Benedito Rodrigues da Silva Neto
(Organizador)

Conceitos Básicos da **Genética**

Atena Editora
Ano 2019

Benedito Rodrigues da Silva Neto
(Organizador)

Conceitos Básicos da Genética

Atena Editora
2019

2019 *by Atena Editora*
Copyright © Atena Editora
Copyright do Texto © 2019 Os Autores
Copyright da Edição © 2019 Atena Editora
Editora Executiva: Profª Drª Antonella Carvalho de Oliveira
Diagramação: Geraldo Alves
Edição de Arte: Lorena Prestes
Revisão: Os Autores

O conteúdo dos artigos e seus dados em sua forma, correção e confiabilidade são de responsabilidade exclusiva dos autores. Permitido o download da obra e o compartilhamento desde que sejam atribuídos créditos aos autores, mas sem a possibilidade de alterá-la de nenhuma forma ou utilizá-la para fins comerciais.

Conselho Editorial
Ciências Humanas e Sociais Aplicadas
Prof. Dr. Álvaro Augusto de Borba Barreto – Universidade Federal de Pelotas
Prof. Dr. Antonio Carlos Frasson – Universidade Tecnológica Federal do Paraná
Prof. Dr. Antonio Isidro-Filho – Universidade de Brasília
Prof. Dr. Constantino Ribeiro de Oliveira Junior – Universidade Estadual de Ponta Grossa
Profª Drª Cristina Gaio – Universidade de Lisboa
Prof. Dr. Deyvison de Lima Oliveira – Universidade Federal de Rondônia
Prof. Dr. Gilmei Fleck – Universidade Estadual do Oeste do Paraná
Profª Drª Ivone Goulart Lopes – Istituto Internazionele delle Figlie de Maria Ausiliatrice
Prof. Dr. Julio Candido de Meirelles Junior – Universidade Federal Fluminense
Profª Drª Lina Maria Gonçalves – Universidade Federal do Tocantins
Profª Drª Natiéli Piovesan – Instituto Federal do Rio Grande do Norte
Profª Drª Paola Andressa Scortegagna – Universidade Estadual de Ponta Grossa
Prof. Dr. Urandi João Rodrigues Junior – Universidade Federal do Oeste do Pará
Profª Drª Vanessa Bordin Viera – Universidade Federal de Campina Grande
Prof. Dr. Willian Douglas Guilherme – Universidade Federal do Tocantins

Ciências Agrárias e Multidisciplinar
Prof. Dr. Alan Mario Zuffo – Universidade Federal de Mato Grosso do Sul
Prof. Dr. Alexandre Igor Azevedo Pereira – Instituto Federal Goiano
Profª Drª Daiane Garabeli Trojan – Universidade Norte do Paraná
Prof. Dr. Darllan Collins da Cunha e Silva – Universidade Estadual Paulista
Prof. Dr. Fábio Steiner – Universidade Estadual de Mato Grosso do Sul
Profª Drª Girlene Santos de Souza – Universidade Federal do Recôncavo da Bahia
Prof. Dr. Jorge González Aguilera – Universidade Federal de Mato Grosso do Sul
Prof. Dr. Ronilson Freitas de Souza – Universidade do Estado do Pará
Prof. Dr. Valdemar Antonio Paffaro Junior – Universidade Federal de Alfenas

Ciências Biológicas e da Saúde
Prof. Dr. Benedito Rodrigues da Silva Neto – Universidade Federal de Goiás
Prof.ª Dr.ª Elane Schwinden Prudêncio – Universidade Federal de Santa Catarina
Prof. Dr. Gianfábio Pimentel Franco – Universidade Federal de Santa Maria
Prof. Dr. José Max Barbosa de Oliveira Junior – Universidade Federal do Oeste do Pará

Atena Editora
Ano 2019

Profª Drª Natiéli Piovesan – Instituto Federal do Rio Grande do Norte
Profª Drª Raissa Rachel Salustriano da Silva Matos – Universidade Federal do Maranhão
Profª Drª Vanessa Lima Gonçalves – Universidade Estadual de Ponta Grossa
Profª Drª Vanessa Bordin Viera – Universidade Federal de Campina Grande

Ciências Exatas e da Terra e Engenharias
Prof. Dr. Adélio Alcino Sampaio Castro Machado – Universidade do Porto
Prof. Dr. Eloi Rufato Junior – Universidade Tecnológica Federal do Paraná
Prof. Dr. Fabrício Menezes Ramos – Instituto Federal do Pará
Profª Drª Natiéli Piovesan – Instituto Federal do Rio Grande do Norte
Prof. Dr. Takeshy Tachizawa – Faculdade de Campo Limpo Paulista

Conselho Técnico Científico
Prof. Msc. Abrãao Carvalho Nogueira – Universidade Federal do Espírito Santo
Prof. Dr. Adaylson Wagner Sousa de Vasconcelos – Ordem dos Advogados do Brasil/Seccional Paraíba
Prof. Msc. André Flávio Gonçalves Silva – Universidade Federal do Maranhão
Prof.ª Drª Andreza Lopes – Instituto de Pesquisa e Desenvolvimento Acadêmico
Prof. Msc. Carlos Antônio dos Santos – Universidade Federal Rural do Rio de Janeiro
Prof. Msc. Daniel da Silva Miranda – Universidade Federal do Pará
Prof. Msc. Eliel Constantino da Silva – Universidade Estadual Paulista
Prof.ª Msc. Jaqueline Oliveira Rezende – Universidade Federal de Uberlândia
Prof. Msc. Leonardo Tullio – Universidade Estadual de Ponta Grossa
Prof.ª Msc. Renata Luciane Polsaque Young Blood – UniSecal
Prof. Dr. Welleson Feitosa Gazel – Universidade Paulista

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

C744 Conceitos básicos da genética [recurso eletrônico] / Organizador Benedito Rodrigues da Silva Neto. – Ponta Grossa (PR): Atena Editora, 2019.

Formato: PDF
Requisitos do sistema: Adobe Acrobat Reader.
Modo de Acesso: World Wide Web
Inclui bibliografia.
ISBN 978-85-7247-421-4
DOI 10.22533/at.ed.214192106

1. Genética – Estudo e ensino. 2. Genética e melhoramento. I.Silva Neto, Benedito Rodrigues da.

CDD 576

Elaborado por Maurício Amormino Júnior | CRB6/2422

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

APRESENTAÇÃO

Há exatos dezenove anos, mais precisamente na data de 21 de junho de 2000, um dos anúncios mais esperados nos últimos tempos pela comunidade científica era feito: simultaneamente nos Estados Unidos e em Londres o presidente Bill Clinton e o primeiro ministro Tony Blair divulgaram, o que segundo eles seria uma nova era para a humanidade, o sequenciamento do genoma humano. O “rascunho da vida” como denominaram traria novas expectativas quanto à doenças incuráveis, desafios éticos, novas propostas tecnológicas para a pesquisa, mas principalmente uma acessibilidade muito maior ao conceito de genética para a população.

Desde então uma revolução molecular pôde ser observada, novos conceitos adentraram às salas de aula, novos equipamentos evoluíram os laboratórios de pesquisa, novos e milhares de artigos passaram a publicar quase que “em tempo real” as descobertas no campo ambiental, microbiológico, industrial e da saúde. Podemos dizer também que a genética chegou como nunca às mesas das famílias, deixando de ser um assunto apenas dos cientistas.

Portanto a literatura aqui apresentada e intitulada “Conceitos básicos da genética” torna-se relevante não apenas por abordar assuntos relativos à comunidade acadêmica, mas principalmente por demonstrar a diversidade de áreas que hoje utilizam das ferramentas genéticas e moleculares em seus estudos que estão diretamente relacionados ao dia-a-dia da população.

Cada vez mais, o acelerado mundo das descobertas científicas caminha a passos largos e rápidos no sentido de transformar a pesquisa básica em aplicada, portanto é relevante destacar que investimentos e esforços nessa área contribuem grandemente com o desenvolvimento de uma nação. A genética como sabemos possui um campo vasto de aplicabilidades que podem colaborar e cooperar grandemente com os avanços científicos e tecnológicos.

Esperamos que seja apenas o primeiro de muitos outros livros na área, já que a cada dia novas tecnologias genéticas tornam-se acessíveis e novas descobertas são possíveis. Parabenizamos cada autor pela teoria bem fundamentada aliada à resultados promissores, e principalmente à Atena Editora por permitir que o conhecimento seja difundido e disponibilizado para que as novas gerações se interessem cada vez mais pelo ensino e pesquisa em genética.

SUMÁRIO

CAPÍTULO 1 1

FERRAMENTAS GENÔMICAS E GEOGRÁFICAS PARA AVALIAR A DIVERSIDADE E ESTRUTURA GENÉTICA DE POPULAÇÕES SUÍNAS

Elizabete Cristina da Silva
Samuel Rezende Paiva
Concepta Margaret McManus Pimentel
Victor Hugo de Vasconcelos Calado

DOI 10.22533/at.ed.2141921061

CAPÍTULO 2 12

A ABORDAGEM DE GENÉTICA SOB O OLHAR DOS DISCENTES DE ENFERMAGEM DE UMA INSTITUIÇÃO DE ENSINO SEMIPRESENCIAL NO MUNICIPIO DE ANANINDEUA, ESTADO DO PARÁ

Letícia Gomes de Oliveira
Maria Josilene Castro de Freitas
Brena Yasmim Barata Nascimento
Shirlene de Nazaré Costa da Silva
Leandro Neves da Silva Costa
Dolanno Ferreira Alves
Adan Rodrigues de Oliveira
Joycianne Rodrigues Parente
Karina Guedes Lima
Abigail das Mercês do Vale Batista
Dayara de Nazaré Rosa de Carvalho

DOI 10.22533/at.ed.2141921062

CAPÍTULO 3 17

A GENÉTICA TOXICOLÓGICA E O BIOENSAIO *Allium cepa*

Schirley Costalonga
Maria do Carmo Pimentel Batitucci

DOI 10.22533/at.ed.2141921063

CAPÍTULO 4 25

ANÁLISES GENÉTICAS NÃO INVASIVAS E SUA CONTRIBUIÇÃO PARA A GENÉTICA DA CONSERVAÇÃO DE FELINOS BRASILEIROS

Andiara Silos Moraes de Castro Souza
Bruno Henrique Saranholi
Pedro Manoel Galetti Jr

DOI 10.22533/at.ed.2141921064

CAPÍTULO 5 40

AVALIAÇÃO DA DISCIPLINA DE GENÉTICA HUMANA FRENTE ÀS DIRETRIZES CURRICULARES NACIONAIS PARA O CURSO DE GRADUAÇÃO EM MEDICINA

Sulyanne Saraiva de Almeida
Alcivan Batista de Morais Filho
João Paulo da Silva Liberalino
Sandy Albuquerque Silveira
Bruna Prado de Oliveira
Thales Allyrio Araújo de Medeiros Fernandes

DOI 10.22533/at.ed.2141921065

CAPÍTULO 6 54

CITOGENOTOXICIDADE E MUTAGENICIDADE DO SULFATO DE COBRE EM DIFERENTES VARIEDADES DE *allium cepa* LINN

Júlio Brando Messias
Rosanne Lopes de Brito
Gerusa Tomaz de Aquino Beltrão
Inalda Maria de Oliveira Messias
Mônica Simões Florêncio
Betty Rose de Araújo Luz
Sura Wanessa Nogueira Santos Rocha
Mércia Cristina de Magalhães Caraciolo
João Ferreira da Silva Filho

DOI 10.22533/at.ed.2141921066

CAPÍTULO 7 65

COMO SURGEM NOVAS ENZIMAS? EVOLUÇÃO MOLECULAR DE NOVAS CÓPIAS GÊNICAS NA SUPERFAMÍLIA DAS RODANASES EM DIPTERA

Luana Sousa Soares
Iderval da Silva Júnior Sobrinho

DOI 10.22533/at.ed.2141921067

CAPÍTULO 8 83

DIVERSIDADE GENÉTICA EM *Hoplias malabaricus* (BLOCH, 1794) REVELA DIFERENTES LINHAGENS EM BACIAS MARANHENSES

Walna Micaelle de Moraes Pires
Maria Claudene Barros
Elmary da Costa Fraga

DOI 10.22533/at.ed.2141921068

CAPÍTULO 9 98

DNA BARCODING CONFIRMA A OCORRÊNCIA DE ESPÉCIES AMAZÔNICAS NA ICTIOFAUNA DO RIO TURIAÇU, MARANHÃO/BRASIL

Bruno Rafael da Silva Teixeira
Maria Claudene Barros
Elmary da Costa Fraga

DOI 10.22533/at.ed.2141921069

CAPÍTULO 10 111

EVALUATION OF HETEROLOGOUS PROTEIN EXPRESSION AT DIFFERENT CONCENTRATIONS OF MGSO4 AND IPTG IN ESCHERICHIA COLI W110

Yago Queiroz dos Santos
Gabriella Silva Campos Carelli
Bruno Oliveira de Veras
Joelton Igor Oliveira da Cruz
Geovanna Maria Medeiros Moura
Antônio Moreira Marques Neto
Anderson Felipe Jácome de França

DOI 10.22533/at.ed.21419210610

CAPÍTULO 11 119

ANÁLISE DA IMPORTANCIA DE ESTUDOS DO GENE MDR1 E SEU PAPEL NO DESENVOLVIMENTO DE MULTIRESISTENCIA A FÁRMACOS PARA TRATAMENTO DE CANDIDÍASE

Lucas Lopes Lima
Benedito R. Da Silva Neto

DOI 10.22533/at.ed.21419210611

CAPÍTULO 12 128

EVALUATION OF PLASMA MIRNAS FOR EARLY DIAGNOSIS OF BREAST CANCER

Alexis Germán Murillo Carrasco
Stefano Giannoni Luza
Oscar Acosta Conchucos
José Manuel Cotrina Concha
Alfredo Aguilar Cartagena
Lia Pamela Rebaza Vásquez
Ricardo Miguel Fujita Alarcón
José Luis Buleje Sono

DOI 10.22533/at.ed.21419210612

CAPÍTULO 13 139

POLIMORFISMO DO GENE GOLA-DRB.2 EM REBANHOS CAPRINOS LEITEIROS

Luciana Florêncio Vilaça Lopes
Elizabete Cristina da Silva
Elizabete Rodrigues da Silva
Severino Benone Paes Barbosa
Ângela Maria Vieira Batista
Kleber Régis Santoro

DOI 10.22533/at.ed.21419210613

CAPÍTULO 14 151

IDENTIFICAÇÃO E CARACTERIZAÇÃO MOLECULAR DE PEIXES DA APA DO INHAMUM, LESTE MARANHENSE, BRASIL

Renato Corrêia Lima;
Marcelo Silva de Almeida;
Maria Claudene Barros;
Elmary da Costa Fraga;

DOI 10.22533/at.ed.21419210614

CAPÍTULO 15 169

MIRNAS: UMA CLASSE DE PEQUENOS RNAS REGULATÓRIOS

Juliana Santana de Curcio
Kleber Santiago Freitas e Silva
Lívia do Carmo Silva
Amanda Alves de Oliveira
Thaynara Gonzaga Santos
Lucas Weba Soares

DOI 10.22533/at.ed.21419210615

CAPÍTULO 16 179

O CICLO CELULAR E SEUS MECANISMOS DE CONTROLE: UMA REVISÃO

Schirley Costalonga
Maria do Carmo Pimentel Batitucci

DOI 10.22533/at.ed.21419210616

CAPÍTULO 17 191

OSTEOSSARCOMA PEDIÁTRICO

Natália Paiva do Nascimento
Thauanna Alves Meira
Mariana Camargo Maschietto

DOI 10.22533/at.ed.21419210617

CAPÍTULO 18 202

PHYLOGENETIC ANALYSIS AND IDENTIFICATION OF A CELLULASE PRODUCING BACILLUS SP. STRAIN BY 16S RRNA SEQUENCING

Yago Queiroz dos Santos
Anderson Felipe Jácome de França
Bruno Oliveira de Veras
Gabriella Silva Campos Carelli
Geovanna Maria Medeiros Moura
Joelton Igor Oliveira da Cruz
Fernanda Granja da Silva Oliveira
João Ricardhis Saturnino de Oliveira
Luciclaudio Cassimiro de Amorim
Elizeu Antunes dos Santos

DOI 10.22533/at.ed.21419210618

CAPÍTULO 19 210

POLIMORFISMOS GENÉTICOS E DOENÇAS HUMANAS NA ERA DA BIOINFORMÁTICA

Kleber Santiago Freitas e Silva
Juliana Santana de Curcio
Lucas Weba Soares
Lívia do Carmo Silva
Amanda Alves de Oliveira
Thaynara Gonzaga Santos

DOI 10.22533/at.ed.21419210619

CAPÍTULO 20 226

QUIMIOPROTEÔMICA: DESCOBRINDO MOLÉCULAS BIOATIVAS E SEUS ALVOS

Lívia do Carmo Silva
Kleber Santiago Freitas e Silva
Juliana Santana De Curcio
Lucas Weba Soares

DOI 10.22533/at.ed.21419210620

SOBRE O ORGANIZADOR 240

CAPÍTULO 18

PHYLOGENETIC ANALYSIS AND IDENTIFICATION OF A CELLULASE PRODUCING BACILLUS SP. STRAIN BY 16S RRNA SEQUENCING

Yago Queiroz dos Santos
Institute of Tropical Medicine, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil.
Department of Biochemistry, Federal University of Rio Grande do Norte,
Natal, Rio Grande do Norte, Brazil.

Anderson Felipe Jácome de França
Multicampi School of Medical Sciences, Federal University of Rio Grande do Norte, Brazil.

Bruno Oliveira de Veras
Department of Biochemistry, Federal University of Pernambuco, Recife, Pernambuco, Brazil.

Gabriella Silva Campos Carelli
Department of Biochemistry, Federal University of Rio Grande do Norte,
Natal, Rio Grande do Norte, Brazil.

Geovanna Maria Medeiros Moura
Institute of Tropical Medicine, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil.

Joelton Igor Oliveira da Cruz
Institute of Tropical Medicine, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil.

Fernanda Granja da Silva Oliveira
Department of Biochemistry, Federal University of Pernambuco, Recife, Pernambuco, Brazil.

João Ricardhis Saturnino de Oliveira
Department of Biochemistry, Federal University of Pernambuco, Recife, Pernambuco, Brazil.

Luciclaudio Cassimiro de Amorim
Department of Biochemistry, Federal University of Pernambuco, Recife, Pernambuco, Brazil.

Elizeu Antunes dos Santos
Institute of Tropical Medicine, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil.
Department of Biochemistry, Federal University of Rio Grande do Norte,
Natal, Rio Grande do Norte, Brazil.

ABSTRACT: The microorganisms belonging to genus *Bacillus* include a wide spectra and ubiquitous group of bacteria that can be found from the forest soil, to marine ecosystems occurring in association with a variety of aquatic organisms such as scleractinian corals present in the intertidal boulders. Therefore, these microorganisms are exposed to various abiotic stresses that cause ecological selection for a physiologically adapted microbiota to such extremes of temperature and salinity. In this work, we isolated and characterized a cellulase from a marine bacterial strain and indentified its specie by 16S rRNA sequencing followed by a BLAST analysis. The cellulolytic strain called SR22 showed to be a gram-positive spore-forming bacilli, facultative anaerobe, and catalase positive, as well as negative for indole, H_2S production, and citrate utilization; those findings led us to consider the isolate

belonging to the genus *Bacillus*, which was confirmed by the phylogenetic analysis, which revealed that the SR22 strain formed a clade with *Bacillus subtilis*. Its nucleotide sequence was deposited in GenBank as Accession No. MH119099 and the degree of sequence similarity of strain SR22 to Bacillus sp. was 99%. Taken together, the present data indicate the present celulase-positive indentified strain as a potential and useful candidate for industrial applications that employs celulase degrading processes like second-generation bioethanol and paper industries being still necessary further studies to complete characterize this microorganism secretome.
KEYWORDS: Endoglucanase, Marine bacteria, rRNA

INTRODUCTION

Microorganisms are present in the most diverse ecosystems, from the terrestrial to the aquatic, besides performing terminal functions on biogeochemical cycles underneath and above the surface of mineral and living beings. The main way of bacterial interaction with the environment involves the production of chemical substances like enzymes, which act mainly as biological barriers against the growth of other microorganisms (Kazeem *et al.*, 2017).

According to Rosenberg (Rosenberg *et al.*, 2007) the marine environment includes a mega diversity of microorganisms that constitute a complex and intricate system of these ecological relations with the other biota elements and represent an important source of biomolecules, but it's still poorly explored comparing to others habitats, and their microorganisms are still to be described. As a consequence, natural microbial products are promising sources for the bioprospecting of new molecules with potential applications in medicine (pharmaceuticals), agriculture (agrochemicals) and in biological processes (chemical biology) studies (Prakash *et al.*, 2014).

The earliest research on the marine ecosystem and its ecological diversity began in the middle of the last century, gaining more visibility in the present day. Introducing itself as a hostile environment of particular characteristics, the marine ecosystem harbours different ecological niches that produce particular macromolecules in response to variations in this environment of pH, temperature, pressure and salinity. Approximately 30.000 macromolecules isolated from these niches have already been discovered and catalogued, however, these data correspond to only 5% of the studied sources of this ecosystem, where only a few are explored and validated (Indraningrat, Smidt e Sipkema, 2016; Ye *et al.*, 2015).

Currently recognized as the "Cradle "The seas and oceans represent an important source with potential for application in the cosmetic, nutritional, agrochemical and therapeutic industries, especially in the attempt to obtain and elucidate the anticancer properties of these compounds. By the end of the 1990s the number of molecules discovered was approximately 500 per year. Currently 28.175 chemical classes with hundreds of new compounds are discovered annually. Although increasing the number

of researches for discoveries of new marine compounds this ecosystem is little explored.

Different aspects are considered in the study of products from marine species, such as chemical diversity found from different ecological niches, function in nature and strategies for preclinical or clinical use. The first proposal of an anticancer compound was approved in 1969, known and applied nowadays the Citarabina or Ara-C, was developed to be a synthetic analogue of nucleoside C found in species of sea sponges, *Tethya crypta*. After this first proposal new molecules, from different marine niches, were launched, such as Trabectedina from tunicate *Ecteinascidia turbinate*, Eribulin from the metabolite produced by marine sponges *Halichondria okadai* and Ziconotide with analgesic function obtained from studies in *Conus magus* (Verma *et al.*, 2007).

The discovery of novel bioactive marine natural products (MNPs) is based on a sequence of methods under the organism to be worked through the process of isolation, extraction, characterization and purification of the molecule of interest. Although many methods still employed are considered standard in the identification of new molecules some cultivate the problematic of low efficiency, costly and that demand of spaced times to obtain satisfactory results. The process of isolation of microorganisms that have a symbiosis relationship with marine eukaryotic organisms has been one of the major problems present during the process of obtaining molecules from these agents, such as some bacteria of the genus *Prochlorococcus* that present a compromised growth in synthetic media, due to the absence of heterotrophic bacteria in co-cultures. Techniques that employ concepts of genomics, metagenomics, proteomics, bioinformatics, expression systems have been used as alternative ways to discover and obtain bioactive molecules from marine organisms (Batool *et al.*, 2016; Dustan, 1973).

Enzymes are great biological catalysts that plays unique role on accelerating the speed of chemical reactions being essential for the maintenance for the homeostasis of biological systems which, when under uncatalyzed conditions, would take so long to happen that they would make the structuring of complex organisms thermodynamically unfeasible. In addition to forming the basis of metabolic systems, enzymes provide enormous opportunities for industries to perform biocatalytic conversions with high yield and reliability (Adrio e Demain, 2014).

Hydrolytic enzymes are widely used in different processes in major industries such as pharmaceuticals, textiles, detergents, food and biofuels, employing different classes of enzymes such as proteases, pectinases, lipases, cellulases (Santos, Dos *et al.*, 2018; Veras, Queiroz, Gomes, *et al.*, 2018). In a field so required by new bioactive molecules, microorganisms belonging to new environments are emerging as potential sources for new research and industry employment of brand-new molecules due the metabolic versatility and stability in extreme environmental conditions (temperature, salinity and so on) where these organisms inhabit. Thus, the present work seeks mainly to isolate bacterial strains from marine organisms present in the coastal Northeast region of Brazil in order to characterize and purify enzymes synthesized by these microbes,

with potential biotechnological applications (Veras, Queiroz, Diniz, *et al.*, 2018).

MATERIALS AND METHODS

Isolation of bacteria

The bacterial isolates were from aseptically collected tissues from *Siderastrea stellata* colonies (Verrill, 1868) on the coral reefs of Cabo Branco-Paraiba, Brazil (7°08'50"S,34° 47'51"W), being removed according to Dustan methodology (Dustan, 1973). For bacterial isolation, tissues from the ecto and mesoderm of the Anthozoa were suspended in sterile saline solution, then inoculated into culture medium Sea Agar (5.0 g/L^{-1} peptone, 0.1 g/L^{-1} extract yeast, 15.0 g/L^{-1} agar) and incubated at 55°C until adequate growth. The strains were growth for 48 h at 40 °C on pH 7,0 in carboxymethylcellulose (CMC) agar plates (containing 0.1%, CMC; 0.05%, $NaNO_3$; 0.1%, K_2HPO_4; 0.05%, $MgSO_4 \cdot 7H_2O$; 0.001% $FeSO_4 \cdot 7H_2O$; 0.1% Yeast extract; 1.5% agar) and then overlaid with 0.1% congo-red solution for 30 min and washed with 1 M NaCl for equal time as well as stained with 0.2% potassium iodine for 5 min, bacterial colonies showing clear zones were considered to be cellulase producers and selected for agro-waste degradation experiments. (Kasana *et al.*, 2008).

BACTERIAL MOLECULAR IDENTIFICATION

In order to identify the cellulase producer isolates, 16S rRNA gene sequence was amplified from extracted DNA. Bacterial universal primers 26F (5'-GAGTTTGATCMTGGCTCAG) and 1492R (5' -ACGGCTACCTTGTTACGACTT-3') were used to amplify the 16S rDNA gene by Polymerase Chain Reaction (PCR) performed in MWG-Biotech Primus 96 Plus Thermal Cycler (Primus, USA). Each reaction mixture (50 µL) contained reaction buffer, 2 mM MgCl2, 0.2 µM of each primer, 0.2 mM of dNTPs, 1U of DNA polymerase and 40 ng of genomic DNA. The amplification products were directly purified from the PCR reaction using the Wizard® SV Genomic DNA Purification System. The purified reactions were sequenced using an ABI-PRISM 3100 Genetic Analyzer automatic sequencer (Applied Biosystems, USA).

PHYLOGENETIC ANALYSIS

The obtained 16S rRNA gene sequence for isolates was compared to deposited sequences in the GenBank database (NCBI). For the local alignment, BLASTn tool (NCBI) was used. MEGA 7.0 software was used for multiple sequence monitoring and for the construction of a dendrogram by the Neighbour-Joining method (Hogg e Lehane, 1999; Mahajan *et al.*, 2013).

RESULTS AND DISCUSSION

Bacterial strains associated with coral tissues were obtained from the collection

of corals in works prior to this study and deposited in stock in the marine Agar culture medium (composed of filtered sea water from the study region, 0, 5% Peptone (Difco), 0.1% Yeast Extract (Difco) and 1.5% Agar.

Bacterial isolates from corals presenting different health conditions were divided into two groups named “SS” and “SR” plus an individualized numbering for each isolate strain. The isolates from coral samples affected by the depigmentation received the SR code with a numbering associated with each strain. In total, 29 SS strains and 40 SR strains coral were isolated.

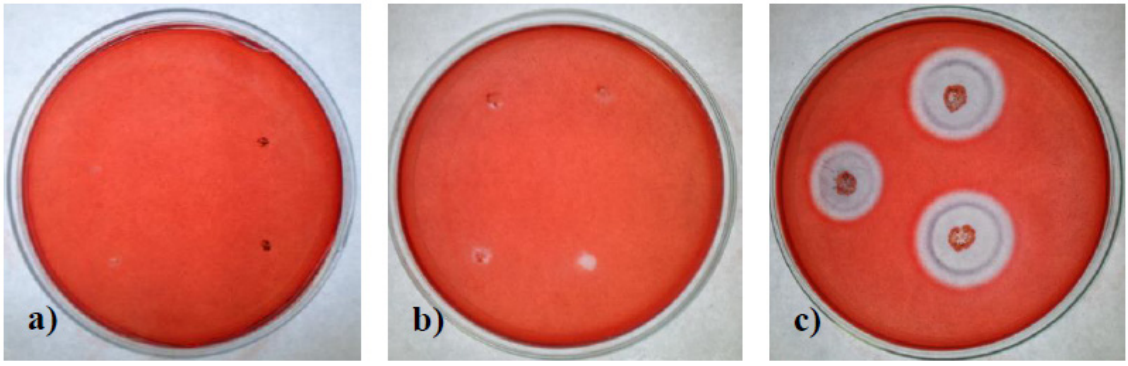

Figure 1. Aspects of the cellulolytic activity test: a) negative result (there is no halo formation); b) positive result demonstrating degradation of the substrate around the colony (although not significant for prospecting purposes; c) positive result with evident halo of more than 3 cm of diameter.

Among the bacteria of the SS lineage listed, three presented considerable cellulolytic activity while the others formed only a faint halo evidencing activity, often restricted only to the colony area; the proteolytic activity (data not shown) was more widely observed with five strains showing conspicuous activity while five others also exhibited the formation of considerable degradation halos of the protein substrate, in addition to the other strains where the degradation was restricted to the colony region.

In the SR line, seven presented intense cellulolytic activity with the others presenting activity only in the region of the colony; in relation to the proteolytic activity (data not shown), five strains had a significant halo of degradation while nine presented a positive result, although attenuated when compared to the other five strains. For classification purposes, lineages that produced degradation halos with diameters greater than 1 cm were considered as a significant positive activity, being considered as high activity the halos with diameters of approximately three centimeters or higher (Figure 1).

Among SR isolates, one strain showed to be a gram-positive spore- forming bacilli, facultative anaerobe, and catalase positive, as well as negative for indole, H_2S production, and citrate utilization. The same morphological characteristics were found by previous studies with cellulolytic *Bacillus* sp. strains Rastogi et al. (2010 e Rawat; Tewari (2012) e Srivastava et al. (2018)Bacteroidetes, Chloroflexi, Deinococcus-Thermus, Firmicutes, and Proteobacteria were identified. Eight isolates capable of

degrading cellulose, carboxymethyl cellulose (CMC.

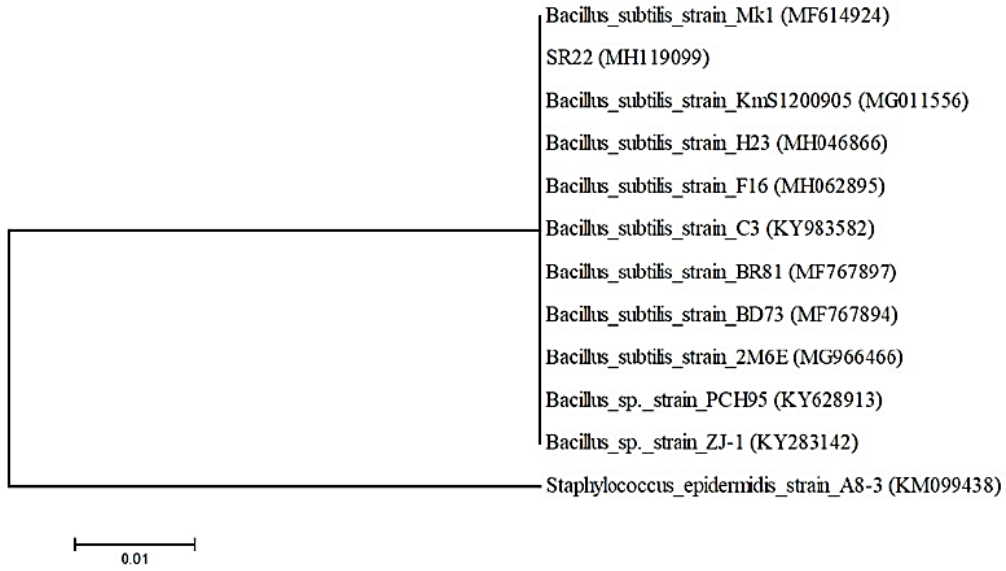

Figure 2. Phylogenetic tree of SR isolate labelled as “22” and other related species based on 16S rDNA sequences, constructed using the Neighbour-Joining principle. The scale bar represents 0.01 substitutions per site. The evolutionary distances were computed using the Kimura two-parameter method. Bootstrap values are indicated at the branches from 1.000 replications. GenBank accession numbers of the sequences are given in parentheses.

Those findings led us to consider the isolate belonging to the genus Bacillus, which was confirmed by the phylogenetic analysis, which revealed since the strain formed a clade with *Bacillus subtilis* sequences (Figure 2). Its nucleotide sequence was deposited in GenBank as Accession No. MH119099 and the degree of sequence similarity of strain was 99%.

CONCLUSIONS

Besides the expression of extracellular enzymes, the secretion of bactericidal compounds by microorganisms competing for the same ecological niche with other prokaryotes is also responsible for modulating a large part of the structure of the associated microbiota, changes in the environmental and physiological conditions of the host can be decisive events triggering succession, where the antagonistic strains to a certain pathogen is eliminated allowing the colonization of hosts.

When subjected to a new temperature condition, corals are therefore suffering from the phenomenon of ecological succession due to the decrease in the fitness of their microbiota associated with the concomitant elevation of competition for adhesion sites and virulence in the case of some pathogens.

This process leads to an insertion of other microorganisms into the coral microbiota, causing an increase in diversity and destabilizing the mutual equilibrium between prokaryotes and the host, which in turn becomes even more susceptible due to the aggression of the pathogens now present and the development of virulence

in previously non-pathogenic strains of stressors that culminate in further favoring the succession of the original microorganisms leading to a vicious circle capable of chronically compromising coral homeostasis leading to a qualitative deficit in the mutualistic association between coral and a photobiont component of the association that is very often algae of the genus Symbiodinium (commonly referred to as zooxanthellae) causing bleaching (Rosenberg *et al.*, 2007).

REFERENCES

ADRIO, J. L.; DEMAIN, A. L. Microbial Enzymes: Tools for Biotechnological Processes. **Biomolecules**, p. 117–139, 2014.

BATOOL, T. *et al.* A Comprehensive Review on l-Asparaginase and Its Applications. **Applied Biochemistry and Biotechnology**, v. 178, n. 5, p. 900–923, 7 mar. 2016.

DUSTAN, P. Distribution of zooxanthellae and photosynthetic chloroplast pigments of the reef-building coral Montastrea. **Bulletin of Marine Science**, v. 29, n. 1, p. 79–95, 1973.

HOGG, J. C.; LEHANE, M. J. Identification of bacterial species associated with the sheep scab mite (Psoroptes ovis) by using amplified genes coding for 16S rRNA. **Applied and environmental microbiology**, v. 65, n. 9, p. 4227–9, set. 1999.

INDRANINGRAT, A. A. G.; SMIDT, H.; SIPKEMA, D. Bioprospecting sponge-associated microbes for antimicrobial compounds. **Marine Drugs**, v. 14, n. 5, p. 1–66, 2016.

KASANA, R. C. *et al.* A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. **Current Microbiology**, v. 57, n. 5, p. 503–507, 2008.

KAZEEM, M. O. *et al.* Prospecting Agro-waste Cocktail: Supplementation for Cellulase Production by a Newly Isolated Thermophilic B. licheniformis 2D55. **Applied Biochemistry and Biotechnology**, v. 182, n. 4, p. 1318–1340, 2017.

MAHAJAN, R. V. *et al.* A rapid, efficient and sensitive plate assay for detection and screening of l-asparaginase-producing microorganisms. **FEMS Microbiology Letters**, v. 341, n. 2, p. 122–126, 2013.

PRAKASH, S. *et al.* Optimization and partial purification of a protease produced by selected bacterial strains grown on trash fish meal substrate and its antagonistic property against bacterial pathogens. **Biocatalysis and Agricultural Biotechnology**, v. 3, n. 4, p. 288–295, 2014.

RASTOGI, G. *et al.* Characterization of thermostable cellulases produced by Bacillus and Geobacillus strains. **Bioresource Technology**, v. 101, n. 22, p. 8798–8806, 2010.

RAWAT, R.; TEWARI, L. Purification and characterization of an acidothermophilic cellulase enzyme produced by Bacillus subtilis strain LFS3. **Extremophiles**, v. 16, n. 4, p. 637–644, 2012.

ROSENBERG, E. *et al.* The role of microorganisms in coral health, disease and evolution. **Nature Reviews Microbiology**, v. 5, n. 5, p. 355–362, 26 maio 2007.

SANTOS, Y. Q. DOS *et al.* A new salt-tolerant thermostable cellulase from a marine bacillus sp. Strain. **Journal of Microbiology and Biotechnology**, v. 28, n. 7, p. 1078–1085, 2018.

SRIVASTAVA, A. *et al.* Screening of biologically active microbial strains having therapeutic

applications. **Indian journal of experimental biology**, v. 56, n. April, p. 244–251, 2018.

VERAS, B. O. DE; QUEIROZ, Y.; GOMES, A.; *et al.* Production of Antileukemic Enzyme L-asparaginase from Marine Bacteria Associated. **Australian Journal of Basic and Applied Sciences**, v. 12, n. 11, p. 87–91, 2018.

VERAS, B. O. DE; QUEIROZ, Y.; DINIZ, K. M.; *et al.* Screening of protease , cellulase , amylase and xylanase from the salt-tolerant and thermostable marine Bacillus subtilis strain. **F1000Research**, n. 0, p. 1–7, 2018.

VERMA, N. *et al.* L-Asparaginase: A Promising Chemotherapeutic Agent. **Critical Reviews in Biotechnology**, v. 27, n. 1, p. 45–62, 2007.

YE, J. *et al.* Anticancer agents from marine sponges. **Journal of Asian Natural Products Research**, v. 17, n. 1, p. 64–88, 2015.

SOBRE O ORGANIZADOR

Benedito Rodrigues da Silva Neto - Possui graduação em Ciências Biológicas pela Universidade do Estado de Mato Grosso (2005), com especialização na modalidade médica em Análises Clínicas e Microbiologia. Em 2006 se especializou em Educação no Instituto Araguaia de Pós graduação Pesquisa e Extensão. Obteve seu Mestrado em Biologia Celular e Molecular pelo Instituto de Ciências Biológicas (2009) e o Doutorado em Medicina Tropical e Saúde Pública pelo Instituto de Patologia Tropical e Saúde Pública (2013) da Universidade Federal de Goiás. Pós-Doutorado em Genética Molecular com concentração em Proteômica e Bioinformática. Também possui seu segundo Pós doutoramento pelo Programa de Pós-Graduação Stricto Sensu em Ciências Aplicadas a Produtos para a Saúde da Universidade Estadual de Goiás (2015), trabalhando com Análise Global da Genômica Funcional e aperfeiçoamento no Institute of Transfusion Medicine at the Hospital Universitatsklinikum Essen, Germany. Palestrante internacional nas áreas de inovações em saúde com experiência nas áreas de Microbiologia, Micologia Médica, Biotecnologia aplicada a Genômica, Engenharia Genética e Proteômica, Bioinformática Funcional, Biologia Molecular, Genética de microrganismos. É Sócio fundador da “Sociedade Brasileira de Ciências aplicadas à Saúde” (SBCSaúde) onde exerce o cargo de Diretor Executivo, e idealizador do projeto “Congresso Nacional Multidisciplinar da Saúde” (CoNMSaúde) realizado anualmente no centro-oeste do país. Atua como Pesquisador consultor da Fundação de Amparo e Pesquisa do Estado de Goiás - FAPEG. Coordenador do curso de Especialização em Medicina Genômica e do curso de Biotecnologia e Inovações em Saúde no Instituto Nacional de Cursos. Como pesquisador, ligado ao Instituto de Patologia Tropical e Saúde Pública da Universidade Federal de Goiás (IPTSP-UFG), o autor tem se dedicado à medicina tropical desenvolvendo estudos na área da micologia médica com publicações relevantes em periódicos nacionais e internacionais.arroz, milheto, sorgo, plantas de cobertura e integração lavoura pecuária. E-mail para contato: alan_zuffo@hotmail.com

Agência Brasileira do ISBN
ISBN 978-85-7247-421-4