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Enzymatic Activity in Plant Lipids by the Lipase/Galactolipase from *Burkholderia lata* BL02

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ABSTRACT
This study focuses on the extracellular lipase/galactolipase from *Burkholderia lata* BL02, highlighting its unique properties and potential applications. The enzyme was produced through submerged fermentation, reaching peak enzymatic activity of 132 U/mL and 396 U/mg at 96 hours. The purification process involved a novel single-step method using DEAE-Cellulose, resulting in high recovery (53.9%). The enzyme displayed higher activity on substrates rich in unsaturated fatty acids, showcasing its versatility in hydrolyzing various substrates efficiently. Particularly, it exhibited remarkable galactolipase activity (2700 U/mg) when applied to plant-derived galactolipids in situ, suggesting potential applications in waste treatment and high-value product conversion. This study sheds light on the untapped potential of *B. lata* BL02 lipase/galactolipase, both in terms of its unique characteristics and diverse applications, making it an enticing subject for further research and exploration.

Keywords-biocatalysis, novel enzyme, cellulose, biotechnological applications, galactolipids

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I. INTRODUCTION

Based on the abundance of vegetation on Earth's surface, galactolipids (GLs) represent the most predominant class of lipids in the biosphere, primarily found in the leaves of higher plants. Chloroplasts and thylakoids are characterized by the presence of high proportions of GLs, making them particularly vital in the photosynthetic membranes of plants, algae, and some bacteria[1], [2]. GLs differ from other lipid types, such as triglycerides, due to the attachment of one (monogalactosyldiacylglycerols, MGDG) or two (digalactosyldiacylglycerols, DGDG) galactose molecules to glycerol, giving the molecule a polar region. They are also characterized by one or two unsaturated long-chain fatty acids ranging from C16 to C20 [3].

The enzyme monogalactosyldiacylglycerol synthase 1 (MGD1) [EC 2.4.1.46] catalyzes the synthesis of the majority of monogalactosyldiacylglycerol (MGDG) in the inner membrane of chloroplasts. It transfers a galactose residue from the donor substrate, UDP-α-D-galactose, to the acceptor substrate, a diacylglycerol (DAG), to form MGDG (Galβ-DAG). Once assembled, MGDG is transported via an as-yet-unknown mechanism to the outer membrane, where the synthesis of digalactosyldiacylglycerol (DGDG) takes place, playing a significant role in membrane function and thermostability. The content of DAG is very low in the chloroplast membrane (<1 mol%), suggesting it is rapidly converted to MGDG [4].

Lipases [EC 3.1.1.3] catalyze the hydrolysis of ester bonds in triacylglycerols, preferably at positions sn-1 and sn-2, but their substrate selectivity is often broad [5]. Specifically, galactolipases [EC 3.1.1.26] are enzymes that hydrolyze ester bonds in galactolipids, such as MGDG and DGDG, producing free fatty acids, galactose, and glycerol as products. Recently, galactolipases and their reaction products have gained significant interest in the food, cosmetic, and pharmaceutical industries, as well as the chemical industry, owing to their useful biochemical properties and bioactive potential [6].

The current biochemical knowledge about galactolipases remains quite limited, with scant information available on their structure and characterization, and their detailed functions still need to be fully elucidated [6]–[11]. The application

of galactolipases has not yet been described, and it could be intriguing for the hydrolysis of the cell membrane and the extraction of polyunsaturated fatty acids from its components.

II. METHODS

The bacterial strain *Burkholderia lata* BL02 was isolated in our laboratory in a fungal culture contamination and identified by morphological and molecular methods (16S RNA) at Fundação André Tosello (FAT), Campinas/SP – Brazil[12].

2.1 Enzyme Production and Purification

2.1.1 Production of lipase in submerged fermentation (SmF)

The lipase from *B. lata* LBBIO-BL02 was produced through submerged fermentation in an optimized culture medium [10]. The bacteria cultivation was started with a pre-inoculum in 250 mL Erlenmeyer flasks containing 50 mL of Luria-Bertani medium with an incubation time of about 48 h (10^8 cells/mL) at 35 ºC, and pH 8.0 and with 180 rpm orbital shaking. After this, 1 mL of the culture was transferred to 500 mL Erlenmeyer flasks containing 100 mL of fermentation medium maintained under the same conditions.

The optimized culture medium was composed of K_2HPO_4 (1.0 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), NaCl (0.38 g/L) and $FeSO_4 \cdot 7H_2O$ (0.01 g/L) using chicken fat (12.5 ml/L) and Ammonium Phosphate (15 g/L) as a carbon and nitrogen source. Bacterial growth was monitored during the cultivation by counting cells in a Neubauer chamber.

2.1.2 Enzyme Purification by Single-Step Methodology Using DEAE-Cellulose

The enzyme was purified in a single-step process following a protocol exclusively developed for this lipase/galactolipase and filed for patent application with the National Institute of Research and Innovation (INPI) under the number BR 10 2019 006621 0[12].

2.1.3 Protein Assay

Protein concentration was determined according to the procedure described by Bradford [13] using BSA as a standard protein.

2.1.4 SDS-PAGE Electrophoresis

Analytical protein electrophoresis in polyacrylamide gel in the presence of sodium dodecyl sulfate (SDS-PAGE) was carried out using the Laemmli method [14].

2.2 Characterization of galactolipase activity

The enzyme's characteristics were studied using the previously purified enzyme to understand its biochemical properties and features for potential uses.

2.2.1 Lipase Activity

The lipase activity of the enzyme was measured by the hydrolysis of *p*-nitrophenylpalmitate (*p*NPP) as first described by Winkler andStuckmann[15]. One unit of lipaseactivity is defined as the release of 1 mmol/min of *p*-nitrophenol (*p*NP). The molar extinction coefficient of pNP (1.5×10^4 mol/L x cm) was used to correlate the concentration ofproduct from the absorbance readings.

2.2.2 Hydrolysis of Different Substrates

The hydrolytic capacity of the *B. lata* BL02 enzyme was investigated across various substrates, encompassing model lipids: Triolein (TC18) and Tributyrin (TC4); plant oils: Olive, Soybean, Sunflower, Flaxseed, Crambe, Canola, Used Cooking Oil, Palm, Corn, and Margarine (Claybom®); and animal fats: Chicken, Pork, Fish, Butter, and Cream. The utilized substrates display a diverse fatty acid composition (Table 1).

The reaction mixture was comprised of 0.2 mol/L substrate, 6% (w/v) Triton X-100, and 74% (v/v) citrate-phosphate buffer at 0.05 mol/L and pH 7.0. To this mixture, 1 mL of enzyme solution was added to 5 mL of enzyme reaction medium, followed by incubation for 30 minutes at 55 °C and 300 rpm. After incubation, 16 mL of a 1:1 (v/v) mixture of acetone and ethanol was added, and this solution was titrated with NaOH 0.1 mol/L. One unit of enzyme activity was equivalent to 1 mmol of fatty acid released per minute.

2.2.3 Galactolipase Activity

To measure the galactolipase activity of the purified *B. lata* LBBIO-BL02 enzyme, natural substrates were utilized: Spinach (*Spinacia oleracea*), Grass (*Brachiaria* sp.) and Sugarcane (*Saccharum officinarum*) green leaves. The leaves were boiled for 5 minutes to deactivate endogenous lipolytic enzymes, then cut into 1-2 mm pieces and lyophilized.

Hydrolysis assays of galactolipids were conducted in 30 mL glass vials containing 100 mg of pre-treated leaves and 9 mL of Tris buffer (pH 8.0, Tris 50 mmol/L, NaCl 150 mmol) with the addition of 1 mL of enzyme solution (5 mg/mL)r. The mixtures were incubated at 55 °C under orbital agitation (250 rpm) for 24 hours. Using the same

protocol, transesterification assays of galactolipids were also performed using a Tris buffer solution with the addition of methanol at 1 and 3 mol/L.

After the reaction time, a 500 µL sample was remove from each tube, mixed with 3.5 mL of FOLCH reagent ($CHCl_3$:CH_3OH, 2:1, v/v), vortexed for 1 minute, and centrifuged for 5 minutes (4000 rpm). The organic phase was collected and added to a test tube, dried with $MgSO_4$, filtered (0.45 µm), and evaporated under a stream of N_2. Finally, the dried extract was dissolved in 3 mL of FOLCH and analyzed by HPTLC. In each case, the experiments were performed in triplicate using enzyme-free controls to verify the inactivation of endogenous enzymes in the leaves through the absence of free fatty acids and/or ester formation.

III. RESULTS

3.1.1 Strain identification

Morphologically, the strain introduced in the form of large Gram-negative bacilli and the sequencing of the 16S RNA, allowed the identification of the strain as *Burkholderia lata*. The strain was deposited at the culture collection of the Faculdade de Ciências e Letras de Assis – São Paulo State University under LBBIO-BL02 code. This species was differentiated from *Burkholderia cepacia* complex (Bcc) in 2009 [16], and there are no records in the literature of galactolipase production by this strain.

3.1.2 Enzyme Production in submerged fermentation

After submerged fermentation under optimized conditions, the enzymatic activity of the fermentation broth reached 396.3±9.0 U/mg. In the literature, various strains such as *B. cepacia*[17], *B. cepacia* ATCC 25609, *B. multivorans* V2[18],as well as other lipase-producing bacteria like *Pseudomonas aeruginosa*[19] and *Bacillus sphaericus* MTCC 7526 [20], exhibited average enzymatic activities of 51.0, 0.23, 1.76, 0.28, and 182.8 U/mg, respectively. These findings underscore the superior overproduction capability of our *B. lata* BL02 strain, as previously documented by our research group [10]. This further highlights the advantageous position of *B. lata* BL02 wild strain in comparison to other microorganisms.

3.1.3 Purification via Non-Conventional Single-Step Method using DEAE-Cellulose

Through a non-conventional single-step purification method utilizing DEAE-Cellulose, which has been developed and patented by our research group, it was feasible to purify the *B. lata* BL02 enzyme in both small (5 mL) and large volumes (500 mL), in solution, directly from the lipid-depleted culture supernatant, eliminating the necessity for chromatographic columns.

The purified lipase exhibited a purification factor of 46.5, with a specific activity of 18,422 U/mg, and an activity recovery of 53%. SDS-PAGE electrophoresis revealed the presence of a singular 32 kDa band (Fig. 1), closely aligned with the molecular weights of many well-known *Burkholderia* sp. lipases, which generally fall within the range of 29 to 35 kDa [21]–[26].

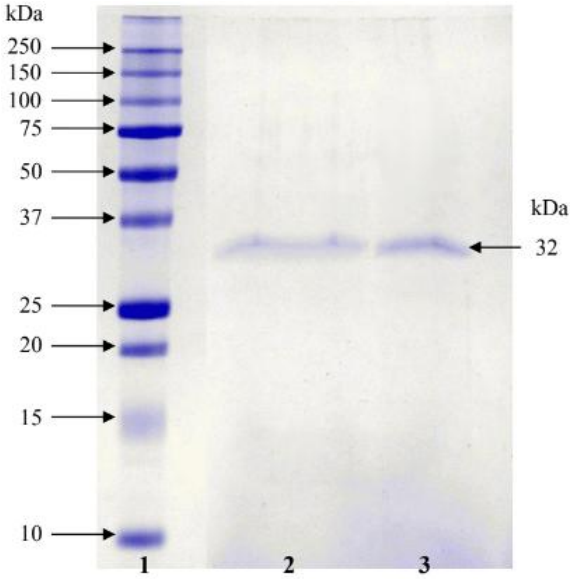

Figure 1: SDS-PAGE Electrophoresis. Lane 1: Molecular weight markers; Lane 2: Purified lipase with DEAE-Cellulose, loading of 100 µg of protein; Lane 3: Purified lipase with DEAE-Cellulose, loading of 45 µg of protein. Stained with Coomassie Blue.

Therefore, this protocol offers ease (single step), cost-effectiveness (cellulose is widely available globally), and efficiency in enzyme recovery.

3.1.4 Hydrolysis of Different Substrates

The purified enzyme from *B. lata* BL02 was employed in hydrolysis assays. The hydrolysis results for the different substrates are detailed in Table 1. The most significant observation from this experiment was that all tested triacylglycerols, both natural and synthetic with varying fatty acid compositions, were hydrolyzed by the *B. lata* enzyme, confirming that the purified enzyme exhibits true lipase behavior.

Table 1. Activity of purified lipase from *Burkholderia lata* BL02 against natural and synthetic substrates.

Substrates	**Purified Enzyme (U/mg)**	**a 18:1$^{\Delta 9}$**	**b 18:2$^{\Delta 9,12}$**	**c 18:3$^{\Delta 9,12,15}$**	**d 22:1$^{\Delta 13}$**	**e 16:0**	**f 18:0**	**g 20:5$^{\Delta 5,8,11,14,17}$**	**h 22:6$^{\Delta 4,7,10,13,16,19}$**
*p*NPP	14,291.3 ± 324	-	-	-	-	100	-	-	-
Tributyrin 97%	27,571.5 ± 137	-	-	-	-	-	-	-	-
Triolein 98%	**21,771.5 ± 321**	98.0	-	-	-	-	-	-	-
Butter	**12,444.4 ± 302**	20.4	2.0	1.5	-	32.1	9.7	-	-
Heavy Cream	10,079.2 ± 324	22.8	1.6	0.7	-	30.6	12.2	-	-
Lard	8,328.5 ± 374	40.4	12.4	2.2	-	22.4	15.3	-	-
Soybean Frying Oil	10,393.9 ± 602	21.0	54.0	6.0	0.3	16.0	4.7	-	-
Chicken Fat	13,246.5 ± 360	43.0	14.0	0.7	-	25.0	-	-	-
Palm Oil	11,395.2 ± 407	40.0	17.0	-	-	34.0	-	-	-
Margarine	4,539.2 ± 321	13.5	14.3	-	-	5.5	5.1	-	-
Corn Oil	11,148.5 ± 297	35.0	45.0	1.0	-	10.0	3.3	-	-
Soybean Oil	13,360.8 ± 489	21.0	54.0	6.0	0.3	16.0	4.7	-	-
Linseed Oil	19,393.1 ± 626	11.0	15.0	40.0	-	2.0	-	-	-
Olive Oil	8,173.9 ± 525	70.0	10.0	0.2	-	16.0	3.4	-	-
Fish Oil	**15,520.8 ± 586**	10.6	1.3	0.2	-	15.5	3.6	16.3	10.6
Crambe Oil	5,863.1 ± 621	18.0	9.0	6.0	56.0	-	-	-	-
Sunflower Oil	12,679.9 ± 468	16.0	71.4	-	-	6.0	5.9	-	-
Canola Oil	9,034.6 ± 321	60.8	20.8	-	-	5.4	2.7	-	-

Fatty acids: a) oleic; b) linoleic; c) α-linolenic; d) erucic; e) palmitic; f) stearic; g) eicosapentaenoic (EPA); h) docosahexaenoic (DHA). Assay conditions: 55°C. Substrate concentration: 200 mmol/L. 0.05 M Tris buffer pH 7.0.

Among the natural substrates, linseed, fish, and soybean oils displayed elevated activities with the purified enzyme. Notably, linseed oil, containing 40% α-linolenic acid (18:3$^{\Delta 9,12,15}$) in its composition, exhibited the highest enzymatic activity (19,393.1±626 U/mg).

3.1.5 Galactolipase activity

Galactolipids, also known as galactoglycerolipids, are estimated to be the most abundant class of lipids found on Earth's surface and consequently serve as the primary storage of fatty acids [27], [28]. As there is no large-scale process for the easy recovery of galactolipids dispersed within plant materials, particularly in the forms of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), enzymes with galactolipase activity can play a crucial role in recovering fatty acids from galactolipids through hydrolysis, alcoholysis, or in situ transesterification reactions. Thus, the *B. lata* BL02 lipase was assessed for its in situ galactolipase activity on Spinach (*Spinacia oleracea*),Grass (*Brachiaria* sp.) and Sugarcane (*Saccharum officinarum*) green leaves samples for hydrolysis and methanolysis reactions.

The Fig. 2 illustrates the products of MGDG and DGDG lipolysis in spinach leaves. After a 10-minute reaction, both hydrolysis and alcoholysis proved efficient. The *B. lata* lipase/galactolipase managed to hydrolyze around 60% of the galactolipids in the sample (line 2), reaching 2700 U/mg. Methanolysis transformed approximately 20% of the substrate into esters. However, as the reaction occurred in an aqueous medium, hydrolysis prevailed with a 30% conversion, resulting in an overall substrate conversion of 50% when using 3 mol/L of methanol.

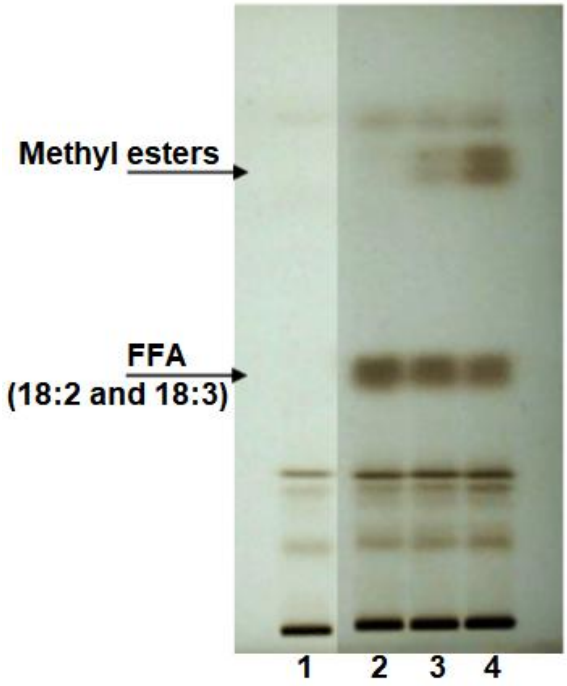

Figure 2: Hydrolysis and alcoholysis of natural galactolipids from spinach (MGDG and DGDG) by *Burkholderia lata* BL02 galactolipase. Lanes: 1: Blank; 2: hydrolysis reaction; 3: methanolysis with 1 mol/L alcohol; 4: methanolysis with 3 mol/L alcohol. The galactolipid lipolysis reactions were followed by lipid extraction and separation on HPTLC plates on silica using dual migration: 30 mm in Hexane/Diethyl Ether/Phosphoric Acid (60:40:1, v/v/v) and 60 mm in Hexane. Assay conditions: 55 °C, pH 8.0, and 250 rpm for 10 minutes. FFA: Free Fatty Acids.

The hydrolysis activity of *B. lata* BL02 galactolipase against galactolipids was remarkable, reaching 2700 U/mg using spinach leaves (*Spinacia oleracea*) as a substrate. When compared to pancreatic enzymes known for their galactolipid activity [29], [30] and other lipases [31], *B. lata*BL02 galactolipase stood out (Table 2), exhibiting specific activity against galactolipids that was only lower than recombinant human and porcine pancreatic lipases and at least 2.5 times higher than any microbial lipase.

Thus, *B. lata* galactolipase emerges as a significant alternative for utilizing fatty acids present in the form of plant galactolipids. When utilizing grass family leaves, known for their thick cell walls and silica content, the galactolipase still displayed substantial activity of 700 U/mg and 380 U/mg for Grass (*Brachiaria* sp.) and Sugarcane (*Saccharum officinarum*) green leaves samples, respectively.

IV. DISCUSSION

IV.1.1 Enzyme production

The enzyme was produced through submerged fermentation in stirred flasks by the *Burkholderia lata* BL02 strain, with enzymatic activity peaking at 96 hours, reaching 132 U/mL and 396 U/mg of lipolytic activity. Low-cost byproducts, such as chicken fat, and ammonium phosphate were used as carbon and nitrogen sources, as previously reported by Oliveira et al. [10]. This microorganism demonstrates potential as a lipase producer for the market, owing to its high enzymatic activity achieved in a cost-effective fermentation medium. Therefore, investigating the application of this enzyme, which combines high catalytic activity with low production costs, holds significant importance.

IV.1.2 Enzyme purification

The extracellular enzyme of *Burkholderia lata* BL02 was purified to homogeneity in a single step using DEAE-Cellulose, eliminating the need for chromatographic columns. In comparison with the purification processes of most other purified lipases, the purification of the *B. lata* lipase was notably straightforward and achieved a high recovery rate (53.9%). Lower purification yields, such as 4.8% for the *B. cepacia* ATCC 25416 lipase [24], 3.9% for the *Burkholderia* sp. GXU56 lipase, and 0.96% for the *B. multivorans* V2 lipase [18], have also been reported. Rahman et al. [32] achieved a higher recovery rate of 52% for the *Pseudomonas* sp. S5 lipase, but this involved using affinity

chromatography in combination with ion-exchange chromatography.

Table 2. Specific activity (U/mg) of lipases on triacylglycerol (tributyrin, 4:0) and galactolipids (MGDG and DGDG) compared to *Burkholderia lata* BL02 lipase.

Enzyme	Enzymatic activity (U/mg)	
	Tributyrin (4:0)	Galactolipids (MGDG and DGDG)
Burkholderia lata BL02 Lipase	8,345 ± 132	2,700 ± 143
rGPLRP2[a]	2,700 ± 300	9,795 ± 105
rHPLRP2[b]	1,250 ± 150	4,762 ± 85
*Fusarium solani*Cutinase	2,596 ± 96	1,284 ± 45
Thermomyces lanuginosus Lipase	7,834 ± 850	1,122 ± 51
Candida antarctica A Lipase	309 ± 11	176 ± 7
*Rhizomucormiehei*Lipase	413 ± 44	126 ± 4
*Candida rugosa*Lipase	753 ± 44	20 ± 1
*Rhizopus oryzae*Lipase	3,375 ± 270	41 ± 3
*Pseudomonas glumae*Lipase	1,179 ± 35	0
Pseudomonas cepacia Lipase	86 ± 8	0
*Penicillium camembertii*Lipase	875 ± 10	0
Yarrowialipolytica LIP2 Lipase	8,102 ± 590	0
Candida antarctica B Lipase	670 ± 15	0

a: protein related to guinea pig pancreatic lipase; b: protein related to human pancreatic lipase. Except for the lipase from this study produced by *B. lata* BL02, the enzymatic activities for the other enzymes were obtained from Amara *et al.*[31].

It is common that the purification of lipases/galactolipases often requires multiple steps, including one or more chromatographic steps [33]. Consequently, a series of processes or combinations of purification steps become necessary, raising costs, and reducing overall process yield. When comparing all these examples from the literature, the straightforward purification with relatively high recovery becomes highly promising for large-scale applications in lipase purification.

IV.1.3 Strain activity against different substrates

The substrates used in this study can be divided into three groups: plant origin, animal origin, and synthetic. Among the substrates of vegetable origin, modified lipid margarine stands out, even though it exhibits the lowest hydrolysis rate. Despite undergoing hydrogenation of part of its unsaturated fats and containing up to 7% trans fatty acids, margarine is a complex mixture of vegetable fats that retains its potential for interaction with the enzyme's active site, leading to favorable hydrolysis outcomes [34].

Additionally, significant attention is drawn to used soybean frying oil. Continuous frying processes, commonly employed in industries, subject oils to hydrolysis, oxidation, rancidity, and triglyceride molecule polymerization[35]. The outcome is the generation of polar secondary compounds, known as total polar compounds; these compounds are more polar than triglycerides and are non-volatile[36]. Despite these complex transformations, the enzyme effectively hydrolyzed used soybean oil, displaying only a 22% decline in activity in comparison to refined soybean oil. In terms of practical applications, the enzyme holds substantial potential for treating waste in industries employing continuous frying processes or converting discarded materials into high-value-added products.

Regarding animal-origin substrates, it is evident that the highest specific activity increases were observed for fish oil. Fish oil has the highest percentage of unsaturated fatty acids among animal-origin substrates, followed by chicken oil. The unique composition of fish oil, primarily consisting of polyunsaturated fatty acids (PUFA) like docosahexaenoic acid (DHA, $22{:}6^{\Delta 4,7,10,13,16,19}$) and eicosapentaenoic acid (EPA, $20{:}5^{\Delta 5,8,11,14,17}$), accounts for its distinct behavior.

For lipases, natural substrates are typically long-chain triacylglycerols, with the capability to act on short-chain chains as well, whereas esterases are limited to short chains[37]. It is essential to emphasize that most lipases can hydrolyze the same substrates as esterases, but the reverse is not true [38]. Therefore, the enzyme produced by the *B. lata* BL02 strain is a genuine lipase, hydrolyzing triolein ($18:1^{\Delta 9}$).

This study highlights the significant capacity and versatility of the lipase from *B. lata* BL02 in efficiently hydrolyzing various substrates, thus opening a wide range of possibilities for applications that harness its hydrolytic properties.

IV.1.4 Galactolipase activity

The current understanding of galactolipase biochemistry remains limited, unlike the well-characterized lipases and phospholipases that find widespread application in biotechnological processes [9], [39], [40]. This limited knowledge arises partly from the lack of commercially available galactolipid substrates on a large scale, which are cost intensive. Additionally, only a few laboratories have delved into developing assays to quantify galactolipase-specific activities [29]–[31], [41]–[43]. Moreover, although the existence of galactolipid hydrolases in plants and animals has been recognized for some time, their levels are typically low, and their purification from natural sources is challenging [42], [44]–[46]. Notably, galactolipase activities have also been identified in microorganisms, as examples listed in Table 2. The selection of galactolipase-producing strains, their purification, and subsequent kinetic and biochemical characterization contribute to advancing knowledge in a highly significant realm of biotechnology: enzymatic technology.

The natural *B. lata* BL02 lipase/galactolipase exhibited a remarkable activity against spinach (*Spinacia oleracea*) galactolipids in-situ, reaching an impressive 2700 U/mg, a level of activity that stands out in comparison to other lipases. Furthermore, its surprising activity when tested on grasses family leaves as substrates is even more remarkable, with no literature data available for comparison. This novel finding presents unexploited potential for utilization, warranting further exploration of its applications.

V. CONCLUSION

The findings of this study underscore the novelty of the *B. lata* BL02 enzyme, an unreported biocatalyst with unique characteristics. The methodological approach applied for its purification, characterized by novelty, simplicity, and cost-effectiveness, presents the prospect of extending its utility to the purification of lipases from diverse microbial sources. Beyond exhibiting high lipase activity across various substrates, this enzyme also demonstrates high galactolipase activity in the in-situ hydrolysis of plant-derived galactolipids (spinach, grass, and sugarcane). This characteristic positions it with potential applications in these realms, thus substantiating the rationale for further exploration of these attributes and potentials in forthcoming studies. The emergence of this new enzyme underscores its significance and offers a compelling pathway for continued research and development.

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REFERENCES

[1] P. Dörmann and C. Benning, Galactolipids rule in seed plants, TRENDS in Plant S cience, 7(3), 2002, 112–118.

[2] G. Hölzl and P. Dörmann, Progress in Lipid Research Structure and function of glycoglycerolipids in plants and bacteria Georg Ho, Prog Lipid Res, 46, 2007, 225–243.

[3] L. Christensen, Galactolipids as Potential Health Promoting Compounds in Vegetable Foods, Recent Patents on Food, Nutrition &Agriculturee, 1(1), 2009, 50–58.

[4] J. Lyu, R. Gao, and Z. Guo, Galactosyldiacylglycerols: From a Photosynthesis-Associated Apparatus to Structure-Defined In Vitro Assembling, J Agric Food Chem, 69(32), 2021, 8910–8928.

[5] K. E. Jaeger and M. T. Reetz, Microbial lipases form versatile

tools for biotechnology, Trends Biotechnol 16(9), 1998, 396–403.

[6] S. Hashiro, K. Fujiuchi, D. Sugimori, and H. Yasueda, A novel galactolipase from a green microalga *Chlorella kessleri*: purification, characterization, molecular cloning, and heterologous expression, Appl MicrobiolBiotechnol 102(4), 2018, 1711–1723.

[7] K. de G. Daiha, R. Angeli, S. D. de Oliveira, and R. V. Almeida, Are Lipases Still ImportantBiocatalysts? A Study of Scientific Publications and Patents for Technological Forecasting," PLoS One 10(6), 2015.

[8] R. Gupta, N. Gupta, and P. Rathi, Bacterial lipases: an overview of production, purification and biochemical properties, Appl MicrobiolBiotechnol 64(6), 2004, 763–81.

[9] K. E. Jaeger, S. Ransac, B. W. Dijkstra, C. Colson, M. Van Heuvel, and O. Misset, Bacterial lipases, FEMS Microbiol Rev 15(1), 1994, 29–63.

[10] B. H. Oliveira, R. É. Santos, L. E. A. Loiola, and V. M. G. Nascimento, Overproduction and properties of lipase by a wild strain of *Burkholderia lata* LBBIO-BL02 using chicken fat, Ann Microbiol 65(2), 2015, 865–877.

[11] B. H. Oliveira, Produção e Purificação da Lipase de*Burkholderia lata*LBBIO-BL02, sua Caracterização Cinética e Aplicação em Reações de Biocatálise de Interesse Farmacológico e Industrial, doctoral diss., São Paulo State University (UNESP), 2017.

[12] B. H. Oliveira and V. M. G. Nascimento, Método de Purificação de Lipases Bacterianas,Patent BR 10 2019 006621 0 A2, 2019.

[13] M. M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal Biochem 72, 1976, 248–54.

[14] U. K. Laemmli, Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4, Nature 227, 1970, 680–685.

[15] U. K. Winkler and M. Stuckmann, Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*, J Bacteriol 138(3), 1979, 663–70.

[16] E. Vanlaere, A. Baldwin, D. Gevers, D. Henry, E. De Brandt, J. J. LiPuma, E. Mahenthiralingam, D. P. Speert, C. Dowson, P. Vandamme, Taxon K, a complex within the *Burkholderia cepacia* complex, comprises at least two novel species, *Burkholderia contaminans* sp. nov. and *Burkholderia lata* sp. Nov., Int J Syst EvolMicrobiol 59, 2009, 102–11.

[17] P. Rathi, R. K. Saxena, and R. Gupta, A novel alkaline lipase from *Burkholderia cepacia* for detergent formulation, Process Biochemistry 37(2), 2001, 187–192.

[18] V. Dandavate, J. Jinjala, H. Keharia, and D. Madamwar, Bioresource Technology Production, partial purification and characterization of organic solvent tolerant lipase from *Burkholderia multivorans* V2 and its application for ester synthesis, Bioresour Technol 100(13), 2009, 3374–3381.

[19] C. Joshi and S. K. Khare, Purification and characterization of *Pseudomonas aeruginosa* lipase produced by SSF of deoiled Jatropha seed cake, Biocatal Agric Biotechnol 2(1), 2013, 32–37.

[20] B. Joseph and P. W. Ramteke, Extracellular solvent stable cold-active lipase from psychrotrophic*Bacillus sphaericus* MTCC 7526: partial purification and characterization, Ann Microbiol 63(1), 2012, 363–370.

[21] S. Dalal, P. K. Singh, S. Raghava, S. Rawat, and M. N. Gupta, Purification and properties of the alkaline lipase from *Burkholderia cepacia* A.T.C.C. 25609, Biotechnol Appl Biochem 51(1), 2008, 23–31.

[22] D.-S. Park, H.-W. Oh, S.-Y. Heo, W.-J. Jeong, D. H. Shin, K. S. Bae, and H.-Y. Park., Characterization of an extracellular lipase in *Burkholderia* sp. HY-10 isolated from a longicorn beetle, J Microbiol 45(5), 2007, 409–17.

[23] P. Ungcharoenwiwat and A. H-Kittikun, Purification and characterization of lipase from *Burkholderia* sp. EQ3 isolated from wastewater from a canned fish factory and its application for the synthesis of wax esters, J Mol Catal B Enzym 115, 2015, 96–104.

[24] X. Wang, X. Yu, and Y. Xu, Homologous expression, purification and characterization of a novel high-alkaline and thermal stable lipase from *Burkholderia cepacia* ATCC 25416, Enzyme Microb Technol 45(2), 2009, 94–102.

[25] J. Yang, D. Guo, and Y. Yan, Cloning, expression and characterization of a novel thermal stable and short-chain alcohol tolerant lipase from *Burkholderia cepacia* strain G63, J Mol Catal B Enzym 45, 2007, 91–96.

[26] C. Yao, Y. Cao, S. Wu, S. Li, and B. He, An organic solvent and thermally stable lipase from *Burkholderia ambifaria* YCJ01: Purification, characteristics and application for chiral resolution of mandelic acid, J Mol Catal B Enzym 85-86, 2013, 105–110.

[27] A. A. Benson, R. Wiser, R. A. Ferrari, and J. A. Miller, Photosynthesis of Galactolipids, J Am Chem Soc 80(2), 1958, 4740.

[28] G. Hölzl, S. Witt, A. A. Kelly, U.Zähringer, D. Warnecke, P.Dörmann, and E. Heinz, Functional differences between galactolipids and glucolipids revealed in photosynthesis of higher plants, Proceedings of the National Academy of Sciences 103(19),2006, 7512–7517.

[29] L. Andersson, C. Bratt, K. C. Arnoldsson, B.Herslöf, N. U. Olsson, B.Sternby, and A. Nilsson, Hydrolysis of galactolipids by human pancreatic lipolytic enzymes and duodenal contents, J Lipid Res 36(6), 1995, 1392–400.

[30] L. Andersson, F. Carrière, M. E. Lowe, Å. Nilsson, and R. Verger, Pancreatic lipase-related protein 2 but not classical pancreatic lipase hydrolyzes galactolipids, Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism 1302, 1996, 236–240.

[31] S. Amara, D.Lafont, G.Parsiegla, V. Point, A. Chabannes, A. Rousset, and F. Carrière, The galactolipase activity of some microbial lipases and pancreatic enzymes, European Journal of Lipid Science and Technology 115, 2013, 442–451.

[32] R. N. Z. R. A. Rahman, S. N. Baharum, M. Basri, and A. B. Salleh, High-yield purification of an organic solvent-tolerant lipase from *Pseudomonas* sp. strain S5, Anal Biochem 341 (2), 2005, 267–274.

[33] C. H. Tan, P. L. Show, C. W. Ooi, E. P. Ng, J. C. W. Lan, and T. C. Ling, Novel lipase purification methods - a review of the latest developments, Biotechnol J 10(1), 2015, 31–44.

[34] T. J. Silva, D. Barrera- Arellano, and A. P. Badan Ribeiro, The impact of fatty acid profile on the physicochemical properties of commercial margarines in Brazil, J Am Oil Chem Soc 99 (6), 2022, 469–483.

[35] A. Cherif and A. Slama, Stability and Change in Fatty Acids Composition of Soybean, Corn, and Sunflower Oils during the Heating Process, J Food Qual 22, 2022, 1–17.

[36] B. Hu, R. Wu, J. Sun, H. Shi, C. Jia, R. Liu, and J. Rong, Monitoring the oxidation process of soybean oil during deep-frying of fish cakes with 1H nuclear magnetic resonance, Food Chem X 17, 2023, 100587.

[37] M. Salameh and J. Wiegel, Lipases from Extremophiles and Potential for Industrial Applications, Adv Appl Microbiol 61(6), 2007, 253–283.

[38] K. Jaeger, B. W. Dijkstra, and M. T. Reetz, Bacterial Biocatalysts: Molecular Biology, Three-Dimensional Structures, and Biotechnological Applications of Lipases, Annu. Rev. Microbiol 53, 1999, 315–351.

[39] K.-E. E. Jaeger and T. Eggert, Lipases for biotechnology, CurrOpinBiotechnol 13(4), 2002, 390–397.

[40] A. K. Singh and M. Mukhopadhyay, Overview of fungal lipase: a review, Appl BiochemBiotechnol 166(2), 2012, 486–520.

[41] S. Amara, D. Lafont, B. Fiorentino, P. Boullanger, F. Carrière, and A. De Caro, Continuous measurement of galactolipid hydrolysis by pancreatic lipolytic enzymes using the pH-stat technique and a medium chain monogalactosyl diglyceride as substrate, BiochimBiophys Acta 1791(10), 2009, 983–990.

[42] S. Amara, N.Barouh, J. Lecomte, D.Lafont, S. Robert, P. Villeneuve, A. De Caro, F. Carrière, Lipolysis of natural long chain and synthetic medium chain galactolipids by pancreatic lipase-related protein 2, BiochimBiophys Acta Mol Cell Biol Lipids 1801(4), 2010, 508–516.

[43] R. Jallouli, H. Othman, S. Amara, G.Parsiegla, F. Carriere, N.Srairi-abid, Y. Gargouri, S.Bezzine, The galactolipase activity of *Fusarium solani* (phospho)lipase, Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1851(3),2015, 282–289.

[44] E. P. Hasson and G. G. Laties, Separation and Characterization of Potato Lipid Acylhydrolases, Plant Physiol 57, 1976, 142–147.

[45] T. Sakaki, T. Kato, and H. Saji, Lipid acyl-hydrolase in leaves of different kidney bean (*Phaseolus vulgaris* L.) cultivars : Purification and characterization of a kidney bean lipid acyl-hydrolase , and foliar lipid changes in the cultivars with ozone exposure, Plant Science 172, 2007, 462–472.

[46] B. Sias, F. Ferrato, P.Grandval, D. Lafont, P.Boullanger, A. De Caro, B. Leboeuf, R. Verger, F. Carrière, Human Pancreatic Lipase-Related Protein 2 Is a Galactolipase, Biochemistry 43, 2004, 10138–10148.