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Improving Fungal Product Yield through Fast, Eco-friendly, and Cost-effective Chrom Genic Media Technology

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AD Shaban and KFK conceptualized the research work. Authors AD Shaaban and SJL performed the methodology. All authors have read and agreed to the published version of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The cost of commonly available culture media is a source of concern for many researchers, highlighting the need to explore more cost-effective alternatives or reduce the use of additives. Despite limited research in this area, efforts have been made to identify alternative culture media that are economically viable for cultivating microorganisms. This study explored the potential of beetroot extract as a cost-effective medium with col-or-inducing properties for fungal growth and as a chromogenic medium for testing lipase enzyme production efficiently as one of the rapid differential tests. The results indicated that Beetroot Agar Medium (BAM) offered significant cost

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savings in fungal cultivation while facilitating rapid fungal growth compared to other expensive and time-consuming media. Within a 3-4 day incubation period at a temperature range of 25-27 ˚C, all isolated fungi exhibited robust growth. Furthermore, the medium successfully served as a chromogenic differentiating medium by inducing fungi to produce lipase. These findings have broad implications for bio-technology and industrial applications, providing a sustainable and efficient alternative for microbial cultivation.

Keywords: A chromogenic medium; BAM; eco-friendly; fungi; productivity.

1. INTRODUCTION

Microorganisms are known to have various uses and benefits in different life applications, and they are employed in different industrial processes [1]. The field of application includes industrial processes such as the food industry, medicine, agriculture, the chemical industry, the energy industry, biomass conversion, and other sectors [2]. Most of these processes harness the ability of microorganisms to produce cellular biomass [3], proteins [4], as well as primary and/or secondary metabolites [5]. Traditional growth media for edible or medicinal fungi encompass a variety of substrates [6]. Since available culture and differentiation media for microorganisms are generally expensive, it is imperative to identify cheaper alternatives or reduce additives, thus making culture media more cost-effective for obtaining affordable products [7]. Limited research has been conducted to address this issue.

As a cost-effective alternative for cultivating fungi, some studies have explored the use of economical materials [8]. An attempt was made to develop a medium for enhancing the germination of bacterial spores and the growth of bacterial, yeast, and fungal cell cultures using various ingredients, including bananas or any member of the Musa genus, which was patented in the United States by Lyte [9] in the year 2020. In another patent registered in the People's Republic of China by Kelly and Langham [10], cocoa was added to improve the utilization of fungal bacterial strains in the substrate.

Many researchers have approached the development of alternative media for fungal growth to reduce the cost for researchers by utilizing industrial media. Inexpensive sources like Chickpea Dextrose Agar, Bean Dextrose Agar, Carrot Dextrose Agar, Papaya Dextrose Agar, Czapek Dox Agar, and Sabouraud's Dextrose Agar have been employed for fungal growth [11]. In general, the development of cost-effective and rapid fungal growth media, in addition to reducing the cost of fungal tests, remains a crucial objective. This study aimed to create an economical medium for fungal growth using Beetroot Agar Medium (BAM) while also utilizing this medium as a screening (chromogenic) medium for expedient assays in the synthesis of lipase by fungi.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Waksman's technique was employed to separate soil-based fungus samples. One gram of every sample was placed in ten milliliters of sterile distilled water (SDW) and violently stirred for five minutes to achieve the initial dilution (10-1). Then, to make the second dilution (10-2), 1 milliliter of the first dilution was combined with nine milliliters of SDW; lastly, to make the final dilution, 1 milliliter of the previous dilution was blended with nine milliliters of SDW [12].

2.2 Media Preparation

Potato Dextrose Agar (PDA): The medium used for fungal development was prepared by dissolving 39 grams of PDA powder (HIMEDIA, India) in one liter of distilled water. After that, further heat is applied to the mixture until the dissolution process is complete. Following the completion of the dissolution process, 0.05 grams of chloramphenicol were injected to the medium. Following this, the whole mixture is sterilized utilizing an autoclave for full dissolution so as making it suitable for the cultivation of fungi.

BAM for fungi growth and lipase test: Very carefully, a protocol had been used in carrying out the process of the development of a growth medium for fungi. Five hundred grams of Beta vulgaris were first put in a liter of distilled water, weighing about 50% percent (formula1) and left to settle for about 20 minutes. Further, heating of the mixture was done for about 15 minutes, after which it was filtered using medical gauze. After

filtering, 0.05 grams of chloramphenicol were added to the liquid so that bacteria growth in the medium would remain low. The mixture Was then cooked under certain circumstances to make sure it's well mixed, and 20 grams of the agar were added to it. Finally, the mixture was sterilized in an autoclave with a maintenance temperature of 121 ˚C for 15 minutes. This was only done to make sure the process of sterilization has been completed properly. In addition, the isolated fungi were measured for the produced lipase using the formulated medium by adding one ml of castor oil before the medium was set for sterilization in order for the fungi to be induced to produce lipase in the medium.

As a result, this process has come to be one of the standardized procedures, which can sustain the growth of fungi and also help in measuring the productivity of lipase in standardized growth medium. Hence, this process was done very precisely and in full compliance with all protocols practiced by the scientific community.

The total volume of the mixture (%)= (1)
Tn pieces * size cm^3/piece / 1000 cm^3

$$\text{Total volume of the beetroot pieces} = \frac{500\ \text{g}}{1\ \frac{g}{cm^3}} = 500\ \text{cm}^3$$

$$\text{Volume of each piece of beetroot} = 4\ \text{cm}^3$$

$$\text{Total number (Tn) of beetroot pieces added to the water}$$

$$= \frac{500\ \text{cm}^3}{4\ \text{cm}^3} = 125\ \text{pieces}$$

$$\text{The total volume of the water and the pieces together} = 1\ \text{L}\ (1000\ cm^3)$$

$$\text{Volume of the beetroot pieces relative to the total volume of the mixture}$$

$$= \frac{125\ \text{pieces} * 4\ \frac{\text{cm}^3}{\text{pieces}}}{1000\ \text{cm}^3} = 0.5 = 50\%$$

2.3 Fungal Isolates

Three replicates of each dilution were dispensed onto Petri dishes using sterile pipettes to inoculate one milliliter of the suspension of each concentration. The dishes containing the soil samples were subsequently filled with the dissolved PDA medium and allowed to solidify. These dishes were then incubated at 25°C for a period of three – five days. After the incubation period, all dishes were examined using the naked eye, a dissecting microscope, and a light microscope. The purified isolates were preserved and stored at a temperature of 4°C. To facilitate their identification, all fungal isolates were transferred to PDA plates for purification and isolation.

2.4 Morphological Identification of Fungi

Identification of the fungal isolates was done based on the description of their detailed morphological features like shape, color, expansion of the colony, its margin, characteristic aerial mycelium, and color of the reverse side, pigment production among others. The structures of fungi were made visible after adding lactophenol cotton blue, and microscopic examination was conducted. The taxonomic keys guided the identification of the fungal isolates.

2.5 Detection of Fungal Growth and Lipase Production

Fungal growth in the incubation of the ready medium was observed when the medium was exposed to approximately 25°C for about three to four days. On the contrary, lipase production was determined on the basis of lipolysis activity evidenced by the clarity zone in the medium around the fungal colony. This method avoided the use of chemicals and media that would be expensive in order to obtain the lowest cost and results in the least time.

The lipolysis was sequentially obtained by the following process: at the beginning, a disc was removed from a pre-identified fungal culture, and the latter was set right in the centre of a Petri dish, in which the lipase test medium was added. The medium was incubated at 27°C for four days. This process was carried out by cultivating three replicates for each pre-diagnosed fungal species and using three control dishes that were devoid of any fungal culture.

Generally, such a rigorous approach to procedures assured the accurate detection of fungal growth and lipase production in the medium and allowed the reduction of financial and time costs of such procedures. The ratio of lipolysis activity around the colonies was then calculated using formulae (2–5), taking into account the diameters of fungal colonies and the precipitations, which indicate lipolysis activity.

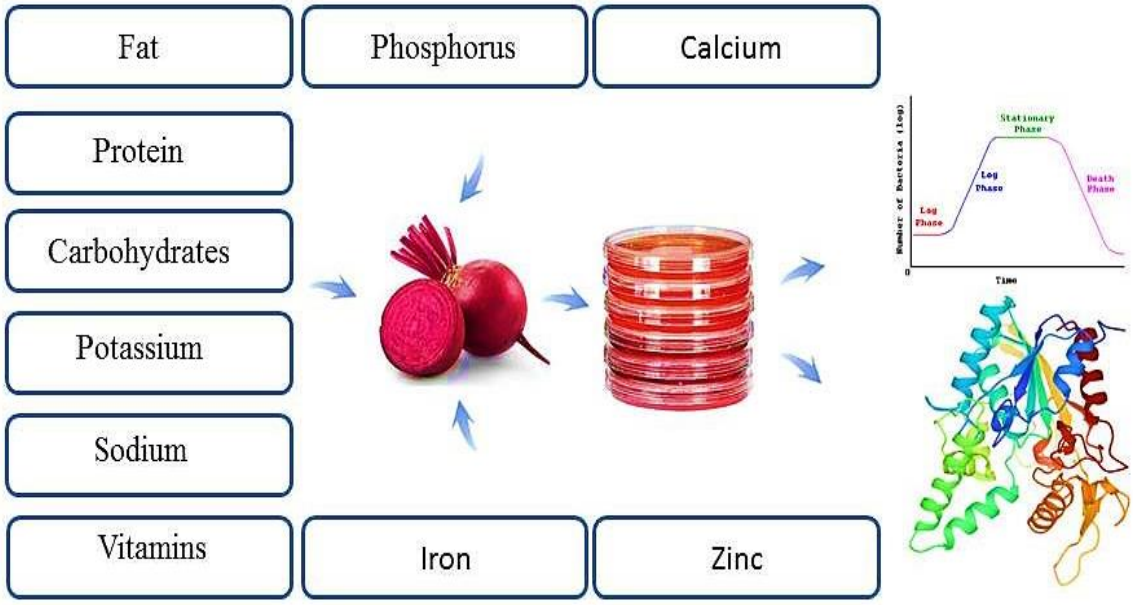

Fig. 1. Composition of beetroot (Deshmukh et al., 2018) and practical steps for the current Study

$$\text{CGD (mm)} = \frac{(\sum R - \text{CGD})}{4} \quad (2)$$

$$\text{LD (mm)} = \frac{(\sum R - \text{LD})}{4} \quad (3)$$

$$\text{LP} = \frac{(\sum R - \text{LD})}{(T - \sum R - LD)} * 100 \quad (4)$$

$$\text{LP}/CGD = \frac{(\sum R - \text{LD})}{\text{CGD}} * 100 \quad (5)$$

Where:

R = rate,
ΣR = summation rate,
CGD = colony growth diameter (mm),
ΣR-LD = 4-sides for lipolytic activity (mm),
ΣR-CGD = 4-sides for colony growth diameter (mm),
T = total,
LP = lipolytic percentage.

3. RESULTS

3.1 Isolation of Fungi

Over 100 fungal isolates were obtained through soil dilution method and were classified into six genera and ten species. Most of the fungi belonged to the Deuteromycetes group, which includes *Alternaria* sp (Alsp), *A. fumigatus*(Asfu), *A. niger*(Asni), *A. terreus*(Aste), *A.flavus*(Asfl), *A.ochraceus*(Asoc), *Mucor* sp(Musp), *Fusarium* sp(Fus), *Penicillium* sp(Pesp), and yeast(Yesp). The dominant fungal species was *Aspergillus* spp (Assp). In addition to these species, some previously identified fungi such as *Alternaria solani*(Also) were tested , which was identified in the biology department of the College of Education-Qurna, Basra University.

3.2 Fungi Growth on BAM and Testing their Ability to Produce Lipase

All the isolated fungi exhibited robust growth in the medium after three - four days of incubation. They developed extensive mycelium within a temperature range of 25-27 °C. The highest growth was observed in *Fusarium* sp., with a diameter of more than 65mm in a petri dish, followed by *Alternaria solani* at about 45mm, and then *A. flavus* and *A. niger*, both at approximately 40mm (see Figs. 2 and 3). The ability of the fungi to produce lipase varied from weak to medium, and high to very high. All filamentous fungi demonstrated a medium to very high capacity for lipase production. For instance, *Aspergillus* sp. displayed the highest ability in lipase production (see Figs. 4 and 5).

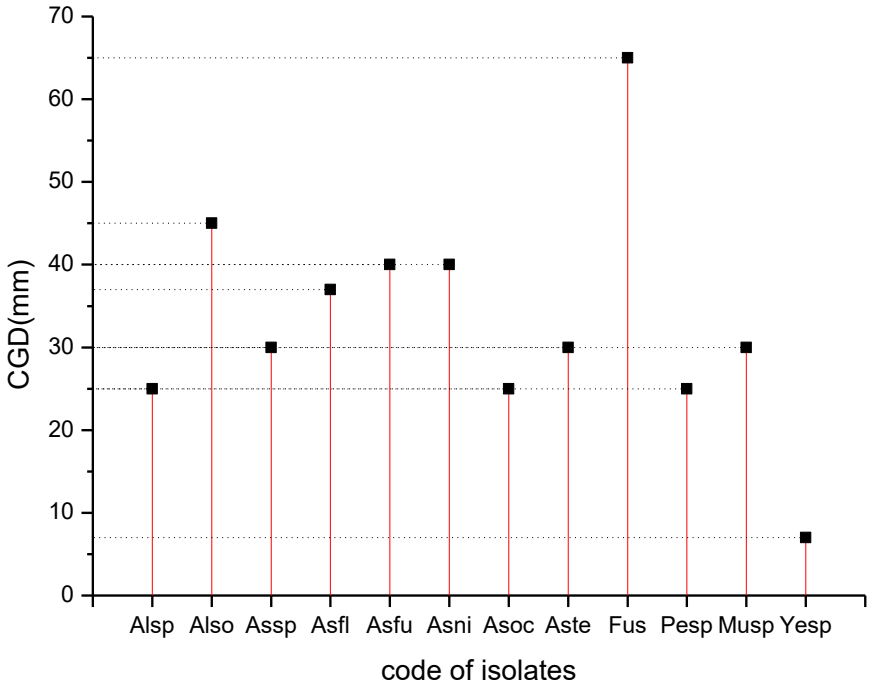

Fig. 2. CGD production for fungi on BAM after about 3-4 day

Table 1. Statistical results of CGD production for fungal species after about 3-4 day

Replicates	Count	Mean	Median	Std Dev	Min/Max	Variance	Range	Sig.
R1	12	32.67	30.00	13.23	10 / 65	175.56	55	P<0.05
R2	12	34.33	30.00	12.32	4 / 65	151.56	61	
R3	12	31.83	30.00	12.17	7 / 65	148.27	58	

Where, Count: Number of observations in each group. Mean: The mean (average) value for each group. Median: The median, which represents the middle value when the data is sorted. Std Dev: The standard deviation, a measure of the spread or dispersion in the data. Min/Max: The minimum and maximum values in each group. Variance: The variance, which is the average of the squared differences from the Mean. Range: The range, which is the difference between the maximum and minimum values. It indicated the spread of data from the lowest to highest value

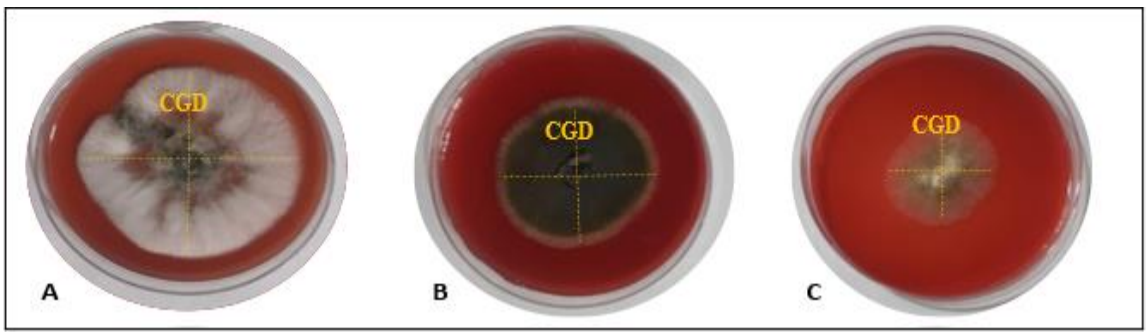

Fig. 3. Development of fungi on BAM after 3-4 days (A= Fus, B= Also, C= Aste)

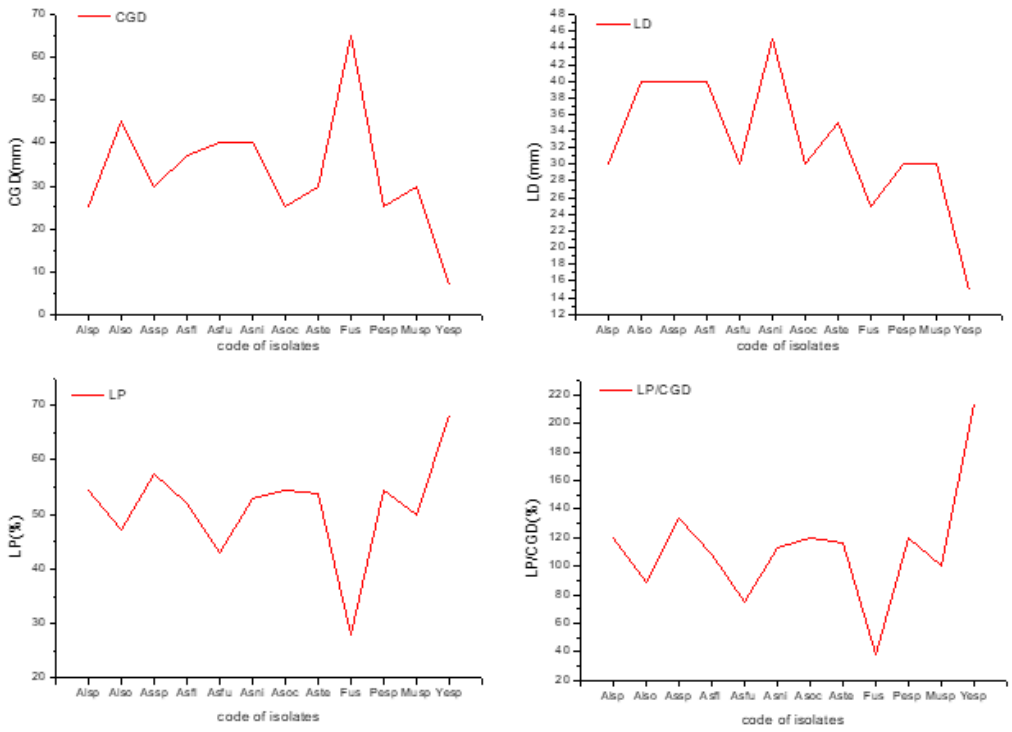

Fig. 4. LD, LP, and LP/CGD production for fungi on BAM after four day

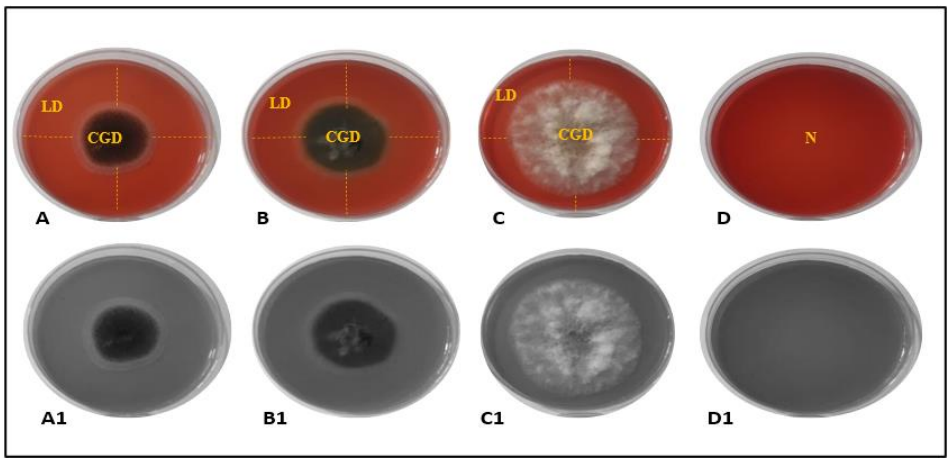

Fig. 5. The precipitation zone (lipolysis activity) of fungi on BAM after four days (A= Asni. B= Also, C= Fusp, D= Negative Control, A1, B1, C1, and D1 under grayscale)

4. DISCUSSION

Microbes, particularly fungi, play a pivotal role in diverse scientific applications. To facilitate their growth and maintenance, the culture medium employed in laboratory settings serves as a crucial source of essential nutrients, as reported by Nurfarahin, Mohamed [13]. Nutrient agar, revered for its versatility and capacity to support the growth of a wide spectrum of microorganisms, stands as a globally recognized and widely utilized medium. For the isolation and cultivation of fungi, predominantly the PDA, as elucidated by the research of Wantini and Octavia [14], has become a cornerstone in laboratory practices.

However, there have been financial challenges in schools, laboratories, and research centers when it comes to the use of ready-made cultural

media. This financial constraint thus triggered conscientious exploration of alternative culture media development and, in particular, the substitutes, namely PDA and nutrient agar, as reviewed in great detail by Uthayasooriyan, Pathmanathan [15].

For example, using locally available and cheap alternatives to nutrient media for the growth of bacteria and fungi, a concept propagated by Uthayasooriyan et al. in 2016, provides an alternative approach that is practically possible within budgetary limitations. This was witnessed by Ravimannan, Arulanantham [16], as they demonstrated that the practice of buying commercial culture media was highly expensive and unsustainable for most laboratories that suffer from funding deficits. In a nutshell, various researchers have again echoed this fact in a bid to establish the possibility of getting a cheap but quality food medium for the growth of fungi. In this regard, such a medium, upon use, facilitates the production of different goods or provides a base for production after a short cultivation period at a temperature of 27°C for a period of 3 to 4 days. This distinction in the results of our study may be due to the fact that the medium used is rich in nutrients, this opinion is consistent with what Deshmukh, Inka [17] and Mohammed, Al-Mugdadi [18] concluded that beetroot are a rich nutritional substance. Where, Beta vulgaris (Beetroot) is the most well-known and commonly cultivated fruit from the Chenopodiaceae family. Beetroot is a rich source of nutrients including vitamins B and C, minerals, fiber, proteins, and a variety of bioactive phenolic substances [19].

The crux of the matter is that microorganisms, be they bacteria or fungi, fundamentally require specific nutrients, an energy source, and an environment tailored to their unique needs in order to thrive and reproduce. In this context, Basic Nutrient Agar (BAM) emerges as a particularly appealing choice, as it offers the essential nutrients requisite for the flourishing of microorganisms. This medium not only supports the robust development of fungi but also engenders the generation of substantial biomass.

The importance of suitable growth media is of paramount significance, a fact well displayed in the realm of mycological research. Commercially available, pre-fabricated culture media have been in use for a long time and with excellent results. The spiraling cost associated with these media is a big barrier for those institutions that are being faced with financial constraints. Thus, the development of an economical nutritional medium which possesses high nutritional properties is very crucial. This is somewhat what has been echoed by efforts of such researchers as Ezeilo, Wahab [20], Hasanin and Hashem [21], Ilmi, Putri [22], and Pandey and Gaire [11] in unveiling the urgency with the energies of enforcing a solution.

Chromogenic isolation media emerge as a promising alternative in the quest for effective microbiological culture systems. These media have demonstrated superior efficacy in detecting fungi within mixed cultures when compared to traditional counterparts, resulting in cost savings by diminishing the need for confirmation tests, as elucidated by the research of Perry [23]. The realm of diagnostic and clinical microbiology has witnessed an increased adoption of chromogenic culture media in recent years, a phenomenon evidenced by the research of Perry and Freydière [24]. The burgeoning diversity in the range of culture media available to clinical laboratories, a development outlined in the research led Perry, Asir [25], has significantly enhanced the precision of infection detection [26].

Not only identifying pathogens, the chromogenic media can various enzymatic and chemical products. This inclusion, such as Congo red or any other colored readily available for procurement, enables such versatility as enlightened by insights of Kadhim and Alrubayae [27].

Chromogenic media are based on synthetic chromogenic enzyme substrates to selectively identify pathogenic species or groups, differentiated by their characteristic enzyme activity. However, although neither inherently species-specific nor discriminative, such enzyme activity was a manifestation of the importance of the judicious choice of complementary enzyme substrates and selection agents, as emphasized by Perry [23] and Pala, Sirec [28].

Within the scope of the present study, the adoption of an eco-friendly and cost-effective fungal medium derived from beetroot carries profound significance. This unique medium assumes a pivotal role in facilitating a crucial industrial test—the detection of lipase production by stimulating this medium, the fungi stimulate for lipase production by adding oil to this medium. It is noteworthy that all fungal strains under investigation exhibit the capability to

produce lipase, as evidenced by the formation of a precipitation zone, indicative of lipolysis activity, within a mere four days of incubation. The productive capacity of filamentous fungi within this context spans a spectrum from moderate to exceptionally high, affirming their competence as proficient lipase producers. Notably, *Aspergillus* sp. emerges as the most prolific lipase producer among the studied fungi, and this opinion is fully consistent with Kadhim and Alrubayae [27], Kavitha, Shankari [29], Ma, Kexin [30], Siódmiak, Dulęba [31], and Liu, Li [32].

Lipases, characterized by their enzymatic activity as EC 3.1.1.3, inherently catalyze the hydrolysis of triglycerides into glycerol and free fatty acids, particularly at the oil-water interface in the presence of emulsions. Additionally, lipases play a pivotal role in highly enantioselective esterification and transesterification reactions, particularly in anhydrous conditions, to yield esters. Owing to their capacity to catalyze specific bio-transformations across a spectrum of reaction conditions, lipases have found diverse applications across commercial sectors, including pharmaceuticals, food, and chemicals, a testament to their versatility, as illuminated by the works of Colacicco, Ciliberti [33] and Treichel, de Oliveira [34].

Finally, the quest for cost-effective and versatile culture media for microorganism cultivation represents a formidable challenge in the domain of microbiology. The prospect of utilizing alternative, economically viable mediums sourced from local materials, alongside the exploration of chromogenic media, has emerged as a promising avenue to alleviate the financial constraints confronting laboratory research. The specific case of an eco-friendly fungal medium derived from beetroot, designed to facilitate the cost-effective detection of lipase production, underscores the potential for innovative solutions in the realm of industrial testing and bioprocessing. These research endeavors collectively contribute to the advancement of scientific knowledge, optimizing laboratory practices to cater to the needs of both resource-constrained and well-funded research institutions.

5. CONCLUSIONS

The study highlights the potential of Beetroot Agar Medium (BAM) as a versatile and cost-efficient tool for researchers in the field of microbiology. BAM affordability, rapid growth-inducing properties, and chromogenic capabilities make it a valuable addition to the repertoire of culture media options available to researchers. Eventually, facilitating advancements in microbiological research while addressing economic and environmental concerns.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wangpraseurt D et al. Biomimetic 3D living materials powered by microorganisms. Trends Biotechnol. 2022;40(7):843-857. DOI:https://doi.org/10.1016/j.tibtech.2022.01.003
2. Yadav AN et al. Recent Advancement in White Biotechnology Through Fungi. 2020, New York: Springer Nature Switzerland A&G. 2020;511-518.
3. Chu R et al. A review on co-cultivation of microalgae with filamentous fungi: Efficient harvesting, wastewater treatment and biofuel production. Renew. Sust. Energ. Rev. 2021;139(1):110689-110696. DOI:https://doi.org/10.1016/j.rser.2020.110689
4. Sperschneider J et al. Improved prediction of fungal effector proteins from secretomes with EffectorP 2.0. Mol. Plant Pathol. 2018;19(9):2094-2110. DOI: https://doi.org/10.1111/mpp.12682
5. Keller NP. Fungal secondary metabolism: regulation, function and drug discovery. Nat. Rev. Microbiol. 2019;17(3):167-180. DOI: https://doi.org/10.1038/s41579-018-0121-1

6. Miller AH, Rubin JS. Nutritionally and botanically enhanced mycelial mass, in United States Patents USA. 2021;1-6.
7. Kadhim KF, AL-Rubayae IM. Alternative fuel production from four Oleaginous fungal species in Basra using waste industrial media. AIP Conf Proc. 2023;2845(1):040001.
DOI: https://doi.org/10.1063/5.0157398
8. Shareef SA. Formulation of alternative culture media from natural plant protein sources for cultivation of different bacteria and fungi. Zanco J. Pur. App. Sci. 2019;31(4):61-69.
DOI:http://dx.doi.org/10.21271/ZJPAS.31.4.7
9. Lyte M. Media compositions for promoting bacterial and fungal growth, in United States Patents. USA; 2020.
10. Kelly BJ, Langham JP. Mycelium product and the method for preparing mycelium product by cocoa and other agricultural substrates, in United States Patents. USA. 2019;1-5.
11. Pandey P, Gaire SP. Effect of different media on mycelium growth of Sclerotium rolfsii Sacc. invitro condition. Int. J. Environ. Agric. Biotech. 2019;4(4):1195-1198.
DOI: https://dx.doi.org/10.22161/ijeab.4445
12. Waksman SA. A method for counting the number of fungi in the soil. J. Bacteriol. 1922;7(3):339-341.
DOI:https://doi.org/10.1016/j.tibtech.2022.01.003
13. Nurfarahin AH, Mohamed MS, Phang LY. Culture medium development for microbial-derived surfactants production—An overview. Mol. 2018;23(5):1049.
DOI:https://doi.org/10.3390/molecules23051049
14. Wantini S, Octavia A. Perbandingan pertumbuhan jamur *Aspergillus flavus* pada media PDA (potato dextrose agar) dan media alternatif dari singkong (*Manihot esculenta* Crantz). J. Media Analis Kesehat. 2018;6(2):625-631.
15. Uthayasooriyan M et al. Formulation of alternative culture media for bacterial and fungal growth. Der Pharm. Lett. 2016;8(1):31-436.
DOI:http://repo.lib.jfn.ac.lk/ujrr/handle/123456789/3910
16. Ravimannan N et al. Alternative culture media for fungal growth using different formulation of protein sources. Ann. Biol. Res. 2014;5(1):36-39.
17. Deshmukh G et al. Application of beetroot as natural coloring pigment and functional ingredient in dairy and food products. Int. J. Curr. Microbiol. Appl. Sci. 2018;7(12):2010-2016.
DOI:https://doi.org/10.20546/ijcmas.2018.712.231
18. Mohammed AA et al. Beetroot juice as an alternative growth and maintenance medium for six isolates of Mycobacterium spp. J. Pharm. Sci. Res. 2018;10(8):1881-1884.
19. Punia Bangar S et al. Bioactive potential of beetroot (*Beta vulgaris*). Int. Food Res. J. 2022;158:111556.
DOI:https://doi.org/10.1016/j.foodres.2022.111556
20. Ezeilo UR et al. Fungal-assisted valorization of raw oil palm leaves for production of cellulase and xylanase in solid state fermentation media. Waste Biomass Valorization. 2020;11(7):3133-3149.
DOI: https://doi.org/10.1007/s12649-019-00653-6
21. Hasanin MS, Hashem AH. Eco-friendly, economic fungal universal medium from watermelon peel waste. J. Microbiol. Methods. 2020;168(1):105802.
DOI:https://doi.org/10.1016/j.mimet.2019.105802
22. Ilmi M et al. Use of mung bean sprout (Tauge) as alternative fungal growth medium. J. Phys. Conf. Ser. 2019;1241(1):012015-012022.
DOI: https://doi.org/10.1088/1742-6596/1241/1/012015
23. Perry JD. A Decade of development of chromogenic culture media for clinical microbiology in an era of molecular diagnostics. Clin. Microbiol. Rev. 2017;30(2):449-479.
DOI: https://doi.org/10.1128/cmr.00097-16
24. Perry JD, Freydière AM. The application of chromogenic media in clinical microbiology. J. Appl. Microbiol. 2007;103(6):2046-2055.
DOI:https://doi.org/10.1111/j.1365-2672.2007.03442.x
25. Perry JD et al. Evaluation of a chromogenic culture medium for isolation of Clostridium difficile within 24 Hours. J. Clin. Microbiol. 2010;48(11):3852-3858.
DOI: https://doi.org/10.1128/jcm.01288-10

26. Reddi N, Sanapala S, Budumuru R. Early detection of drug resistant enterobacteriaceae in urinary tract infections using chromogenic agar medium in a Tertiary Care Hospital, Kakinada, Andhra Pradesh, India. J. Clin. Diag. Res. 2022;11(4):MO20-MO23. DOI:https://doi.org/10.7860/jcdr/2018/35432.11983
27. Kadhim KF, Alrubayae IM. Study of lipase production and lipids accumulation of oleaginous fungi isolated from oil-rich soil in Basrah. Sci. J. Med. Res. 2019; 3(12):123-127.
28. Pala L, Sirec T, Spitz U. Modified enzyme substrates for the detection of bacteria: A review. Mol. 2020;25(16):3690. DOI:https://doi.org/10.3390/molecules25163690
29. Kavitha K, Shankari K, Meenambiga S. A review on extraction of lipase from Aspergillus Species and its applications. Research Journal of Pharmacy and Technology. 2021;14(8):4471-4475. DOI: http://dx.doi.org/10.52711/0974-360X.2021.00777
30. Ma X et al. Optimization of low-temperature lipase production conditions and study on enzymatic properties of aspergillus Niger. Iran. J. of Chem. and Chem. Eng. 2021;40(4):1364-1374. DOI:https://doi.org/10.30492/ijcce.2021.529010.4694
31. Siódmiak T et al. The high 'lipolytic jump' of immobilized amano a lipase from aspergillus niger in developed 'ESS Catalytic Triangles' containing natural origin substrates. Catalysts. 2022;12(8): 853. DOI: https://doi.org/10.3390/catal12080853
32. Liu YW et al. Interaction between Aspergillus oryzae lipase and chitosan: The underlying mechanism and complex characterization. Food Hydrocoll. 2023; 137;108382. DOI:https://doi.org/10.1016/j.foodhyd.2022.108382
33. Colacicco M et al. Study of lipase production by Yarrowia lipolytica grown in high concentration of hydrophobic carbon sources. Chem. Eng. Trans. 2022;93(1): 247-252. DOI: https://doi.org/10.3303/CET2293042
34. Treichel H et al. A review on microbial lipases production. Food Bioproc Tech. 2010;3(2):182-196. DOI: https://doi.org/10.1007/s11947-009-0202-2

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