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Evaluation of Antimicrobial Activity of Aryl/Alkyl Cyanamides and Substituted Tetrazole Compounds

Shaik Mohammed Jasmine, Seelam Mohan, Kota Neela Mani Kanta, Kiranmai Chadipiralla, Karumanchi Deepthi, Praveena Ketha, Prasada Rao Kammela & Uday Sankar Allam

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Abstract

Cyanamides, tetrazole and their derivatives have pharmaceutically attracted much attention because of their wide range of biological activities such as antihypertensive, anti alergic, anti-asthmatic, antimicrobial, antiviral, anti-inflammatory, anti-neoplastic and anticonvulsant activities. The aryl/alkyl cyanamides and substituted tetrazole copounds were synthesized through cobalt-promoted one-pot reaction of isothiocyanates previously in our laboratory. In the present study, 11 synthesized aryl/alkyl cyanamides and substituted tetrazole compounds were tested for their antimicrobial activity using Kirby–Bauer disc diffusion method against the reference Gram-negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603) and Gram-positive (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* 6A1) bacterial strains. Among the tested compounds, 1-(4-chlorophenyl)-1*H*-tetrazol-5-amine (**XI**) followed by 4-amino benzonitrile (**IX**) and 1-(4-methylphenyl)-1*H*-tetrazol-5-amine (**VII**) were found to be efficient in inhibiting bacteria at tested concentrations with wider zones. Further modification of tetrazole moiety may help to find even more valuable biological activities.

Research Article

In Silico Modeling and Docking Analysis of CTX-M-5, Cefotaxime-Hydrolyzing β-Lactamase from Human-Associated *Salmonella* Typhimurium

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Abstract

Objective: To computationally model the CTX-M-5 β-lactamase and establish its structure, which is exclusively present in human-associated *Salmonella*.

Methods: The CTX-M-5 aminoacid sequence (Uniprot ID:O65975) of *Salmonella enterica* subsp. enterica serovar typhimurium was retrieved from UniProt database and subjected to homology modeling using MODELLER 9v7. The homology models were duly validated using RAMPAGE tool by generating Ramachandran plots, ERRAT graphs, and ProSA score. DoGSiteScorer server and ConSurf server were used to detect the cavities, pockets, and clefts to identify conserved amino acid sites in the predicted model. Subsequently, the modeled structure was docked using CLC Drug Discovery Workbench against proven drugs and known inhibitors.

Results: Obtained high-quality homology model with 91.7% of the residues in favorable regions in Ramachandran plot and qualified in other quality parameters. Docking studies resulted in a higher dock score for PNK (D-benzylpenicilloic acid) molecule when compared to other reported inhibitors.

Conclusion: This in silico study suggests that the compound PNK could be an efficient ligand for CTX-M-5 β-lactamase and serve as a potent inhibitor of CTX-M-5.

Keywords

Extended-spectrum β-lactamase (ESBL), *Salmonella typhimurium*, CTX-M-5, Homology modeling, Docking, PNK

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Introduction

Salmonella enterica subsp. enterica serovar typhimurium (aka *S. typhimurium*), a member of the *Enterobacteriaceae* family, is a gram-negative, facultative anaerobe that causes self-limiting gastroenteritis in humans. On the other hand, infection is systemic in murine models that resemble typhoid fever in humans caused by *Salmonella enterica* subsp. enterica serovar typhi. *Salmonella* is one of the four major causes of diarrheal diseases and foodborne infections across the globe.[1] The World Health Organization reports 93,000,000 cases of enteric infections and 155,000 mortality cases annually.[2] Currently, drugs used in the treatment of gastroenteritis lead to the increasing emergence of antibiotic resistance and the most rampant antibiotic-resistant mechanism is the production of β-lactamases by gram-negative bacteria.[3] β-lactamases inactivate β-lactam group of antibiotics by hydrolyzing the amide bond of a four-membered β-lactam ring. Based on sequence variation, about

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ORIGINAL ARTICLE

KAP Analysis on Food Safety among University Students of South India

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ABSTRACT Food-borne diseases represent a growing worldwide public health problem. Young adults have been reported to have inadequate food safety knowledge, poor attitude and inappropriate behavior. The present study was taken up to assess the level of food safety characteristics and investigate the association between the living status and knowledge, attitude and practices of the South Indian University students. A quantitative online questionnaire was used to collect the data from the 4 different university students of South India. The online questionnaire was shared with students via different social media groups and E-mail. 465 students from various institutions in south India has responded for the food safety questionnaire, among them 211 students are day scholars, 53 students stay as paying guest and 201 are staying at hostels. Comparison of food safety knowledge among living status of students (Day Scholar, Hosteller and Paying Guest) was performed using X2 test and ANOVA. The study reports indicate that the students living status has influence on the knowledge towards food safety ($p< 0.000$), students living as paying guest (Wt. Mean = 1.41) scored well on knowledge comparatively with day scholars (Wt.Mean = 1.37) and hostellers (Wt. Mean = 1.34). However there is no considerable difference among students towards attitude and practices of food safety ($p>0.000$).The study results indicated that majority of the students irrespective of their living status have positive attitude towards food safety and have good practices. Therefore, the findings of the present study indicate that educational interventions in the form of courses and workshops can be effective in improving food safety knowledge in students especially among the hostlers.

Keywords: Food safety, University students, Knowledge, Attitude

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BACKGROUND

Food borne diseases (FBD) comprises a broad spectrum of illnesses and arises due to consumption of unsafe food or water contaminated with toxic chemicals and harmful microorganisms. Food borne diseases represents a growing global public health problem causing more than 200 diseases ranging from severe diarrhea to cancer [1,2]. Food borne diseases may lead to miscarriage in pregnant women, long-lasting disability and death of both adults and new born babies. Enterohaemorrhagic *Escherichia coli*, *Salmonella sps.*, *Listeria sp.* *Vibrio cholera* and Campylobacter sp, are some of the common food borne bacterial pathogens that affect millions of people annually across the world. According to WHO 2017 statics, an estimated 600 million people (1 in 10 people) fall ill due to consumption of contaminated food and of which, 420000 die each year with a total loss of 33 million healthy life years

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Original Article

Evaluation of detection of severe acute respiratory syndrome coronavirus-2 by chip-based real-time polymerase chain reaction test (truenat™ beta CoV) in multi-sample pools

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Abstract

Introduction: Systematic testing for Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV-2) using molecular diagnostic tools to identify individuals with coronavirus disease 2019 (COVID-19) infection, and tracing their primary and secondary contacts is important to curb its spread. With resource limitations on testing individual samples, testing of pooled samples provides alternative approach to increase testing capacity. Present aimed at assessing the detection of SARS-CoV-2 RNA in pooled samples using chip-based real-time polymerase chain reaction Test (Truenat™ Beta CoV).

Materials and Methods: Pooled sample size of five was used from laboratory confirmed COVID-19 positive and negative samples. SARS-CoV-2 positive nasopharyngeal specimens of known samples from high, medium, low, and very low viral load were mixed with SARS-CoV-2 negative nasopharyngeal specimens of known samples in 1:4 ratio, followed by analysis using Truenat. Furthermore, each sample in that pool was tested individually. Pooled sample testing was also done on the samples of unknown status.

Results: The results of the present study showed cycle threshold (Ct) values of pooled sample with SARS-CoV-2 positive RNA of high, medium, low, and very low viral load were 16.8, 24.22, 28.2, and 33.43, compared to Ct values of individual samples of 16.43, 22.0, 28.00, and 33.00, respectively.

Conclusion: These results suggest that the Ct values of pooled samples were in agreement with Ct values of individual samples indicating the validity of pooled sample testing for screening SARS-CoV-2 using Truenat.

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The following core competencies are addressed in this article: Medical knowledge, Patient care and procedural skills systems-based practice, Practice-based learning and improvement.

Keywords: Corona virus disease 2019, cycle threshold value, real-time polymerase chain reaction, sample pooling, severe acute respiratory syndrome corona virus-2, Truenat™ Beta CoV

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19) has been creating havoc across the world. The viral disease was first identified in Wuhan city of Hubei province in China on December 12, 2019 and since then globally 298,915,721, laboratory confirmed cases of COVID-19, including 5,469,303 deaths were reported as of January 9, 2022.[1,2] SARS-CoV-2 has been continuously emerging into new variants through mutations in the spike gene of the SARS-CoV-2. The mutations may lead to increased transmissibility, high infectivity, and less responsive to treatments for COVID-19. Alpha, beta, and delta SARS-CoV-2 are few such SARS-CoV-2 variants, which have been associated with the new waves of infection in the recent past.[3] A new SARS-CoV-2 variant named Omicron variant (B.1.1.529 variant) with more than 30 mutations and with high transmissibility rate has been identified by the World Health Organization (WHO) in November 2021.[4]

To date, no specific, definitive approved antiviral therapeutic drugs with proven clinical efficiency available against SARS-CoV-2 infection and the only way is to contain the disease by following nonpharmaceutical interventions or mitigation measures as suggested by WHO.[5] The effective mitigation involves rapid laboratory based diagnostic testing to identify the COVID-19 infection, and hence, the WHO Director-General Ghebreyesus advised all countries to test, test, test as a best way to contain the pandemic.[6]

Studies conducted on the occurrence of asymptomatic COVID-19 cases suggest that around 10%–30% of COVID-19 cases reported were asymptomatic.[7-9] Hence, rapid and accurate identification of presymptomatic and asymptomatic cases is very crucial in the effective control of silent spread COVID-19. Systematic testing of the suspected individuals using molecular diagnostic tools is important for the identification of COVID-19 patients and tracing COVID-19 patient close contacts to curb the spread of the virus. However, a major hindrance of containing COVID-19 in several developing nations is the lack of large scale diagnostic testing and done only on symptomatic patients due to resource constraints such as poor laboratory capabilities, high cost and in some places overwhelmed on testing laboratories.

Truenat™ Beta CoV is a chip-based real-time polymerase chain reaction (PCR) test for the detection of coronavirus RNA in human nasopharyngeal and oropharyngeal specimens.[10] TrueNat is an indigenous diagnostic test originally designed (Truenat *Mycobacterium tuberculosis* [MTB]™) for the detection of drug-resistant MTB isolates in sputum specimens.[11] Later, the TrueNat system has been validated by the India's apex biomedical research body, i.e., *Indian Council of Medical Research* (ICMR), New Delhi and approved it as point of care test for the first line screening test for diagnosis of COVID-19 in April, 2020. Truenat Beta CoV test is stand on the detection of the E gene, which is commonly present in SARS-CoV causes SARS and SARS-CoV-2 causes COVID-19. Hence, individuals tested positive with Truenat Beta CoV (E gene screening assay) may be confirmed with S gene or RdRP gene or N gene reverse transcription (rRT)-PCR for the accurate identification of SARS-CoV-2.[12-14] TrueNat is an easy, quick, user friendly, and robust real-time PCR-based diagnosis technique which can be used in even at resource-limited settings.[15-17]

Pooled sample testing involves mixing of specimens from multiple individuals in a single tube and screening through rRT-PCR. If the pool test results positive then pool de-convolution will be done i.e., rRT-PCR will be done for the individual samples of the positive pool. Pooled sample testing was first introduced in 1943 by Robert Dorfman to large scale screening of syphilis in US military men.[18] Since then, the Dorfman approach has been applied in screening infectious diseases such malaria,[19]

influenza[20] and Chlamydia[21] and during early stages of HIV pandemic.[22] Pooled sample testing approach has been demonstrated successfully for screening of SARS-CoV-2 in Germany,[23] USA,[24,25] and Israel[26] India[27,28] and been implemented for large scale screening of COVID-19. ICMR also suggested testing laboratories to use sample pooling for molecular testing in areas with low (<2%) infection positivity and for surveillance or survey studies in areas with 2%–5% infection positivity from the existing data.[29] The overall success rate of pool or group testing depends upon the incidence of the infectious disease in the population, specificity, sensitivity, and detection limits of the diagnostic test employed.[24,30]

The number of COVID-19 tests conducted per million population in India is low compared with the figures reported for UK (29, 412), US (36,961), Russia (50,381), and Italy (51 347). There is an urgent for increasing the molecular confirmatory tests to identify the asymptomatic carriers of COVID-19 to curb further spread of disease.[31] In this context, the present study is aimed to assess the detection of SARS-CoV-2 RNA in pooled samples from multiple individuals using Chip-based Real Time PCR Test (Truenat™ Beta CoV) while maintaining the reliability of the test and conserving the resources.

MATERIALS AND METHODS

The prospective evaluation study was carried out at nodal COVID-19 testing laboratory of Damien Foundation TB research centre. The center is a TB culture and drug susceptibility testing (C and DST) referral laboratory accredited by the National Mycobacteriology Accreditation System of Central TB Division Ministry of Health, Govt. of India. Government of Andhra Pradesh approved and converted Damien TB centre into COVID-19 nodal testing laboratory. The study was carried out on the left over samples received at nodal testing laboratory, Nellore, Andhra Pradesh, India, under BSL2 laboratory facilities. Nasopharyngeal swabs received in Trueprep® Auto Transport medium swab specimen tube containing viral lysis media (VLM) at nodal testing laboratory were processed following the recommended protocols of ICMR, New Delhi, Govt. of India. Initially, evaluation was done using known samples from laboratory confirmed COVID-19 positive and negative patient samples received during April and May, 2020. The present evaluation study was considered exemption as it was taken up as a part of the COVID-19 testing study. Further the results of the study were not considered for declaring patient status. The authors declare that the study was found to be noninterventional, and exempt by the local Ethics Committee.

Nucleic acids extraction (RNA) extraction

Pooled sample size of 5 was used with a VLM sample volume of 100 µl from each sample to make the final volume of l. SARS-CoV-2 positive nasopharyngeal specimens of high, medium, low, and very low viral load (as specified by the Manufacturer) were mixed with SARS-CoV-2 negative nasopharyngeal specimens of known samples in 1:4 ratio (1 each positive nasopharyngeal specimens and 4 negative nasopharyngeal specimens) and isolation of RNA was done. 500 µL of individual sample VLM was also taken into the lysis buffer tube and extraction of RNA was done separately. RNA extraction was done using Trueprep AUTO Universal Cartridge based Sample Prep Kit and Trueprep AUTO Universal Cartridge ®based Sample Prep Device following the manufacturers' instructions (Molbio Diagnostics Pvt. Ltd. Goa, India).

RNA isolated from pooled samples and individual samples were run on Truelab™ Real Time micro PCR Analyzer following the manufacturer's instructions. Truenat™ Beta CoV/Truelab™ Real-Time micro PCR Analyzer works on the principle of real time rRT PCR based on Taqman chemistry. Briefly, 6 l of the purified RNA isolated from the pooled and individual samples were added to the microtube containing the freeze dried PCR components (Supplied by Manufacturer) and allowed to stand for 30–60 s to get a clear solution. 6 l of the clear solution of template and PCR reagents were loaded onto the reaction well of the Truenat™ Beta CoV chip. Output is read as cycle threshold (Ct), a number of amplification cycles needed to cross the background signal. A clear horizontal amplification curve occurs in case of negative samples as there is no PCR amplification. The amount of target nucleic acid sequence present in the test sample is inversely proportional to the Ct values. After the completion of test run, the results are displayed are as "Detected" or "Not Detected" in case of positive and negative samples, respectively. Truelab™ PCR Analyzer also gives the viral load as "HIGH," "MEDIUM," "LOW," or "VERY LOW" for the COVID-19 positive samples. The reliability of the Truenat™ Beta CoV was assessed from the amplification of internal positive control (IPC) (human RNase P gene) to

know proper sample collection, RNA purification and rRT-PCR. The human RNase P gene is a full process internal control which goes through every step the test specimen undergoes – from RNA isolation to rRT-PCR step thereby corroborate the test from specimen to result. RNase P will co amplifies along with positive samples case and shift or absence Ct value of RNase P beyond a preset range in case of negative samples cancels the test run. The validity of the test run is also displayed based on amplification of RNase P and the test results can be acquired to the laptop/desktop via Wifi network or can be taken as printout using the Truelab™ micro PCR printer.[14]

The study analyzes the test results of individual and pooled sample data for any significant difference in testing methods using Mann–Whitney *U*-test. The test was conducted using SPSS 16.0 version (SPSS, Inc., Chicago, USA).

RESULTS

Optimal pool testing was initially evaluated using online Shiny App for pooled testing tool as explained at https://www.chrisbilder.com/shiny. Although accurate COVID-19 prevalence rate in Nellore District of Andhra Pradesh, India is not known, the observed disease prevalence rate from samples received the different locations of Nellore has been considered as 2%. Optimum pooling size suggested by ICMR was evaluated with the following assumptions and parameters were given as inputs for shiny tool for hierarchical two stage pooling algorithm, TrueNat testing specificity and sensitivity as 100%, COVID-19 prevalence rate as 2% and pool size of 5. The algorithm results indicated that the two-stage hierarchical testing reduces the expected number of tests by 70% with pool size of 5 when compared to individual testing (https://www.chrisbilder.com/shiny/).

In the present study, threshold cycle (Ct) values E-gene of SARS-CoV-2 was evaluated in pooled sample and unpooled (Original) samples of known and unknown cases for the suitability of the pooled sample testing. Based on literature and test results of the Shiny algoritham pool size of 5 was chosen for testing. Comparison of Ct values between the pooled samples mixed in the ratio of one positive nasopharyngeal swab with the four negative nasopharyngeal swabs and individual of the pool indicate that pool size of five is good pool size for the accurate detection of corona virus. Four different pools were taken with VLM from patients with high, medium, low and very low viral load in 1: 4 ratio i.e., one each E-positive sample and four E-gene negative samples. One control pool with all 5 from E-gene negative samples was used as control. Pooling of samples was done before RNA isolation and then RNA isolation, rRT-PCR was done on Truenat™ Beta CoV/Truelab™ Real Time micro PCR Analyzer. RNA also isolated from COVID-19 positive patients and rRT-PCR was done on Truenat™ Beta CoV/Truelab™ Real Time micro PCR Analyzer separately. The Ct values for E-gene ranges from 16.8 to 33.43.

The Ct values of pooled samples from positive sample from high, medium, low, and very low viral load samples were 16.8, 24.22, 28.2, and 33.43, respectively [Table 1 and Figures 1 and 2]. Similarly, the Ct values of original samples of the pool from high, medium, low, and very low viral load samples were 16.43, 22.00, 28.00, and 33.00, respectively [Table 1 and Figures 1 and 2]. The Ct values of E-gene in original positive specimens and pools were below 35 and considered as positive. The difference in Ct values between pooled and original individual positive samples (Ct_{Pool}-$Ct_{Original\ Positive\ Sample}$) shows that there is only minimal difference and the values high, medium, low, and very low viral load samples were 0.37, 2.22, 0.20, and 0.43, respectively [Table 1 and Figure 3]. The data collected are subjected to Mann–Whitney *U*-Test for determining the difference in threshold cycles between individual sample and pooled sample data. The test results reveal that there is insignificant difference in threshold cycle values among the individual and pooled sample data mean ranks (Mean Rank: Individual –5.00, Pooled −4.00, U = 6.00, $P = 0.564$). Hence, it is evident that there is no difference in the pooled sample testing and testing samples individually for COVID-19 [Table 2]. No E-gene amplification was observed in negative control

Table 1: Comparison of Ct values between the unspooled and pooled samples mixed in one positive nasopharyngeal swabs with the four negative nasopharyngeal swabs

Sample number	E gene		
	Ct value of pooled samples (1 positive: 4 negative)	Ct value of individual or original sample	Difference in Ct value
High viral load	16.8	16.43	0.37
Medium viral load	24.22	22.0	2.22
Low viral load	28.8	28.0	0.8
Very low viral load	33.43	33.0	0.43

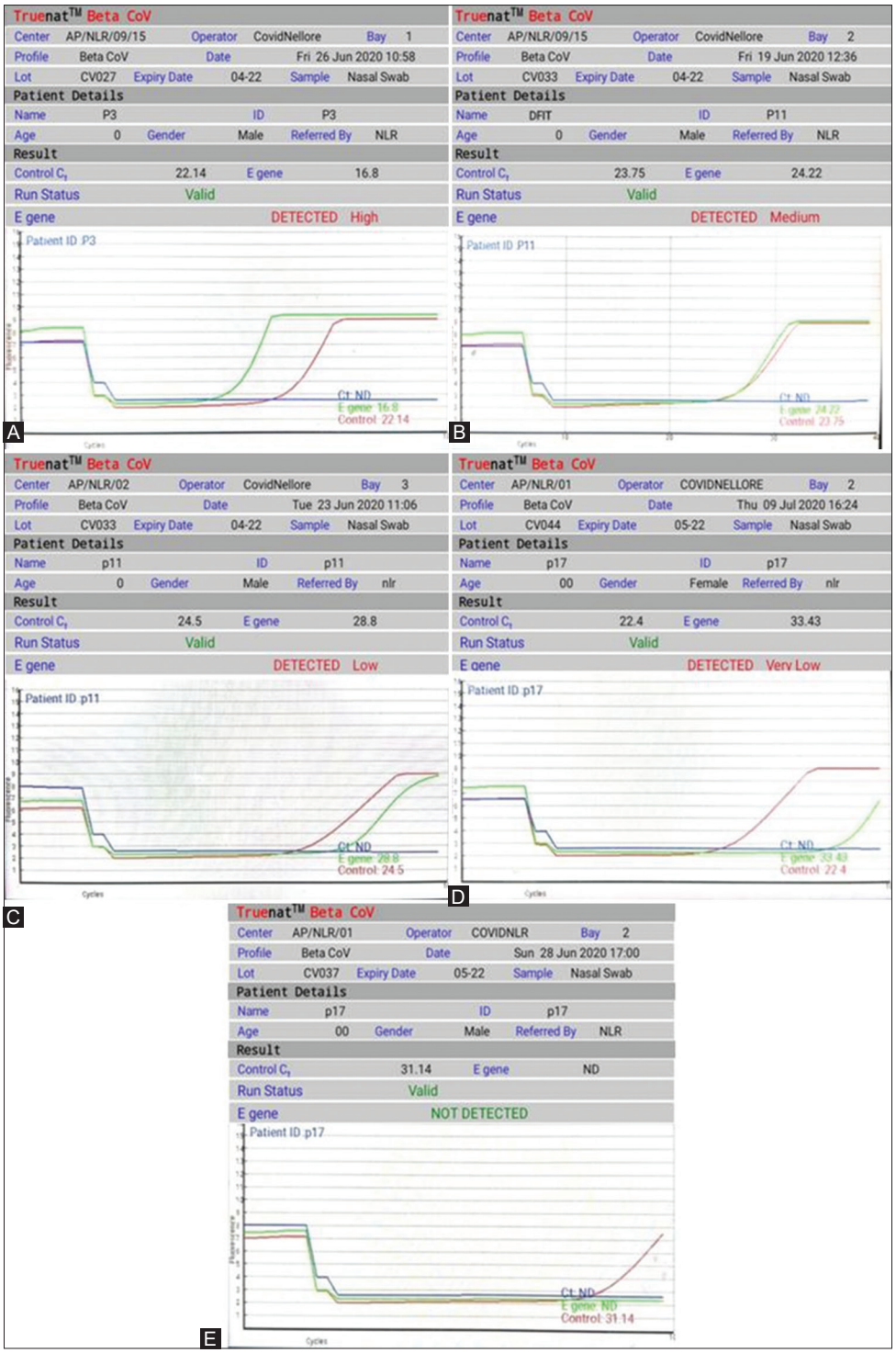

Figure 1: Screenshots of pooled sampling rRT-PCR test results formats as displayed on Real Time Quantitative micro Truelab™ PCR Analyzer. rRT-PCR: Real-time reverse transcription polymerase chain reaction (A: High Viral Load, B: Medium Viral Load, C; Low Viral Load, D: Very low Viral Load & E: negative Control)

Table 2: Mann–Whitney U-test analysis of pooled sample and individual sample testing

Group	Threshold cycles Ct – (mean rank)	Mann–Whitney U (statistic)	Asymptotic Significant (two-tailed)
Individual	5.00	6.00	0.564
Pooled	4.00		

pool with all 5 nasopharyngeal swabs from individuals with negative status. The pooled sample testing done with all 5 negative samples remain valid as there was clear amplification of IPC. No false-positive results were seen in our study. Subsequently, we applied

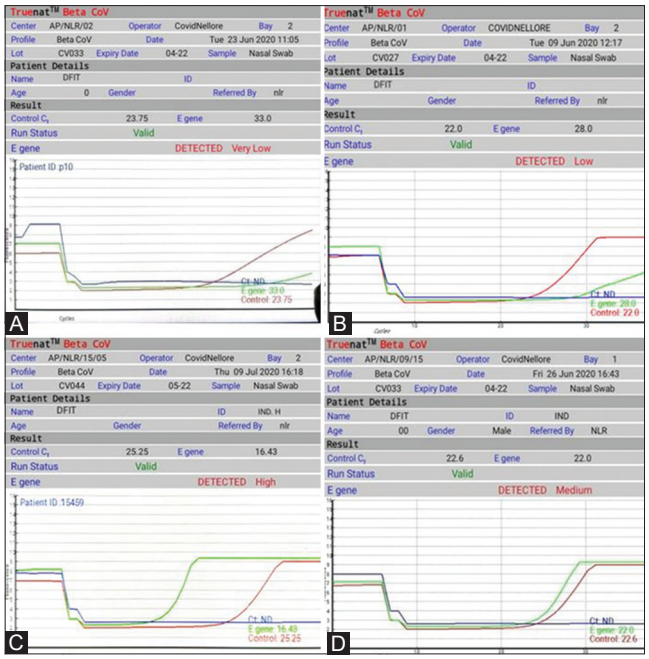

Figure 2: Screenshots of individual sample rRT-PCR test results formats as displayed on Real Time Quantitative micro Truelab™ PCR Analyzer. rRT-PCR: Real-time reverse transcription polymerase chain reaction. (A: High Viral Load, B: Medium Viral Load, C; Low Viral Load, D: Very low Viral Load)

pooled sample testing for the detection of E-gene in samples from patients with unknown status. Total 500 nasopharyngeal swabs collected during May 15 to June 25, 2020, were pooled in groups of 5 to create 100 pools. Then, the RNA isolation from the pools was done and rRT-PCR was done on TrueNat system. Five pools tested positive out of 100 pools, subsequent testing respective individual samples of positive pools detected 7 positive individuals with a prevalence rate of 1.4%. Overall, time taken for the pooled sample testing and identifying positive individual is only 4 h.

DISCUSSION

During the present COVID-19 pandemic, availability of RNA extraction kits and rRT-PCR testing kits has become a most important limiting factor in screening the large number of people to know the disease prevalence status and accurately determine the disease prevalence rate. In The sufficient COVID-19 testing laboratories and enough trained technical persons are lacking in majority of the states in India are not having sufficient number of. As a result enormous number of ($\sim 10^3$–10^4) samples to be tested are in waiting list in each nodal COVID-19 testing laboratory. Due to limited availability of testing, COVIID-19 diagnostic tests are done only to patients who have

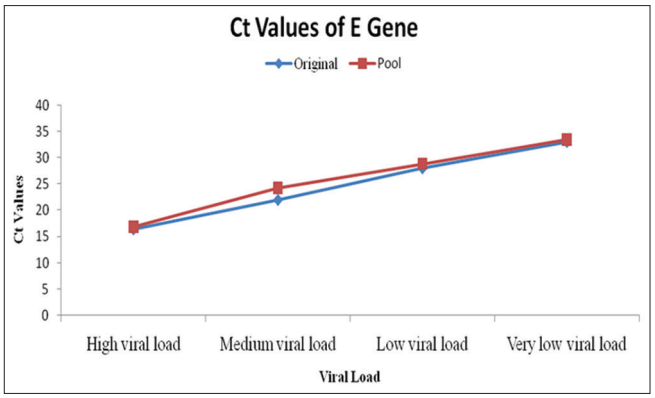

Figure 3: Graphical representation of Ct values between the original and pooled samples mixed in one positive Nasopharyngeal swabs with the four negative nasopharyngeal swabs. Ct: Cycle threshold

visible symptoms, travel history and to certain extent primary contacts. In addition, due to delay in testing, the chances of spread of COVID-19 might increase and makes situation the worst. Moreover, the recent studies indicate that 20%–30% COVID-19 cases are asymptomatic and a few are mild cases. Testing every individual who is in close vicinity of the COVID-19 patient, and all primary and secondary contacts would enable to reduce the rapid spread of COVID-19. Further, the time taken for testing the suspected cases and associated cost is also very high. RT-qPCR study conducted by Yelin *et al*., reported that the SARS-CoV-2 RNA of a single positive specimen can be detected even in pooled sample size up to 32, with an expected false negative rate of 1 out of 10 (10%). Pure RNA instead of original sample specimens was mixed in multi pool study carried out by Yelin *et al*.[26] Similarly, using Pooling-Based Efficient SARS-CoV-2 Testing, Shental *et al*., identified 1–5 SARS-Cov-2 positive carriers in 48 pools grouped from 384 samples providing an 8 fold increase in the COVID-19 testing efficiency.[9]

In this context, pooled sample testing method reduces the time for screening large of individuals, reagents required for testing and most importantly the cost. The average cost for testing one sample by TrueNat testing method is INR1700 (22.48$). Use of sample pooling (pool size of 5), the cost incurred for testing one individual sample is only INR 340 (4.5$) and pooled sample testing reduces the cost by 80%. The results of the present study suggest that COVID-19 screening using pooled sample testing method detects even if the viral load is very low as indicated by the TrueNat without increasing the additional number of cycles and with satisfactory diagnostic accuracy. We

pooled sample prior to RNA extraction, doing so, bottlenecks associated with the RNA extraction will be reduced. As pool testing follows standard approved proved equipment, reagents and protocols, pooled testing method can be adapted in existing COVID-19 testing laboratories. Immediate implementation of pooled testing may increases the current screening capacity and thus increasing the testing of large number of individuals in the community and hence reducing the community transmission. Success of sample pooling depends upon quality and adequate sample collection, efficient extraction and sensitive detection methods. The pool sample testing has some limitations such as intricate work flow, availability of the skilled lab workers, collection and appropriate storage of samples. Further, sample pool testing can only be done when the prevalence of disease positivity is low in the community and hence, pool sampling can't be used during the peak of pandemic. Lastly occurrence of false negative results is high especially with low viral load samples or samples with borderline Ct values.

CONCLUSIONS

The findings of the present study suggest that the Ct values of pooled samples were in agreement with Ct values of individual samples indicating the validity of pooled sample testing for screening SARS-CoV-2 using Truenat. Further, a very low viral load of SARS-CoV-2 in multipool nasopharyngeal samples can be detected using Chip-based Real Time PCR Test (Truenat™ Beta CoV). Testing of pooled samples provides alternative approach to increase testing capacity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Research quality and ethics statement

The authors of this manuscript declare that the applicable EQUATOR Network (https://www.equator-network.org/) reporting guidelines were followed. The authors also declare that the study was found to be exempt by the local Ethics Committee.

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JOURNAL ARTICLE

Immobilization stress exacerbates arsenic-induced reprotoxic effects in adult rats

Kaduru Venkaiah, Thathapudi Daveedu, Papisetty Prathima, Ramanadhapuram Pavani, Sadepalli Sukeerthi, Malapati Hanuma Reddy, Jangampalli Adi Pradeepkiran ✉, Sri Bhashyam Sainath ✉

Toxicology Research, Volume 11, Issue 3, June 2022, Pages 426–436, https://doi.org/10.1093/toxres/tfac022
Published: 09 May 2022 **Article history** ▾

Abstract

Objective

The central objective of this study was to investigate the cumulative effects restraint stress and sodium arsenite on reproductive health in male rats.

Methods

Healthy male Wistar rats were allocated into 4 groups (n = 8). Animals in group 1 served as controls and did not subjected to any stress. Rats in groups 2, 3, and 4 were subjected to either restraint stress (5 h/day) or maintained on arsenic (25 ppm) via drinking water or both for 65 days. After completion of the experimental period, all the rats were analyzed for selected reproductive endpoints.

Results

Restraint stress or sodium arsenite treatment increased serum corticosterone levels, reduced testicular daily sperm count, epididymal sperm viability, motility, membrane integrity, and decreased testicular steroidogenic enzymes such as 3β- and 17β-hydroxysteroid dehydrogenases associated with reduced serum testosterone levels, deteriorated testicular architecture, and reduced activity levels of testicular superoxide dismutase and catalase accompanied by elevated lipid peroxidation levels. In rats subjected to restraint stress and sodium arsenite, a significant decrease in selected sperm qualitative and quantitative parameters, serum testosterone levels were observed as compared with rats subjected to sodium arsenite alone. A significant increase in the levels of lipid peroxidation with a concomitant decrease in the activities of antioxidant enzymes was observed in the testis of rats subjected to both restraint stress and sodium arsenite treatment as compared with sodium arsenite alone intoxicated rats. Surprisingly, serum corticosterone levels were significantly elevated in rats following both stressors as compared with arsenic alone treated rats. Analysis of atomic absorption spectroscopy revealed that the accumulation of arsenic in the testis of arsenic-treated and arsenic plus immobilization stress groups was significant as compared with controls.

Conclusions

Based on the findings, it can be concluded that deterioration of male reproductive health could be accelerated in arsenic intoxicated rats following restraint stress.

Keywords: arsenic, immobilization stress, spermatogenesis, testicular steroidogenesis, rats

Issue Section: Paper

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In Vitro anti-Protozoan Activity of Methanolic Extracts of *Caralluma procumbens* Against *Tritrichomonas foetus*.

Rajani Vemula[1], Gayathri Pachipala[1], P.V.B Reddy[3], Sathish Kota [4], M. Raman [4], Uday Sankar Allam[1], Kota Neela Manikanta[2] & Kiranmai Chadipiralla[1*]

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Abstract

Tritrichomonas foetus is a flagellated venereal parasite that causes Trichomoniasis. Among various infections, *Tritrichomonas* infection is a major threat to animal husbandry contributing to heavy economic loss due to fetal deaths or abortions. Plants are a great source of a variety of secondary products that consist of different bioactive compounds with medicinal properties. Species of *Caralluma* are known to be sources of potential therapeutic molecules. However, no studies have been carried out on the anti-protozoan activity *of Caralluma procumbens* against *Tritrichomonas foetus*. Hence the effect of different concentrations of *Caralluma* extract on the growth and survival of *Tritrichomonas foetus* was examined. Methanolic extract of *Caralluma procumbens*, at the concentrations of 5 and 10 mg/ml inhibited the growth of *Tritrichomonas foetus* completely after 24 hours of incubation. A concentration of 2 mg/ml inhibited 80% growth of *Tritrichomonas foetus* after 48 hours of incubation with *Caralluma* extract. The results of the current study suggest that *C. procumbens* could be suitable for treatment and prevention of protozoan induced Trichomoniasis and prove to be an effective therapeutic strategy without any nonspecific effects. Further studies in this line will help in identification of effective structural bioactive components present in the methanol extract of *C. procumbens*.

Keywords: Bioactive Compounds, *Caralluma procumbens*, Methanol Extract, *Tritrichomonas foetus, Trichomoniasis*.

Introduction

Tritrichomonas foetus is a single cell flagellate parasite, known for causing infections in the reproductive tracts of bovine and intestinal tract of cats (1). *Tritrichomonas* belongs to the kingdom Protoctista. These are spindle shaped flagellate parasites with their size ranging from 5 to 25µm. *T. foetus* consists of three anterior flagella, one posterior flagellum with an undulating membrane (2). It is also known as venereal pathogen of the cattle that spreads through sexual intercourse. It has been recognized as major threat in the cat families specifically in the domesticated ones. This protozoan is an enteric pathogen usually residing in the inner lining of the colon (3). *Ttitrichomonas foetus* is also known to cause Feline Trichomonias, a large-bowel disease in cats (4,5). *T. foetus* has been reported to induce spontaneous abortions in the first trimester of pregnancy in cats. Further it is also known to be a causative factor responsible for infertility (1, 6).

In addition to cat, this pathogen is also known to infect cattle. Symptoms of *T. foetus*

Egyptian Journal of Biological Pest Control

REVIEW ARTICLE Open Access

Recent advances in engineering crop plants for resistance to insect pests

Shilpa Kamatham[1], Sandhya Munagapati[2], Kota Neela Manikanta[3], Rohith Vulchi[4], Kiranmai Chadipiralla[1], Sri Hari Indla[1] and Uday Sankar Allam[1*]

Abstract

Background: While the rapidly increasing global population has led to a dramatically increased demand for the agricultural production, there have been heavy economic losses owing to various pest attacks on different food crops. The advancement of various biotechnological techniques have come as a boon in addressing the global concern and leads to the development of novel varieties that have proven to be highly economical, pesticide resistant and environmentally safe.

Main body: The present review was aimed to update the recent developments that have taken place in the field of crop production. Major focus was laid predominantly on such genes that have demonstrated positive effects and proved to be of commercial success at the market primarily due to the development of pest-resistant transgenic food crops with expression of *Bacillus thuringiensis* toxins. This technology has been effective against a wide range of pests including coleopterans, lepidopterans, hemipterans, dipterans, strongylida (nematodes) and rhabditida. In similar lines various plant derived toxic proteins were also discussed along with different genes that code for insect resistant proteins such as δ-endotoxins and secreted toxins. This article also helps in understanding the structural features of the genes that are endowed with insect resistance followed by their mechanism of action on pests. Further the role of secondary metabolites in controlling the pests was addressed. The Pros and Cons of existing tools of insect pest management were demonstrated.

Conclusions: Novel technologies are necessary in crop improvement to progress the pace of the breeding programs, to confer insect resistance in crop plants. Therefore, the future aim of crop biotechnology is to engineer a sustainable, multi-mechanistic resistance to insect pests considering the diversity of plant responses to insect attack.

Keywords: Genetic engineering, Insect resistance, Toxins, CRISPR/Cas9, RNAi, Phytophagous, Stacked traits

Background

Genetic engineering is a deliberate process of making changes to the characteristics of an organism by changing its genetic material. Genetic engineering in crop plants mainly offers two advantages i.e., (1) combining several individual, commercially useful genes to form gene cassettes and (2) reducing the time to introgression of these genes into a single genetic background. Since the "first report of genetically modified plants appeared in 1984 (Horsch et al. 1984), there has been a very rapid progress directed at using this novel technology for the practical ends of crop improvement. Protection of crop plants from insect pests was quickly seized upon as a major goal of plant genetic engineering (National Council 2000). The potential size of this market attracted major attention of a number of commercial organizations and the potential economic importance of this sector of biotechnology is "finally becoming more widely recognized (Burke and Thomas 1997). The practical application of plant genetic engineering involves two equally important technologies; cellular and molecular biology. The list of crop species

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Science of The Total Environment
Volume 797, 25 November 2021, 149044

An ancestral nuclear receptor couple, PPAR-RXR, is exploited by organotins

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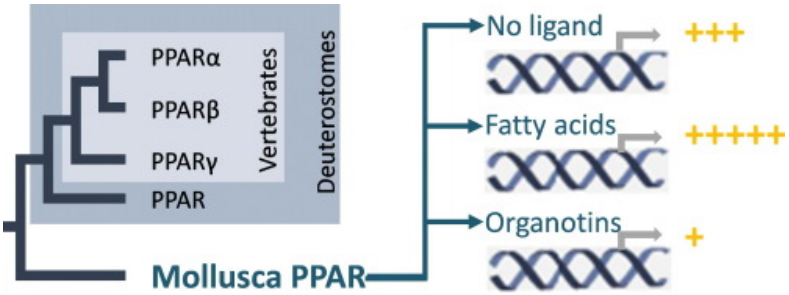
Highlights

- First functional characterization of a Mollusca PPAR
- TBT and TPT represses the Molluscan heterodimer PPAR/RXR.
- PPAR tyrosine 277 is involved in the response to TBT.

Abstract

Environmental chemicals have been reported to greatly disturb the endocrine and metabolic systems of multiple animal species. A recent example involves the exploitation of the nuclear receptor (NR) heterodimeric pair composed by PPAR/RXR (peroxisome proliferator-activated receptor/retinoid X receptor), which shows lipid perturbation in mammalian species. While gene orthologues of both of these receptors have been described outside vertebrates, no functional characterization of PPAR has been carried in protostome lineages. We provide the first functional analysis of PPAR in *Patella* sp. (Mollusca), using model obesogens such as tributyltin (TBT), triphenyltin (TPT), and proposed natural ligands (fatty acid molecules). To gain further insights, we used site-directed mutagenesis to PPAR and replaced the tyrosine 277 by a cysteine (the human homologous amino acid and TBT anchor residue) and an alanine. Additionally, we explored the alterations in the fatty acid profiles after an exposure to the model obesogen TBT, *in vivo*. Our results show that TBT and TPT behave as an antagonist of *Patella* sp. PPAR/RXR and that the tyrosine 277 is important, but not essential in the response to TBT. Overall, these results suggest a relation between the response of the mollusc PPAR-RXR to TBT and the lipid profile alterations reported at environmentally relevant concentrations. Our findings highlight the importance of comparative analysis between protostome and deuterostome lineages to decipher the differential impact of environmental chemicals.

Graphical abstract



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Keywords

PPAR; Lipid metabolism; Mollusca; Organotins; *Patella sp*

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Regional Studies in Marine Science
Volume 48, November 2021, 102019

Green treatment of chromium contaminated water using *Spongomorpha indica*

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https://doi.org/10.1016/j.rsma.2021.102019

Highlights

- *Spongomorpha indica* was found to be reliable biosorbent for removal of Cr(VI) from wastewater.
- Maximum biosorption of 95.29% Cr(VI) removal was obtained using RSM optimization.
- Isothermal and Kinetic studies disclosed that Cr(VI) biosorption is due to chemisorption.
- FTIR analysis of biosorbent revealed that functional groups like α, β unsaturated ketones and alkenes are involved in metal binding.

Abstract

The current study presents chromium (VI) biosorption performance of *Spongomorpha indica* from synthetic medium. *Spongomorpha indica*, naturally available green marine alga was selected as an adsorbent. The influence of three process parameters i.e., initial pH, initial chromium (VI) ions concentration and *Spongomorpha indica* biomass dosage, on the performance of biosorbent was studied. CCD of RSM was adopted to optimize process parameters and the results were analyzed using ANOVA to determine significance of influential parameters and their interaction effect on process efficiency. The predicted optimum values i.e., the biosorption 95.29%, pH 5.62, initial Cr(VI) concentration 31.82 mg/L and *Spongomorpha indica* biomass dosage 0.04 g/L through statistical optimization were found to be approximately equal to the confirmation experiment measured values. The results of kinetic studies revealed that the mechanism involved in metal capturing by biosorbent is ionic interaction between metal cations and various surface ionic groups of biomass with heterogeneous mass transfer. Equilibrium isotherm studies disclosed that Langmuir model was well correlated to equilibrium data. FTIR and SEM analysis were recorded for surface characterization of *Spongomorpha indica*.

Graphical abstract

Potential of artificial intelligence to accelerate diagnosis and drug discovery for COVID-19

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ABSTRACT

The coronavirus disease (COVID-19) pandemic has caused havoc worldwide. The tests currently used to diagnose COVID-19 are based on real time reverse transcription polymerase chain reaction (RT-PCR), computed tomography medical imaging techniques and immunoassays. It takes 2 days to obtain results from the RT-PCR test and also shortage of test kits creating a requirement for alternate and rapid methods to accurately diagnose COVID-19. Application of artificial intelligence technologies such as the Internet of Things, machine learning tools and big data analysis to COVID-19 diagnosis could yield rapid and accurate results. The neural networks and machine learning tools can also be used to develop potential drug molecules. Pharmaceutical companies face challenges linked to the costs of drug molecules, research and development efforts, reduced efficiency of drugs, safety concerns and the conduct of clinical trials. In this review, relevant features of artificial intelligence and their potential applications in COVID-19 diagnosis and drug development are highlighted.

Subjects Bioinformatics, Drugs and Devices, Computational Science
Keywords Machine learning, Neural networks, Drug discovery, Reverse transcriptase polymerase chain reaction, Artificial intelligence, Computed tomography, SARS CoV-2, Pharmacogenomics, Homology modeling, Protein prediction

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Additional Information and Declarations can be found on page 9

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INTRODUCTION

The COVID-19 pandemic is a worldwide health crisis. The causative agent for COVID-19 disease is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (*Singhal, 2020*). Various vaccines and drugs are being tested in trials for the treatment of COVID-19 disease and released in to the market for emergency purpose. SARS-CoV-2 infects the respiratory tract, mainly the lungs that leads to acute respiratory syndrome and finally death in severely ill and co-morbid patients. One challenge facing the health care workers is identifying people with COVID-19 (*Wu et al., 2020*). The disease is diagnosed using the test reverse transcriptase polymerase chain reaction (RT-PCR), which is the preferred method. However, the time taken to run the test and return the results is 2 days. Furthermore, there is an under-supply of RT-PCR test kits, lack of advanced technologies

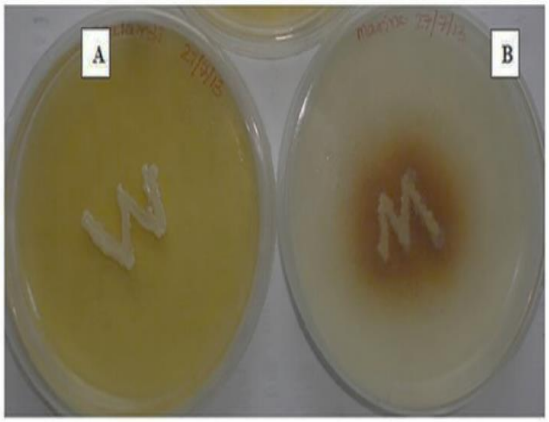
A blue enzyme from marine bacterium for green technological applications

Abraham P. Karlapudi & Vidya P. Kodali

Pages 167-168 | Received 06 Apr 2021, Accepted 16 Jun 2021, Published online: 13 Jul 2021

Abstract

Laccases are the green tools that can find potential applications in various industries. There are many reports available on laccases from plants and fungal sources but very few reports are available on bacterial laccases. Bacterial laccases show broad range of substrate specificity and it is easy to isolate and purify the bacterial extracellular laccases as compared to fungal laccases. Therefore, there are many advantages of bacterial laccases over fungal laccases.



Keywords: Laccase | *Acinetobacter* | marine bacterium

Journal of Genetic Engineering and Biotechnology

RESEARCH Open Access

Antioxidant potential and optimization of production of extracellular polysaccharide by *Acinetobacter indicus* M6

Ch. Ravi Teja[1], Abraham P. Karlapudi[2], Neeraja Vallur[3], K. Mamatha[1], D. John Babu[2], T. C. Venkateswarulu[2] and Vidya Prabhakar Kodali[1]*

Abstract

Background: Extracellular polysaccharides (ECPs) produced by biofilm-producing marine bacterium have great applications in biotechnology, pharmaceutical, food engineering, bioremediation, and bio-hydrometallurgy industries. The ECP-producing strain was identified as *Acinetobacter indicus* M6 species by 16S rDNA analysis. The polymer produced by the isolate was quantified and purified and chemically analyzed, and antioxidant activities have been studied. The face-centered central composite design (FCCCD) was used to design the model.

Results: The results have clearly shown that the ECP was found to be endowed with significant antioxidative activities. The ECP showed 59% of hydroxyl radical scavenging activity at a concentration of 500 μg/mL, superoxide radical scavenging activity (72.4%) at a concentration of 300 μg/mL, and DPPH$^{\cdot}$ radical scavenging activity (72.2%) at a concentration of 500 μg/mL, respectively. Further, HPLC and GC-MS results showed that the isolated ECP was a heteropolymer composed of glucose as a major monomer, and mannose and glucosamine were minor monomers. Furthermore, the production of ECP by *Acinetobacter indicus* M6 was increased through optimization of nutritional variables, namely, glucose, yeast extract, and $MgSO_4$ by "Response Surface Methodology". Moreover the production of ECP reached to 2.21 g/L after the optimization of nutritional variables. The designed model is statistically significant and is indicated by the R^2 value of 0.99. The optimized medium improved the production of ECP and is two folds higher in comparison with the basal medium.

Conclusions: *Acinetobacter indicus* M6 bacterium produces a novel and unique extracellular heteropolysaccharide with highly efficient antioxidant activity. GC-MS analyses elucidated the presence of quite uncommon (1→4)-linked glucose, (1→4)-linked mannose, and (→4)-GlcN-(1→) glycosidic linkages in the backbone. The optimized medium improved the production of ECP and is two folds higher in comparison with the basal medium. The newly optimized medium could be used as a promising alternative for the overproduction of ECP.

Keywords: Extracellular polysaccharide, Antioxidant activity, Response surface methodology, Monosaccharide composition

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Future Journal of Pharmaceutical Sciences

REVIEW Open Access

A review on importance of bioactive compounds of medicinal plants in treating idiopathic pulmonary fibrosis (special emphasis on isoquinoline alkaloids)

Sai Sushma Dudala[1], T. C. Venkateswarulu[1], Sushma Chandulee Kancharla[2], Vidya Prabhakar Kodali[3*] and D. John Babu[1*]

Abstract

Background: Idiopathic pulmonary fibrosis (IPF) is a fatal lung disease of unknown cause which disrupts the normal lung architecture and functions by deregulating immune responses and ultimately leads to the death of the individual. A number of factors can lead to its development and currently there is no cure for this disease.

Main text: There are synthetic drugs available to relieve the symptoms and decelerate its development by targeting pathways involved in the development of IPF, but there had also been various side effects detected by their usage. It is known since decades that medicinal plants and their compounds have been used all over the world in natural medicines to cure various diseases. This review article is focused on the effects of various natural bioactive compounds of 26 plant extracts that show prophylactic and therapeutic properties against the disease and so can be used in treating IPF replacing synthetic drugs and reducing the side effects.

Short conclusion: This review includes different mechanisms that cause pulmonary fibrosis along with compounds that can induce fibrosis, drugs used for the treatment of pulmonary fibrosis, diagnosis, the biochemical tests used for the experimental study to determine the pathogenesis of disease with a special note on Isoquinoline alkaloids and their role in reducing various factors leading to IPF thus providing promising therapeutic approach.

Keywords: Pulmonary Fibrosis, Idiopathic, Isoquinoline, Berberine

Background

Fibrosis is the surplus development of fibrous connective tissue in an organ that interferes or inhibits the normal function and architecture of the underlying organ or tissue. Fibrosis arises from a single cell line called fibromas which are benign tumours and are composed of fibrous or connective tissue. Rising from mesenchymal tissue, they can grow at any organ or tissue [1]. The formation of fibromas in the lungs is termed pulmonary fibrosis, which is also known as idiopathic pulmonary fibrosis (IPF). IPF is a disease or condition which arises spontaneously for which the cause is unknown. IPF is a progressive, age-related, devitalizing lung disorder that is fatal with a high mortality rate. Different disorders can arise during the wound healing process of the damaged or scarred lung tissue which can be characterized as fibroblasts differentiation, infiltration of inflammatory cells, extracellular matrix remodelling and collagen deposition [2]. In general, the extracellular matrix (ECM) is mainly constituted by collagens and it gives strength to the tissues. Amino acids such as glycine, proline and lysine

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Chapter 15 - Fiber degradation strategies of bacteria in rumen ecosystem

Satyanagalakshmi Karri, Manohar Babu Vadela, Vijay A.K.B. Gundi

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Abstract

The degradation of fiber by microbiota is an important and unique process occurring in ruminants. The fibrolytic bacterial consortia, primarily *Fibrobacter succinogenes* and *Ruminococcus* species, have different strategies for fiber degradation. It is very important to understand the enzymes and encoding genes of cellulolytic bacteria and the mechanism of cellulosome, a significant integral part of the fibrolysis. In this chapter, we discuss cellulolytic strategies, followed by rumen bacterial populations, molecular identification, and omics application in substrate degradation mechanism exploitation.

Keywords

Rumen bacteria; Cellulosome; FibroChip; Fiber-degrading enzymes

Journal of Genetic Engineering and Biotechnology

RESEARCH Open Access

Molecular characterization of coat color gene in Sahiwal versus Karan Fries bovine

Talla Sridhar Goud[1,2], Ramesh Chandra Upadhyay[1], Vijaya Bhaskar Reddy Pichili[3], Suneel Kumar Onteru[4] and Kiranmai Chadipiralla[2*]

Abstract

Background: *Melanocortin-1-receptor* gene (*MC1R*) plays a significant role in signaling cascade of melanin production. In cattle, the coat colors, such as red and black, are an outcome of eumelanin and pheomelanin pigments, respectively. The coat colors have become critical factors in the animal selection process. This study is therefore aimed at the molecular characterization of reddish-brown coat-colored Sahiwal cattle in comparison to the black and white-colored Karan Fries.

Results: The Sequence length of the *MC1R* gene was 954 base pairs in Sahiwal cattle. The sequences were examined and submitted to GenBank Acc.No. MG373575 to MG373605. Alignment of both (Sahiwal and Karan Fries) protein sequences by applying ClustalO multiple sequence alignment programs revealed 99.8–96.8% sequence similarity within the bovine. *MC1R* gene phylogenetic studies were analyzed by MEGA X. The gene *MC1R* tree, protein confines, and hereditary difference of cattle were derived from Ensemble Asia Cow Genome Browser 97. One unique single-nucleotide polymorphism (c.844C>A) (SNP) was distinguished. Single amino acid changes were detected in the seventh transmembrane structural helix region, with SNP at p.281 T>N of *MC1R* gene in Karan Fries cattle.

Conclusions: In this current research, we first distinguished the genomic sequence of the *MC1R* gene regions that showed evidence of coat variation between Indian indigenous Sahiwal cattle breed correlated with crossbreed Karan Fries. These variations were found in the *Melanocortin 1 receptor* coding regions of the diverse SNPs. The conclusions of this research provide new insights into understanding the coat color variation in crossbreed compared to the Indian Sahiwal cattle.

Keywords: Coat color, *Melanocortin 1 receptor* gene, Karan Fries cattle (Bos taurus taurus), Sahiwal (Bos taurus indicus), SNPs

Background

Coat color is a part of the major essential features for identifying the modern breeds of cattle. Each breed has its own specific phenotypic, physiological, hormonal, and metabolic functions to sustain and adapt to diverse agro-geographical and tropical climatic conditions. Coloration is an effective part of variable phenotypical traits in a diversity of animals, and there are several hypotheses for its function, such as camouflage and signaling of diverse detectable progression [1–3]. In mammals, coat color has been associated with their production and environmental adaptation. Darwin for the first time stated that a broad range of domestic animals shares multiple phenotypic characteristics, the most evident of which is a wide variety of coat colors [4, 5]. In vertebrates, the only character other than variable coat colors that occur The Sequence length of the *MC1R* gene was 954 base pairs. Coat color phenotypes are useful in the identification of diverse cattle breeds and other livestock. The coat color in animals is dependent on the percentage of eumelanin coating for black-brown pattern [6] to that of the pheomelanin coating for yellow-reddish pattern [7].

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Traditional Rice Beer of Assam, North East India: Traditionalism, Ethnobiology and its Pharmacomedicinal Trends

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Abstract

Assam which is an integral part of the North East India, well recognized for its tribal ethnicity. Several tribal communities reside in Assam and also in other parts of North East India. The major ethnic groups and tribes are Ahom, Bodo, Mishing, Karbi, Rabha etc. Among various practices that are followed by the tribes, the preparation of traditional rice beer is considered as a spiritual practice and plays an important role in their socio-economic development. Although rice is the primary ingredient used in the preparation of the rice beer, the method of preparation, the herbal components used during the preparation process vary among the various tribes. These traditional rice beers that are prepared are reported to be possessed with diverse medicinal properties and therapeutic values and hence, are considered as nutraceuticals. Owing to the uniqueness in their preparation, the type of herbal components present and the subtle differences that lie among the methods of preparation, traditional rice beer brewing techniques have gained scientific attention. This review article focuses on documentation of the differential methodologies and the type of the plant products that are used in the preparation of the rice beer.

Key words: Nutraceuticals, Ethnic groups and tribes, traditional rice beer, North-east India

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Functional Characterization of traditional rice based alcoholic beverages of Assam, North East India

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Abstract:
In North-East India, different types of alcoholic beverages hor arak, horalank, jou, jougwran, xaajpani, noginapong, sai mod and choko are traditionally prepared by tribal people viz., Karbi, Mishing, Bodo, Rabha, Ahom. They use it in a traditional manner and also play an important role in contributing to livelihoods by enhancing their income generation. Rice and certain selected medicinal plants are used as substrates in preparation of these beverages and hence possess high value among the tribal communities. Present study was aimed at determining the nutritional, nutraceutical and biochemical properties of various beverages prepared by different tribal communities. Qualitative and quantitative screening was performed using standard protocols. Phenolic and flavonoid contents were estimated spectrophotometrically using Folin- ciocalteu and aluminium chloride method, respectively. Alcohol content was determined using pycnometer. Electrolytes were calorimetrically estimated using electrolyte kit. The sai mod of Mishing community was found to be the sweetest. A blended sweet and sour taste was sensed in horalank and noginapong. Sai mod was found to contain highest carbohydrate content and electrolytes, such as sodium, potassium and chlorine. Titratable acidity was highest for horalank. Jougwran showed highest amount of reducing sugar and protein content. Glucose and ascorbic acid were highest in noginapong. Traditional use of medicinal plants during the preparation of these beverages could be attributed to their bioactive potential and health promoting properties.
Keywords: Traditional Rice Beer, Assam, ethnic, bioactive, characterization, health effect

Original Article | Published: 07 July 2020

Testosterone recuperates deteriorated male fertility in cypermethrin intoxicated rats

Vasudha Katragadda, Meghapriya Adem, Reshma Anjum Mohammad, Sainath Sri Bhasyam & Kishori Battini

Toxicological Research **37**, 125–134 (2021)

Abstract

The present study investigates the protective effects of testosterone against reproductive toxicity induced by cypermethrin (50 mg/kg body weight) in rats. Significant reduction in the testicular and accessory sex organ weights were observed in cypermethrin-treated rats over controls. Cypermethrin intoxication significantly reduced testicular daily sperm count, epididymal sperm count, sperm motility, sperm viability and HOS-tail coiled sperm accompanied by significant reduction in the activity levels of testicular steroidogenic enzymes such as 3β- and 17β- hydroxysteroid dehydrogenases in rats as compared to controls. Further, qPCR studies indicated that the mRNA expression levels of steroidogenic acute regulatory protein (StAR) significantly decreased in cypermethrin-treated rats over controls. Molecular docking analysis indicated that the binding affinity of cypermethrin (– 11.2 kcal/mol) towards StAR protein was greater as compared to its natural ligand, cholesterol (– 8.2 kcal/mol) suggesting improper cholesterol channeling across the testis. Significant reduction in the circulatory levels of testosterone was also recorded in cypermethrin-exposed rats. An increase in pre- and post-implantation loss was observed in rats cohabited with cypermethrin-treated rats. On the other hand, testosterone (4.16 mg/kg body weight) treatment ameliorated cypermethrin-induced reprotoxic effects in rats. To conclude, cypermethrin-induced deterioration of suppressed reproductive performance in male rats could be linked to its antiandrogenic effects and on the other hand, testosterone-mediated protection of male reproductive health in cypermethrin-treated rats at least in part occurs via restoration of testosterone biosynthesis, spermatogenesis and sperm maturation events.

Original Article | Published: 03 March 2020

α-lipoic acid protects testis and epididymis against linuron-induced oxidative toxicity in adult rats

P. Prathima, K. Venkaiah, T. Daveedu, R. Pavani, S. Sukeerthi, M. Gopinath & Sri Bhashaym Sainath

Toxicological Research **36**, 343–357 (2020)

Abstract

Linuron is well known for its antiandrogenic property. However, the effects of linuron on testicular and epididymal pro- and antioxidant status are not well defined. On the other hand, α-lipoic acid is well known as universal antioxidant. Therefore, the purpose of this study was twofold: firstly to investigate whether linuron exposure alters antioxidant status in the testis and epididymis of rats and if so, whether the supplementation of α-lipoic acid mitigates linuron-induced oxidative toxicity in rats. To address this question, α-lipoic acid at a dose of 70 mg/Kg body weight (three times a week) was administered to linuron exposed rats (10 or 50 mg/Kg body weight, every alternate day over a period of 60 days), and the selected reproductive endpoints were analyzed after 60 days. Respective controls were maintained in parallel. Linuron at selected doses reduced testicular daily sperm count, and epididymal sperm count, sperm motility, sperm viability, and number of tail coiled sperm, reduced activity levels of 3β- and 17β-hydroxysteroid dehydrogenases, decreased expression levels of StAR mRNA, inhibition of testosterone levels, and elevated levels of testicular cholesterol in rats over controls. Linuron intoxication deteriorated the structural integrity of testis and epididymis associated with reduced the reproductive performance over controls. Conversely, α-lipoic acid supplementation enhanced sperm quality and improved the testosterone synthesis pathway in linuron exposed rats over its respective control. Administration of α-lipoic acid restored inhibition of testicular and epididymal enzymatic (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidise) and non-enzymatic (glutathione content), increased lipid peroxidation and protein carbonyl content produced by linuron in rats. α-lipoic acid supplementation inhibited the expression levels of testicular caspase-3 mRNA levels and also its activity in linuron treated rats. To summate, α-lipoic acid-induced protection of reproductive health in linuron treated rats could be attributed to its antioxidant, and steroidogenic properties.

ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy
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One-pot synthesis of thiazolo[3,2-*a*]pyrimidine derivatives, their cytotoxic evaluation and molecular docking studies

Thuraka Sekhar [a], Pinnu Thriveni [a], Annavarapu Venkateswarlu [a], Thathapudi Daveedu [b], Kotha Peddanna [c], Sri Bhashyam Sainath [b]

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Highlights

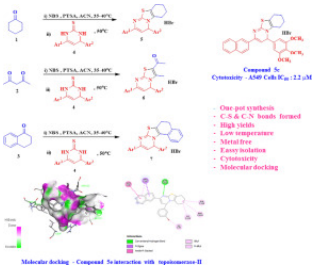
- The synthesized novel thiazolo[3,2-*a*]pyrimidine derivatives exhibited more potent anticancer activity.
- Molecular docking of synthesized compounds against topoisomerase-II reveals, the compounds showed best docking score than the standard doxorubicin.
- The significant features of this synthesis methodology are novel, simple, inexpensive experimental procedure, Short reaction time, Good yield, Low temperature and Metal free synthesis.
- The present research results may be considered for designing new class of drugs in anticancer treatment
- Thiazolo[3,2-*a*]pyrimidine derivatives were synthesized in one-pot approach and the structure of thiazolopyrimidines were characterized using FT-IR, ^{1}H NMR, ^{13}C NMR and HRMS techniques.

Abstract

An economical, simple and efficient one-pot method has been developed for the synthesis of thiazolo[3,2-*a*]pyrimidine hydrobromide derivatives. 2,4-diaryl-6,7,8,9-tetrahydro-4*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimidine hydrobromides were synthesized by the α-bromination of cyclohexanone with N-Bromosuccinamide (NBS) and followed by cyclization with 3,4-dihydropyrimidine-2(1*H*)-thiones, respectively, in the presence of p-toluenesulfonic acid (PTSA) in acetonitrile. However when cyclohexanone was replaced by acetyl acetone and alpha-tetralone gave the corresponding 1-(3-methyl-5,7-diaryl-5*H*-thiazolo[3,2-*a*]pyrimidin-2-yl)ethan-1-one hydrobromide and 9,11-diaryl-6,11-dihydro-5*H*-naphtho[1′,2′:4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide derivatives, respectively. The significant features of this method are novel, simple, inexpensive experimental procedure, short reaction time, and good yield. The some of the synthesized compounds were evaluated for cytotoxic activity against human lung adenocarcinoma cell line (A549), human breast carcinoma cell line (MCF-7), human cervical cancer cell line (HeLa) and human neuronal carcinoma cell lines (SKNSH). Tested compounds **5(b-e)** showed the excellent anticancer activity against various cell lines. Particularly compound **5c** with IC_{50} value of 2.2±0.6μM against A549 and compound **5e** with IC_{50} value of 5.6±0.4μM against HeLa showed best cytotoxic effects. Furthermore, Molecular docking study was performed for some of the synthesized compounds **5(b-e)** against topoisomerase-II by using Auto dock method. Docking results of the compounds **5c**, **5d**, and **5e** exhibited higher cytotoxic activity than the standard doxorubicin.

Graphical abstract

An efficient one-pot method has been developed for the synthesis of novel series of 2,4-diaryl-6,7,8,9-tetrahydro-4*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide, 1-(3-methyl-5,7-diaryl-5*H*-thiazolo[3,2-*a*]pyrimidin-2-yl)ethan-1-one hydrobromide and 9,11-diaryl-6,11-dihydro-5*H*-naphtho[1′,2′:4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide derivatives by the α-bromination of ketone (Cyclohexanone/acetyl acetone/alpha-tetralone) with N-Bromosuccinamide (NBS) and followed by cyclization with 3,4-dihydropyrimidine-2(1*H*)-thiones, respectively, in the presence of p-toluenesulfonic acid (PTSA) in acetonitrile. The significant features of this method are novel, simple, inexpensive experimental procedure, short reaction time, and good yield. The some of the synthesized compounds were evaluated for the cytotoxic activity against human lung adenocarcinoma cell line (A549), human breast carcinoma cell line (MCF-7), human cervical cancer cell line (HeLa) and human neuronal carcinoma cell lines (SKNSH). Tested compounds **5(b-e)** showed the excellent anticancer activity against various cell lines. Particularly compounds **5c** and **5e** with IC_{50} values of 2.2±0.6μM, and 5.6±0.4μM showed best cytotoxic effects against A549 and HeLa cancer cell lines. Furthermore, Molecular docking study was performed for synthesized compounds **5(b-e)** against topoisomerase-II by using Auto dock method. Docking results of the compound **5e** exhibited highest docking score than the standard doxorubicin.



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Keywords

Cytotoxicity; Molecular docking; NBS; PTSA; Thiazolo[3,2-*a*]pyrimidines; One-pot synthesis

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Chemosphere

Volume 249, June 2020, 126148

Phenotypic and transcriptomic changes in zebrafish (*Danio rerio*) embryos/larvae following cypermethrin exposure

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Abstract

Cypermethrin is one of the widely used type-II pyrethroid and the indiscriminate use of this pesticide leads to life threatening effects and in particular showed developmental effects in sensitive populations such as children and pregnant woman. However, the molecular mechanisms underlying cypermethrin-induced development toxicity is not well defined. To address this gap, the present study was designed to investigate the phenotypic and transcriptomic (next generation RNA-Seq method) impact of cypermethrin in zebrafish embryos as a model system. Zebrafish embryos at two time points, 24h postfertilization (hpf) and 48 hpf were exposed to cypermethrin at a concentration of 10μg/L. Respective control groups were maintained. Cypermethrin induced both phenotypic and transcriptomic changes in zebrafish embryos at 48 hpf. The phenotypic anomalies such as delayed hatching rate, increased heartbeat rate and deformed axial spinal curvature in cypermethrin exposed zebrafish embryos at 48 hpf as compared to its respective controls. Transcriptomic analysis indicated that cypermethrin exposure altered genes associated with visual/eye development and gene functional profiling also revealed that cypermethrin stress over a period of 48h disrupts phototransduction pathway in zebrafish embryos. Interestingly, cypermethrin exposure resulted in up regulation of only one gene, *tnnt3b*, fast muscle troponin isoform 3T in 24 hpf embryos as compared to its respective controls. The present model system, cypermethrin exposed zebrafish embryos elaborates the toxic consequences of cypermethrin exposure during developmental stages, especially in fishes. The present findings paves a way to understand the visual impairment in sensitive populations such as children exposed to cypermethrin during their embryonic period and further research is warranted.

Graphical abstract

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Research Article

Recovery of Prenatal Baicalein Exposure Perturbed Reproduction by Postnatal Exposure of Testosterone in Male Mice

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Baicalein (BC), a flavonoid, which lacks the qualities of reproductive health and shows adverse effects, is tested in this study. Inseminated mice were injected with 30, 60, and 90 mg BC/Kg body weight on gestation days 11, 13, 15, and 17. The F1 BC-exposed males of each dosage were divided into six groups. First three groups ($n = 6$ from each BC dosage) were used for assessment of reproductive performance, the others ($n = 4$ from each BC dosage) were administered with testosterone 4.16 mg/kg body weight on postnatal days 21, 31, and 41. The reproductive health of adult F1 males at the age of 55 and 60 was tested. Prenatal BC exposure showed reduced fertility after cohabitation with control females. The BC exposure significantly reduced the body weight, tissue indices, and sperm parameters (motility, count, viability, and daily sperm count) and altered the sperm membrane in a hypoosmotic swelling test. A downward trend was observed in testicular steroidogenic marker enzymes (3β- and 17β-steroid dehydrogenases) and serum testosterone, whereas increase in serum titers of FSH and LH along with altered the testicular histology. Conversely, testosterone (4.16 mg/kg body weight) partially recovered reduced male reproductive health by BC. BC impaired male reproductive health due to low levels of testosterone is reverted by external testosterone is evidenced in this study.

1. Introduction

The development of male reproductive tract is very sensitive to changes in hormones including estrogens, and thus minute changes can adversely affect the reproductive functions. In male, the correlation between reproduction and estrogen signalling occurs via the expression of estrogen receptors in the testis and accessory sex organs at all developmental stages: fetal, neonatal, and adult periods [1]. Earlier, it has been shown that the phytoestrogen-induced developmental and reproductive toxicity occurs via estrogen signalling [2, 3]. Previously, the reproductive toxic effects of phytoestrogen genistein, diadzein, and coumestrol have been demonstrated [4]. Studies of Brooks and Thompson [5] also indicated that the phytoestrogens can interfere with the steroidogenic pathway and subsequently affect androgen synthesis. A reduction in the testosterone levels has also been reported in coumestrol-exposed rats [6]. Neonatal studies

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Biochemical and molecular characterization of lactase producing bacterium isolated from dairy effluent

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ABSTRACT

In the present study, the microbial source for potent lactase producers was explored to supplement the lactase intolerant individuals. Dairy effluent was screened for lactase producing bacteria by conventional microbiological methods. Among the positive isolates, one isolate VUVD001 was found to be a strong producer of lactase enzyme and this strain identified by 16S rDNA analysis. The lactase producing bacterium is identified as *Bacillus subtilis* by biochemical and 16S rDNA analysis. The VUVD001 strain found to survive in a temperature range of 20–55 °C, pH range of 5–8 and a salt concentration up to 8%. Further, the partially purified enzyme preparation was tested by zymogram analysis for activity testing using x-gal and the enzyme activity of cell free supernatant was estimated to be 15.10 U/ml at 37 °C, 4% NaCl and pH of 7.0. Our finding reports a new isolate *Bacillus subtilis* VUVD001 strain which can be used as potential strain for commercial production of lactase.

1. Introduction

Lactose intolerance is the inability to hydrolyze dietary lactose due to the deficiency of lactase (Schrezenmeir and deVrese, 2001; Jost et al., 1998). About, 75 percent of population in the world was suffered from lactose intolerance, which significantly decreases their quality of life (Pribila et al., 2000; Venkateswarulu et al., 2017c; Peele et al., 2018). The lactase deficiency could be resolved by supplementary probiotics. The microbial production of lactase enzyme was found in yeast, fungi and bacteria (Rosenberg, 2006; He et al., 2008). Currently, the dairy industry uses probiotic strains namely, *Lactobacillus* and *Bifidobacterium* for the production of lactase (Ganeva et al., 2001). However, *Kluyveromyceslactis* is the most important strain for lactase production due to their dairy environmental habitat. But, its major drawback is lower thermostability (Chen et al., 2008). The bacterium *Lactobacillus acidophilus* isolated from fermented ragi (finger millet) produces thermostable lactase enzyme and this enzyme prevents microbial contamination in milk processing (Akolkar et al., 2006). Thus, the bacterial strains have considerable industrial potential for large scale production. The bacterial sources for production of lactase were extensively used for hydrolysis of lactose because of easy fermentation, high activity and good stability of enzyme (Picard et al., 2005; Natarajan et al., 2012; Venkateswarulu et al., 2017b). Lactase producing bacterial strains was used in treatment of milk based products in dairy industry (Sen and Srinivasa Babu, 2005). The enzyme activity was affected by different process variables like type of strain, cultivation conditions and the C:N ratio in the medium (Jurado et al., 2004). In the present study, isolation of lactase producing bacteria from a commercial dairy farm was attempted and also focused on lactase enzyme production from gram positive organism. The main aim of the present work is to screen the dairy effluent for isolation of potential microbial strain for bulk production of lactase such that the produced lactase from this isolate could be used as biotherapeutic agent for treating the patients with lactose intolerance.

2. Materials and methods

2.1. Screening and isolation of lactase producing bacteria

The bacterial strain, isolated from Sangam dairy industry effluent, Vadlamudi Village, Guntur (District), Andhra Pradesh, India.

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Short communication

Evaluation of anti-cancer, anti-microbial and anti-biofilm potential of biosurfactant extracted from an *Acinetobacter M6* strain

Abraham Peele Karlapudi [a,*], Venkateswarulu T.C. [a], Krupanidhi Srirama [a], Rohini Krishna Kota [a], Indira Mikkili [a], Vidya Prabhakar Kodali [b]

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ABSTRACT

Biosurfactants are amphiphilic compounds produced by bacteria either extracellularly or as a part of the cell membrane. Biosurfactants have had a profound impact on medical and pharmaceutical biotechnology. In our previous work, we isolated a new biosurfactant produced by *Acinetobacter indicus M6* which reduces the surface tension of water from 72.0 to 39.8 mN/m and which showed thermophilic, halophytic and acidophilic stability. The chemical nature was found to be a class of glycolipoprotein. Here, our research presents the extracted biosurfactant's anti-proliferative activity against lung cancer cells (A549), and anti-microbial and anti-biofilm activity against MRSA. The anti-tumour activity of biosurfactant against lung cancer cells was evaluated in terms of cell viability at different concentrations. The results showed a decrease in the percentage of lung cancer viable cells with increasing biosurfactant concentrations and incubation time, with a significant decrease being observed at 200 μg/ml concentration leading to cell proliferation inhibition at G1 phase. Treatment of biofilms for seven days at 500 μg/ml resulted in up to 82.5% biofilm disruption.

1. Introduction

Biosurfactants are amphiphilic surface-active compounds produced extracellularly by microbes with a profound impact on medicine, food and bioremediation fields. Biofilms comprise an exopolysaccharide (EPS) sheath which protects bacteria from unfavourable conditions. Chemical surfactants have been predominant on the market, while attention has more recently been diverted to extracting lower toxicity and higher biodegradability biosurfactants (Peele, 2016). Biosurfactants exhibit an interesting biological activity profile, and may be useful as anticancer drugs. The molecules are progressing to become highly suitable drug candidates against many infectious diseases; biosurfactants have anti-proliferative *in vitro* activity against human lung cancer cell lines as well as antimicrobial effects against selected pathogens (Karlapudi et al., 2018). Although many studies have found biosurfactants to be potential drug candidates in the antimicrobial field, their role has been poorly explored in the area of cancer biology. Here, our research explored biosurfactant molecules and resulted in designing powerful non-toxic and biocompatible anticancer agents. Biosurfactants inhibited proliferation of A549 lung cancer cells. The antiproliferative potency had no influence on non-tumour cell cultures. The evaluation of biosurfactants as an active compound showed that they inhibit DNA synthesis in cancer cells and are non toxic. *In vitro* evaluation of antiproliferative activity against cancer cell lines and cytotoxicity against normal cells was performed. In the next step, in order to identify the molecular mechanism involved in the anti cancer action of the biosurfactant and its effects on DNA synthesis, cell cycle progression was examined.

2. Materials and methods

2.1. Biosurfactant production and recovery

The high-yielding biosurfactant strain *Acinetobacter indicus M6* was used to produce biosurfactant (Accession No: KR559749). *Acinetobacter indicus M6* culture was grown in LB medium and

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Original Research

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Effect of cypermethrin on reproductive efficacy in zebrafish (*Danio rerio*): *In-vivo* and *in-silico* studies

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Authors Info

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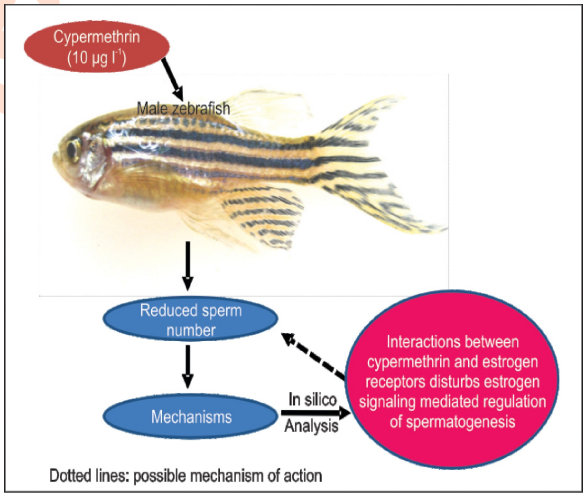
Abstract

Aim : The aim of the present study was to evaluate the reproductive efficacy of male and female zebrafish following cypermethrin exposure.

Methodology : The adult zebrafish (*Danio rerio*) of both sexes were exposed to cypermethrin at three selected concentrations 0.1, 1.0 and 10 μgl^{-1} over a period of 21days. After completion of experimental period, the reproductive endpoints such as fecundity, hatchability, testis and ovarian histology and plasma vitellogenin levels were selected and determined in this study.

Results : Cypermethrin exposure did not affect the cumulative fecundity rates in experimental fishes over controls. However, cypermethrin at 10 μgl^{-1} showed a significant reduction in the sperm number in male fishes over control. On the other hand, the same concentration of cypermethrin did not show significant changes in the plasma vitellogenin levels of both male and female fishes over their respective controls. Analysis of testicular and ovarian architectures of male and female zebrafish exposed to cypermethrin at 10 μgl^{-1} showed no marked differences over controls. In addition, molecular docking studies revealed that the binding energy between the cypermethrin and zebrafish estrogen receptor (zfER) β1 was almost similar to the binding energies exhibited by reference molecules, estradiol and ethinyl estradiol with zfERβ1. Further, binding energies between the ligands (cypermethrin and its metabolites phenoxybenzaldehyde and 3-phenoxybenzoic acid) with zfERα were low as compared to the binding energies between the reference molecules and zfERα.



Interpretation : *In-vivo* studies indicated that cypermethrin at 10µg l^{-1} leads to spermatotoxicity in zebrafish and *in silico* analysis showed that the cypermethrin at least in part interfere with the signalling of zfERα.

Key words: Cypermethrin, Estrogen receptors, Spermatogenesis, Zebrafish

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RECOVERY OF DIMINISHED SPERMATOGENESIS BY RESVERATROL AGAINST THE PYRETHROID, LAMBDA CYHALOTHRIN-INDUCED REPRO-TOXICITY IN ALBINO RATS

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Department of Biotechnology [2], Vikrama Simhapuri University, Kakutur, Nellore - 524320, Andhra Pradesh, India.

Keywords:

Lambda-cyhalothrin, Resveratrol, Rats, Spermatogenesis, Oxidative stress

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ABSTRACT: Lambda-cyhalothrin [-cyano-3-phenoxybenzyl 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate] is one of the type II synthetic broad-spectrum pyrethroids used to protect the crops against insects. The aim of the present study was to evaluate the protective effect of resveratrol on male reproductive health in lambda-cyhalothrin exposed rats. Exposure of male rats to lambda-cyhalothrin resulted in a significant reduction in the reproductive organ weights accompanied by a reduction in the testicular daily sperm count and epididymal sperm count, sperm motility, and sperm viability. Further, a significant decrease in the activity levels of superoxide dismutase and catalase with a significant increase in the levels of lipid peroxidation were observed in the testis of lambda-cyhalothrin administered rats over the controls. Moreover, the integrity of testicular architecture was deteriorated in lambda-cyhalothrin exposed rats. Conversely, supplementation of resveratrol enhanced the activity levels of testicular enzymatic antioxidants and inhibited lipid peroxidation levels in lambda-cyhalothrin exposed rats as compared to its respective controls. Significant increase in the selected epididymal sperm variables accompanied by the restoration of testicular architecture was recorded in resveratrol plus lambda-cyhalothrin treated rats over lambda-cyhalothrin exposed rats. On the other hand, no changes were observed in the selected reproductive endpoints in resveratrol administered rats over controls. To conclude, resveratrol could plausibly inhibit lambda cyhalothrin-induced testicular oxidative stress and improves the sperm quality and quantity in rats.

INTRODUCTION: Male infertility is a serious ongoing problem all over the world. Several studies claimed that the exposure of humans to a range of environmental contaminants, including pyrethroids causes several male reproductive disorders [1]. Pyrethroids are used to protect the crops against insects.

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Type II pyrethroids belong to the broad-spectrum potent organic insecticides which contain cyhalothrin rings and widely used in agricultural, veterinary and household applications [2]. Lambda-cyhalothrin (LCT), is one of the synthetic type II pyrethroid insecticides extensively used to control pests in food crops, non-food crops and against to kill disease vectors such as insect, ticks and flies [3].

Due to its wide usage, LCT has been detected in vegetables and fruits [4], milk and blood of dairy cows [5] as well as in cattle meat [6]. Although LCT exhibits low mammalian toxicity, several studies indicated that LCT exposure in mammals might cause genotoxicity, neurotoxicity, and mutagenicity

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Original Article

Detection and mode of action of retinoids on ovarian development in the mud crab, *Scylla serrata*

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Abstract: In the current study, the retinoic acid isomers such as 9-*cis* retinoic acid and all-*trans* retinoic acid were detected in the mature ovaries of mud crabs, *Scylla serrata* using HPLC analysis. Given the detection of retinoids in the ovaries, an attempt has been made to elucidate the possible role of retinoic acid in the regulation of reproduction in mud crabs. Injection of 9-*cis* retinoic acid induced ovarian maturation in intact mud crabs as evidenced by a significance elevation in the ovarian index (226.76%, $P<0.001$), and oocyte diameter (150.61%, $P<0.001$) accompanied by accumulation of yolk globules in the oocytes as compared to the untreated crabs. Further, a significant increase (258.63%, $P<0.0001$) in the circulatory ecdysteroid levels were also observed in 9-*cis* retinoic acid injected mud crabs over vehicle injected crabs. From the results, it can be postulated that retinoic acid-induced stimulation of ovarian maturation at least in part mediates ecdysteroids in the mud crabs.

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Ecdysteroids
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Ovaries
Oocyte diameter
Retinoic acid

Introduction

It is well-known that the ovarian development in decapods is primarily under the control of two antagonistic hormones viz., gonad/vitellogenin inhibiting hormone (GIH or VIH) of the major neuro-endocrine system located in the eyestalks, X-organ-sinus gland complex (XO-SG) and gonad/vitellogenin stimulating hormone (GSH or VSH) secretion from the brain and the thoracic ganglia, respectively (Subramoniam, 2017). In addition, ecdysteroids of Y-organs and methyl farnesaote of mandibular organs also play a crucial role in the regulation of crustacean ovarian development (Swetha et al., 2011). However, their precise crosstalk during ovarian maturation is not well-defined (Rotllant et al., 2018). Therefore, understanding the molecules that coordinate the crosstalk between the endogenous hormones are instrumental to gain insights into the regulatory effects of peptides, steroids and the terpenoids during the ovarian development of decapod crustaceans (Sainath et al., 2013).

Vitamin A (retinol: ROL) is a multifaceted molecule with wide array of functions in vertebrates. The biologically active metabolite of vitamin A, retinoic acid (RA) acts as one of the signaling molecules in the coordination of reproductive regulators in vertebrates (Andre et al., 2014). In addition, the role of RA in the regulation of a range of physiological processes such as embryonic development and organogenesis, tissue homeostasis, cell proliferation, differentiation, embryonic growth and development and vision is well acknowledged (Theodosiou et al., 2010; Clagget-Dame and Knutson, 2011; Macejova et al., 2016). Most notably, the biological functions of RA are operated via genomic actions where RA through the ligation of its cognate nuclear receptors [wherein 9-*cis* retinoic acid (9CRA) exerts its genomic actions via retinoic acid X receptors (RXRs) and retinoic acid receptor (RAR), whereas all-*trans* retinoic acid (ATRA) exerts its genomic action via RARs]. After ligand bounded, the RAR/RXR or RXR/RXR specifically binds the retinoid response elements on the DNA and

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Review

The evolutionary road to invertebrate thyroid hormone signaling: Perspectives for endocrine disruption processes ☆

S.B. Sainath [a b], A. André [a], L. Filipe C. Castro [a c l], M.M. Santos [a c l]

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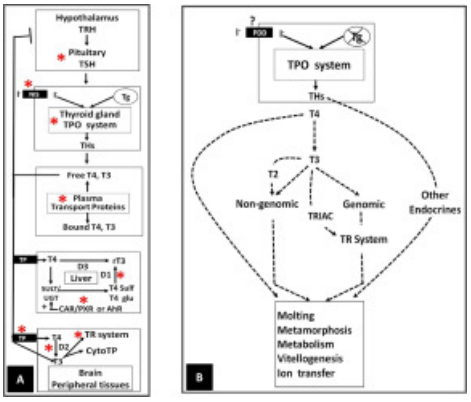
Abstract

Thyroid hormones (THs) are the only iodine-containing hormones that play fundamental roles in chordates and non-chordates. The chemical nature, mode of action and the synthesis of THs are well established in mammals and other vertebrates. Although thyroid-like hormones have been detected in protostomes and non-chordate deuterostomes, TH signaling is poorly understood as compared to vertebrates, particularly in protostomes. Therefore, the central objective of this article is to review TH system components and TH-induced effects in non-vertebrate chordates, non-chordate deuterostomes and protostomes based on available genomes and functional information. To accomplish this task, we integrate here the available knowledge on the THs signaling across non-vertebrate chordates, non-chordate deuterostomes and protostomes by considering studies encompassing TH system components and physiological actions of THs. We also address the possible interactions of thyroid disrupting chemicals and their effects in protostomes and non-chordate deuterostomes. Finally, the perspectives on current and future challenges are discussed.

Graphical abstract



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Karbala International Journal of Modern Science 4 (2018) 361–368
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Research Article

Isolation and characterization of bacteriocin producing *Enterococcus casseliflavus* and its antagonistic effect on *Pseudomonas aeruginosa*

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Available online 27 September 2018

Abstract

The discovery of antimicrobial bacteriocin molecules with broad-spectrum activity against bacterial infections caused by spoilage microorganisms is vital for food safety. In this study, a potential bacteriocin-producing bacterium was isolated, screened and confirmed as *Enterococcus casseliflavus* MI001 by 16s rDNA sequencing. The bacteriocin peptide was purified and analysed by MALDI-TOF, which revealed the presence of a bacteriocin ABC transporter protein with a molecular mass of 22.53 kDa. The purified bacteriocin gave a final yield of 2.0%, with a specific activity of 15,000 AU/mg. The bacteriocin showed antagonistic activity against *Pseudomonas aeruginosa* and has potential as an antioxidant.

Keywords: Bacteriocin; Probiotic; *Enterococcus casseliflavus*; Antimicrobial activity; Antioxidant activity

1. Introduction

Probiotics are beneficial bacteria that provide health benefits for the host when consumed at appropriate dosages [1]. Microbial flora play a key role in metabolic, physiological and immunological processes by producing and regulating immune cells in the intestine [2]. They benefit the host by increasing resistance to pathogen colonization, aiding the development of mucosal immunity, enhancing secretion of mucin and preventing the overgrowth of intestinal pathogens. Genera of *Lactobacillus, Bifidobacterium* and yeast are widely used as probiotics. Additional probiotic genera used in human therapeutic applications include *Enterococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Bacteroides* spp., *Propionibacterium* spp. *and Escherichia coli* [3]. Previous studies have reported that *Enterococci* are normal residents of the gastrointestinal tract [4] and *Enterococcus faecium* and *Enterococcus faecalis* are the predominant species in humans [5]. Many studies have reported that *in vitro* and *in vivo* properties vary

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Research Paper | Published: 29 June 2018

Purification and Lignocellulolytic Potential of Cellulase from Newly Isolated *Acinetobacter indicus* KTCV2 Strain

Abraham Peele Karlapudi, T. C. Venkateswarulu, Krupanidhi Srirama, Vijaya Ramu Dirisala, Bala Pratyusha Kamarajugadda, Rohini Krishna Kota & Vidya Prabhakar Kodali

Iranian Journal of Science and Technology, Transactions A: Science **43**, 755–761 (2019)

Abstract

A novel approach of cellulase-based co-culture producing bioethanol using low-cost nutrient medium has been employed for the study, and docking strategies provided the information of cellobiose and cellotetraose inhibitors during cellulase production. The *Acinetobacter* species was isolated from termite gut and confirmed in 16s rDNA analysis. SDS-PAGE reveals the molecular weight of purified cellulose was 45 kDa. Cellulose activity was achieved maximum of 1.2 IU/mL at 48th hour of incubation, and about 80% of polysaccharides were converted into simple sugars. The enzyme activity was optimum at different physiological conditions like temperature at 37 °C, pH 7.0 and with 4% concentration of banana peduncle extract powder. Upon reaching maximum cellulolytic activity of 0.594 IU/mL, the percentage of ethanol produced from cellulosic hydrolysate using *S. cerevisiae* reached maximum ethanol (18.3 g/L) during 48 h of incubation. Cellulase produced by *Acinetobacter indicus* KTCV2 strain exhibits a short incubation period (48 h) and produces cellulase in broader pH and temperature ranges. Banana peduncle offers a cheapest raw material composed of cellulosic biomass in agricultural practices, which served as a good substrate for the production of ethanol.

Short Communication | Published: 11 November 2018

Production of polyhydroxybutyrate from *Acinetobacter nosocomialis* RR20 strain using modified mineral salt medium: a statistical approach

A. R. Reddy, K. A. Peele, S. Krupanidhi, K. V. Prabhakar & T. C. Venkateswarulu

International Journal of Environmental Science and Technology **16**, 6447–6452 (2019)

Abstract

Eco-friendly biopolymers, polyhydroxybutyrate, have been produced by many kinds of bacteria that possess most important applications in food packaging industries and also in medical field. In the present work, PHB production was economized through the statistical optimization of nutritional components. The preferable carbon and nitrogen sources chosen for the enhanced production of PHB were molasses and ammonium sulphate. The relative rate of production has been studied by understanding the complex interactions of variables using face-centred central composite design. The submerged fermentation with *Acinetobacter nosocomialis* RR20 produced PHB yield of 7.82 g/L at optimized conditions with a notable value change of eightfold increase in comparison with minimal salt medium and these findings have showed that the designed medium was significant in terms of higher of PHB production.

Current Trends in Biotechnology and Pharmacy
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First page : (64) Last page : (71)
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Characterization of Bacteriocin Producing Probiotic Properties of *Enterococcus casseliflavus* MI001 Isolated from Curd Sample

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[2]Department of Biotechnology, Vikrama Simhapuri University, Nellore-524001, Andhra Pradesh, India

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Online published on 30 December, 2019.

Abstract

The identification of bacteriocin with a wide activity spectrum as a consequence of bacterial infections and spoilage microorganisms addresses an important aspect of food safety. In this study, the potential bacteriocin-producing bacterium was preliminarily confirmed as *Enterococcus* species and the isolate was identified as *Enterococcus casseliflavus* MI001. The probiotic properties of *E. casseliflavus* MI001 was studied for acid and bile tolerance tests. In addition, the aggregation and co aggregation ability of the strain to protect the host from colonization was studied. Further, the strain was also found antibiotic susceptibile against commonly available antibiotics.

Top

Keywords

Bacteriocin; Probiotic; *Enterococcus casseliflavus;* Antimicrobial activity.

Beni-Suef University Journal of Basic and Applied Sciences

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Research | Open Access | Published: 12 September 2019

Characterization of bacteriocin ABC transporter ATP-binding protein produced by a newly isolated *Enterococcus casseliflavus* MI001 strain

Indira Mikkili, Venkateswarulu TC, Abraham Peele Karlapudi, Vidya Prabhakar Kodali & Krupanidhi Srirama

Beni-Suef University Journal of Basic and Applied Sciences **8**, Article number: 5 (2019)

1143 Accesses | **4** Citations | Metrics

Abstract

Background

ATP-binding cassette (ABC) transporters constitute one of the largest transporter protein families and play a role in diverse biological processes.

Results

In the present study, bacteriocin isolated from the *Enterococcus casseliflavus* MI001 strain was identified as an ABC transporter ATP-binding protein. The optimal conditions for the production of bacteriocin were found to be at 35 °C, a pH 5.5, and an incubation time of 24 h. Purification was performed using ammonium sulphate precipitation, gel filtration, and DEAE ion exchange chromatography. The bacteriocin was purified with an eightfold purification scheme resulting with a specific activity of 15,000 AU/mg. The NMR spectrum of purified bacteriocin revealed the presence of amino acids, namely lysine, methionine, cysteine, proline, threonine, tryptophan, and histidine. Further, the bacteriocin ABC transporter showed antimicrobial activity against food spoilage microorganisms.

Conclusions

The ABC transporter ATP-binding protein could be used as a potential alternative for food preservation, and it may be considered as a bio-preservative agent in food processing industries.

1 Introduction

Living organisms depend on various means of transport for the uptake of external nutrients and sequestration of waste products into the surrounding environment [3]. ATP-binding cassette (ABC) transporters are

Animal Biotechnology ›
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302 Views | 6 CrossRef citations to date | 0 Altmetric

Articles

Identification and sequence characterization of melanocortin 1 receptor gene (*MC1R*) in *Bos indicus* versus (*Bos taurus X Bos indicus*)

Talla Sridhar Goud, Ramesh Chandra Upadhyay, Suneel Kumar Onteru, Vijaya Bhaskar Reddy Pichili & **Kiranmai Chadipiralla**

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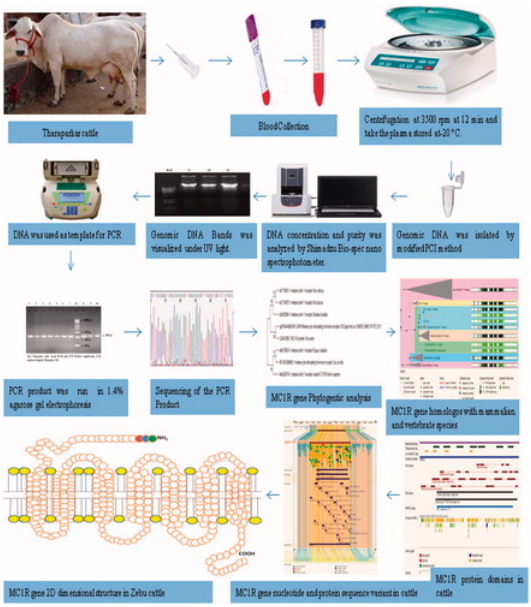
Abstract

Melanocortin 1 receptor (*MC1R*) plays a vital role in melanogenesis and determines coat color of mammals. Polymorphic variants in *MC1R*, causing coat color variation, were described in few mammals; however, such studies were not done in cattle. The objective of the study was to explore the association of *MC1R* gene polymorphism within Tharparkar (*Bos indicus*) and Karan Fries (*B. indicus X Bos taurus*) cattle. Genomic DNA isolated from blood samples of Tharparkar breed by modified Phenol: Chloroform; Isoamyl alcohol method. Using genomic DNA as template for PCR, *MC1R* gene was amplified and sequenced. The sequences were analyzed and submitted to Genbank with Acc.No MG373615-MG373644. Comparison of sequence alignment with other bovine species using ClustalW revealed 99–96% similarity. *MC1R* gene phylogenetic analyses were analyzed using MEGA X. The *MC1R* gene tree, protein domains and genetic variation of cattle were retrieved from Ensemble Asia Cattle Genome Browser. Eight single nucleotide polymorphisms (SNPs) (c.296T > C, c.583T > C, c.663C > T, c.830T > C, c.853G > A, c.880G > A, c.906C > G, c.927C > T) in CDS reveal high genetic variability. Subsequent to amino acid changes p.L99P, p.F195L, p.F277S, p.A285T and p.D293N, p.R302S, respectively found in seven-transmembrane. Mutations appeared in *MC1R* of *B. taurus* with white and black coat color as compared to *B. indicus* with white coat.



Keywords: *Bos indicus* Coat color melanocortin 1 receptor *Bos taurus* SNPs

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Disclosure statement

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Petroleum

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Role of biosurfactants in bioremediation of oil pollution-a review

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ABSTRACT

The energy resources mainly petroleum and petroleum hydrocarbons are major pollutants of the environment. The oil and oil products contamination may cause severe harm and hence. the attention has been remunerated in the development of alternative technologies for elimination of these contaminants. Biosurfactants were used in the remediation of oil pollution due to advantages such as biodegradability and low toxicity. The biosurfactants are produced from low cost substrates like agro-industrial wastes which reduce the cost of production. Biosurfactants and bioemulsifiers are amphiphilic compounds and are produced as extracellular or a part of the cell membrane by bacteria. The insight view, how hydrocarbons are degraded by microorganisms and thereby reduce the damage of ecosystem is highly essential to target the problem. Biofilms are the bacterial communities which protects the bacterial cells from various adverse conditions. The present review describes the biosurfactants and its synthesis from bacteria and also emphases on the role of surfactants in oil remediation.

1. Introduction

1.1. Hydrocarbon contamination sites

Rapid growth of industries leads to the environmental pollution and other environmental hazards. One of the prevalent ecological hazards is petroleum pollution, which show harmful effects on all aquatic living organism's particularly microbial population. The first step in this effect is hydrocarbon transportation to the surface of the microbial cell from oil phase to cell surface through the contact and then transportation across the cell membrane. Even though a great amount of work was done in this area, n-alkane transportation into the bacterial cell and assimilation mechanism of the hydrocarbons in the microbial cells were poorly understood [1]. It has already been reported that some bacterial populations exhibited resistance to oil transportation and also few bacterial population efficiently degrade oils/hydrocarbons. Two different types of interactions normally observed in the processes of oils/hydrocarbon biodegradation. Oil adhesion, pseudo-solubilization and degradation of hydrocarbons to form small droplets of oils are the sequential steps involved in one of the mechanisms. Microbial cells adhere to the drops of hydrocarbons whose size was less than the cells and the substrate uptake has taken place by active transport or by diffusion at the point of interference between cells and hydrocarbons [2]. Bioemulsifiers that reduce the surface tension are termed as biosurfactants. Biosurfactants may be located inside the cells (intracellular) or secreted outside the cells (extracellular) [3]. There are many reports available on bacterial biosurfactants, but the spectrum of activity depends on their chemical composition. A strain of *Pseudomonas aeruginosa* was reported to produce the rhamnolipid type biosurfactant which was mono as well as di-rhamnolipid [4]. It has been proved that the rhamnolipids and its producing microorganisms specifically degraded hexadecane, hence there is a clean correlation exists between the type of surfactant and the type of hydrocarbon/oil that gets degraded. It

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Research Article

Isolation and characterization of bacteriocin producing *Enterococcus casseliflavus* and its antagonistic effect on *Pseudomonas aeruginosa*

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Abstract

The discovery of antimicrobial bacteriocin molecules with broad-spectrum activity against bacterial infections caused by spoilage microorganisms is vital for food safety. In this study, a potential bacteriocin-producing bacterium was isolated, screened and confirmed as *Enterococcus casseliflavus* MI001 by 16s rDNA sequencing. The bacteriocin peptide was purified and analysed by MALDI-TOF, which revealed the presence of a bacteriocin ABC transporter protein with a molecular mass of 22.53 kDa. The purified bacteriocin gave a final yield of 2.0%, with a specific activity of 15,000 AU/mg. The bacteriocin showed antagonistic activity against *Pseudomonas aeruginosa* and has potential as an antioxidant.

Keywords: Bacteriocin; Probiotic; *Enterococcus casseliflavus*; Antimicrobial activity; Antioxidant activity

1. Introduction

Probiotics are beneficial bacteria that provide health benefits for the host when consumed at appropriate dosages [1]. Microbial flora play a key role in metabolic, physiological and immunological processes by producing and regulating immune cells in the intestine [2]. They benefit the host by increasing resistance to pathogen colonization, aiding the development of mucosal immunity, enhancing secretion of mucin and preventing the overgrowth of intestinal pathogens. Genera of *Lactobacillus, Bifidobacterium* and yeast are widely used as probiotics. Additional probiotic genera used in human therapeutic applications include *Enterococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Bacteroides* spp., *Propionibacterium* spp. *and Escherichia coli* [3]. Previous studies have reported that *Enterococci* are normal residents of the gastrointestinal tract [4] and *Enterococcus faecium* and *Enterococcus faecalis* are the predominant species in humans [5]. Many studies have reported that *in vitro* and *in vivo* properties vary

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SHORT COMMUNICATION

In silico sgRNA tool design for CRISPR control of quorum sensing in *Acinetobacter* species

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KEYWORDS
AbaI;
Acinetobacter baumannii;
CHOPCHOP;
Crispr-cas9;
sgRNA

Abstract CRISPR genome editing utilizes Cas9 nuclease and single guide RNA (sgRNA), which directs the nuclease to a specific site in the genome and makes a double-stranded break (DSB). Design of sgRNA for CRISPR-Cas targeting, and to promote CRISPR adaptation, uses a regulatory mechanism that ensures maximum CRISPR-Cas9 system functions when a bacterial population is at highest risk of phage infection. *Acinetobacter baumannii* is the most regularly identified gram-negative bacterium infecting patients. Recent reports have demonstrated that the extent of diseases caused by *A. baumannii* is expanding and, in a few cases, now surpasses the quantity of infections caused by *P. aeruginosa*. Most *Acinetobacter* strains possess biofilm-forming ability, which plays a major role in virulence and drug resistance. Biofilm bacteria use quorum sensing, a cell-to-cell communication process, to activate gene expression. Many genes are involved in biofilm formation and the mechanism to disrupt the biofilm network is still not clearly understood. In this study, we performed in silico gene editing to exploit the *AbaI* gene, responsible for biofilm formation. The study explored different tools available for genome editing to create gene knockouts, selecting the *A. baumannii AbaI* gene as a target.

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Optimization of process parameters for Poly Hydroxy Butyrate Production from Isolated *Acinetobacter nosocomialis* RR20 through Submerged Fermentation

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Abstract

Poly Hydroxy Butyrate member of polyhydroxyalkanoates family and is generally used as an alternative to polypropylene based plastic. Production of Poly Hydroxy Butyrate, biodegradable polymer from industrial wastes has several advantages such as recycle of waste and the production of high valuable products. It has been isolated from various sources till date. In this study, different strains of isolated bacteria were evaluated for their PHB productivity, but *Acintobacter nosocomialis* RR20 strain resulted highest production. Therefore, optimization of process parameters was determined in batch fermentation mode using one-parameter -at a-time approach for enhanced production of PHB. The influence of physical and chemical variables namely incubation temperature, incubation time, inoculums size, pH, carbon source, nitrogen source and mineral salts were studied for improving the production of PHB. Maximum PHB was found 4.17 g/L at optimized conditions of incubation period 48 h, temperature 37 °C, pH 7.0, inoculums size of 4%, 30g/L molasses, 3g/L Ammonium sulphate and $MgSO_4$ 0.3g/L.

Key words: PHB, *A.nosocomialis RR20*, Shake flask culture, Process variables

Introduction

In our day-to-day life polypropylene based synthetic polymers have become an integral part. The compounds produced from fossil resources like polyvinylchloride, polyhomopropylene, polyethylene and others include desirable properties like durability and resistance to degradation. The non-biodegradable plastics pose serious threat to the surroundings by accumulating in the global environment at a rate of 25 million tons per year (1). Poly-3-hydroxybutyrate (PHB) is the most predominant member of the family of PHAs (2,3). PHB is a biodegradable polymer and serves as a source for biodegradable plastics. Poly-3-hydroxybutyrate (PHB) is similar compound like Polyhydroxyalkanoate (PHA), emerged recently as an alternative for synthetic plastics as its structural properties are similar to polypropylene (4-6) and yet it is completely biodegradable (7-9).PHA's are widely used in the manufacturing of surgical pins, sutures, staples, wound dressing, stimulation of bone growth, replacement of bones, blood vessel replacements, and packaging industry (includes films, bags etc)(10-12). Polyhydroxybutyrate (PHB) is a biodegradable polymer synthesized and accumulated in cells as intracellular granules (inclusion bodies) by a diverse group of bacteria (13-15) because of nutritional limitation or excess carbon in the growth media and they are biodegraded by the bacteria itself. In previous studies many researchers reported the production of PHB from different bacterial species such as *Bacillus* sp., *Pseudomonas* sp., *Methylobacterium* sp.,

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Novel extraction of high quality genomic DNA from frozen bovine blood samples by using detergent method

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Abstract
DNA is the prerequisite for life's inception that transfers hereditary information, past several years; various types of commercial kits are made available which vary depending on the type of the biological sample being used. The present study is focused on developing an improvised methodology for the isolation of genomic DNA from stored bovine blood samples. DNA was isolated by using the conventional Phenol: Chloroform: Isoamyl alcohol (PCI) method and Detergent method. The aim of the study was to make a comparative analysis and evaluation of these two methods to identify the one that gives a superior quality and quantity of genomic DNA. Total (*n=48*) each duplicate blood samples from three different buffalo(*Bubalus bubalis*) breeds Banni, Surti, Murrah, three zebu cattle (*Bos indicus*) breeds Kankerj, Gir, Sahiwal were collected from the jugular vein. The quantity, purity of the genomic DNA was assessed based on the total DNA yield, purity ratios, spectral profile, agarose gel electrophoresis analysis and polymerase chain reaction amplification of MC1R gene product without any inhibitors. The results of our study suggest that detergent method is also suitable for extraction of genomic DNA from the bovine blood and results were significant (**P>0.05*). The total mean yield was found to be 329.05±11 µg/5ml for all six breeds while the PCI method was employed. The total mean yield of the gDNA for all six breeds was 406.6±43 µg/5ml of blood when the detergent method was used. One way ANOVA test showed that the total DNA yield varied depending on the isolation method used. The DNA yield obtained from the DG method was (***P< 0.001*) significant as compared to the PCI method *(**P<0.01*).
Keywords: DG method, gDNA, Melanocortin-1-receptor gene, PCI method, Total lymphocytes.

Introduction

In current modern era growth of molecular biology, animal biotechnology, veterinary and agricultural science large practical applications of their achievement have been observed. Genomic research area benefits for the large genomic population studies for human, animal, plant and other mammalian species. The ruminant livestock in sustainable agricultural systems plays a major significant role (Hackmann and Spain, 2010). Genomic DNA considers as a major important molecule in genetic testing (Bakker, 2006).

Isolation of genomic DNA is a most important step in a variety of clinically related studies including genetics, genomics, gene polymorphism, DNA fingerprinting and gene sequencing. These studies exploit a number of techniques to facilitate which include, restriction fragment length polymorphism (RFLP), real-time polymerase chain reaction (RT-PCR), Sanger-sequencing and microarrays and Next-generation sequencing (Bakker, 2006). Whole blood is one of the accessible sources to obtain the genomic DNA. Consistency, possibility, and reproducibility of molecular inheritance studies are often limited by the primary step of the DNA isolation. To obtain a large amount of genomic DNA from cells and tissue is often a laborious task. DNA isolation methods should be ideally is efficient, reliable, quick and reproducible. Whole blood is a vital resource for the isolation of genomic DNA from bovine and yak (Neary *et al*., 2014).

In molecular biology studies, which includes the breed recognition, food nutritional functions and food traceability, genetic difference along with animals, marker-assisted studies of breeding, in genetic hybridization studies requires high-quality genomic DNA for southern blotting test (Murphy *et al*., 2002). The source of genomic DNA from bovine mainly blood and other tissue parts which require professional people and it was a laborious process to get the samples.

In the present research, various techniques available to isolate the genomic DNA from blood and also other biological materials like nails, meat, semen, and hair

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α-lipoic acid inhibits oxidative stress in testis and attenuates testicular toxicity in rats exposed to carbimazole during embryonic period

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ARTICLE INFO

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ABSTRACT

The aim of this study was to evaluate the probable protective effect of α-lipoic acid against testicular toxicity in rats exposed to carbimazole during the embryonic period. Time-mated pregnant rats were exposed to carbimazole from the embryonic days 9–21. After completion of the gestation period, all the rats were allowed to deliver pups and weaned. At postnatal day 100, F1 male pups were assessed for the selected reproductive endpoints. Gestational exposure to carbimazole decreased the reproductive organ indices, testicular daily sperm count, epididymal sperm variables *viz.*, sperm count, viable sperm, motile sperm and HOS-tail coiled sperms. Significant decrease in the activity levels of 3β- and 17β-hydroxysteroid dehydrogenases and expression of StAR mRNA levels with a significant increase in the total cholesterol levels were observed in the testis of experimental rats over the controls. These events were also accompanied by a significant reduction in the serum testosterone levels in CBZ exposed rats, indicating reduced steroidogenesis. In addition, the deterioration of the testicular architecture and reduced fertility ability were noticed in the carbimazole exposed rats. Significant reduction in the activity levels of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase and reduced glutathione content with a significant increase in the levels of lipid peroxidation were observed in the testis of carbimazole exposed rats over the controls. Conversely, supplementation of α-lipoic acid (70 mg/Kg body-weight) ameliorated the male reproductive health in rats exposed to carbimazole during the embryonic period as evidenced by enhanced reproductive organ weights, selected sperm variables, testicular steroidogenesis, and testicular enzymatic and non-enzymatic antioxidants. To conclude, diminished testicular antioxidant balance associated with reduced spermatogenesis and steroidogenesis might be responsible for the suppressed reproduction in rats exposed to the carbimazole transplacentally. On the other hand, α-lipoic acid through its antioxidant and steroidogenic properties mitigated testicular toxicity which eventually restored the male reproductive health of carbimazole-exposed rats.

1. Introduction

Antithyroid drugs continue to be the first-line treatment to manage hyperthyroidism and also to control thyroid function before surgery. The mode of action of antithyroid drugs is via blockage of iodine organification and iodine-tyrosine coupling, thereby biosynthesis of thyroid hormones (THs) from the thyroid gland. Antithyroid drugs such as polypropylthiouracil (PTU), carbimazole (CBZ) and its metabolite methimazole (MMI) are widely used all over the world [1]. Despite their promising therapeutic potential, the clinical success of antithyroid drugs is often limited by deleterious side effects such as nephrotoxicity, neurotoxicity, hepatotoxicity, acute pancreatitis, thyroid carcinoma and testicular toxicity [2–6]. Further, there is a major concern towards the usage of antithyroid drugs in pregnant women, because these drugs can readily cross the placenta and affect fetal development [1]. Rodent studies indicated that perinatal exposure to CBZ causes disorganization of thyroid gland [7] and administration of PTU and MMI during the pre- (via the placenta) and peri-natal (via the placenta and through the milk) periods not only resulted in neurotoxicity [8] but also caused testicular and epididymal toxicity accompanied by the reduced serum testosterone levels [9–13]. Further, studies of Calanis-Continente et al. [4] reported the clinical history of a woman with a necrotizing

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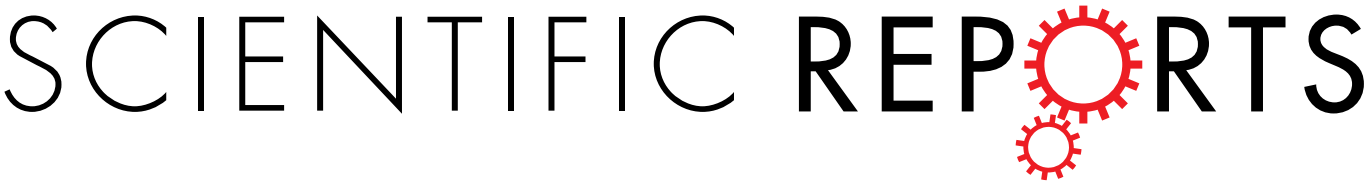

OPEN

Novel 1, 4-dihydropyridines for L-type calcium channel as antagonists for cadmium toxicity

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The present study, we design and synthesize the novel dihydropyridine derivatives, i.e., 3 (a-e) and 5 (a-e) and evaluated, anticonvulsant activity. Initially due to the lacuna of LCC, we modeled the protein through modeller 9.15v and evaluated through servers. Docking studies were performed with the synthesized compounds and resulted two best compounds, i.e., 5a, 5e showed the best binding energies. The activity of intracellular Ca^{2+} measurements was performed on two cell lines: A7r5 (rat aortic smooth muscle cells) and SH-SY5Y (human neuroblastoma cells). The 5a and 5e compounds was showing the more specific activity on L-type calcium channels, i.e. A7r5 ($IC_{50} = 0.18 \pm 0.02$ and $0.25 \pm 0.63\,\mu g/ml$, respectively) (containing only L-type channels) than SH-SY5Y (i.e. both L-type and T-type channels) ($IC_{50} = 8 \pm 0.23$ and $10 \pm 0.18\,\mu g/ml$, respectively) with intracellular calcium mobility similar to amlodipine. Finally, both *in silico* and *in vitro* results exploring two derivatives 5a and 5e succeeded to treat cadmium toxicity.

Channels are the building blocks for many metabolic regulations and act as check boundaries for the entry of many nutrients and minerals. Toxic metals may also enter through these channels and causes adverse metabolic regulations thereby leading to cell poison. The Entry of heavy metals like cadmium through channel plays the crucial role and key finding in cadmium toxicity. Present scenario, the understanding the cadmium entry through channels and targeting these channels with drug discovery process are very attractive and novel to treat the toxicity. Cadmium (Cd) is an extremely toxic metal commonly found in industrial workplaces. It is also a food contaminant and a major component of cigarette smoke. Cd can enter the brain parenchyma and neurons, causing neurological alterations in humans and animal models, leading to lower attention, hypernociception, olfactory dysfunction, and memory deficits[1].

Cd influx mediates voltage-gated calcium channels (VDCCS) in excitable cells[2] including mammalian neurons and also Cd uptake in non-excitable tissues[3]. They are transmembrane proteins, which are playing an integral role in the entry of Ca^{2+} permeation in excitable cells and also in controlling synaptic transmission, muscle contraction, gene transcription, cell division, cell death, hormone (or) Neuro transmitter and signal transduction pathways[4]. L-type Ca^{2+} channels (LCCs) are multi subunit complex and heteromeric proteins consisting of the pore forming appha-1 ($\alpha 1$) subunit, disulfide-linked transmembrane complex of alpha-2 ($\alpha 2$), intra cellular beta (β) subunit, delta (Δ) and a gamma (γ) subunit characteristics of skeletal muscle Ca^{2+} channels in a 1:1:1:1 ratio[5,6]. The pore forming $\alpha 1$ subunit directs the channel activity that displays the major pharmacological and electrophysiological properties. The pore forming$\alpha 1$ subunit of LCCs folds from a single polypeptide chain, composed of four distinct repeats (I-IV), each repeat formed by six transmembrane segments (S1-S6). S1-S4segments have a voltage sensing domain, an outer helix S5, an inner helix S6 and a membrane dividing P-loop between S5-S6. The EEEE ring (selecting filter glutamates) in four repeats (I-IV) of LCC[5,6]. This ring in P-loops forms the selectivity filter for metal ions. Calcium channel blockers proceed onion conducting cell membrane channels.

The 1,4-dihydropyridine (DHP) class of calcium channel blockers are widely utilized in the treatment of cardiovascular diseases such as hypertension, angina pectoris, and other spastic smooth muscle disorders[1]. The SAR of calcium channel blockers signifies the presence of ester linkage and electron withdrawing groups like

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RESEARCH ARTICLE

α-Lipoic acid inhibits testicular and epididymal oxidative damage and improves fertility efficacy in arsenic-intoxicated rats

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Abstract

The present study evaluates the protective effect of α-lipoic acid (LA) against arsenic-induced testicular and epididymal oxidative damage in rats. Arsenic caused significant reduction in the reproductive organ weights, serum testosterone levels, testicular daily sperm count, epididymal sperm count, sperm motility, sperm viability, and sperm membrane integrity. Significant reduction in the activity levels of superoxide dismutase, catalase, and glutathione levels with a concomitant increase in the lipid peroxidation and protein carbonyl content in the testis and the cauda epididymis of arsenic-exposed rats. Arsenic intoxication also enhanced the testicular caspase-3 mRNA levels, disorganization of testicular and cauda epididymal architecture as well as increased arsenic content in the testis and the cauda epididymis of rats. Arsenic exposure also deteriorated fertility ability in male rats over controls. Conversely, α-LA negated the testicular and cauda epididymal oxidative stress and restored the male reproductive health in arsenic-exposed rats.

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PRELIMINARY STUDIES ON THE EFFECT OF RETINOIDS ON OVARIAN MATURATION IN SELECTED EDIBLE CRUSTACEANS OF AQUACULTURE IMPORTANCE

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ABSTRACT

Eyestalk ablation (removal of eyestalks) is a classical technique performed by aqua-farmers to induce seed in hatchery industry. However, this operational technique often leads to deterioration of seed quality and also leads to mortality in the brood stock. Among several strategies to overcome this problem, endocrine manipulation approach is well acknowledged. The present study was aimed to investigate the effect of retinyl palmitate on ovarian growth in selected fresh water crabs, *Oziotelphusa senex senex* and prawns, *Macrobrachium malcomsonii*. Intermolt stage female crabs were allocated into four groups (n= 15 per group). Crabs in group 1 served as controls (no treatment). Whereas, crabs in groups 2, 3 and 4 were treated as experimental groups and received different doses of retinyl palmitate (1, 5 and 10 µg/g, respectively) on days 1, 7 and 14 over a period of 21 days. Similarly, female prawns were subdivided into controls (no treatment) and experimental groups (n= 20 per group). Prawns in experimental group received retinyl palmitate at a dose of 10 µg/g body weight and followed same experimental regimen as that of crabs. Injection of retinyl palmitate to crabs and prawns showed a significant increase in the weights of ovaries, with a significant increase in the oocyte diameter of treated crabs and prawns over their respective controls. Retinoid-induced reproductive effects in crabs were dose-dependent. Further, histological studies of ovaries revealed that the crabs and prawns injected with retinyl palmitate showed vitellogenic stage as evidenced by accumulation of yolk globules in the occyte, whereas such changes were not observed in untreated crabs and prawns. From these preliminary results, it can be suggested that retinyl palmitate may be used as a tool to promote ovarian maturation in edible crustaceans. However, in-depth studies such as understanding the portfolio of retinoid signalling cascade and its crosstalk with endogenous hormonal factors are needed to develop an alternative strategy against eyestalk ablation to induce seed in hatchery industry.

***Keywords*:** Aquaculture, crustaceans, eyestalk ablation, hormone manipulation, sustainable hatchery industry

1. *Introduction*

Crustaceans such as shrimps, prawns, lobsters, crayfishes and crabs play an important role in the aquaculture industry, gaining popularity day-by-day in recent years. The increasing demand for high-protein food from aquatic sources and also to find an alternate for fisheries has given rise to a worldwide expansion of shellfish culture. The development of intensive aquaculture has opened a new field in the engineering area and would be a challenge for biotechnological research. Animal species important for aquaculture have evolved many complex reproductive strategies, although most of them are still poorly understood. Indeed, reproduction and other physiological aspects have been studied in only a limited number of species.

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Isolation and Identification of PolyHydroxyButyrate (PHB) producing bacteria from Sewage sample

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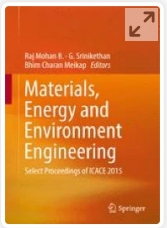
Abstract

Plastics and synthetic polymers are synthesized from nonrenewable resources like petrochemicals and persist in the environment long after intended use, resulting into problems of solid waste management and global environmental pollution. Hence, an alternative source such as Polyhydroxyalkanoates that are biodegradable, linear polyesters produced primarily by bacteria which can be used as an effective thermoplastic, and has many characteristics similar to those of standard commercial plastics like polypropylene. Aliphatic polyester, poly3-hydroxy butyrate was discovered and identified as a granular component in bacterial cells. PHB can grow in a wide variety of natural environments and is the reserve polymer found in many species of bacteria found in nature, e.g. in soil, sea water, sewage waste or compost. In this present study high PHB producing strains were isolated from sewage sample. Five strains were showing PHB granules with Sudan Black B staining. The five strains were labeled as strain 2, 4, 5, 9 and 11. Further, they were morphologically and biochemically characterized. Growth profiles were studied for all these strains and were found that the PHB was produced maximum after 48 hrs at 37°C of incubation. Strain 2 showed high PHB production among the five strains isolated. The sugarcane molasses used in the medium for PHB production accounted for the least production cost.

Top

Keywords

Polyhydroxy Butyrate, Biopolymer, Thermoplastic, Municipal sewage.



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Optimization Study of Cadmium Biosorption on Sea Urchin Test: Application of Response Surface Methodology

D John Babu, Y Prasanna Kumar, Pulipati King & K Vidya Prabhakar

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Abstract

Cadmium (Cd) is an occupational environmental contaminant gets transported through air, water and soil. The nephrotoxicity of Cd is well authenticated. Hence, to study the individual and interaction effects of different physicochemical parameters namely, temperature, pH, biomass dosage, initial Cd ion concentration and to determine the optimum values of process conditions to maximize the removal of Cd from the aqueous synthetics solutions, Box–Behnken design using response surface methodology was employed. Regression analysis indicated that quadratic model was highly significant. Maximum Cd removal of 89.143 %with sea urchin test as biosorbent was obtained using optimization by response surface methodology at optimum conditions of pH 5.97, temperature 31.98 °C, initial concentration of Cd, 20.56 mg/l and biosorbent loading of 0.5 g/l.

Keywords

Biosorption **Cadmium** **Sea urchin test**

Response surface methodology **Box-Behnken design**

Design of an economically feasible nutrient medium for microorganisms using banana waste

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Abstract

The management of the generated waste is a major problem in developing countries. The waste generated in the agroprocessing has been used as sources of nutrients for the microbes. In the present study, a medium has been formulated with banana fruit stalk and the microbial growth was monitored. Growth and biomass production was examined on banana stalk agar (BSA) and broth (BSB), banana stalk dextrose agar (BSDA) and broth (BSDB) using commercial potato dextrose agar (PDA) and broth (PDB) as control. It was observed that the good microbial growth was observed when compared to that of other conventional growth media. The weight of the Aspergillus niger biomass in BSDB was 1.8 g after 4 weeks of growth and the weight of the biomass in PDB was 0.9 g. The remarkable growth on BSDB may be attributed that the banana stalk is highly rich in nutrients.

Keywords

banana waste, economically feasible, nutrient medium, microorganisms, fungal cultures

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Optimization of Variables for Lactase Production from Isolated *Bacillus subtilis strain*VUVD001 Through Submerged Fermentation

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Abstract

Lactase enzyme is commercially important and is generally used for lactose hydrolysis in milk and whey. To date, it has been isolated from various sources. In this study, different strains of isolated bacteria were evaluated for their lactase productivity, but *Bacillus subtilis* VUVD001 resulted with the highest production. Therefore, optimal physical conditions were determined in batch fermentation process using one-variable-ata-time approach for the production of lactase. The influence of some physical conditions namely pH, incubation temperature and time, inoculum size on enzyme production were studied for higher yield. Maximum activity of lactase in shake flask culture was found 15.27 U/ml at optimized conditions of incubation period 36 h, temperature 37°C, pH 7.0 and inoculums size of 5%.

Top

Keywords

Lactase, *Bacillus subtilis*VUVD001, Shake Flask Culture.

Top

Original Article | Published: 30 June 2017

Optimization of nutritional components of medium by response surface methodology for enhanced production of lactase

T. C. Venkateswarulu, K. Vidya Prabhakar & R. Bharath Kumar

3 Biotech **7**, Article number: 202 (2017)

Abstract

Lactase has excellent applications in dairy industry and commercially this enzyme is produced from bacterial sources but not in high yields. In this work, the production of lactase was improved by designing of nutrient components in fermentation medium by one factor at a time. Lactose and yeast extract were selected as preferable carbon and nitrogen sources for lactase production with tryptophan and $MgSO_4$ showing enhanced production. Statistical analysis proved to be a useful and powerful tool in developing optimum fermentation conditions. The individual and interactive role of lactose, yeast extract, magnesium sulfate, and tryptophan concentration on lactase production was examined by central composite design. Submerged fermentation with *Bacillus subtilis* strain VUVD001 produced lactase activity of 63.54 U/ml in optimized medium. The activity was threefold higher in comparison to an unoptimized medium. This result confirmed that the designed medium was useful for producing higher yields of lactase.

Original Article | Published: 29 June 2017

Modeling and optimization of fermentation variables for enhanced production of lactase by isolated *Bacillus subtilis* strain VUVD001 using artificial neural networking and response surface methodology

T. C. Venkateswarulu, K. Vidya Prabhakar, R. Bharath Kumar & S. Krupanidhi

3 Biotech **7**, Article number: 186 (2017)

Abstract

Modeling and optimization were performed to enhance production of lactase through submerged fermentation by *Bacillus subtilis* VUVD001 using artificial neural networks (ANN) and response surface methodology (RSM). The effect of process parameters namely temperature (°C), pH, and incubation time (h) and their combinational interactions on production was studied in shake flask culture by Box–Behnken design. The model was validated by conducting an experiment at optimized process variables which gave the maximum lactase activity of 91.32 U/ml. Compared to traditional activity, 3.48-folds improved production was obtained after RSM optimization. This study clearly shows that both RSM and ANN models provided desired predictions. However, compared with RSM (R^2 = 0.9496), the ANN model (R^2 = 0.99456) gave a better prediction for the production of lactase.

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Immunological and Antioxidant Response of *Litopenaeus vannamei* fed With *Lactobacillus sp*ecies under WSSV challenge

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Abstract

Two-phased experiment was conducted to investigate the effects of dietary supplementation of probiotic bacterium on shrimp physiology. In first phase shrimp fed with probiotic supplemented feed for 21 days, in second phase challenged with White spot syndrome virus (WSSV) and their physiological responses were investigated for days post-challenged. The probiotic bacteria *Lactobacillus sps* was added to the formulated basal diet at 5%, 10% and 15%concentration. Growth, immune response, antioxidant status of shrimp were evaluated. The results showed that dietary supplementation of probiotics in shrimp had significant ($P<0.01$) impact on growth. The treated shrimp groups showed significant increase in THC, percentage of phagocytosis and phenoloxidase enzyme activity. IgG, IgA, and IgM like substances in the haemolymph of treated shrimp were significantly increased when compared to control. Higher levels of these substances were observed in 10% treated shrimp than other two groups. The antioxidant enzymes like catalse and superoxide dismutase enzymes also significantly increased in probiotic treated shrimp when compared to control. In addition, dietary supplementation of all the three concentrations of *Lactobacillus sps* probiotic bacterium was effective in improving the resistance of shrimp against WSSV as they had higher THC, higher percentage of phagocytosis, phenoloxidase enzyme and immunoglobin like substances level.

PLOS ONE

RESEARCH ARTICLE

Oral nicotine aggravates endothelial dysfunction and vascular inflammation in diet-induced obese rats: Role of macrophage TNFα

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Abstract

Obesity and cigarette smoke are major cardiovascular (CV) risk factors and, when coexisting in the same individuals, have additive/synergistic effects upon CVD. We studied the mechanisms involved in nicotine enhancement of CVD in Sprague Dawley rats with diet–induced obesity. The rats were fed either a high fat (HFD) or normal rat chow diet with or without nicotine (100 mg/L in drinking water) for 20 weeks. HFD rats developed central obesity, increased systolic blood pressure (SBP), aortic superoxide (O_2^-) production, and impaired endothelial nitric oxide synthase (eNOS) and endothelium-dependent relaxation to acetylcholine (EDR). Nicotine further increased SBP, O_2^- and impaired eNOS and EDR in obese rats. In the peritoneal macrophages from obese rats, tumor necrosis factor (TNF) α, interleukin 1β and CD36 were increased, and were further increased in nicotine-treated obese rats. Using PCR array we found that 3 of 84 target proinflammatory genes were increased by 2–4 fold in the aorta of obese rats, 11 of the target genes were further increased in nicotine-treated obese rats. HUVECs, incubated with conditioned medium from the peritoneal macrophages of nicotine treated-obese rats, exhibited reduced eNOS and increased NADPH oxidase subunits gp91phox and p22phox expression. Those effects were partially prevented by adding anti-TNFα antibody to the conditioned medium. Our results suggest that nicotine aggravates the CV effects of diet–induced obesity including the oxidative stress, vascular inflammation and endothelial dysfunction. The underlying mechanisms may involve in targeting endothelium by enhancement of macrophage-derived TNFα.

Introduction

Cigarette smoke is the most common cause of preventable morbidity and mortality worldwide, and an independent risk factor for cardiovascular (CV) diseases and type 2 diabetic mellitus[1, 2]. We and others have demonstrated the importance of chemically stable compounds, present