UNIVERSIDADE ESTADUAL DE MONTES CLAROS

Ronize Viviane Jorge de Faria

Perfil fenotípico, enzimático e antagônico de isolados de actinobactérias endofíticas do Cerrado

Montes Claros
2017

Ronize Viviane Jorge de Faria

Perfil fenotípico, enzimático e antagônico de isolados de actinobactérias endofíticas do Cerrado

Dissertação apresentada ao Programa de Pós-graduação em Ciências em Saúde - PPGCS, da Universidade Estadual de Montes Claros – Unimontes, como parte das exigências para obtenção do título de Mestre em Ciências da Saúde.

Área de Concentração: Saúde Coletiva.

Orientadores: Prof[a]. Dra. Ana Cristina de C. Botelho
Prof. Dr. Sérgio Avelino Mota Nobre

Montes Claros
2017

F224p

Faria, Ronize Viviane Jorge de.
Perfil fenotípico, enzimático e antagônico de isolados de actinobactérias endofíticas do Cerrado [manuscrito] / Ronize Viviane Jorge de Faria. – 2017.
55 f. : il.

Inclui Bibliografia.
Dissertação (mestrado) - Universidade Estadual de Montes Claros - Unimontes,

Programa de Pós-Graduação em Ciências da Saúde/PPGCS, 2017.

Orientadora: Profa. Dra. Ana Cristina de Carvalho Botelho.
Coorientador: Prof. Dr. Sérgio Avelino Mota Nobre.

1. *Candida* spp. 2. Actinobactérias. 3. Endofíticos. 4. Antibiose. 5. Enzimas. I. Botelho, Ana Cristina de Carvalho. II. Nobre, Sérgio Avelino Mota. III. Universidade Estadual de Montes Claros. IV. Título.

Catalogação: Biblioteca Central Professor Antônio Jorge.

UNIVERSIDADE ESTADUAL DE MONTES CLAROS-UNIMONTES

Reitor: Professor João dos Reis Canela

Vice-reitor: Professor Antonio Alvimar Souza

Pró-reitor de Pesquisa: Professor Virgílio Mesquita Gomes

Coordenadoria de Acompanhamento de Projetos: Karen Torres C. L. de Almeida

Coordenadoria de Iniciação Científica: Sônia Arrudas

Coordenadoria de Inovação Tecnológica: Dario Alves de Oliveira

Pró-reitor de Pós-graduação: Professor Hercílio Martelli Júnior

Coordenadoria de Pós-graduação Lato-sensu: Felipe Fróes Couto

Coordenadoria de Pós-graduação Stricto-sensu: Maria de Fátima Rocha Maia

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Coordenadora: Professora Marise Fagundes Silveira

Subcoordenador: Professor Luis Fernando Rezende

UNIVERSIDADE ESTADUAL DE MONTES CLAROS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Unimontes

MESTRANDO(A): RONIZE VIVIANE JORGE DE FARIA

TÍTULO DO TRABALHO: "Perfil fenotípico, enzimático e antagônico de isolados de actinobactérias endofíticas do cerrado".

ÁREA DE CONCENTRAÇÃO: Saúde Coletiva.

LINHA DE PESQUISA: Epidemiologia Populacional e Molecular.

BANCA (TITULARES)	**ASSINATURAS**
PROFª. DRª. ANA CRISTINA DE CARVALHO BOTELHO ORIENTADOR/PRESIDENTE	
PROF. DR. SÉRGIO AVELINO MOTA NOBRE	
PROF. DR. MAURO APARECIDO SOUSA XAVIER	
PROF. DR. ANDRÉ LUIZ SENA GUIMARÃES	

BANCA (SUPLENTES)	**ASSINATURAS**
PROF. DR. SÍLVIO FERNANDO GUIMARÃES DE CARVALHO	
PROFª. DRª. LETÍCIA ANTUNES ATHAYDE	

[X] **APROVADA** [ ] **REPROVADA**

Hospital Universitário Clemente Farias – HUCF
http://www.unimontes.br / ppgcs@unimontes.br
Telefone: (0xx38) 3224-8372 / Fax: (0xx38) 3224-8372
Av. Cula Mangabeira, 562, Santo Expedito, Montes Claros – MG, Brasil – Cep: 39401-001

AGRADECIMENTOS

Agradeço a Deus por me fazer persistir e lutar por meus sonhos.

Aos meus pais, que sempre foram exemplo de bondade, dignidade, honestidade.

Aos meus irmão, representantes de grandes apoios na minha vida.

Ao meu amor, amigo e companheiro Edmilson Faria por ser meu grande incentivadordesde o princípio, por sempre acreditar em mim, pelo apoio, pela amizade pelaconvivência e pelo amor e carinho que sempre me dedicou.

Aos meus filhos, João Pedro e Marcos Philipe, responsáveis pelas minhas maiores alegrias,"Por vocês e com vocês luto pelo futuro".

Aos meus orientadores Prof[a]. Dra. Ana Cristina de Carvalho Botelho e Prof. Dr. Sérgio Avelino Mota Nobre, mestres e amigos. Agradeço pelas oportunidades, pela convivência e pelo apoio.

À equipe do Laboratório de Epidemiologia e Biocontrole de Microrganismos (LEBM), pela valorosa contribuição na elaboração deste trabalho.

Á secretária da pós-graduação Du Carmo, pela solicitude sempre presente.

A todos que direta ou indiretamente, apoiaram a realização deste estudo. Muito obrigada!

RESUMO

A savana brasileira, denominada Cerrado, é caracterizada por apresentar tipologia diversificada de vegetação, sendo muitas delas utilizadas na medicina popular como uma forma alternativa de cuidados com a saúde, entretanto, pouco estudadas cientificamente. As plantas podem servir como reservatório para diversos microrganismos, que classificados como endófitos podem produzir uma infinidade de metabólitos, tanto primários quanto secundários. As actinobactérias endofíticas fazem parte de um grupo alvo de estudos que buscam novos produtos de interesse industrial como antibióticos, enzimas, antifúngicos, e outras substâncias bioativas. Este trabalho teve como objetivo avaliar o perfil fenotípico dos isolados de actinobactérias endofíticas do Cerrado, a capacidade de sintetizar enzimas extracelulares e metabólitos ativos contra espécies de *Candida albicans, C. krusei, C. tropicalis, C. glabrata* e *C. parapsilosis* isoladas da cavidade oral de pacientes irradiados, portadores de neoplasias malignas de cabeça e pescoço com histórico de resistência ou dose de tolerância aos antifúngicos comerciais. Os isolados da bacterioteca do Laboratório de Epidemiologia e Biocontrole de Microrganismos (LEBM) da Universidade Estadual de Montes Claros foram identificados através de microbiologia clássica utilizando análises micromorfológicas das estruturas reprodutivas. A caracterização enzimática foi avaliada através das expressões amilolítica, celulolítica, esterásica, proteolítica, lipolítica e pectinolítica. Doze gêneros de actinobactérias foram reconhecidos*: Actinopolysora, Micromonospora, Terrabacter, Nocardia, Saccharopolyspora, Streptosporangium, Thermoactinomyces, Streptoalloteichus, Streptoverticillium, Streptomyces, Nocardioides, Nocardiopsis*. Os extratos metabólicos de 88% destas actinobactérias apresentaram ação inibitória superior a 70% frente a alguma das espécies de *Candida* spp. utilizadas. Foi observado que o caldo de cultivo Starch-Nitrate-caseína (SCN) mostrou ser um substrato estimulador no processo biossíntese de substâncias antibióticas produzidas por actinobactérias. Cinco isolados de actinobactérias foram superiores ás demais, contudo com intensidades de inibição dependente da espécie de *Candida* desafiada. Relacionada à atividade enzimática, 94% expressaram amilases, 88% lipases e gelatinases, 71% esterases, 59% celulases, 24% caseinases e 6% expressaram pectinases.

Palavras-chave: *Candida* spp, Actinobactérias, Endofíticos, Antibiose, Enzimas.

ABSTRACT

The Brazilian savanna, denominated Cerrado is characterized by diverse typology of vegetation, with many of them used in folk medicine as an alternative form of health care, however, little studied scientifically. Plants can serve as a reservoir for various microorganisms that classified as endophytes can produce a multitude of metabolites, both primary and secondary. The entophytic actinobacteria are part of a target group of studies that seek new products of industrial interest as antibiotics, enzymes, antifungals, and other bioactive compounds. This work aimed to evaluate the phenotypic profile of actinobacteria isolated entophytic from the Cerrado, the ability to synthesize extracellular enzymes and active metabolites against species of *Candida albicans, C. krusei, C. tropicalis, C. glabrata* and *C. parapsilosis* isolated from the oral cavity of irradiated patients, with head and neck malignancies with a history of resistance or tolerance dose to commercials antifungals. Actinobacteria collection from Epidemiology and Biocontrol of Microorganisms Lab, Universidade Estadual de Montes Claros, were morphotyping regarding reproductive structures. The enzymatic characterization was evaluated through the amylolytic, cellulolytic, sterolytic, lipolytic, proteolytic and pectinolytic expressions. Twelve genera of actinobacteria were recognized *Actinopolysora, Micromonospora, Terrabacter, Nocardia, Saccharopolyspora, Streptosporangium, Thermoactinomyces, Streptoalloteichus, Streptomyces, Streptoverticillium, Nocardioides, and Nocardiopsis*. The metabolic extracts of 88% of actinobacteria strains presented more than 70% inhibitory action against at least one of the species of *Candida* spp. challenged. It was observed that the Starch-Nitrate-casein broth (SCN) showed to be better stimulating substrate in the biosynthesis process of antibiotic substances produced by actinobacteria. Five actinobacteria isolated were superior to the others, however with inhibition intensities dependent on the Candida species challenged. Related to the enzymatic activity, 94% expressed amylase, 88% lipase and gelatinase, 71% esterase, 59% cellulase, 24% caseinase and 6% pectinase.

Key words: *Candida* spp., Actinobacteria, Endophytes, Antibiosis, Enzymes.

LISTA DE ILUSTRAÇÕES

SUMÁRIO

1 INTRODUÇÃO

1.1 Biodiversidade do Cerrado

O Brasil está entre o grupo de países com os mais altos níveis de diversidade biológica, em decorrência dos diferentes biomas e ecossistemas que o caracterizam. Possui a mais rica diversidade genética vegetal do mundo distribuída em seis biomas terrestres. Detém dois *hotspots* de biodiversidade globalmente reconhecidos, a Mata Atlântica e o Cerrado (1). Contudo, esta grande biodiversidade é ainda desconhecida e poucos estudos exploram o potencial de uso dos recursos genéticos dos biomas brasileiros (2). Dessa forma, as plantas constituem ainda uma fonte importante para a descoberta de novas substâncias biologicamente ativas.

O Cerrado é um termo comumente utilizado para designar o conjunto de ecossistemas como: savanas, matas, campos e matas de galeria que ocorrem no Brasil Central (3). Este é considerado o segundo maior bioma do país, sendo superado em área apenas pela Amazônia (Figura 1) (4).

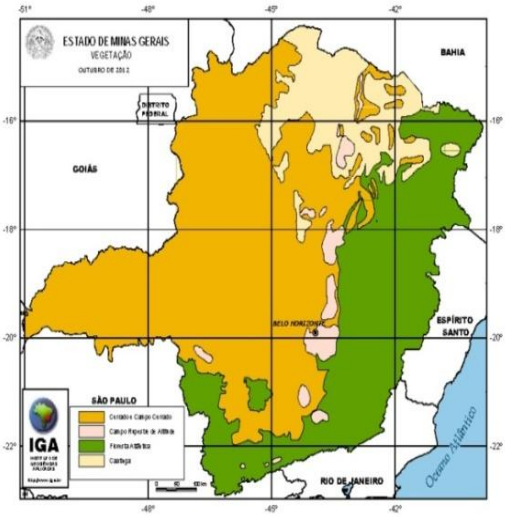
Figura 1 - Área de abrangência do Bioma Cerrado no território brasileiro, 2011

Fonte: BRASIL, 2011 (4)

O Cerrado ocupa 24% do território nacional, isto é, aproximadamente um quarto do território brasileiro; cerca de dois milhões de hectares abrigando um rico patrimônio de recursos naturais renováveis, adaptados às duras condições climáticas, edáficas e hídricas (5). Abrange o Distrito Federal, Goiás, Mato Grosso, Mato Grosso do Sul, Tocantins, Maranhão, Bahia, Piauí, Minas Gerais, São Paulo e Paraná (6).

Em Minas Gerais, o Cerrado cobre o correspondente a 57% do território estadual, exibindo fases de transição de difícil caracterização. É o maior bioma do Estado que aparecem especialmente nas bacias dos rios São Francisco e Jequitinhonha. Neste bioma, normalmente o clima é quente e as estações, seca e chuvosa são bem definidas (Figura 2) (7).

Figura 2 - Área de abrangência do Bioma Cerrado no território mineiro, 2012



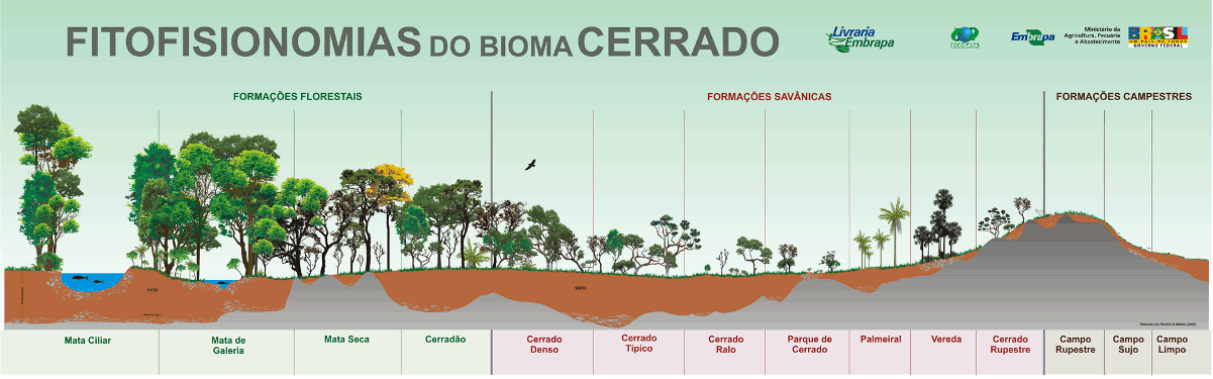
Fonte: IGA, 2012 (8)

A vegetação do bioma do Cerrado, em decorrência de sua extensão e diversidade de solo e clima, não possui uma fisionomia única. Ao contrário, ela é bastante diversificada, apresentando desde formas campestres bem abertas, como os campos limpos de Cerrado, até formas relativamente densas, florestais, como os cerradões (Cerrado *Lato sensu*). Entre esta dualidade fisionômica, encontra-se toda uma gama de formas intermediárias, com fisionomia de savana, às vezes de carrasco, como os campos sujos, os campos Cerrados, (Cerrado *Stricto sensu*) (9).

As formações florestais compreendem as fitofisionomias Mata Ciliar, Mata de Galeria, Mata Seca e o Cerradão. As formações savânicas são compostas pelo Cerrado Denso, Cerrado

Típico, Cerrado Ralo, Cerrado Rupestre, Veredas, Parque Cerrado e Palmeiral. As formações campestres englobam as fitofisionomias de Campo Sujo, Campo Limpo e o Campo Rupestre (Figura 3) (9).

Figura 3 - Fitofisionomias que ocorrem no domínio do Bioma Cerrado



Fonte: Embrapa (10)

Por essas razões é considerado um dos biomas mais ricos, mas também um dos mais ameaçados do mundo (1). Apresenta rica biodiversidade, com milhares de espécies de plantas vasculares, sendo grande parte destas correspondentes a plantas endêmicas, que se encontram ameaçadas de extinção, devido, principalmente, a ações antrópicas (11).

Apesar da alta biodiversidade e os poucos estudos científicos voltados para as espécies vegetais nativas deste bioma, muitas delas são utilizadas na medicina popular e tem sido muito aceita como uma forma alternativa de cuidados com a saúde mesmo sem evidências em relação a sua eficácia (12).

1.2Microrganismos endofíticos e Actinobactérias

Pesquisas mostram que as plantas podem servir como reservatório para diversos microrganismos. Estima-se que cada espécie vegetal possua microrganismos endofíticos ainda não classificados e com propriedades pouco conhecidas, mas potencialmente de interesse aplicado (13). Grande importância vem sendo dada a estes microrganismos endófitos que foram mencionados pela primeira vez no início do século XIX, porém, apenas no final dos anos 70 do século XX, começaram a serem tratados com maior ênfase em trabalhos

científicos. Esses microrganismos incluem principalmente bactérias e fungos que vivem no interior das plantas, habitando de modo geral suas partes aéreas, como folhas e caules, sem causar aparentemente nenhum dano a seus hospedeiros (14).

Levando em conta as estratégias de vida dos endófitos, estes podem ser classificados em obrigatórios ou facultativos, sendo que os obrigatórios são estritamente dependentes da planta hospedeira para seu crescimento e sobrevivência, enquanto que os facultativos possuem uma fase de seu ciclo vital no interior da planta hospedeira e outra fase da vida fora dela (15).

Por muitos anos, a prospecção de fármacos esteve voltada para extratos de plantas e microrganismos do ar e do solo, porém, a frequência em encontrar microrganismos que produzam tais compostos nesses ambientes tem decrescido havendo a necessidade de se explorar novos habitats como o interior de plantas superiores e com o objetivo de encontrar endófitos que possam produzir substâncias bioativas (16). Estes microrganismos desempenham funções importantes no processo de adaptação da planta e capacidade de produzir uma infinidade de metabólitos, tanto primários quanto secundários, os quais apresentam diferentes aplicações biotecnológicas, movimentando um mercado de dezenas de bilhões de dólares em todo o mundo (17).

Nesta perspectiva, pesquisas vêm demonstrando a importância da utilização de microrganismos endofíticos como fontes relativamente espontâneas e potenciais de produtos naturais modernos para exploração na medicina, agricultura e indústria; por se tratarem de sintetizadores químicos dentro da planta. Muitos deles são capazes de sintetizar compostos bioativos que podem ser usados pelas plantas para defesa contra patógenos, sendo algumas destas combinações de grande utilidade para a descoberta de drogas inovadoras de interesse farmacológico (18,19).

Uma das descobertas mais importantes com microrganismos endofíticos foi o isolamento do microrganismo *Taxomyces andreanae* produtor de taxol, um diterpenóide considerado uma droga anticancerígena potente, porém, a provisão desta droga esteve limitada pela exploração destrutiva da árvore (*Taxus brevifolia*) usada como a fonte principal do taxol. Sobretudo, com a descoberta do *Taxomyces andreanae* produtor da substância bioativa, este problema pôde ser resolvido (19,20,21). Assim, com a utilização de microrganismos endofíticos na produção de determinadas drogas fitoquímicas, muitos problemas poderiam ser evitados, como o lento

crescimento das plantas produtoras destes compostos, ou até mesmo o impacto ambiental causado pela extração dos mesmos (18).

Outros endofíticos são relatados, como *Fusarium subglutinans* que produz subglutinol com função imunosupressiva, tendo como planta hospedeira *Triptergium wilfordii* (22); *Paenibacillus amylolyticus*, que produz e ativa a enzima pectinaliase, tendo como planta hospedeira *Coffea arábica* (23); *Pestalotiopsis microspora* presente nos tecidos de *Terminalia Morobensis*, que produz compostos antioxidantes (24).

A produção de moléculas bioativas por microrganismos endofíticos, que eram somente encontradas em determinadas espécies vegetais, comprova a teoria de que durante a colonização da planta hospedeira, esses microrganismos se adaptam ao microambiente vegetal e assimilam parte do DNA vegetal em seu genoma, constituindo um processo de co-evolução, no qual os endófitos adquirem de seus hospedeiros a capacidade de biossíntese de compostos bioativos (25).

Um grupo de microrganismos que tem recebido especial atenção e está inserido na classe de microrganismos endofíticos são as actinobactérias. As actinobactérias compreendem um grupo heterogêneo de bactérias filamentosas, que filogeneticamente pertencem ao ramo das bactérias Gram-positivas com alto teor de guanina e citosina em seu DNA. Sua característica principal é a formação de filamentos ramificados ou hifas em algum ponto do ciclo de vida em diversas taxas, persistindo como um micélio estável ou fragmentando-se em bacilos ou cocos (26). Esses microrganismos estão taxonomicamente classificados dentro do domínio Eubactéria, na divisão Actinobactéria, classe Actinobactéria e ordem *Actinomycetales* (27).

Este grupo de bactérias tem sido muito explorado devido à sua aplicação biotecnológica, sendo alvo de diversos estudos que buscam novos produtos de interesse industrial como enzimas, antibióticos, antifúngicos, anticancerígenos e outras substâncias bioativas (28). As actinobactérias constituem uma grande capacidade quanto à produção de metabólitos secundários, isto é, compostos gerados no decorrer do seu desenvolvimento. Na maioria das vezes, essas biomoléculas exibem interessantes atividades biológicas, sendo as antibióticas a mais explorada e conhecida, proporcionando um versátil emprego das mesmas na medicina, indústria e agricultura (29,30).

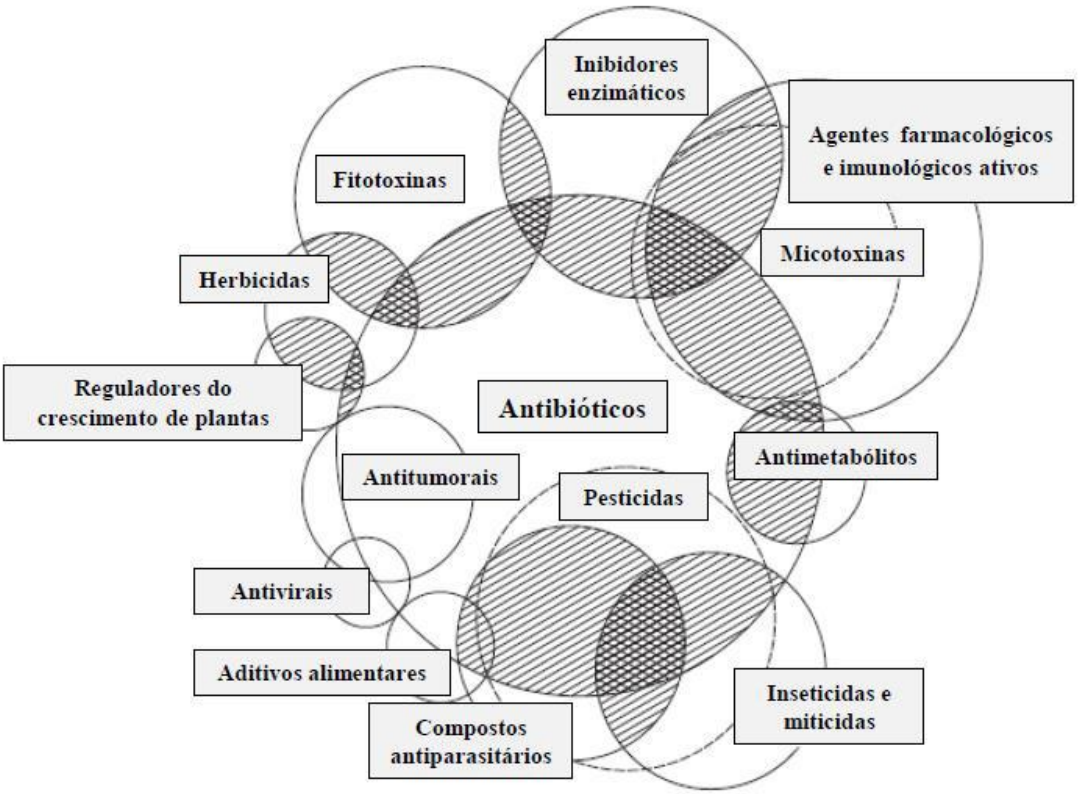
Entre os maiores produtores de antibióticos estão os fungos e as actinobactérias. As actinobactérias constituem a maior fonte de antibióticos, sendo que cerca de dois terços destes são sintetizados por estes microrganismos. Os antibióticos produzidos por estas bactérias apresentam grande variedade de estruturas químicas, incluindo aminoglicosídeos, antraciclinas, glicopeptídeos, β-lactâmicos, macrolídeos, nucleosídeos, peptídeos, polienos, poliéteres e tetraciclinas, dentre os quais podemos destacar cloranfenicol, eritromicina, neomicina, novobiocina, estreptomicina, tetraciclina, e gentamicina (31).

O gênero *Streptomyces* spp. destaca-se nesta ordem quanto à produção destas substâncias. O número de compostos antimicrobianos declarados provenientes de espécies desse gênero por ano aumentou quase exponencialmente em cerca de três décadas, demonstrando, assim, que esse grupo de microrganismos tem representado uma fonte promissora para a produção de novos agentes antimicrobianos. Devido a sua alta capacidade de produzir uma grande variedade de metabólitos (antibióticos, vitaminas e enzimas), os *Streptomyces* spp. são responsáveis por diversos antibióticos comercialmente importantes (32).

1.3 Metabolismo secundário e atividade antimicrobiana

Os metabólitos secundários são compostos não essenciais para o crescimento e reprodução dos diferentes organismos vivos, sendo derivados dos metabólitos primários. A gama de possíveis atividades biológicas que os metabólitos secundários, especialmente os microbianos, podem apresentar é muito ampla sendo: antibióticos (ações antibacteriana, antifúngica, antiviral, antiprotozoárica e antihelmíntica), pigmentos, toxinas, efetores na competição ecológica e na simbiose, feromônios, inibidores de enzimas, agentes imunomoduladores e quimioterápicos, antagonistas ou agonistas de receptores, pesticidas, herbicidas, aditivos alimentares, surfactantes, ou promotores do crescimento de animais e plantas (Figura 3) (33,34,35).

Figura 4 - Funções dos metabólitos microbianos bioativos



Fonte: Adaptado de Bérdy, 2012 (36)

O metabolismo secundário é geralmente acarretado por exaustão de nutrientes, biossíntese ou adição de um indutor e/ou decréscimo na taxa de crescimento. Esses eventos geram sinais que incitam uma série de cascatas regulatórias as quais resultam nas diferenciações químicas (idiofase) e morfológicas (morfogênese) (34). A produção destes metabólicos comumente coincide ou, sutilmente, antecede o desenvolvimento das hifas aéreas e a fase estacionária nos cultivos microbianos em meios sólidos e líquidos, respectivamente (37). Os microrganismos que produzem grande variedade dessas substâncias metabólicas estão provavelmente refletidas nas diferenças de habitats e estratégias de sobrevivência dos mesmos (38).

Os denominados antibióticos, produtos desse metabolismo secundário, são pequenas moléculas microbianas bioativas que sob uma determinada concentração exibem antibiose, ou seja, a capacidade de um organismo e seus metabólitos de prejudicar ou inibir o crescimento de outro (39).

O estimulo à intensiva busca por novos isolados com atividade biológica, se deu com o trabalho pioneiro do grupo de Waksman (40), que demonstrou que as actinobactérias eram capazes de produzir antibióticos úteis para aplicação na prática médica, sendo que milhares de novas moléculas e microrganismos deste grupo já foram estudados (41).

Estima-se que o grupo das actinobactérias seja responsável pela produção de milhares de antibióticos já conhecidos, superando a produção deste por fungos e outras bactérias. As vias biossintéticas de produção dos antibióticos foram evoluindo nas actinobactérias por cerca de um bilhão de anos (42). Dentre este grupo de bactérias, destaca-se o gênero *Streptomyces* sp., onde grande porcentagem dos antibióticos produzidos por este gênero, são comercializados e utilizados na saúde humana e animal e na agricultura (43).

1.4 Infecções fúngicas e resistência a drogas

Os antimicrobianos têm sido amplamente utilizados como opções terapêuticas para o tratamento de determinadas doença provocadas por bactérias e fungos. Entretanto, é universalmente aceito que, o uso indiscriminado dessas drogas tem acarretado na diminuição contínua da sensibilidade dos microrganismos aos antimicrobianos. O potencial surgimento de tolerância e resistência se faz a partir da utilização indiscriminada de qualquer agente terapêutico, através da coexistência de microrganismos com a espécie humana, por milhares de anos, que têm aperfeiçoado uma série de mecanismos para enfrentamento que os capacitam a sobreviver às condições de adversidade e na presença de agentes tóxicos (44,39).

As infecções fúngicas passaram a ter uma grande importância nos últimos anos, devido ao seu aumento progressivo na incidência. Apesar do elevado número de antimicóticos disponíveis, estes ainda encontram-se em desvantagem, quando comparados às drogas antibacterianas. Além disso, a resistência aos antifúngicos tem representado um grande desafio para a clínica médica (45).

As infecções provocadas por espécies de *Cândida* spp. vem se destacando nas últimas décadas, pela alta frequência com que infectam e colonizam o hospedeiro humano. O gênero compreende espécies leveduriformes, que residem como comensais, fazendo parte da

microbiota normal dos indivíduos saudáveis. Todavia, quando há uma ruptura no balanço normal da microbiota ou o imunocomprometimento do hospedeiro, as espécies deste gênero tendem a manifestações agressivas, tornando-se patogênicas (46).

Candida albicans é considerada a principal espécie do gênero representando cerca de 90% dos processos infecciosos de candidíase. Algumas características, ou fatores de virulência, são responsáveis pela patogenicidade de *C. albicans* como adesão a substratos inertes e biológicos, formação de tubo germinativo, variabilidade fenotípica e genotípica, variabilidade antigênica e imunomodulação do hospedeiro (47).

Infecções por *Candida* spp. envolvem um espectro amplo de doenças superficiais e invasivas oportunistas, acometendo pacientes expostos a uma grande diversidade de fatores de risco. As infecções sistêmicas por estas leveduras podem comprometer vísceras como resultado de disseminação hematogênica, complicações infecciosas que são geralmente documentadas em pacientes críticos, portadores de doenças degenerativas e/ ou neoplásicas (48).

Processos infecciosos resistentes a antibióticos estão relacionados às espécies de *Candida albicans,* porém,infecções por outras espécies de *Candida* não-*albicans* tem sido elevado. Dentre essas espécies se destacam *C. krusei*, *C. tropicalis*, *C. parapsilosis*, *C.glabrata* e *C. guillermondii* (49,50). Alterações no alvo molecular do fármaco, produção de enzimas fúngicas que degradam as drogas e uma redução na concentração intracelular do fármaco são mecanismos moleculares envolvidos na resistência a antifúngicos (51). Isso representa um sério problema de saúde pública, porque os microrganismos desenvolvem resistências a múltiplas drogas.

O aumento de espécies de *Candida* spp. resistentes aos antifúngicos representa um enorme risco à saúde pública, necessitando de medidas adequadas para controle de infecções. Contudo, estes problemas de resistência, a sensibilidade do paciente e incapacidade de controlar certas doenças infecciosas tem dado um impulso para a busca contínua de novos antibióticos em todo o mundo (52).

1.5 Actinobactérias produtoras de enzimas com potencial biotecnológico

Depois dos antibióticos, as enzimas, são os produtos mais produzidos pelas actinobactérias (53). As enzimas são catalisadores de reações químicas, envolvendo reações com substratos orgânicos e inorgânicos; geralmente de natureza protéicas, altamente específicas. Devido sua atividade e versatilidade, executam uma variedade de transformações de forma seletiva e rápida, em condições brandas de reação. As atividades enzimáticas não requerem altas temperaturas nem valores extremos de pH, podendo ser regulada com relativa facilidade, por alteração da natureza do meio de reação, como, pH ou a adição de algum efetor (54).

Os microrganismos são as mais importantes fontes de produção enzimática, evidenciando assim as actinobactérias, pela capacidade na produção de uma proporção considerável de enzimas com alto potencial tecnológico. São amplamente utilizadas no processamento de alimentos (pectinase, protease, celulase, oxidoredutase), na fabricação de detergentes (protease, lipase, celulase, oxidoredutase),nas indústrias farmacêuticas (lipase,esterase), têxteis (amilase, celulase, oxidoredutase), em terapia médica (L-asparaginase, urato oxidase), na biologia molecular (endonucleases, Taqpolimerase, etc.) e bioquímica (colesterol esterase, urato oxidase, L-glutamato oxidase, fosfolipase D, colina oxidase) (53,55). O gênero *Streptomyces* spp. destaca-se entre os actinomicetos pela capacidade de produzir uma grande variedade de enzimas com aplicação industrial (56).

Entre as várias enzimas de importância industrial destacam-se:

- Amilases: são responsáveis pela degradação da molécula de amido e estão amplamente distribuídas na natureza, podendo este ser encontrado principalmente em sementes de cereais como milho, cevada, trigo, arroz e em tubérculos ou raízes como batata e mandioca (57). Elas hidrolisam moléculas de amido em vários produtos e progressivamente em polímeros menores, compostos de unidades de glicose (58), requerendo para este completo processo de hidrólise a combinação de enzimas incluindo α-amilases, β-amilases, gluco-amilases e isoamilases (59). A ocorrência de amilases em actinomicetos é uma característica comumente observada em *Nocardia* spp. e *Streptomyces* spp. (60). A hidrólise do amido compreende cerca de um terço do consumo de enzimas no mundo, onde programas para selecionar novos microrganismos para a produção têm aumentado consideravelmente (61).

- Celulases: A celulose é um dos biopolímeros mais abundantes e pode ser hidrolisada, através do rompimento das ligações β-1,4-glicosídicas de suas microfibrilas, tendo como produto final a glucose (62). A atividade celulolítica se refere a um sistema enzimático que consiste de três tipos de celulases: endoglucanases, exoglucanases e β-glicosidases. As celulases são amplamente utilizadas na indústria têxtil, e contam com aproximadamente 14% do mercado de enzimas industriais (63). Embora os microrganismos degradadores de celulose estejam distribuídos em grupos diversificados, os sistemas de celulases de bactérias e fungos têm sido mais estudados que os outros sistemas. Os gêneros que apresentam atividade celulolítica dentre as actinobactéria são *Microbispora*, *Streptomyces*, *Thermoactinomyces* e *Thermomonospora*, incluindo mesofílicas e termofílicas (64-66,67,68).

- Esterases: As esterases são definidas como enzimas que hidrolisam os triglicerídeos em pequenas cadeias e preferem substratos solúveis em água, podendo catalisar a quebra e a formação da ligação éster (69). Estão amplamente distribuídas na natureza podendo ser encontradas nos animais, nas plantas e nos microrganismos. Estas enzimas possuem propriedade que as tornam atrativas como biocatalizadores na obtenção de compostos oticamente puros em reações de síntese da química fina (70). As esterases desempenham ainda um papel importante na síntese de alguns medicamentos de importância clínica, tais como as esterases de *Trichosporon brassicae* e *Rhodococcus* sp., *Bacillus circulans* que podem produzir em ampla escala, compostos de uso terapêutico. Esterases de *Pseudomonas* sp. são utilizados na produção de drogas como ibuprofeno, usado como anti-inflamatório (71).

- Lipases: As lipases (triacilglicerol acilhidrolases) constituem um importante grupo de enzimas que estão associadas ao metabolismo e à hidrólise dos lipídios, com considerada significância fisiológica e potencial industrial, pois catalisam a hidrólise de triacilglicerol a diacilglicerol, monoacilglicerol, ácidos graxos e glicerol na interface entre a fase aquosa e lipídica (72,73). São amplamente utilizadas nas indústrias de laticínios, agroquímicas, cosméticas e processamentos farmacêuticos (74). Muitas lipases comercialmente importantes são de origem microbiana, dentre eles destacam-se *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Candida tropicalis*, *Candida rugos*, *Aspergillus niger*, *Aspergillus oryza*e e dentre as actinobactérias, o gênero *Streptomyces* sp. (75).

- Pectinases: São produzidas por um grande número de bactérias, leveduras e fungos, insetos, nematódeos e plantas, a fim de degradar ou modificar o heteropolissacarídeo pectina (76). A biodegradação eficiente da pectina, um polissacarídeo construído principalmente pela ligação α-1,4-do ácido D-galacturônico e seu metilesteres, ocorre como resultado da ação sinergística de uma ampla diversidade de enzimas pectinolíticas (77,78). Possuem importância industrial, principalmente para aumentar a eficiência de filtração e clarificação de sucos de frutos, na maceração, liquefação e extração de tecidos vegetais (indústria têxtil), podendo em alguns casos ser responsáveis pela patogênese em plantas (76). Pesquisas envolvendo enzimas pectinolíticas em actinobactérias, ainda que de forma limitada, tem revelado principalmente a presença de pectato liases (79). Para o gênero *Streptomyces* sp. e alguns termofílicos do gênero *Thermomonospora* sp. tem revelado a presença de pectinas as quais foram purificadas e caracterizadas (80).

- Proteases: São enzimas capazes de quebrar ligações peptídicas de cadeias protéicas, estão amplamente distribuídas na natureza e ainda estão associadas a importantes processos biológicos tais como: a digestão protéica, coagulação sangüínea e morte celular (81). Para a avaliação da atividade proteolítica, podem ser utilizados diversos tipos de substratos, como a caseína, azocaseína, gelatina, peptídeos e albuminas (82,83). As proteases possuem uma vasta aplicação comercial e industrial, estando entre os três maiores grupos de enzimas industriais (81). Elas são usadas em alimentos, produtos farmacêuticos, e na indústria do couro e têxtil (84,85).

Levando-se em conta os aspectos abordados, a descoberta e o desenvolvimento de novos antimicrobianos são essenciais no combate contra patógenos resistentes, pela investigação de novas biomoléculas mais efetivas. Destaca-se os microrganismos endofíticos, especialmente actinobactérias, como uma fonte promissora de grande interesse para a indústria farmacêutica e biotecnológica, bem como a produção deenzimas extracelulares como agentes catalisadores capazes de modificar extensivamente estruturas que vão desde propriedades toxicológicas de contaminantes até o desenvolvimento de fitoterápicos enzimáticos.

Considerando as argumentações expostas, este trabalho teve como objetivo avaliar o potencial biotecnológico de actinobactérias residentes no interior de plantas nativas do Cerrado, como agentes de síntese de substâncias antibióticas ativas contra diferentes espécies de *Candida* spp. obtidas da cavidade oral de pacientes irradiados e portadores de neoplasias malignas, e como fontes de enzimas de valor industrial.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar o perfil fenotípico, enzimático e antagônico dos isolados de actinobactérias endofíticas residentes em áreas do Cerrado.

2.2 Objetivos específicos

- Identificar fenotipicamente as actinobactérias endofíticas isoladas de espécies vegetais do Cerrado.
- Avaliar substrato para extração de produtos do metabolismo das actinobactérias endofíticas do Cerrado.
- Avaliar atividades antifúngicas dos extratos metabólicos das actinobactérias contra leveduras patogênicas à espécie humana.
- Mensurar o efeito inibitório dos extratos metabólicos frente a diferentes espécies fúngicas patogênicas.
- Caracterizar as actinobactérias endofíticas quanto à atividade de enzimas amilolíticas, lipolíticas, esterásicas, pectinolíticas, proteolíticas e celulolíticas.

3 PRODUTO CIENTÍFICO

3.1 Produto Científico 1: Perfil fenotípico, enzimático e antagônico de isolados de actinobactérias endofíticas do Cerrado, formatado segundo as normas para publicação do periódico Brazilian Journal of Microbiology, enviado em um periódico.

Profile enzymatic and biological trait of actinobacteria isolated entophytic antagonistic of the Cerrado

Prospecting of Cerrado actinobacteria

Ronize Viviane Jorge de Faria[1]*

Thais Tiemi Yoshinaga[1]

Ana Cristina de Carvalho Botelho[2]*

Sérgio Avelino Mota Nobre[1]*

[1]Epidemiology and Biocontrol of Microorganisms Laboratory, Universidade Estadual de Montes Claros, Montes Claros – Brasil.

[2]Faculdades Integradas Pitágoras

* Graduate Program in Health Sciences at the Universidade Estadual de Montes Claros

SUMMARY

The entophytic actinobacteria are part of a target group of studies that seek new products of industrial interest as antibiotics, enzymes, antifungals, and other bioactive compounds. This work aimed to evaluate the phenotypic profile of actinobacteria isolated entophytic from the Cerrado, the ability to synthesize extracellular enzymes and active metabolites against species of *Candida albicans, C. krusei, C. tropicalis, C. glabrata* and *C. parapsilosis* isolated from the oral cavity of irradiated patients, with head and neck malignancies with a history of resistance or tolerance dose to commercials antifungals. Actinobacteria collection from Epidemiology and Biocontrol of Microorganisms Lab, State University of Montes Claros, were morphotyping regarding reproductive structures. The enzymatic characterization was evaluated through the amylolytic, cellulolytic, sterolytic, lipolytic, proteolytic and pectinolytic expressions. Twelve genera of actinobacteria were recognized *Actinopolysora, Micromonospora, Terrabacter, Nocardia, Saccharopolyspora, Streptosporangium, Thermoactinomyces, Streptoalloteichus, Streptomyces, Streptoverticillium, Nocardioides, and Nocardiopsis*. The metabolic extracts of 88% of actinobacteria strains presented more than 70% inhibitory action against at least one of the species of *Candida* spp. challenged. It was observed that the Starch-Nitrate-casein broth (SCN) showed to be better stimulating substrate in the biosynthesis process of antibiotic substances produced by actinobacteria. Five actinobacteria isolated were superior to the others, however with inhibition intensities dependent on the Candida species challenged. Related to the enzymatic activity, 94% expressed amylase, 88% lipase and gelatinase, 71% esterase, 59% cellulase, 24% caseinase and 6% pectinase.

Key words: *Candida* spp., Actinobacteria, Entophytes, Antibiosis, Enzymes.

1. INTRODUCTION

One of the fungal infections, caused by *Candida* spp. involve a broad spectrum of surface diseases and invasive infections, affecting patients exposed to a wide range of risk factors. Systemic infections by *Candida* spp. may compromise viscera because of hematogenous dissemination, infectious complications that are usually documented in critical patients with degenerative and/or neoplastic diseases.[1] Normally affects immunocompromised individuals with cancer and chemotherapy intensive treatment, who made widespread use of antibiotics, or using immunosuppressive drugs for organ transplant, parenteral nutrition, in addition to HIV-positive patients.[2,3] The emergence of antifungal resistant yeast populations has represented a great challenge for the medical clinic.[4]

The soil ecosystem traditionally is the preferred environment for bioprospecting of antimicrobial drugs, however new ways have been considered, among them the inner of plant tissues.[5] So the prospecting involving entophytic microorganisms has become a promising source of secondary metabolites with antibacterial, antifungal, antitumor, antimalarial, among othersactions.[6,7]

The actinobacteria are widely distributed in terrestrial biomes with different environmental conditions such as soil type, pH, and humidity, among others. These bacteria produce a wide variety of secondary metabolites, probably reflecting differences in habitats and survival strategies.[8] these are prokaryotes valuable biotechnologically to play important roles in the process of adaptation of the plant, in addition to the high capacity of producing a diversity of secondary metabolites.[8]They are the subject of several studies that seek new products of industrial or commercial interest as enzymes, antibiotics, antifungal, anticancer and other bioactive substances.[9] The bacteria of the *Actinomycetales* order are responsible for producing more than 45% of microbial metabolites of pharmaceutical access. In this order, the genus Streptomyces is responsible to produce more than 70% of 10,000 biotechnological products secondary metabolites actives, discovered from actinobacteria.[10]

After the antibiotics, these microorganisms more often produce enzymes, products. Highlight to those used in food processing (pectinase, protease, cellulase, oxidoreductase), in the manufacture of detergents (protease, lipase, cellulase, oxidoreductase), pharmaceutical industries (lipase, esterase), textiles (amylase, cellulase, oxidoreductase), on medical therapy (L-asparaginase, urate oxidase), in molecular biology (endonucleases, Taq polymerase, etc.) and biochemistry (cholesterol esterase, ureato oxidase, L-glutamate oxidase, phospholipase D, choline oxidase).[11,12]

The Cerrado is referred to as a diverse biome quite adapted to soil and climate conditions of stress resulting from spontaneous combustion and burning, acid soils with high aluminum levels normally related to higher temperatures, with rainy summers and dry winters well define.[13] Despite the significant biological diversity in the Cerrado, the bioprospecting in this biome is still incompatible with the potential already recognized in folk medicine, especially with herbal medicines.[14]

This work had objective to study the actinobacteria isolated from native plants of the Cerrado and the biotechnological potential of uses, against different species of *Candida* obtained from oral cavity of irradiated patients with malignant neoplasms, as well as source of enzymes of commercial value.

2. MATERIALS AND METHODS

2.1 Origin of microbial isolates

17 Actinobacteria were isolated from the inner tissues of native Cerrado plant species, belonging to the Laboratory of Epidemiology and Biocontrol of Microorganism (LEBM) of the Universidade Estadual de Montes Claros, Brasil. The isolates were stored at -80°C.The plant species were: *Acrocomia aculeata* (Macaúba) in the region of Montes Claros-MG on the campus of UNIMONTES, *Solanum lycocarpum* (Lobeira), *Vergonia polysphaera* (Assapeixe), *Dipteryx alata* (Barú), *Ficusdoliaria* (Gameleira), *Acanthospermum hispidum* (Espinho de agulha), *Guazuma ulmifolia* (Mutamba), *Caryocar brasiliense* (Pequi).As standard procedure for regeneration of the isolates, 100µL aliquots of the conservation suspension were used and spread in Czapek-Dox Agar (CZA) and the plates were incubated (BOD 411-D Nova Ética) at 28ºC and under photoperiod of 12 hours for 10 days.

Three species *C. albicans* (Csp 22/09); *C. tropicalis* (Csp 11/09); *C. glabrata* (Csp 16/09); *C. krusei* (Csp 21/09) and *C. parapisilosis* (Csp 60/10) were isolated from the oral cavity of irradiated patients with malignant neoplasm. The strains were characterized as to the level of sensitivity to fluconazole (FL), Ketoconazole (KE), Itraconazole (IT), Amphotericin B (APB, polyene) and Flucytosine (FC, pyrimidine) through E-test. (Probac. Brazil, São Paulo, SP, Brazil).

2.2 Morphological characterization of actinobacteria

The isolates were stained by the Gram method and, those typical of actinobacteria, subsequently examined in microcultivo.[15] Two distinct procedures conducted for microcultivo, both in CZA and incubated at 28° C (411 BOD-D Nova Ética). Observations and records made in optical microscope (Nikon E200) with magnification of 400x. Micromorphological definitions established between 10 and 21 days of incubation.[16] The observations made permanent in polyacrylamide resin, and the fresh preparations stained with lacto phenol cotton blue. Observed and recorded characteristics such as branch of the mycelium on the substrate, mycelium formation, fragmentation and spore production, these attributes used to distinguish isolates based on identification keys.[16,17]

2.3 Evaluation of antifungal activity of extracellular products of actinobacteria

The production of metabolic extracts (ME) of the actinobacteria made in erlenmeyers flasks of 250 ml containing 20 ml of broth CZA or Starch-Nitrate-Casein (SNC), distinctly. The cultivation were done in orbital shaking incubator (SOLAB SL222), at 28° C, 120 rpm for 13 days. The spore suspension was obtained from colonies after 10 days of cultivation in CZA plate at 28ºC (BOD 411-D Nova Ética). After cultivation, 1 mL of spore solution (0.85% NaCl; 0.1% Tween 80) were added homogeneited and the spore containing solution was recovered from the plate. After the period of fermentation, bacterial cells and supernatant were separated by centrifugation at 12,000 rpm for 10 minutes. The supernatant containing ME was filtered on 0.22 µm membrane (Millipore, Millex ®). Were 34 extracellular metabolic extracts, being 17 produced in CZA (ME1) and 17 in SNC (ME2). The suspension of the *Candida* specie were prepared from colonies grown in 24 hours in Sabouraud Dextrose Agar (DSA-Oxoid, England) at 28 ºC. The yeasts colonies were suspended in sterile solution (NaCl 0.85% m/v) and calibrated in scale 0.5 McFarland ($\approx 5 \times 10^6$ cfu.mL^{-1}) measurement at 530nm wave length. Then, dilution 1:100 in 0.85% g.L^{-1} NaCl followed by 1:20 dilution in liquid medium RPMI 1640 (Fluka); to obtain concentration of 2.5×10^3 yeast.mL^{-1}.[18]Were carriedouto the antibiosis test were performed in sterile microplate 96 wells.[19]The wells were filled with 100µL of the specific ME and the 100µL of suspension yeast prepared as above. The microplates were incubated at 37°C for 24 hours. The statistical model applied was completely randomized design in factorial scheme (34 ME x 5 species of *Candida* spp.) with three replicates, each well a repeat. Treatments controls added, as well as the sterility of each.

The intensity of inhibition was estimated spectrophotometrically at 620nmwave length in Elisa reader apparatus (model EP-Reader-End Plate). The values of absorbances were considered in the calculations of the percentage of inhibition generated from each in front of each species of yeast, whereas the ratio between the growths observed in treatments with and without the presence of ME, as alleged inhibiting factors.[20] The percentage of inhibition was calculated using the formula: for each *Candida* and ME.

$$Inb_{n-z}(\%) = \left[\frac{(AE_nCsp_z - AE_n)}{ACsp_z}\right] \times 100$$

At where:

Inb_{n-z} *(%)* = Intensity of inhibition of *n-ME* on species *z-Candida*;

AE_nCsp_i = Absorbance in *n-ME* treatment vs species *z-Candida*

$ACsp_z$= Absorbance observed for *z-Candida* species in the absence of *ME*

AE_n= Absorbância do extrator isoladamente

2.4 Evaluation of the enzymatic activity from Actinobacteria

The activity of enzymes: amylase, cellulose, esterase, lipase, protease and pectinase were measured by the presence and intensity of expression. The actinobacteria grown as described in iten 2.3, whose colonies were inoculated in media semisolid (0.3% w/v) with vigorous homogenization. With the aid of an automatic pipette, three aliquots of 10μL each were standardized arranged, about the specific means for evaluation of each enzyme. The enzymatic activity was measured at 4, 11 and 18 days post inoculation. The tests were randomized considering three replications for each actinobacteria-cultivation time. Amilolítica activity was measured as described by Nogueira and Cavalcanti (1996):[21] inoculating 10μL of actinobacteria suspension isolated on Nutrient Agar plates (NA) containing 0.2% (w/v) of soluble starch, pH 6.0. The revelation of the halo of hydrolysis was done with Lugol's iodine.[21]Celulolítica activity was measured in the midst of carboxymethylcellulose (CMC) 1%, with incubation at 28° C, and the revelation with Congo red dye (0.1 g/100 ml^{-1}), pH 8.0, followed by solution of NaCl (0.5 M) pH 8.0.[22] Esterásica activity was evaluated in growth medium containing (g. L^{-1}): Peptone (5 g); Yeast extract (1 g); NaCl (5 g); Agar (15 g); $CaCl_2$ (0, 1 g), supplemented in a Tween 80 final concentration of 1% v/v. Positive results is indicated formation of opaque halo around the colonies.[23]The proteolytic activity was done by two procedures: (i) caseinase activity was held in culture medium containing skimmed-milk powder (5% w/v), and the revelation was made by measuring the translucent halo around the circle of the colonies.[24] (ii) gelatinase activity of in Nutrient Gelatin Agar (NGA: nutrient agar, 23 g; gelatin, 8 g), and the revelation highlighted with saturated ammonium sulphate solution.[25] The lipolytic activity was measured using the growth medium containing (g. L^{-1}): Bacto peptone (10 g), NaCl (5 g), $CaCl_2$, anhydrous (0.086g), agar (15 g), pH 7.4; After sterilization and cooling added 1 ml of Tween 80 in final concentration of 1% (v/v). The plates were incubated at 28° C, and lipolytic activity assessed by observation of the calcium crystals halo around the colonies.[26] Pectinolítica activity evaluated in Soy Agar Tryptone (TSA) supplemented with 1% of citrus pectin, and the revelation with $CaCl_2$ solution (1M). The positive activity was indicated by the presence of translucent halo around the colonies.[24]The intensity of the expression of enzymes was expressed by the Enzymatic Index (EI), being the ratio of the measurement of the halo of hydrolysis divided by and the diameter of the colony at the specificied time.[27]

It was assigned of enzymes as Normal expression time (NE), Early expression time (EE) and Late expression time (LE), consideruy to the records from the scientific literature and the common period of expression of the respectives enzymes. Hence, the name 'Normal' correlates directly to the period commonly referred to in specialized publications: (i) amylase and pectinase (usually = 4 days of cultivation);[21,24] (ii) caseinase, cellulase, esterase and lipase (normally = 11 days of cultivation);[22,23,24,26] (iii) gelatinase (normally = 18 days of cultivation).[26]

186 **2.5 Data Analysis**

187 The statistical analyses were performed by Minitab ® software (Minitab Statistical Software,
188 Release 15 for Windows, State College, Pennsylvania). The data was regard to the assumptions for
189 parametric analysis and subsequently conducted analyses of variance (ANOVA), Cluster analysis and
190 Student's t test, using the significance level $\alpha = 0.05$.

191

192 **3. RESULTS AND DISCUSSION**

193

194 **3.1 Morphological characterization of the isolates**

195 Twelve genera of actinobacteria residents internally in native Cerrado plant tissues were
196 morphologically recognized using selective culture media and microculture (Table 1).

197 Diverse morphotypes were identified, from fully fragmented to filamentous. Forms this
198 morphological variety is cited in the literature, which often refers to the actinobacteria as a diverse and
199 versatile group.[28]

200

201 Table 1 – Morphological and presumptive identification of actinobacteria genus isolated from inner of
202 plants tissues, native to the Cerrado.

Isolate Code	Morphotyping			Genus Presumptive
	Color of the colony	Melanoides Pigments	Microscopic Structures *	
Act1	Brown	Absent	RA	*Actinopolysora*
Act2	Brown	Absent	RA	*Actinopolysora*
Act3	Brown	Absent	RA	*Micromonospora*
Act4	Beige	Absent	R	*Terrabacter*
Act5	White	Present	B	*Nocardia*
Act6	Green	Absent	RA	*Saccharopolyspora*
Act7	White	Absent	RA	*Streptosporangium*
Act8	Green	Absent	RA	*Thermoactinomyces*
Act9	Green	Absent	RA	*Streptoalloteichus*
Act10	Green	Absent	RA	*Streptoverticillium*
Act11	Greyed out	Present	S	*Streptomyces*
Act12	Beige	Absent	R	*Terrabacter*
Act13	White	Absent	B	*Nocardioides*
Act14	Yellow	Absent	RA	*Saccharopolyspora*
Act15	Beige	Absent	R	*Terrabacter*
Act16	Pink	Present	F	*Nocardiopsis*
Act17	Green	Absent	RA	*Streptoalloteichus*

203 * F (Flexibilis); R (Rectus); RA (Retinaculum-Apertum); S (Spira); B (Bacillus)
204

205 It was observed a predominance of the genus *Terrabacter* (17%), followed by the genera
206 *Actinopolysora* (12%), *Saccharopolyspora* (12%) and *Streptoalloteichus* (12%), *Micromonospora*
207 (6%), *Streptomyces* (6%), *Nocardia* (6%), *Streptosporangium* (6%), *Streptoverticillium* (6%),
208 *Thermoactinomyces* (6%), *Nocardioides* (6%) and *Nocardiopsis* (6%).

As noticed in our studies, other reports show that the morphological diversity of the Actinobacteria are based primarily on their reproductive strategies that lead to formation of a variety of structures of spores, such as the arthrospores (*Streptomyces*), aleuriospores (*Micromonospora*), endospores (*Thermoactinomyces*) and zoospores (several members of *Actinoplanaceae*, *Geodermatophilus*, *Oerskovia*, *Kitasatoa*).[29]

Three isolates (Act5, Act11 and Act16) presented production of melanóides pigment in the CZA medium. The morphotyping, at the 21 days, of cultivation showed severeil types of macroscopic structures grown in CZA medium (Figure 1).Of the Isolates observed 14 showed Branched aerial mycelia, being: Act11 spiraled, Act1, Act2, Act3, Act6, Act7, Act8, Act9, Act10 Act14 and mycelia Act17, snared, and mycelia Act13 Act5 Act16 rod-shaped and flexible, while the mycelium Act4, Act12 and Act15 presented straight and non-air mycelia. Such structural features express faithfully the order *Actinomycetales* as described by literature.

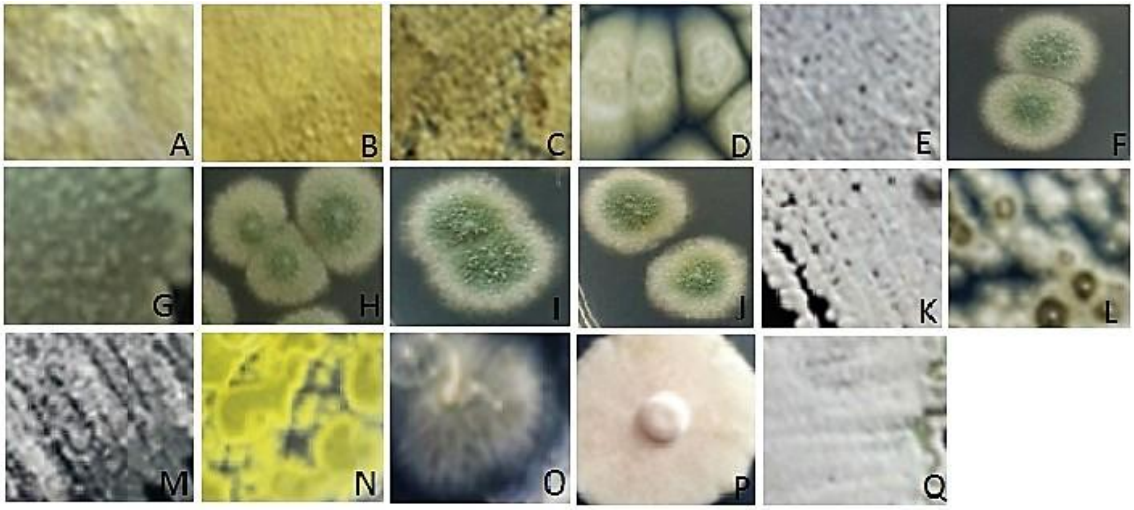


Figure 1 - Macroscopic morphology of colonies of endophytic actinobacteria from the Cerrado's plants: (A) *Actinopolysora* sp., (B) *Actinopolysora* sp., (C) *Micromonospora* sp., (D) *Terrabacter* sp., (E) *Nocardia* sp., (F) *Saccharopolyspora* sp., (G) *Streptosporangium* sp., (H) *Thermoactinomyces* sp., (I) *Streptoalloteichus* sp., (J) *Streptoverticillium* sp., (K) *Streptomyces* sp., (L) *Terrabacter* sp., (M) *Nocardioides* sp., (N) *Saccharopolyspora* sp., (O) *Terrabacter* sp., (P) *Nocardiopsis* sp. and (Q) *Streptoalloteichus* sp.

The Figure 2 shows the morphotyping of five actinobacteria strains selected by the highest rates of inhibition against the species of *Candida* spp., through optical microscopy. Variations in edafoclimatic conditions can favor, selectively, certain physiological types, having the dry soils and warm a large occurrence of actinobacteria, being the predominant genera and commonly isolated, *Nocardia*, *Streptomyces* and *Micromonospora*.[30,31] Even if there is an analogy from the ground of the Savannah, our results showed different results in a smaller number for these genres, indicating that not all are fit biologically to colonize plant tissues, without causing diseases such as endophytic.

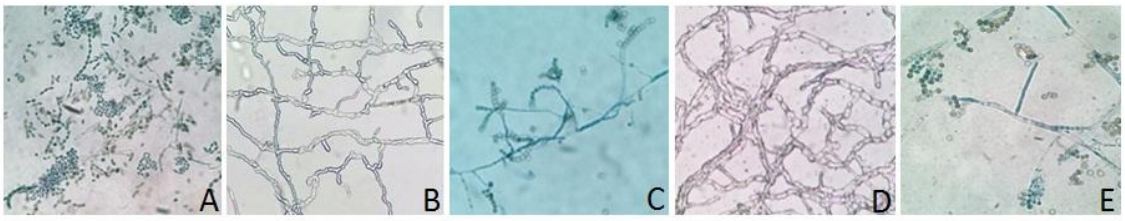


Figure 2 - Micromorphology, recorded in microcultivos, of the residents in the Cerrado biome actinobacteria and selected as inhibitory substances producing species of *Candida* spp. A - Act3 (*Micromonospora* sp.), B - Act12 (*Terrabacter* sp.), C - Act10 (*Streptoverticillium* sp.), D - Act15 (*Terrabacter* sp.), E - Act17 (*Streptoalloteichus* sp.). 400 x Magnification

3.2 Screening of strains producing inhibitory substances against *Candida* spp.

The choice of isolated *Candida* species and was based on the responses of sensitivity to antifungal drugs: fluconazole (FL), Ketoconazole (KE), Itraconazole (IT), Amphotericin B (APB) and flucytosine (FC), being recognized as resistant or tolerant dose to some of these substances.

On cluster analysis of these yeasts regarding sensitivity to these antifungals, as well as the metabolic extracts (ME) from actinobacteria, supernatant showed grouping pattern similar between *Candida glabrata* and *C. tropicalis* (Similarity = 94%), closing of *C. krusei* and highlighting of other species in this study (Figure 3A). These species of *Candida* presented the same similarity when grouped by levels of sensitivity to antifungal trade (Figure 3B).

The different between behavior of *Candida albicans* and *C. tropicalis* in face of the ME of the actinobacteria (Figure 3A), suggest the existence of distinct biochemical mechanisms to deal with the suppressive effect of antifungals, deserving differentiated attention regarding the following results and monitoring of populations resistant to antifungal drugs. An important characteristic of *C. tropicalis* is related to the high mortality rate (59.0 to 85.7%) and it is fast dissemination in immunocompromised patients, especially in individuals with haematological complications and other disseminated infections.[32]

Studies indicate that *C. tropicalis* and *C. albicans* is pathogenic species in the oral and vaginal cavity, responsible for more than 70% of the fungal infections in these areas.[33]

These results are associated with the enzyme, profile metabolic pathways and genetic mechanisms characteristic of the species.34-36

The isolates of Actinobacteria that stood out as promising, regardless of the medium and the species of *Candida* spp. challenged, can be recognized in figures 4A and 5: Act17, 15, 4, 10 and 3, and the intensity of their inhibitory in variable with the growth medium used.

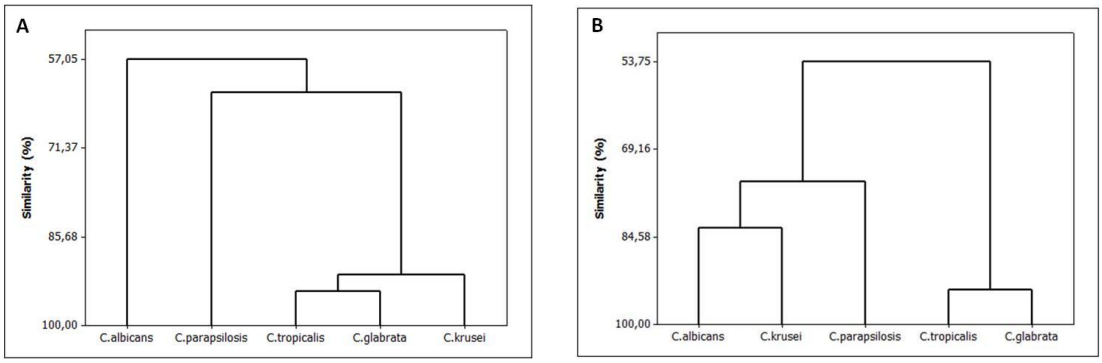


Figure 3-Cluster analysis of *Candida* spp. isolates considering the sensitivity level to ME from actinobacteria isolates (Panel A) and commercial antifungals: Fluconazole, Itraconazole, Cetaconazol, Flucytocine and Amphotericin B(Panel B).

The 17 isolate characterized to being to genus *Streptoalloteichus* produced inhibitory substances, in both media of fermentation, which inhibited all species intensities of *Candida* spp. tested in different medium. On average 48% inhibited yeast being the highest inhibition on *C. parapsilosis* (100%), with ME1 and the minimum, about *C. krusei* (6.3%) with ME2(Figure 4C). Studies point out that species of the genus *Streptoalloteichus* produce Kedarcidin, which is a cromoprotein with amino acid residues that displays activity both as anticancer and antibiotic.[37,38] Literature does not address specifically the adversarial process of metabolic extracts of this genre in front of other microorganisms, however reports that these actinobacteria is known to produce the Polyhydroxyalkanoates (PHAs), which are biodegradable and renewable polymers.[39] These polymers can used as a substrate for the production of D-β-hydroxybutyrate synthesis of antibiotics, vitamins, aromatics and pheromones.[40]

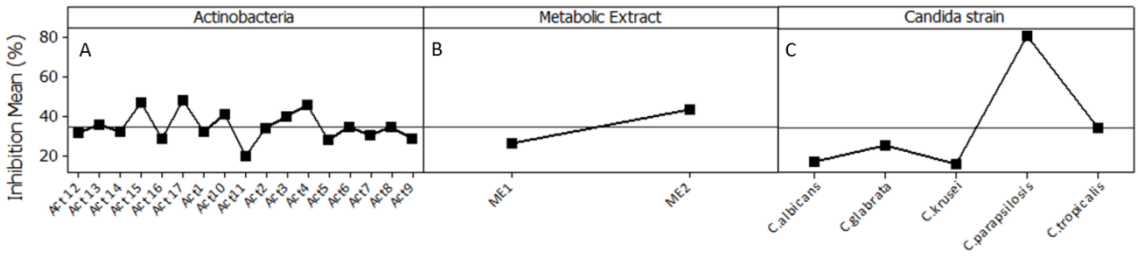


Figure 4 - Mean of the dependent effects involved in the inhibition test: A – Medium promoted by inhibition in the actinobacteria regardless of the means and of the species of *Candida* challenged (n = 30), B – regardless of actinobactéria and species of *Candida* challenged, C – regardless of the actinobactéria and of the growth medium.

The ME produced by *Terrabacter* sp., who are isolated Act4 and Act15 inhibited three species of *Candida* spp. when Czapek-Dox broth produced (ME1) and all when cultivated in the SNC (ME2) broth.

The ME from the isolated Act15 inhibited on average 47% of tested yeasts, being the highest inhibition on *C. parapsilosis* (100%) with ME1 and ME2, and the hower minimum on *C. albicans* (0.0%) with ME1 and ME2. The ME from the isolated Act4 inhibited on average 45.6% of yeasts,

being the highest inhibition on *C. parapsilosis* (98.6%), and the minimum about ME1 *C. albicans* and *C. krusei* (0.0%) with ME1 and on *C. albicans* (0.0%) with ME2.

Although they belong to the same genre and the slower growth among the Actinobacteria evaluated, these isolates are very promising showing minor variations in inhibition in relation to the yeasts challenged. Similar data was found in out actinobacteria isolated studies by Rodrigues (2006),[41] in the process of composting, in which it found that the genus *Terrabacter* represented 8% of bioactive metabolites, with emphasis on the biocidal effectiveness against microorganisms of clinical importance as *Enterobacter cloacae*, and additionally the genre of greatest inhibition to *C. albicans*.[41] The largest number of isolates with greater potential for the antimicrobial activity, can be assigned because the genre *Terrabacter* be characterized by adaptations to dryness, radiation and high salinity,[42]andthese conditions are also observed in the Cerrado.

The ME from the isolated Act10 (*Streptoverticillium* sp.) showed inhibition average of 41% of on yeasts, Being the maximum inhibition on *C. parapsilosis* (85%), with EM1, and the minimum on *C. albicans* (0.0%) with the same ME. Itis described in the literature that *Streptoverticillium cinnamoneum* var. *scleroticum*, isolated from soil samples, produces a substance named sclerotia, which under specific environmental conditions in liquid or solid, broth presents a promising antifungal and antibacterial activity.[43]And her example is the occurrence of antifungal activities and antitumor of secondary metabolic produced by *Streptoverticillium* sp. isolated from soil samples, which 2 isolated substances showed antimicrobial activity against *C. albicans, C. krusei, C. parapsilosis, C. utilis, C. tropicalis, C. lipolytica* and *C. guillermondii*.[44] However our results demonstrated that the actinobacteria from this genre (Act10) produced showed inhibition of 89% and 26% to *C. albicans* and *C. krusei* when grown in SCN broth, respectively. No inhibition was detected to the same *Candida* using ME from CZA medium cultivation. (Figure 5A and G).

The In the isolated Act3 (*Micromonospora* sp.) inhibited 40% on average of the yeasts, being the highest inhibition on *C. parapsilosis* (96%) with ME2, and the minimum on *C. albicans* and *C. krusei* (0.0%) with ME1.Studies show that species from this genus are promising bioactive compounds producers, including gentamicin and caliqueamicina.[10,45,46] New studies on *Micromonospora* sp. confirm the high antibacterial activity against several important human pathogens, such as *Mycobacterium abscessus, Mycobacterium smegmatis, Bacillus subtilis, Staphylococcus aureus, Proteus vulgaris, Salmonella, Pseudomonas aeruginosa,* and *Escherichia coli*.[47]

It was meaningful and statistically significant superiority of the SCN medium in stimulating the biosynthesis of the antibiotic substances (Figure 4B), suggesting that the excretion of bioactive substances by the actinobacteria has been more potentized by substrate components ME2 in relation to the components of ME1 substrate. Studies have shown the influence of cultures with carbon or nitrogen sources more complex and claim this interference on the growth and production of bioactive metabolites.[48,49] The glucose is one of the essential carbon source for the growth of microorganisms, however there is disagreement among scholars about itis use as substrates for production of secondary

metabolites. There are claims that this substance represents an excellent source of C for cell growth and it has influence in the production of various antibiotics.[48] Others found that cell growth not related to the production of secondary metabolites, a substrate can enhance growth of the microorganism, but the production of bioactive compounds may not significantly influenced.[50] The use of monomeric and polymeric energy sources like starch have treated as relevant when the goal is the biosynthesis of antibiotics.[51]This study showed the superiority of the culture medium SCN, whose main source of C is starch, which surpassed, for the great majority of actinobacteria the CZA, whose source is glucose(Figure 4B).

Sensitivity to ME distinct and was statistically superior to the *Candida parapsilosis* and conversely *C. albicans*, *C. Krusei* and *C. glabrata* (Figure 4C) and noted greater resistance by *Candida albicans, C. krusei* to ME the actinobacteria. Opposite behavior observed for *C. parapsilosis* that showed the highest sensitivity.

It is worth noting the importance in this yeast inhibition, since *C. krusei* spp. has been recognized as a potentially pathogen resistant, in addition, have the ability to form biofilms in biomedical devices that come into contact with the skin, mucosa or inert.[52]

3.3 Profile of enzyme expression of isolated

The intensity and diversity of enzyme is expression varied among isolates of actinobacteria, and all these have expressed at least one of the enzymes studied, therefore Enzymatic Index (EI $\geq$ 1), and indicating qualitative activity.[53,54] So assumed high potential and biotechnology value for some of the actinobacteria isolated from plants of cerrado.

Figure 6 deals with the average values of EI for each decoupled factor, especially without considering the interactions among actinobactéria, time of expression and enzyme. Table 2 provides qualitative results of expression of each one of the enzymes in the respective actinobacteria.

The most versatile actinobacteria were: Act11 Act13 and 6 of 7 expressed enzymes measured, followed by Act7, Act8, Act9, Act10, Act16, Act17 expressed 5, Act1, Act2, Act3, Act6, Act14 Act15 and that expressed 4, followed by Act4 and Act5 that expressed 3, and strain Act12 which featured only 2 enzymes, and low enzyme expression of the latter can be attributed to the slow growth when compared to the other actinobacteria (Figure 6A and Table 2).

Considering the moment of the expression of the enzymes, performed greater intensities of enzymatic activities expressed in Regular time (NE), followed by Late expressions (LE) and Early (EE), in lower intensity (Figure 6B).

The enzymes that showed higher expression were amylases, lipases and gelatinases, regardless of the time of cultivation and actinobactéria producer (Figure 6C).

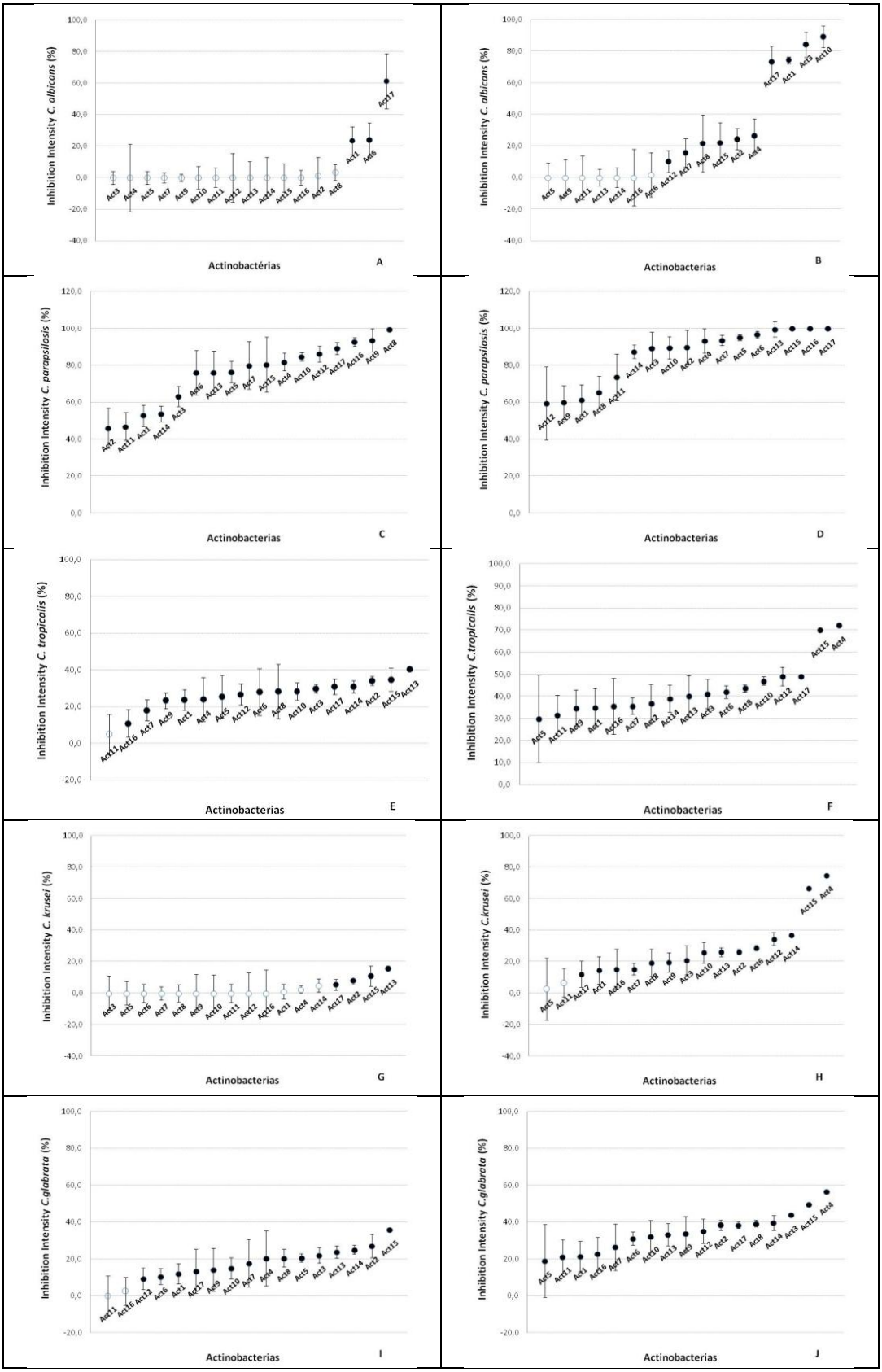


Figure 5 - Intensity inhibition (%) of *Candida* spp., provided by entophytic actinobacteria ME native plants of the Cerrado obtained. Frames: A, C, E, and I – cultivation in Czapek-Dox broth and B, D, F, H and J – cultivation in SCN broth. (●) positive Inhibition (○) without inhibition. Bars = Average standard deviation.

The hydrolysis capacity of the isolates Act11 (*Streptomyces*), Act13 (*Nocardioides*) and Act5 (*Nocardia*), deserves to be highlighted because they present higher mean EI, (Figure 6A), although the latter showed only 3 enzymes (Table 2).

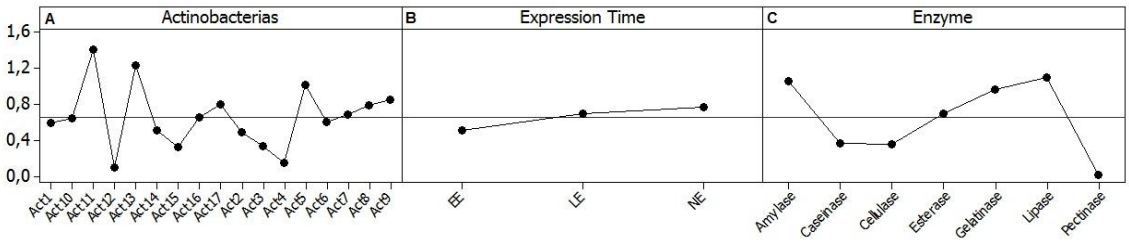


Figure 6 – Average of the dependent effects involved in the assay of enzyme activity: (A) Average of enzymatic expressions promoted by actinobacteria regardless of the time of expression and the type of enzyme expressed (n = 189), (B) Regardless of the actinobactéria and the type of enzyme expressed, (C) Regardless of actinobactéria and time of expression.

Table 2 - Presence of enzyme activities observed to entophytic actinobacteria, in function of growing time. Early expression (EE), Regular expression (NE) and Late expression (LE). (+) Activity observed and (-) absence of activity.

Strain Code	Genus Morphotyped	Enzymes-expression time																				
		Amylase			Caseinase			Cellulase			Esterase			Gelatinase			Lipase			Pectinase		
		NE	LE	LE	EE	NE	LE	EE	NE	LE	EE	NE	LE	EE	EE	NE	EE	NE	LE	NE	LE	LE
		4	11	18	4	11	18	4	11	18	4	11	18	4	11	18	4	11	18	4	11	18
Act1	*Actinopolyspora*	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-
Act2	*Actinopolyspora*	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	-	-	-
Act3	*Micromonospora*	+	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	+	-	-	-	-
Act4	*Terrabacter*	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
Act5	*Nocardia*	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-
Act6	*Saccharopolyspora*	+	+	+	-	-	-	-	-	-	-	+	+	-	+	+	-	+	+	-	-	-
Act7	*Streptosporangium*	-	+	+	-	-	-	+	+	-	-	+	+	+	+	+	-	+	+	-	-	-
Act8	*Thermoactinomyces*	+	+	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	-	-	-
Act9	*Streptoalloteichus*	+	+	+	-	-	-	+	+	-	-	+	+	+	+	+	+	+	+	-	-	-
Act10	*Streptoverticillium*	-	+	+	-	-	-	-	+	+	-	+	+	-	+	+	-	+	+	-	-	-
Act11	*Streptomyces*	+	+	+	+	+	+	+	-		+	+	+	+	+	-	+	+	+	-	-	-
Act12	*Terrabacter*	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-
Act13	*Nocardioides*	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	-	-	-
Act14	*Saccharopolyspora*	+	-	-	-	+	+	-	-	-	-	-	-	+	+	+	-	-	-	+	+	+
Act15	*Terrabacter*	+	-	-	-	-	-	+	-	-	-	-	-	+	+	+	-	-	+	-	-	-
Act16	*Norcadiopsis*	+	-	-	-	-	-	+	+	+	-	+	+	-	-	+	-	-	+	-	-	-
Act17	*Streptoalloteichus*	+	+	+	-	-	-	+	+	-	-	+	+	+	+	+	+	+	+	-	-	-
Regular expression time advocated in the literature		4 days			11 days			11 days			11 days			18 days			11 days			4 days		

Whereas the enzymes amylase, lipase, and gelatinase which were expressed in greater quantity, as well as the isolated Act5, Act11 and Act13 we can affirm that the expression of amylase and lipase was common to all regardless of the time of cultivation, differing itself from the expression

of gelatinase, where Act5 was unable to synthesize it. Among these isolates the Act11 (*Streptomyces* spp.) showed the highest expression of gelatinases (EI = 3.9) (Data not presented). Other studies found that *Streptomyces* spp. isolated from soil of the savanna was able to hydrolyze casein, gelatin and being a common feature of this genre, corroborating with our findings.[55] Bacterial Proteases have special relevance as commercial enzymatic in detergents, being your basic property used for generation of protein hydrolysate's of high nutritional value.[56] The genus *Streptomyces* stands out as the synthesis of these enzymes, since they release in extracellular medium, which itis generally regarded as safe, when applied to the administration of drugs and nutrients.[57]

Among the three selected isolates, all starch was hydrolyzed up to 18 days of cultivation. The isolated Act11 along with the Act16 were those who had higher activity of this enzyme (CI = 2.90 ± 0,156, $p \leq 0.05$). However, it should be emphasized that the Act16 expresses this enzyme until the fourth day as well on the isolates 14, 15 and 3.

The Act6, Act7 and Act10 from the 11th day of cultivation. The Act12 was the only one who did not express this enzyme, which explains the slow growth of this microorganism in the SCN, broth. The occurrence of amylases in actinobacteria is a feature commonly found in *Nocardia* spp. and *Streptomyces* spp.,[58] showing for this first genre high activity amilolítica that according to reports 100% of the isolates hydrolyzed starch after cultivation of 7 days.[41] As regards the lipolytic activity, the isolatesAct5 *Nocardia* spp. (EI = 3.51), Act11 *Streptomyces* spp. (EI = 2.1), and Act13*Nocardioides* spp. (EI = 2.4) were the most intensely expresses lipase, producing them from 4th to 18th day. According to the literature, little information is available on the lipase (carboxylic acid ester hydrolase) in actinobacteria. Most of the data refers to cholesterol esterase, which can also reveal lipolytic activity.[59] Studies have shown that microbial enzymes have excelled in use in clinical diagnosis and therapeutic such as cholesterol esterase obtained from species of *Nocardia* sp. which have been widely used in enzyme assays.[60] Considering the isolated qualified as promising substances inhibitory to producers *Candida* spp., therefore the isolatesAct17, Act15, Act10, Act4 and Act3 some considerations must made. None of these produced caseinase unlike the actinobacteria 5, 11 and 13, which were the only ones to produce this enzyme. It should be emphasized the presence of casein in the SCN medium, and this variable is associated to the metabolism of these bacteria in the most efficient broth observed in the antibiosis assays for the control of *Candida* spp..

Associations between enzyme activity and microbial antagonism referred to in the literature showing a synergistic relationship between them, in which the fungal cell wall digestion by the secreted enzyme, including chitinase, glucanase and peroxidase. Individually, all these enzymes exert antifungal activity, but their actions are frequently synergistic with antibiotics.[61] Our studies have not been evaluated these enzymes, but the composition of the substrate of culture medium can assist in expression of enzymes associated with antibiotic or antifungal substances biosynthesis.

CONCLUSION

The study of entophytic microbiota of native species of the Savannah are scarce, so a habitat unexplored, and so very promising to bio-prospecting of natural products.

The taxonomic identification of entophytic actinobacteria in this strate was able to detect the presence of 12 diverse genres with predominance of the genus *Terrabacter*.

The Starch-Nitrate-casein (SCN) broth proved to be a potent substrate to obtaining secondary metabolites produced by actinobacteria. The isolates that inhibit the greater number of yeasts can be targets for future studies aimed at the characterization of the compounds with antifungal activity and improvemment of cultivation technology for including culture medium composition.

The metabolic extracts produced by entophytic actinobacteria from this biome point to the presence of promising substances with potential antifungal. In the study, it was observed that 88% of the metabolic extracts showed inhibitory effect hisher 70% to some species of *Candida* spp.. The actinobacteria 17, 15, 10, 4 and 3 that produced the most suppressive metabolites of Candida spp.

Considering the enzymatic activities, the expressions of amylases, lipases and gelatinases were the most recurrent and most intensely produced by actinobacteria 5, 11 and 13.

The promising anti-fungal metabolism-producing actinobacteria 17, 15, 10, 4 and 3 did not express caseinase biosynthesis, unlike the actinobacteria 5, 11 and 13 that were the only ones to produce this enzyme, which could be exploited in the induction process in culture for the production of antibiotic substances in SCN medium.

REFERENCES

1. Colombo AL, Guimarães T. Epidemiologia das infecções hematogênicas por *Candida* spp. Rev Soc Bras Med Trop. 2003;6:599-607.
2. Wanke B, Lazéra MS, Nucci M. Fungal infections in the immunocompromised host. Mem. Inst. Oswaldo Cruz. 2000; 95(Suppl.1):153-158.
3. Harvey RA, Champe PC, Fisher BD. Microbiologia ilustrada. 2ª ed. Porto Alegre: Artmed; 2008.
4. Batista JM, Birman EG, Cury AE. Suscetibilidade a antifúngicos de cepas de *Candida albicans* isoladas de pacientes com estomatite protética. Rev. Odontol. Univ. São Paulo. 1999; 13:343-348.
5. Strobel GA. Rainforest endophytes and bioactive products. Critical Reviews in Biotechnology. 2002; 22:315-333.
6. Strobel GA, Daisy B, Castillo U, Harper J. Natural products from entophytic microorganisms. Journal of Natural Products. 2003; 67:257-268.
7. Wiyakrutta S, Sriubolmas N, Panphut W, et al. Entophytic fungi with anti-microbial, anti-cancer and anti-malarial activities isolated from Thai medicinal plants. World Journal of Microbiology & Biotechnology. 2004, 20(3):265-272.
8. Harvey A. Strategies for discovering drugs from previously unexplored natural products. DDT, Brasília. 2000; 5:294-300.
9. Anderson AS, Wellington EMH. Ther taxonomy of *Streptomyces* and related genera. International Journal of Systematic and Evolutionary Microbiology. 2001; 51:797-814.

10. Bérdy JJ. Bioactive microbial metabolites. Antibiot. 2005, 58:1–26.
11. Peczynska-Czoch W, Mordarski M. Actinomycete's enzymes. In: Actinomycetes in Biotechnology, M. Goodfellow, S. T. Williams, and M. Mordarski (Eds) Academic. 1988:219-284.
12. Nielsen RI, Oxenb K. Enzymes from fungi: their technology and uses. Myxologist. 1998; 12: 69-71.
13. Ratter JA, Ribeiro JF, Bridgewater S. The Brazilian Cerrado vegetation and threats to its biodiversity. Annals of Botany, London. 1997; 80:223-230.
14. Roesler R. Estudo de frutas do Cerrado brasileiro para avaliação de propriedade funcional com foco na atividade antioxidante. Tese de doutorado, Campinas (UNICAMP), Programa de Pós-Graduação em Ciência de Alimentos, 2009.
15. Anvisa. Agência Nacional de Vigilância Sanitária. Procedimentos Laboratoriais: da Requisição do Exame à Análise. Disponível em: Microbiológicawww.anvisa.gov.br/servicosaude/microbiologia/mod_3_2004.pdf. Acesso: 13/12/16.
16. Holt JG, Williams ST, Sharpe ME. Bergey's Manual of Systematic Bacteriology Baltimore. Williams's e Wilkins. 1989; 4:2300-2648.
17. Williams ST, Goodfellow M, Alderson G. Genus Streptomyces Waksman and Henrici 1943, 339AL. In: Williams ST, Sharpe ME, Holt JG (Eds.) Bergey's Manual of Systematic Bacteriology, Vol. 4, Williams and Wilkins, Baltimore. 1989:2452-2492.
18. CLSI. Manual Clinical and Laboratory Standards Institute. Reference methods for broth dilution antifungal susceptibility tests for yeasts: approved standards, CLSI document M27-A3, Wayne, PA., 2008.
19. Duarte MCT, Figueira GM, Sartoratto A, Rehder VLG, Delarmelina C. Anti-Candida activity of Brazilian medicinal plants. Journal of Ethnopharmacology. 2005; 97:305-311.
20. Gudiña EJ, Rocha V, Teixeira JA, Rodrigues LR. Antimicrobial and antiadhesive properties of a biosurfactant isolated from *Lactobacillus paracasei* ssp. Paracasei A20. Letters in Applied Microbiology. 2010; 50:419-424.
21. Nogueira EBS, Cavalcanti MAQ. Cellulolytic fungi isolated from processe doats. Revista de Microbiologia, São Paulo. 1996; 27:7-9.
22. Barbosa EC, et al. Isolamento, identificação e avaliação das atividades enzimática e antibacteriana de micro-organismos endofíticos de Hyptis suaveolens (l.) Poit. Enciclopédia Biosfera, Centro Científico Conhecer – Goiânia. 2015; 11:36-55.
23. Haba E, et al. Isolation of lipase-secreting bacteria by deploying used frying oil as selective substrate. Enzyme and Microbial Technology. 2000; 26:40–44.
24. Minotto E, et al. Enzyme Characterization of Entophytic Actinobacteria Isolated From Tomato Plants. Journal of advanced scientific research. 2014, 5(2):16–23.
25. McDade JJ, Weaver RH. Rapid Methods for the Detection of Gelatin Hydrolysis. Journal of Bacteriology. 1959; 77(1):60–64.
26. Sierra G. A simple method for the detection flipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek. 1957; 23:15–22.
27. Hankin L, Anagnostakis SL. The Use of Solid Media for Detection of Enzyme Production by Fungi. Mycology. 1975; 67:597–607.
28. Connell ND. Expression systems for use in actinomycetes and related organisms. Current opinion in biotechnology Cleveland, 2001; 12:446-449.
29. Ensign JC. Formation, properties and germination of actinomycete spores. Ann Rev.Microbial. 1978; 32:185-219.

30. Basilio A, González I, Vicente MF, et al. Patterns of antimicrobial activities from soil actinomycetes isolated under different conditions of pH and salinity. Journal of Applied Microbiology. 2003; 95:814–823.
31. Pelczar M, Reid R, Chan EC. S. Microbiologia. São Paulo: McGraw-Hill Ltda., 1981.
32. Chang MR, Correia FP, and Costa LC, et al. Candida bloodstream infection: data from a teaching hospital in Mato Grosso do Sul, Brazil. Rev Inst. Med Trop. São Paulo. 2008, 50(5):265-268.
33. Alves PM, et al. Atividade antifúngica do extrato de Psidium guajava Linn (goiabeira) sobre leveduras do gênero Candida da cavidade oral: uma avaliação in vitro. Rev. Bras. Farmacogn., João Pessoa. 2006; 16:192-196
34. Souza SO, Campos TR. Candida albicans: Fatores de Virulência e de Resistência e o uso de derivados vegetais como alternativa de tratamento da candidíase oral. Dissertação de Trabalho de Conclusão de Curso. Universidade Estadual de Goiás, Anápolis, 2011.
35. Kelly S, Lamb D, Kelly D, Manning N, Loeffler J, Hebart H, et al. Resistance to fluconazole and cross-resistance to amphotericin B in *Candida albicans* from AIDS patients caused by defective sterol Delta 5,6- desaturation. FEBS Lett. 1997, 400(1):80-2.
36. Yang YL, Cheng MF, Chang YW, Young TG, Chi H, Lee SC, et al. Host factors do not influence the colonization or infection by fluconazole resistant Candida species in hospitalized patients. J Negat Results Biomed. 2008; 7:12.
37. Lam KS, Hesler GA, Gustavson DR, et al. Kedarcidin, a new chromoprotein antitumor antibiotic. I. Taxonomy of producing organism, fermentation and biological activity. J Antiobiot. 1991; 44:472–478.
38. Hofstead SJ, Matson JA, Malacko AR, Marquardt H. Kedarcidin, a new chromoprotein antitumor antibiotic. II. Isolation, purification and physicochemical properties. J Antiobiot. 1992; 45:1250–1254.
39. Madiso LL, Huisman GW. Metabolic engineering of poly (3 hydroxyalkanoates): from DNA to plastic. Microbial. Mol. Biol. Rev. 1999; 63(1):21–53.
40. Zhao K, Tian G, Zheng Z, Chen JC, Chen GQ. Production of D-(–)-3-hydroxyalkanoic acid by recombinant *Escherichia coli*.FEMS Microbial. Lett. 2003, 218(1): 59–64.
41. Rodrigues K. Identificação, produção de antimicrobianos e complexos enzimáticos de isolados de actinomicetos. Porto Alegre, 129p. Dissertação de Mestrado em Microbiologia Agrícola e do Ambiente - Faculdade de Agronomia. Universidade Federal do Rio Grande do Sul, 2006.
42. Bull AT. "Actinobacteria of the extremobiosphere," in Extremophiles Handbook, edK. Horikoshi (Springer). 2011:1203–1240.
43. Paradkar VR, Gupte TE, Joshi AP, Naik SR. A novel *Streptoverticillium cinnamoneum* vary scleroticum producing a polyene antibiotic. Dordrecht. 1998, 14(5):705-709.
44. Lima CSA, Silva RF, Abreu SMB, Sena KXFR, Nascimento SC, Amorim ELC. Atividade antimicrobiana e antitumoral de *Streptoverticillium* sp. (DAUFPE 13. 729). Rev. Lect. 2002;20 (2):161-165.
45. Igarashi Y, Ogura H, Furihata K, Ok N, Indananda C, Thamchaipenet A. Maklamicin, and antibacterial polyketide from and entophytic *Micromonospora* sp. J.Nat. Prod.2011a;74:670–674.
46. Furumai T, Igarashi Y, Higuchi H, Saito N, Oki T. Kosinostatin, a quinocycline antibiotic with antitumor activity from *Micromonospora* sp. EP-A0468. J Antibiot. 2002; 55:128–133.
47. Talukdar M, Das D, Bora C, Bora TC, Deka Boruah HPD, Singh AK. Complete genome sequencing and comparative analyses of broad-spectrum antimicrobial-producing *Micromonospora* sp. HK10. Gene. 2016; 594:97–107.
48. Sanchez S, Demain AL. Metabolic regulation of fermentation processes. Enzyme Microb. Technol. 2002; 31:895-906.

49. Mukhopadhyay T, Garrison NK, Hinton DM, et al. Identification and characterization or bacterial endophytes or rice. Micopathology. 1996; 134:151-159.
50. Takur D, Bora TC, Bordoloi GN, Mazumda S. Influence of nutrition and culturing conditions for optimum growth and antimicrobial metabolite production by *Streptomyces*. J Med Myc. 2009, 19(3):161-167.
51. Huck TA, Porter N, Bushell ME. Positive selection of antibiotic-producing soil isolates. Jornal of General Microbiology. 1991; 137:2321-2329.
52. Quindós G, Villar-Vidal M, Eraso E. Actividad de lamicafungina contra las biopelículas de *Cándida*. Rear. Iberoam Micol. 2009; 26:49-55.
53. Lin JE, Chang DCN, Shen GJ. Correlations among several screening methods used for identificatifying wood-decay fungi that can degrade toxic chemicals. Biotech. 1971; 5:275-280.
54. Fungaro MHP, Maccheroni Jr W. Melhoramento genético para produção de enzimas aplicadas a Indústria de Alimentos. In: Melo, I. S.; Valadares-Inglis M.C.; Nass L. L. and Valois A.C.C., ed. Recursos Genéticos e Melhoramento-Microrganismo. Jaguariúna, São Paulo, Brasil: Embrapa Meio Ambiente. 2002:426-453.
55. Azeredo LAI. Et al. Protease from *Streptomyces* sp. isolated from Brazilian Cerrado soil. Optimization of culture medium employing statistical experimental design. Applied Biochemistry and Biotechnology, Clifton. 2003; 105:749-755.
56. Gupta R, Beg QK, Lorenz P. Bacterial alkaline proteases: molecular approaches and industrial applications. Applied Microbiology and Biotechnology. 2002, 59(1):15-32.
57. Ghorbel S, Kammoun M, Soltana H, Nasri M, Hmidet N. *Streptomyces flavogriseus* HS1: Isolation and Characterization of Extracellular Proteases and their compatibility with laundry detergents. BioMed Research International. 2014:1-8.
58. Vigal T, et al. Cloning characterization and expression of an alpha amylase gene from *Streptomyces griseus* IMRU 3570. Molecular General Genetic, Berlin. 1991; 225:278-288.
59. Toshio K, Suzuki H, Asano K, Matsuzaki M, Nakamura S. Cholesterol esterase produced by *Strptomyces lavendulae*. II. Purification and properties as a lipolitic enzyme. Chemical and Pharmaceutical Bulletin. 1979; 27:1704-1707.
60. Wong CM, Wong KH, Chen XD. Glucose oxidase: natural occurrence, function, properties and industrial applications. Applied Microbiology and Biotechnology. 2008; 78:927-938.
61. Lorito M, Peterbauer C, Hayes CK, Harmam GE. Synergistic interaction between fungal cell wall degrading enzymes and different antifungal compounds enhances inhibition of spore germination. Microbiology. 1994; 140:623-629.

4 CONCLUSÕES

Estudos da microbiota endofítica de espécies nativas do Cerrado são escassos, assim um habitat pouco explorado, contudo bastante promissor quando se refere a bioprospecção de produtos naturais.

A identificação taxonômica das actinobactérias endofíticas aqui estudada, ainda que em nível de gênero, esclareceu a presença de 12 gêneros com predomínio do gênero *Terrabacter*.

O caldo de cultivo Starch-Nitrate-caseín (SCN) mostrou ser um potente substrato estimulador no processo de obtenção de metabólicos secundário produzidos por actinobactérias. Os isolados que inibiram o maior número de leveduras podem ser alvos de futuros estudos visando à caracterização dos compostos com atividades antifúngicas.

Os extratos metabólicos produzidos por actinobactérias endofíticas deste bioma apontam para a presença de substâncias promissoras com um grande potencial antifúngico. Neste estudo foi observado que 88% dos extratos metabólicos apresentaram efeito inibitório superior a 70% frente a espécies de *Candidas* spp.. Destaque para as actinobactérias 17, 15, 10, 4 e 3 que foram as que produziram metabólitos mais supressoras de *Candida* spp.

Considerando as atividades enzimáticas, as expressões de amilases, lipases e gelatinases foram as mais recorrentes e mais intensamente produzidas pelas actinobactérias 5, 11 e 13, independente do tempo de cultivo.

As actinobactérias 17, 15, 10, 4 e 3, promissoras produtoras de metabólicos antifúngicos não expressaram biossíntese de caseinase, ao contrário das actinobactérias 5, 11 e 13 que foram as únicas a produzirem esta enzima, podendo esta característica ser explorada em processo de indução em cultivo visando produção de substâncias antibióticas em meio SCN.

REFERÊNCIAS

1. Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J. Biodiversity hotspots for conservation priorities. Nature. London. 2000;403:853-858.

2. Bertoncello R. et al. A phytogeographic analysis of cloud forests and other forest subtypes amidst the Atlantic forests in south and southeast Brazil. Biodiversity and Conservation. 2011;20(14):3413-3433.

3. Ratter JA, Ribeiro JF, Bridgewate S. The Brazilian Cerrado vegetation and threats to its biodiversity. Annals of Botany, London. 1997;80:223-230.

4. Brasil. Ministério do Meio Ambiente. Quarto relatório nacional para a convenção sobre diversidade biológica. Brasília. 248 p. 2011.

5. Borlaug NE. Feeding a world of 10 billion people: themiracle ahead. In: R. Bailey (ed.). Global warming and othereco-myths. Competitive Enterprise Institute, Roseville, EUA. 2002:29-60.

6. Brasil. Ministério de Minas e Energia. Balanço energético nacional, Brasília, 59p. 2010.

7. Instituto Estadual de Florestas de Minas Gerais-IEF. [acesso em 2017 jan 20] Disponível em: http://www.ief.mg.gov.br/florestas.

8. Mapa, clima, vegetação e forma de relevo de Minas Gerais. [acesso em 2017 jan 20] Disponível em: http://www.estadaomg.com.br/2015/08/climavegetacao-e-relevo-de-minas-gerais.html

9. Ribeiro JF, Walter BMT. Fitofisionomias do bioma Cerrado. In: Sano SM, Almeida SP. (Ed.) Cerrado: ambiente e flora. Planaltina, DF: EMBRAPA-CPAC, 1998;3:87-166.

10. Cobertura vegetal. [acesso em 2017 jan 23]. Disponível em: http://www.wwf.org.br/natureza_brasileira/areas_prioritarias/cerrado/bioma/cobertura_vegetal/

11. Klink AC, Machado RB. Conservation of the Brazilian Cerrado. Conservation Biology, Boston, 2005;19:707-713.

12. Roesler, R. Estudo de frutas do Cerrado brasileiro para avaliação de propriedade funcional com foco na atividade antioxidante [tese]. Campinas: UNICAMP, 2009. Programa de Pós-Graduação em Ciência de Alimentos.

13. Neto PASP, Azevedo JL, Araujo WL. Microrganismos endofíticos. Biotecnol Cienc Desenvol. 2002;29:62-76.

14. Azevedo JL. Microrganismos endofíticos. In: MELO IS, Azevedo JL. (Ed.) Ecologia microbiana. Jaguariúna: EMBRAPA, 1998. p. 117-137.

15. Hardoim PR, Van Overbeek LS, Van Elsas JD. Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol. 2008;16:463–471.

16. Qin S, Xing K, Jiang JH, Xu LH, Li WJ. Biodiversity, bioactive natural products and biotechnological potential of plant associated endophytic actinobacteria. Appl Microbiol Biotechnol. 2011;89:457-473.

17. Azevedo JL, Araujo WL. Diversity and applications of endophytic fungi isolated from tropical plants. In: Ganguli BN. Desmuckh SK. Fungi: Multifacetated Microbes. New Dehli: Anamaya Publication. 2006:189-207.

18. Lin X, Lu C, Huang Y, Zheng Z, Su W, Shen Y. Endophytic fungi from a pharmaceutical plant, Camptotheca acuminata: isolation, identification and bioactivity. World Journal of Microbiology and Biotechnology, Dordrecht, 2007;23:1037-1040.

19. Guo B, Wang Y, Sun X, Tang K. Bioactive natural products from endophytes: a review. Applied Biochemistry and Microbiology, Moscow. 2008;44:136-142.

20. Gangadevi V, Muthumary J. Taxol, an anticancer drug produced by an endophytic fungus *Bartalinia robillardoides* Tassi, isolated from a medicinal plant, Aegle marmelos Correa ex Roxb. World Journal of Microbiology and Biotechnology, Dordrecht. 2008;24:717-724.

21. Wang J, Li G, Lu H, Zheng Z, Huang Y, Su W. Taxol from *Tubercularia* sp. strain TF5, na endophytic fungus of *Taxus mairei*. FEMS Microbiology Letters, Oxford. 2000;193:249-253.

22. Lee JC, Lobkovsky E, Pliam NB, Strobel GA, Clardy J. Subglutinols A and B: immunosuppressive compounds from the endophytic fungus *Fusarium subglutinans*. The Journal of Organic Chemistry, Washington. 1995;60:7076-7077.

23. Sakiyama CCH, Paula EM, Pereira PC, Borges AC, Silva DO. Characterization of pectin lyase produced by an endophytic strain isolated from coffee cherries. Letters in Applied Microbiology, Oxford. 2001;33:117-121.

24. Harper JK, Arif AM, Ford EJ. Pestacin: a 1,3-dihydro isobenzofuran from Pestalotiopsis microspora possessing antioxidant and antimycotic activities, Tetrahedron, Oxford. 2003;59:2471-2476.

25. Kour A, Shawl AS, Rehman S, et al. Isolation and identification of na endophytic strain of *Fusarium oxysporum* producing podophyllotoxin from *Juniperus recurva*. World Journal of Microbiology and Biotechnology, Dordrecht. 2008;24:1115-1121.

26. Goodfellow M. The Actinomycetes I. Supragenic Classification of actinomycetes. In: Bergey's Manual of Systematic Bacteriology. (Williams, S. T.; Sharpe, M. E. and Holt, J. G. Eds.). 1989;4:2333-2339.

27. Garrity GM, Bell JÁ, Lilburn TG. Taxonomic Outline of the Prokaryotes Bergey's Manual of Systematic Bacteriology.2 ed. New York: Springer-Verlag, 2003.

28. Anderson AS, Wellington EM. Hither taxonomy of Streptomyces and related genera. Internacional Journal of Systematic and Evolutionary Microbiology. 2001;51:797-814.

29. Groth I, Vettermann R, Schuetze B, Schumann P, Saiz-Jimenez C. Actinomycetes in Karstic caves of northern Spain (Altamira and Tito Bustillo). J Microbiol Meth. 1999;36:155-122.

30. Wohlleben W, Mast Y, Muth G, Rottgen M, Stegmann E, Weber T. Synthetic biology of secondary metabolite biosynthesis in actinomycetes: engineering precursor supply as a way to optimize antibiotic production. FEBS Lett. 2012;586:2171-2176. 34-31

31. Okami Y, Hotta K. Search and discovery of new antibiotics. In Goodfellow M, Williams ST, Mordarski M (eds.), Actinomycetes in Biotechnology, Academic Press, Londres. 1988:33-68.

32. Pacheco Da Rosa J,Korenblum E, et al. Streptomyces lunalinharesii strain 235 shows the potential to inhibit bacteria involved in biocorrosion processes. BioMed Res. Internat. 2013.

33. Vining LC. Functions of secondary metabolites. Annu Rev Microbiol. 1990;44:395-427.

34. Demain AL. Induction of microbial secondary metabolism. Internatl Microbiol. 1998;1:259-264.

35. Bérdy J. Bioactive microbial metabolites. J Antibiot. 2005;58:1-26.

36. Bérdy J. Thoughts and facts about antibiotics: Where we are now and where we are heading. J Antibiot. 2012;65:385-395.

37. Bibb MJ. Regulation of secondary metabolism in streptomycetes. Curr Opin Microbiol. 2005;8:208-215.

38. Harvey A. Strategies for discovering drugs from previously um explored natural products. DDT, Brasília. 2000;5:294-300.

39. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Ver. 2010;74:417-433.

40. Waksman SA, Woodruff HB. The soil as a source of microorganisms antagonistic to disease-producing bacteria. J. Bacteriol. 1940;40,581-600

41. Chater KF. Streptomyces inside-out: a new perspective on the bactéria that provide us with antibiotics. Philosophical Translactions of th Royal Society B. 2006;361:761-798.

42. Baltz RH. Antimicrobials from actinomycetes: back to the future. Microbe. 2007.

43. Procópio REL, Silva IR, Martins MK, Azevedo JL, Araújo JM. Antibiotics produced by Streptomyces. The Brazil. J. Infect. Dis. 2012;16(5):466-471.

44. Woo PCY, Lau SKP, Huang Y, Yuen KY. Genomic evidence for antibiotic resistance genes of actinomycetes as origins of antibiotic resistance genes in pathogenic bacteria simply because actinomycetes are more ancestral than pathogenic bacteria. Med Hypotheses. 2006;67:1297-1304.

45. Batista JM, Birman EG, Cury AE. Suscetibilidade a antifúngicos de cepas de *Candida albicans* isoladas de pacientes com estomatite protética. Rev Odontol Univ São Paulo. 1999;13:343-348.

46. Barbedo LS, Sgarbi DBG. Candidíase. Jornal Brasileiro de Doenças Sexualmente transmissiveis, Rio de Janeiro, 2010.

47. Álvares CA, Svidzinski TIE, Consolaro EL. Candidíase vulvovaginal: fatores predisponentes do hospedeiro e virulência das leveduras. Jornal Brasileiro de Patologia e Medicina Laboratorial, Rio de Janeiro. 2007;43(5):319- 27.

48. Colombo AL, Guimarães T. Epidemiologia das infecções hematogênicas por Candida spp. Rev Soc Bras Med Trop, 2003.

49. Bassetti M, Taramasso L, Nicco E, Molinari MP, Mussap M, Viscoli C. Epidemiology, species distribution, antifungal susceptibility and outcome of nosocomial candidemia in a tertiary care hospital in Italy. Plos One. 2011;6:1-6.

50. Viriato A. Terpenoides com atividade antifúngica para *Candida Berkhout*, causadoras de infecções hospitalares. O mundo da saúde. 2014;38(1):40-50.

51. Pemán J, Zaragoza R, Quindós G, et al. Clinical factors associated with a *Candida albicans* Germ Tube Antibody positive test in Intensive Care Unit patients. BMC Infectious Diseases, Valencia. 2011;11(60):1-7.

52. Arasu MV, Duraipandiyan V, Agastian P, Ignacimuthu S. In vitro antimicrobial activity of *Streptomyces* spp. ERI-3 isolated from Western Ghats rock soil (India). Journal de Mycologie Médicale. 2009;19:22-28.

53. Peczynska-Czoch W, Mordarski M. Actinomycetes enzymes, In: Actinomycetes in Biotechnology. M. Goodfellow ST. Williams M. Mordarski (eds) Academic. 1988:219-284.

54. Wong C, Whitesides M. Tetrahedron organic chemistry series. 1a ed. Pergamon, Enzymes in synthetic organic chemistry. 1994;12.

55. Nielsen RI, Oxenb K. Enzymes from fungi: their technology and uses. Myxologist. 1998;12:69-71.

56. Padilha G. Biologia Molecular de Streptomycese aplicações industriais. Em Melo, I. S. e Azevedo, J. L. Ecologia Microbiana. Embrapa, Jaguariúna. 1998:327-347.

57. Moraes LMP. Amilases. In: SAID S, PIETRO R. Enzimas como agentes Biotecnológicos. Ribeirão Preto: Legis Summa, 2004.

58. Dastager SG, Dayanand A, Li W, et al. Proteolytic activity from a alkali-thermotolerant *Streptomyces gulbargensis* sp. nov. Current Microbiology. 2008;57:638-642 SAIR.

59. Poonam N, Dalel S. Enzyme and microbial systems involved in starch processing. Enzyme Microbial Technology, New York. 1995;17:770-778.

60. Vigal T, et al. Cloning characterization and expression of an alpha amylase gene from *Streptomyces griseus* IMRU 3570. Molecular General Genetic, Berlin. 1991;225:278-288.

61. Van Der Maarel M, et al. Properties and applications of starch converting enzymes of the α-amylase family. Journal Biotechnology, Oxford. 2002;94:137-15.

62. Bayer EA, Lamed R. The cellulose paradox: pollutant par excellence and/or a reclaimable natural resource? Biodegradatio. 1992;3:171-188.

63. Miettinen-oinonen A. Cellulases in the textile industry. Industrial Enzymes. Springer. 2007:51-63.

64. Crawford DL, Mccoy E. Cellulases of *Thermomonospora fusca* and *Streptomyces thermodiastaticus*. Applied Microbiology, Washington. 1972:150-152.

65. Gallagher J, et al. Production of cellulase and β-glucosidase activity durying growth of the actinomycete *Micromonospora chalcae* on cellulose-containig media. Biotechnology Letters, Dordrecht. 1996;18:537-540.

66. Ishaque M, Kluepfel D. Cellulase complex of a mesophilic Streptomyces strain. Canadian Journal of Microbiology, Ottawa. 1980;26:183-189.

67. Tuncer M, et al. Optimization of extracellular endoxylanase, endoglucanase and peroxidase production by Streptomyces sp. F2621 isolated in Turkey. Journal of Applied Microbiology, Oxford. 2004;97:783-791.

68. Yazdil MT, et al. Cellulase production by *Neurospora crassa*: Purification and characterization of cellulolytic enzymes. Enzyme and Microbial Technology, New York. 2000;12:120-123.

69. Bornscheuer UT. Microbial carboxyl esterases: classification, properties and application in biocatalysis. FEMS Microbiology Reviews, Amsterdam. 2002;26:73-81.

70. Jaeger KE, Reetz MT. Microbial lipases form versatile tools for biotechnology. Trends Biotechnol. 1998;16:396-403.

71. Kim GJ, et al. Purification and characterization of an erythromycin resistent Pseudomonas sp. GD 100. FEMS Microbiology Letters, Amsterdam. 2002;210:239-244.

72. Veeraragavan K. A simple and sensitive method for the estimation of microbial lipase activity. Analytical Biochemistry, New York. 1990;186:301-305.

73. Thomson CA, Delaquis PJ, MAZZA G. Detection and measurement of microbial lipase activity: a review. Critical Reviews in Food Science and Nutrition, Cleveland. 1999;39:165-187.

74. Liese A, Seelbach K, Wandrey C. (Ed.). Industrial biotransformation. Weinheim: Wiley-VCH, 2000.

75. Sztajer H, Maliszewska I, Wieczorek J. Production of exogenous lipase by bacteria, fungi and actinomycetes. Enzyme and Microbiol Technology, New York. 1988;10:492-497.

76. Whitaker JR. Microbial pectolytic enzymes. In: Microbial Enzymes and Biotechnology (eds. Fogarty WM, Kelly CT) Appl. Science, London e New York. 1991:133-175.

77. Osborne JM, Dehority BA. Synergism in degradation and utilization of intact forage cellulose, hemicellulose and pectin by three pure cultures of ruminal bacteria. Applied and Environmental Microbiology. 1989;55: 2247-2250.

78. Voragen FGJ, Heutink R, Pilnik W. Solubilization of apple cell walls with polysaccharide degrading enzymes. Journal of Applied Biochemistry. 1980;2:452-468.

79. Spooner FR, Hammerschmidt RJr. Characterization of extracellular pectic enzymes produced by *Streptomyces*species. Phytopathology, Saint Paul. 1989;79:1190.

80. Stutzenberger FJ. Inducible thermoalkalophilic polygalacturonate lyse from *Thermomonospora fusca*. Journal of Bacteriology, Washington. 1987;169:2774-2780.

81. Romero FJ, García LA, Salas JA, Díaz M, Quirós LM. Production, purification and partial characterization of two extracellular proteases from *Serratia marcescens* grown in whey. Process Biochemistry. 2001;36:507-515.

82. Ladd JN, Butler JHA. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biology and Biochemistry. 1972;4:19-30.

83. Ross DJ, Speir TW, Giltrap DJ, Mcneilly BA, Molly LF. A principal component analysis of some biochemical activities in climosequence of soils. Soil Biology and Biochemistry. 1975;7:349-355.

84. Cowan D. Industria l enzyme technology. Trends in Biotechnology, Amsterdam. 1996;14:177-178.

85. Mozersky S, Marmer W, Dale AO. Vigorous proteolysis: Relining in the presence of an alkaline protease and bating (Post- Liming) with an extremophile protease. Jalca. 2002;97:150-155.

ANEXO

ANEXO A – Normas para publicação no periódico Brazilian Journal of Microbiology

ELSEVIER

BRAZILIAN JOURNAL OF MICROBIOLOGY

GUIDELINES TO AUTHORS

SCOPE OF THE JOURNAL

Brazilian Journal of Microbiology, published by the Brazilian Society of Microbiology, publishes original research papers and reviews, covering all aspects of Microbiology. The publication fee for this journal is **USD 300** for non-Brazilian citizens, and, **R$ 840,00** for Brazilian citizens.

The following categories of papers are acceptable for publication in Brazilian Journal of Microbiology:

- ***Research paper:*** the research paper reports results of original research, which has not been published elsewhere.
- ***Short Communication:*** a Short Communication is new and significant findings. Submit form is the same way as research paper. They receive the same review, they are not published more rapidly than research paper.
- ***Short-review:*** Review articles should deal with microbiological subjects of broad interest (ONLY BY INVITATION).
- ***Letter to the editor:*** Letters to the Editor are intended only for comments on final, typeset articles published in the journal (manuscripts posted online are not accepted) and must cite published references to support the writer's argument.

Your manuscript must be written clearly, in **comprehensible English.**

The text submitted for publication has to be English reviewed before **submission**. **To submit the manuscript, you must attach the issued certificate in supplementary files.**

SECTIONS

Biotechnology and Industrial Microbiology

Bacterial Fermentation

- biosynthesis and bioconversion of natural products, including antibiotics, xenobiotics, and macromolecules produced by bacteria.
- molecular aspects of bacterial biotechnology

Fungal Fermentation

- biosynthesis and bioconversion of natural products, including antibiotics, xenobiotics, and macromolecules produced by fungi
- molecular aspects of fungal biotechnology

Food Microbiology

Technology

- applications of microorganisms (bacteria and fungi) for food production

Safety and Quality

- food borne diseases
- food spoilage
- microbial ecology in foods

Clinical Microbiology

Bacteria, Fungi, and Virus

- Laboratory diagnosis of human infections and the role of the laboratory in both the management of infectious diseases and the elucidation of the epidemiology of infections.
- Microbial resistance and mechanisms of antimicrobial agents.

Environmental Microbiology

Microbial Ecology

- ecology of natural microbial assemblages, microbial diversity of natural environments such as water, soil, sediments and higher organisms
- microbial interactions
- environmental aspects of public health
- biodegradation
- bioremediation

Bacterial and Fungal Molecular Pathogenesis

- Genetic, biochemical, and structural basis of bacterial and fungal pathogenesis

Bacterial and Fungal Physiology

- Biochemistry, biophysics, metabolism, cell structure, stress response, growth, differentiation, and other related process of bacteria and fungi

Veterinary Microbiology

- control and/or treatment of animals
- animal pathogen diagnostics
- veterinary or zoonotic pathogens

SUBMISSION OF A MANUSCRIPT

Submission of a manuscript to Brazilian Journal of Microbiology is understood to imply that it has not

previously been published (except in an abstract form) and that it is not being considered for publication elsewhere.

Upon receipt of a manuscript all authors will receive an electronic message acknowledging the receipt.

Responsibility for the accuracy of the manuscript content lies entirely with the authors.

PUBLICATION OF A MANUSCRIPT

Manuscripts are accepted for publication after having been critically reviewed by at least two referees, indicated by the Editors.

The suggestions and recommendations of the reviewers and Editors will be forwarded electronically to the corresponding author, who should return the reviewed manuscript to the Editors within the stipulated date, via online system. Whenever applicable, the corresponding author should explain or comment each modification introduced in the text.

The corresponding author will receive an electronic message whenever the manuscript moves from one status to the next.

Membership in Brazilian Society for Microbiology is not a pre requisite for submission of a manuscript for publication.

Nonmember scientists from Brazil and other countries are invited to submit papers for analysis.

ETHICS

When the study, described in the manuscript, is related to experiments carried out with human beings and/or animals, author(s) must inform, within the text, if the research project has been approved by the Research Ethics Committee of their institution, according to the Declaration of Helsinki (http://www.fcm.unicamp.br/fcm/sites/default/files/declaracao_de_helsinque.pdf). Experimental studies involving animals should follow the guidelines established by the "Guide for the Care and Use of Laboratory Animals" (http://www.ncbi.nlm.nih.gov/books/NBK54050/) (Institute of Laboratory Animal Resources, National Academy of Sciences, Washington, D. C. 1996), and the *Princípios Éticos na Experimentação Animal do Colégio Brasileiro de Experimentação Animal* (COBEA) (Ethical Principles for Animal Experimentation of the Brazilian College of Animal Experimentation - http://www.cobea.org.br/conteudo/view?ID_CONTEUDO=65).

PREPARATION OF A MANUSCRIPT

The manuscript should be submitted as one single WORD file. This single file should include: the whole text, figures, tables, etc. Only manuscripts written in English will be considered.

For **research papers**, the WORD file should contain:

- Title (100 characters)
- Running title (40 characters)
- Authors and Affiliations
- Abstract (up to 200 words)
- Three to five key-words
- Introduction
- Materials and Methods
- Results
- Discussion
- Acknowledgements (optional)
- References

For **short communications**, the WORD file should contain:

- Title
- Running title
- Authors and Affiliations
- Abstract (up to 50 words)
- Three to five key-words
- Text not divided in topics
- Acknowledgements (optional)
- References

For **short-review**, the WORD file should contain:

- Title (100 characters)
- Running title (40 characters)
- Authors and Affiliations
- Abstract (up to 200 words)
- Three to five key-words
- Text
- Acknowledgements (optional)
- References

For **Letter to the Editor** the WORD file should contain:

- Title (100 characters)
- Running title (40 characters)
- Authors and Affiliations
- Text (no more than 500 words and must be typed double-spaced)
- References

Author affiliations should be presented in decreasing hierarchical order (e.g. Harvard University, Harvard Business School, Boston, USA) and should be written as established in its own language (e.g. Université Paris-Sorbonne; Harvard University, Universidade de São Paulo).

All manuscripts should be typed double-spaced with 3 cm margins and pages should be numbered sequentially. The lines in each page of the manuscript should be numbered too. The Editors recommend that a manuscript should be critically read by someone fluent in English before submission.

Manuscripts written in poor English will not be accepted.

Research papers and *short-review* consist of 20 pages, including references, tables and figures.

Abbreviations of terms and symbols should follow the recommendations of IUPAC-IUB Commission

(*Comission on Biochemical Nomenclature, Amendments and Corrections*) and the units are to be used according to SI (*International Systems of Units*).

Suggested Reviewers

Authors may submit suggestions of reviewers to evaluate the manuscripts. The following information must be provided: reviewer name, e-mail address, and the home institution.

Use of plant extracts in microbiological experiments

Articles that present studies with plant extracts, or other complex substances, will be accepted only after identification of compounds.

ORGANIZATION

The full **Title** of the article should be as brief as possible, not exceed 100 characters including spaces, should not contain abbreviations, and be truly indicative of the subject of the paper. Authors should suggest a **Running title** that appears in the page header which should not exceed 40 characters, including spaces.

Expressions like "Effects of", "Influence of", "Study on", etc, should be avoided. Care should be exercised in preparing the title since it is used in literature retrieval systems.

The **Abstract** should summarize the basic content of the paper. The abstract should be meaningful without reference to the text. An abstract should not contain references, tables or unusual abbreviations. Abstracts are reprinted by abstracting journals and therefore will be read by persons who do not have access to the entire paper.

The **Introduction** should provide the reader with sufficient information so that the results reported in the paper can be properly evaluated without referring to the literature. However, the introduction should not be an extensive review of the literature. The introduction should also give the rationale for and objectives of the study that is being reported.

The **Materials and Methods** section should provide enough information for other investigators to repeat the experiments.

Repetition of details of procedures which have already been published elsewhere should be avoided. If a published method is modified, such modification(s) must be described in the paper. Sources of reagents, culture media and equipment (company, city, state, country) should be mentioned in the text. Names that are registered trade marks should be so indicated. Subheading often makes this section easier to read and understand.

The **Results** section should, by means of text, tables and/or figures, give the results of the experiments, extensive interpretation of results has to be avoid but do so in the *Discussion* section. Tables and figures should be numbered using Arabic numerals. All tables and figures must be mentioned in the text.

The approximate location of tables and figures in the text should be indicated.

The **Discussion** section should discuss the results in relation to the literature cited.

In-text citations: Indicate references by (consecutive) superscript arabic numerals in the order in which they appear in the text. The numerals are to be used outside periods and commas, inside colons and semicolons. For further detail and examples you are referred to the AMA Manual of Style, A Guide for Authors and Editors, Ninth Edition, ISBN 0-683- 40206-4, copies of which may be ordered from Lippincott Williams & Wilkins (http://www.lww.com/index.html).

Data references: This journal encourages you to cite underlying or relevant datasets in your manuscript by citing them in your text and including a data reference in your Reference List. Data references should include the following elements: author name(s), dataset title, data repository, version (where available), year, and global persistent identifier. Add [dataset] immediately before the reference so we can properly identify it as a data reference. This identifier will not appear in your published article. [dataset] 5. Oguro M, Imahiro, S Saito, S Nakashizuka, T. Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley. Data, v1; 2015. http://dx.doi.org/10.17632/xwj98nb39r.1

Reference list: Number the references in the list in the order in which they appear in the text.

General rules from the 10th edition

- Items are listed numerically in the order they are cited in the text
- Include up to 6 authors
- For more than six, provide the names of the first three authors and then add et al

Examples:

1. Paivio A, Jansen B, Becker LJ. Comparisons through the mind's eye. Cognition. 1975;37(2): 635-647.
2. Yuen AWC. Lamotrigine: a review of antiepileptic efficacy. Epilepsia. 1994;35(suppl 5):S33-S36.
3. Glaser R, Bond L, eds. Testing: concepts and research. Am Psychol. 1981;36 [special issue].
4. Yasuda N, Takagi S-i, Toriumi A. Spectral shape analysis of infrared absorption of thermally grown silicon dioxide films. Appl Surf Sci. 1997;117-118(June (II)):216-220.
5. Assink EHM, Verloop N. Het aanleren van deel–geheel relaties [Teaching part–whole relations]. Pedagogische Studiën. 1977;54:130-142 [in Dutch].
6. H1 Collaboration. Nucl Phys B. 1997;504:3.
7. Weikert S, Freyer D, Weih D, et al. Rapid Ca2+ -dependent NO-production from central nervous system cells in culture measured by NO-nitrite/ ozone chemoluminescence. Brain Res. 1997;748: 1-11.
8. VanDecar JC, Russo RM, James DE, Ambeh WB, Franke M. Aseismic continuation of the Lesser Antilles slab beneath northeastern Venezuela. J Geophys Res. 2003;108:2043. doi:10.1029/2001J B000884.

9. Strunk Jr W, White EB. The Elements of Style. 3rd ed. New York: MacMillan; 1979 [chapter 4].
10. College Bound Seniors. Princeton, NJ: College Board Publications; 1979.
11. Luria AR. The Mind of a Mnemonist [Solotarof L, Trans.]. New York: Avon Books; 1969 [Original work published 1965].
12. Letheridge S, Cannon CR, eds. Bilingual Education: Teaching English as a Second Language. New York: Praeger; 1980.
13. Chaddock TE. Gastric emptying of a nutritionally balanced liquid diet. In: Daniel EE, ed. Proceedings of the Fourth International Symposium on Gastrointestinal Motility. Vancouver, British Columbia, Canada: Mitchell Press; 1974:83-92.
14. Adams MJ, Briscoe BE, Sinha SK. Interface friction and energy dissipation in soft solid processing applications. In: Dowson D, Taylor CM, Childs THC, Godet M, Dalmas G, eds. Dissipative Processes in Tribology. Amsterdam: Elsevier; 1994:223-234. Dowson D, ed. Tribology Series; vol. 27.
15. Wilson JG, Fraser FC, eds. Handbook of Teratology. Vols 1-4. New York: Plenum Press; 1977-1978.
16. Sluzki CE, Beavin J. Symmetry and complementarity. In: Watzlawick P, Weakland JH, eds. The Interactional View. New York: Norton; 1977:71-87. Reprinted from: Acta Psiquiatr Psicol Am Lat. 1965;11:321-330.
17. Yu F, Wu X-S. Phys Rev Lett. 1992;68:2996. Available from: hep-th/9112009.
18. Douglis F, Ball Th. Tracking and viewing changes on the web. In: Proc. 1996 USENIX Technical Conference; 1996.
19. See the references in: Buchdahl HA. The Concepts of Classical Thermodynamics. First published by Cambridge University Press; 1966. Also available electronically as The Concepts of Classical Thermodynamics [Last updated 1999]. This reference discusses the basic concepts in a very thorough manner. Its literature list is a main entry point into the discipline.
20. Cancer Research UK. Cancer statistics reports for the UK.; 2003 Accessed 13.03.03.

ACKNOWLEDGMENTS: This section is optional. It acknowledges financial and personal assistance.

TABLES: should be inserted in the text according to which they are cited, and numbered sequentially in Arabic number. The title of a table should be placed in the top of it and should be brief but fully descriptive of the information contained. Headings and subheadings should be concise with columns and rows of data carefully centered below them. Should be of sufficient quality to ensure good reproduction. Please, open the following link to see the requirements to obtain the adequate resolution. (http://www.ncbi.nlm.nih.gov/pmc/about/image_quality_table.html)

FIGURES: should be inserted in the text according to which they are cited, and numbered sequentially in Arabic number. Data presented in the tables should not be repeated in the figures. The legend of the figures should be placed at their bottom. Should be of sufficient quality to ensure good reproduction. Please, open the following link to see the requirements to obtain the adequate resolution. (http://www.ncbi.nlm.nih.gov/pmc/about/image_quality_table.html)

PHOTOGRAPHS: Should be of sufficient quality to ensure good reproduction. Please, open the following link to see the requirements to obtain the adequate resolution. (http://www.ncbi.nlm.nih.gov/pmc/about/image_quality_table.html)

CONFLICTS OF INTEREST

It is Brazilian Journal of Microbiology policy that everyone involved in the publication process (authors, reviewers, editorial board members, and editorial staff) must be free from conflicts of interest that could adversely influence their judgment, objectivity or loyalty to the article and assignments. The BJM recognizes that any potential conflict of interest raised must be disclosed promptly to Editor. Conflicts of interest in publishing can be defined as conditions in which an individual holds conflicting or competing interests that could bias editorial decisions. Conflicts of interest may be only potential or perceived, or they may be factual. Personal, political, financial, academic, or religious considerations can affect objectivity in numerous ways.

AUTHORS' COPYRIGHT

Upon receipt of the galley proofs for approval, authors of approved manuscripts should fax or email the Author´s Copyright Statement to the BJM (55-11-3037-7095, bjm@sbmicrobiologia.org.br). The statement (see text below) must be signed by at least one of the authors (who agrees to inform the other authors, if any).

TRANSFER OF AUTHORS' COPYRIGHT

"The undersigned author(s) state(s) that the article being submitted is original, does not infringe copyright laws or any other third-party property rights, has not been previously published, and is not being considered for publication elsewhere. The author(s) confirm(s) that the final version of the manuscript has been reviewed and approved by all authors. All manuscripts published become the permanent property of the Brazilian Journal of Microbiology and can not be published without authorization in writing from its Editors."

Article No. _____

Title of the article: "_____
_____"

Name(s) of the author(s) Signature(s)

_____ _____

_____ _____

_____ _____

Date: ____/_____/_____