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Exploring microbial oil from oleaginous actinobacteria *Streptomyces fradiae* for biodiesel application

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Abstract
Streptomyces fradiae is a commonly used microorganism for designing the targeted antibiotic drugs. It has many medicinal properties, due to the presence of antibiotics, antioxidants and other bioactive compounds. The lipid producing efficiency of *Streptomyces fradiae* in biodiesel feedstock production is explored in this study, the fermentation of *Streptomyces fradiae* and *Aspergillus fumigatus* was successful using waste rice wash water (RWW). In this communication, the efficiency of most widely used algae *Aspergillus fumigatus* in biodiesel feedstock production is compared with *Streptomyces fradiae* **JJ1 MK733985.1.** The result showed that *Streptomyces fradiae* grown in RWW produced a lipid content of 15% from biomass and, the microbial oil yield was 7.5g/L. Whereas, the results of *Aspergillus fumigatus* showed a lipid content of 12% from biomass and the microbial oil yield was 4.8g/L. According to the findings, *Streptomyces fradiae* was comparatively more beneficial than *Aspergillus fumigatus* in microbial oil production.

Keywords
Microbial oil; *Streptomyces fradiae*; *Aspergillus fumigatus*; Biodiesel feedstock.

1.Introduction

Climatic change across the globe has led to numerous green gas emissions from fossil fuel combustion resulting in global warming that remains uncontrolled due to increasing air pollutants. To reduce green gas emissions immediate action has to be taken to avoid depletion of fossil fuel by implementing alternate fuel from renewable resources. Biodiesel is an important liquid fuel, produced from microbial communities are beneficial in providing a non-toxic, biodegradable, and ecofriendly environment **(Lund et al., 2014).** Commercialization of biodiesel is generated based on the Fatty acid methyl ester (FAME) profile, supporting the properties of oxidative stability, fuel viscosity, cetane number etc., **(Ferruh et al., 2020)**

Many types of actinobacteria have the efficiency to accumulate more than 20% of microbial oil in the form of intracellular lipids under nitrogen limiting conditions **(Mhlongo et al., 2021)**. It is evident that many researches confirmed the presence of lipids (microbial oil) in many fungi including *Aspergillus fumigatus.* The level of lipid accumulation exceeds 20% from fungal biomass are stored in the form of triacylglycerol (TAG) **(Abu-Elreesh et al., 2014)**. Many industrial bio processes require the use of

complex media for cultivation of microorganisms **(Boortseva et al., 2018).** Incorporation of these oleaginous microbial systems in an organic waste nutrient supply (RWW) ensures sustainable biodiesel feedstock production **(Meng., et al 2009).** Initially, the raw materials from the substrate get degraded by the oleaginous microorganism and get converted to volatile fatty acids (VFAs) that finally form the lipids **(Fontanille et al., 2012 and, Moon et al., 2013).** Actinobacteria grow faster than microalgae, with less chance of contamination possibilities when compared with microalgae, this unique property makes actinobacteria suitable for microbial oil production. Utilization of wastes from the organic matter serves as a substrate in biodiesel feedstock production thus, evolves the microbial resources in designing new platform for energy research **(Arvanitoyannis et al., 2008, Jin et al., 2007 and Ezeonu 2014)**. The mechanism of lipid accumulation in oleaginous microorganisms follows the fatty acid biosynthetic pathway that produces acetyl-CoA and NADPH as a precursor before TAG synthesis. Lipids are the primary storage compounds in most bacteria but a majority of the bacterial species accumulate complex lipids or polymeric lipids such as poly-hydroxyalkanoate (PHA) and poly (β hydroxybutyrate) (PHB), that cannot be used for biodiesel production. Only a few bacteria including *Actinomyces, Arthrobacter, Dietzia, Gordonia, Mycobacterium, Nocardia, Rhodococcus,* and *Streptomyces* species accumulate microbial oil (lipids) inside their cell mycelium. These oils are stored in the form of TAG and wax esters (WE) that can be used as biodiesel feedstock **(Schreiberova et al., 2010 and Debora et al., 2020).** Lipogenesis occurs when the entire nitrogen source in the growth medium gets completely utilized by the microbe in the presence of an excessive carbon source that induces the cell to undergo continuous biochemical reactions leading to *de novo* fatty acid biosynthesis in the presence of the enzyme fatty acid synthetase (FAS). The whole mechanism continues repeatedly inside the cytosol. Lipid production can be enhanced by treating the oleaginous microorganisms with stress-related studies in the microbial cell by implementing metabolic engineering techniques that help compile the desired fatty acids profile suitable for biodiesel feedstock production **(Ahmed et al., 2020).** This study focuses on isolation of two different oleaginous microorganisms, one is the most widely used algae *Aspergillus fumigatus* commonly known for biodiesel feedstock production. The other microbe is an actinobacteria, *Streptomyces fradiae* isolated from the soil sample. Finally, the efficacy of both the microbial oil was investigated based on their fatty acid composition and fuel properties.

2. Materials and methods:

2.1 Isolation of oleaginous microorganisms

The soil sediments from the sea shores of Indian east coast Mamallapuram beach, Tamil Nadu, India, were collected and processed for immediate actinobacteria isolation. Finally, 53 isolates of actinobacteria was successfully isolated using Actinobacteria Isolation Media (AIM) based on previous research protocols **(Janice et al., 2024).** All the 53 isolates were stored carefully in slants of Starch Casein Agar (SCA) and, preserved at 4°C. *Aspergillus fumigatus* were sourced from the same soil sediment sample used for actinobacteria isolation. 1g of collected sample was serially diluted by adding 9ml of distilled water.1ml of each dilution was spread on potato dextrose agar (PDA) plates supplemented with 100mg/L chloramphenicol, and all the plates were incubated for 7 days at room temperature. Pure fungal colonies were picked and inoculated on PDA slants and, preserved at 4°C. **(Haliru and Adebayo, 2012)**

2.2 Identification of oleaginous *Streptomyces fradiae*

The identification of the potential strain among the 53 isolates was determined by previously published process of Sudan black B staining experiment. Fixed smears of every isolates were mounted on 0.3% Sudan black B stain (0.3g in 100ml of 70% alcohol) followed by immersion of xylene and methylene blue as the counterstain. The mounts were observed under a light microscope. Molecular identification of the strong strain was carried out using the primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') /

1492R (5'-AGAGTTTGATCMTGGCTCAG-3') 16S Recombinant RNA (rRNA) sequencing technique. The results identified that the potential isolate is ***Streptomyces fradiae*** **JJ1 MK733985.1 (**99% sequence similarity with ***Streptomyces fradiae*** **RSU15)** based on the 16s rRNA gene sequence analysis (GenBank accession number KP698740.1).

2.3 Biomass production

Fermentation of *Streptomyces fradiae* and *Aspergillus fumigatus* in RWW grew well after 5th day of incubation. The biomass was produced as pellets with natural flocculation allowed biomass separation without difficulties. After harvest of biomass Bligh and dyer method **(Ulmer et al., 2018)** was employed for microbial oil extraction. The fermentation of *Streptomyces fradiae* using RWW reduces the risk of contamination with accelerated biomass production **(Ravi kumar et al., 2024).** The total lipid content in extracted microbial oil was estimated using phosphor-vanillin assay. Finally the extracted microbial oil is transesterified to fatty acid methyl esters (FAME) and analyzed in GC-Mass.

3. Results and discussion

3.1 Microbial oil analysis

According to the Sudan black B staining results, depicted in **Fig.1A** confirmed the presence of lipid bodies all over the vesicles. The total microbial oil yield from *Streptomyces fradiae* grown in RWW was found to be 7.5g/L with 15% lipid content from biomass whereas; *Aspergillus fumigatus* produced 4.8g/L of microbial oil with 12% lipid content from biomass is represented in **Fig.4A.** The results of the biochemical estimation of microbial oil synthesized by *Streptomyces fradiae* and *Aspergillus fumigatus* is shown in **Table 1 and 2.** The microbial oil produced by *Streptomyces fradiae* in RWW is picturized in **Fig.2B**. Fermentation of *Aspergillus fumigatus* in RWW is depicted in **Fig.5A** and, the extracted microbial oil is depicted in **Fig.5B**. The large scale bioreactor set up with 10 liter fermentation capacity was used for microbial oil production is depicted in **Fig.3.** The result of GC-Mass indicating the presence of esters in microbial oil required for the conversion of FAME is depicted in **Fig.7.** The composition of essential fatty acids required for biodiesel production is depicted in **Fig.4B**

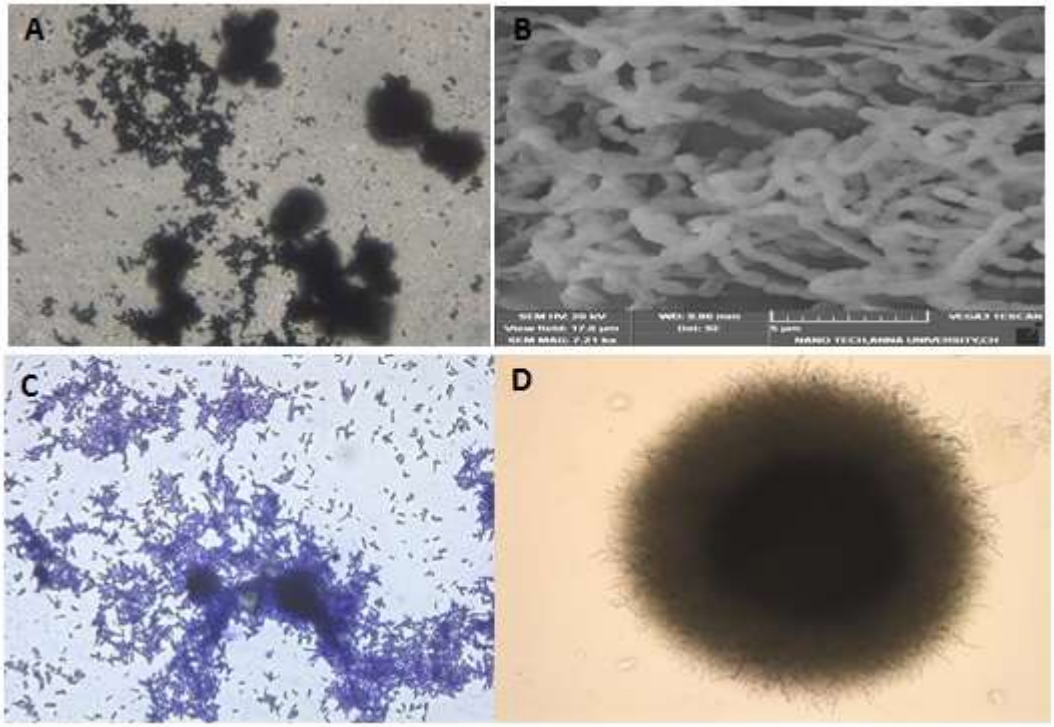

Fig 1.A Light microscopic view of lipid bodies of ***Streptomyces fradiae*** **JJ1 MK733985.1**
Stained with Sudan black B stain

Fig 1.B Scanning electron microscopic morphology of ***Streptomyces fradiae*** **JJ1 MK733985.1** showing smooth spore surface

Fig 1.C Gram positive confirmation of ***Streptomyces fradiae*** **JJ1 MK733985.1**

Fig.1.D Light microscopic view of *Streptomyces fradiae* **JJ1 MK733985.1**

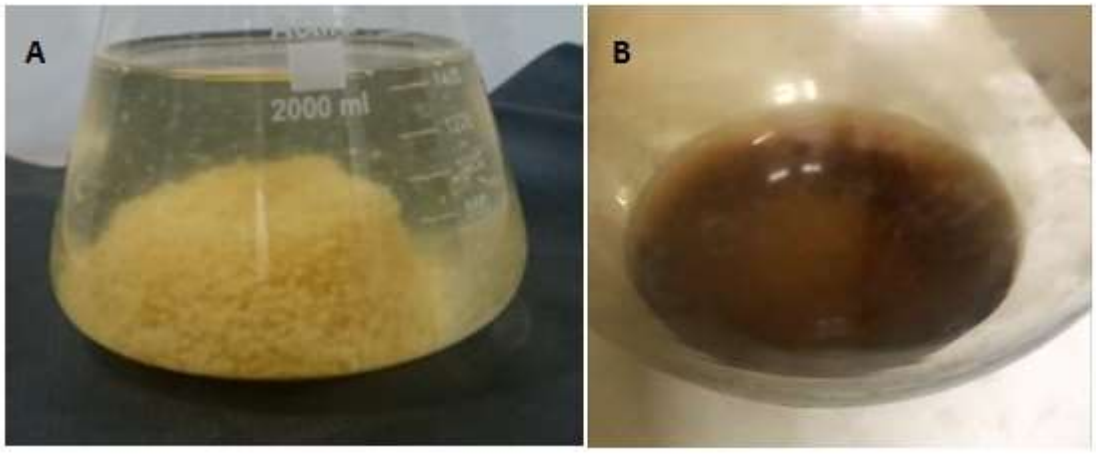

Fig.2A Flocculation efficiency of biomass produced by ***Streptomyces fradiae*** **JJ1 MK733985**

Fig. 2B Microbial oil produced by ***Streptomyces fradiae*** **JJ1 MK733985.1**

Fig.3 Large scale production of microbial oil

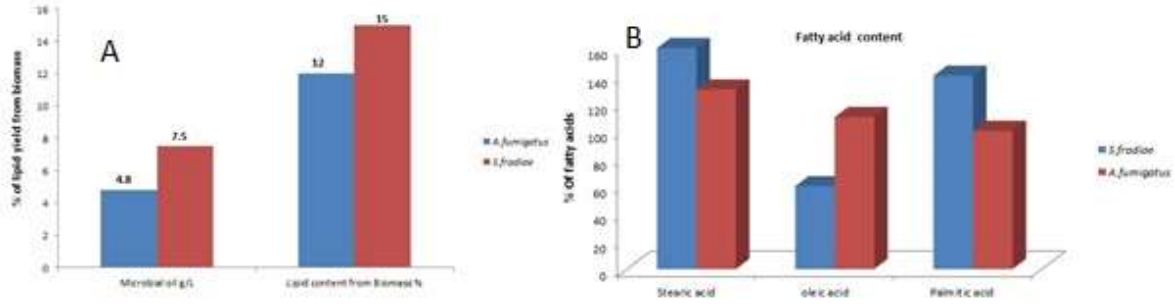

Fig.4.A Total lipid content from biomass produced by ***Streptomyces fradiae*** **JJ1 MK733985 and** *Aspergillus fumigatus*

Fig.4B. Fatty acid profile of ***Streptomyces fradiae*** **JJ1 MK733985 and** *Aspergillus fumigatus*

Fg.5A Cultivation of *Aspergillus fumigatus* in RWW
Fig.5B. Microbial oil produced by *Aspergillus fumigatus* in RWW

3.2 Microbial oil characterization

The acid value (AV) for *S.fradiae* oil and *A.fumigatus* oil was determined using the following formula below.

Acid value (mg KOH/g) = Vol. of titrant × Normality of KOH × 56.1/ Wt. of sample (g)

The peroxide value (PV) for *S.fradiae* oil and *A.fumigatus* oil was determined using the following formula obtained by the titrimetric methods.

Peroxide Value (mEq peroxide /kg oil) = Titre value × Normality of sodium thiosulfate×1000/ Wt. of sample (g)

Based on the results of **Table 2** the acid value and peroxide value of microbial oil is higher indicates the faster autoxidation property of fatty acids on elevated storage conditions. This also indicates the presence of high levels of unsaturated fatty acids that can undergo autoxidation in a minimal time period **(Langseter et al., 2021)**. The higher concentration of unsaturated fatty acids is positively correlated with oil rancidity **(Feiner et al., 2016 and Negash et al., 2019). Table 1**

Constant burette readings

Constant burette readings (ml)	*S.Fradiae* oil	*A.Fumigatus* oil
Acid value	3.1	2.8
Peroxide value	18.1	17.6

Table 2

Biochemical analysis of *S.Fradiae* oil and *A.Fumigatus* oil

Sample	*Acid value* (mg KOH/g oil)	Peroxide Value (mEq of peroxide/kg oil
S.Fradiae oil	0.46	47.02
A.Fumigatus oil	0.48	48.03

3.3 Morphological characterization

The morphology of *Streptomyces fradiae* was analyzed based on their mycelial and cellular morphology observed under a light microscope depicted on **Fig.1D** The colony morphology of *Streptomyces fradiae* indicates the presence of aerial and substrate mycelium is depicted in **Fig6.** The mycelial morphology was observed in scanning electron microscopy (SEM) is depicted on **Fig.1B**. The gram staining technique further confirmed that *Streptomyces fradiae* belongs to gram positive group of bacteria is depicted in **Fig1C.** The biomass flocculation efficiency of *Streptomyces fradiae* after fermentation is depicted in **Fig.2A**.

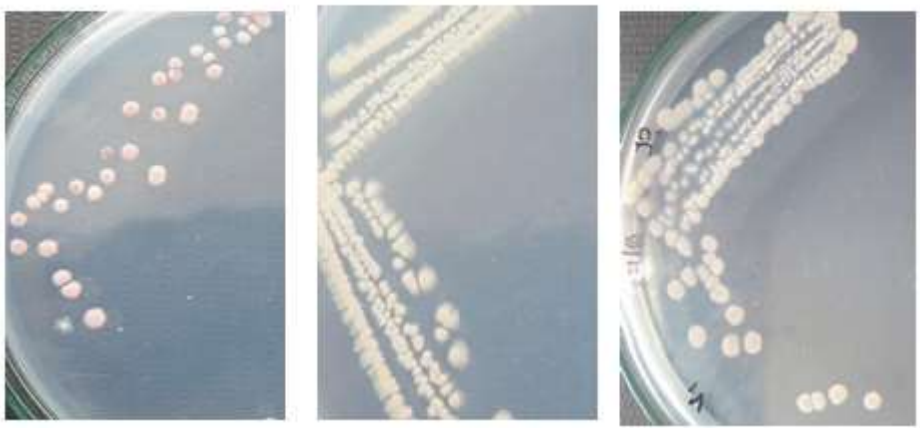

Fig.6 Cultural colony morphology of *Streptomyces fradiae* **JJ1 MK733985.1**

4. Conclusion

This study outlines the efficiency of oleaginous microorganism *Streptomyces fradiae* JJ1 MK733985.1 to produce microbial oil more efficient than the commonly used algae *Aspergillus fumigatus* in biodiesel feedstock production. RWW is proved to be the best alternative medium to grow both algae and actinobacteria in an inexpensive method to produce biodiesel directly from starch. From the analysis of this study, it is evident that incorporation of RWW during fermentation supported the biomass growth and increased the lipid content to15% with a microbial oil yield of 7.5g/L. The higher acid values and peroxide values of both the microbial oil indicate faster autoxidation of microbial oil. The addition of antioxidants in microbial oil may help to prevent autoxidation and block the formation of free radicals. Further analysis of our microbial oil helps to identify the therapeutic values and other possible applications.

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