ISBN: 978-1-63802-003-5

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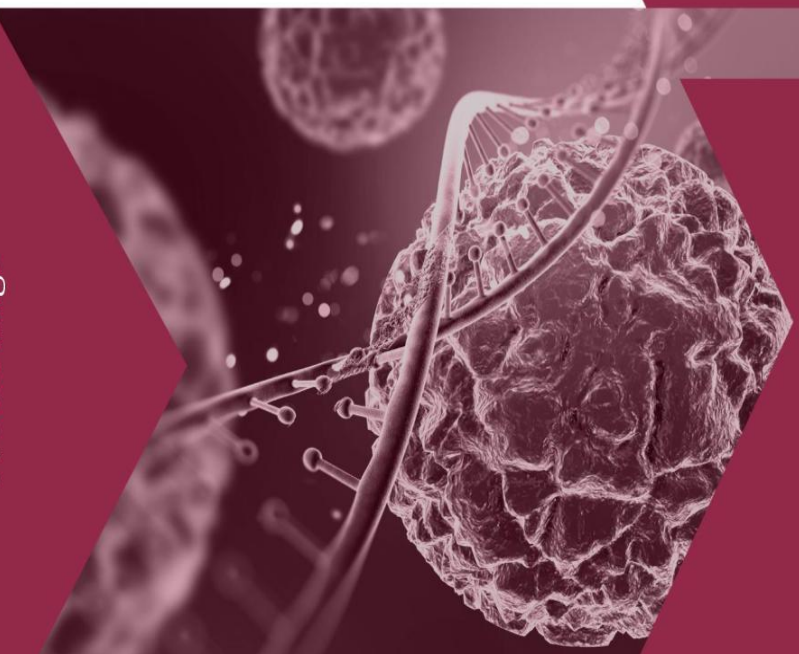
Proceedings



World Advanced Science LLC. (WAS)

2nd International Conference on Biological, Biomedical Sciences, Biotechnology and Applications

(BBSBA2021)

2021

World Advanced Science LLC (WAS), 2701 Centerville Road,
New Castle County, Wilmington, Delaware 19808, USA
http://worldascience.com/Press/proceedings.php

PROCEEDING OF THE

2nd International Conference on Biological, Biomedical Sciences, Biotechnology and Applications

The 2nd International Conference on Biological, Biomedical Sciences, Biotechnology and Applications

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Book and Cover design by Designer
ISBN: 978-1-63802-003-5

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OBJECTIVES

BBSBA2021 has featured a cutting edge research on Biosciences and Applications. Speakers have covered a range of mechanisms underlying Biological sciences and their Applications. New findings in biology and interdisciplinary science have been highlighted, along with emerging technologies in bioengineering, structural biology, and imaging for high-resolution studies.

All papers were reviewed using a blind review process: names and affiliations of the authors were removed from the manuscript, and reviewers did not know each other's identities, nor did the authors receive information about who had reviewed their manuscript.

Conference Chair, BBSBA2021

Prof. AlaaEddeen M. Seufi

Professor of Molecular Genetics

EIC: WAS Science Nature Journal ***(WASSN) ISSN: 2766-7715***

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BBSBA2021 PUBLICATION PARTNERS

The following are some of the selected journals:

Bioscience Research (BR)

Indian Journal of Science & Technology

Web of Science (ISI)

Advances in Animal and Veterinary Sciences (AAVS)

Complex Adaptive Systems Modeling (CASM)

Journal of Animal Health and Production (JAHP)

WAS Science Nature (WASSN)

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MESSAGE FROM THE HOSTING PARTNER BBSBA2021

The World Advanced Science (WAS) LLC. is a proud hosting partner of the ***2nd International Conference on Biological, Biomedical Sciences, Biotechnology, and Applications*** **held Virtually on 30th December 2021.** This is our second time collaborating with **WAS Press** to organize this important conference. We are excited to be a part of a conference that allows scholars and practitioners from around the world, especially Saudi Arabia, to present their research and practical ideas to enhance Life Sciences.

We were founded in 2018 as a developing publisher in the USA. We launched our first Open Access, Multidisciplinary ***WAS Science Nature journal (WASSN), having ISSN: 2766-7715***. We plan to publish additional WAS Journals, Books, and Proceedings that will cover a wide range of fields shortly. WAS intended to offer many online Webinars, Training Courses, and Workshops to prepare instructors and educators for future promising jobs.

This International Conference on Life Sciences fits with the mission of WAS to enhance and improve scientific practice worldwide. More information about WAS can be obtained from our

website (https://worldascience.com/waspress/index.php/press/) or by emailing to: **wasmngeditor@worldascience.com**, WAS Press Principal and WAS Manager OR to: **worldascience@gmail.com**, WAS Press Technical Support.

MESSAGE FROM THE CONFERENCE CHAIR, BBSBA2021

Hello and welcome, everybody.

It is my great pleasure to serve as a Conference Chair for the second time for the ***International Conference on Biological, Biomedical Sciences, Biotechnology and Applications***, organized by WAS LLC., and WAS Press. Both conferences were very lively events where Life Science researchers and practitioners from around the world came together to discuss a wide array of important issues in BioSciences.

I want to thank all members of our Organizing Committee, Keynote Speakes, Presenters, and Attendees for supporting all aspects of BBSBA2021 Planning and Success. This year we are proud to have distinctive keynote speakers who shared their knowledge and explained many lighting ideas about the hottest topics of research and innovations. We had Sixty Nine attendees and presenters from different countries.

We believe that we have all benefitted from our combined participation in BBSBA2020. I hope the conference allowed us to engage with our peers, discuss our ideas for research and practice, and enhance opportunities for collaboration with other scientists.

We also invite you to attend our coming ***3rd International***

Conference on Biological, Biomedical Sciences, Biotechnology, and Applications. **BBSBA2022** is intended to be held in Dubai, UAE, on July 23-24, 2022**.** We hope you will spend some time exploring **Dubai**, one of the most dazzling cities worldwide. Meanwhile, we will keep you updated timely with our news and events.

Thank you,

Conference Chair BBSBA 2021

Prof. AlaaEddeen M. Seufi

Professor of Molecular Genetics

EIC: WAS Science Nature Journal (WASSN) ISSN: 2766-7715

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ABSTRACTS &POSTERS

Pyrethroid resistance and kdr genotype in Anopheles Mosquitoes: a study of the Sudan savannah region of Jigawa State, Nigeria

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ABSTRACT

Malaria is a public health problem that affects the globe. The disease is transmitted by Anopheles mosquitoes. In Africa, malaria mortalities were recorded primarily in children under the age of five and pregnant women. Nigeria accounts for up to 27% and 24% of Africa and global malaria deaths, respectively. Insecticide-based control measures are key control strategies that have helped in targeting disease vectors. The most widely and effective insecticide-based control measures are indoor residual spraying with insecticides and the use of insecticide-treated materials (bed nets). Pyrethroids are the only World Health Organization (WHO) approved class of insecticide for the treatment of bed nets. The study aimed at assessing pyrethroid resistance and kdr genotype in Anopheles mosquitoes of the study area. Larval samples were collected (April 2020 and November / December 2020) and were

reared to adults. Samples were morphologically and molecularly identified, WHO bioassay was conducted using permethrin and deltamethrin. Kdr mutations were assayed using PCR technique. Morphological identification showed the abundance of Anopheles gambiae complex, molecular identification gave varying percentages of An. gambiae s.s, An. coluzzii, and An. arabiensis. Percentage knockdown and mortality were higher using deltamethrin compared to permethrin. A higher negative kdr mutation genotype (70%) was observed in the resistance population compared to the susceptible population with 50% negative kdr mutation genotype. KDR mutation genotype frequency of Anopheles specie from the study sites indicates the possibility of another form of resistance mechanism other than kdr mutation. Resistance might also be attributed to activities occurring in the study sites and or time of sample collection.
Keywords: Pyrethroids; kdr mutation genotype; Malaria; deltamethrin; permethrin.

Antimicrobial resistance and genes encoding for virulence factors among community-associated uropathogenic *E. coli* in Western Saudi Arabia

Sara Arafa[1*], Wafa Alshehri[2], Najla Obaid[1], Sameer Organji[1], Khaled Elbanna[3], Hussein Abulreesh[1]

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ABSTRACT

Background: Urinary tract infections (UTIs) remain a common and leading cause of morbidity worldwide, with more than 150 million people affected annually, resulting in a social and healthcare burden of around 3.5 billion US Dollars per year in the USA alone. Aims: To explore the prevalence of multiple resistant community-acquired UTI-associated E. coli and their virulence factors in Western

Saudi Arabia. Methods: A total of 1000 urine samples were examined for the presence of E. coli by selective plating on MacConkey, CLED, and sheep blood agar. Antimicrobial susceptibility patterns were carried out by means of Vitek2 Compact (MIC) and the disc diffusion method on Mueller-Hinton agar. Detection of genes encoding for virulence factors (kpsMTII, traT, sat, csgA, vat, and iutA) were achieved by PCR. Results: The overall prevalence of UTI-associated E. coli was low (5%, n =1000), with a higher prevalence in samples of female origin. Resistance to norfloxacin was exhibited by 82% of the isolates, followed by 60% resistance to ampicillin. No resistance was exhibited to imipenem, meropenem, and ertapenem. In general, about 50% of the isolates showed multiple resistance patterns (resistance to more than 3 antimicrobial classes). All 50 uropathogenic E. coli carried kpsMTII, iutA, traT, and csgA genes, while 98% of the isolates carried sat gene, vat gene was found in 38% of the isolates. Conclusion: Although a low prevalence of uropathogenic *E. coli* is reported in this study, the isolates were found to be highly virulent. This is the first report to explore the virulence of uropathogenic E. coli in Saudi Arabia.

Keywords: Uropathogenic *E. coli*; Antimicrobial resistance; Virulence factors; Urinary tract infection

Antimicrobial Resistance and Genes Encoding for Virulence Factors among Community-associated Uropathogenic *E. coli* in Western Saudi Arabia

Sara H. Arafa*, Wafa A. Alshehri, Najla A. Obied, Sameer R. Organji, Khaled Elbanna, Hussein H. Abulreesh
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INTRODUCTION

Urinary tract infections (UTIs) remain a common and leading cause of morbidity worldwide, with more than 150 million people affected annually, resulting in a social and healthcare burden (Flores-Mireles *et al.*, 2015). UTIs are either community-associated or hospital-acquired UTIs (CA-UITs or HA-UTIs, respectively). The aetiology of UTIs varies because different bacterial, fungal, and parasitic agents are the cause of the infections. Among causative bacterial agents, *Escherichia coli* is the most frequently reported (Sokhn *et al.*, 2020).

The aim of this work was to explore the prevalence of multiple resistant community-acquired UTI-associated *E. coli* and their virulence factors in Western Saudi Arabia.

MATERIALS AND METHODS

STEPS

1st SAMPLE COLLECTION
A total of 1000 urine samples were examined for the presence of *E. coli* by selective plating on MacConkey, CLED, and Sheep blood agar.

2nd IDENTIFICATION OF PRESUMPTIVE UTI-ASSOCIATED *E. coli*
Presumptive *E. coli* isolates were subject to a set of biochemical tests that were performed to identify. Isolates were identified manually by API 20E strip for Gram-negative bacteria (BioMérieux Inc., Marcy-l' Etoil, France) and automated by (Vitek 2) rapid system (BioMérieux Inc).

3rd ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST)
Antimicrobial susceptibility patterns were carried out by means of Vitek2 Compact (MIC) and disc diffusion method on Mueller-Hinton agar using the disk diffusion (Kirby Bauer's) technique following the (CLSI) (CLSI, 2021) guidelines.

4th DNA EXTRACTION
Bacterial DNA was extracted by means of boiling, a loopful of *E.coli* colonies growing on EMB plates were added to a 50 µl of sterile distilled water. The mixture was boiled for 5 min and then centrifuged at 1260 RCF for 5 min. The supernatant was then used as template DNA for PCR.

5th PCR DETECTION
The PCR reaction mixture (15 µl final volume) contained: 2 µl template DNA (~100 ng/ µl), 1 µl of each primer (10 moles/µl), and 6 µl DNase-free distilled water was added to 6 µl of Master Mix. The following temperature cycles were utilized for DNA amplification: initial denaturation at 94 °C for 5 min followed by 32 cycles of denaturation (94 °C, 50 s), annealing (62 °C,1 min for *kpsMTII*, and *traT*, 85 °C for *iutA*, 60 °C for *csgA*, 59 °C for *sat*, 58 °C for *vat*), and extension (72 °C, 1 min for all), with a final extension step (72 °C, 8 min for all). The amplified DNA was separated via gel electrophoresis on (1% agarose) and visualized under UV transillumination.

RESULTS

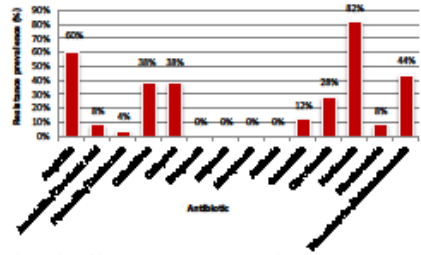

Fig 1. Prevalence of drug-resistance among *E. coli* in UTIs – Total: 50 of positive strains

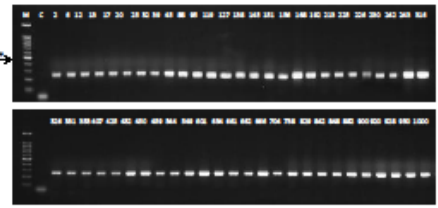

Fig 2. Agarose Gel Electrophoresis shows positive results of *kpsMTII* virulence gene at (270 bp) in UPEC. Where, Lane (M) DNA marker, Lane (C) negative control, and Lanes (407, 459): positive (*Klebsiella pneumoniae*), and Lane (920): positive (*Klebsiella oxytoca*)

DISCUSSION

All positive UTI cultures were caused by UPEC isolates, and this high prevalence of UPEC is in accordance with the world trend and previous studies conducted in Saudi Arabia (Ahmad, 2019). Females may be at a higher risk of developing UTIs than males (Medina and Castillo-Pino, 2019). In the present study, we observed high rates of resistance to norfloxacin (82%), followed by ampicillin (60%). Other studies in Saudi Arabia have revealed similar observations (Ahmad, 2019) With the emergence of ESBL-producing UPEC strains, the use of carbapenems (e.g., imipenem, ertapenem, and meropenem) has been recommended for the treatment of UPEC because the majority of the strains show susceptibility to these agents (Terlizi *et al.*, 2017). We observed complete susceptibility to these agents among our isolates, which is in accordance with the observations from a study by Ahmad (2019). A prevalence of 100% (n = 50) was found for *csgA*, *iutA*, *traT* and *kpsMTII*, and the prevalence rates of *vat* were 38%. Although a low prevalence of uropathogenic *E. coli* is reported in this study, the isolates were found to be highly virulent. This is the first report to explore the virulence of uropathogenic *E. coli* in Saudi Arabia.

ACKNOWLEDGMENTS

We thank Hayat Ashi for help with sampling

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Terlizzi ME., Gribaudo G., Maffei ME. (2017) Uropathogenic Escherichia coli (UPEC) infections: virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies. Frontiers in Microbiology 8, Article 1566. DOI: 10.3389/fmicb.2017.01566.

Phenotypic and molecular characterization of uric acid degrading bacteria

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ABSTRACT

Background: Uricase is a peroxisomal (oxyreductase) enzyme that catalyzes the oxidative opening of the purine ring of the urate pathway to produce allantoin, carbon dioxide, and hydrogen peroxide. Uric acid results from the decomposition of living and dying cells from the transformation of their nucleic acids, especially adenine and guanine. In addition, it is derived from proteins. This enzyme plays an important therapeutic role in the medical field, such as biochemical diagnosis, as it is used as a reagent to detect uric acid in the blood serum, and uricase can be used as a protein drug to reduce toxic urinary accumulation in diseases such as gout, bedwetting, and hyperuricemia.

Aim of the work: This study aimed to isolate and examine highly efficient urease-producing strains, characterize the phenotype and genotype of uricase-producing strains, study the factors that affect uricase secretion from the most promising strains, and finally, biochemical and molecular characterization of uricase from the most promising

strains.

Experimental work and Results: In this study, 56 uricase-producing isolates were isolated from different samples collected from Makkah and Al-Taif city using mineral salt medium supplemented with uric acid. Partial characterization for all isolates was conducted. In general, all isolates were partially identified and were short rods, Gram-negative, motile, spore-forming, oxidase, catalase-positive. Based on uricase activity detected by forming clear zone around colonies, top fifth strains were selected and completely identified using VITEK kit and sequencing 16s rRNA gene. Based on phenotypic characterization, the most promising isolates of UR1, UR2, were identified by VITEK as Pseudomonas oleovorans and isolated UR3, UR4 and UR5 as Alcaligenes faecalis with similarity of 95, 95, 96, 96 and 97 %, respectively. While 16s rRNA sequencing identified isolates UR1, UR2, UR3, as Alcaligenes faecalis and isolates UR4 and UR5 ad Alcaligenes aquatica, 94 99, 97, 96, 97%, respectively. To improve the uricase secretion, isolate UR1 was selected, and different production factors were studied using the uric acid medium. The uricase activity of strain UR1 was measured qualitatively using the clear zone technique of uric acid agar plates. The results indicate that strain UR1 inducted the maximum uricase activity at 37°C, pH 7.2 after 48h period. Furthermore, the extracellular uricase was purified to electrophoretic homogeneity using Sephadex column and biochemically characterized. For this, the extracellular uricase was purified from 250 ml culture supernatant of UR1 grown for

36h at 37°C in uric acid mineral salt medium containing 0.15 % (w/v) uric acid as the sole nitrogen source. The highest level of uricase activity was 4.98 units/ml/min after the purification step by Sephadex column. SDS-PAGE analysis revealed that the molecular size of the partially purified urinary enzyme was 43 kDa.

Conclusion: From these results, it could be concluded that the most promising uricase-producing bacterial strains were isolated from Makkah soil samples. The extracellular purified uricase of isolate UR1 could be potentially used as a reagent to detect uric acid in the blood serum, and antigout, bedwetting and hyperuricemia.

Keywords: Uric acid; Extracellular Uricase; Genotypic characterization; 16s rRNA sequencing; Gout; Bedwetting; Hyperuricemia

Phenotypic and molecular characterization of uric acid degrading bacteria

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Abstract

In this study, 56 uricase producing isolates were isolated from different samples collected from Makkah and Al-Taif city using mineral salt medium supplemented with uric acid. Partial characterization for all isolates was conducted. In general, all isolates were partially identified and were short rods, Gram-negative, motile, spore-forming, oxidase, catalase-positive. Based on uricase activity detected by forming clear zone around colonies, top fifth ifrains were selected and completely identified using VITEK kit and sequencing 16s rRNA gene. Based on phenotypic characterization, the most promising isolates of UR1, UR2, were identified by VITEK as Pseudomonas oleovorans and isolates UR3, UR4 and UR5 as Alcaligenes faecalis with similarity of 95, 95, 96, 96 and 97 %, respectively. While 16s rRNA sequencing identified isolates UR1, UR2, UR3, as Alcaligenes faecalis and isolates UR4 and UR5 as Alcaligenes Aquatica, with 94, 99, 97, 96, 97%, respectively.
To improve the uricase secretion, isolate UR1 was selected, and different production factors were studied using uric acid medium. The uricase activity of strain UR1 was measured qualitatively by clear zone technique using uric acid agar plates. The results indicate that strain UR1 inducted the maximum uricase activity (65mm) at 37°C, pH 7.2 after 48h period. Furthermore, the extracellular uricase were purified to electrophoretic homogeneity using Sephadex column and biochemically characterized. For this, the extracellular uricase was purified from 250 ml culture supernatant of UR1 grown for 36h at 37°C in uric acid mineral salt medium containing 0.15 % (w/v) uric acid as sole nitrogen source. The highest level of uricase activity was 4.98 units/ml/min after purification thgy by Sephadex column. SDS-PAGE analysis revealed that the molecular size of the partially purified urinary enzyme was 46 kDa.

Introduction

Uricase is a peroxisomal (oxyreductase) enzyme that catalyzes the oxidative opening of the purine ring of the urate pathway to produce allantoin, carbon dioxide and hydrogen peroxide. Uric acid results from the decomposition of living and dying cells from the transformation of their mucleic acids, especially adenine and guanine, in addition, it is derived from proteins (Retailleau et al;2004 & Collings et al;2010). This enzyme plays an important therapeutic role in the medical field, such as biochemical diagnosis, as it is used as a reagent to detect uric acid in the blood serum, and uricase can be used as a protein drug to reduce toxic urinary accumulation in diseases such as gout, bedwetting and hyperuricemia (Abdel-Fattah et al;2005).

Methodology

Samples were isolated according to the basics of isolation from soil, liquid materials and plants, as well as bacteria were identified according to Bergey's guide and using the VITEK device. The instructions shown on each package of the nutrient medium preparations were followed for cultivation and purification of the isolates.

Result and Discussion

After examining the isolated samples of soil, poultry and pigeon waste, which were 56 samples, the code UR1 - UR56 was taken. The samples shown in Table1 were the highest in the emergence of the clear zone around the colonies. By using the standard physiological induction techniques and by using the VITEK device, it was found that the present bacteria are Alcaligenes faecalis, and the table also shows the best nitrogen and carbon sources for these bacteria according to the laboratory results.

Table 1: General Characteristics of Uricase Producing Bacterial Isolates (Cell)

Isolate code	Cell Shape	Sporulation	Motility	Nitrogen source utilization (Diameter mm)				Carbon source utilization (Diameter mm)					
				Casein	Peptone	Urea	CMC	Maltose	Dextrose	Fructose	D-Glucose	Sucrose	Fructose
UR 1	Irregular	- Ve	+ Ve	70	60	12	55	75	50	60	60	70	60
UR 2	Irregular	- Ve	+ Ve	70	70	10	60	70	50	70	55	60	60
UR 3	Irregular	- Ve	+ Ve	70	60	10	70	70	45	70	70	60	70
UR 4	Irregular	- Ve	+ Ve	70	65	8	70	68	45	65	65	69	65
UR 5	Irregular	- Ve	+ Ve	70	70	15	70	70	25	70	70	70	70
UR 6	Irregular	- Ve	+ Ve	65	70	10	55	65	50	60	62	65	60
UR 7	Irregular	- Ve	+ Ve	70	70	8	60	65	60	65	60	70	65
UR 8	Irregular	- Ve	+ Ve	70	65	7	50	70	50	70	55	65	70
UR 9	Irregular	- Ve	+ Ve	55	34	5	50	70	60	70	70	75	70
UR 10	Irregular	- Ve	+ Ve	70	60	10	70	70	55	60	60	70	60
UR 18	Irregular	- Ve	+ Ve	55	32	5	65	70	50	70	70	70	70

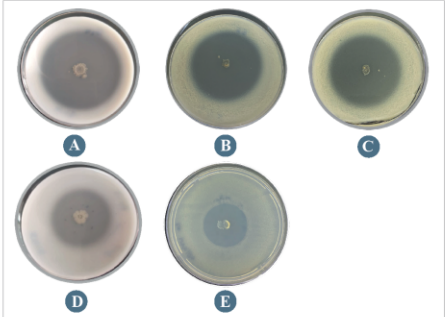

Fig. (1) Uricase activity of the most promising uricase producing strains as indicated by clear zone diameter on uric acid medium where A (UR1) ,B (UR2), C (UR3), D (UR4) and E (UR5)

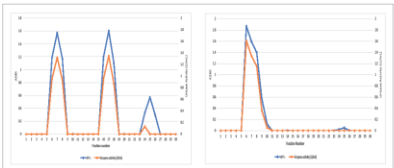

Fig (2). Purification of the uricase using column chromatography

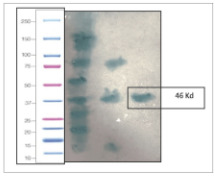

Fig (3). Gel electrophoresis band that showing the molecular weight of uricase enzyme producing by isolate bacteria where The purified enzyme was collected, lyophilized and analyzed. The molecular weights, determined by SDS-PAGE.

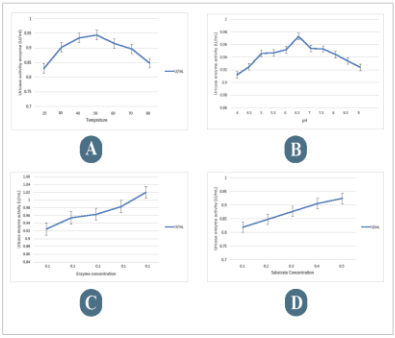

Fig (4) Factors affecting enzyme productivity and activity: A- Effect of temperature, B- Effect of pH , C- Effect of enzyme concentration and D- Effect of different substrate concentration.

Conclusion

From these results, it could be concluded that the most promising uricase producing bacterial strains were isolated from Makkah soil samples. The extracellular purified uricase of isolate UR1 could be potentially used as a reagent to detect uric acid in the blood serum, and antigout, bedwetting and hyperuricemia.

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Biotechnological and Taxonomic Studies of some Alginate producing Bacteria

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ABSTRACT

Introduction: Alginate is an important polysaccharide consisting of monomeric acid (M) and guluronic (G) monomers that various genera of brown seaweed can produce and few genera of bacteria Pseudomonas, Rhizobium, and Azotobacter.

Background: Due to its biocompatibility, viscosifying, and gelling properties, non-toxic and relatively low cost, alginate obtained from brown seaweed has been extensively investigated and widely used in medical, pharmaceutical, foods, and agriculture applications. Seaweed alginates are extensively used in biomedical applications as an immobilization material, but it suffers from problems with mechanical stability, low purity and homogeneity, wide pore size distribution, and osmotic swelling during physiological conditions. So, bacterial alginate could be the best choice for alginate production compared to algal alginate.

Aim of the study: This work aims to (i) solation and screening of highly efficient alginate producing strains, (ii)

identify the most promising alginate producing strains based on phenotypic and genotypic characterization (iii) study the factors effect and optimization of alginate production from the most promising strains, (iv) production of alginate from alternative agricultural wastes, (v) characterization and identification of the obtained alginate.

Experimental and Results: In this study, forty-seven strains were isolated from different soil and rhizosphere samples collected from Makkah using Ashby's medium. All high viscose colonies were picked and purified by subculturing many times on an agar medium and partially characterized. The most promising isolates Az14, Az19, Az33, and Az75 were selected, and complete identification was conducted by VITEK and 16s rRNA sequencing gene. All isolates were Gram-negative, aerobes, some motile and some non-motile, non-spore former, oxidase and catalase-positive. The phenotypic and genotypic characterization identified isolates Az14, Az19, Az33 and Az75 as Enterobacter rabaei, Roseateles terrace, Rhizobium pusense and Rhizobium pakistanense with similarity percentage of 96.55%, 99.56%, 94.59% and 99.35%, respectively.

To optimize alginate production, strain Az19 was chosen, and different physiological parameters, including incubation time, temperature, pH and salt concentration and production from sugar, were conducted using Ashby's broth. Also, molasses as a low-cost carbon source were also investigated. To purify and identify the bacterial alginate, the promising isolate Az19 were grown on Ashby's broth

medium at the growth optimum conditions, then the culture was centrifuged, and two volumes of chilled ethanol extracted the yield of alginate, and pellets were dried in a hot air oven at 60°C, the weighted. The data obtained revealed that isolate Az19 inducted 8 g/l after 10 days incubation at 37o C, pH 7.0, and NaCl (1%). Finally, the yield products obtained from the most promising strains were purified and identified as alginate by Fourier transform infrared spectroscopy (FTIR). Mannitol appears to be the best carbon source, and ammonium sulphate at concentration 0.5% was the nitrogen source of choice for optimal alginate production.

Conclusion: From this study, it could be concluded that forty-seven strains were isolated and partially identified. The most promising strain was selected and completely characterized by VITEK and 16s rRNA sequencing. Among those, strain Az19 inducted 8 g/L pure alginate could be used in many applications.

Keywords: Bacterial alginate; Polysaccharides; Medical applications; Food industry applications; Viscosifying and gelling properties; Drug's delivery; Phenotypic and genotypic characterization; Makkah

Biotechnological and Taxonomic Studies of some Alginate producing Bacteria

Amjad Alharbi* [1,2], Hussein H Abulreesh[1,2], Mohamed R Shaaban[3,4], Wafa Alshehri[5], Ashjan Khalel[6], Rakan Felemban[7],Khaled Elbanna[1, 2,8]

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Abstract

In this study, forty-seven strains were isolated from different soil and rhizosphere samples collected from Makkah using Ashby's medium. All highly viscose colony were picked and purified by subculturing many times on agar medium and partially characterized. The most promising isolates Az14, Az19, Az33 and Az75 were selected, and complete identification were conducted by VITEK and 16s rRNA sequencing gene. All isolates were Gram-negative, aerobes, some motile and some none-motile, non-spore former, oxidase and catalase positive. The phenotypic and genotypic characterization identified isolates Az14, Az19, Az33 and Az75 as *Enterobacter rabaei, Roseateles terrace, Rhizobium pusense* and *Rhizobium pakistanense* with similarity percentage of 96.55%, 99.56%, 94.59% and 99.35%, respectively. To optimize alginate production, strain Az19 was chosen, and different growth conditions were conducted. Data obtained revealed that, isolate Az19 inducted 8 g/l using Ashby's medium supplemented with mannitol at 37° C, pH 7.0 and NaCl (1%) after 10 days shaking incubation. Finally, the yield products obtained from the most promising strains were purified and identified as alginate by Fourier transform infrared spectroscopy (FTIR).

Introduction

Alginate is an important polysaccharide consists of monomeric acid (M) and guluronic (G) monomers can be produced by various genera of brown seaweed and few genera of bacteria such Pseudomonas, Rhizobium and Azotobacter. Due to its biocompatibility, viscosifying and gelling properties, non-toxic and relatively low cost, alginate obtained from brown seaweed has been extensively investigated and widely used in medical, pharmaceutical, foods and agriculture applications. Seaweed alginates are extensively used in biomedical applications as an immobilization material, but it suffers from problems with mechanical stability, low purity and homogeneity, wide pore size distribution and osmotic swelling during physiological conditions. So, bacterial alginate could be the best choice for alginate production compared to algal alginate.

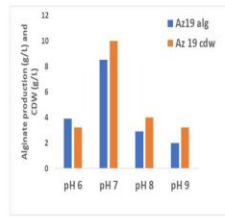

Figure 2a: Effect of pH) on Alginate production from isolate Az19

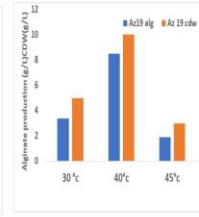

Figure 2b: Effect temperature on Alginate production from isolate Az19

Experimental & Results

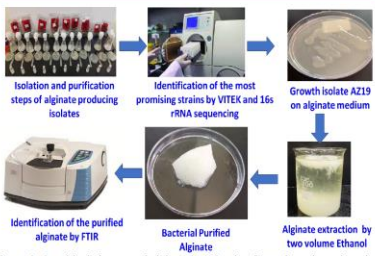

Figure 1: Graphical abstract of alginate production from the selected strains

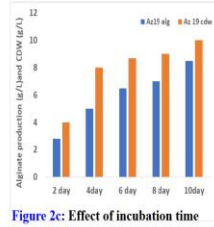

Figure 2c: Effect of incubation time (day) on Alginate production from isolate Az19

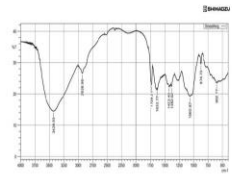

Figure 3: FT-IR spectrum of the purified Alginate of strain Az19

Table 1: Partial characterization of Alginate producing strains:

Characteristics	Alginate producing isolates.		
	Az19	Az33	Az75
Colony morphology:			
Form	circular	circular	circular
Color	Ceramic	White	Ceramic
Texture	Slimy	Slimy	Slimy
Cell morphology			
Cell Shape	ovoid's	Roods	Roods
Gram stain	-	-	-
Sporulation	-	-	-
Motility	-	-	-
Carbon source utilization:			
Glucose	+	+	+
glycerol	+	+	+
Sucrose	+	+	+
Lactose	+	+	+
Maltose	+	+	+
Mannitol	+++	+++	+++

Conclusion

From this study it could be concluded that, forty-seven strains were isolated and partially identified. The most promising strain were selected and completely characterized by VITEK and 16s rRNA sequencing. Among those, strain Az19 inducted 8 g/L pure alginate could be used in many applications.

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Biotechnological and taxonomic studies on poly glutamic producing bacteria

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ABSTRACT

Introduction: Biopolymer from microbial source has recently received much attention due to the awareness of the environmental problem that inevitably affects human health.

Background: Poly glutamic acid (PGA) is a naturally occurring, infrequent anionic polymeric compound composed of extensively viscous homo-polyamide of D and L- Glutamic acid units, and it is an extracellular polymer that is completely biodegradable and nontoxic to humans. PGA has been successfully used as thickener, cryoprotectant, humectant, drug carrier, biological adhesive, heavy metal absorbent, etc., with biodegradability in the fields of food, cosmetics, medicine, drugs, and water. Among other novel applications, it can be used for protein crystallization as a soft tissue adhesive and a non-viral vector for safe gene delivery.

Aim of the work: This study aimed to isolate and screen for highly efficient polyglutamic acid-producing strains, phenotypic and genotypic characterization of PGA producing strains, studying the factors and optimization of PGA production from selected strains and finally

characterization and identification of the obtained PGA.

Experimental and Results: In this study, fifty PGA producing isolates from different samples were isolated and screened using PGA medium. Based on the fermentation batch for PGA production, strains PG14, PG21, PG 22, PG 37 and PG49 recorded the highest PGA amount, which was 40.8, 29.40, 30.0, 32.2 and 50 g/litter, respectively. All isolates were partially identified, and they were long rods, Gram-positive, motile, spore formers, oxidase, and catalase positive. The most promising isolates were selected and completely identified using 50 CHB API kit, VITEK, and 16s rRNA sequencing. Based on phenotypic and genotypic characterizations, isolates PG14, PG21, PG 22, PG, 37 and PG 49 were identified as B. licheniformis with similarity 98, 96, 98, 99% and 97%, respectively. For optimization of PGA production, isolate PG 49 was chosen and different production factors were studied using PGA medium. Surprisingly, the maximum PGA production (50 g/L) was obtained when sodium glutamate, citric acid, ammonium sulfate used with a concentration of 60, 30, 5 g/L, respectively. Furthermore, strain PG 49 exhibited maximum PGA yield (50 g/L) at pH 7.4, temperature (37oC) after an incubation period of 36h. Furthermore, the monomer of glutamic acid of PGA was identified and detected by high-performance liquid chromatography.

Conclusion: In this work, fifty PGA-producing isolates were isolated from different soil and rhizosphere samples of Makkah. The most promising PGA producers were phenotypically and genotypically characterized. Also, it was

found that sodium glutamate, citric acid, ammonium sulfate, were the most significant factors increasing PGA production. Furthermore, the monomer of sodium glutamate of PGA was identified and detected by high-performance liquid chromatography (HPLC).
Keywords: Microbial Biopolymer; poly Glutamic acid (PGA); Medical Applications; Phenotypic and Genotypic characterization; Optimization conditions; Growth factors; 16s rRNA sequencing

Biotechnological and taxonomic studies on poly glutamic producing bacteria

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[2]Research Laboratory Unit, Faculty of Applied Science, Umm Al-Qura university, Makkah, Saudi Arabia.

[3]Deprtment of Agricultural Microbiology, Faculty of Agriculture, Fayoum University, Fayoum, Egypt.

Introduction

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Aim of Work.

This study aimed to isolation and screening for highly efficient polyglutamic acid producing strains, phenotypic and genotypic characterization of PGA producing strains , studying the factors and optimization of PGA production from selected strains and finally characterization and identification of the obtained PGA

Marital and Methods

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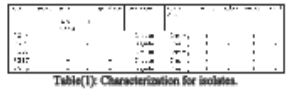
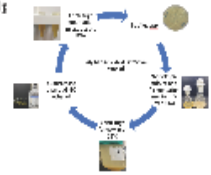


Table(1): Characterization for isolates.

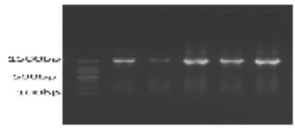
Isolate PG 49 chosen and different production factors were studied using PGA medium . Shake flask fermentation was used in this study to produce maximum production. Compared with other methods, it is the most cost-effective and advantageous, like inexpensive raw material and minimal environmental ;



Figure(1) : Poly Glutamic Acid extraction method

Results

All isolates were partially identified, and they were long rods, Gram-positive, motile, spore formers, oxidase, and catalase-positive. The most promising isolates were selected and completely identified using 50 CHB API kit, VITEK, and 16s rRNA sequencing.



Figure(2) : PCR for 16s Gene

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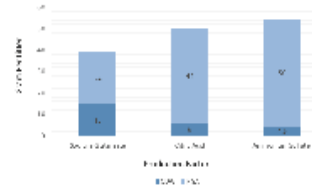

Figure (3): showing the tested factors comparing to the production.

Conclusion

In this work, fifty PGA producing isolates were isolated from different from soil and rhizosphere samples of Makkah. The most promising PGA producers were phenotypically and genotypically characterized. Also, it was found that glutamic acid, citric acid, ammonium sulfate, were the most significant factors increasing PGA production. Furthermore, the monomer of glutamic acid of PGA was identified and detected by high performance liquid chromatography (HPLC).

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Acknowledgements

I would like to express my deep gratitude to Professor Khaled Elbanna and Professor Hussein Abulreesh my research supervisors, for their patient guidance, enthusiastic encouragement and useful critiques of this research work. Also, My special thanks are extended to the staff of Microbiology Laboratory in East Jeddah General Hospital and lab Director Dr.Reema Sotah.

Phenotypic and genotypic characterization of some

melanin producing microorganisms

Areej Alharbi[1*], Hussein Abulreesh[1], Mohamed Shaaban[1], Wafa Alshehri[2], Ashjan Khalel[3], Khaled Elbanna[4]

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ABSTRACT

Introduction: Melanin's are natural pigments of polyphenol compounds that have an indole ring as their monomeric base, which is present in animals, plants, and most microorganisms. They are the dark-colored negatively charged high molecular weight pigments which are formed due to polymerized phenolic and/or indole compounds.

Background: Melanin pigments produced from microorganisms are considered more attractive and promising because they are more efficient and cost-effective than the chemical synthesis of pigments. Microorganisms are also more feasible sources of pigments than pigments extracted from plants and animals because they do not have seasonal constraints, do not compete for limited farming land with actual foods, and can be easily produced in the cheap culture medium with high yields. Microorganisms don't cause the problems of seasonal variations and are selected arsenals as they modify them according to the medium and conditions provided to them. Melanin's have great application potentials in the medicine,

agriculture, cosmetics, and pharmaceutical industries. Besides radioprotective and photo protection, melanin possesses broad biological activities such as radical scavenging, antioxidant, antitumor, antiviral, antimicrobial, liver protecting and antivenin activity, anti-inflammatory and as immune-stimulating agent.

Aims of the work: This work aims to: (i) isolation and screening for melanin-producing strains, (ii) Phenotypic and genotypic characterization of melanin producing strains, (iii) studying the factors and optimization of melanin production from selected strains, (iv) molecular detection of melanin encoding gens by RT-PCR (screening for tyrosinase gene), (v) characterization and identification of melanin.

Experimental and Results: In this study, forty thermophilic melanin-producing bacteria were isolated using medium supplemented with L tyrosine as melanin precursor substrate. All isolates were partially identified, and the most promising strains ME 13, ME19, ME17, ME25, were selected and complete identification by VITEK and 16s rRNA gene sequencing were conducted. All isolates were rods, Gram-positive, motile, spore former and non-spore formers, catalase and oxidase positive. Based on phenotypic and genotypic characterization, strains ME 13, ME 25, were identified as Bacillus badius strain M9-20, Brevibacillus borstelensis strain S4T209, with similarities of 100% and 97%, respectively. For optimization of the growth and melanin production from the most promising strains, isolate ME13 was chosen, and different growth parameters

were monitored using melanin broth media. To extract melanin pigment, ME13 was inoculated on melanin media (pH 7) and incubated at 50oC for 4 days, and melanin pigment was extracted from the culture supernatant. The obtained results reveal that, strain ME13 recoded the maximum growth and melanin production at 50oC, pH7 after 96h to 120h. The preliminary fermentation data indicate that strains ME13 recoded the maximum pigment yield (2.302 g/l). Finally, melanin produced from the most promising strain of ME17 was purified, dried, and identified by Uv-vis spectroscope analysis and by Fourier transform infrared spectroscopy (FTIR).

Conclusion: From these results, it could be concluded that melanin-producing bacterial isolated from Makkah soil samples could be promising isolates used for melanin production and could be used in different applications as radical scavenging, antioxidant, antitumor, antiviral, and antimicrobial.

Keywords: Melanin; microbial melanin; thermophilic bacteria; Phenotypic and Genotypic characterization.

Phenotypic and genotypic characterization of some melanin producing microorganisms

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Abstract

In this study, forty thermophilic melanin producing bacteria were isolated using medium supplemented with L tyrosine as melanin precursor substrate. All isolates were partially identified, and the most promising strains ME 13, ME19, ME17, ME25, were selected and complete identification by VITEK and 16s rRNA gene sequencing were conducted. All isolates were rods, Gram-positive, motile, some spore former and some non-spore formers, catalase and oxidase positive. Based on phenotypic and genotypic characterization strains ME 13, ME 25, were identified as *Bacillus badius* strain M9-20, *Brevibacillus borstelensis* strain S4T209, with similarity of 100% and 97%, respectively. For optimization of the growth and melanin production from the most promising strains, isolate ME13 was chosen, and different growth parameters were monitored using melanin broth media. To extract melanin pigment, ME13 was inoculated on melanin media (pH 7) and incubated at 50°C for 4 days, and melanin pigment was extracted from the culture supernatant .The obtained results reveal that, strain ME13 recoded the maximum growth and melanin production at 50oC, pH7 after 96h to 120h. The preliminary fermentation data indicate that strains ME13 recoded the maximum pigment yield (2.302 g/l). Finally, melanin produced from the most promising strain of ME17 was purified, dried and identified by Uv-vis spectroscope analysis and by Fourier transform infrared spectroscopy (FTIR).

Introduction

Melanin's are natural pigments of polyphenol compounds that have an indole ring as their monomeric base which presence in animals, plants and in most of the microorganisms. They are the dark colored negatively charged high molecular weight pigments which are formed due to polymerized phenolic and/or indole compounds. Melanin pigments produced from microorganisms is considered more attractive and promising because it is a more efficient and cost-effective process than chemical synthesis of pigments. Microorganisms are also more feasible sources of pigments in comparison to pigments extracted from plants and animals because they do not have seasonal constraints, do not compete for limited farming land with actual foods, and can be produced easily in the cheap culture medium with high yields. Microorganisms don't cause the problems of seasonal variations and are selected arsenals as they modify them according to the medium and conditions provided to them. Melanin's have great application potentials in the medicine, agriculture, cosmetics, and pharmaceutical industries. Besides radioprotective and photo protection, melanin possesses broad biological activities such as radical scavenging, antioxidant, antitumor, antiviral, antimicrobial, liver protecting and antivenin activity, anti-inflammatory and as immune stimulating agent.

Methodology

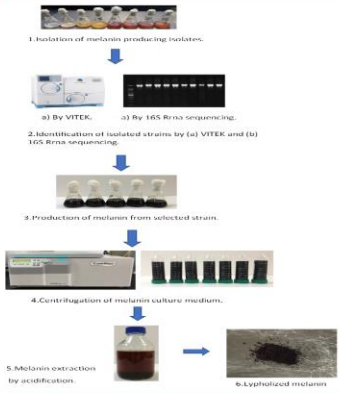

Figure (1): Graphical abstract of melanin production from selected isolate.

Characteristics	Melanin producing isolates			
	ME13	ME 19	ME17	ME25
Colony morphology Color	Brown	Black	Brown	Brown
Texture	Dry	Dry/Skinned	Smooth	Smooth
Cell characteristics				
Cell Shape	Bacilli	Filamentous	Bacilli	Bacilli
Gram stain	+	+	+	+
Sporulation	+	+	+	+
Motility	+	+	+	+
Oxidase	+	+	+	+
Catalase	+	+	+	+
Indol	-	-	-	-
KOH	-	-	-	-

Table (1):Partial characterization of melanin producing isolates

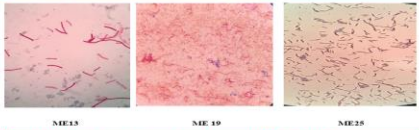

Figure (2): Cell morphpology of melanin producing isolates: ME13,ME 19 and ME 25.

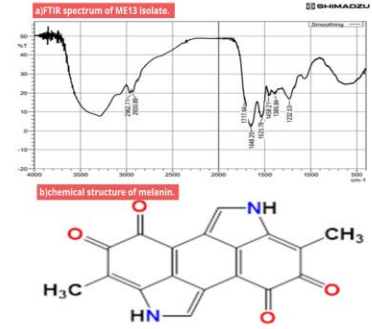

Figure (3): FT-IR spectrum of purified melanin from the selected isolate. a)FTIR spectrum of ME13 isolate.b)chemical structure of melanin.

	Item	Value
2	Sample name	28/2
3	Sample ID	3
4	Optics	
5	Intensity Mode	% Transmittance
6	Apodization	Happ-Genzel
7	No.of Scans	50
8	Resolution	2 cm-1

	Peak	Intensity	Corr. Intensity	Base(H)	Base(L)	Area	Corr.Area
1	1232.53	16.57	6.26	1309.69	1180.46	10387.232	406.610
2	1386.84	19.61	0.67	1391.66	1379.13	1004.375	5.423
3	1458.21	18.43	1.40	1463.03	1454.35	701.395	6.159
4	1523.79	7.98	0.47	1525.72	1511.25	1319.065	6.760
5	1648.20	2.68	0.25	1649.17	1639.52	935.217	0.666
6	1717.64	16.18	1.42	1731.14	1714.75	1339.909	14.785
7	2930.89	20.56	2.25	2947.28	2885.56	4743.079	55.515
8	2962.71	19.94	2.28	3001.29	2947.28	4221.783	54.302

Conclusion

From this study it could be concluded that, forty strains were isolated and partially identified. The most promising strain were selected and completely characterized by VITEK and 16s rRNA sequencing. Among those, strain ME13 inducted (2.302 g/l) pure melanin and could be used in many applications.

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Phenoloxidases characterization in the red palm weevil; *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) using catechol as a substrate

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ABSTRACT

Phenoloxidases (EC.1.14.18.1) are implicated in the immune response of insects to microorganisms, so oxidative enzymes such as phenoloxidases (PO) from the seventh instar larvae of the red palm weevil ; Rhynchophorus ferrugineus (Oliv.) was partially isolated to characterize its activity using catechol as the substrate. The mitochondrial fraction of larval homogenate was used as enzyme source for partial kinetic studies. The initial velocity (Vo) of PO reaction was calculated to be 333 m O.D. units min -1 mg protein -1 . Enzyme catalysis was linearly proportional to enzyme crude protein conc. up to 200 ug contained in 1 ml of the reaction mixture. Michaelis-Menten kinetics of PO activity was evaluated by constructing Lineweaver Burk double reciprocal plot. Michaelis constant (Km) was 1.6 X10-2 M, and the maximum velocity (V max) of PO reaction was 500 O.D.min-1 mg protein-1. The effect of pH, temperature, and substrate concentration on enzyme reaction was tested.

Generally, each variable was chosen while other conditions were at optimum found primarily. The reaction rate was optimal at 10-1 N catechol, neutral medium (pH 7) and 40 C . PO kept its most activity when incubated at 60 C for 15 min before reaction initiation. Phenoloxidases efficiently catalyze catechol, probably facilitating their study as one of the innate defense reactions in insects.

Keywords: Phenoloxidases; Rhynchophorus ferrugineus; Phenoloxidases characterization.

Acknowledgments

The WAS LLC. and WAS Press would like to acknowledge their thanks to everybody who contributed to the success of the ***2nd International Conference on Biological, Biomedical Sciences, Biotechnology and Applications (BBSBA2021)***, especially the members of the organizing committee for their endless efforts during the preparation and employment of the conference. Gratitudes should extend to keynote speakers, presenters, and all attendees.

Herein, we take the leed to invite all of you to prepare your valuable contributions to be shared in the ***3rd International Conference on Biological, Biomedical Sciences, Biotechnology and Applications (BBSBA2022)***, planned to be held on 23-24 July 2022 in Dubai, UAE.

Thank you for sharing our success, and we are expecting the continuity of your cooperation. Additionally, we are waiting to receive your valuable contributions and see you in Dubai the next July

2022.

Sincerely Yours;

BBSBA2022 Organizers