Karine Dalazoana
(Organizadora)

A Produção do Conhecimento nas Ciências Biológicas 2

Atena Editora
Ano 2019

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Atena Editora
2019

2019 *by Atena Editora*

Editora Executiva: Profª Drª Antonella Carvalho de Oliveira
Diagramação: Lorena Prestes
Edição de Arte: Lorena Prestes
Revisão: Os Autores

Atena Editora
Ano 2019

Dados Internacionais de Catalogação na Publicação (CIP)
(eDOC BRASIL, Belo Horizonte/MG)

P964 A produção do conhecimento nas ciências biológicas 2 [recurso eletrônico] / Organizadora Karine Dalazoana. – Ponta Grossa, PR: Atena Editora, 2019. – (A produção do conhecimento nas ciências biológicas; v. 2)

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader.
Modo de acesso: World Wide Web.
Inclui bibliografia
ISBN 978-85-7247-638-6
DOI 10.22533/at.ed.386192309

1. Ciências biológicas. 2. Biologia – Pesquisa – Brasil. I.Dalazoana, Karine.

CDD 574

Elaborado por Maurício Amormino Júnior – CRB6/2422

Atena Editora
Ponta Grossa – Paraná - Brasil
www.atenaeditora.com.br
contato@atenaeditora.com.br

Atena Editora
Ano 2019

APRESENTAÇÃO

A Produção do Conhecimento nas Ciências Biológicas 2 é uma obra que tem por objetivo dar continuidade à divulgação dos estudos realizados na área das Ciências Biológicas em diversas instituições de ensino e pesquisa no Brasil.

O segundo volume traz onze artigos, que versam sobre temas de grande relevância científica, alinhados com as demandas atuais de conhecimento, com enfoque nas áreas de biologia molecular, microbiologia, biorremediação, epidemiologia, botânica, zoologia, ensino de ciências e campos correlatos.

A pesquisa nas ciências biológicas oferece uma amplitude de vertentes de estudo e busca compreender o funcionamento do mundo microbiológico, promover a manutenção dos ecossistemas naturais, a conservação de paisagens e de espécies em risco ou ameaçadas, compreender o processo de evolução das espécies, o desenvolvimento de tecnologias sustentáveis e, o mais importante, levar todo o conhecimento produzido à sociedade, de modo a contribuir com o desenvolvimento regional resultando na melhoria da qualidade de vida da população.

A pesquisa nas ciências biológicas tem a preocupação de buscar sempre alternativas sustentáveis para a manutenção da qualidade de vida das populações humanas e a conservação das populações naturais com a manutenção de hábitat, garantindo assim o seu potencial biótico e o fluxo gênico. Tais estratégias, seja com espécies de micro-organismos ou componentes da fauna e da flora, garantem a conservação da biodiversidade brasileira e todas as suas peculiaridades.

Mais além, é necessário divulgar as descobertas científicas e aplicá-las de modo a otimizar as experiências da vida cotidiana. Nesse sentido o ensino de ciências se presta como ferramenta de grande valia, capacitando alunos como multiplicadores de boas práticas para a conservação da biodiversidade e manutenção dos recursos naturais.

Espera-se que a Produção do Conhecimento nas Ciências Biológicas 2 venha contribuir para com os pesquisadores na área da Biologia e, além disso, possa contribuir com a sociedade, uma vez que os conhecimentos produzidos nos centros de ensino superior do Brasil não devem ficar restritos aos muros das instituições e sim subsidiar práticas viáveis ambientalmente, socialmente e economicamente.

Boa leitura.

Karine Dalazoana

SUMÁRIO

CAPÍTULO 1 1

A PRODUÇÃO DE ÁCIDO KÓJICO POR *Aspergillus flavus*

Hellen Kempfer Phillippsen
Alberdan Silva Santos

DOI 10.22533/at.ed.3861923091

CAPÍTULO 2 9

AVALIAÇÃO DO CRESCIMENTO RADICULAR DA LEGUMINOSA *Canavalia ensiformis L.* CULTIVADA EM SOLOS CONTAMINADOS POR MEDICAMENTOS

Maise Menezes dos Santos Souza
Juliana do Nascimento Gomides

DOI 10.22533/at.ed.3861923092

CAPÍTULO 3 22

CARACTERIZAÇÃO E DIVERSIDADE DOS CRIADOUROS ENCONTRADOS COM FASES EVOLUTIVAS DE *Aedes aegypti* (LINNA.US, 1762) E *Aedes albopictus* (SKUSE, 1894) (Díptera: Cullicidae), VETORES DAS ARBOVIROSES NO MUNICÍPIO DO IPOJUCA - PE/BRASIL

Hallysson Douglas Andrade de Araújo
Jussara Patrícia Monteiro Vasconcelos
Robson Ramos Lima de Melo
Anderson Artenis dos Santos Francelino
Odilson Bartolomeu dos Santos
Andrea Lopes de Oliveira
Juliana Carla Serafim da Silva

DOI 10.22533/at.ed.3861923093

CAPÍTULO 4 30

CONSTRUÇÃO DE UM MODELO NIR (ESPECTROSCOPIA NO INFRAVERMELHO PRÓXIMO) PARA PREDIÇÃO DE PRODUÇÃO E CONSUMO DE POLISSACARÍDEOS EXTRACELULARES DURANTE A FERMENTAÇÃO DOS RESÍDUOS DA PALMA DE ÓLEO POR *Pleurotus Ostreatus*

Jhonatas Rodrigues Barbosa
Ivonete Quaresma da Silva de Aguiar
Maurício Madson dos Santos Freitas
Raul Nunes de Carvalho Junior
Marcos Enê Chaves Oliveira

DOI 10.22533/at.ed.3861923094

CAPÍTULO 5 40

FAUNA DIGITAL DO RIO GRANDE DO SUL: CRIANDO UMA PLATAFORMA DIGITAL PARA DIVULGAÇÃO CIENTÍFICA

Filipe Ferreira da Silveira
Maria João Veloso da Costa Ramos Pereira
Gabriel Matte de Oliveira
Heitor Jardim Ferreira
Rafaella Migliavacca Marchioretto

DOI 10.22533/at.ed.3861923095

CAPÍTULO 6 53

HISTOLOGIA DA VIDA: UMA PROPOSTA DE ATIVIDADE CONTEXTUALIZADA PARA O ENSINO

Luciano Cardoso Santos
Cristina Luísa Conceição de Oliveira

DOI 10.22533/at.ed.3861923096

CAPÍTULO 7 59

Licania tomentosa (Benth.) FRITSCH: DA ARBORIZAÇÃO URBANA À FITOTERAPIA, REVISÃO DE LITERATURA

Jonathan Augusto da Silva
Maria Ágda Correia Lemos
Maria Lúcia Vieira de Britto Paulino
Helane Carine de Araújo Oliveira
Heloísa Helena Figuerêdo Alves
Karulyne Silva Dias
Mayara Andrade Souza
Thiago José Matos Rocha
Jessé Marques da Silva Júnior Pavão
Joao Gomes da Costa
Aldenir Feitosa dos Santos

DOI 10.22533/at.ed.3861923097

CAPÍTULO 8 71

PERCEPÇÕES DE ESTUDANTES DO 4º AO 9º ANO DO ENSINO FUNDAMENTAL
EM DUAS ESCOLAS PÚBLICAS DE ESPERA FELIZ, MG SOBRE OS MORCEGOS *(Chiroptera, Mammalia)*

Maria Joventina Ferreira Bendia
Viviane da Silva de Oliveira
Alessandro Brinati
Luciane da Silva Oliveira

DOI 10.22533/at.ed.3861923098

CAPÍTULO 9 77

SCIENTIFIC PROSPECTION OF THE MOLECULAR CHARACTERIZATION OF LIPASE *RHIZOMUCOR MIEHEI* FREE AND IMOBILIZED FORM

Fabiana Borralho Frazão
Ricardo Henrique Nascimento Frazão
Isadora Fontenelle Carneiro de Castro
Emygdia Rosa do Rêgo Barros Pires Leal
Marcelo Souza de Andrade
Adeilton Pereira Maciel

DOI 10.22533/at.ed.3861923099

CAPÍTULO 10 88

TRANSMISSÃO E IMPORTÂNCIA DE STAPHYLOCOCCUS COAGULASE NEGATIVA RESISTENTE A METICILINA

Nahara Cralcev Maróstica
Álex Aparecido Rosini Silva
Natália Reiko Sato Miyasaka

DOI 10.22533/at.ed.38619230910

CAPÍTULO 11 93

USO DE TANINO, ÓLEOS FUNCIONAIS E FRACIONANDO DE LEVEDURAS COMO SUBSTITUTOS DA VIRGINIAMICINA EM GRÃOS INTEIROS DE MILHO

Marcelo Penha Silva
Wallace Vieira da Silva

DOI 10.22533/at.ed.38619230911

SOBRE A ORGANIZADORA..... 101

ÍNDICE REMISSIVO 102

CAPÍTULO 9

SCIENTIFIC PROSPECTION OF THE MOLECULAR CHARACTERIZATION OF LIPASE *Rhizomucor miehei* FREE AND IMOBILIZED FORM

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RESUMO: Este artigo teve por objetivo fazer uma prospecção científica dos avanços da caracterização molecular da lipase *Rhizomucor miehei* livre e imobilizada. A prospecção científica foi realizada entre os meses de outubro de 2018 à janeiro de 2019, por meio de buscas nas bases de artigos nos portais de periódicos Web of Science, Scopus, Scientific Eletronic Library Online (Scielo) e Periódicos Capes. A plataforma online Periódico Capes (1.025) é a que detém o maior número de publicações considerando o termo "Rhizomucor miehei lipase", seguido do Web of Science (360) e da *Scopus* (307). Quanto a base de dados Scielo, esta é que menos fornece informações sobre a lipase Rhizomucor miehei de forma livre ou até mesmo inexistindo dados sobre a forma imobilizada. Quanto os métodos de caracterização molecular, verificou-se que a Reação em Cadeia da Polimerase – PCR foi a mais dotada nas pesquisas, sendo publicados um total de 197 artigos distribuídos entre as quatro bases de dados. Os resultados da prospecção demonstraram que ainda existem poucas pesquisas com enfoque na relação da biologia molecular com o processo de imobilização de lipases. Porém, houve um percentual de crescimento desde 1988 até 2018 nas pesquisas com a temática "Rhizomucor miehei lipase". Portanto, conclui-se que o Brasil está no ranking dos países que mais publicam sobre esta lipase fúngica, caracterizando o grande potencial em biodiversidade e biotecnológico que este país apresenta.
PALAVRAS-CHAVE: Prospecção. Enzima. Biologia molecular. Métodos de imobilização. Suportes.

ABSTRACT: This article aimed to make a scientific prospection of the molecular characterization of *Rhizomucor miehei* lipase

free and immobilized form. The scientific prospection was carried out from October 2018 to January 2019, through searches of articles bases in the Web of Science, Scopus, Scientific Electronic Library Online (Scielo) and Capes Journals portals. The Capes Online Journals platform (1.025) has the largest number of publications considering the term "*Rhizomucor miehei* lipase", followed by Web of Science (360) and Scopus (307). As for the Scielo database, this is the one that provides information about *Rhizomucor miehei* lipase free form or even lacking data on the immobilized form. As for the molecular characterization methods, it was verified that the Polymerase Chain Reaction - PCR was the most endowed in the research, and a total of 197 articles distributed among the four databases were published. The results of the prospection showed that there are still few researches focusing on the relationship between molecular biology and the lipase immobilization process. However, there was a percentage of growth from 1988 to 2018 in the researches with the theme "*Rhizomucor miehei* lipase". Therefore, it is concluded that Brazil is in the ranking of the countries that publish the most about this fungal lipase, characterizing the great potential in biodiversity and biotechnology that this country presents.
KEYWORDS: Prospection. Enzyme. Molecular biology. Methods of immobilization. Brackets.

1 I INTRODUCTION

The multidisciplinary area of biocatalysis is currently undergoing extensive development. Research in various fields of chemistry and molecular biology has as its main objective, the development of new catalysts for industrial use. Despite the great development of molecular biology techniques aiming to obtain enzymes with their specificities altered, the exploration of biodiversity with the selection of new microorganisms and enzymes is still the most frequently used method for the development of biocatalysis on an industrial scale. Enzyme cloning and expression in host microorganisms that are easy to grow are of more immediate usage, because they lead to the production of biocatalysts in quantities which are compatible with their use in industrial processes (CONTI et al., 2001).

The enzymes, known industrially as biocatalysts, are generally proteins which are formed by long chains of amino acids with peptide bonds. For thousands of years, enzymes have been used in traditional processes. These biocatalysts can be extracted from animal, plant and microorganism tissues (SENAI, 2009).

The Enzyme Commission of the International Union of Biochemistry and Molecular Biology (IUBMB) divides the enzymes into six main groups, oxidoreductases (1), transferases (2), hydrolases (3), lyases (4), isomerases (5) and ligases (6) in which subclasses are included according to the type of catalyzed reaction. In such a system, each enzyme receives its recommended name, usually formed by the name of the catalyzed substrate and the suffix -*ase*. Each enzyme also receives its systematic name consisting of four digits, each number describing the enzyme in a

progressively more detailed way. (IUBMB, 2013).

Lipases, which are classified as EC 3.1.1.3, in which: EC stands for Enzyme Commission of the IUBMB: first digit (3 – Hydrolase Class); second digit (1 – Esterase Subclass); third digit (1 - Carboxylic ester hydrolase sub-subclass) and fourth digit (3 - Triacylglycerol lipase) (LOPES et al., 2011).

Lipases (Glycerol ester hydrolases; EC 3.1.1.3) present promising applications in a wide range of biotechnological activities and industrial processes, including flavor enhancement in the food industry, biodiesel production and use in the pharmaceutical industry. These enzymes hydrolyze triacylglycerols to fatty acids, glycerol and partial acylglycerols. This reaction is reversible; thus, lipases also catalyze the formation of glycerol acylglycerols and free fatty acids by means of esterification. Other valuable properties of most lipases are the ability to catalyze enzymatic interesterification reactions rearranging a triglyceride molecule, as well as those of transesterification between oily compounds, alkyl or aryl esters, and alcohols. Lipases can also be used to accelerate the degradation of fatty residues and polyurethane (TAKÓ et al., 2017).

From the industrial point of view, lipases are considered very important due to their catalytic properties and easy production on a large scale. The lipases can be produced by several microorganisms such as *Bacillus* sp., *Candida rugosa*, *Candida antartica*, *Burkholderia cepacia*, *Aspergillus* sp., *Rhizopus* sp., *Penicillium* sp., *Rhizomucor miehei* among others (SOUZA et al., 2013; CARVALHO et al., 2015).

Thermophilic fungi are important microorganisms producing thermostable enzymes that can be used in temperature bioprocesses. For a long time, *R. miehei* as a thermophilic fungus has been used mainly as a producer of proteases and industrial lipases. The aspartic protease produced by *R. miehei* has been widely used as a substitute for calf chymosin in industrial cheese manufacturing. However, *R. miehei* lipases are quite studied and commercially available in the soluble and immobilized forms, with very high activity and good stability (Morgenstern et al., 2012; ZHOU et al., 2014).

The extracellular lipase enzyme (EC 3.1.1.3) from *Rhizomucor miehei* (RML) was first described in the 1970s and, in subsequent years, has been reported for its application in the food industry. This lipase is a highly versatile biocatalyst, also widely used in the agrochemical industry, in the field of biodiesel, biofuel, as well as in the pharmaceutical industry. However, the application of RML in industries is still limited due to its high price (HE et al., 2015; LI et al., 2016).

Rhizomucor miehei lipases are extracellular proteins of fungal origin constituted of 269 amino acids having a molecular mass of 31.6 kDa and an isoelectric point (pI) of 3.8 (HE et al, 2015;. Rodrigues; Fernandez Lafuente, 2010). Nevertheless, in the studies of ZHOU et al. (2014), the molecular mass of *R. miehei* was estimated to be approximately 55 kDa by means of the SDS-PAGE type of electrophoresis. In the case of filamentous fungi, the molecular mass of lipases generally ranges between 25 and 70 kDa.

The catalytic sites of the *R. miehei* lipase are formed by the residues of serine 144, histidine 257 and aspartate 203 amino acids. The backbone of serine 82 and leucine 145 residues constitute the “oxyanion cavity”. The hydrophobic lid region (lid) consists of amino acids residues ranging from position 85 to position 91 (OLIVEIRA et al., 2018).

Currently, this enzyme is marketed in a soluble form (Palatase 200L) or in an immobilized form (Lipozyme RM IM) by manufacturers such as Sigma and Novozymes (OLIVEIRA et al., 2018). There is still a great potential for the improvement of RML’s performance. In addition to this, the cost of this enzyme itself corresponds to the majority (more than 90%) of the total costs of the enzymatic process. Thus, the challenge in the field of research is to increase the expression of this enzyme and to reduce its production costs (HE et al., 2015).

The immobilized form of *R. miehei* lipase is especially attractive in biotechnological processes in order to meet the requirements for industrial application, such as reactions of biotransformation. The improvement of catalytic efficiency leads to an increase in the value added in the face of the principles of green chemistry and sustainability (CARVALHO et al., 2015).

In addition, in comparison to *R. miehei* free lipase, the immobilized lipase has a good capacity for reuse and stability, resulting in cost savings (LI et al., 2016).

Enzymatic immobilization may be defined as: enzymatic molecules confined to a solid matrix/support other than that in which the substrate or products are present. That is, by binding the enzymes or inserting them into a suitable carrier material. Various materials may be used as a support to immobilize the enzymes, but inert polymers and inorganic materials are commonly used. The ideal carrier must be of a low cost, inert, stable, exhibiting physical strength, capacity to increase enzyme specificity, capacity to reduce product inhibition and to prevent non-specific adsorption and bacterial contamination (SIRICHA, VL et al., 2016).

The physical, chemical and morphological modifications of the carriers, by means of the application of additives, can produce imobilized biocatalysts with higher catalytic efficiency due to the minimization of the diffusional effects of substrates and products during the reaction, besides the improvement of the operational stability in continuous and discontinuous processes, and, for this reason, it also arouses the industrial interest for these biocatalysts (CARVALHO et al., 2015 , OLIVEIRA et al., 2018).

The current methods of enzymatic immobilization can be divided into three categories: chemical interaction (adsorption, ionic bonds, covalent bonds), physical retention (encapsulation) and cross-linking binding (SANTOS et al., 2014).

Because of the advantages provided, the application of *Rhizomucor miehei* lipase in its process of immobilization and molecular characterization has become a very promising field of research in the world market. In the present work, a review and scientific prospecting study was carried out in order to map the existing

publications in the area and analyze the perspectives on the subject.

2 I MATERIAL AND METHODS

In the present study a literature review was carried out to identify researches that used molecular biology techniques and the *Rhizomucor miehei* lipase immobilization process.

The scientific prospection was carried out from October 2018 to January 2019, through searches on articles databases in the Web of Science, Scopus, Scientific Electronic Library Online (Scielo) and Capes periodicals portals.

The searched terms were chosen based on keywords that frequently appear in articles related to the area and in evaluating the relevance of the results of previously searched terms. The keywords searched were "*Rhizomucor miehei* lipase", "*Rhizomucor miehei* lipase and Immobilization", "*Rhizomucor miehei* and lipase immobilized and molecular characterization or molecular biology" among others. All the terms were used for the international databases and their respective translations in Portuguese were used for the searches in the Brazilian database. The expressions were selected so that they varied from more general terms to more specific terms, in order to compare and map the scientific and technological productions of effluent treatment with other applications of lipase enzymes.

Inclusion criteria was defined as: articles published between 1992 and 2018, since initial surveys identified that in the period prior to 1992 there is a shortage of articles that describe in greater detail the molecular characterization and immobilization of *R. miehei* lipase. Finally, the scientific works, where more accurate expressions were detected, were organized based on the countries of origin and also on the temporal distribution of their publications.

3 I RESULTS AND DISCUSSION

The publication of articles is a means of democratizing science and reveals to society the scientific content of research being carried out by groups, institutions and companies; which allows other scientists to use it and evaluate it on other aspects (BROFMAN, 2012). Interest in fungal lipase can be assessed by the increasing number of articles published and indexed in the Web of Science database. The results of the bibliometric analysis for the search for the keyword "*Rhizomucor miehei* lipase", in the last 27 years, are presented in Figure 1.

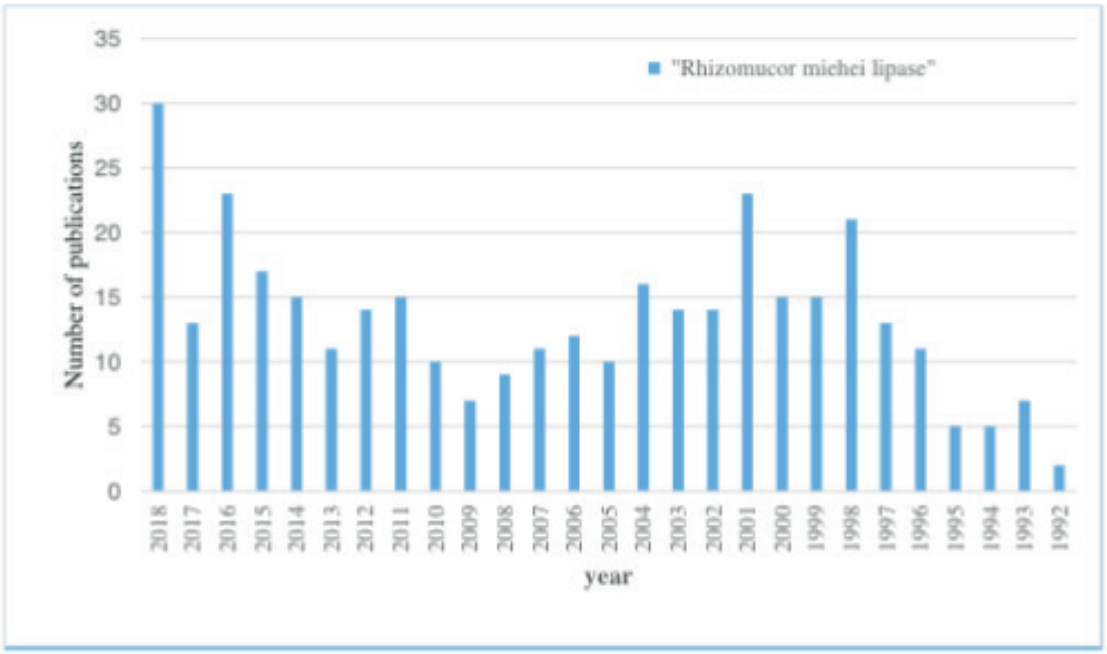

Figure 1: Evolution of the number of records of articles published in Web of Science on the keywords "*Rhizomucor miehei* lipase", from the period from 1992 to 2018.

Source: Own authorship (2019).

According to Figure 1, publications for the general topic "*Rhizomucor miehei* lipase" increased by 91.06% in the year 2018, in which it evolved from 2 manuscripts in the year 1992 to 356 articles published by 2018.

After analyzing the documents, it was found that the United States and China had the largest number of publications on *Rhizomucor miehe*i lipase (Figure 2), with 40 and 39, respectively, representing about 21.94% of all publications.

Considering only the publications with authors and co-authors of Brazilian institutions, Brazil will be in the 12th place in the list of countries with publications in the topic "*Rhizomucor miehei* lipase", with 16 (4.44%) articles published until this year.

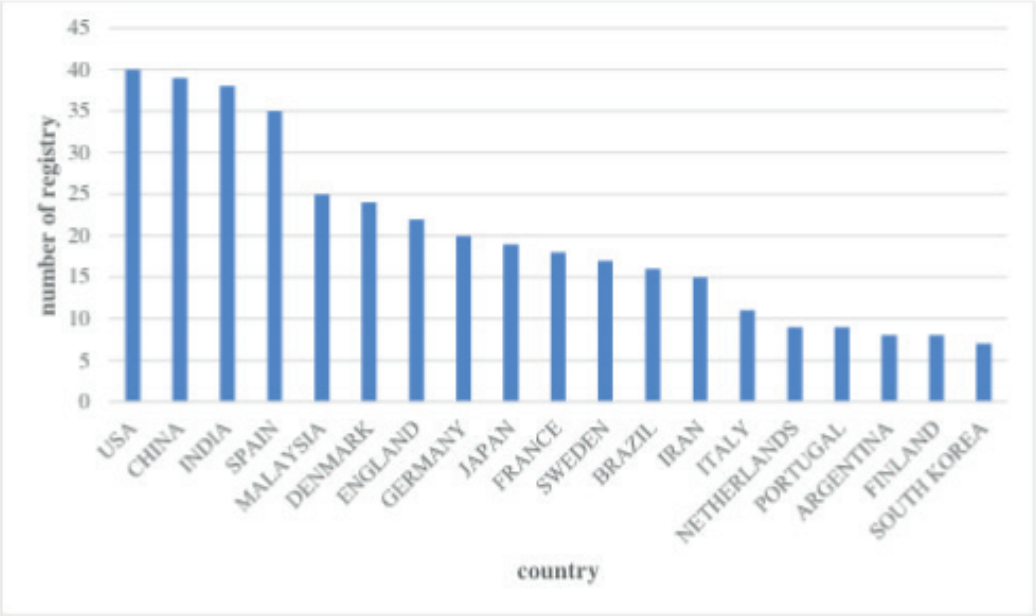

Figure 2: Participation of countries with the largest number of articles publications related to *Rhizomucor miehei* lipase in the Web of Science, from 1992 to 2018.

Source: Own authorship (2019).

Scientific publications in indexed journals generate recognition and a higher rate of approval of projects by the development agencies, especially with regard to research in Brazil. However, in addition to publishing results to disseminate knowledge to society, the researcher must ensure the protection of research and the availability of its results as assets for society (Giese et al., 2010; Federman 2010).

Scientific prospection of the molecular characterization of *Rhizomucor miehei* lipase in free and immobilized form

In analyzing the results for molecular characterization of lipase, it is possible to observe the difference in the number of scientific productions when compared to the term "*Rhizomucor miehei* lipase" with the term "*Rhizomucor miehei* lipase and molecular characterization OR molecular biology" (Table 1), using the Boolean operators properly in the journals database of Scopus, Capes Periodical, Scielo and Web of Science. The Capes Online Journals platform holds the largest number of publications for both terms.

KEYWORDS	JOURNALS DATABASE			
	PERIODIC CAPES	WEB OF SCIENCE	SCIELO	SCOPUS
“Rhizomucor miehei lipase”	1025	360	1	307
“Rhizomucor miehei lipase” and immobilization and molecular	293	4	0	9
“Rhizomucor miehei lipase” and immobilization and “molecular biology”	37	0	0	0
“Rhizomucor miehei lipase” and immobilization	361	68	0	87
“Rhizomucor miehei lipase” and (“molecular characterization” OR “molecular biology”)	90	2	0	1
Total	**1806**	**434**	**1**	**395**

Table 1. Number of articles published on lipase on free and immobilized form Rhizomucor *miehei* and its relationship with molecular biology in the journal databases, from 1992 to 2018.

Source: Own authorship (2019).

Considering *R. miehei* free form, the keyword “*Rhizomucor miehei* lipase”, according to table 1, the Capes Online Journals platform (1025) is the one with the largest number of publications, followed by the Web of Science (360) and Scopus (307). However, the keywords “*Rhizomucor miehei* lipase” and “Immobilization” show that for immobilized lipase presents a smaller number of publications on the Capes Online Journals platform (361), Web of Science (68) and Scopus (87).

And, by relating the terms “Rhizomucor miehei lipase” and immobilization and “molecular biology” (table 1), it can be seen that Capes Journal is the only database that holds 37 publications. Demonstrating few published papers showing the relationship in the area of molecular biology with this lipase immobilized. Also considering the term “Rhizomucor miehei lipase” and “molecular characterization OR molecular biology”, Capes Journal (90) again holds the largest number of publications, followed by Web of Science (2) and Scopus (1). As for the Scielo database, this is the least that provides information about Rhizomucor miehei lipase (only 1 scientific article). It is noteworthy that this represents a small amount of scientific articles that involve the molecular characterization of this lipase.

By relating the term “*Rhizomucor miehei* lipase” with molecular biology techniques, it was found that Polymerase Chain Reaction (PCR) was the most endowed in the research, and a total of 58 published articles distributed among the four databases (Table 2).

KEYWORDS	JOURNALS DATABASE			
	PERIODIC CAPES	WEB OF SCIENCE	SCIELO	SCOPUS
"*Rhizomucor miehei* lipase" and "electrophoresis"	3	3	0	9
" *Rhizomucor miehei* lipase" and "Polymerase chain reaction"	54	1	0	3
" *Rhizomucor miehei* lipase" and "Reverse transcriptase Chain Reaction"	0	0	0	0
" *Rhizomucor miehei* lipase" and "PCR Multiplex"	0	0	0	0
" *Rhizomucor miehei* lipase" and "Restriction fragment length polymorphisms"	0	0	0	0
" *Rhizomucor miehei* lipase" and "Molecular Markers"	2	0	0	0
TOTAL	**59**	**4**	**0**	**12**

Table 2: Total number of articles published on the key word Rhizomucor miehei lipase and its relationship with molecular methods on the basis of journals, from 1992 to 2018.

According to table 2, the Capes Online Journals Plataform (59) is the one that holds the largest number of publications, for all the terms researched.

The Polymerase Chain Reaction (PCR) technique uses an enzyme called DNA polymerase capable of producing multiple copies of double-stranded DNA molecules and involves repeating a three-step cycle: denaturation, annealing, and synthesis. PCR is considered a sensitive and specific technique and made possible the analysis and detection of DNA from any organisms, even from small amounts. By allowing large amounts of DNA copies to be obtained from genes of interest, PCR became a technique of great importance in different areas (Hepp and Nonohay 2016).

Second, according to table 2, the technique most used in lipase *R. miehei* searches is electrophoresis, and a total of 15 published articles were distributed among three databases (Capes Journal, Web of Science and Scopus).

Electrophoresis is a molecular technique that allows visualization of DNA by separating molecules based on their size through an electric field. In this method, the DNA is applied to a gel formed by a polymer (agarose or polyacrylamide) and a buffer (chemical solution capable of transmitting electricity and keeping the constant pH). Due to the electronegative character, when submitted to an electric current, the DNA is attracted to the positive pole, migrating through the gel. The smaller the molecule, that is, fewer nucleotides, the faster the migration will be and the DNA will travel a greater distance inside the gel. In this way, the electric current separates DNA molecules by size, from the smallest to the largest. Molecules of the same size migrate together, stopping at the same position on the gel and forming a region called a band, visible through dyes that bind to DNA (Hepp and Nonohay 2016).

It is worth mentioning that the relationship of this lipase with the Reverse

Transcriptase Chain Reaction, PCR Multiplex and Restriction fragment length polymorphisms (Table 2) techniques, was not cited in any database. This shows that, until now, the application of these techniques in molecular studies of this lipase is nonexistent.

As for the Scielo database, it does not provide information on *Rhizomucor miehei* lipase with the respective molecular techniques.

4 I CONCLUSION

The results of the prospection showed that there is still few researches focusing on the relationship between molecular biology and the lipase immobilization process. However, there was a percentage of growth from 1988 to 2018 in the researches with the theme "*Rhizomucor miehei* lipase", these results show trend and perspectives of growth of the number of publications for the future. Brazil is in the ranking of the countries that publish the most about this fungal lipase, characterizing the great potential in biodiversity and biotechnology that this country presents.

ACKNOWLEDGMENTS

To the Foundation for Research and Scientific and Technological Development of Maranhão (FAPEMA) for the financing and granting of the doctoral scholarship.

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ÍNDICE REMISSIVO

A

Ácido kójico 1, 3, 4, 6, 7
Antimicrobianos 88, 91
Arboviroses 22, 23, 26, 27, 28
Aspergillus Flavus 1, 7, 8

B

Biologia molecular 77

C

Chrysobalanaceae 59, 60, 61, 62, 65, 68, 69, 70
Conservação 40, 41, 42, 44, 46, 47, 50, 51, 71, 75, 76
Contextualização 53, 54, 57, 58
Crescimento vegetativo 9, 11
Criadouros 22, 23, 24, 25, 26, 27, 28, 29

D

Divulgação científica 40, 44, 45, 48

E

Educação ambiental 43, 51, 52, 71, 72, 75, 76
Ensino de biologia 53
Enzima 2, 7, 77, 91
Estafilococos 88, 92

F

Fases larvais 23
Fauna 40, 41, 44, 47, 49, 50, 51, 52, 63, 74, 76
Feijão-de-porco 9, 11, 14, 15, 16, 17, 18, 19, 20

H

Histologia 1, 5, 30, 31, 35, 53, 54, 55, 56, 57, 58, 96, 100

I

Ipojuca 22, 23, 24, 25, 27, 28

L

Licania tomentosa 59, 60, 61, 63, 64, 65, 66, 67, 68, 69, 70

M

Metabólitos secundários 60, 61, 67
Métodos de imobilização 77
Mídias digitais 40
Modelo de predição NIR 30

N

Nitrogênio 6, 9, 15, 19, 31, 32, 36, 63, 93, 95, 96, 98

O

Oiti 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69

P

Paracetamol 9, 10, 12, 14, 15
Pleurotus Ostreatus 30, 31, 32, 35, 36, 37, 38, 39
Polissacarídeo extracelular 30, 36
Poluentes do solo 9
Preservação 11, 42, 43, 45, 71, 72, 74, 75
Produtos 2, 4, 10, 32, 42, 93
Prospecção 77, 86

Q

Quirópteros 71, 72, 73, 74, 75, 76

R

Remediação ambiental 9
Resistente 61, 65, 88, 89, 91

S

Substituição 2, 93, 98
Suportes 77

T

Tratamento 1, 2, 3, 9, 12, 14, 15, 16, 17, 18, 19, 33, 35, 57, 59, 62, 88, 90, 92, 93, 96, 97, 98

V

Vetores 22, 23, 24, 26, 27, 28

Agência Brasileira do ISBN
ISBN 978-85-7247-638-6