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Molecular detection of *Lac* and *Porin* genes in *Proteus spp*. to azo dyes decolorize and degradation

A Thesis

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By

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بِسْـــــــــــمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
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صدق الله العلي العظيم

سورة النحل:
آية 78

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We, are the examining committee, certify that we have read this thesis entitled **" Molecular detection of *Lac* and *Porin* genes in *Proteus spp*. to azo dyes decolorize and degradation "** and examined the student **" Mohammed khudhair Abbas Abtan "** in its content. In our opinion, it meets the standards of a thesis for the degree of Master of Science in Biology and accepted with "**Excellent**" degree.

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Dedication

whom ... their prayers is the secret of my success

and their satisfaction with me is my goal and my hope,

they are the most beautiful thing my eyes have seen...

my dear father And my dear mother

whom ... are more precious to me than myself

Whom helped me in my distress

My wife and daughter Fattouma

the luminous lamps that illuminated the path for me and increased me
Ambitious and optimistic... my brothers

the shining sun of knowledge over time...

my teachers - may God protect them

I dedicate this fruit of my humble effort out of loyalty and gratitude

Acknowledgements

Praise be to God, Lord of the Worlds, and prayers and peace be upon His faithful Messenger, our master Muhammad, may God's prayers and peace be upon him, his pure family, and his faithful companions and those who followed them in goodness until the Day of Judgment.

After that, I thank God, the Mighty and Sublime, for the extent of His grace, favor, and kindness, for He eases every difficulty and removes all troubles, and that the pen is puzzled and the mind stands helpless, and all words cannot fulfill thanks, praise and praise to Him, Glory be to Him.

To the absent, in front of me, Al-Hujjah Ibn Al-Hassan ((May God Almighty hasten his honorable reappearance))

I extend my thanks and gratitude to the Presidency of the University of Babylon, the Deanship of the College of Science, the Presidency of the Department of Sciences and its affiliates of professors and teachers.

As I put the final touches in preparing this letter, I would like to extend my deepest thanks and great gratitude and appreciation to my supervisors , prof. Dr. Iyad Muhammad Jabr and Assistant Professor Souad Ghaly, College of Science, University of Babylon for their supervision in building this modest scientific effort.

They have been the best help for me for a long time Research with continuous supervision, close follow-up and valuable advice.

I do not forget to thank my fellow graduate students in the department, for their brotherly treatment and scientific care, so may God reward them on my behalf.

To all these and to others who extended a helping hand to me, I say: May God reward you with good...

In conclusion, it is my pleasure to invoke the Almighty God to bear the burden of gratitude and appreciation for my father, mother, brothers, wife, daughter, Fattouma, which I cannot bear in terms of recompense and reward for all of them, and to make this work purely for his honorable face, and praise be to God, Lord of the Worlds .

Summary

This study included, decolorizing bacteria were isolated from water and soil samples, 30 environmental specimens (15 soil samples) were collected from different agriculture places of the gardens scattered in the medical city/ Baghdad teaching hospital , as well as (15 wastewater samples) from Al-Saqlawiyah drainage station, northwest of Baghdad, at the period from January 2022 to February 2022. Two bacterial species namely, *Proteus vulgaris and Proteus. mirabilis* were identified according to cultural , microbiological, biochemical test and by VITEK 2 system compact. Phenotypic characterization and phylogenetic analysis based on 16S rRNA sequence comparisons indicate that these strains belonged to the genus *Proteus spp.* The degradation efficiency of these two microorganisms was compared through optimization of pH, temperature, incubation time, initial dye concentration.

Proteus vulgaris and Proteus mirabilis were able to degrade 65% and 84.69% Direct yellow, 97.61% and 100% Disperse Blue, 48.56% and 78.01% Reactive Green , 63.35% and 70.7% Direct Brown and 70.04% and 75.1% Reactive Brown dyes of 90mg/L concentration respectively. under optimum condition pH , temperature and time were found to be 7, 37 ºC within 24 hours respectively .

A PCR primer pair was developed and tested to identify and quantify a wide variety of bacterial genes of the P class dye in complicated matrices. This strain's genomic analysis revealed the existence of genes encoding enzymes involved in decolorization methods, such as *Lac* and *Porin* azoreductase Clade III, which cleaves the dye's azo bond, and enzymes involved in deamination and isomerization of intermediate compounds.

The FT-IR analysis confirmed that microorganisms were able to degrade toxic azo dyes into a non-toxic product as proved through structural modifications to analyze chemical functions in materials by detecting the vibrations that characterize chemical bonds,

All results of FT-IR results of current study , were demonstrated that bioadsorption procedure happened because of the existance of hydroxyl, carbonyl, amide, and carboxyl combinations these mean that the azo dyes after treatment were bind with the functional group mentioned above leading to shifting the absorbance from site to another. Variation in spectra portrays alterate in active gatherings of cells after azo dyes adsorption, makes us suggested the likelihood that bioadsorption could be occurred over ion-exchange operation instead of collection.Current study showed the bonds of functional groups, for example, Ion-exchange between H of carboxyl (- COOH),hydroxyl (- OH) and amine (- NH2) gathering of cells and azo dyes are principally included with bioadsorption surface for it.

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Chapter One

Introduction

Introduction

Organic substances with the functional group RN=NR′, in which R and R′ are typically aryl, are known as azo dyes. They are a class of economically significant azo compounds or substances with the bond C-N=N-C (Figure :1). Azo dyes are frequently used to color meals, leather goods, and fabrics (Bheemaraddi *et al.*, 2014).

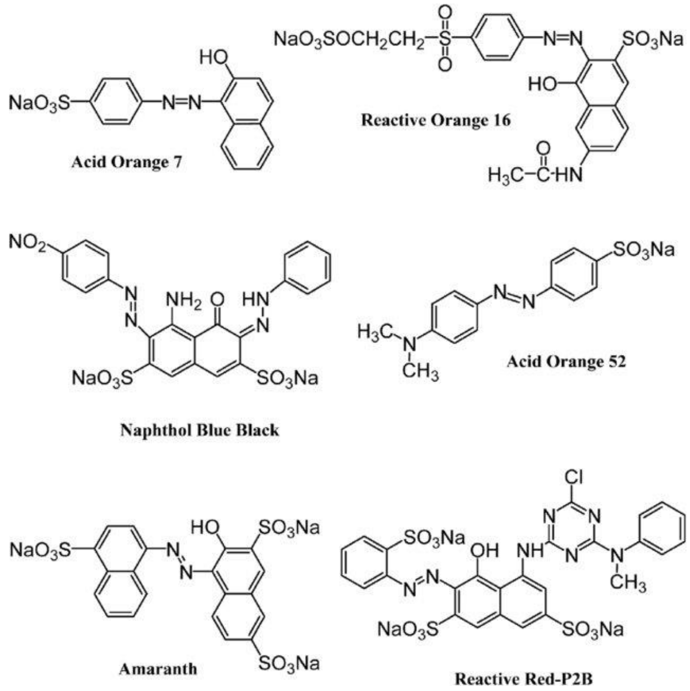


Figure 1:Azo dyes types (Qurrat-Ul-Ain, 2020)

Environmental impact of Azo dyse .

Because of its chemical makeup, which includes aromatic rings, azoic linkages, and amino groups, azo dyes are one of the more harmful families of synthetic dyes that are emitted in effluents from textile manufacturers.

The culinary, pharmaceutical, cosmetic, textile, and leather sectors all employ azo dyes extensively. They are artificial substances distinguished by one (monoazo) or several intramolecular non linkages. Azo dyes can be degraded by liver cells and skin surface bacteria via azoreductases of intestinal microflora if they are systemically absorbed. This metabolism produces potentially dangerous aromatic amines (Sigurdson *et al.*, 2017).

Genotoxic effects of several dye classes

By far the most significant category of dyes, azo dyes account for more than half of yearly global output. It should come as no surprise that azo dye toxicity has been thoroughly researched. Bladder cancer rates among employees who made dyes were found to be rising as early as 1895. Numerous research has now been undertaken to demonstrate the azo dyes' potential for toxicity. Levine's writings as well as those of Brown and DeVito40 provide a more comprehensive grasp of the issue. For water-soluble dyes, the carcinogen is a metabolite of the dye. A particularly significant family of dyes for coloring hydrophilic fabrics like cotton and viscose rayon are water-soluble azo dyes based on H-acid, J-acid, and Gamma-acid. Given that cotton is the most popular textile material worldwide, these water-soluble colors are utilized in exceptionally large quantities. The dyes are easily separated into two categories:

1-those that have the potential to produce a carcinogenic metabolite.

2. those that are not. The metabolites of benzidine and 2-naphthylamine, not the dyes themselves, are what caused bladder cancer in the employees who handled colors based on these substances.

Numerous anaerobic bacteria may break the azo linkage(s) to create aromatic amines. These bacteria were identified from the caecal or fecal contents of experimental animals and people. These processes are catalyzed by azoreductase(s), which are discovered to be oxygen-sensitive and need flavins for maximum activity. Different food components, such as cellulose, proteins, fibers, antibiotics, or supplementation with live cultures of lactobacilli, had an impact on the azoreductase activity in a variety of intestinal preparations (Li HH *et al.*, 2019).

The aim of the study

Isolate and identify bacteria capable of degrading azo dyes.

Objectives

1- Morphological and biochemical characterization of the strain.

2-Isolation and screening of dye degrading bacteria with molecular characterization by 16S rRNA.

3- Optimization of bacterial isolates for dye decolorization.

4- Phylogenetic characterization of the strain.

5-Measure the effect of environmental parameters on dyes degradation.

6-Use the FTIR spectrum to approve that bacterial isolated degrades toxic azo dyes into a non-toxic product.

Chapter Two

Literature Review

2-Literature Review

2-1 Azodyes Structure and degradation

Synthetic dyes from textile industries are common contaminants of water and possess serious environmental health hazards. A complex mixture of stubborn molecules makes up the textile industry's effluent, which may be hazardous to both the environment and human health. Due to their great effectiveness and cheap implementation and operating costs, biological treatments—particularly activated sludge—are typically used for these wastewaters. However, nothing is known about the microbiota of activated sludge. *Acidobacteria, Bacillus, Clostridium, Pseudomonas, Proteobacteria,* and *Streptococcus* are the main microorganisms found in activated sludges, with *Bacillus* and *Pseudomonas* being particularly important for dye degradation. As a result, the procedure is not performed under ideal circumstances (Didier *et al.*, 2021).

The environmental significance of microbial dye detoxification research of past >3 decades is critically evaluated with reference to dye structure and the possible influence of microbial interactions in different environments. In the absence of ecosystem-based studies, the results of laboratory-based studies on dye degradation, metabolite production and their genotoxic impact on model organisms are used to predict the possible fate and consequences of azo dyes/metabolites in the environment (Rawat *et al.*, 2016).

Industrialization increases use of dyes due to its high demand in paper, cosmetic, textile, leather and food industries. This in turn would increase wastewater generation from dye industrial activities. Various dyes and its structural compounds present in dye industrial wastewater have harmful effects on plants, animals and humans. Synthetic dyes are more resistant than natural dyes to physical and chemical methods for remediation which makes them more difficult to get decolorize. Microbial degradation has been researched and reviewed largely for quicker dye degradation. Genetically engineered microorganisms (GEMs) play an important role in achieving complete dye degradation (Varjani *et al.*, 2020).

The bacterial metabolism of azo dyes is initiated in most cases by a reductive cleavage of the azo bond, which results in the formation of (usually colorless) amines. These reductive processes have been described for some aerobic bacteria, which can grow with (rather simple) azo compounds. These specifically adapted microorganisms synthesize true azoreductases, which reductively cleave the azo group in the presence of molecular oxygen.(Stolz , 2001).

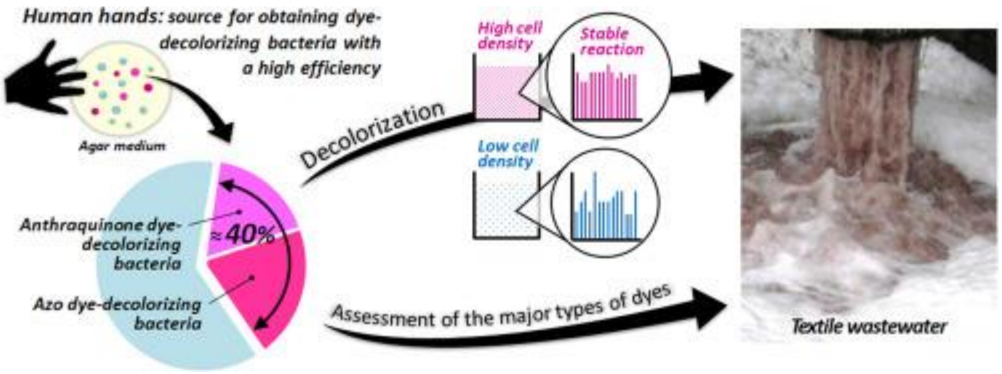
In this study ,288 strains were examined of azo-dye degrading bacteria to identify efficient strains and determine incubation times required for decolorization. Initial enrichment cultures were carried out using a mixture of four structurally different dyes (Acid Red 88, Reactive Black 5, Direct Red 81, and Disperse Orange 3) as the sole source of C and N to isolate the bacteria from soil, activated sludge, and natural asphalt. Six strains were selected for further study based on their prolific growth and ability to rapidly decolorize the dyes individually or in mixtures (Azeem *et al.*, 2008).

Bacterial community composition based on 16S rRNA gene analysis revealed that mixed cultures SB4 composed of six bacterial strains namely Bacillus sp. V1DMK, *Lysinibacillus sp*. V3DMK, Bacillus sp. V5DMK, *Bacillus sp*. V7DMK, *Ochrobacterium sp*. V10DMK, *Bacillus sp*. V12DMK. SB4 grew well in minimal medium containing low amount of glucose and yeast extract (YE) (1 g/L) and decolorized 200mg/L of RV5 within 18 h under static condition. Mixed cultures SB4 decolorized wide range of azo dyes and maximum rate of decolorization was observed at 37 °C and pH 7.0.(Jain *et al.*, 2012).

This study indicate That three cyanobacteria demonstrated the ability to decolorize and lessen the toxicity of textile effluent. On sludge, cyanobacteria did not effectively colour the water due to their sluggish development. The only strain of cyanobacteria that totally destroyed the indigo pigment was *Phormidium. autumnale* UTEX1580. Using *Allium* sp., the micronucleus test was used to assess the potential for mutagenicity. After the therapy, there was no evidence of mutagenicity. Anthranilic acid and isatin, two metabolites formed during the breakdown, were not harmful after the therapy. The capacity of the cyanobacteria to break down the colours in a textile effluent means that they may be employed in the tertiary treatment of effluents containing refractory substances (Dellamatrice *et al.*, 2016) .

According to molecular research, in anaerobic or low oxygen circumstances, two groups of enzymes involving flavin-dependent and flavin-free azoreductases can carry out the first reductive step. Broad specificity peroxidases, laccases, and tyrosinases are generally used in the second stage, which is carried out by oxidative enzymes. This study focuses in particular on how these enzymes are described in terms of their enzyme kinetics and the environmental requirements for bioreactor systems to treat azo dyes found in wastewater(Mahmood *et al.*, 2015).

in wastewater treatment facilities, physical or chemical techniques are still used to remove colours. However, there are now many research on degradation based on the employment of bacteria, an area that has been extensively investigated. However, there has not been much advancement in the application of biological techniques to deal with this environmentally harmful waste. The biggest dyes class, azo dyes (Chengalroyen& Dabbs, 2012) .



Figure(2.1) Potential use of bacteria collected from human hands for textile dye decolorization .

The environment and living things across the world are severely impacted by textile waste that is dumped into water and soil. There is an urgent need for innovative, environmentally sound, and financially feasible treatment technologies. In this work, it was determined if under ideal physicochemical circumstances, non-adapted *Aeromonas hydrophila* MTCC 1739 and textile-effluent adapted *Aeromonas hydrophila* SK16 bacteria could decolorize and break down three azo dyes. High performance liquid chromatography (HPLC) for degraded metabolites analysis based on retention time, FTIR (fourier transform infrared spectroscopy) for functional group identification, and GC-MS (gas chromatography-mass spectrometry) for proposing degradative pathways based on mass/charge as well as molecular weight all supported effective biodegradation of these dyes into simpler metabolites (Srinivasan and Sadasivam, 2020).

(do Nascimento *et al.*, 2018), in this work, the degradation of Remazol Yellow Gold RNL-150% and Reactive Turquoise Q-G125 were investigated using AOP: photolysis, UV/H_2O_2, Fenton and photo-Fenton. It was found that the photo-Fenton process employing sunlight radiation was the most efficient, obtaining percentages of degradation above 87%. The ideal conditions for the degradation of the dyes were determined from a factorial design 23 and study of the [H_2O_2] ([H_2O_2] equal to 100 $mg·L^{-1}$); [Fe] equal to 1 $mg·L^{-1}$ and pH between 3 and 4. In the kinetic study, a degradation of more than 97% was obtained after 150 min for the chromophoric groups and 91% for the aromatic compounds. The experimental data obtained presented a good fit to the nonlinear kinetic model. The model of artificial neural networks multilayer perceptron (MLP) using the software Statistic 8.0 enabled the modeling of the degradation process and showed a better prediction of the data. The toxicity to the seeds of Lactuca sativa to the bacteria *Escherichia coli* and *Salmonella enteritidis* allowed to evaluate the effectiveness of the process. The results of this study suggest that the use of photo-Fenton process with sunlight radiation is an effective way to degrade the dyes under study. Using Statistic 8.0, the multilayer perceptron (MLP) model of artificial neural networks allowed for the modeling of the deterioration process and demonstrated improved data prediction. The ability to assess the method' efficacy was made possible by the microorganisms *Escherichia coli* and *Salmonella enteritidis*' to Lactuca sativa seeds toxicity . The findings of this study indicate that solar radiation combined with the photo-Fenton process is an efficient method for deteriorating the dyes being investigated.

Azo dyes have a high level of resistance to biodegradation and light-induced fading. These dyes cannot be effectively removed from wastewater streams by the advanced oxidative pre-treatment techniques currently in use, and post-treatment oxidative dye removal is problematic because it may leave many byproducts with unknown toxicity profiles in the effluent water or result in pricey complete mineralization. Combining photocatalysis and biodegradation may be able to solve these issues. Self-assembly was used to create a new visible-light sensitive hybrid dye removal agent that contained calcium alginate beads as well as photocatalysts (g-C3N4-P25) and photosynthetic microorganisms(Zhang *et al*, 2017), This system successfully decolored synthetic dye-contaminated wastewater and reduced its COD, demonstrating the benefits of combining photocatalysis and biocatalysis for wastewater purification. It also achieved a removal efficiency of 94 percent for the dye reactive brilliant red X-3b and reduced the COD of synthetic wastewater samples by 84.7 percent. The combination appears to break down X-3b by first turning it into aniline and phenol derivatives, whose aryl moieties are subsequently attacked by free radicals to generate alkyl derivatives, limiting the buildup of aromatic hydrocarbons that may potentially restrict microbial activity. The photosynthetic bacteria ultimately destroy these alkyl intermediates.

2.2 Decolorization Gene for Bacterial spp.

Many strains with the capacity to decolorize a range of azo and triphenylmethane dyes have been found thanks to the biodegradative ability of certain actinomycetes. An *Amycolatopsis sp.* isolate that shown the capacity to remove the color amido black, an azo dye. A 4.5 kbp fragment coding for the putative decolorization gene was discovered through the screening of a DNA library. Seven open reading frames encoding a polyprenyl synthetase, cupin-2 conserved barrel domain, transcriptional regulator, membrane protein, DNA damage inducible gene, and two putative proteins were predicted as a result of the sequencing of this gene fragment. The potential enzyme of interest was found as a gene encoding a protein with 312 amino acids that shares 77% of its amino acids with a conserved putative protein in Amycolatopsis mediterranei(Chengalroyen *et al.,* 2013).

(Tang *et al.,* 2018)found that since efficient azo-dye degradation bacteria have been isolated and identified, bioaugmentation with certain microbial strains has shown to be a successful method for accelerating azo dye bioremediation. However, the widespread use of the known mesophilic azoreducing bacteria was constrained by the azo dye wastewater emitted at high temperatures. Here, we offer the whole genome sequence of Novibacillus thermophiles SG-1 (=KCTC 33118T =CGMCC 1.12363T), a thermophilic bacteria capable of degrading azo dye. A circular chromosome with a length of 3,629,225 base pairs and a G + C composition of 50.44 percent makes up the whole genome of strain SG-1. According to a genome study, the strain SG-1 has genes for riboflavin biosynthesis proteins, which might produce riboflavin and serve as electron shuttles to carry electrons to extracellular azo dye during the decolorization process.

(Yassin *et al.*, 2021) study of bacteria that produce thermostable amylase in harsh settings is essential to overcoming many industrial difficulties. Extremophiles live in the Afar region in Ethiopia because it is one of the hottest and saltiest places on Earth. The objective of this study was to identify the amylase-producing bacteria that were isolated from soil samples in the Afdera, Afar Region. As a result, the collected soil samples yielded a total of 49 bacterial isolates. Out of them, three isolates (M2, M8, and M13) were chosen based on the average clear zone creation diameter and iodine solution decolorization duration. The isolates' physical and biochemical traits allowed for the genus Bacillus to be determined. The three bacterial isolates' possession of the amylase gene was validated by PCR amplification and identification of the gene encoding amylase.

The widespread use of synthetic dyes in a variety of sectors endangers aquatic life by acting as contaminants in the environment. In thes sdudy, been evaluated the *Salinivibrio kushneri* HTSP, a halophilic bacterium isolated from a saltpan, for its capacity to decolorize and bioremediate manmade colors. This strain's genomic analysis revealed the presence of genes encoding decolorization processes, such as FMN-dependent NADH azoreductase Clade III, which cleaves the dye's azo bond, and deamination and isomerization of intermediate compounds. As several peaks for distinct vibrations of aromatic rings, aliphatic groups (-CH2, -CH3), and functional groups (-NH, -SO3H, and -SO3 -) in decolorized solutions were altered and lost, the results suggested that dyes broke down during decolorization. This work has demonstrated that *Salinivibrio kushneri* HTSP has the ability to decolorize dyes at larger concentrations and at a faster rate than other bacterial strains that have been previously described. (John *et al.*, 2020) .

In Pakistan's textile exports from Faisalabad account for 55% of all exports of textiles. These textile manufacturing facilities dump their untreated effluents to the environment, which has resulted in a significant pollution with synthetic azo dyes. The goal of the current study was to assess the role of an azoreductase-encoding gene (azrS) from the previously characterized azo dye-decolorizing bacterial strain *Bacillus* sp. MR-1/2 in a high copy number host system (pUC19-T7-Top-T) of *Escherichia coli* strain DH5. This was followed by in-silico prediction of the function of the azoreductase enzyme (Az After 72 hours of incubation, the azrS-containing recombinant cells removed color from congo red and reactive black-5 dyes at a rate that was noticeably higher than that of wild-type MR-1/2 and *E. coli* DH5 (Abbas *et al.*,2020). In addition, the recombinant strain was shown to significantly reduce the values of all parameters investigated in real wastewater, including pH, electrical conductivity, turbidity, TSS, and COD. bioinformatics analysis suggested that the derived azoreductase protein of strain MR-1/2 is related with the strain's capacity for dye decolorization via NAD(P)H-ubiquinone: oxidoreductase activity. In addition, discovered that while the deduced protein had many characteristics with closely similar proteins in the protein databank, strain MR-1/2 enzyme activity indicated certain distinct characteristics. It was determined that the recombinant strain may be tested in pilot-scale studies for the treatment of textile effluent (Guang *et al.*, 2020).

the found that a significant barrier to azo dye decolorization is the high pH and salinity of textile effluent. Under order to discolor Methanil Yellow G, a mixed bacterial consortia ZW1 was enhanced in salty (10 percent salinity) and alkaline (10.0 pH) conditions (MY-G) (Figure 2.2) . Among the members of consortium ZW1, Halomonas (49.8%), Marinobacter (30.7%), and Clostridiisalibacter made up the majority (19.2 percent). Physicochemical influences, the degradation process, and metagenome analyses were all thoroughly explored. Adding yeast extract and the co-carbon source resulted in a 93.3 percent decolorization of 100 mg/L MY-G after 16 hours (compared with 1.12 percent for control). The ideal pH, salinity, temperature, and starting dye concentration were, respectively, 8.0, 5–10%, 40 °C, and 100 mg/L. The normal dye-related degradation enzymes worked best at salinities of 10%. Five more direct and acidic dyes may be variably decolored by Consortium ZW1 in a little amount of time. Phototoxicity studies showed that MY-G degradation products have been detoxified. The consortium ZW1 presented MY-G degradation route combining UV-vis, FTIR, and GC-MS detection. Additionally, a metagenomic method was employed to clarify the probable functionality of the genes involved in MY-G biodegradation. These findings show that halo-alkaliphilic consortia have a wide range of potential applications in the bioremediation of dyeing wastewater.

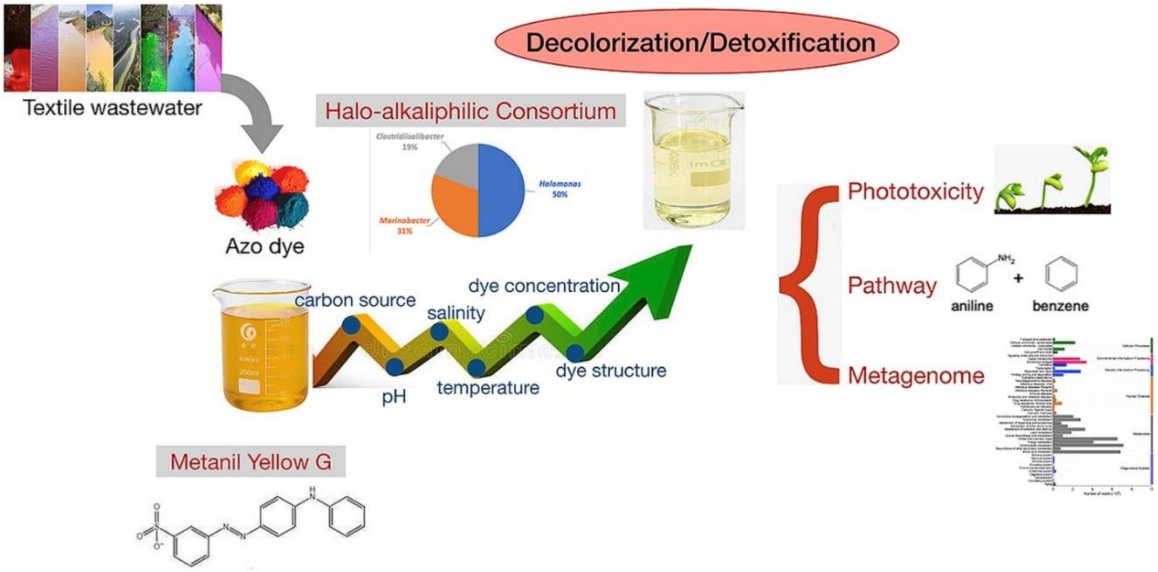


Figure (2.2): Systematic examinations of the decolorization and detoxification of azo dye by the halo-alkaliphilic bacterial consortium: performance, route, and metagenome (Guang *et al.*,2020).

In Serbia, industrial and agricultural soil yielded 100 wild-type strains of *Bacillus sp*., which were then tested for laccase activity. Three strains demonstrated strong laccase activity toward ABTS at temperatures between 65 and 80 °C. *Bacillus* amyloliquefaciens 12B, the strain with the greatest temperature optimum, produced a novel laccase gene that was cloned and expressed in *Escherichia coli*. While fungal laccases were unable to operate on this substrate at pH levels higher than 4.0 and were immediately inactivated at temperatures higher than 45 °C, recombinant laccase destroyed dye Reactive blue 52 at pH 7.0 and pH 4.0 as well as at increased temperature. By using HPLC-DAD to track dye degradation, the precipitate that resulted was examined using FTIR spectroscopy (Lončar *et al.,* 2013).

Azo dyes are more widely employed in the textile sector as a coloring agent . It produces a significant volume of effluent that contains dye, which is poisonous and endangers all forms of life, including humans. The biological treatment of dye effluent is currently receiving greater attention due to the azoreductase enzyme's ability to break down the azo link, which causes the dye to decolorize and degrade. Reactive Yellow-145 (RY-145) dye was degraded and decolored using a bacterial consortium of *E. asburiae* and *E. cloacae* (1:1 ratio) (Figur 2.3). To find the ideal conditions for maximal color degradation and decolorization, dye concentration, temperature, pH, and medium have all been optimized. Under static conditions at 500 mg.L^{-1} concentration, 35 °C, and pH 7.0 over a 12-hour contact time, the mixed consortium (10 percent) demonstrated 98.78 percent decolorization of the RY-145 dye. The degradation of the dye was shown by FTIR analysis, which also revealed the creation of novel functional groups in the treated dye, including O-H stretch at 1361 cm1, C-H stretch at 890 cm1, N-H stretch at 1598 cm1, and aromatic C-H at 671 cm1 (Asha *et al.*,2022) .

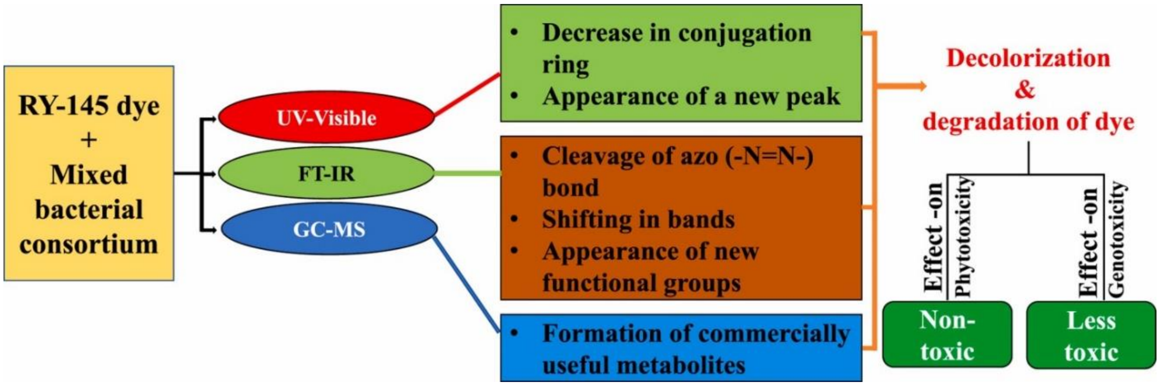


Figure 2.3: Reactive Yellow-145 azo dye biodegradation utilizing a bacterial consortium: A deterministic analysis based on phytotoxicity and genotoxicity research (Asha *etal.*,2022).

In thes study, the decolorization mechanism, the influence of various factors on the decolorization percentage, and the toxicity analysis of Reactive Black 5 both before and after decolorization were examined . strain MEH038S cells were decolored shows that reactive black 5 was decolored throughout the degradation process. The decolorization was caused by an FMN-dependent NADH-azoreductase gene, which was validated by the findings and subsequently assigned to Staphylococcus sp.(Fazeliyan *et al.*, 2021).

The work by (Yogesh *et al.,* 2012) focuses on the collaboration EDPA's decolorization of the di-azo dye Acid Maroon V using two isolates collected from a polluted location. Based on 16S rDNA sequencing, the two isolates Enterobacter dissolvensAGYP1 and *Pseudomonas aeruginosa* AGYP2 were identified. Under static incubation conditions, 93 percent of the dye (100 mg/L) underwent decolorization in Mineral Salt Medium after 20 hours. With maximal activity at pH 7.0, the consortium maintained its decolorization activity throughout a wide pH range (6.0–9.0). Additionally, consortia decolored Acid Maroon V at levels between 100 and 2000 mg per liter.

Azo dyes are persistent pollutants that pose a serious threat to the environment. They are poisonous, carcinogenic, mutagenic, and teratogenic. Using a probiotic consortia, Remazol Brillant Orange 3R (RBO 3Rdecolorization)'s and mineralization effectiveness were investigated (Lactobacillus acidophilus and Lactobacillus plantarum). Under shaking conditions in a Mineral Salt Medium (MSM) solution with a pH of 11.5 and a temperature of 25°C, the biodegradation of RBO 3R (750 ppm) was examined. FTIR and UV-Vis studies further supported the bio-decolorization process. Under ideal circumstances, the bacterial community was able to thoroughly (> 99 percent) decolorize the dye in 12 hours. At 750 ppm, the color removal was 99.37 percent. The Lactobacillus genes were found using the multiplex PCR method. Toxicological investigations of RBO 3R before and after biodegradation were investigated using phytotoxicity, cytotoxicity, mutagenicity, and biototoxicity endpoints. A toxicity test revealed that the RBO 3R dye was detoxified as a result of biodegradation.(Ayed *et al.*, 2019) .

(Maqbool *et al.*, 2016)studied the ability of 220 bacteria isolated from textile wastewater to decolorize each of the four reactive dyes—reactive red-120, reactive black-5, reactive yellow-2, and reactive orange-16—in the presence of a combination of four different heavy metals—Cr, Zn, Pb, and Cd—commonly found in textile effluents—was examined in this study. The isolate ZM130, which was determined to be *Pseudomonas* aeruginosa strain ZM130 by 16S rRNA gene analysis, was shown to be the most effective in decolorizing reactive dyes in the presence of the combination of heavy metals.

2-3 Environmental factors effecting Azo dyes reduction by use Bacterial Sp.

(Chang *et al.*, 2001) used a *Pseudomonas luteola* strain with azoreductase activity to get rid of reactive red 22's hue from tainted solutions. In order to identify the critical elements that significantly influence the effectiveness of azo-dye decolorization, the effects of substrate concentrations, medium compositions, and operation parameters (such as pH, temperature,.) on the azo dye decolorization by a *Pseudomonese* . *Luteola* strain were systematically investigated. High-performance liquid chromatography (HPLC) and mass spectrometry were used to examine the metabolites produced by bacterial decolorization (MS). According to the findings, *Pseudomonese*. *Luteola* ability to decolorize reactive red 22 was delayed by the proportion of dissolved oxygen and glucose. The ideal temperature for azo-dye decolorization was 37 °C, and decolorization happened more quickly at pH 7-9. Tryptone and yeast extract significantly accelerated the decolorization . The relationship of a given decolorization rate with substrate concentration may be adequately explained by the Michaelis-Menten model (reactive red 22 or yeast extract). The growth phase had nothing to do with how well *Pseudomonese*. *luteola's* intact cells decolored the azo dye, but the azoreductase activity of the cell-free extract declined in the following order: late-stationary phase > early-stationary phase > mid-log phase.

Due to the difficulties of O_2 reduction by the conduction band electron on WO, photocatalytic degradation of organic substrates over WO(3) in an aerated aqueous solution proceeds very slowly . In this study, we present evidence that H_2O_2 considerably speeds up the photodegradation of phenol and the azo-dye X3B in water when exposed to UV or visible light. More significantly, compared to pure WO(3) (HW) made concurrently, an iron-containing WO(3) (FeW) produced by the thermal breakdown of a ferrotungstenic acid showed a significantly greater activity(Bi D,& Xu , 2011).

(Onder et al., 2011) A study evaluating the decolorization of azo-naphthol blue-black dye using the enzyme horseradish peroxidase was conducted. To better understand the process factors including pH, temperature and reaction time, studies are performed. By using a UV-Vis spectrophotometer and LC-MS data, the dye's enzymatic decolorization was investigated. High azo-dye decolorization was achieved by optimizing temperature and pH conditions, it was discovered that azo-dye removal was the greatest in a pH range of 4-6. Approximately 80–90% of the dye's pigment was removed after 5 hour of treatment. The decolorization of the azo dye with enzyme was caused by the reduction of the azo bonds, according to the LC-MS and spectrophotometric investigations.

A *Proteus mirabilis* bacteria was discovered in acclimated sludge from a wastewater treatment facility for dyeing. This strain quickly turned a dark crimson azo dye solution white (RED RBN). Also investigated was the decolorizing procedure as it relates to biodegradation and biosorption. Although *P. mirabilis* showed strong growth in shaking culture, anoxic static cultures were superior for color removal. 6.5-7.5 ph and 30-35 ºC were the ideal values . Even at high azo dye concentrations, the organism showed outstanding color removing abilities. At a dye concentration of 1.0 g L^{-1}, more than 95% of the azo dye was degraded in under 20 hours. Decolorization appears to be predominantly driven by enzymatic action , with a little amount of biosorption to inactive microbial cells (13–17%) (Chen *et al.*,1999).

(Mohanty & Kumar,2021) study the decolorization of the dye Indanthrene Blue RS, as well as the optimization of the process variables required for the bacterial consortia to decolorize the dye successfully. From the textile wastewater sample obtained from a local textile processing unit outlet and dye-contaminated soil from Odisha, India, the pure culture of strains TS8, PMS, and NCH have been isolated. *Bacillus flexus* TS8, Proteus mirabilis PMS, and *Pseudomonas* aeruginosa NCH were developed into a bacterial consortium, or BP. To achieve the highest decolorization efficacy, the physicochemical parameters were tuned. Through the use of UV-vis, FT-IR, and GC-MS analyses, it was determined that Indanthrene Blue RS was degrading and that metabolites were forming.

(Olukanni *etal.*, 2010) confirmed that Reactive Blue 13 (RB13), a sulphonated reactive azo dye, was removed and biodegraded using the *Proteus mirabilis* LAG bacterium strain, which was obtained from the soil of a municipal waste disposal site close to Lagos, Nigeria. Within 5 hours, this strain lightened RB13 (100mg/l). Fourier transform infrared spectroscopy (FTIR), which showed the elimination of several peaks, especially those of the aromatic C-H bending at 600-800 cm, suggested the production of aromatic amine prior to mineralization (-1). The dye metabolite was examined using gas chromatography-mass spectroscopy (GCMS), which revealed the existence of sodium-2(2-formyl-2-hydroxyvinyl) benzoate with a tropylium cation as its base peak. This demonstrated that the naphthalene rings in RB13 had broken. The presence of azoreductase and laccase activity indicated that azo bonds had been reduced enzymatically prior to mineralization. Additionally, phytotoxicity experiments showed that this strain of P. mirabilis LAG detoxified RB13 into non-toxic breakdown products.

Chapter Three

Materials & Methods

3.Materials and Methods

3.1. Materials

3.1.1 Equipment and Instruments of Laboratory .

Table (3.1) shows the laboratory instruments and equipment that were used in this study and their sources.

Table (3.1): Equipment and Instruments of Laboratory .

NO.	Equipment & instruments	Company / Country
1	Autoclave	HIRAYAMA / USR
2	Sensitive balance	Sartorius /Germany
3	Cabinet hood	BioLAB/ Korea
4	Burner	Memmert/ Germany
5	Incubator	Memmert GmbH/ German
6	Water Distillator	GFL/ Germany
7	Micropipettes (Different sizes)	Eppendroff/ Germany
8	Refrigerator	Kelon /Korea
9	Beakers, Flasks & Cylinders (Different sizes)	Superior/ Germany
10	Tips (Different sizes)	Jippo / Japan
11	Petri dish	Sterial/Jordan
12	Swabs	AFCOSWAB /Jordan
13	Plane tubes(10ml)	DMD-DISPO/ Jordan
14	Platinum wire loop & Platinum wire needle	Himedia/India
15	Light Microscope	Olympus/ Japan
16	Slides & Coverslips	OEM/ China
17	Disposable syringes (5ml)	Mediplast /U.A.E
18	Parafilm	ParafilmM/USA

19	0.22 µm Millipore filter papper	Bio-Hit/ Finland
20	pH meter	Martini /USA
21	Shaker incubator	Gyromax/USA
22	Test tube Racks	Barcopharma/ China
23	Centrifuge	Selecta p / Germany
24	UV. Visible Spectrophotometer	CECIL 1000 series /France
25	FTIR-8400 spectroscopy	Shimadzu/Japan
26	Rotary Evaporator	Rotavapor-RE- Buchi/USA
27	Electrophoreses	Clarivate /UK
28	1.5 ml Eppendorf tubes	Sigma-Aldrich /USA
29	UV.transmission	Vilber Lourmat Sté /Farance
30	Vortex	Fisher Scientific/ USA
31	Thermostatic Incubator	Zxinstrument/Chain
32	Power Supply	Biorad/ USA
33	Applied Biosystems™ ProFlex™ PCR System	Fisher Scientific/ USA
34	Vitek 2 system	Biomerieux / France
35	PCR tube	Eppendorf /Germany

3.1.2: Biological and Chemical Materials .

The biological and chemical materials used in this study are listed in (Table 3.2)

Table (3. 2) Biological and Chemical materials

NO.	Equipment & instruments	Company / Country
1	Nutrient broth & Nutrient agar	Pronadisa/ Spain
2	MacConkey agar, Simon citrate agar, Blood agar, Mannitol Salt Agar, MR-VP broth & Kligler iron agar	Himedia/ India
3	Urea agar base	Sigma/USA
4	Brain heart infusion (BHI) broth	Pronadisa/ Spain
5	Gram Stain Solutions	Fluka / Switzerland
6	Normal saline	Mediplast /U.A.E
7	Human blood	AL-Yarmouq blood bank /Iraq
8	Kovac's reagent	Fluka chemika/ Switzerland
9	Peptone broth medium	Mast/U.K.
10	Urea , Agar, Absolute ethanol (%99), Glycerol Methyl Red Reagent , Voges-Proskauer Reagents, Oxidase Reagent & Catalase Reagen	BDH /England
11	Sodium Hydroxide (NaOH) Hydrochloric Acid (HCL)	Crescent/SaudiArabia
12	azo dyes (Direct Yellow, Disperse blue, Reactiv green, Direct Brown & Reactive Brown)	China
13	Agarose	Carl Roth/Germany
14	Red safe staining souluion	Intron / Korea
15	FavorPrep Blood/ Cultured Cells Genomic DNA Extraction Mini Kit	Korea
16	6X Loading dye	Intron / Korea
17	TBE10X	Intron / Korea
18	Ladder 100	Kapa /USA

3.2 Methods

3.2.1. Preparation of Solutions & Culture media

Solutions and both traditional and differential culture media have been prepared in compliance with the orders of the manufacturers. The culture media sterilization was done by autoclave at 121 ° C, under 1bar pressure for 15-20 min. Filtration using 0.22 µm Millipore filters were used for heat-sensitive solutions such as urea solution. The pH of the solutions was adjusted using (1M) NaOH or (1M) HCL.

3.2.1.1 McFarland Standard Solution

(0.5) McFarland standard solution tube with concentration of 1.5 x 10^8 CFU/ml was obtained from the supplier company (biomerieux)and used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial testing.

3.2.1.2 (40%) Urea Solution

Urea solution prepared by dissolving 20g of urea in 50ml distilled water and sterilized by filtration using 0.22 µm Millipore.

3.2.1.3 Gram Stain Solutions

They are used as supplied from the manufacturing company (Fluka). Which are four solutions; crystal violet, iodine, absolute alcohol, and safranine. These solutions used for microscopic examination and identification of bacterial cells by observing the shape, arrangement of cells and type of reaction with Gram-stain , Gram + (Purple) or Gram - (Red) (Mahon *et al*., 2018).

3.2.1.4 Aluminum sulfate or alum [$Al_2(SO_4)_3 .14H_2O$] Stock Solution

It was prepared by dissolving 10 g of alum into 1000ml of distilled water (Zane ,2004).

3.2.1.5 [1 N] Sodium Hydroxide Solution (NaOH)

It was prepared by dissolving 40 g of NaOH in appropriate volume of distilled water, then the volume was completed to 1000 ml (Nikolskij, 1964).

3.2.1.6 [0.25] M Hydrochloric Acid Solution (HCl)

This solution was prepared by adding 5.2 ml of 37% HCl to appropriate volume of distilled water, then volume was completed to 250 ml with distilled water (Nikolskij, 1964).

3.2.1.7 Nutrient agar

To isolate, cultivate and to save bacterial strains (Mahon *et al*., 2018).

3.2.1.8 Nutrient Broth

This medium was used to grow and preserve the bacterial isolates. This medium was prepared according to the method recommended by the manufacturing company (Himedia) (Mahon *et al*., 2018).

3.2.1.9 Brain heart infusion (BHI) broth medium

This product was developed by the manufacturer and was used for the activation of bacterial isolates (Mahon *et al*., 2018).

3.2.1.10 Methyl red Voges Proskauer (MR-VP) broth media

This medium was prepared according to instructions of company by dissolving 17 g of the powder in 1000 mL of distilled water. Mix thoroughly and sterilized by autoclave at 121˚C after setting the pH at 7 (Ph=7.0). After cooled at 50˚C distributed the medium into sterilized test tubes. This medium was developed to enable both the MR and the VP tests to be performed in the same medium, although in different tubes (Mahon *et al*., 2018).

3.2.1.11 MacConkey agar medium

Isolate Gram-negative bacilli and separate lactose fermented from non-lactose fermented bacteria (Mahon *et al*., 2018).

3.2.1.12 Blood agar medium

Blood agar base was processed according to the manufacturing company, autoclaved at (121 ° C \ for 15 minutes), then cooled to 50 ° C, and human blood was added .This method was used to cultivate bacterial strains and to determine their ability to produce blood hemolysis (Mahon *et al*.,2018).

3.2.1.13 Peptone Water Medium:

This medium was prepared according to the method suggested by the manufacturing company. It was used for the demonstration of the bacterial ability to decompose the amino acid tryptophan to indole. The positive result was formation of red ring at the top of broth (MacFaddin, 2000).

3.2.1.14 Simon Citrate agar medium

This method has been used to determine the capacity of the bacteria to citrate consumption (Mahon *et al.*, 2018).

3.2.1.15 Kligler Iron Agar

This medium was prepared according to the method suggested by the manufacturing company. This media use to determining bacterial ability to glucose and lactose fermentation and possible hydrogen sulfide (H_2S) production as a first step in the identification of gram negative bacilli (MacFaddin, 2000).

3.2.1.16 Urea Agar

This medium was prepared according to instructions of company (Sigma) by dissolving 24 g of Urea Agar Base in 950 ml of distilled water, Ph was setting (Ph=7.1) then sterilized by autoclave at 121˚C for 15 minutes. Cooled at 50˚C and aseptically add 50 ml of sterile 40% urea solution (3.2.1.2) to the agar base. Mixed well and distributed the medium into sterilized test tubes and allowed to solidify in slant form. This medium was used to detect bacterial ability to produce urease enzyme (MacFaddin, 2000).

3.2.1.17 Motility Medium

Motility medium was prepared by dissolving (0.5gm) agar with (100ml) of nutrient broth, then dispensed into sterile test tubes (5ml in each tube) using. This medium used to detect the bacterial motility (Aygan and Arikan, 2007).

3.2.2 Specimens collection

30 environmental specimens (15 soil samples) were collected from different agriculture places of the gardens scattered in the medical city/ Baghdad

teaching hospital, as well as (15 wastewater samples) from Al-Saqlawiyah drainage station, northwest of Baghdad, at the period from January 2022 to February 2022. Each specimen was collected in sterile screw-cap container and immediately transferred under cooling conditions to the laboratory for analysis. Samples were tested within 24 hrs. of collection.

3.2.3 Isolation and Identification of *Proteus spp.*

Each sample was cultured in a brain heart infusion broth medium and incubated at 37 ºC for 24 hours to promote bacterial growth. After that they were streaked on the general and differential culture media, also incubated for 24 hours at 37 ºC. The positive growth was diagnosed depending on morphological (Shape, swarming, fishy odor and lactose or non-lactose fermenter on MacConkey agar), microscopical features and biochemical tests; also, we used vitek II system in the identification of bacterial isolates .

3.2.3.1 Colonial morphology identification

The culture characteristics were studied after culturing and purifying the bacterial isolates on different culture media such as the MacConkey agar medium, the blood agar medium etc...,. The study included all of the shape, size, texture, color, edges and heights of isolated bacterial colonies that recorded after incubation for 24 hr. at 37 ºC (Wanger *et al*., 2017).

3.2.3.2 Microscopic identification

Smears were prepared from a single colony of young bacterial isolate grown on nutrient agar medium for 18- 24 hours, and fixed on a clean and dry slide, then dyed with Gram stain and examined under a light microscope to see the shape, arrangement and colors of the cells according to their interaction with the gram stain (Levinson,2016).

3.2.3.3 Biochemical Identification

3.2.3.3.1 Oxidase test

A colony growing on nutrient agar medium for 24 hours was transferred onto sterilized filter paper by wooden chopsticks, then a drop of oxidase reagent is added to it, and the color changed to purple in (30-60) seconds is evidence of the production of the oxidase enzyme (Hemraj *et al*., 2013).

3.2.3.3.2 Catalase test

A colony growing on nutrient agar medium for 24 hours was transferred onto a sterile, dry glass slide with wooden chopsticks, then one drop of 3% catalase reagent was added to it. The appearance of bubbles on the surface of the glass slide is evidence of the production of the enzyme catalase (Hemraj *et al*., 2013).

3.2.3.3.3 IMVIC tests

3.2.3.3.3.1 Indole test

This test was carried out by inoculating test tubes containing peptone water medium with bacterial colonies, and then incubating for 24 hours at a temperature of 37 ºC, then 1-2 drops of Kovacs reagent were added to the medium. The appearance of a red ring on the surface of the median indicates a positive test (Hemraj *et al*., 2013).

3.2.3.3.3.2 Methyl Red Test

This is a qualitative test of the acidity. The tubes of the (MR-VP broth) were inoculated with the selected bacterial colonies and incubated at 37ºC for 24 hrs., then (5 drops) of methyl red reagent are added to it. The cultured MR-VP broth was tested for complete fermentation of glucose, which low pH down to 5, a bright red color is a positive test, a yellow or orange color is a negative test (Hemraj *et al*., 2013).

3.2.3.3.3.3 Voges –Proskauer test

This test is used to detect bacterial ability to ferment glucose and produce acetoin. The tubes of (MR-VP broth) are inoculated with tested bacterial culture and are incubated at 37 ºC for 24 hrs., then the result is read by adding 0.6 ml of

VP1 reagent which is a (α- naphthol dissolved in Absolut ethanol) and 0.2 ml of VP2 reagent which is a (KOH dissolved in distilled water; appearance of red color after 15 min. due to partial analysis of glucose, which produces acetoin or acetyl Methyl–Carbinol (Hemraj *et al*., 2013).

3.2.3.3.3.4 Citrate utilization test

This test was used for the purpose of knowing the ability of bacterial isolates to consume citrate as the sole source of carbon and energy and on the consumption of ammonium salts as a source of nitrogen .This assay was performed by streaking a loopful of colony onto a petri dish with simmon citrate medium or simmon's citrate agar slant then incubating for 24-48 hours at 37°C.The change of the color of the medium from green to blue with the appearance of bacterial growth is in indication of a positive result while green color of the medium remains the same it is a negative result (Hemraj *et al*., 2013).

3.2.3.3.4 Hemolysin production test

Bacterial isolates were cultured on plates containing blood agar medium, the plates were incubated at 37°C for 24 hours, then the results were read by noting the type of decomposition (alpha, beta or gamma) (Hemraj *et al*., 2013).

3.2.3.3.5 Kliger's iron test

This test was used for the differentiation of members of the Enterobacteriaceae based on the fermentation of lactose, glucose, and the production of H2S. Kligler iron agar (KIA) slant prepared as mentioned in (3.2.1.15) was inoculated with tested bacterial culture by streaking on the surface and stabbing in bottom of the medium, incubated at 37°C for 24hrs., the positive result was noticed by changing the color from red to yellow as a result for acid production from sugar fermentation ,the results of sugar fermentation during this test are read as in table (3.3), space at the bottom of the tube and the split in the agar in the middle of the tube indicates gas (CO_2) production from sugar aerobic fermentation, black residue appeared at the bottom of the tube indicates hydrogen sulfide (H_2S) production .Sometimes the butt portion will be entirely black. In such a case, it is assumed that the butt portion of the tube is acidic (yellow color is masked due to the H_2S production). While the negative result observed by keeping the medium on its red color, bubbles and black residue does not form.

Table (3.3): Indicator results for sugar fermentation during Kliger's iron test

NO.	Slant Color	Bottom Color	Fermentation Result
1.	Red (Alkaline Slant)	Yellow (Acid Bottom)	(K/A) Only Glucose Fermentation (positive result)
2.	Yellow (Acid Slant)	Yellow (Acid Bottom)	(A/A) Glucose & lactose Fermentation (positive result)
3.	Red (Alkaline Slant)	Red (Alkaline Bottom)	(K/K) No Fermentation (negative result)

3.2.3.3.6 Urease Production Test

This test was used to detect bacterial capacity to produce urease enzyme which hydrolyze urea to ammonia and carbon dioxide. Urea agar slant (3.2.1.16) was inoculated by streaking with a sterile loop with tested bacteria and incubated at 37ºC for 24hrs. Pink color indicates positive result for the test; while yellow color indicates negative result (MacFaddin *et al*., 2000).

3.2.3.3.7 Motility test

This test was done by using the tube that contained semisolid media (motility medium that prepared as mentioned in (3.2.1.17). The media were inoculating with tested bacteria by a stabbing method and is incubated at 37ºC for 24hrs. , the disseminating of growth out of the stab line was an indication of positive result (Aygan and Arikan, 2007).

3.2.3.4 Identification of *Proteus spp* using the Vitek II system

This system was used according to manufacturing company (bioMérieux, France) by inoculation of bacteria on MacConkey agar plate, then incubation at (37 ºC /24 hours). A bacterial suspension was prepared from the above growth by transferring 1-3 colonies to test tubes containing (3 ml) from normal saline and the suspension turbidity was adjusted to a McFarland standard of (0.5) (Fritsche *et al*., 2011).

3.2.4 Preparation of dyes solutions

The commercial dye powders were pasted with Double Distilled water (ddH_2O), dissolved, and then diluted to prepare stock solutions of 1000mg/L. Three different concentrations 30 mg/L, 60 mg/L, and 90mg/L were used for decolorization studies (Parmar and Shukla, 2015). ddH_2O can also be used instead of Distilled water (dH_2O) to reduce the cost of treatment, but the ddH_2O provides more sterilization conditions.

3.2.5 Preparation of inoculum for liquid culture

A young and pure culture of previously identified bacterial isolate was used to prepare the inoculum. Bacterial isolates were sub-cultured on nutrient agar and incubated at (37 ° C for 24 hrs.), then adding at least 3 to 5 well isolated colonies to plane tubes containing (3 ml) from normal saline for the emergence of the turbid growth equal to the normal turbidity standard solution (McFarland standard tube number 0.5 with concentration of 1.5 x 10^8 CFU/ml) (Parmar and Shukla, 2015).

3.2.6 Dye decolorization experiments

Decolorization experiment was performed in 250 mL Erlenmeyer flasks containing 100 mL of nutrient broth and was inoculated with 1mL of fresh bacterial inoculum (*Proteus vulgaris or P. mirabilis*) that previously prepared at (3.2.3). Then 50 mL of the dye solution (Direct Yellow, disperse blue, Reactive green, Direct Brown and Reactive Brown) of a specific concentration (90mg/L ,60 mg/L & 30 mg/L) was added. A flask containing only nutrient broth and the dye solution but no bacterial culture was used as control. The flasks were incubated at 37°C and shaking (120 rpm) conditions for 24 h (Parmar and Shukla, 2015).

After incubation at different time intervals (12 hrs., 18 hrs. and 24 hrs.) an aliquot (5 mL) of the culture was withdrawn, alum stock solution (3.2.1.4) was added (in a percent of 1%, which is equivalent to 50 µl) and centrifuged at 10,000 rpm for 20 min to separate the bacterial cell mass. Decolorization was determined using a UV/V which is Spectrophotometers by measuring the change in the absorbance value of the culture supernatant at the wavelength of maximum absorption (λ max) specific to each dye (Parmar and Shukla, 2015), Direct Yellow = 404 nm, disperse blue = 557 nm, Reactive green = 628 nm, Direct Brown = 467nm and Reactive Brown= 469 nm). Percentage of decolorization was calculated from the difference between initial (before incubation) and final (after growth) absorbance values. To calculate decolorization percentage the following equation was considered: (Erysipelothrix, 2012).

Decolorization efficiency (%) = 100x (ODi - ODt) / ODi

ODi refers to the initial absorbance and ODt refers to the absorbance after incubation for an incubation time t . Further, the decolorization efficiency of azo dyes by *Proteus sp.* was studied at different pH ranges by adjusting the pH of the nutrient broth medium to at (6 ,7 and 8) .Also, the nutrient broth medium was inoculated with the selected *Proteus sp.* and incubated at different temperatures (30, 37 and 42) °C to determine the optimum temperature for Azo dyes decolorization.

Whereas biodegradation was monitored by Fourier Transform Infra-Red (FTIR) Spectrophotometer. The functional group characterization of the dyes before and after decolorization was studied. The samples prepared at the same concentrations were inoculated with the desired microbial inoculum to perform decolorization. These samples were centrifuged at 10 000rpm for 20 min. The degraded products present in culture supernatant were extracted using an equal volume of ethyl acetate. The extracts were dried over anhydrous $Na2SO4$ and evaporated to dryness in a rotary evaporator. The FT-IR analysis of degraded products was carried out using Shimadzu FTIR-8400S, Japan in the mid-infrared region of 500-4000 cm-1 with 16-scan speed. IR spectrum was recorded and compared with the control dye (Khan and Joshi, 2020; Parmar and Shukla, 2015)

3.3 DNA extraction

3.3.1 Protocol

1.Transfer the appropriate number of cell (up to 5 x 10^9) to a 1.5 ml microcentrifuge tube, and Centrifuge at 14000rpm for 1 min, and remove the supernatant carefully and completely.

2. Add 200 µl lysozyme Buffer to the sample and mix by vortexing. or pipetting Incubate in room temperature for 10 min to lyse the sample.
3. Add 200 µl FABG Buffer to the sample ,and mix thoroughly by vortexing by 5 sec.
4. Incubate at 70 ºC for 10 minutes to Lysate the sample.
5. Preheat required Elution Buffer in a 70 ºC water bath for DNA Elution.

DNA Binding

6. Add 200 µl of ethanol (96~100%) to the sample and vortex for 10 sec. Pipette the sample to mix well if there is any precipitate formed.

Place a FABG Column to a Collection Tube. Transfer the sample mixture carefully to FABG Column. Centrifuge at speed 14,000 rpm for 1 min. Discard the Collection tube and place the FABG Column to a new collection Tube.

Column Washing

8. Add 400 µl of W1 Buffer to the FABG Column and centrifuge for 30 sec at speed 14,000 rpm . Discard the flow-through and place the FABG Column back to the Collection Tube.
9. Add 600 µl of Wash Buffer to the FABG Column and centrifuge for 30 sec at speed 14,000 rpm.Discard the flow-through and place the FABG Column back to the Collection Tube.

- Make sure that ethanol has been added to Wash Buffer when first open.

10. Centrifuge for an additional 3 min at speed 14,000 rpm to dry the column.

- Important Step! This step will avoid the subsequent enzymatic reactions from being inhibited by residual liquid. Elution

11. Place the dry FABG Column to a new 1.5 ml microcentrifuge tube.
12. Add 100 µl of Preheated Elution Buffer or TE to the membrane center of FABG Column.

- Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and absorbed completely.

13. Incubate the FAGB Column at 37 ºC for 10 min in an incubator.
14. Centrifuge for 1 minute at full speed 14,000 rpm to elute the DNA .

- Standard volume for elution is 100 µl. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total volume could be 200 µl. 20. Store the DNA fragment at 4ºC or -20ºC.

3.3.2 Determination of DNA Concentration:

3.3.2.1 Gel Electrophoresis to Analyze DNA Quality:-

1- An agarose solution was prepared by dissolve 1g of agarose powder in 100 ml of 1x TBE in the (100) ml flask, agarose was melted in hot block until the solution became clear.

2- The agarose solution was made cool to about (50- 55^{0}C), swirling the flask occasionally to cool evenly.

3- Red stain (3 µl) was added to the warm gel then sealed the ends of the casting tray with two layer of tape.

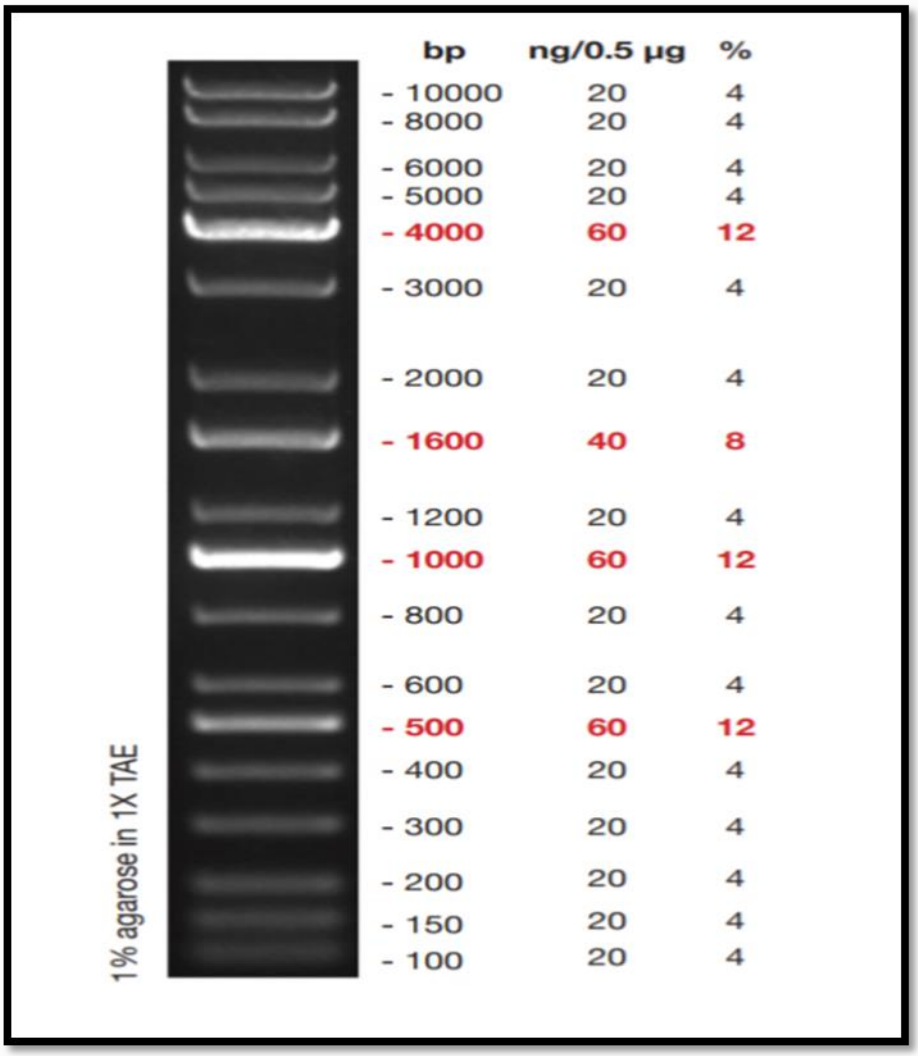
4- The combs were placed in the gel-casting tray.

5- Melted agarose solution was poured into the casting tray.

6- The agarose was allowed to solidify at room temperature, the comb pulled out carefully and the tape was removed. The gel was placed onto the electrophoresis chamber that was filled with TBE (1x) buffer.

7- DNA samples (5µl) were mixed with (3µl) DNA loading buffer and loaded in agarose gel wells.

8- The agarose gel electrophoresis was completed at 70V, 65Amp for 1hour.The DNA was observed by viewed under UVtrans illuminator.



SiZer 100bp DNA Markers (Kapa /USA)

3.3.3. Primer pairs (16S rRNA) for phylogenetic analysis .

The primer pair (Table 3.3), used in this study , were lyophilized, they dissolved in the free ddH2O to give a final concentration of 100 pmol/µl as stock solution and keep a stock at -20 to prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free ddH2O water to reach a final volume 100 µl .

Table (3.4): Sequences of primer that use to detect 16s rRNA gene.

Primer	Sequence	Primer sequence	Tm (°C)	GC%	Size of Product (bp
16s RNA	**F**	**5'- AGAGTTTGATCCTGGCTCAG- 3'**	**54.3**	**50.0**	**1250bp**
	R	**5'- GGTTACCTTGTTACGACTT- 3’**	**49.4**	**42.1**	

Table (3.5): The Components of the Maxime PCR PreMix kit (i-Taq)

Component	Reaction size 20 µl
i-Taq TM DNA Polymerase(5U/µl)	2.5 U
dNTPs	2.5mM each
Reaction Buffer(10x)	1x
Gel Loading buffer	1x

Table (3.6): Reaction components of PCR.

Component	25 µL (Final volume)
Taq PCR PreMix	**5µl**
Forward primer	**10 picomols/µl (1 µl)**
Reverse primer	**10 picomols/µl (1 µl)**
DNA	**1.5µl**
nuclease free water	**16.5 µl**

Table (3.7): The optimum condition of detection

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min	1 cycle
2-	Denaturation -2	95°C	45sec	35 cycle
3-	Annealing	57°C	45sec	
4-	Extension-1	72°C	1min	
5-	Extension -2	72°C	5 min.	1 cycle

3.3.4. Primers used in the study

Table (3.8): The sequence of primers that used this study to gene Lac .

Primer	Sequence	Primer sequence	Size of Product (bp)
Lac	**F**	**5'- ATGCCRCTGTGGCTGGARCARGTNCA - 3'**	**580-600 bp**
	R	**5'- TTRATCCARATCAGNSWNGCCAT - 3'**	

3.3.5. The primers preparation

The primers were lyophilized, they dissolved in the free ddH2O to give a final concentration of 100 pmol/µl as stock solution and keep a stock at -20 to prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free ddH2O water to reach a final volume 100 µl .

Table (3.9): Reaction components of PCR.

Component	25 µL (Final volume)
Taq PCR PreMix	**5µl**
Forward primer	**10 picomols/µl (1 µl)**
Reverse primer	**10 picomols/µl (1 µl)**
DNA	**1.5µl**
nuclease free water	**16.5 µl**

Table (3.10): The optimum condition of detection

No.	Phase	Tm (^{o}C)	Time	No. of cycle
1-	Initial Denaturation	95^{o}C	5 min	1 cycle
2-	Denaturation -2	95^{o}C	sec45	35 cycle
3-	Annealing	56^{o}C	sec45	
4-	Extension-1	72^{o}C	1min	
5-	Extension -2	72^{o}C	5 min.	1 cycle

Table (3.11): The sequence of primers that used this study to gene Porin .

Primer	Sequence	Primer sequence	Size of Product (bp)
Porin	**F**	**5'- ATGATGAAACGYAAYCTGCTNGC- 3'**	**950-1200 bp**
	R	**5'- AAYTGRTAVGTCAGVCCCAGVCC- 3’**	

Table (3.12): Reaction components of PCR.

Component	25 µL (Final volume)
Taq PCR PreMix	**5µl**
Forward primer	**10 picomols/µl (1 µl)**
Reverse primer	**10 picomols/µl (1 µl)**
DNA	**1.5µl**
nuclease free water	**16.5 µl**

Table (3.13): The optimum condition of detection

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min	1 cycle
2-	Denaturation -2	95°C	sec45	35 cycle
3-	Annealing	53°C	sec45	
4-	Extension-1	72°C	1min	
5-	Extension -2	72°C	5 min.	1 cycle

Chapter Four

Results & Discussion

4. Results and Discussion

4.1. Isolation of dye decolorizing *Proteus* isolates

In this study, only **8** *Proteus* isolates (**5** *P.mirabilis* isolates and **3** of *P.vulgaris*) were detected in 30 environmental samples (15 soil samples) which were collected from different agriculture places of the gardens scattered in the medical city/ Baghdad teaching hospital, as well as 15 wastewater samples from Al-Saqlawiyah drainage station, northwest of Baghdad, at the period from January 2022 to February 2022.

4.1.1. Characterization and screening of *P. mirabilis* & *P. vulgaris*

All bacterial isolates of *P. mirabilis* and *P. vulgaris* were characterized with morphological, Microscopical, biochemical tests and Vitek II system.

4.1.1.1 Phenotypic Characterization and Microscopic Identification

Identification of *P.mirabilis* and *P. vulgaris* was done by the bacteriological methods including colonial morphology (Shape, swarming, odor and lactose fermentation MacConkey agar and blood agar microscopic examination that include the morphology of bacterial cells by Gram- stain to observe a shape, arrangement of cells and type of reaction with Gram-stain. The current study found that isolates of *P. mirabilis* were unable to lyse blood cells and produced gamma hemolysis or non- hemolysis on blood agar media (figure 4.1 B). While grown on the MacConkey agar medium, observed the non- lactose fermenter isolates that appeared as pale, convex, and circular and smooth colonies, with special fish-like odour (figure 4.1 A).When part of a suspected colony grown on MacConkey agar was smeared on a microscopical slide and stained by gram staining technique, cells were found as small- short rods, grouped mainly singly, and gram-negative When hanging drop preparation was made, cells were motile which mean that they possess factors of movement, the flagella (figure 4.1 C).

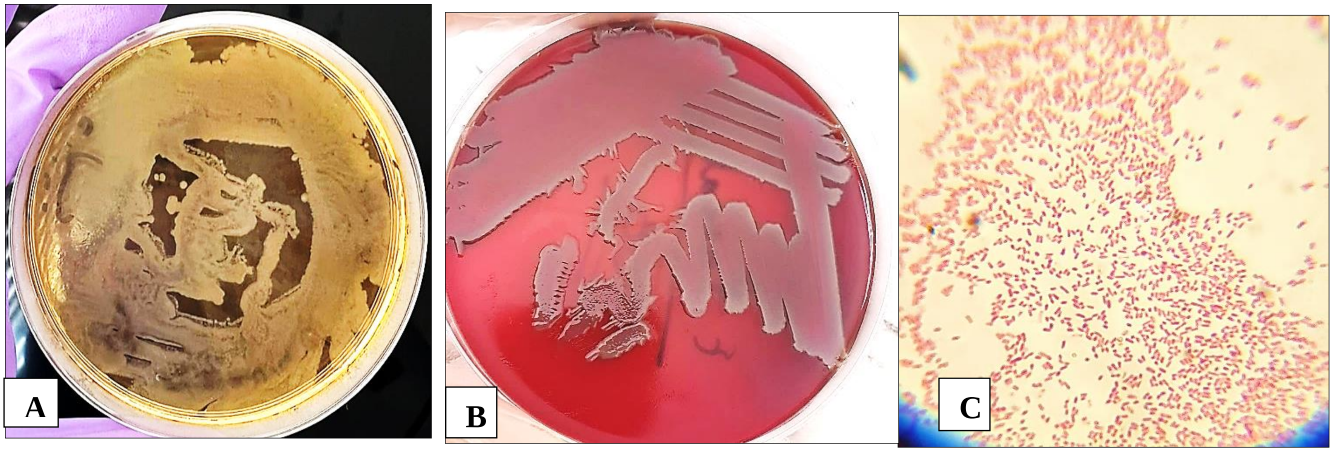


Figure (4.1): (A): Pale Colonies of *P. mirabilis* grew on when grown on MacConkey agar at 37°C for 24 hrs.; **(B)** : Colonies of *P. mirabilis* (gamma hemolytic) grown on Blood agar at 37°C for 24 hrs. **(C):** Microscopic examination of *P. mirabilis* after performing the dying process with a gram stain, that showed small, short, rod -shaped, Gram-negative (red color) bacterial cells.

The isolates of the *Proteus vulgaris* under study, when grown on the MacConkey agar medium, showed small, circular, pale colonies, and non-fermented lactose sugar with fishy odour (figure 4.2 B) .They appeared on the blood agar medium in the form of an alpha-hemolysis (α-hemolysis) because they produced hemolysin enzyme. Hemolysin production considered as essential virulence factors as it contributes in tissue damage by forming pores in biological membranes. When alpha-hemolysis is present, the agar under the colony is light and greenish, so sometimes called green hemolysis because of the color change in the agar (figure 4.2 A). The results of the microscopic examination showed that the bacteria cells were rod-shaped , arranged in pairs, or they could be single and Gram negative (figure 4.2 C).

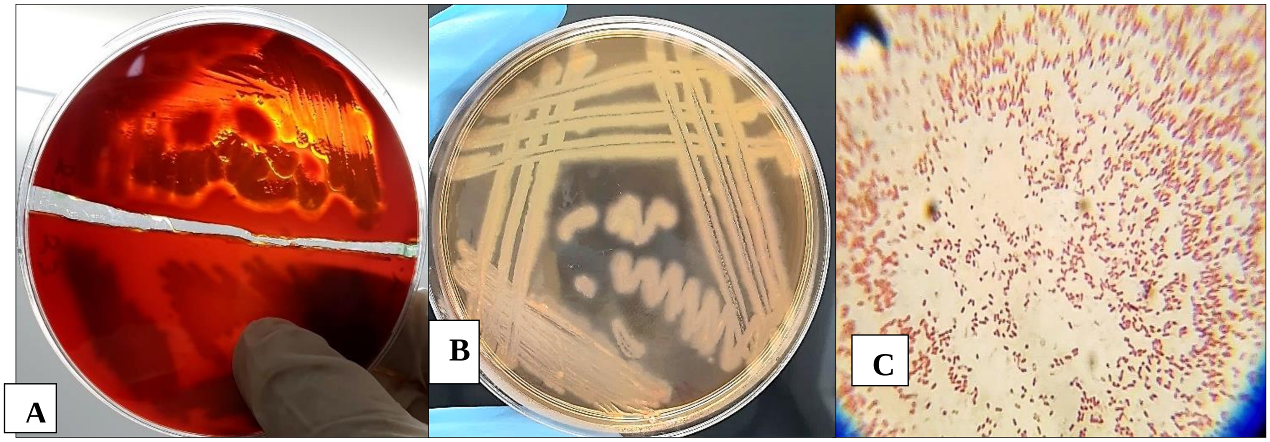


Figure (4.2): (A): Colonies of *P. vulgaris* (alpha hemolytic) grown on Blood agar at 37°C for 24 hrs. **(B)** : Colonies of *P. vulgaris* (small, circular, pale colonies, and non-fermented lactose sugar grown on MacConkey agar at 37°C for 24 hrs. **(C):** Microscopic examination of *P. vulgaris* after performing the dying process with a gram stain, that showed rod-shaped , single or pairs, Gram-negative (red color) bacterial cells

In this study, *Proteus* isolates (both of *Proteus mirabilis* and *Proteus vulgaris*) were exhibited swarming motility by all isolates when grown on agar plates. Figure (4.3).

Figure (4.3): (A): Swarming phenomenon of *P. mirabilis* had grown on nutrient agar at 37°C for 24 hrs. **(B)** : Swarming phenomenon of *P. vulgaris* had grown on nutrient agar at 37°C for 24 hrs.

4.1.1.2 Biochemical Identification

Several biochemical tests were done to characterize *Proteus spp,* the results of biochemical tests are summarized in the table (4.1). According to the biochemical properties, two species of *Proteus* were identified in this study; they were *P. mirabilis* and *P. vulgaris*. All the (5) isolates of *P. mirabilis* and (3) isolates *P. vulgaris* were positive for the catalase test and the appearance of bubbles of oxygen gas on the glass slide or agar plate was indicates the ability of these bacteria to produce catalysis enzyme that analyzed the hydrogen peroxide reagent into water and oxygen (figure 4.4 A). But all were negative to the oxidase test and Voges – Proskauer test. Remarkably, one test was carried out to distinguish between these isolates, it was indole production. *P. mirabilis* displayed negative result for indole production because a yellow ring appeared on the surface of the peptone water medium when Kovacs reagent was added (figure 4.4 B), while *P. vulgaris* showed positive result as a result of the appearance of a red to pink ring on the surface of the peptone water median after added drops of Kovacs reagent , this refers to the capacity of these bacteria to produce the tryptophanase enzyme that analyzes tryptophan into Indole (figure 4.4 C).

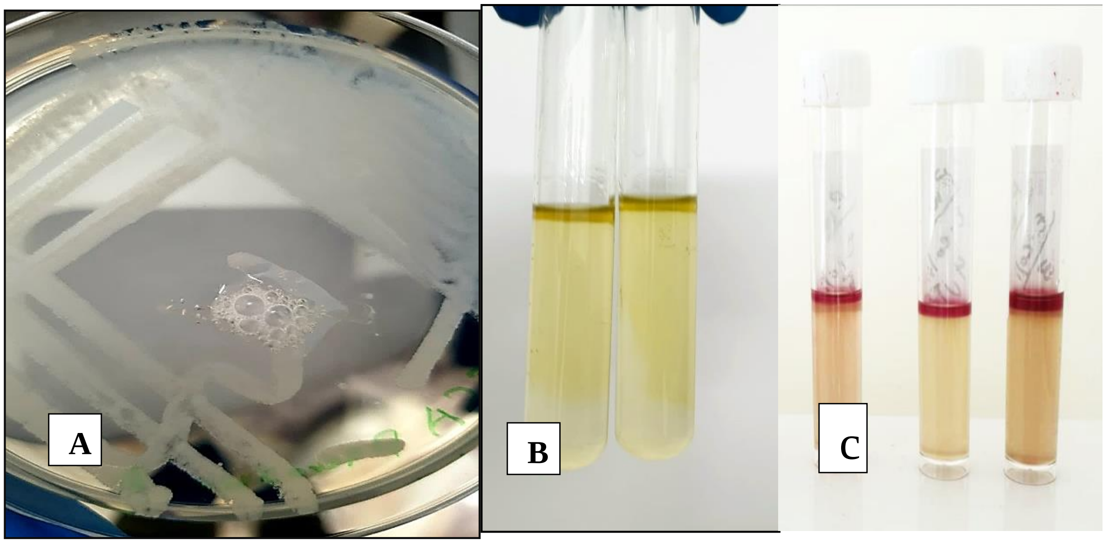


Figure (4.4) : (A): Biochemical identification of ***Proteus spp**:* Positive **catalase test** (appearance of bubbles) ; **(B)** : Negative **indole test of *P. mirabilis*** (appearance of yellow ring**)** on the surface of the peptone water broth medium & **(C)** : Positive **indole test of *P. vulgaris*** (appearance of red to pink ring**)** on the surface of the peptone water broth medium**.**

Table (4.1): Biochemical tests for characterization of *proteus spp.*

Proteus spp	**Biochemical Tests**								
	Catalase	**Oxidase**	**Indole**	**Methyl red**	**VP**	**Citrate**	**KIA**	**Urease**	**Motility**
P. mirabilis	+	-	-	+	-	+	K/A	+	+
P. vulgaris	+	-	+	+	-	+	K/A	+	+

(+) positive result, (-) negative result, (VP) Voges –Proskauer test, (KIA) Kliger Iron Agar test, (K/A) Alkaline Slant/Acid Bottom.

Table (4.1) shows that the *P.mirabilis* and *P.vulgaris* isolates were given clearly positive results for to Citrate utilization test to the change of the color of the Simon citrate agar medium from green to blue due to the ability of bacterial isolates to consume citrate as the only source of carbon (figure 4.5 A).As well as both *proteus* isolates were able to produce urease enzyme and thus changed the color of the of Urea agar slant from yellow to pink (figure 4.5 A).The production of urease enzyme is a hallmark of infections with *Proteus spp*., in addition this enzyme is considered as one of the most significant virulence factors of *Proteus spp*., which has been involved in the pathogenesis of several disease namely pyelonephritis and development of infection induced urinary stones, because it catalyzes the formation of kidney and bladder stones or to encrust or obstruct indwelling urinary catheters (Newsholme and Leech, 2011). Motility test of the indicated that they were motile.

In addition, they showed positive results to methyl red test and Kligler iron test by forming alkaline slant / Acidic bottom with H_2S gas production as a result of only glucose fermentation without fermentation of lactose (figure 4.5 B)

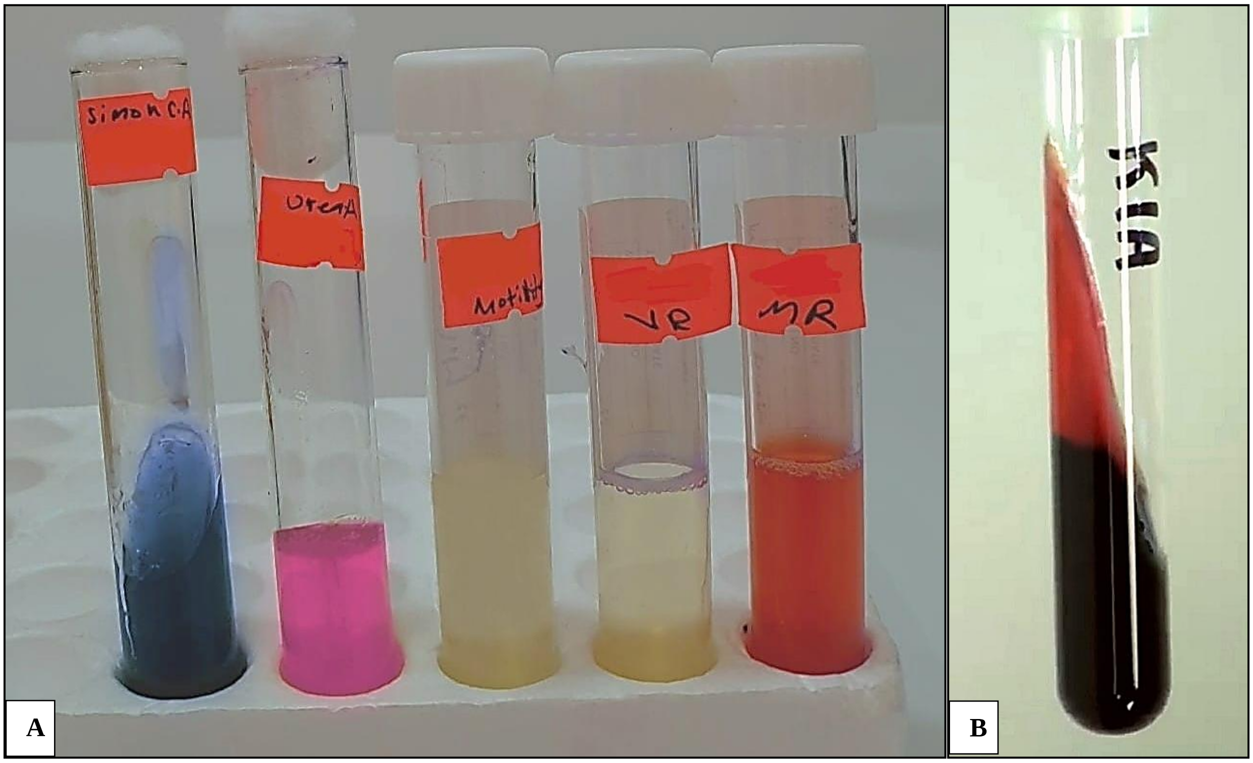


Figure (4.5): (A) The results of some biochemical tests of ***Proteus spp***;(from left - right) **Citrate utilization (+)** [changed the color of the of the Simon citrate agar from green to blue] ,**Urease test (+)** [changed the color of the of Urea agar slant from yellow to pink] , **Motility test (+)** [turbidity appeared in the medium] ,**Vogas Proskauer test** (-) and **methyl red test (+)**, (B)**:** The results of **Kliger Iron Agar test** by ***Proteus spp:*** (K/A) Alkaline Slant (red color / Acidic bottom with H2S production and no gas production.

4.1.1.3 Vitek II Identification

The appendix (1) and appendix (2) show the diagnostic result using the Vitek II system for one isolate from *P. mirabilis* and one from *P. vulgaris* with a probability of (99%). Vitek-II system which is an automated microbiology system used for bacterial identification and to confirm the results of morphological, microscopical and biochemical identification.

4.2 Dey Decolorization using bacterial isolates

There is an increasing interest in bacteria as biodegrading agents due to their potential to transform and degrade most synthetic dyes effectively (Alghazeer *et al*.,2019; Arora *et al*.,2014 and Asad *et al*.,2007). Bacterial degradation of azo dyes is generally considered a specific reaction by azo reductase under aerobic condition. The efficient removal of these dyes is necessary and significant for environmental protection (Ahmed and Suad, 2014). In the present study the effect of *Proteus mirabilis* and *P. vulgaris* culture on decolorization of azo dyes (Direct Yellow dye, disperse blue dye, Reactive green dye, Direct Brown dye and Reactive Brown dye) was tested under static aerobic condition with dye concentration of (30, 60 and 90 mg/L) at different incubation time (12h.,18 h. and 24h.). *P. mirabilis* was the best decolorizer compared with *P. vulgaris* bacteria. As shown in figure (4.6).

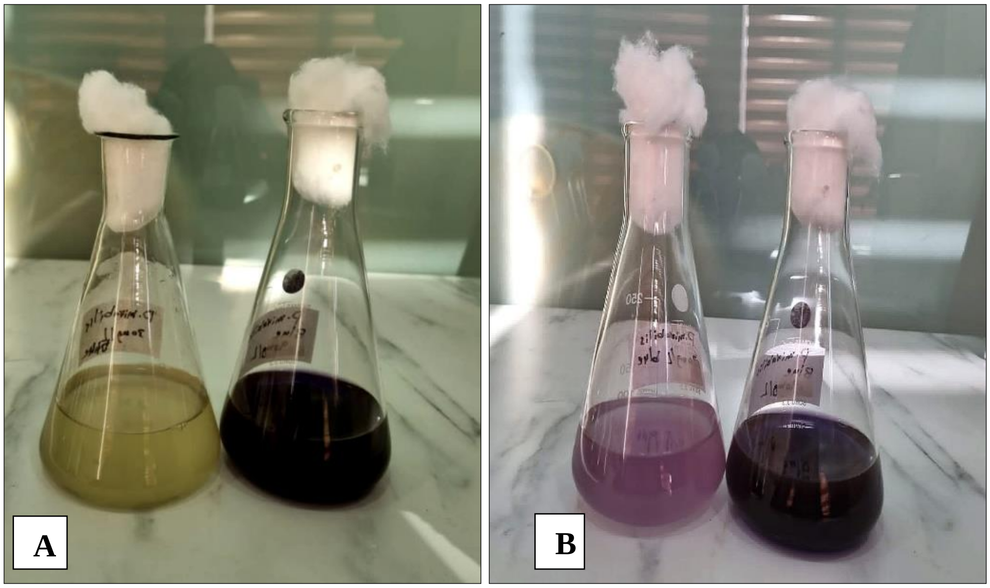


Figure (4.6): (A) Effect of *Proteus mirabilis* on decolorization of disperse blue at a concentration of 90 mg/L and Ph=7 after incubation at 37°C for 24 hrs. (B): Effect of *Proteus vulgaris* on decolorization of disperse blue at a concentration of 90 mg/L and Ph=7 after incubation at 37°C and shaking (120 rpm) for 24 hrs.

4.2.1 Effect of dye concentrations at different incubation time on decolorization

In this study , it has been observed that whenever an increase in the dye concentration, the decolorization efficiency of the bacteria decreased .This is due to the presence of the higher amount of dye required to be degraded. Thus, the dye solution showed maximum decolorization at the lower concentration of 30 mg/L, (Table 4.2 and table 4.3), e.g. , the decolorization of Direct Yellow by the *P. mirabilis* was decreased slowly with increasing dye concentration. It could effectively decolorize up to 30 mg/L (100%) within 24 h and was decreased to 92.81% and 84.69% when dye concentration increased to 60 mg/L and, 90 mg/L respectively. likewise a high decolorization rate (94.01%) Direct Yellow was obtained by *P. vulgaris* at 30 mg/L within 24 h. and was decreased to 70.01% and 65% when dye concentration increased to 60 mg/L and, 90 mg/L respectively.

Table (4.2): Decolorization percentage at various dye concentrations (mg/L) at different treatment period by *P.mirabilis* isolates .

Dye	Con. (mg/L)	*P.mirabilis*/Time		
		12h.	18h.	24h.
		De%	De%	De%
Direct Yellow	30	37.02%	79.01%	100%
	60	35.00%	48.01%	92.81%
	90	31.04%	45.18	84.69%
LSD(0.05) (con.*time) =10.124				
Disperse Blue	30	59.01%	71.24%	100%
	60	48.01%	65.02%	100%
	90	41.02%	64.01%	100%
LSD(0.05)(con.*time) =12.171				
Reactive Green	30	32.05%	42.08%	100%
	60	25.84%	41.21%	80.04%
	90	14.32%	30.14%	78.01 %
LSD(0.05)(con.*time) =9.223				
Direct Brown	30	71.54%	90.07%	100%
	60	56.20%	60.63%	83.77%
	90	23.51%	47.51%	70.70%
LSD(0.05)(con.*time) =8.112				
Reactive Brown	30	62.47%	77.30%	100%
	60	48.04%	62.09%	94.36%
	90	43.50%	55.92%	75.01%
LSD(0.05)(con.*time) =11.674				

Con. = concentration , De = decolorization

Table (4.3): decolorization percentage at various dye concentrations (mg/L)) at different treatment period by *P. vulgaris* isolates .

Dye	con. (Mg/L)	*P. vulgaris* /Time		
		12h.	18h.	24h.
		De%	De%	De%
Direct Yellow	30	33.01%	55.01%	94.01%
	60	28.10%	46.64%	70.01%
	90	22.02%	42.02%	65%
	LSD(0.05)(con.*time) =7.245			
Disperse Blue	30	40.52%	71.24%	100%
	60	41.93%	61.01%	100%
	90	38.02%	51.41%	97.61%
	LSD(0.05)(con.*time) =11.552			
Reactive Green	30	30.17%	40.01%	81.25%
	60	20.25%	29.34%	61.17%
	90	12.02%	17.90%	48.56%
	LSD(0.05)(con.*time) =8.447			
Direct Brown	30	56.22%	68.05%	95.90%
	60	40.03%	50.71%	72.03%
	90	22.70%	36.43%%	63.35%
	LSD(0.05)(con.*time) =12.634			
Reactive Brown	30	56.58%	67.87%	93.02%
	60	43.88%	58.28%	86.03%
	90	41.23%	47.90%	70.04%
	LSD(0.05)(con.*time) =7.752			

Con. = concentration , De = decolorization

Time is one major contributory factor on decolorization of dyes with different bacterial isolates. The decolorization of tested dyes differed when treated with bacterial isolates at different incubation time. Generally, decolorization trends were quite high within 24h in most of the cases studied (Figure 4.7), e.g. , the data in figure (4.8) indicated that the decolorization was completed (100%) for Disperse Blue dye at about 24 h regardless of the dye concentration when treated with *P. mirabilis*.

Figure (4.7): Effect of Incubation time on decolorization of azo dyes by *Proteus mirabilis* at a concentration of 90 mg/L and Ph=7 after incubation at 37°C and shaking (120 rpm) for (A) 0 hrs. , (B) 12 hrs. , (C) 18 hrs. and (D) 24 hrs.

On the other hand the data in figure (4.9) indicated that the decolorization was completed (100%) for Disperse Blue dye at about 24 h in the dye concentration 30 mg/L and 60 mg/L when treated with *P. vulgaris* but at the dye concentration 90 mg/L the decolorization rate was recorded at 97.61% .

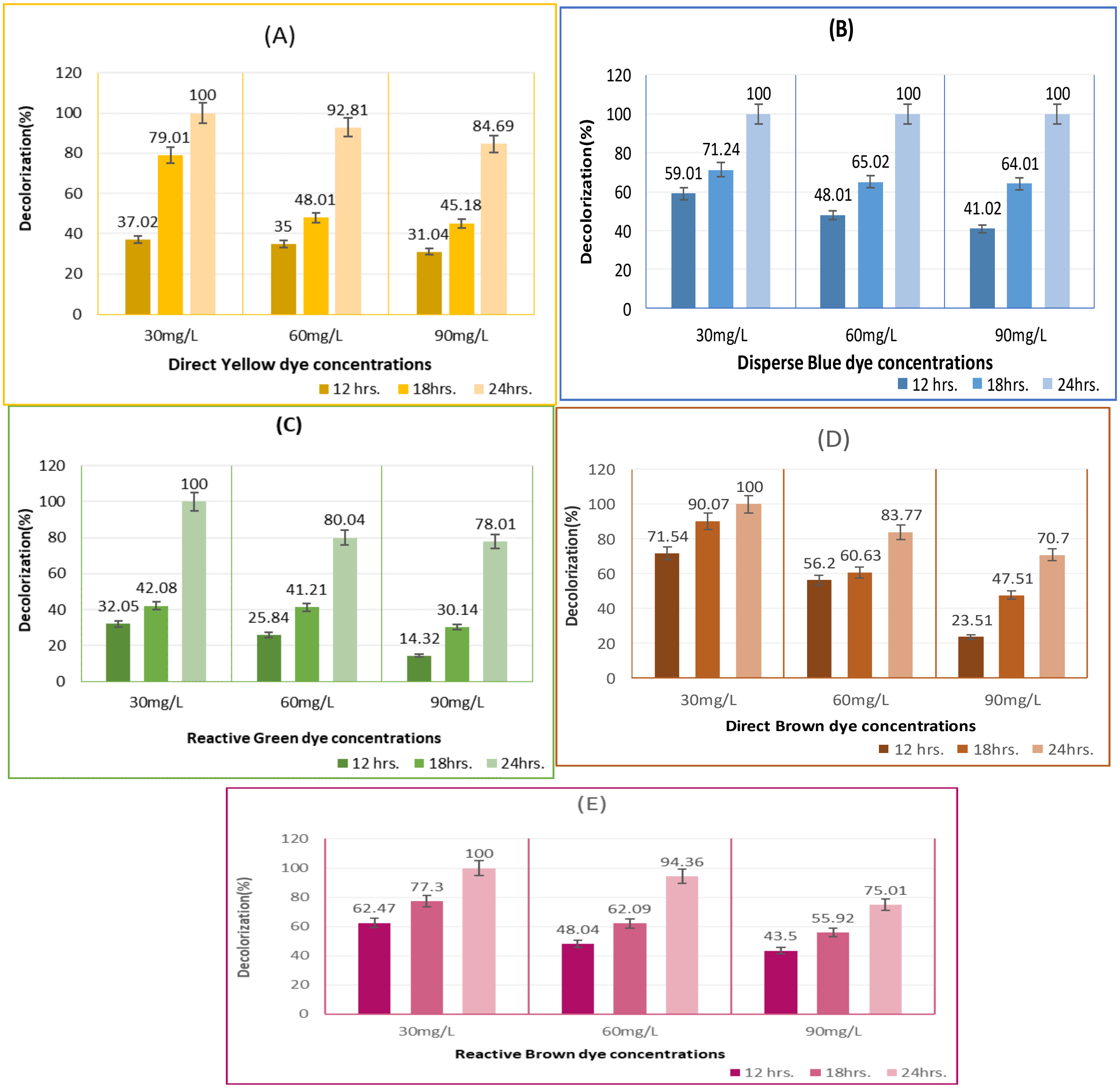


Figure (4.8): Decolorization percentage by *P. mirabilis* isolates for azo dyes (A) Direct Yellow,(B) Disperse Blue, (C) Reactive Green, (D) Direct Brown & (E)Reactive Brown at various time (hrs.)

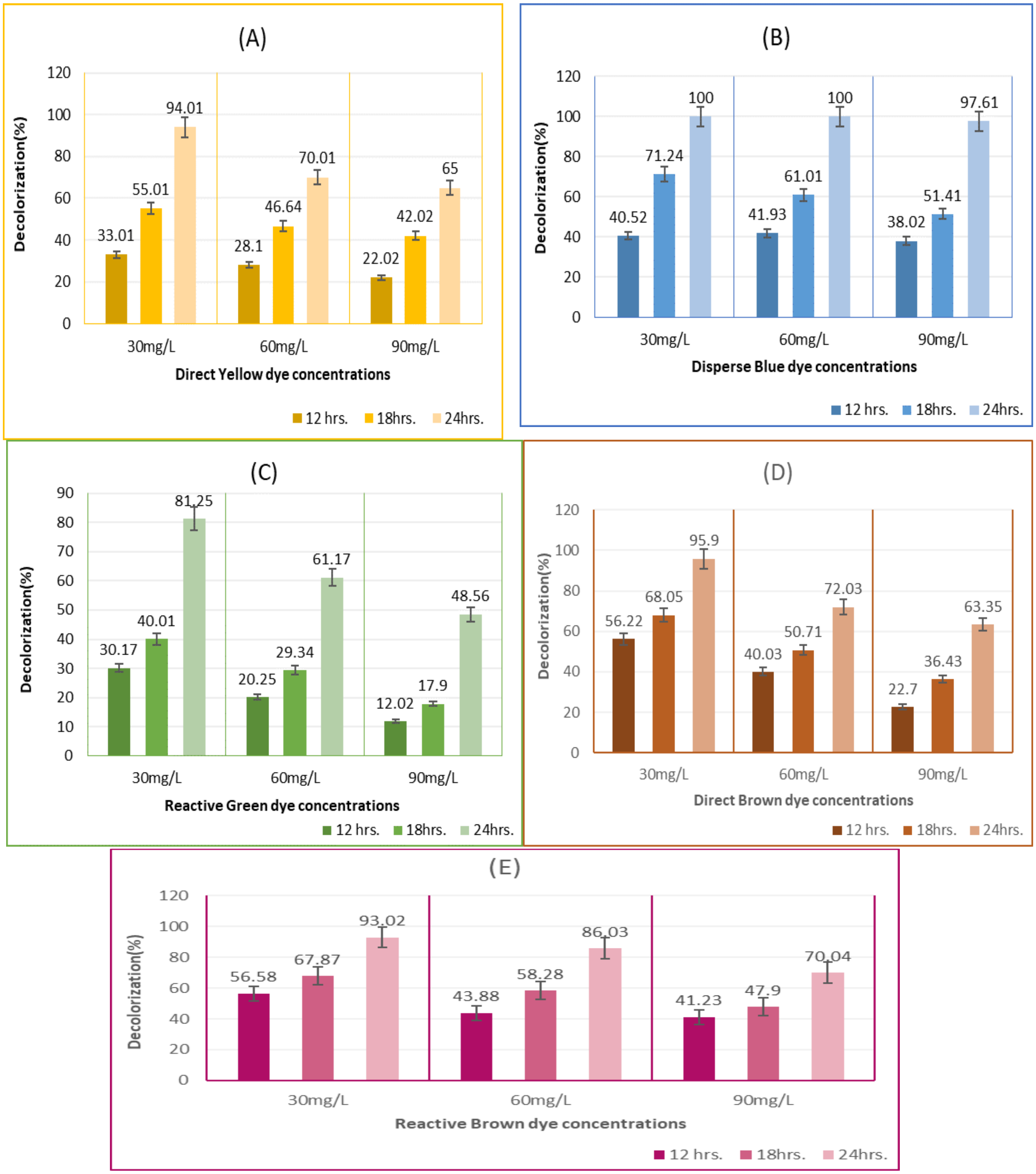


Figure (4.9): Decolorization percentage by *P. vulgaris* isolates for azo dyes (A) Direct Yellow,(B) Disperse Blue, (C) Reactive Green, (D) Direct Brown & (E)Reactive Brown at various time (hrs.)

4.2.2 Effect of pH on decolorization

The effect of pH on decolorization of Azo dyes by *Proteus* spp. was determined over a wide range of pH 6 ,7 and 8. The data in (Table 4.4) and Appendix (3.4.5.6.7.8.9.10.11.12) clearly indicate that pH= 7 was the optimum and either the lower or the higher pH decreased the efficiency of bacterial decolorization significantly, e.g. , the *P.mirabilis* isolates showed the maximum of 100% decolorization for Disperse Blue dye at pH= 7.0 even at a high concentration of this dye (90mg/L), but following the increase from either side of neutral pH, the percentage of decolorization decreased 61.80% on the alkaline side (pH=8) , while the percent decolorization decreased to 40.8% on acidic side (pH=6) . Similar optimum pH was observed in the decolorization of the same dye by *P. vulgaris* as shown in (Table 4.4). So, we can say that the neutral pH would be more favorable for decolorization of the azo dyes and is suitable for industrial applications.

Table (4.4): Effect of pH on decolorisation of Azo dyes (in a different concentrations) by *P. mirabilis* isolates and *P. vulgaris*.

Dye	Con. (mg/L)	λmax (nm)	***P. mirabilis*/pH**			***P. vulgaris*/pH**		
			6	7	8	6	7	8
			De%	De%	De%	De%	De%	De%
Direct Yellow	30	404	35.37%	100%	80.02%	31.04%	93.02%	51.01%
	60	404	33.19%	90.01%	40.82%	27.02%	67.02%	44.%
	90	404	30.64%	82.25%	38.95%	21.01%	62.02%	38%
			LSD(0.05)(bacteria*con.*time)=13.442					
Disperse Blue	30	557	55.15%	100%	72.17%	43%	100%	70.03%
	60	557	41.07%	100%	64.72%	37.51%	100%	60%
	90	557	40.80%	100%	61.80%	36.23%	92.91%	49%
			LSD(0.05)(bacteria*con.*time)=10.277					
Reactive Green	30	628	31.77%	100%	43.67%	29.80%	80.22%	38.05%
	60	628	27.49%	79.39%	40.21%	22.80%	59.73%	30.03%
	90	628	17.72%	75.99%	30%	15.40%	47.31%	19.92%
			LSD(0.05)(bacteria*con.*time)=7.241					
Direct Brown	30	467	70.93%	100%	89.90%	52.04%	92.08%	65.01%
	60	467	57.30%	81%	61.07%	40.03%	70.09%	50%
	90	467	24.43%	65.45%	45.18%	21.02%	62.70%	34.16%
			LSD(0.05)(bacteria*con.*time)=12.133					
Reactive Brown	30	469	60.80%	100%	73.08%	55.89%	91.15%	68.07%
	60	469	44.66%	90.71%	58.19%	38.68%	84.64%	52.47%
	90	469	41.51%	72.03%	52.30%	34.91%	63.44%	49.04%
			LSD(0.05)(bacteria*con.*time)=9.417					

Con. = concentration , De = decolorization

4.2.3 Effect of Temperature on decolorization

Temperature is an important environmental factor, and the biodegradation activities of the microorganisms are largely affected by variations in the temperature. Under extreme conditions on either side of the optimum temperature, the activity of microorganisms drastically decreases due to their slow growth and reproduction rate, also causing the deactivation of enzymes that are responsible for the degradative action on dyes. Thus, the biodegradation performance of microorganisms will be the best at optimum temperature needed for their growth, reproduction, and activity (Pearce *et al*., 2003). Among the three temperatures studied (30 ,37 and 42) . 37°C was found to be the optimum (Table.4.5) and Appendix (13.14.15.16.17.18.19.20.21.22) giving higher decolorization rates for all five Azo dyes (Direct Yellow , disperse blue , Reactive green , Direct Brown and Reactive Brown) .

Table (4.5): Effect of Temperature on decolorisation of Azo dyes (in a different concentrations) by *P. mirabilis* isolates and *P. vulgaris*.

Dye	Con. (mg/L)	λmax (nm)	*P. mirabilis*/Temperature			*P. vulgaris/* Temperature		
			30°C	37°C	42°C	30°C	37°C	42°C
			De%	De%	De%	De%	De%	De%
Direct Yellow	30	404	32.31%	100%	74.84%	30%	88.03%	70.04%
	60	404	31.41%	91.22%	42.60%	29.44%	82%	41.01%
	90	404	28.81%	78.19%	39.90%	26.81%	77.02%	38%
			LSD(0.05)(bacteria*con.*time)=12.714					
Disperse Blue	30	557	54.01%	100%	73.01%	51.04%	100%	68%
	60	557	42.01%	100%	65.02%	32.01%	100%	62.01%
	90	557	39%	100%	60.01%	31.02%	92.32%	57.01%
			LSD(0.05)(bacteria*con.*time)=8.132					
Reactive Green	30	628	37.30%	100%	49.01%	33.08%	96.25%	41.14%
	60	628	32.03%	83.03%	38.02%	30.03%	76.44%	36.07%
	90	628	29.92%	75%	30.22%	27.83%	62.61%	28.61%
			LSD(0.05)(bacteria*con.*time)=11.229					
Direct Brown	30	467	67.01%	100%	72.06%	66.05%	88.42%	69.19%
	60	467	63.01%	93%	56.03%	60.03%	77.46%	51.27%
	90	467	25.02%	69.02%	34%	24.64%	66.05%	31.13%
			LSD(0.05)(bacteria*con.*time)=14.154					
Reactive Brown	30	469	53.04%	100%	68.07%	46.07%	93.51%	62.47%
	60	469	41.02%	73.02%	46.05%	35.03%	69.73%	44.23%
	90	469	32%	75.01%	41.73%	30.58%	61.31%	35.77%
			LSD(0.05)(bacteria*con.*time)=9.671					

Con. = concentration , De = decolorization

4.3. Molecular methods for further identification of proteus spp.

DNA was extracted from 6 bacterial isolates after the initial diagnosis based on biochemical and morphological tests and Vitek II system of proteus vulgaris and proteus mirabilis for PCR technique to detect the genes of 16S rRNA. Total DNA was extracted using genomic DNA kit .Then gel electrophoresis was carried out as shown in Figure (4.10).

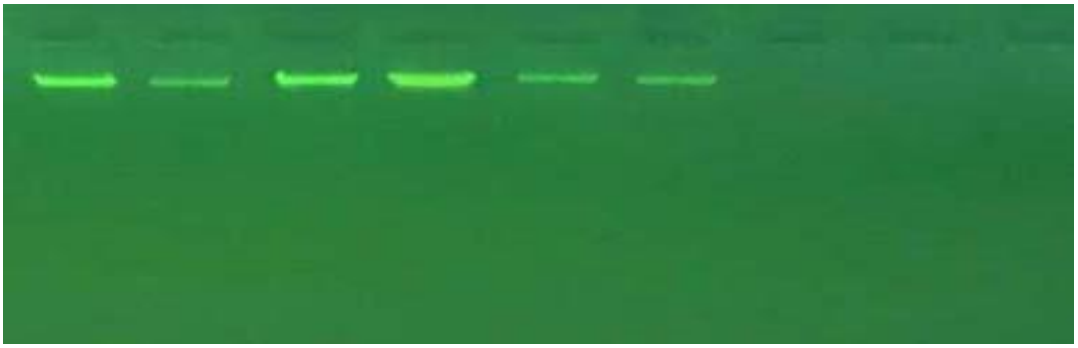


Figure (4-10) Gel electrophoresis of genomic DNA extraction from bacteria 1% agarose gel at 1houre . Lanes 1-6 showed positive results . his process was used to check the purity and integrity of extracted DNA from proteus spp. Isolates .

After the initial diagnosis of *proteus* spp. isolates based on biochemical and morphological tests, it was confirmed by Vitek II system an (appendix 1-2) and PCR-sequencing for 16S rRNA gene, Fig (4.11) . The results of sequencing have been sent to gene bank (NCBI)to see the identity of sequences with reference sequences within NCBI data base. and It was found that all isolates of *proteus* were similar to the isolates identified in the gene bank (NCBI) . (appendix 23.24.25.26.27.28)

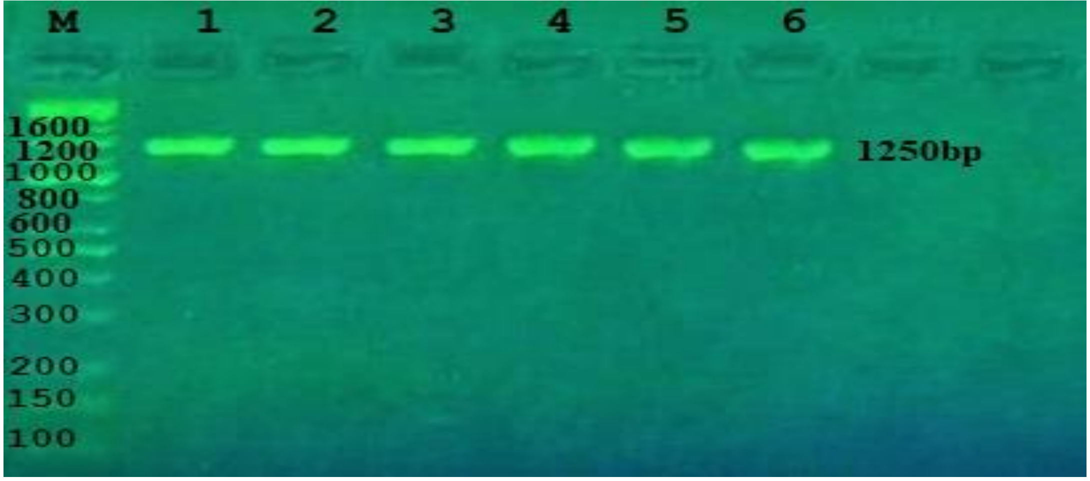


Figure (4-11): Gel electrophoresis of PCR product for detection of 16S rRNA amplicon . using 1.5% agarose at 5 volt/cm2. 1xTBE buffer for 1:30 hours. M: DNA ladder (100).

The ability of Gram-negative bacteria to communicate with each other is important for many cellular functions (Fuqua *et al*., 1996).

The signals might be brand-new quorum sensing signals or they could be released metabolites from the cell(Gwen *et al*.,2002) .

(Allison *et al*., 1992) supposed that *P. mirabilis* uses cell-to-cell signaling for the development of biofilms and swarming . By using some tiny molecule compounds as intermediaries for the transmission of electrons, laccase's decolorization efficiency can be increased (Huang *et al*.,2020).

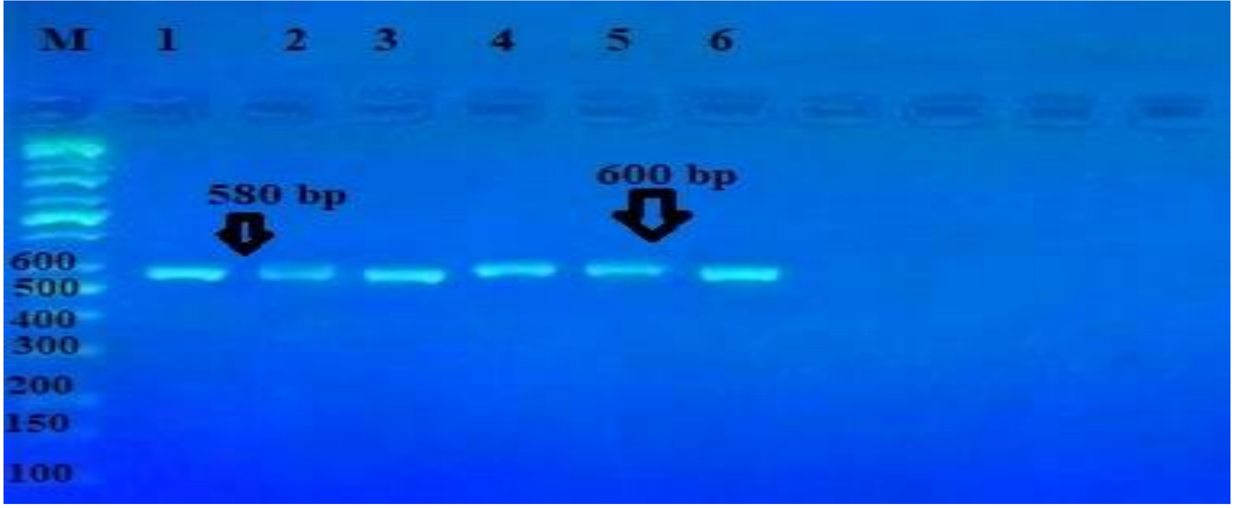


Figure (4-12) PCR product the band size of Lac gene. The product was electrophoresis on 1.5% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100). ((1-3 Proteus vulgaris and 4-6 Proteus mirabilis)).

The results showed that in reconstituted membranes, two main outer membrane proteins of *P.mirabilis* and *P.vulgaris* with apparent molecular weights of 950 and 1200 bp generated hydrophilic holes. They asserted that the two kinds of pores have comparable functional characteristics.

Morganella morganii, *Providencia rettgeri*, *Providencia alcalifaciens*, *Proteus mirabilis*, and *Proteus vulgaris* were all once included in the same genus. showed that P. mirabilis mutants lacking aporin could be quickly isolated on cefoxitin-containing agar medium without the need of any chemical mutagens. At least in the outermembrane permeability of Azodyes, the outer membrane proteins act similarly to the OmpFporin of E. coli.(Mitsuyama *et al*.,1987).

The porin protein class, which forms trimers of identical subunits in the outer membrane, is responsible for the molecular sieving capabilities. Most likely, the porin trimers have a single, big, well-defined pore with a diameter of between 1.2 and 2 nm.(Benz, 1985).

We are unable to maximize the methods for bioremediation of a polluted environment since the processes of bacterial adaptation to the toxic organic pollutant as the extracellular electron acceptor during anaerobic respiration are unclear. Strong viability is achieved through filamentous cells' regulation of gene expression, which improves their capacity for detoxification and destruction (Fang *et al*., 2019) .,(figure 4-32)

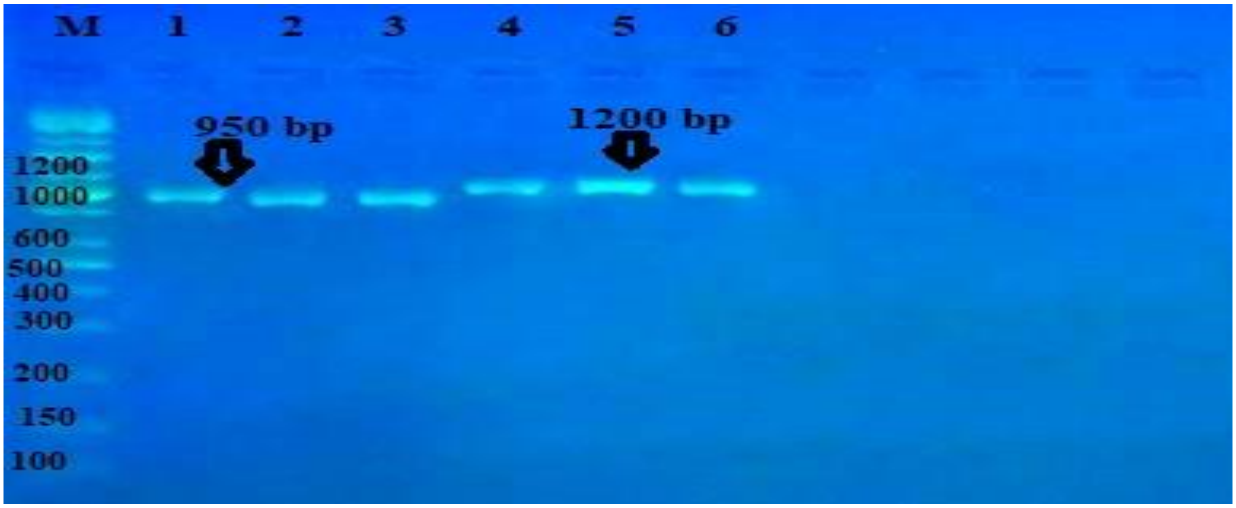


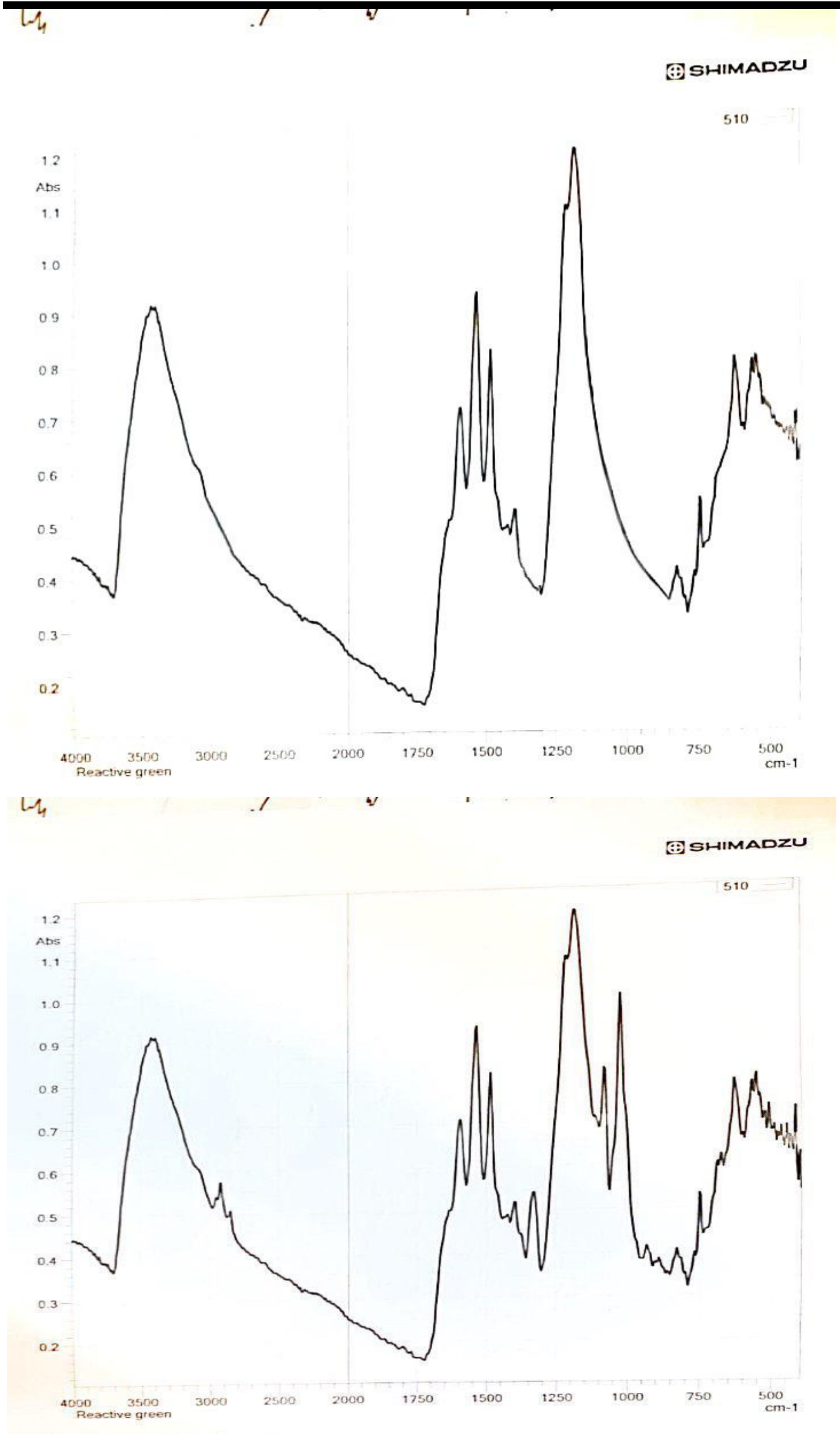
Figure (4-13) PCR product the band size of Porin gene. The product was electrophoresis on 1.5% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100). ((1-3 Proteus vulgaris and 4-6 Proteus mirabilis)).

Curves of the FT-IR examination , found a clear change in the values of wavelengths resulting from the process of linking of dyes bonds found after treatment by *proteus sp.* and Bacterial cell surface demonstrates the task of various groups to particular practical gatherings that have a place with contrast class of macromolecules in the control tests. Just as annoyance in the band structure after dyes treatment. The variation in FTIR peaks and their transmission were because of the reaction with the active sites on the Proteus and lead particles through bioadsorption operation.

Azo dyes were analyzed using FTIR, and the results showed the emergence of a novel O-H stretching that may be caused by oxidation in the dyes and the conversion of the active groups to energy. Additionally, FTIR analysis of dyes revealed that the aromatic cycle band,. Alkanes, carboxylic acids, esters, and lactones were discovered . Changes in chemical properties that might be assessed include the creation or removal of functional groups, as seen by FTIR.

An FTIR examination indicated changes in the samples as well as new peaks in the spectrum that reflected changes to the ether and carbonyl groups.

Because it displays changes like the development and removal of chemical groups and chemical bonds, FTIR analysis or FTIR spectroscopy may be used to detect chemical variations in the building of diverse polymers.



After

Reactive green

Before

Figure (4.14) the result of FTIR examination to Reactive Green dye before and after treatment.

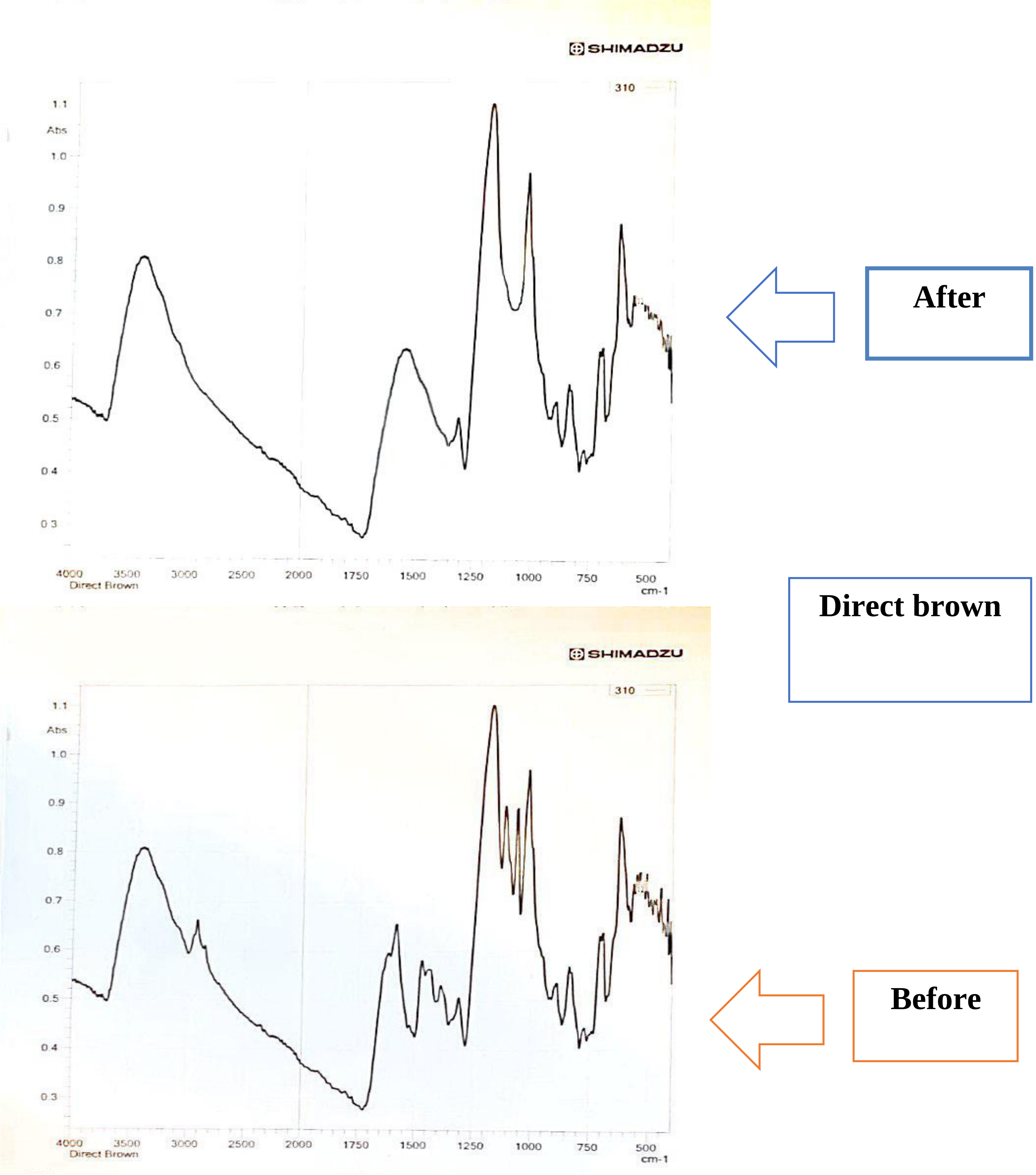
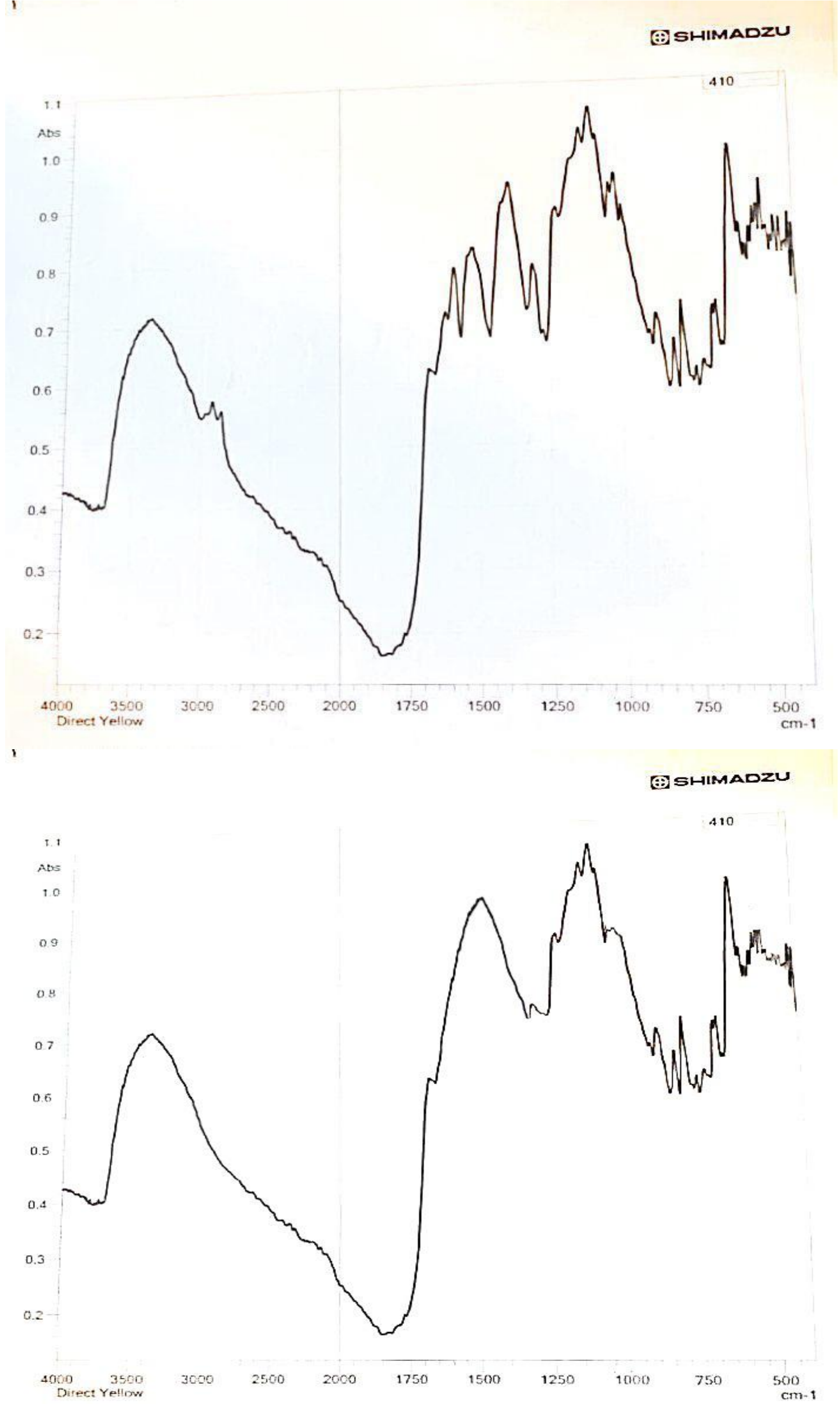


Figure (4.15) the result of FTIR examination to Direct Brown dye before and after treatment.

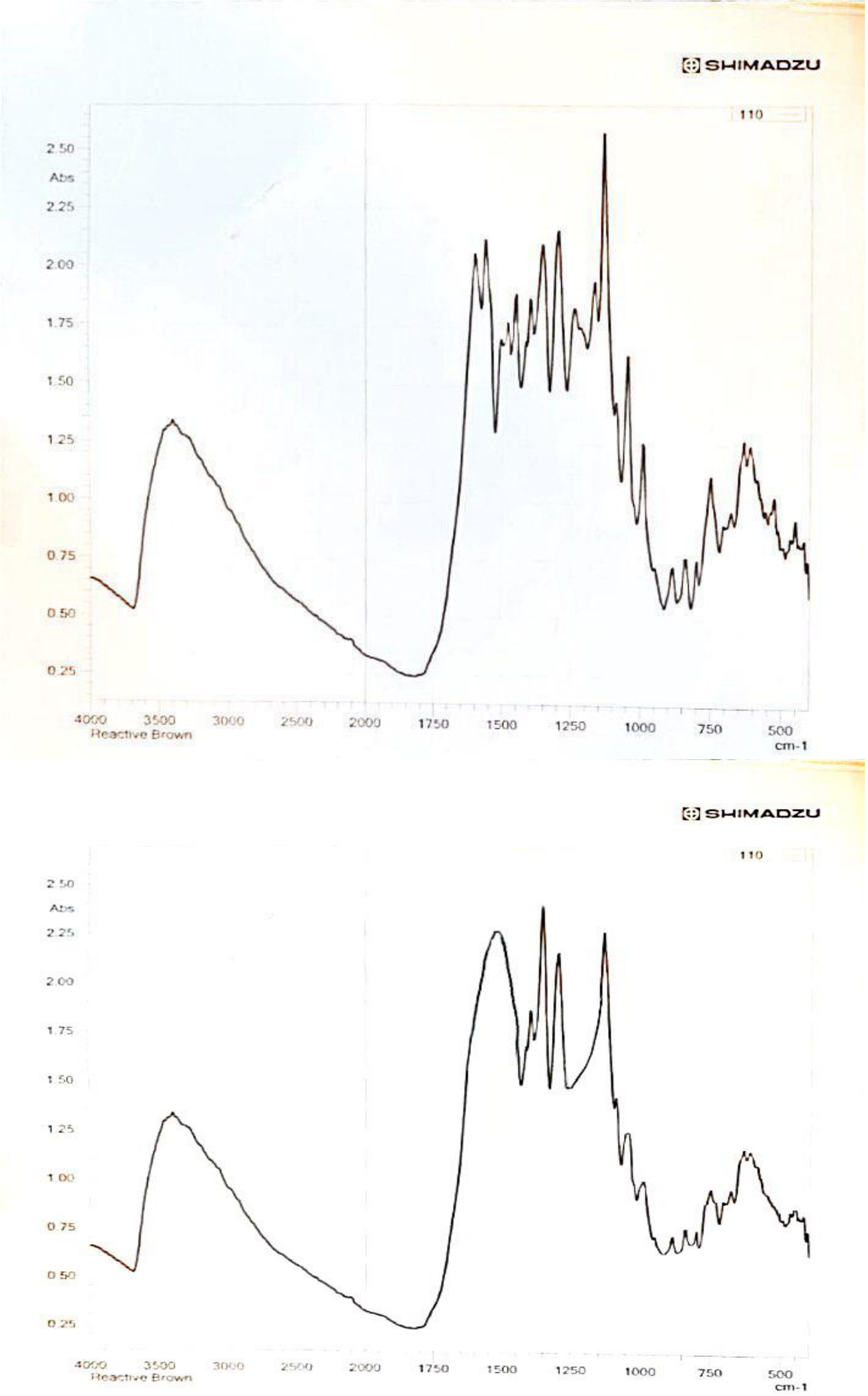


Before

Direct yellow

After

Figure (4.16) the result of FTIR examination to Directe Yellow dye before and after treatment .



Before

Reactive brown

After

Figure (4.17) the result of FTIR examination to Reactive Brown dye before and after treatment.

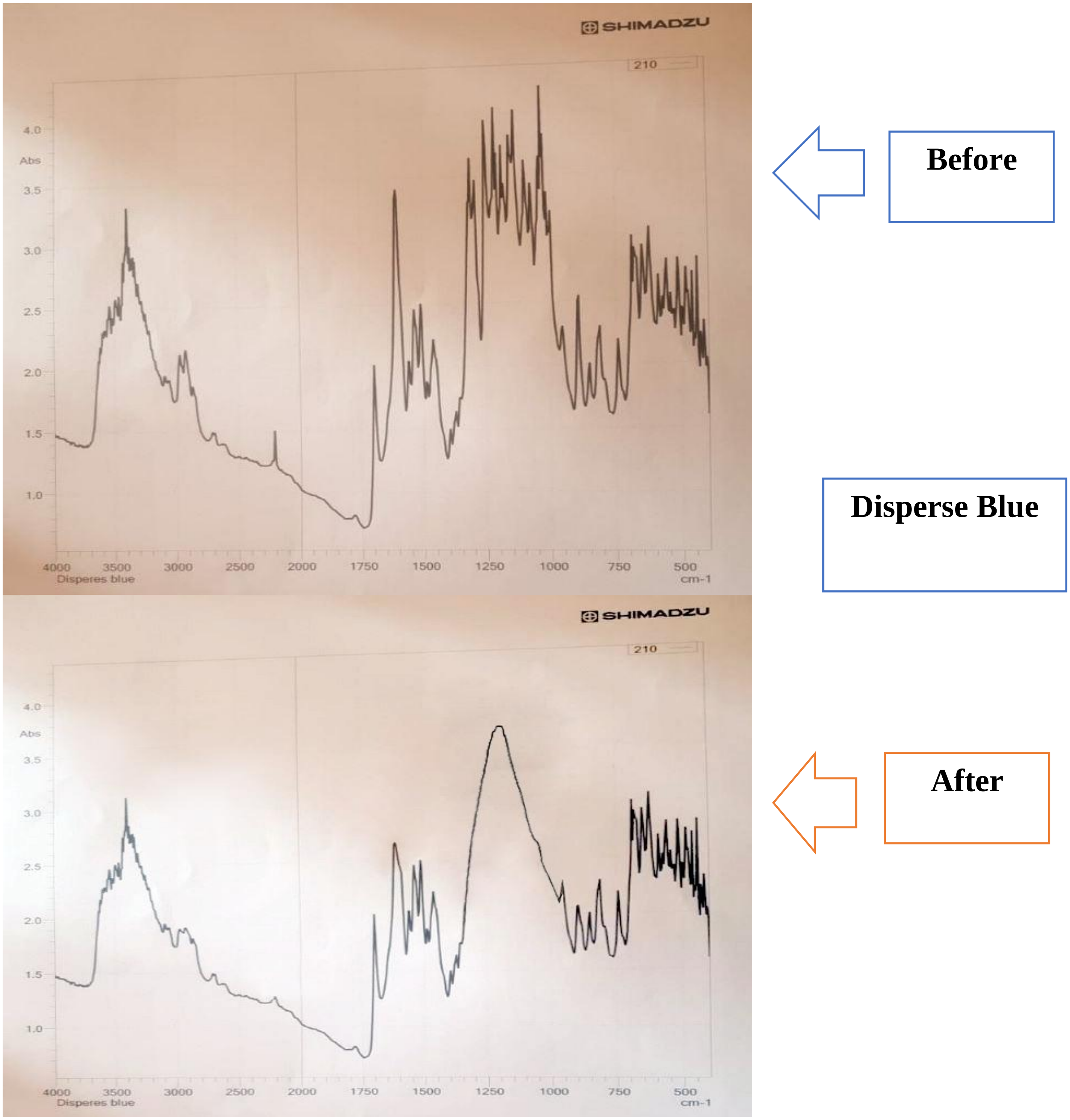


Figure (4.18) the result of FTIR examination to Disperse Blue dye before and after treatment.

4.4 Discussion:

microorganisms have developed a few different ways To break down azo dyes . The ability of the chosen strain determines how well bacteria can break down azo dyes. Microbial biodegradation is cheap and economical with less sludge production (Meerbergen *et al*., 2018) .

A broad variety of microbes has been demonstrated to reductively cleave these azo dyes. Aerobic and anaerobic microorganisms from various settings have the potential to convert azo dyes into genotoxic chemicals.(Lalnunhlimi, and Krishnaswamy, 2016) .

4.4.1 ***Proteus spp*.**

Through the results of the present study, *proteus spp*. were used to show their ability to treat Dyes. the result means that the *proteus* will in general lead to increment azo dyes gathering by biomass and finally increase the removal efficiency to remove the azo dyes from aqueous solution this results is quite with the opinion of (Darnall *et al*., 1986; Aksu *et al*., 1998).

Proteus is frequently used to ponder adsorption of azo dyes(Aksu and Dönmez, 2006; Ruangsomboon and Wongrat, 2006).

(Ting *et al*., 1989) that take-up of elements particles can be partitioned into 2 phases: fast and moderate stage. In the quick phase, element particles are adsorbed upon the outside of the microorganism. In the moderate phase, the element particles transfer over the microorganisms into inside cells.

Bacterial Biomass assumes a basic function for purification polluted water and thusly in the working of water resources (Kuyucak and Volesky, 1989; Corder and Reeves, 1994; Knauer *et al*., 1998). Bacterial cell can be more raised their characteristics of sterilization of impure aqueous systems (Tam *et al*., 1998).

From the result , *Proteus* has been able to adsorb and remediate the Azo dyes and decrease its concentration in aqueous solution because *proteus* has Particular and non-particular positions had been bound azo dyes(Mehta and Gaur, 2001).

Ionic charge and covalent bonds are engaged with biodegradation and the wall of cell components are likewise known to assume a critical function in biodegradation (Crist *et al*., 1981; Mahan *et al*., 1998; Knauer *et al*., 1998). These results are identical to Chong et al. (2000) and Hamdy (2000).

Whole types of *Proteus* that found for amass large amounts of azo dyes, Numerous potential binds positions happen in the cells membrane (Saitoh *et al*., 2001; Tam *et al*., 1998).

Azo dyes represent the significant aqueous toxins that existence in factories sewage (Kuyucak and Volesky,1989). Water contamination is coming about because of an expanded grouping of Azo dyes that causing genuine natural issues in numerous pieces of globe.

Take-up of azo dyes particles by bacteria can show modified strategy for their elimination from sewage. All microorganisms forms can eliminate azo dyes from polluted systems.(Tobin *et al*,1984 ; Volesky & Holan,1995).

From the results that represent the efficiency removal of *Proteus* for the Azo dyes, beacause the ability of these bacterial species to biodegradation that represents a hopeful technique for ecosystems cleaning, among the many of organisms that present naturally in the gushing, *Proteus* can choose for the current investigation, because of the facility they show for elimination of some azo dyes from aqueous solution (Rani faryal *et al*., 2007).

Our study proposed that Azo dyes bioadsorption by some types of *proteus* includes both passive and active uptake. At minimum level of azo dyes focus, Azo particles are acceptable average by the organism and azo dyes take-up happens because of straightforward ion exchange among metal and practical gatherings present on cell divider surface of cells. While, at maximum level of azo dyes, quantity of these dyes has been sorbed more than at minimum levels focuses, where all the bonds positions were liberate for reaction. In addition, the raise of dyes bioadsorption in some types of proteus was required to add to intracellular take-up of azo dyes happening in metabolically dynamic cells in mix with extrcellular bioadsorption (Ezzouhria *et al*,2010).

4.4.2 Azo dyes Removal

Existence of azo dyes in water resources is to make extreme harm for ecosystems, and the poisonous azo dyes are in the limelight because of their real sway on the ecosystems (Diels *et al.*, 2002; Volesky, 1994).. Azo dyes are brought into the waterways from different resources. Elimination of lethal azo dyes from sewage is fundamental from the point of view of ecological contamination control (Guangyu and Thiruvenkatachari, 2003; Say *et al.*, 2003; Svecova *et al*.,2006).

New effecient handling to eliminate azo dyes in watery medium includes the utilization of microorganisms (Yan and Viraraghavan, 2003; Wang and Chen, 2009). Consideration has been centered of late around the use of organisms for dyes elimination from watery medium (Huang and Huang, 1996; Vala, 2010). Bacterial species can aggregate elements by physical, chemical and biological methods(Volesky and May-Phillips,1995;Vankar and Bajpa, 2008).

The high removal efficiency in the current study may return to the ability of functional groups in the surface of beads and the increase of the concentration leads to increase the removal efficiency as a result to increase the dyes ions that lead to produce pressure on the surface of beads and increase the active transport within the beads, the current study is identical to (Velkova *et al*,2018).

The distinctions in bioadsorption ability might be because of the natural capacity of bacteria in addition to its cell memberane sythesis prompting variation in reaction of elements with organisms (Gadd and White, 1993). Many researchers have revealed the bioadsorption capacity of *proteus*. in removing the azo dyes from aqueous solution(Gadd, 1990; Fourest et al., 1996; Sudha Bai and Abraham, 2001 Teskova and Petrov, 2002).

Occurrence of azo dyes in watery ecosystems is recognized to make serious harm to these ecosystems life, in addition to the way that these elements slay organisms through the sewage handling with an ensuing postponement of the procedure of water sanitization (Hussein *et al*,2004). Wastewater treatment techniques, for example, physical and chemical techniques have been broadly used to expel azo dyes from polluted sewage. These operations might be incompetent or costly (Volesky, 1990a; Volesky, 1990b). Bioremidiation techniques, for example, bioadsorption/bioacumulation for the expulsion of azo dyes particles may give an appealing substitutional to physical and chemical strategies (Kapoor and Viraraghavan, 1995).

The low removal efficiency in the current study because the dyes concentration lead to poisoned of the bacteria and increase the probability of kill of bacteria and decrease the dyes that removed from aqueous solution after treatment by bacteria, this means that the mechanisms of bioadsorption(Active & Passive) are stopped and only the adsorption on surface is working. (Hussein et al. 2003).

Utilization of microorganism or its biomss or its outputs for the recuperation of elements from sewage and the bioremediation is the financially savvy and ecological well-disposed procedure, which has been broadly acknowledged by bacterial elements rebioabsorption (Norris,1990).

The high removal efficiency in contrast return to the resistance ability of proteus for dyes, and due to the estrained impacts of dyes expanded as the level of this component was raised in growth media in a significant number of the bacteria. (Nanganuru and Korrapati,2012).

4.4.3 Molecular Characterization and Azo dyes degradation

Microorganisms are everywhere. Their capacity to tolerate tough circumstances and grow even in the most contaminated environments makes them a breeding ground for the discovery of novel enzymes and metabolites that may be used to alleviate environmental concerns.

According to (John *et al*, 2020)a total of 12 genes involved in the dye decolorization pathway was identified. These include genes encoding FMN-dependent NADH azoreductase Clade III, hydroxymuconate delta isomerase, maleylacetoacetate isomerase, cytidine deaminase, cytosine deaminase, ornithine cyclodeaminase, deoxycytidine triphosphate deaminase, tRNA-specific adenosine-34 deaminase, diamino hydroxyl phosphoribosyl amino pyrimidine deaminase, glucosamine-6-phosphate deaminase, adenosine deaminase, and porphobilinogen deaminase.

The genomic analysis of this strain revealed the presence of genes involved in several dye decolorization methods, including as the FMN-dependent NADH azoreductase, which is important in cleaving the dye's azo bonds and turning the dye into aromatic amines(Dave *et al*., 2015). Aromatic amines and other functional groups formed would then be cleaved or reduced further by genes involved in deamination and isomerization processes. We proposed a dye decolorization mechanism based on the genes present in the genome.

The capacity of several bacterial strains to decolorize distinct kinds of dyes via azoreductase activity has already been documented (Eichlerová *et al*., 2006;).

Halomonas elongate, a halophilic bacteria, for example, has been found to decolorize mono and di azo dyes such as methyl red, remazol black, and sulphonyl blue TLE.

4.4.4 Functional Group for Decolorization

FT-IR results of current study, were demonstrated that biodegradation procedure happened because of the existence of hydroxyl, carbonyl, amide, and carboxyl combinations these mean that the dyes ions after treatment were bind with the functional group mentioned above leading to shifting the absorbance from site to another. Comparable fndings were likewise detailed in the writing (Nessim *et al*. 2011; Goher *et al*. 2016; Patel *et al*. 2016).

FT-IR peaks of untreated bioadsorbent demonstrated many particular and sharp adsorption groups of various wavelengths. The FT- IR spectrums of treated proteus demonstrated a few moves in some of distinct wavelengths. Variation in spectra portrays alterate in active gatherings of bacteria after adsorption, make us suggested the likelihood that bioadsorption could be occurred over ion-exchange operation instead of collection.

The current study showed the bonds of functional groups, for example, Ion-exchange between H of carboxyl (- COOH),hydroxyl (- OH) and amine (- NH2) gathering of Bacteria and dyes particles are principally included with bioadsorption surface for it(Fig.4-34,35,36,37,38).

FTIR spectrum of Proteus spp. for treated dyes show the bands of wavelength that shifted to another spectra after treatment of dyes, this means the dyes is binding with the functional groups that record the spectra before treatment such as (O-H,N-H,C=O=C,C-O-H…etc).

Each functional group has spectra of wavelength produced from their vibrate (bonds vibration) and therefore after binding with dyes the vibrate is altered and finally shifting of spectra from sites to another. The current study is identical to (Rao *et al*., 2005 and Akthar *et al*., 1996).

From the peak results of FT-IR in the current study the specrum of wavelength that ranged from (500-4000)cm-1 and the wavelength that records in free and proteus were shifting from one site to another because the binding between the functional groups with lead that cause the vibration changes and therefore the new wavelength was appear, FT-IR spectrum is utilized as to affirm accessibility of restricting locales that discovered (O-H) (N-H)C-OP- H(C=O)(CH2)bonds , a study by Fadl et al(2017)who utilized FT-IR spectrum to determine the nearness of amide, carboxyl, and phosphate bunches in some bacterial species Biomass. A few band changes enabled the creators to anticipate the conceivable contribution of amino, carbonyl, carboxyl, and phosphate bunches in the biosorption of some heavy metals. Curves of the FT-IR examination , found a clear change in the values of wavelengths resulting from the process of linking of dyes bonds found after treatment by *proteus sp.* and Bacterial cell surface demonstrates the task of various groups to particular practical gatherings that have a place with contrast class of macromolecules in the control tests. just as annoyance in the band structure after dyes treatment. The variation in FTIR peaks and their transmission were because of the reaction with the active sites on the Proteus through bioadsorption operation

Conclusions
&
Recommendations

Conclusions

1-Biotreatment is a simple, inexpensive, and efficient method for removing textile colors

2-The bacterial isolates were shown to be an excellent microbial source for waste water treatment.

3-The genotoxic and phytotoxic effects of degraded dye were reduced.

4- *Proteus* spp. have the potential to pave the way for the safe conversion of azo dyes and other xenobiotics.

5-These results indicate that the *Proteus* strain has great potential to degrade dye-contaminated water and soil

Recommendations

1-Utilize the different bacterial isolates like Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli for Azo dyes degradation

2-During the dye decolorization process, it is necessary for detection the activities of oxido-reductive enzymes such as lignin peroxidase, laccase, and azoreductase were observed to be stimulated.

3-Application of Cell Immobilization for azo dyes removal or decolorization

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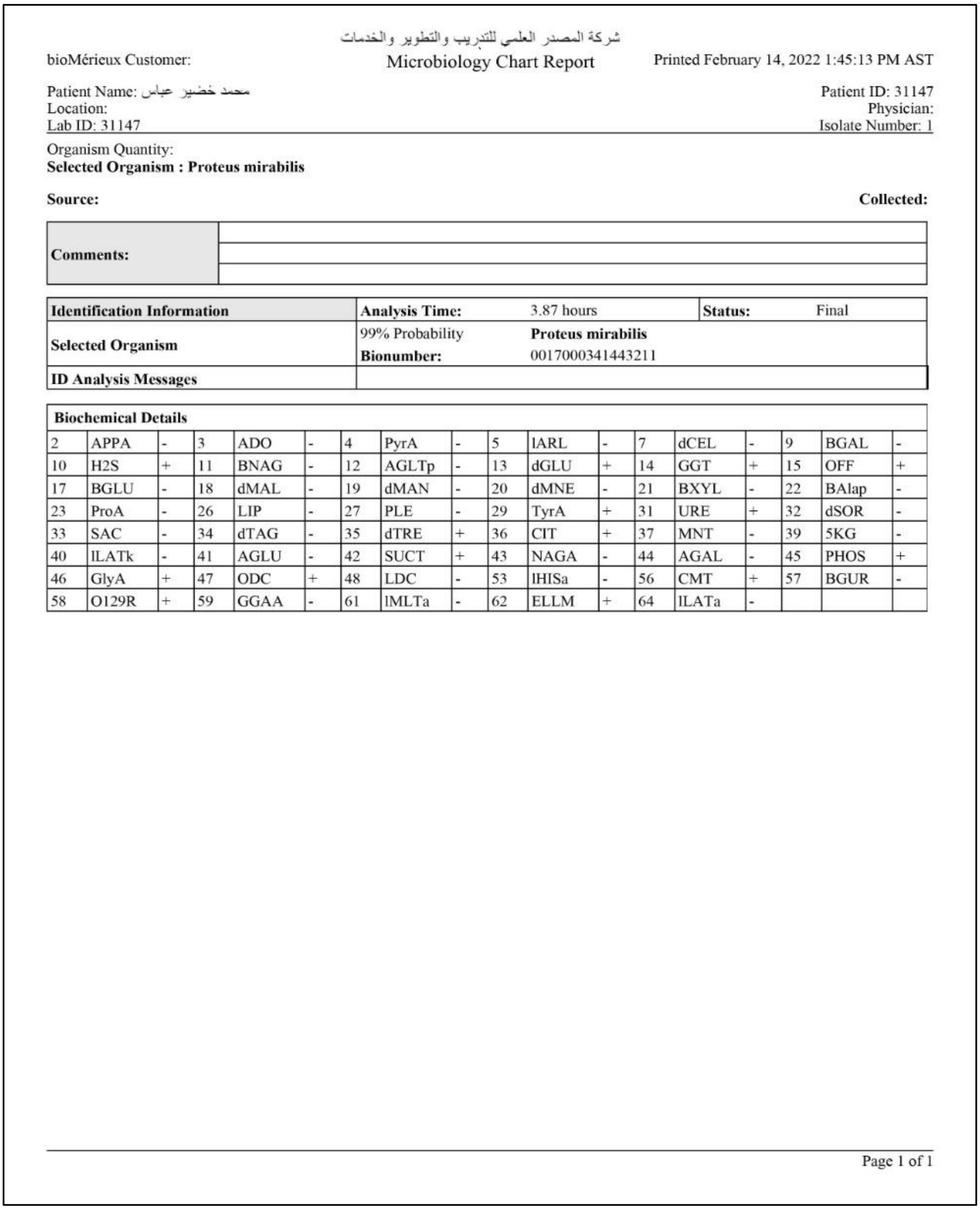
Appendix (1): Vitek Report of *Proteus mirabilis*



شركة المصدر العلمي للتدريب والتطوير والخدمات

bioMérieux Customer:

Microbiology Chart Report

Printed February 14, 2022 1:45:13 PM AST

Patient Name: محمد خضير عباس
Location:
Lab ID: 31147

Patient ID: 31147
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Proteus mirabilis

Source:

Collected:

Comments:	

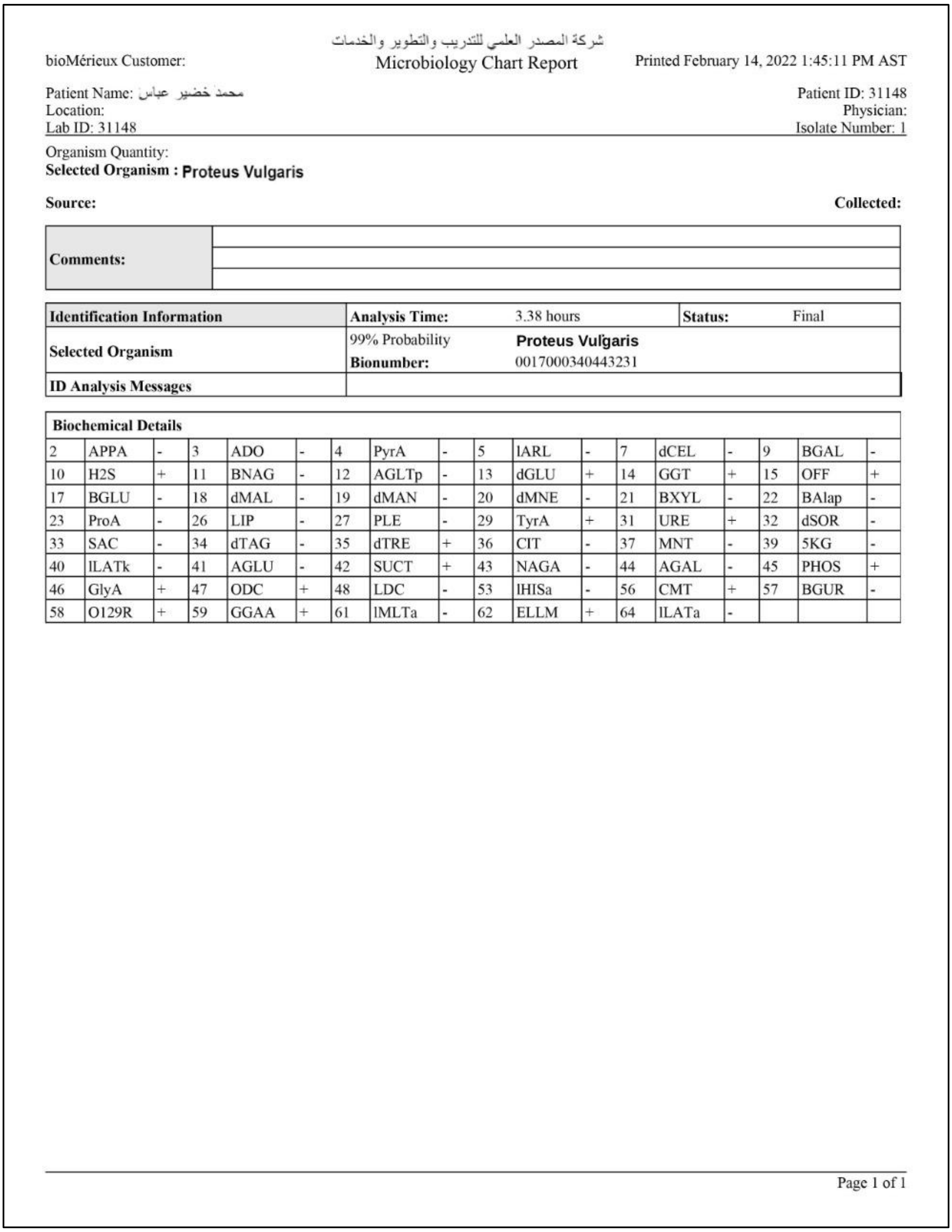
Identification Information	**Analysis Time:**	3.87 hours	**Status:**	Final
Selected Organism	99% Probability **Bionumber:**	**Proteus mirabilis** 0017000341443211		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	+	64	lLATa	-			

Page 1 of 1

Appendix (2): Vitek Report of *Proteus vulgaris*



شركة المصدر العلمي للتدريب والتطوير والخدمات

bioMérieux Customer:

Microbiology Chart Report

Printed February 14, 2022 1:45:11 PM AST

Patient Name: محمد خضير عباس
Location:
Lab ID: 31148

Patient ID: 31148
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Proteus Vulgaris

Source:

Collected:

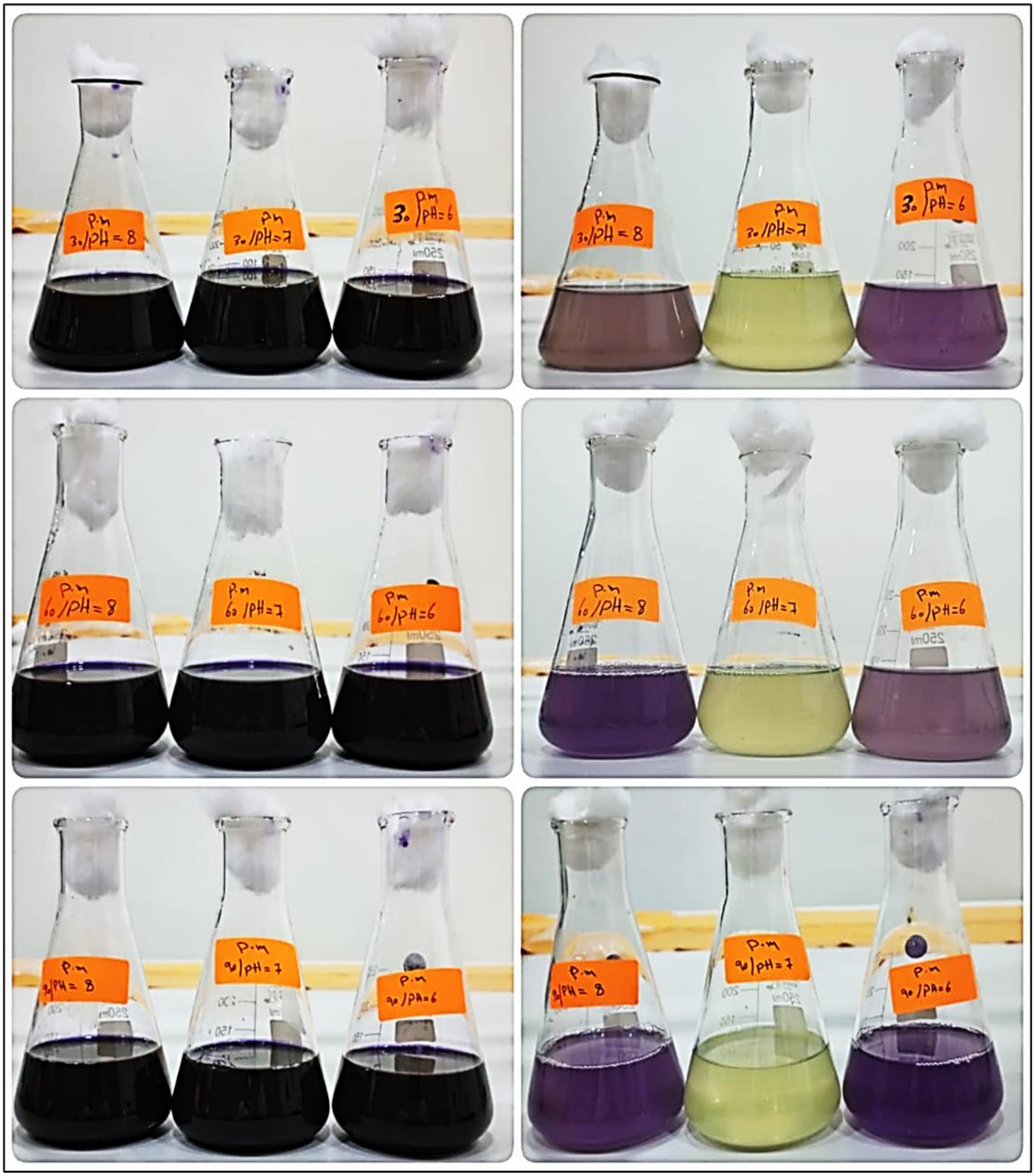
Comments:	

Identification Information	**Analysis Time:**	3.38 hours	**Status:**	Final
Selected Organism	99% Probability **Bionumber:**	**Proteus Vulgaris** 0017000340443231		
ID Analysis Messages				

Biochemical Details																				
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	lMLTa	-	62	ELLM	+	64	lLATa	-			

Appendix (3): Effect of different value pH (6,7&8) on decolorisation of Direct Yellow Azo dye (in a different concentrations 30,60& 90 mg/L) by *P. mirabilis* isolates; pictures on the left before incubation and pictures on the right after incubation at 37°C and shaking (120 rpm) for 24 h.

Appendix (4): Effect of different value pH (6,7&8) on decolorisation of Direct Yellow Azo dye (in a different concentrations 30,60& 90 mg/L) by *P. vulgaris* isolates; pictures on the left before incubation and pictures on the right after incubation at 37°C and shaking (120 rpm) for 24 h.



Appendix (5): Effect of different value pH (6,7&8) on decolorisation of Disperse Blue Azo dye (in a different concentrations 30,60& 90 mg/L) by *P. mirabilis* isolates; pictures on the left before incubation and pictures on the right after incubation at 37°C and shaking (120 rpm) for 24 h

Appendix (6): Effect of different value pH (6,7&8) on decolorisation of Disperse Blue Azo dye (in a different concentrations 30,60& 90 mg/L) by *P. vulgais* isolates; pictures on the left before incubation and pictures on the right after incubation at 37°C and shaking (120 rpm) for 24 h

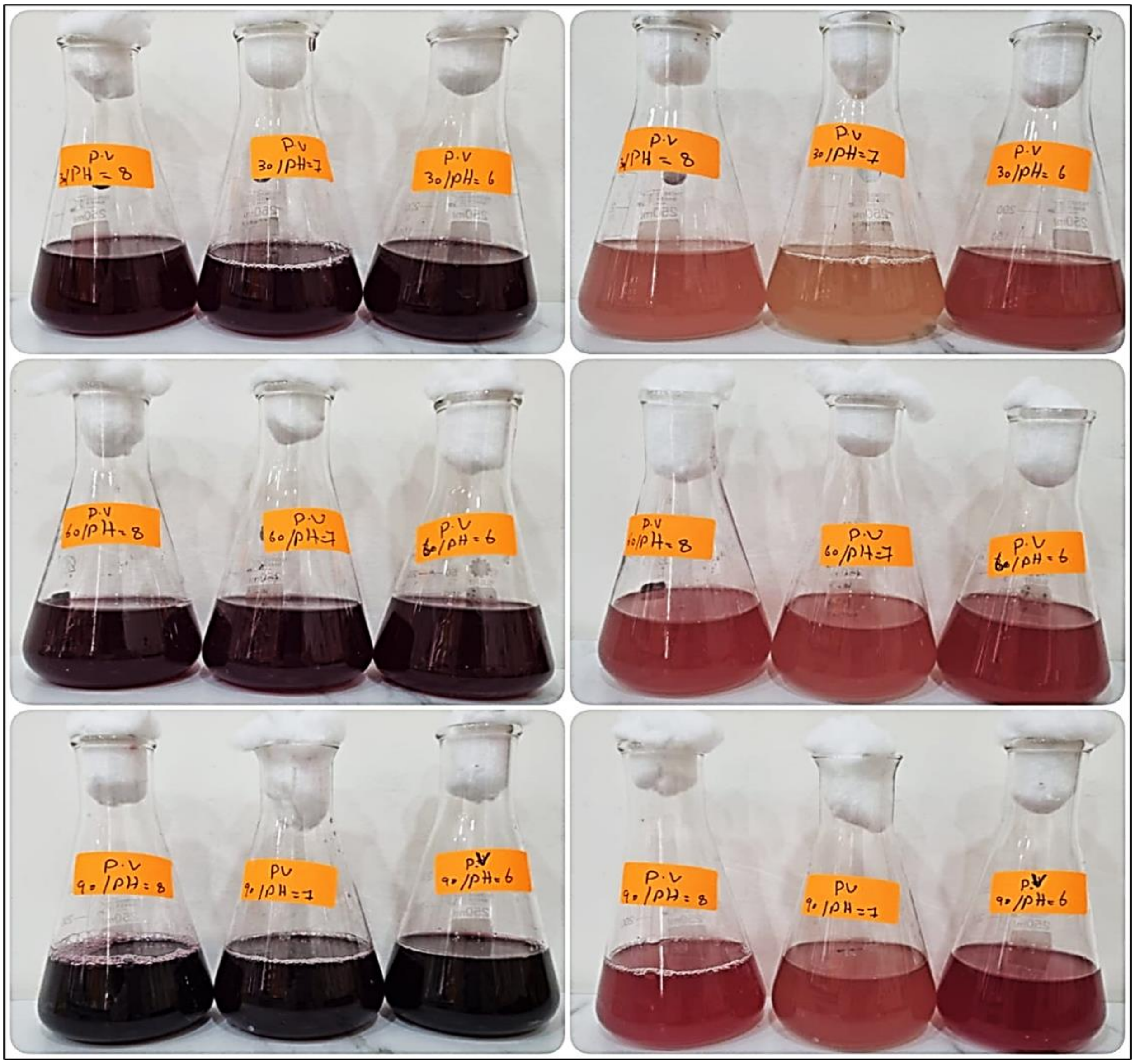
Appendix (7): Effect of different value pH (6,7&8) on decolorisation of Reactive Green Azo dye (in a different concentrations 30,60& 90 mg/L) by *P. mirabilis* isolates; pictures on the left before incubation and pictures on the right after incubation at 37°C and shaking (120 rpm) for 24 h.

Appendix (8): Effect of different value pH (6,7&8) on decolorisation of Reactive Green Azo dye (in a different concentrations 30,60& 90 mg/L) by *P. vulgaris* isolates; pictures on the left before incubation and pictures on the right after incubation at 37°C and shaking (120 rpm) for 24 h.

Appendix (9): Effect of different value pH (6,7&8) on decolorisation of Direct brwon Azo dye (in a different concentrations 30,60& 90 mg/L) by *P. mirabilis* isolates; pictures on the left before incubation and pictures on the right after incubation at 37°C and shaking (120 rpm) for 24 h

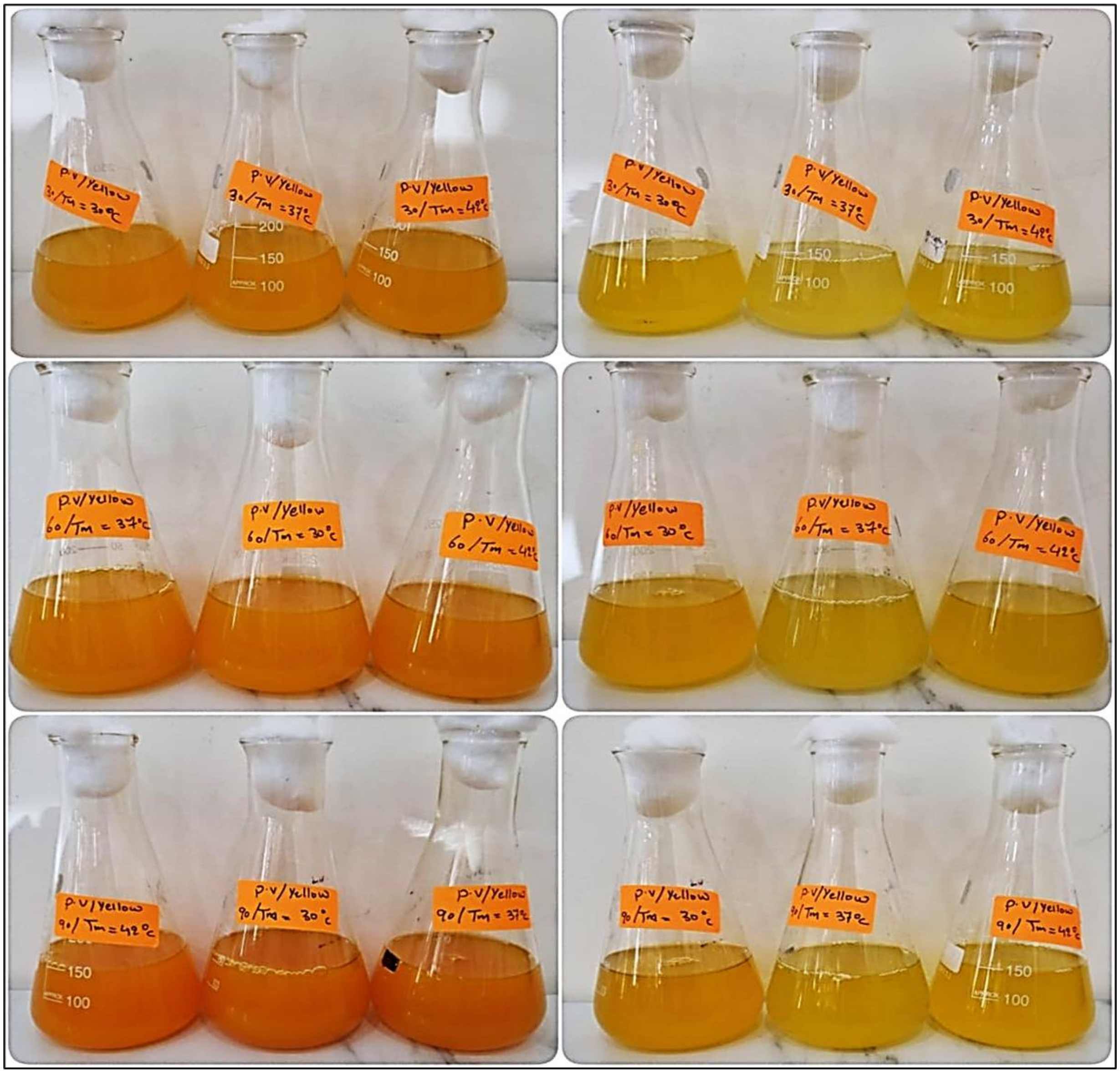
Appendix (10): Effect of different value pH (6,7&8) on decolorisation of Direct brwon Azo dye (in a different concentrations 30,60& 90 mg/L) by *P. vulgaris* isolates; pictures on the left before incubation and pictures on the right after incubation at 37°C and shaking (120 rpm) for 24 h.

Appendix (11): Effect of different value pH (6,7&8) on decolorisation of Reactive brown Azo dye (in a different concentrations 30,60& 90 mg/L) by *P. mirabilis* isolates; pictures on the left before incubation and pictures on the right after incubation at 37°C and shaking (120 rpm) for 24 h



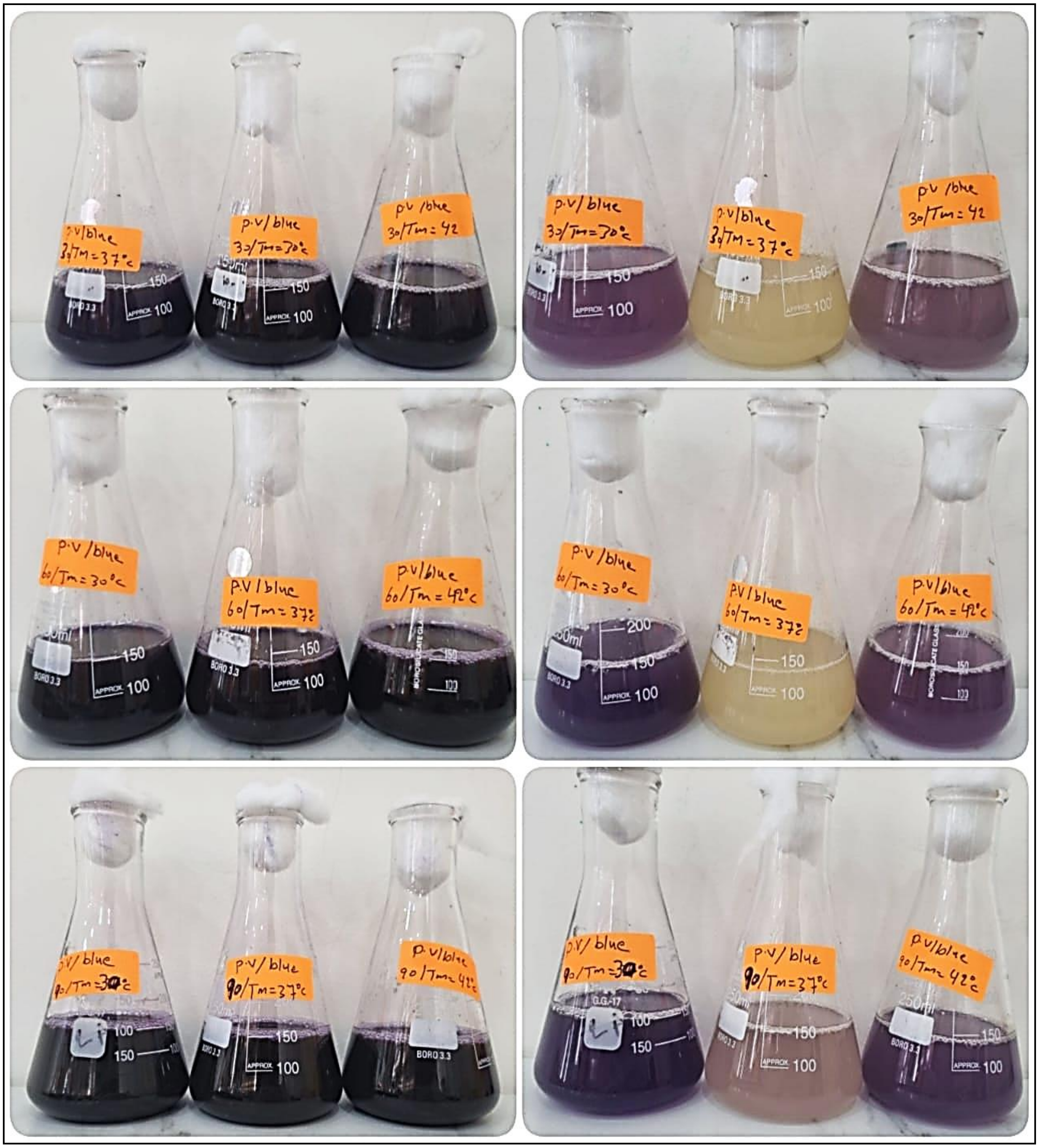
Appendix (12): Effect of different value pH (6,7&8) on decolorization of Reactive brown Azo dye (in a different concentrations 30,60& 90 mg/L) by *P. vulgaris* isolates; pictures on the left before incubation and pictures on the right after incubation at 37°C and shaking (120 rpm) for 24 h

Appendix (13): Effect of Temperature (30,37& 42 °C) on decolorisation of Direct Yellow Azo dye (in a different concentrations 30,60& 90 mg/ml) and pH =7 by *P. mirabilus* isolates; pictures on the left before incubation and pictures on the right after incubation at different temperatures and shaking (120 rpm) for 24h

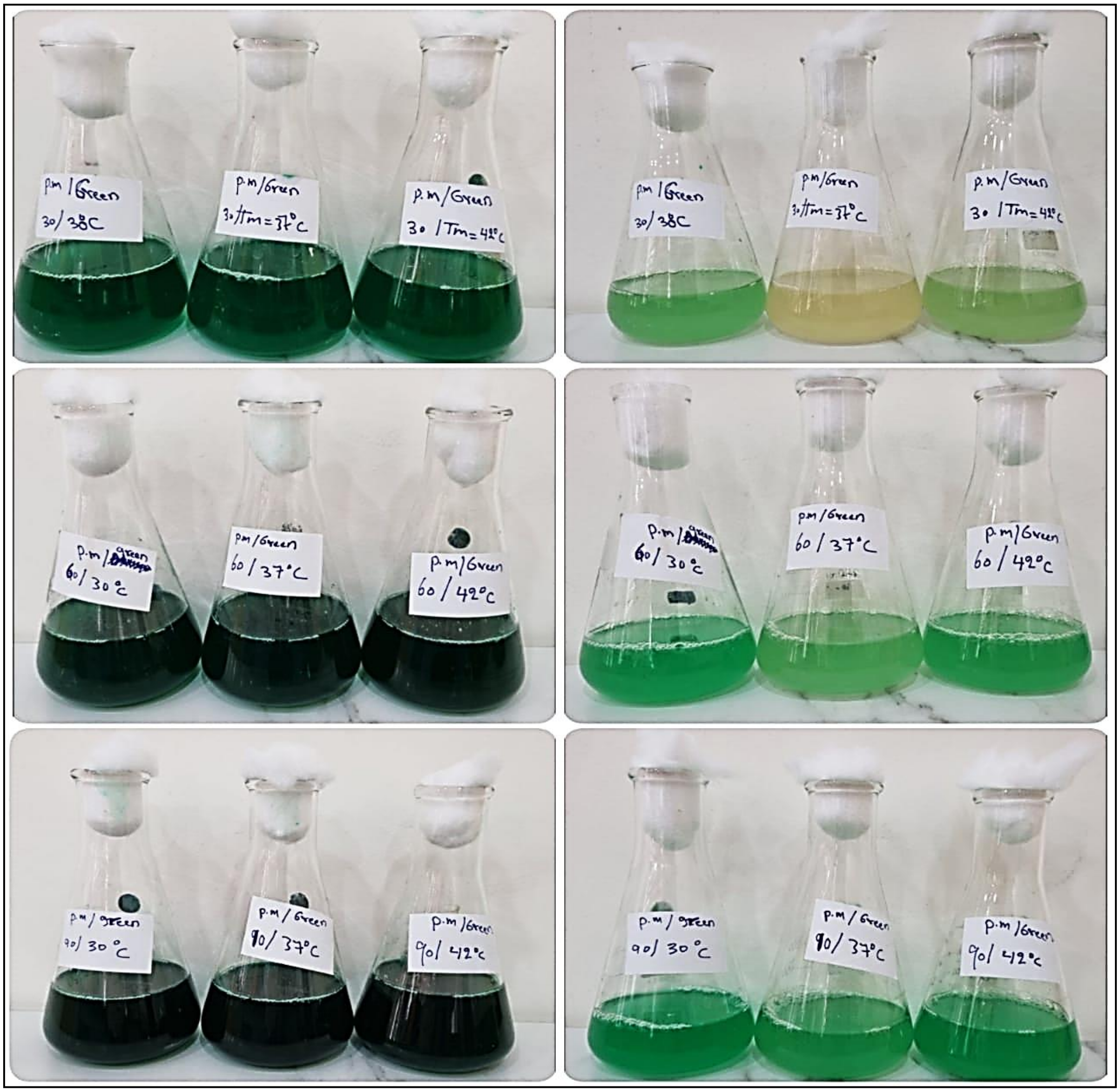


Appendix (14): Effect of Temperature (30,37& 42 °C) on decolorisation of Direct Yellow Azo dye (in a different concentrations 30,60& 90 mg/ml) and pH =7 by *P. vulgaris* isolates; pictures on the left before incubation and pictures on the right after incubation at different temperatures and shaking (120 rpm) for 24 h

Appendix (15): Effect of Temperature (30,37& 42 °C) on decolorisation of Disperse blue Azo dye (in a different concentrations 30,60& 90 mg/ml) and pH =7 by *P. mirabilus* isolates; pictures on the left before incubation and pictures on the right after incubation at different temperatures and shaking (120 rpm) for 24 h



Appendix (16): Effect of Temperature (30,37& 42 °C) on decolorisation of Disperse blue Azo dye (in a different concentrations 30,60& 90 mg/ml) and pH =7 by *P. vulgaris* isolates; pictures on the left before incubation and pictures on the right after incubation at different temperatures and shaking (120 rpm) for 24 h



Appendix (17): Effect of Temperature (30,37& 42 °C) on decolorisation of Reactive Green Azo dye (in a different concentrations 30,60& 90 mg/ml) and pH =7 by *P. mirabilus* isolates; pictures on the left before incubation and pictures on the right after incubation at different temperatures and shaking (120 rpm) for 24 h

Appendix (18): Effect of Temperature (30,37& 42 °C) on decolorisation of Reactive Green Azo dye (in a different concentrations 30,60& 90 mg/ml) and pH =7 by *P. vulgaris* isolates; pictures on the left before incubation and pictures on the right after incubation at different temperatures and shaking (120 rpm) for 24

Appendix (19): Effect of Temperature (30,37& 42 °C) on decolorization of Direct brown Azo dye (in a different concentrations 30,60& 90 mg/ml) and pH =7 by *P. mirabilus* isolates; pictures on the left before incubation and pictures on the right after incubation at different temperatures and shaking (120 rpm) for 24 h.

Appendix (20): Effect of Temperature (30,37& 42 °C) on decolorization of Direct brown Azo dye (in a different concentrations 30,60& 90 mg/ml) and pH =7 by *P. vulgaris* isolates; pictures on the left before incubation and pictures on the right after incubation at different temperatures and shaking (120 rpm) for 24 h.

Appendix (21): Effect of Temperature (30,37& 42 °C) on decolorization of Reactive brown Azo dye (in a different concentrations 30,60& 90 mg/ml) and pH =7 by *P. mirabilus* isolates; pictures on the left before incubation and pictures on the right after incubation at different temperatures and shaking (120 rpm) for 24 h

Appendix (22): Effect of Temperature (30,37& 42 °C) on decolorization of Reactive brown Azo dye (in a different concentrations 30,60& 90 mg/ml) and pH =7 by *P. vulgaris* isolates; pictures on the left before incubation and pictures on the right after incubation at different temperatures and shaking (120 rpm) for 24h

Appendix(23):

1-Proteus vulgaris strain MoAySu-IRAQ-1 16S ribosomal RNA gene, partial sequence
GenBank: OP012843.1
FASTA Graphics
Go to:

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DEFINITION  Proteus vulgaris strain MoAySu-IRAQ-1 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   OP012843
VERSION     OP012843.1
KEYWORDS    .
SOURCE      Proteus vulgaris
 ORGANISM  Proteus vulgaris
 Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
 Morganellaceae; Proteus.
REFERENCE   1  (bases 1 to 801)
  AUTHORS   Mohammed,K.A., Ayad,M.A. and Suad,G.A.
  TITLE     Proteus,16S ribosomal RNA gene, partial sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 801)
  AUTHORS   Mohammed,K.A., Ayad,M.A. and Suad,G.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-JUL-2022) Department of Biology, University of
            Babylon, College of Science, iraq, Babylon 00964, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /strain="MoAySu-IRAQ-1"
                     /isolation_source="soil"
                     /db_xref="taxon:585"
                     /clone="MoAySu-IRAQ-1"
                     /country="Iraq"
                     /collected_by="Mohammed khudhair Abbas AL-Nasrawi, Ayad
                     M.J. Al-Mamoori, Suad Ghali Kadhim Alahmed"
     rRNA            <1..>801
                     /product="16S ribosomal RNA"
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      121 cgctatcgga tgaacccata tgggattagc tagtaggtgg ggtaatggct cagctaggcg
      181 acgatctcta gctggtctga gaggatgatc agccacactg ggactgagac acggcccaga
      241 ctcctacggg aggcagcagt ggggaatatt gcacaatggg cgcaagcctg atgcagccat
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     421 cagcagccgc ggtaatacgg agggtgcaag cgttaatcgg aattactggg cgtaaagcgc
     481 acgcaggcgg tcaattaagt cagatgtgaa agccccсagc ttaacttggg aattgcatct
     541 gaaactggtt ggctagagtc ttgtagaggg gggtagaatt ccacgtgtag cggtgaaatg
     601 cgtagagatg tggaggaata ccggtggcga aggcggcccc ctggacaagg actgacgctc
     661 aggtgcgaaa gcgtggggag caaacaggat tagataccct ggtagtccac gctgtaaacg
     721 atgtcgattt agaggttgtg gtcttgaacc gtggcttctg gagctaacgc gttaaatcga
     781 ccgcctgggg agtacggccg c
//
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Appendix(24):

2- Proteus vulgaris strain MoAySu-IRAQ-2 16S ribosomal RNA gene, partial sequence
GenBank: OP012844.1
FASTA Graphics

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LOCUS       OP012844                801 bp    DNA     linear   BCT 24-JUL-2022
DEFINITION  Proteus vulgaris strain MoAySu-IRAQ-2 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   OP012844
VERSION     OP012844.1
KEYWORDS    .
SOURCE      Proteus vulgaris
  ORGANISM  Proteus vulgaris
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
            Morganellaceae; Proteus.
REFERENCE   1  (bases 1 to 801)
  AUTHORS   Mohammed,K.A., Ayad,M.A. and Suad,G.A.
  TITLE     Proteus,16S ribosomal RNA gene, partial sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 801)
  AUTHORS   Mohammed,K.A., Ayad,M.A. and Suad,G.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-JUL-2022) Department of Biology, University of
            Babylon, College of Science, iraq, Babylon 00964, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..801
                     /organism="Proteus vulgaris"
                     /mol_type="genomic DNA"
                     /strain="MoAySu-IRAQ-2"
                     /isolation_source="soil"
                     /db_xref="taxon:585"
                     /clone="MoAySu-IRAQ-2"
                     /country="Iraq"
                     /collected_by="Mohammed khudhair Abbas AL-Nasrawi, Ayad
                     M.J. Al-Mamoori, Suad Ghali Kadhim Alahmed"
     rRNA            <1..>801
                     /product="16S ribosomal RNA"
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      121 cgctatcgga tgaacccata tgggattagc tagtaggtgg ggtaatggct cacctaggcg
      181 acgatctcta gctggtctga gaggatgatc agccacactg ggactgagac acggcccaga
      241 ctcctacggg aggcagcagt ggggaatatt gcacaatggg cgcaagcctg atgcagccat
      301 gccgcgtgta tgaagaaggc cttgggttg taaagtactt tcagcgggga ggaaggtgat
      361 aaggttaata cccttatcaa ttgacgttac ccgcagaaga agcaccggct aactccgtgc
      421 cagcagccgc ggtaatacgg agggtgcaag cgttaatcgg aattactggg cgtaaagcgc
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      541 gaaactggtt ggctagagtc ttgtagaggg gggtagaatt ccacgtgtag cggtgaaatg
      601 cgtagagatg tggaggaata ccggtggcga aggcggcccc ctggacaaag actgacgctc
      661 aggtgcgaaa gcgtggggag caaacaggat tagataccct ggtagtccac gctgtaaacg
      721 atgtcgattt agaggttgtg gtcttgaacc gtggcttctg gagctaacgc gttaaatcga
      781 ccgcctgggg agtacggccg c
//
```

Appendix(25):

3- Proteus vulgaris strain MoAySu-IRAQ-3 16S ribosomal RNA gene, partial sequence

GenBank: OP012845.1

FASTA Graphics

```
LOCUS       OP012845               801 bp    DNA     linear   BCT 24-JUL-2022
DEFINITION  Proteus vulgaris strain MoAySu-IRAQ-3 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   OP012845
VERSION     OP012845.1
KEYWORDS    .
SOURCE      Proteus vulgaris
  ORGANISM  Proteus vulgaris
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
            Morganellaceae; Proteus.
REFERENCE   1  (bases 1 to 801)
  AUTHORS   Mohammed,K.A., Ayad,M.A. and Suad,G.A.
  TITLE     Proteus,16S ribosomal RNA gene, partial sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 801)
  AUTHORS   Mohammed,K.A., Ayad,M.A. and Suad,G.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-JUL-2022) Department of Biology, University of
            Babylon, College of Science, iraq, Babylon 00964, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /mol_type="genomic DNA"
                     /strain="MoAySu-IRAQ-3"
                     /isolation_source="industrial waste water"
                     /db_xref="taxon:585"
                     /clone="MoAySu-IRAQ-3"
                     /country="Iraq"
                     /collected_by="Mohammed khudhair Abbas AL-Nasrawi, Ayad
                     M.J. Al-Mamoori, Suad Ghali Kadhim Alahmed"
     rRNA            <1..>801
                     /product="16S ribosomal RNA"
ORIGIN
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       61 gaaacggtgg ctaataccgc atgacgtcta cggaccaaag caggggctct tcggaccttg
      121 cgctatcgga tgaacccata tgggattagc tagtaggtgg ggtaatggct cacctaggcg
      181 acgatctcta gctggtctga gaggatgatc agccacactg ggactgagac acggcccaga
      241 ctcctacggg aggcagcagt ggggaatatt gcacaatggg cgcaagcctg atgcagccat
      301 gccgcgtgta tgaagaaggc cttggggttg taaagtactt tcagcgggga ggaaggtgat
      361 aaggttaata cccttatcaa ttgacgttac ccgcagaaga agcaccggct aactccgtgc
      421 cagcagccgc ggtaatacgg agggtgcaag cgttaatcgg aattactggg cgtaaagcgc
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    481 acgcaggcgg tcaattaagt cagatgtgaa agccccgagc ttaacttggg aattgcatct
    541 gaaactggtt ggctagagtc ttgtagaggg gggtagaatt ccacgtgtag cggtgaaatg
    601 cgtagagatg tggaggaata ccggtggcga aggcggcccc ctggacaaag actgacgctc
    661 aggtgcgaaa gcgtggggag caaacaggat tagataccct ggtagtccac gctgtaaacg
    721 atgtcgattt agaggttggg gtcttgaacc gtggcttctg gagctaacgc gttaaatcga
    781 ccgcctgggg agtacggccg c
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Appendix(26):

4- Proteus mirabilis strain MoAySu-IRAQ-4 16S ribosomal RNA gene, partial sequence
GenBank: OP012846.1
FASTA Graphics
Go to:

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LOCUS       OP012846                801 bp    DNA     linear   BCT 24-JUL-2022
DEFINITION  Proteus mirabilis strain MoAySu-IRAQ-4 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   OP012846
VERSION     OP012846.1
KEYWORDS    .
SOURCE      Proteus mirabilis
  ORGANISM  Proteus mirabilis
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
            Morganellaceae; Proteus.
REFERENCE   1  (bases 1 to 801)
  AUTHORS   Mohammed,K.A., Ayad,M.A. and Suad,G.A.
  TITLE     Proteus,16S ribosomal RNA gene, partial sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 801)
  AUTHORS   Mohammed,K.A., Ayad,M.A. and Suad,G.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-JUL-2022) Department of Biology, University of
            Babylon, College of Science, iraq, Babylon 00964, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /clone="MoAySu-IRAQ-4"
                     /country="Iraq"
                     /collected_by="Mohammed khudhair Abbas AL-Nasrawi, Ayad
                     M.J. Al-Mamoori, Suad Ghali Kadhim Alahmed"
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                     /product="16S ribosomal RNA"
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      121 cactatcgga tgaacccata tgggattagc tagtaggtgg ggtaaaggct cacctaggcg
      181 acgatctcta gctggtctga gaggatgatc agccacactg ggactgagac acggcccaga
      241 ctcctacggg aggcagcagt ggggaatatt gcacaaaggg cgcaagcctg atgcagccat
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    481 acgcaggcgg tcaattaagt cagatgtgaa agccccgagc ttaacttggg aattgcatct
    541 gaaactggtt ggctagagtc ttgtagaggg gggtagaatt ccatgtgtag cggtgaaatg
    601 cgtagagatg tggaggaata ccggtggcga aggcggcccc ctggacaaag actgacgctc
    661 aggtgcgaaa gcgtggggag caaacaggat tagataccct ggtagtccac gctgtaaacg
    721 atgtcgattt agaggttgtg gtcttgaacc gtggcttctg gagctaacgc gttaaatcga
    781 ccgcctgggg agtacggccg c
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Appendix(27):

5- Proteus mirabilis strain MoAySu-IRAQ-5 16S ribosomal RNA gene, partial sequence

GenBank: OP012847.1

FASTA Graphics

Go to:

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LOCUS       OP012847                801 bp    DNA     linear   BCT 24-JUL-2022
DEFINITION  Proteus mirabilis strain MoAySu-IRAQ-5 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   OP012847
VERSION     OP012847.1
KEYWORDS    .
SOURCE      Proteus mirabilis
  ORGANISM  Proteus mirabilis
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
            Morganellaceae; Proteus.
REFERENCE   1  (bases 1 to 801)
  AUTHORS   Mohammed,K.A., Ayad,M.A. and Suad,G.A.
  TITLE     Proteus,16S ribosomal RNA gene, partial sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 801)
  AUTHORS   Mohammed,K.A., Ayad,M.A. and Suad,G.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-JUL-2022) Department of Biology, University of
            Babylon, College of Science, iraq, Babylon 00964, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /db_xref="taxon:584"
                     /clone="MoAySu-IRAQ-5"
                     /country="Iraq"
                     /collected_by="Mohammed khudhair Abbas AL-Nasrawi, Ayad
                     M.J. Al-Mamoori, Suad Ghali Kadhim Alahmed"
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                     /product="16S ribosomal RNA"
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      121 cactatcgga tgaacccata tgggattagc tagtaggtgg ggtaaaggct cacctaggcg
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      301 gccgcgtgta tgaagaaggc cttagggttg taaagtactt tcagcgggga ggaaggtgat
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      661 aggtgcgaaa gcgtggggag caaacaggat tagataccct ggtagtccac gctgtaaacg
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Appendix(28):

6- Proteus mirabilis strain MoAySu-IRAQ-6 16S ribosomal RNA gene, partial sequence
GenBank: OP012848.1
FASTA Graphics
Go to:

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VERSION     OP012848.1
KEYWORDS    .
SOURCE      Proteus mirabilis
  ORGANISM  Proteus mirabilis
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales;
            Morganellaceae; Proteus.
REFERENCE   1  (bases 1 to 801)
  AUTHORS   Mohammed,K.A., Ayad,M.A. and Suad,G.A.
  TITLE     Proteus,16S ribosomal RNA gene, partial sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 801)
  AUTHORS   Mohammed,K.A., Ayad,M.A. and Suad,G.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-JUL-2022) Department of Biology, University of
            Babylon, College of Science, iraq, Babylon 00964, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
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                     /isolation_source="industrial waste water"
                     /db_xref="taxon:584"
                     /clone="MoAySu-IRAQ-6"
                     /country="Iraq"
                     /collected_by="Mohammed khudhair Abbas AL-Nasrawi, Ayad
                     M.J. Al-Mamoori, Suad Ghali Kadhim Alahmed"
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                     /product="16S ribosomal RNA"
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      121 cactatcgga tgaacccata tgggattagc tagtaggtgg ggtaaaggct cacctaggcg
      181 acgatctcta gctggtctga gaggatgatc agccacactg ggactgagac acggcccaga
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      301 gccgcgtgta tgaagaaggc cttagggttg taaagtactt tcagcgggga ggaaggtgat
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      541 gaaactggtt ggctagagtc ttgtagaggg gggtagaatt ccatgtgtag cggtgaaatg
      601 cgtagagatg tggaggaata ccggtggcga aggcggcccc ctggacaaag actgacgctc
      661 aggtgcgaaa gcgtggggag caaacaggat tagataccct ggtagtccac gctgtaaacg
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جمهورية العراق
وزارة التعليم والعالي والبحث العلمي
جامعة بابل / كلية العلوم
قسم علوم الحياة

التحري الجزيئي للجينين *Lac* و *Porin* في بكتريا *.Proteus spp*لازالة وتكسيرصبغات الازو

رسالة مقدمة الى

مجلس كلية العلوم – جامعة بابل كجزء من متطلبات نيل درجة الماجستير في العلوم / علوم حياة

من قبل

محمد خضير عباس عبطان

بكالوريوس علوم حياة / جامعة بابل / 2007

باشراف

ا. م. سعاد غالي كاظم الاحمد   أ.د اياد محمد جبر المعموري

1444هـ   2023م

الخلاصة

تضمنت هذه الدراسة عزل بكتيريا إزالة الصبغة من عينات المياه والتربة ، وتم جمع 30 عينة بيئية (15 عينة تربة) من أماكن زراعية مختلفة من الحدائق المنتشرة في المدينة الطبية / مستشفى بغداد التعليمي ، وكذلك (15 عينة مياه عادمة) من محطة تصريف الصقلاوية شمال غرب بغداد في الفترة من كانون الثاني 2022 الى شباط 2022. تم تحديد نوعين من البكتريا هما Proteus vulgaris وProteus mirabilis وفقا للزراعة والميكروبيولوجيا والكيمياء الحيوية ونظام.VITEK 2 .

يشير توصيف النمط الظاهري وتحليل الجيني والتطور بناءً على مقارنات تسلسل 16SrRNA إلى أن هذه السلالات تنتمي إلى جنس *Proteus* spp. تمت مقارنة كفاءة التحلل لهذين الكائنين من خلال تحسين درجة الحموضة ودرجة الحرارة ووقت الحضانة وتركيز الصبغة الأولي كان proteus vulgaris و proteus mirabilis قادرة على تكسر 65٪ و 84.69٪ أصفر مباشر ، 97.61٪ و 100٪ أزرق مشتت ، 48.56٪ و 78.01٪ أخضر رد الفعل ، 63.35٪ و 70.7٪ بني مباشر و 70.04٪ و 75.1٪ صبغات بنية تفاعلية من 90 مجم / لتر تركيز على التوالي. في ظل الظروف المثلى ، وجد أن درجة الحموضة ودرجة الحرارة والوقت كانت 7 ، 37 درجة مئوية خلال 24 ساعة على التوالي.

تم تطوير زوج تمهيدي PCR واختباره لتحديد وقياس مجموعة متنوعة من الجينات البكتيرية لفئة الصبغة في مصفوفات معقدة. كشف التحليل الجينومي لهذه السلالة عن وجود جينات ترميز الإنزيمات المتضمنة في طرق إزالة اللون ، مثل Porin azoreductase Clade III و Lac ، والتي تشق رابطة آزو للصبغة ، والإنزيمات المشاركة في نزع الأمين وأزمرة المركبات الوسيطة.

أكد تحليل FT-IR أن الكائنات الحية الدقيقة كانت قادرة على تحلل أصباغ الآزو السامة إلى منتج غير سام كما ثبت من خلال التعديلات الهيكلية لتحليل الوظائف الكيميائية في المواد عن طريق اكتشاف الاهتزازات التي تميز الروابط الكيميائية ،

أظهرت جميع نتائج FT-IR للدراسة الحالية أن إجراء الامتصاص البيولوجي حدث بسبب وجود تركيبات الهيدروكسيل والكربونيل والأميد والكربوكسيل ، مما يعني أن اصباغ الايزو بعد العلاج كانت مرتبطة بالمجموعة الوظيفية المذكورة أعلاه مما أدى إلى التحول الامتصاص من موقع إلى آخر. يتغير التباين في صور الأطياف في التجمعات النشطة للخلايا بعد امتصاص اصباغ الايزو ، مما يجعلنا نقترح احتمالية حدوث الامتصاص الحيوي خلال عملية التبادل الأيوني بدلاً من التجميع. أظهرت الدراسة الحالية روابط المجموعات الوظيفية ، على سبيل المثال ، التبادل الأيوني بين يتم تضمين H من الكربوكسيل (COOH -) ، الهيدروكسيل (OH -) والأمين (NH2 -) تجمع الخلايا واصباغ الايزو بشكل أساسي مع سطح الامتصاص البيولوجي لذلك.