LIP4 gene expression from Candida viswanathii strain

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Research

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Posted Date: May 20th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-27743/v1

LIP4 gene expression from *Candida viswanathii* strain

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Abstract

Background: Lipases are hydrolases that catalyze the cleavage of triglyceride esters bonds, releasing glycerol and free fatty acids. *Candida* genus can produce distinct isoforms of lipase, among of them, *Candida viswanathii* strain is a potential lipase producer using hydrophobic carbons sources in which produced high level of enzyme under submerged cultivation using olive oil as carbon sources. This enzyme has commercially attractive due to characteristics desired in the industries processes. The genes responsible for encoding the lipases comprise a family called *LIP* gene. *C. viswanathii* not have its genome sequenced and are not available in annotated form through the GenBank nucleotide sequence database for lipase production. The aim of this work was understanding at molecular level the effect of carbon sources to lipase production, to identify and to analyses the gene expression of *CvLIP4* from *C. viswanathii* on different culture media.

Results: *In silico* analysis was carried out with *LIP4* gene from *Candida* species. Degenerate primers were designed and evaluated for expression in different conditions. *CvLIP4* expression was evaluated using carbon sources glucose, tributyrin, triolein and olive oil. Triolein and olive oil were strong inducer for *CvLIP4* gene expression, while tributyrin was a weak inducer and glucose was strong repressor.

Conclusions: These results will contribute to further studies about regulation of the lipase genes expression from *C. viswanathii* and heterologous expression of this enzyme to improve the catalytic conditions in industries processes.

Keywords: gene expression; carbon sources; lipase; polymerase chain reaction

Background

Lipases (E.C. 3.1.1.3) belong to a class of hydrolases and water-soluble enzyme which catalyses the hydrolysis of insoluble triacylglycerols to generate free fatty acids, diacylglycerols, monoacylglycerols and glycerol. They catalyze a variety of reactions including hydrolysis, transesterification and interesterification of other esters as well as the synthesis of esters, and exhibit a range of regio, enantio and stereoselective transformation properties [1]. Lipases are the most popular biocatalysts that have remarkable applications in promoting various biochemical processes in the industry. They are found to be successful in catalyzing numerous processes relevant to the food, pharmaceutical, leather, cosmetic, detergent, medical diagnostics, dairy, beverage, fatty acid, and paper industries [2].

Candida genera can produce several lipases isoform with different catalytic properties. The genes responsible for encoding the lipases comprise a family called *LIP* gene, being composed of several members and with different characteristics, such as the *LIP1* to *LIP8* genes of *C. rugosa*, as well as other sequences of these genes deposited in the GenBank [3, 4]. This gene family comprise a different catalytic behavior in terms of substrate specificity, thermal and pH stabilities. The differences in substrate specificity may be related to specific amino acid variations in the different parts that compose the catalytic machinery. The commercial CRL is actually a mixture of three *Lip* isoenzymes (*Lip1*, *Lip2*, *Lip3*) present in different proportions (e.g., the CRL mixture of Sigma is composed of 73% *Lip1*, 8% *Lip2* and 19% *Lip3*) with *Lip1* being the major component [5].

Candida viswanathii was firstly isolated from wastewater of a Brazilian oil refinery Replan/Petrobras, Paulínia, São Paulo, Brazil) and used for biodiesel and hydrocarbon bioremediation [6]. Almeida et al. [7] described the *C. viswanathii* strain as a potential lipase producer using hydrophobic carbons sources in which produced high level of enzyme under submerged cultivation using olive oil as carbon sources. Biochemical properties of crude lipase showed optimum pH and temperature activities at 4.0 and 40 ^{o}C, high thermal stability and activity in organic solvent [8]. The purified lipase presented high solvent stability and hydrolytic activity on natural triacylglycerols and soy lecithin [9]. *C. viswanathii* was also able to produce high level of lipase using vegetable oils from Brazilian Amazonian with different fatty acids

compositions [10]. The aim of this work was to evaluate at molecular level the effect of carbon sources on *LIP4* isoform expression of *C. viswanathii* by polymerase chain reaction under submerged cultivation conditions.

Methods

Maintenance lineage

C. viswanathii strain is maintained in Laboratory of Biotechnology, Food Analysis and Products (LABAP), Habite – Biotechnology Incubator Companies, Federal University of Tocantins – UFT. *C. viswanathii* was routinely cultivated on potato dextrose agar (PDA) for 3 days at 28 °C and then stored at 4 °C.

Culture conditions

Cultures were also performed in PDA slants at the same conditions for inoculum preparation. Submerged cultivations were carried out in Erlenmeyer flasks (250 mL) containing 60 mL of modified Vogel liquid medium [11] supplemented with 0.2% (w/v) yeast extract and 1.0% (w/v) carbon sources (glucose, tributyrin, triolein or olive oil), pH 6.0. Flasks containing the liquid cultures were autoclaved at 121°C for 20 min and inoculated with 3 mL of cells suspension (10^7 cells/mL). Cultivation was carried out at 28 °C, 180 rpm for 25 h. After cultivations, biomasses were centrifugated at 4,000 rpm, 4 °C for 5 min. One hundred of biomass was macerated using liquid nitrogen and stores at -80 °C.

In silico analyzes

C. viswanathii strain does not have its genome sequenced and are not available in annotated form through the GenBank nucleotide sequence database. A survey of known coding sequences of *LIP4* gene from *Candida* species using GenBank database (www.ncbi.nlm.nih.gov/genbank/). Analysis of the sequence's alignment of *Candida tropicalis* (XM_002548709.1), *Candida albicans* (AF191317.1) and *Candida orthopsilosis* (XM_003871089.1) were performed with the ClustalW program [12], followed by

standard parameters. For the construction of the phylogenetic tree, the MEGA 7.0 program [13] was adopted using the Neighbor-joining comparison model [14], with distance model *p* and pair-wise suppression. The statistical bootstrap method was used for validation of the phylogenetic tree with 10,000 replicates [15].

Design of degenerate primers

The nucleotide sequences of several species of the *Candida* genus were aligned in the same clade of the phylogenetic tree, using the Multiple Sequence Alignment by CLUSTALW program. With the result of the alignment an image was made using the GeneDoc program, where the regions of greater similarity were evaluated. The degenerate primers were designed in the most conserved regions for the *LIP4* gene, where the lowest degree of degeneration was obtained using the Primer Express v2.0 program of Applied Biosystems. For the use of degenerate bases was followed the information of the own manufacturer (https://www.thermofisher.com/br).

Conventional PCR conditions

Deoxyribonucleic acid (DNA) extraction was carried out using Ilustra Nucleon PhytoPure for small samples kit (GE Helthcare), following manufacturer´s protocol. Samples purity were analyzed in spectrophotometer Nanodrop One (Thermo Fischer Scientific) and stored at -20 ºC. Conventional PCR were carried out using kit GoTaq® Hot Start Green Master Mix, 2X (Promega), according manufacturer´s protocol. PCR reactions (T100 Thermal Cycler - Bio-Rad) were performed in reaction mixture in a total volume of 25 µL in an Eppendorf as follow: 12.5 µL GoTaq Hot Start Green Master Mix, 2X, 1.0 µL of primer forward [10 µM], 1.0 µL of primer reverse [10 µM], 1.5 µL of DNA [100.0 ng/µL] and 9.0 µL of nuclease free water. PCR cycles conditions were carried out as follow: 95 ºC for 5 min and 40 cycles with denaturation at 95 ºC for 30 s, annealing at 51.4 ºC for 45 s, and final extension at 72 °C for 1 min.

Design of primers *CvLIP4*

The primers design *LIP4* gene from *C. viswanathii* (named *CvLIP4*) was carried out by analysis of sequencing results obtained previously compared to the sequences used to design the degenerate primers (Table 1). From the sequence of *CvLIP4* to *C. viswanathii* obtained in the sequencing, the primers were designed using the Primer Express v2.0 program of Applied Biosystems.

Table 1

Sequencing

Amplified products were eluted with Illustra™ GFX™ PCR DNA and Gel Band Purification (GE Healthcare) according manufacturer´s protocol. Purified samples 2.0 µL (50 ng/ µL) were transferred to Eppendorf containing 1.0 µL of primer forward [10µM], and 4.5 µL of nuclease free water. These samples were submitted to Myleus Biotechnology (Belo Horizonte, Brazil) for sequencing by capillary electrophoresis, using POP7 polymer and BigDye v3.1.

RNA extraction, DNase treatment and cDNA synthesis

For ribonucleic acid (RNA) total extraction, Trizol™ was used according manufacturer´s protocol. Turbo DNA-Free™ (Applied Biosystems) was also used to eliminate possible trace amounts of contaminating genomic DNA according do kit instructions. The absence of DNA was also verified through PCR, using as template the total RNA. Nucleic acid quantification was assessed spectrophotometrically by measuring the absorbance at 260 nm using a NanoDrop One spectrophotometer (Thermo Fisher Scientific). RNA purity of samples was evaluated through the A260nm/A280nm and A260nm/A230nm ratio. This samples were stored at -80 °C. Samples of treated RNA (1,000 ng/µL) with high-degree purity and integrity were used to cDNA synthesis using High-Capacity cDNA Revere Transcription (Applied Biosystems).

Amplification of *CvLIP4* by conventional PCR

CvLIP4 amplification was performed using GoTaq® Hot Start Green Master Mix 2X (Promega) by conventional PCR according manufacturer´s protocol. For each reaction with 12.5 µL final volume were

added 6.25 µL GoTaq Hot Start reen Master Mix 2X, 0.5 µL primer forward (10 µM), 0.5 µL primer reverse (10 µM), 0.75 µL cDNA (100 ng/µL) and 4.5 µL nuclease free water. PCR was run (T100 Thermal Cycler, Bio-Rad) with an initial step at 95 °C for 5 min and 40 cycles with denaturation at 95 °C for 45 s, annealing at 59 °C for 45 s, extension at 72 °C for 30 s, and final extension at 72 °C for 5 min. Samples were stored at -20 ºC.

Results

C. viswanathii not have its genome sequenced and are not available in annotated form through the GenBank nucleotide sequence database for lipase production. For this study, *in silico* analysis was carried out with *LIP4* gene from *Candida* species (Figure 1A). Namely *CvLIP4* gene product was used to align the degenerate prime designed for *C. viswanathii* strain with *LIP4* gene from *C. orthopsilosis, C. albicans* and *C. tropicalis* (Figure 1B), where the similarity between the sequences in black indicates similarity between the bases of all species, gray indicates that similarity occurs in at least two species and white means that there is no similarity.

Figure 1.

Candida species showed little differences in conserved regions from *LIP4* gene. *CvLIP4* gene was amplified from *C. viswanathii* using degenerated primers (Figure 2). In this experiment, it was observed one band of 1,000 bp (amplicon expected) and one unspecific product; however, the annealing temperature of 51.4 ºC was more specific than 48.3 ºC to produce the interest product.

Figure 2.

The amplification and expression of *CvLIP4* gene was evaluated by cultivation of *C. viswanathii* under minimal salt medium containing sole carbon sources e.g. glucose (G), tributyrin (TB), triolein (TO) and

189 olive oil (O) (Figure 3). In these conditions, *CvLIP4* gene expression is directly associated to the carbon
190 source once the glucose was a strong repressor source, tributyrin a weak inducer source and triolein and
191 olive oil were a strong inducer to *C. viswanathii* lipase production. Triolein and olive oil presented
192 amplification bands with higher intensity when compared to tributyrin and glucose. Triolein and olive oil
193 present oleic acid (C18:1Δ^9) in your fatty acid composition.
194
195 Figure 3.
196
197 **Discussion**
198
199 *Candida* species can express eight *LIP* gene family with a high amino acid identity and similarity among
200 them [5]. In these work, *CvLIP4* from *C. viswanathii* was identify among homologous sequence from
201 *Candida* species and degenerated primers were designed and analyzed in different carbon sources. The
202 highly similarity in sequence but partially different in regions among the yeast species studied in this work
203 can offer distinct biochemical properties such as substrates molecules interactions, sugar content,
204 hydrophobicity and isoelectric point. Brocca et al. [16] extends the family of known *Lip* genes from
205 *Candida rugosa* to seven highly homologous sequences. However, five-lipase genes comprising lipase gene
206 family of *C. rugosa* with 80-88% pair wise identity, in nucleotide sequence. All genes encoding these
207 isozymes were located on the same chromosome, which suggests their origin through gene duplication.
208 The effect of culture conditions on the microbial growth e.g., carbon and nitrogen sources, not only
209 influence the lipase production but also changed the pattern formation of multiple forms of lipase. *C.*
210 *viswanathii* has strong repression of lipase expression compared to olive oil and triolein. The yeast follows
211 a complex pattern of lipase production depending on the presence of multiple lipase encoding genes whose
212 expression is modulate by carbon sources that can act as repressor (e.g. glucose), inducer sources (e.g. oleic
213 acid) or neutral substrates which is employed in two-step fermentation, where the first step of biomass
214 growth is followed by the induction of lipase gene expression [17]. In the first report about lipase production
215 by *C. viswanathii*, Almeida et al. [7] related that this yeast can growth in several pure carbon sources such

as glucose, galactose, mannitol sorbitol and glycerol but was not observed lipase production; on the other hand, pure fatty acids and triacylglycerols were able to promote the microbial growth and lipase secretion. On the other hand, the complex triacylglycerols were also evaluated according to presence of high percentage of long chain fatty acids which observed that presence of oleic acid in the total composition of fatty acids was the most important inducer to lipase production.

Oleic acid in widely described as main inducer source to true lipase production for many microorganisms. Olive oil contains approximately 80% oleic acid and it is considered a good inducer for lipase synthesis by many microorganisms what could be related with the fact of the lipases hydrolyze preferentially fatty acids residues at positions 1 and 3 of the glycerides, and some extracellular lipase requires oleic acid as stabilizer/activator [18]; and also to the presence of several tocopherols and other liposoluble vitamins that are important for microbial growth.

The weak intensity band formation using tributyrin source observed to *CvLIP4* gene expression by *C. viswanathii* can be explained due the specificity of true lipase to long-chain fatty acids ester. Lipases are activated on interfaces of insoluble lipid substrates oil-water, where the catalytic reaction is combined with various interfacial phenomena/processes. The catalytic mechanisms involved for lipase activity depend strongly on the mode of organization of the lipid substrates present in interfacial structures such as membrane bilayers, monolayers, micelles, vesicles, and oil-in-water emulsions [19]. A recombinant *LIP4* isoform from *C. rugosa* (formerly *Candida cylindracea*) was previously related to have higher esterase activity toward long acyl-chain ester and lower lipase activity toward triglycerides [20]. Tributyrin present short-chain fatty acid ester in triacylglycerol which are soluble in water and does not provide an interface oil-water formation. True lipases display a hydrophobic lid structure on active-site that regulate the activity mechanism in the presence of interface oil-water, which rearranges its position leaving an open gate to the active center. Then, the position of the lid marks the difference between the open (active) or closed (inactive) forms of these proteins [21, 22]. On the other hand, Fikers et al. [23] and Lotti et al. [24] reported the repression of lipase production caused by glucose from culture both.

Conclusion

This is the first report on gene expression from lipase family using *C. viswanathii* strain under cultivation growth conditions. These results will contribute to further studies about regulation of the lipase genes and heterologous expression of this enzyme and to understand and improve the catalytic conditions in industries processes.

Acknowledgements

This work was financial supported by National Council of Technological and Scientific Development (CNPq) (Process number 455754/2014-4). A.F. Almeida thank the Produtividade em Pesquisa da UFT grant (Edital 10/2018). Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. R.C. Teixeira thank the National Council of Technological and Scientific Development (CNPq) to Scientific Initiate grant (PIBIC-CNPq).

Author´s contributions

AFA and HGB conceived the study and supervised the experimental work; RCT and NMLO performed the lipase production experiments and determined enzymatic activity; RCT, MMD and HGB performed the in silico analysis, primer design and molecular analysis; RCT, AFA and HGB wrote the main manuscript text, discussions and review. All the authors reviewed and approved the final manuscript.

Funding

National Council of Technological and Scientific Development (CNPq) (Process number 455754/2014-4)

Availability of data and materials

Please contact author for data requests.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

[1] Sharma R, Chisti Y, Banerjee U. Production, purification, characterization, and applications of lipases. Biotechnol Adv 2001;19:627-62.

[2] Sarmah N, Revathi D, Sheelu G, et al. Recent advances on sources and industrial applications of lipases. Biotechnol Prog 2018; 34:5-28.

[3] Longhi S, Fusetti F, Grandori R, et al. Cloning and nucleotide sequences of two lipase genes from *Candida cylindracea*. BBA Gene Struct Expres 1999;21131:227-32.

[4] Lotti M, Grandori R, Fusetti F, et al. Cloning and analysis of *Candida cylindracea* lipase sequences. Gene 1993;124:45-55.

[5] Vanleeuw E, Winderickx S, Thevissen K, et al. Substrate-specificity of *Candida rugosa* lipase and its industrial application. ACS Sust Chem Eng 2019;7;15828−44.

[6] Soares J, Mariano A, de Angelis D. Biodegradation of biodiesel/diesel blends by *Candida viswanathii.* Afr J Biotechnol 2009;8:2774-78.

[7] Almeida A F, Taulk-Tornisielo S M, Carmona E C. Influence of carbon and nitrogen sources on lipase production by a newly isolated *Candida viswanathii* strain. Ann Microbiol 2013;63:1225-34.

[8] Almeida A F, Tauk-Tornisielo S M, Carmona E C. Acid lipase from *Candida viswanathii*: production, biochemical properties, and potential application. Biomed Res Int 2013:435818

[9] Almeida A F, Terrasan C R F, Terrone C C, et al. Biochemical properties of free and immobilized *Candida viswanathii* lipase on octyl-agarose support: Hydrolysis of triacylglycerol and soy lecithin. Process Biochem 2018;65:71-80.

[10] Gomes N B, Dias K B, Netto Teixeira M F, et al. Medium composition and Amazonian oils for lipase production by *Candida viswanathii.* Acta Sci Technol 2018;40:35088

[11] Vogel H J. A convenient growth medium for *Neurospora crassa* (medium N). Microbiol Gen Bul 1956;13:42–43.

296 [12] Thompson J, Higgins D, Gibson T. Clustal-W - improving the sensitivity of progressive multiple
297 sequence alignment through sequence weighting, position-specific gap penalties and weight matrix
298 choice. Nucleic Acids Res 1994;22:4673-80.
299 [13] Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for
300 bigger datasets. Mol Biol Evol 2016;33:1870-74.
301 [14] Saitou N, Nei M. The neighbor-joining method - a new method for reconstructing phylogenetic trees.
302 Mol Biol Evol 1987;4:406-425.
303 [15] Sitnikova T, Rzhetsky A, Nei M. Interior-branched and bootstrap tests of phylogenetic trees. Mol
304 Biol Evol 1995;12:319-33.
305 [16] Brocca S, Grandori R, Breviario D, Lotti M. Localization of lipase genes on *Candida rugosa*
306 chromosomes. Curr Genet 1995;28:454-57.
307 [17] Lotti M, Brocca S, Porro D. High lipase production by *Candida rugosa* is associated with G1 cells.
308 A flow cytometry study. Biotechnol Lett 2001;23:1803-08.
309 [18] Mafakher L, Mirbagheri M, Darvishi F, et al. Isolation of lipase and citric acid producing yeasts from
310 agro-industrial wastewater. N Biotechnol 2010;27:337-40.
311 [19] Aloulou A, Rodriguez J A, Fernandez S, et al. Exploring the specific features of interfacial
312 enzymology based on lipase studies. BBA-Mol Cell Biol L 2006;1761:995-1013.
313 [20] Tang S, Sun K, Sun G, et al. Recombinant expression of the *Candida rugosa LIP4* lipase in
314 *Escherichia coli.* Protein Exp Purif 2000;20:308-13.
315 [21] Fojan P, Jonson P H, Petersen M T, Petersen S B. What distinguishes an esterase from a lipase: a
316 novel structural approach. Biochimie 2000;82:1033-41.
317 [22] Barriuso J, Vaquero M E, Prieto A, et al. Structural traits and catalytic versatility of the lipases from
318 the *Candida rugosa*-like family: A review. Biotechnol Adv 2016;34:874-885.
319 [23] Fickers P, Nicaud J, Gaillardin C, et al. Carbon and nitrogen sources modulate lipase production in
320 the yeast *Yarrowia lipolytica.* J Appl Microbiol 2004;96:742-49.
321 [24] Lotti M, Monticelli S, Montesinos J L, S. Brocca, et al. Physiological control on the expression and
322 secretion of *Candida rugosa* lipase. Chem Phys Lipids 1998;93:143-148.

Figure Caption

Figure 1. Phylogenetic tree of *Candida* species that *Lip4* gene encoded (A). Alignment of LIP4 gene sequences in Candida species. Legend: *CvLIP4*: *C. viswanathii*; *CoLIP4*: *C. orthopsilosis*; *CaLIP4*: *C. albicans*; *CtLIP4*: *C. tropicalis*. Blue line indicate the regions used to design specific primer for *CvLIP4*.

Figure 2. Conventional PCR amplification *LIP4* using degenerate primers. Legend: M: molecular marker weight

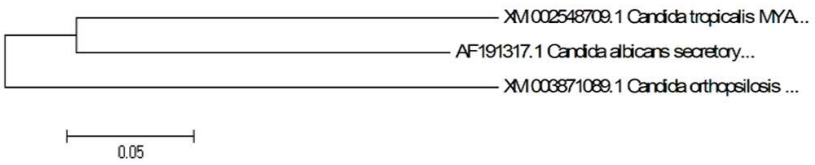
Figure 3. *CvLIP4* gene amplification in different culture media. Legend: TR: tributyrin; TO: triolein; O: olive oil; G: glucose.

Table 1. Degenerate and design primer for *LIP4* gene expression in *C. viswanathii*.

Gene		Primers (5´ - 3´)	MT (ºC)	Amplicon (bp)
LIP4	Degenerate primer	F: AARRTTCARAACTCYTGGCA R: ATKATCCAAGTYAAWGCARC	51.4	1001
CvLIP4	Design primer	F:GCGGAGTTACCCTTTATTGGTGT R:CCACGGACTGAGTTTAAGACAGC	59.0	131

Legend: F: primer forward; R: primer reverse; MT: melt temperature; bp: bases pair

A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AF191317.1 Candida albicans secretory...
XM 003871089.1 Candida orthopsilosis ...
0.05

B

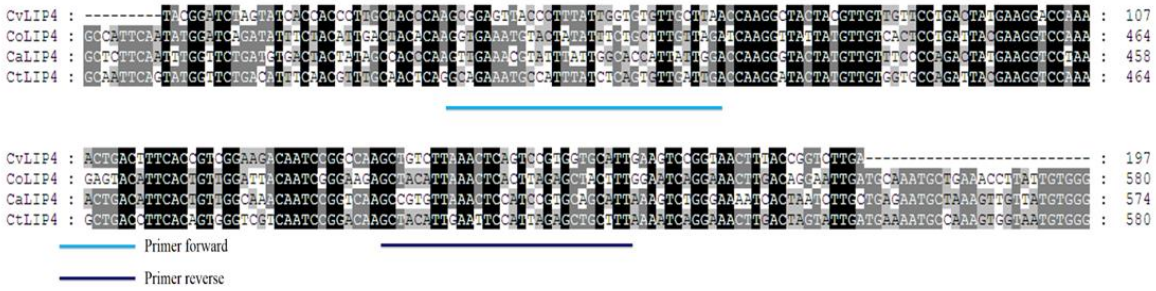

CvLIP4 : ---------TACGGATCTAGTATCACCAACCTTGCTACCCAAGCGGAGTTACCCTTTATTGGTGTGTTGCTTAACCAAGGCTACTACGTTGTTGTTCCCGACTAAGAAGGACCAAA : 107
CoLIP4 : GCCATTCAATATGGATCGATATTTCTACATTGCTACCCAAGGTGAAATGTACTATATTTCTGCTTTGTTAGATCAAGGATTATTATGTTGTCACTCCTGATTAGGAAGGTCCAAA : 464
CaLIP4 : GGTCTTCAATTTGGTTCTGATGTGCTACTTTATGCCACCCAAGTTGAAAGCTATTTATTGGCACCTTTTTTGGACCAAGGCTACTATGTTGTTTCCCCGACTAAGAAGGTCCAAA : 458
CtLIP4 : GCAATTCAGTATGGTTCTGACATTTCTAGCTTTGCTACCCAGCCTGAAATGCCATTTATCTCAGTGTTGATTGACCAAGGATACTATGTTGTGGTGCCTGACTAGAAGGTCCAAA : 464
CvLIP4 : ACTGACTTTCACCGTTGGGAAGACAATCCGGCCAAGCTGTCTTAAACTCAGTCCGTGGCAGCATTGAAGTCGGTAACTTGACCGGTCTTGA---------- : 197
CoLIP4 : GAGTACATTCACTGTTGGATTACAATCGGGAAGAGCTACATTAAACTCAGTTAGAGCTACTTTGGAATCTGGTAACTTGACAGGAATTGATGCAAATGCTGAAACCTTATTGTGGG : 580
CaLIP4 : ACTGACATTCACTGTTGGCAAACAATCCGGTCAAGCCGTGTTAAACTCCATCCGTGGCAGCATTAAAGTCTGGCAAATTGACTAATCTTGCTGAGAATGCTAAAGTTGTATTGTGGG : 574
CtLIP4 : GCTGACGTTCACTGTTGGTCGTCAATCCGGTCAAGCTACATTGAATTCCATTAGAGCTGCTTTAAATTCTGGTAACTTGACTAGTATTGATGAAAATGCCAAGTTGGTAATTGTGGG : 580
Primer forward
Primer reverse

Figure 1.

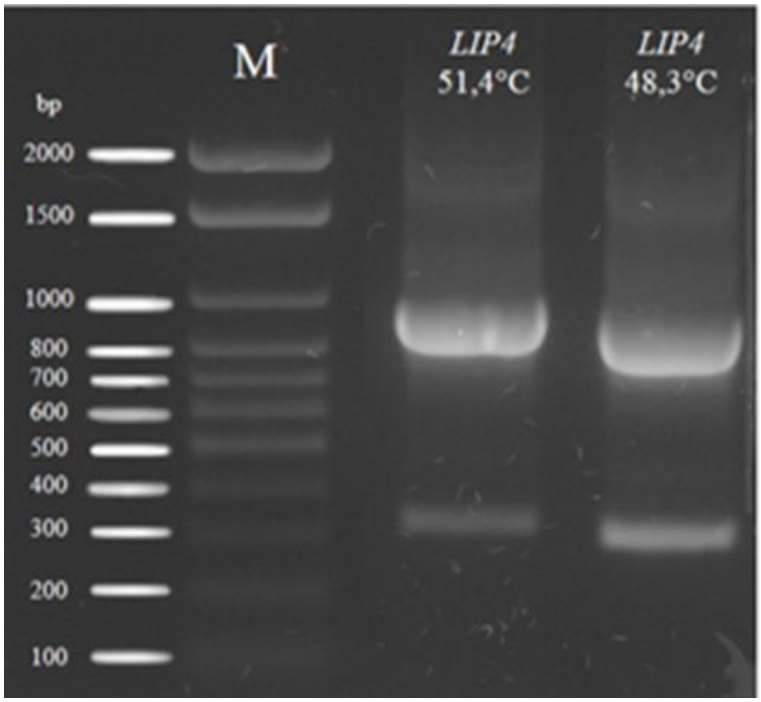
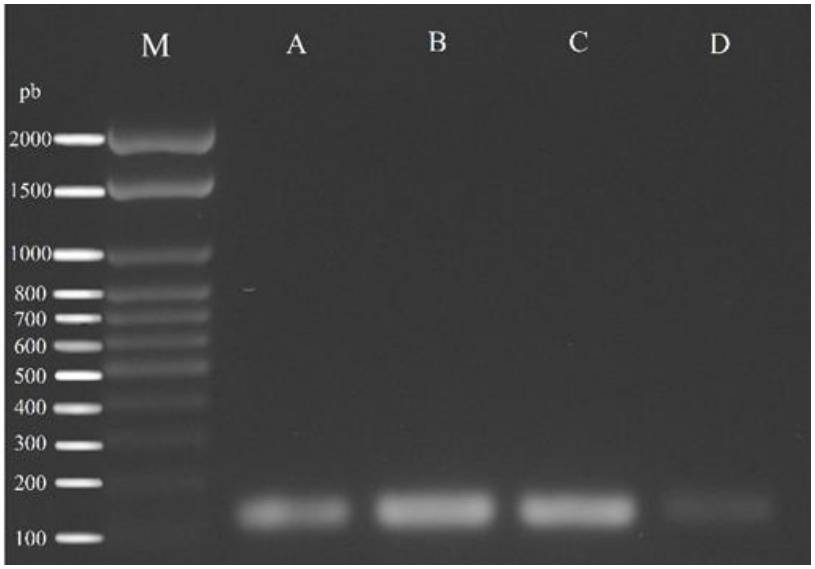

M
LIP4
51,4°C
LIP4
48,3°C
bp
2000
1500
1000
800
700
600
500
400
300
200
100

Figure 2.



420

421 Figure 3.

Figures

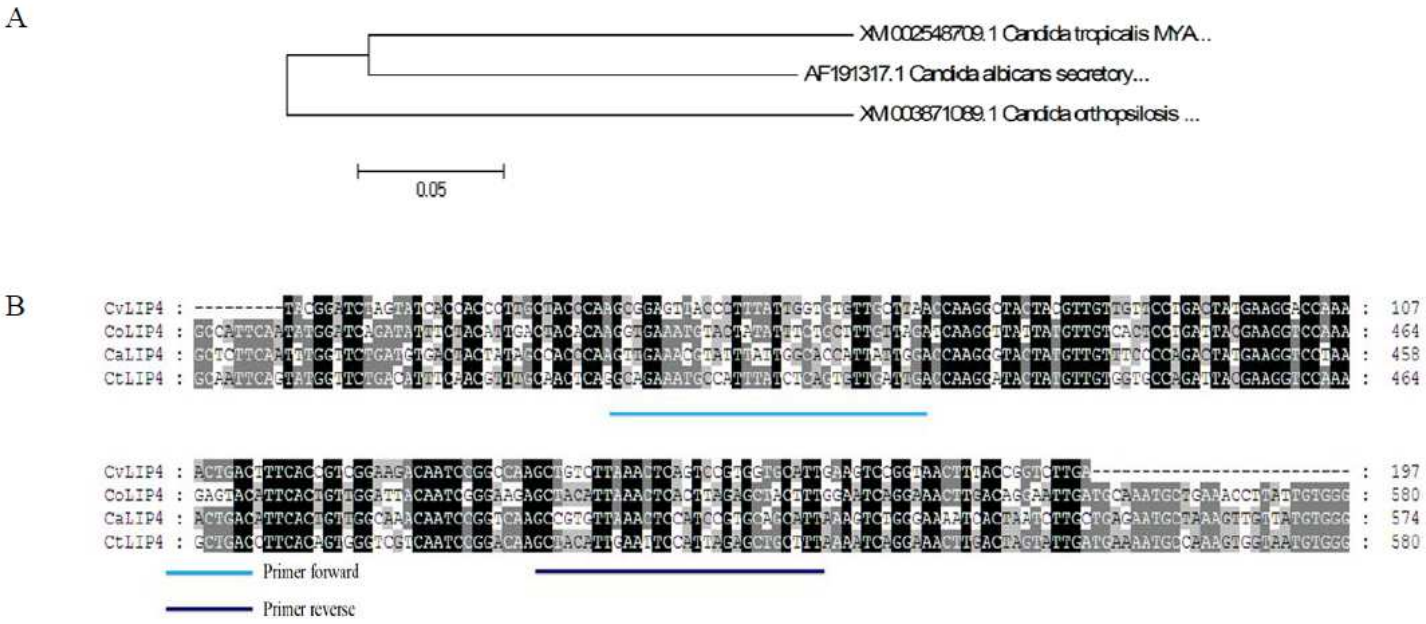


Figure 1

Phylogenetic tree of Candida species that Lip4 gene encoded (A). Alignment of LIP4 gene sequences in Candida species. Legend: CvLIP4: C. viswanathii; CoLIP4: C. orthopsilosis; CaLIP4: C. albicans; CtLIP4: C. tropicalis. Blue line indicate the regions used to design specific primer for CvLIP4.

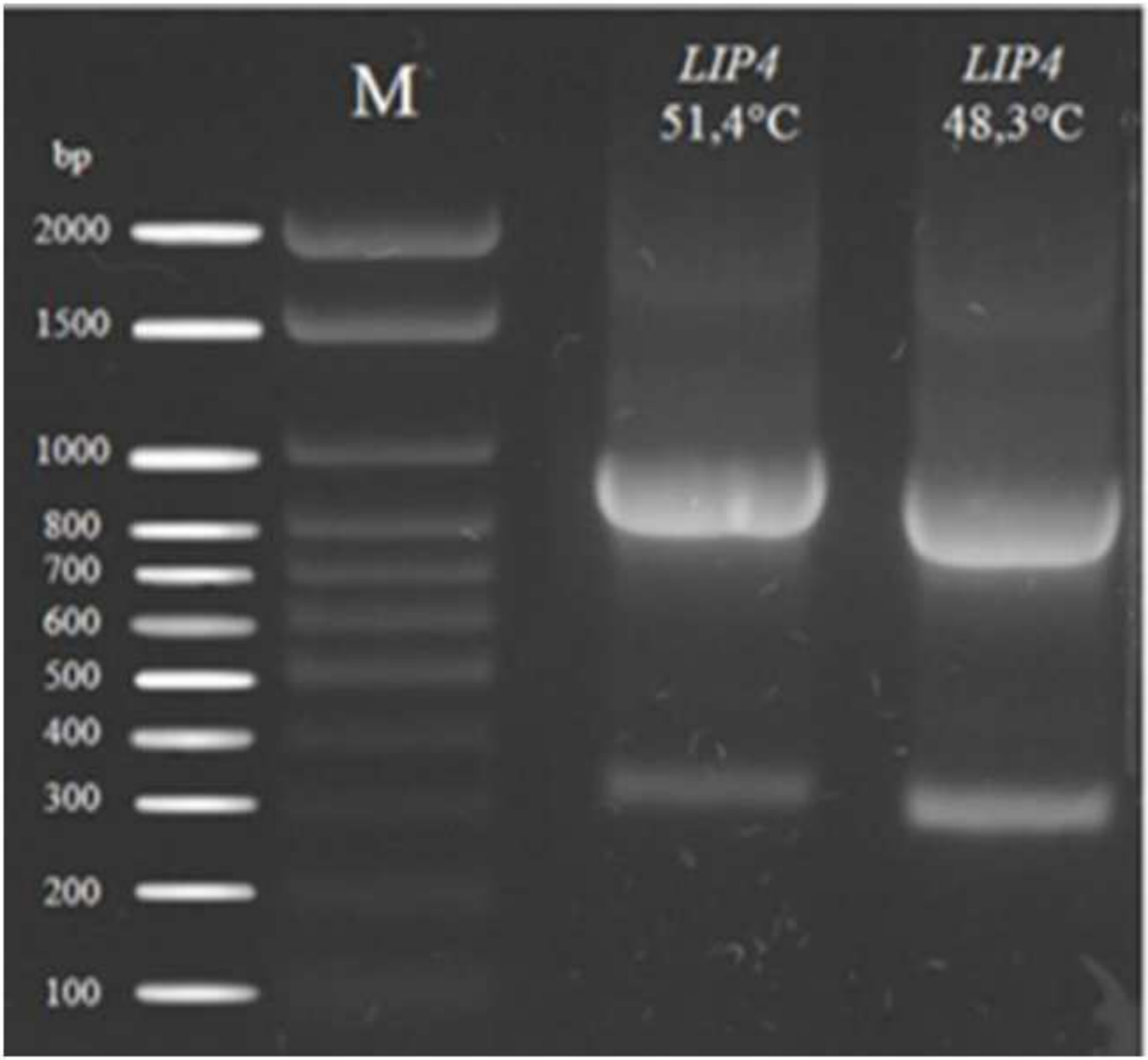


Figure 2

Conventional PCR amplification LIP4 using degenerate primers. Legend: M: molecular marker weight

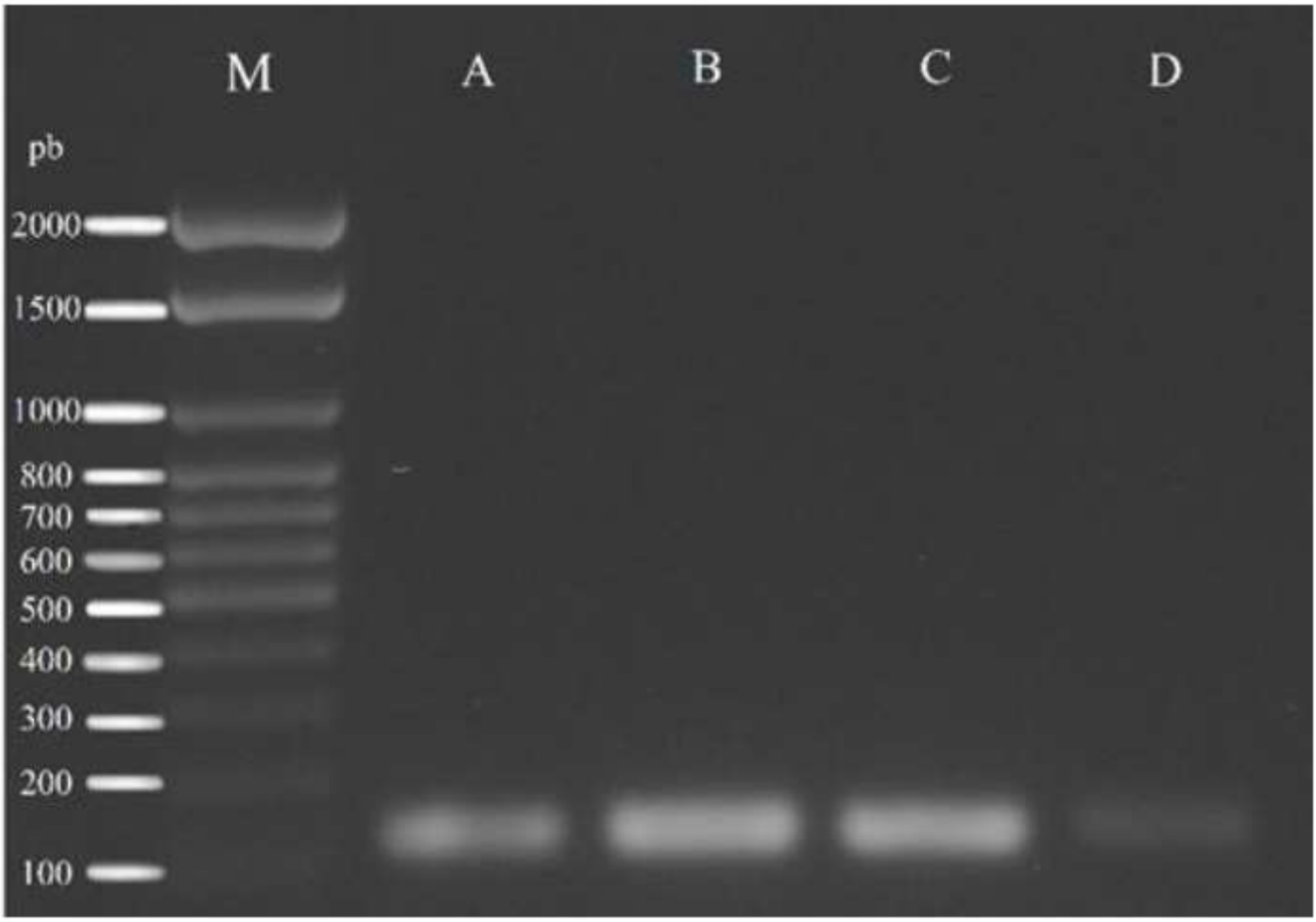


Figure 3

CvLIP4 gene amplification in different culture media. Legend: TR: tributyrin; TO: triolein; O: olive oil; G: glucose.