals of phospholipids, esters of fatty acids were visible in the spectrum of extracted lipids.

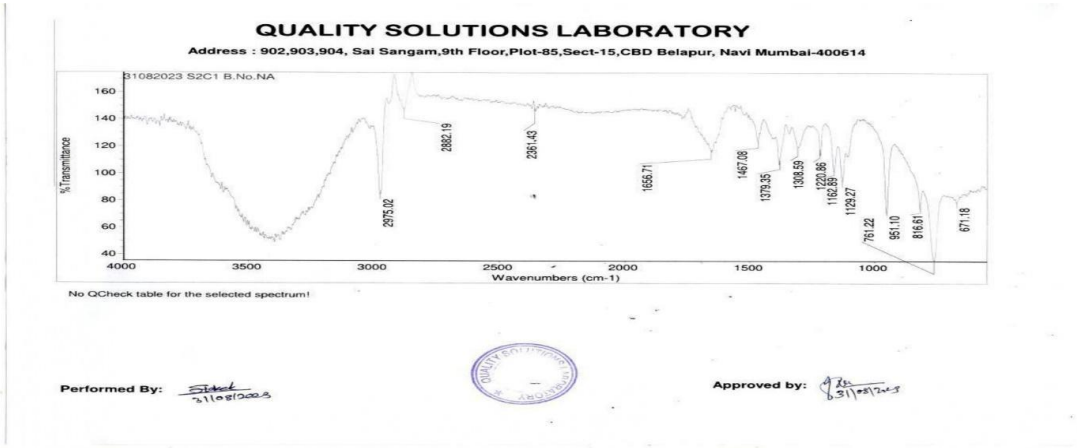
Phosphatidyl ethanolamine, phosphatidyl choline were may be mainly present.

**6.1.**



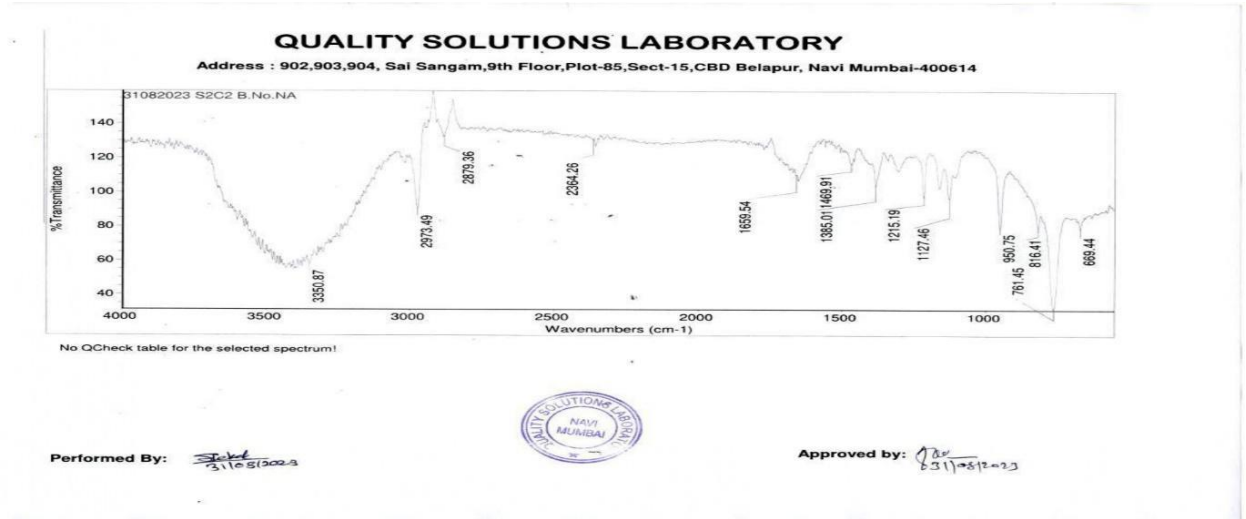

**A) FTIR result of S1C2**

**6.2.**



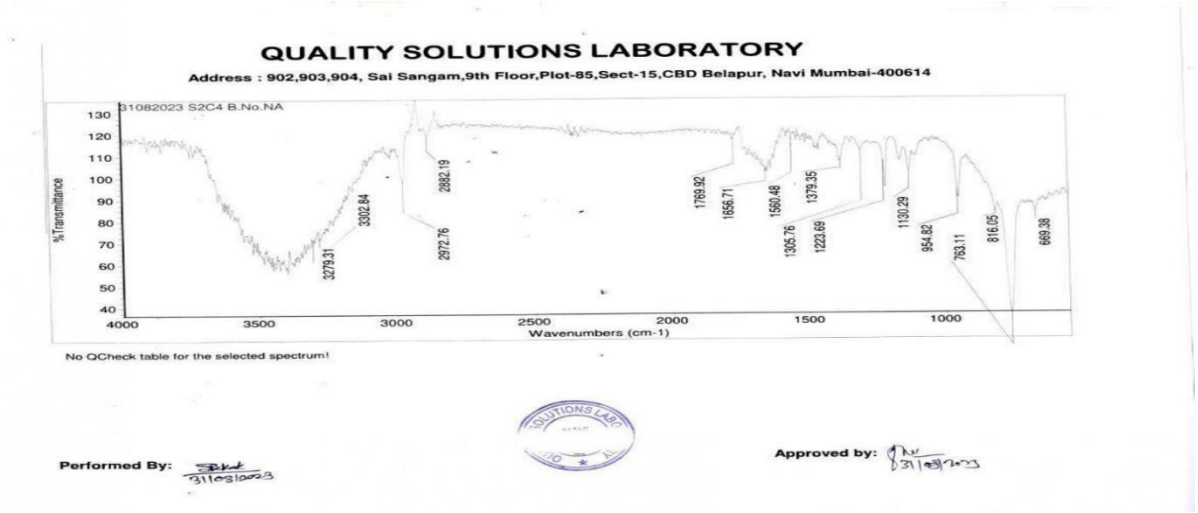

**B) FTIR result of S2C1**

**6.3.**



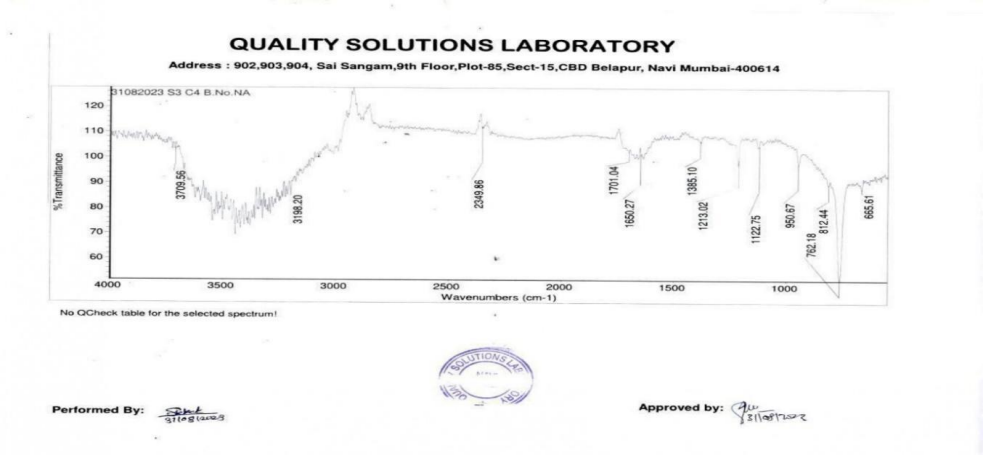
**C) FTIR result of S2C2**

**6.4.**



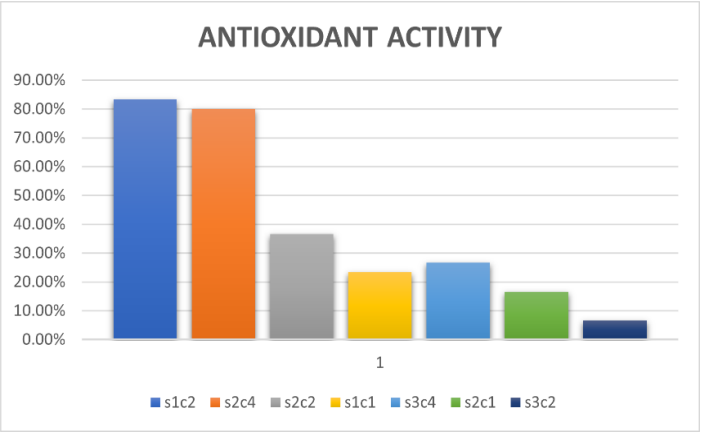

**D) FTIR result of S2C4**

**6.5.**




**E) FTIR result of S3C4**
**Figure No. 7: Characterization by using FTIR**

**7. Assessment of antioxidant potential of extracted lipids:** After performing DPPH assay for assessment of antioxidant potential, the sample S1C2 and S2C4 has 83.3% and 80% antioxidant activity respectively, which is the highest among all samples. The samples S2C2, S1C1, S3C4, S2C1, S3C2 has 36.6%, 23.3%, 26.6%, 16.6%, 6.6% antioxidant activity respectively.



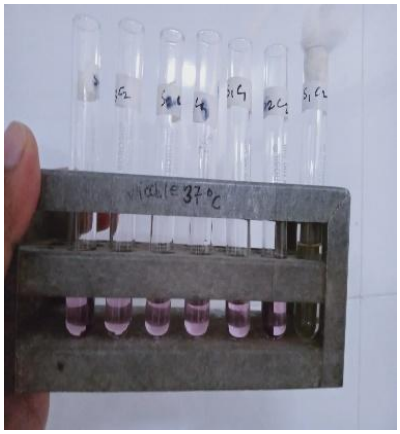



**Figure No. 8: Antioxidant activity of lipids**

**DISCUSSION**:

Soil samples collected from different locations contain diverse bacterial strains that can produce lipids with potential applications in biofuel and biotechnology. Li, S.L. *et al.* has collected 26 soil samples and found 31 isolates which compared to this work in which 12 isolates were found from 3 soil samples. Bajwa *et al* has found 15 isolates, out of which 5 were positive for Sudan black B which compared to this work in which 7 were positive for Sudan black B from 12 isolates. The lipid extraction by Bligh and Dyer method yielded a chloroform phase containing the lipids, which were further analysed by thin layer chromatography (TLC) and Fourier transform infrared spectroscopy (FTIR). The TLC revealed the presence of different types of lipids, such as triacylglycerols (TAG), esters of fatty acids, monoacylglycerols, and phospholipids, based on their Rf values. The FTIR confirmed the presence of these lipids by showing characteristic peaks corresponding to the functional groups of lipids, such as C-H stretching, C-H bending, C-O stretching, N-H stretching, and O-H stretching. The lipid samples also exhibited antioxidant activity, as measured by the DPPH assay, which can be attributed to the presence of unsaturated fatty acids and phenolic compounds in the lipids. The samples S1C2 and S2C4 showed the highest antioxidant activity, followed by S2C2, S1C1, S3C4, S2C1, and S3C2. These results suggest that the bacterial lipids from the soil samples have potential as a renewable source of biofuel and as a natural antioxidant agent. Urval, I.J.B.; Ribeiro, B.G.; Aguiar, J.S.; Rufino, R.D.; Converti, A.; Sarubbo, L.A has extracted lipids from *Bacillus cereus* UCP 1615 and antioxidant potential was recorded 28.45% which is much less compared to the result of this work.

**REFERENCES**:


1. Amer, N. N., Elbahloul, Y., Embaby, A. M., & Hussein, A. (2017). The novel oleaginous bacterium Sphingomonas sp. EGY1 DSM 29616: a value-added platform for renewable biodiesel. *World Journal of Microbiology and Biotechnology*, *33*(7). https://doi.org/10.1007/s11274-017-2305-7
2. Bajwa, K., Bishnoi, N., Toor, M., Gupta, S., Sharma, P., Kirrolia, A., Kumar, S., Sharma, J., & Selvan, S. (2018). Isolation, Screening, Characterization of Indigenous Oleaginous Bacteria: Evaluation of Various Carbon and Nitrogen Sources as Substrates for Single Celled Oil Producing Bacteria. *Asian Journal of Biotechnology and Bioresource Technology*, *3*(1), 1–12. https://doi.org/10.9734/ajb2t/2018/39260
3. Bhutada, G., Kavšček, M., Hofer, F., Gogg-Fassolter, G., Schweiger, M., Darnhofer, B., Kordiš, D., Birner-Gruenberger, R., & Natter, K. (2018). Characterization of a lipid droplet protein from Yarrowia lipolytica that is required for its oleaginous phenotype. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, *1863*(10), 1193–1205. https://doi.org/10.1016/j.bbalip.2018.07.010
4. Bi, K., He, Z., Gao, Z., Zhao, Y., Fu, Y., Cheng, J., Xie, J., Jiang, D., & Chen, T. (2016). Integrated omics study of lipid droplets from Plasmodiophora brassicae. *Scientific Reports*, *6*. https://doi.org/10.1038/srep36965
5. Breil, C., Abert Vian, M., Zemb, T., Kunz, W., & Chemat, F. (2017). "Bligh and Dyer" and Folch methods for solid–liquid–liquid extraction of lipids from microorganisms. Comprehension of solvatation mechanisms and towards substitution with alternative solvents. *International Journal of Molecular Sciences*, *18*(4). https://doi.org/10.3390/ijms18040708
6. Campos, J. M., Montenegro Stamford, T. L., Sarubbo, L. A., de Luna, J. M., Rufino, R. D., & Banat, I. M. (2013). Microbial biosurfactants as additives for food industries. *Biotechnology Progress*, *29*(5), 1097–1108. https://doi.org/10.1002/btpr.1796
7. Cao, Z., Wang, X., Pang, Y., Cheng, S., & Liu, J. (2019). Biointerfacial self-assembly generates lipid membrane coated bacteria for enhanced oral delivery and treatment. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-13727-9
8. Cea, M., Sangaletti-Gerhard, N., Acuña, P., Fuentes, I., Jorquera, M., Godoy, K., Osses, F., & Navia, R. (2015). Screening transesterifiable lipid accumulating bacteria from sewage sludge for biodiesel production. *Biotechnology Reports*, *8*, 116–123. https://doi.org/10.1016/j.btre.2015.10.008
9. Deeba, F., Pruthi, V., & Negi, Y. S. (2016). Converting paper mill sludge into neutral lipids by oleaginous yeast Cryptococcus vishniaccii for biodiesel production. *Bioresource Technology*, *213*, 96–102. https://doi.org/10.1016/j.biortech.2016.02.105
10. Durval, I. J. B., Ribeiro, B. G., Aguiar, J. S., Sarubbo, L. A., Rufino, R. D., & Converti, A. (2021). Application of a biosurfactant produced by bacillus cereus ucp 1615 from waste frying oil as an emulsifier in a cookie formulation. *Fermentation*, *7*(3). https://doi.org/10.3390/fermentation7030189

11. Enshaeieh, M., Nahvi, I., & Madani, M. (2014). Improving microbial oil production with standard and native oleaginous yeasts by using Taguchi design. *International Journal of Environmental Science and Technology*, *11*(3), 597–604. https://doi.org/10.1007/s13762-013-0373-2
12. Fakas, S., Galiotou-Panayotou, M., Papanikolaou, S., Komaitis, M., & Aggelis, G. (2007). Compositional shifts in lipid fractions during lipid turnover in Cunninghamella echinulata. *Enzyme and Microbial Technology*, *40*(5), 1321–1327. https://doi.org/10.1016/j.enzmictec.2006.10.005
13. Fashogbon, R. O., Adebayo, B., Musa, V., & Femi-Ola, T. (2021). Lipolytic Activities of Bacteria and Fungi Isolated from Soil Samples. *Microbiology Research Journal International*, 27–43. https://doi.org/10.9734/mrji/2021/v31i530318
14. Geoffrey, I., Chin, G. J., Lie, W., & Misson, M. (2022). Optimization of lipid production by locally isolated Rhodotorula toruloides using response surface methodology. *Borneo International Journal of Biotechnology (BIJB*, *2*, 31–43. https://doi.org/10.51200/bijb.v2i.3041
15. Grootveld, M., Percival, B. C., Leenders, J., & Wilson, P. B. (2020). Potential adverse public health effects afforded by the ingestion of dietary lipid oxidation product toxins: Significance of fried food sources. *Nutrients*, *12*(4). https://doi.org/10.3390/nu12040974
16. Guo, H., Chen, H., Fan, L., Linklater, A., Zheng, B., Jiang, D., & Qin, W. (2017). Enzymes produced by biomass-degrading bacteria can efficiently hydrolyze algal cell walls and facilitate lipid extraction. *Renewable Energy*, *109*, 195–201. https://doi.org/10.1016/j.renene.2017.03.025
17. Jiru, T. M., & Abate, D. (2014). Oleaginous microorganisms, diversity, lipid biosynthesis pathway and strain improvement. *WebPub Journal of Scientific Research*, *2*(6), 55–65. http://www.researchwebpub.org/wjsr
18. Jones, A. D., Bou Jones, A. D., Boundy-Mills, K. L., Barla, G. F., Kumar, S., Ubanwa, B., & Balan, V. (2019). Microbial lipid alternatives to plant lipids. In *Methods in Molecular Biology* (Vol. 1995, pp. 1–32). Humana Press Inc. https://doi.org/10.1007/978-1-4939-9484-7_1
19. Karim, A., Yousuf, A., Islam, M. A., Naif, Y. H., Faizal, C. K. M., Alam, M. Z., & Pirozzi, D. (2018). Microbial lipid extraction from Lipomyces starkeyi using irreversible electroporation. *Biotechnology Progress*, *34*(4), 838–845. https://doi.org/10.1002/btpr.2625
20. Kharlamenko, V. I., Zhukova, N. v, Khotimchenko, S. v, Svetashev, V. I., & Kamenev, G. M. (1995). *Fatty acids as markers of food sources in a shallow-water hydrothermal ecosystem (Kraternaya Bight, Yankich Island, Kurile Islands)* (Vol. 120).
21. Khobragade, C., Gophane, S. R., Banasavade, V. B., & Marathe, N. (2020). Isolation and characterization of oleaginous yeasts from dairy waste. *Indian Journal of Dairy Science*, *73*(3), 236–241. https://doi.org/10.33785/ijds.2020.v73i03.007
22. Kumar, R. R., Rao, P. H., & Arumugam, M. (2015). Lipid extraction methods from microalgae: A comprehensive review. In *Frontiers in Energy Research* (Vol. 3, Issue JAN). Frontiers Media S.A. https://doi.org/10.3389/fenrg.2014.00061
23. Kurosawa, K., Laser, J., & Sinskey, A. J. (2015). Tolerance and adaptive evolution of triacylglycerol-producing Rhodococcus opacus to lignocellulose-derived inhibitors. *Biotechnology for Biofuels*, *8*(1). https://doi.org/10.1186/s13068-015-0258-3
24. Lin Li, S., Lin, Q., Ran Li, X., Xu, H., Xi Yang, Y., Rong Qiao, D., & Cao, Y. (2012). BIODIVERSITY OF THE OLEAGINOUS MICROORGANISMS IN TIBETAN PLATEAU. *Brazilian Journal of Microbiology*, 627–634.
25. Liu, T., Chen, Z., Xiao, Y., Yuan, M., Zhou, C., Liu, G., Fang, J., & Yang, B. (2022). Biochemical and Morphological Changes Triggered by Nitrogen Stress in the Oleaginous Microalga Chlorella vulgaris. *Microorganisms*, *10*(3). https://doi.org/10.3390/microorganisms10030566
26. Mahan, K. M., Le, R. K., Wells, T., Anderson, S., Yuan, J. S., Stoklosa, R. J., Bhalla, A., Hodge, D. B., & Ragauskas, A. J. (2018). Production of single cell protein from agro-waste using Rhodococcus opacus. *Journal of Industrial Microbiology and Biotechnology*, *45*(9), 795–801. https://doi.org/10.1007/s10295-018-2043-3
27. Mrunalini, B. R., & Girisha, S. T. (2017). Screening and Characterization of Lipid inclusions in Bacteria by Fluorescence Microscopy and Mass Spectrometry as a Source for Biofuel Production. *Indian Journal of Science and Technology*, *10*(21), 1–10. https://doi.org/10.17485/ijst/2017/v10i21/111382
28. Patel, A., Karageorgou, D., Rova, E., Katapodis, P., Rova, U., Christakopoulos, P., & Matsakas, L. (2020). An overview of potential oleaginous microorganisms and their role in biodiesel and omega-3 fatty acid-based industries. In *Microorganisms* (Vol. 8, Issue 3). MDPI AG. https://doi.org/10.3390/microorganisms8030434

29. Patel, A., Mikes, F., & Matsakas, L. (2018). An overview of current pre-treatment methods used to improve lipid extraction from oleaginous microorganisms. In *Molecules* (Vol. 23, Issue 7). MDPI AG. https://doi.org/10.3390/molecules23071562
30. Patel, A., Pruthi, V., & Pruthi, P. A. (2017). Synchronized nutrient stress conditions trigger the diversion of CDP-DG pathway of phospholipids synthesis towards de novo TAG synthesis in oleaginous yeast escalating biodiesel production. *Energy*, *139*, 962–974. https://doi.org/10.1016/j.energy.2017.08.052
31. Qadeer, S., Khalid, A., Mahmood, S., Saleem, A. R., & Anjum, M. (2023). Valorization of Food Waste Slurry as Potential Candidate for Lipid Accumulation: A Concept of Oleaginous Bio-Refinery. *Fermentation*, *9*(2). https://doi.org/10.3390/fermentation9020163
32. Ren, H. Y., Liu, B. F., Ma, C., Zhao, L., & Ren, N. Q. (2013). A new lipid-rich microalga Scenedesmus sp. strain R-16 isolated using Nile red staining: Effects of carbon and nitrogen sources and initial pH on the biomass and lipid production. *Biotechnology for Biofuels*, *6*(1). https://doi.org/10.1186/1754-6834-6-143
33. Shields-Menard,; ), Amirsadeghi, M., & Blake, J. (2017). The Effects of Model Aromatic Lignin Compounds on Growth and Lipid Accumulation of Rhodococcus rhodochrous. In *Janet R. Donaldson* (Issue 1). Rafael Hernandez. http://www.elsevier.com/open-access/userlicense/1.0/
34. Urbano, S. B., di Capua, C., Cortez, N., Farías, M. E., & Alvarez, H. M. (2014). Triacylglycerol accumulation and oxidative stress in Rhodococcus species: Differential effects of pro-oxidants on lipid metabolism. *Extremophiles*, *18*(2), 375–384. https://doi.org/10.1007/s00792-013-0623-8
35. Valdés, G., Mendonça, R. T., & Aggelis, G. (2020). Lignocellulosic biomass as a substrate for oleaginous microorganisms: A review. In *Applied Sciences (Switzerland)* (Vol. 10, Issue 21, pp. 1–43). MDPI AG. https://doi.org/10.3390/app10217698
36. van de Faculteit Geowetenschappen, M., & Rattray, J. E. (n.d.). *GEOLOGICA ULTRAIECTINA Ladderane Lipids in Anammox Bacteria: Occurrence, Biosynthesis and Application as Environmental Markers*.
37. Wang, B., Rezenom, Y. H., Cho, K. C., Tran, J. L., Lee, D. G., Russell, D. H., Gill, J. J., Young, R., & Chu, K. H. (2014). Cultivation of lipid-producing bacteria with lignocellulosic biomass: Effects of inhibitory compounds of lignocellulosic hydrolysates. *Bioresource Technology*, *161*, 162–170. https://doi.org/10.1016/j.biortech.2014.02.133
38. Wu, B., Zheng, D., Zhou, Z., Wang, J. L., He, X. L., Li, Z. W., Yang, H. N., Qin, H., Zhang, M., Hu, G. Q., & He, M. X. (2017). The Enrichment of Microbial Community for Accumulating Polyhydroxyalkanoates Using Propionate-Rich Waste. *Applied Biochemistry and Biotechnology*, *182*(2), 755–768. https://doi.org/10.1007/s12010-016-2359-2
39. Yellapu, S. K., Bharti, Kaur, R., Kumar, L. R., Tiwari, B., Zhang, X., & Tyagi, R. D. (2018). Recent developments of downstream processing for microbial lipids and conversion to biodiesel. In *Bioresource Technology* (Vol. 256, pp. 515–528). Elsevier Ltd. https://doi.org/10.1016/j.biortech.2018.01.129
40. Zhan, J., Hong, Y., & Hu, H. (2016). Effects of nitrogen sources and C/N ratios on the lipid-producing potential of chlorella sp. HQ. *Journal of Microbiology and Biotechnology*, *26*(7), 1290–1302. https://doi.org/10.4014/jmb.1512.12074
41. Zheng, C. (2020). Isolation and Characterization of Lipid-Producing Bacillus sp. TC14. *Journal of Physics: Conference Series*, *1549*(3). https://doi.org/10.1088/1742-6596/1549/3/032027
42. Durval, I. J. B., Ribeiro, B. G., Aguiar, J. S., Sarubbo, L. A., Rufino, R. D., & Converti, A. (2021). Application of a biosurfactant produced by bacillus cereus ucp 1615 from waste frying oil as an emulsifier in a cookie formulation. *Fermentation*, *7*(3). https://doi.org/10.3390/fermentation7030189
43. Cao, Z., Wang, X., Pang, Y., Cheng, S., & Liu, J. (2019). Biointerfacial self-assembly generates lipid membrane coated bacteria for enhanced oral delivery and treatment. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-13727-9
44. Campos, J. M., Montenegro Stamford, T. L., Sarubbo, L. A., de Luna, J. M., Rufino, R. D., & Banat, I. M. (2013). Microbial biosurfactants as additives for food industries. *Biotechnology Progress*, *29*(5), 1097–1108. https://doi.org/10.1002/btpr.1796