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A LOCAL YOGURT WAS SUGGESTED TO BE AN ANTIMICROBIAL AGENT FOR TREATING ORAL INFECTION

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Abstract-*A biological and chemical assays has been applied for studying the activity of an extracted active gradient from Iraqi (Kurdistan) local yogurt and it was used as antibacterial against pathogens isolated from oral infection. Gram (+ve) and gram (-ve) species were isolated and identified using Pincus protocol. The isolates preserved on nutrient agar at 37^{0}C and then cultured on a new culture media. Nutrient broth has been supplemented with 15% glycerol for long preservation so that the isolates maintained frozen for several months. Whey protein was precipitated from the local yogurt using 10% TCA solution. The precipitated protein shows a strong inhibitory effects on the isolates growth, the pathogen Proteus mirabilis.*

Keywords- *Proteus mirabilis isolate from local yogurt, antimicrobial agent.*

I INTRODUCTION

Proteus species are part of the Enterobacteriaceae family of gram-negative bacilli. Proteus organisms are implicated as serious causes of infections in humans, along with *Escherichia*, *Klebsiella*, *Enterobacter*, and *Serratia* species. Proteus species are most commonly found in the human intestinal tract as part of normal human intestinal flora, along with Escherichia coli and *Klebsiella* species, of which *E. coli* is the predominant resident. Proteus is also found in multiple environmental habitats, including long-term care facilities and hospitals. However, Proteus species are not the most common cause of nosocomial infections.

P. mirabilis, is an opportunistic human pathogens. That it includes pathogens responsible for many human urinary tract infections.[1] *P. mirabilis* causes 90% of Proteus infections and can be considered a community-acquired infection. Proteus mirabilis appears as Gram-negative rods after Gram staining under bright-field microscopy with 1000 times magnification. *P. mirabilis* is a Gram-negative, facultative anaerobic, rod-shaped bacterium. It shows swarming motility and urease activity. P. mirabilis causes 90% of all Proteus infections in humans. It is widely distributed in soil and water.[2] The bacteria can be found throughout the renal stones, and these bacteria lurking in the kidney stones can reinitiate infection after antibiotic treatment. Once the stones develop, over time they may grow large enough to cause obstruction and renal failure. Proteus species can also cause wound infections, septicemia, and pneumonia, mostly in hospitalized patients.[3] *P. mirabilis* is generally susceptible to most antibiotics apart from tetracycline and nitro furantoin,[4] but 10–20% of *P. mirabilis* strains are also resistant to first-generation cephalosporins and ampicillin.[5] It is commonly found in the intestinal tracts of humans. *P. mirabilis* is not pathogenic in guinea pigs or chickens. Noteworthy is the ability of this species to

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inhibit growth of unrelated strains, resulting in a macroscopically visible line of reduced bacterial growth where two swarming strains intersect. This line is named the Dienes line after its discoverer by Louis Dienes. [5]

Whey protein is a mixture of globular proteins isolated from whey, the liquid material created as a by-product of cheese production [6]. Whey protein is the collection of globular proteins isolated from whey. The protein in cow's milk is 20% whey protein and 80% casein protein,[7] whereas the protein in human milk is 60% whey and 40% casein.[8] The protein fraction in whey constitutes approximately 10% of the total dry solids in whey. This protein is typically a mixture of beta lactoglobulin (~65%), alpha-lactalbumin (~25%), bovine serum albumin (~8%), and immunoglobulins.[9] These are soluble in their native forms, independent of pH.

II MATERIALS AND METHODS

Biological and Chemical Materials: Crystal violet, Gram's iodine, acetone / ethanol 50:50 v/v, 0.1basic fuchsin solution were used for detection of bacteria.

Culture Media: MacConkey agar, Nutrient agar, Mannitol agar, and Muller Hinton agar were used for bacteria growth.

Collection of samples: Isolated bacteria species used in this study was collected from patients with oral infection, using standard bacteriological methods adopted from that of MacFfaddin (2000).

Preparation of culture and diagnostic media:

Nutrient agar medium*:*

This media is a basic culture medium used in the preparation of blood agar and other media. This medium used for cultivation and maintenance of all isolated bacteria. The medium is composed according to Atals, 1993. Medium containing (per liter of distilled water): 15g agar, 5g peptone, 5g Nacl, 5g yeast extract, 1gm beef extract, at pH 7.4 + 0.2.

MacConkey agar medium: MacConkey agar is a differential and low selectivity medium used to distinguish lactose fermenting from non-lactose fermenting bacteria.

Lactose fermenting bacteria: These were detected as pink to red colonies surrounded by red zone due to lactic acid fermentation.

Non-lactose fermenting bacteria: These were detected as colorless colonies, medium turn to yellow color.

Mannitol salt agar: Mannitol salt agar is a differential and selective plate medium used to isolate staphylococcus aureus. Mannitol is fermented by *Staphylococcus aureus* (yellow in medium) (Finegold and Martin, 1982).
Muller Hinton agar

It is a microbiological growth medium that is commonly used for antibiotic susceptibility testing. It is also used to isolate and maintain *Neisseria* and *Moraxella* species (Henrik Caspar etal, 2002).

Biochemical tests:

Gram stain:

The staining involves 3 major steps that include;

1. Staining with crystal violet (a water soluble dye).
2. De-colorization (using ethanol/acetone).
3. Counterstaining (using Safranin).

Due to the differences in the thickness of the peptidoglycan layer on the cell walls of these bacteria, gram positive bacteria will retain the crystal violet stain after the de-colorization process using ethyl alcohol/ acetone.

After staining the sample with crystal violet, ethyl alcohol is used to decolorize the sample. It achieves its purpose by dehydrating the peptideglycan layer by tightening and shrinking it. In doing so, large crystal violet cannot penetrate the tightened layer of peptidoglycan, and hence it is trapped in the cell wall of gram positive bacteria.

On the other hand, the outer membrane of the gram negative cells cannot retain the crystal violet iodine complex and hence the color is lost. Safranin is a lighter stain as compared to crystal violet and thus it does disrupt the purple coloration in the gram positive cells.

Oxidase test: This test depends on the presence in bacteria of certain oxidase that will catalyase the transport of electron between electron donors in the bacteria and redox dye tetramethyl- p-phenylene diamine. The dye is reduced to a deep purple color.

Preparation of reagents: The following reagents were prepared as described by MacFaddin (2000).

Methyl red reagent: This reagent was prepared by dissolving 0.1 gm f methyl red in 300 ml of 96% ethanol and then completed to 500 ml with D.W. This reagent was used as indicator in methyl red test.

Voges-Proskauer reagents

The reagents were prepared as follows:

Reagent A: 5% naphthol in 96% ethanol.

Reagent B: 40% KOH in D.W.

These reagents were used as indicators in Voges-Proskauer test.

Saline solution: Sodium chloride (0.85 gm) was dissolved in 90 ml D.W. and further completed to 100 ml with D.W (Collee *et al*., 1996).

Catalase test: It is a tetramer of four polypeptide chains, each over 500 amino acids long (Boon EM, Downs A, Marcey D. 2007). It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani P, Fita I, Loewen PC 2004).

Identification of Bacteria isolate: Identification of the bacteria species was achieved by adopting the protocol of (David H. Pincus). Bacteria species was identify using vitek instrument.

Principle of vitek instrument: The VITEK 2 is an automated microbiology system utilizing growth-based technology. The system is available in three formats (VITEK 2 compact, VITEK 2, and VITEK 2 XL) that differ in increasing levels of capacity and automation. Figure (1-1) shows the VITEK 2 compact system. All three systems accommodate the same colorimetric reagent cards that are incubated and interpreted automatically.

Figure 1: VITEK 2 Compact Instrument and Workstation

VITEK 2 Compact

This format focuses on the industrial microbiology-testing environment while also having application for low to middle volume clinical laboratories. Features specifically developed for industrial microbiology include 21 CFR Part 11 compliance (for electronic records and signatures) and a colorimetric reagent card (BCL) used to identify the spore-forming Gram-positive bacilli (i.e., Bacillus and related genera). The other colorimetric reagent cards (GN, GP, YST) apply to all system formats for both industrial and clinical laboratories.

Reagent Cards

The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalization, enzyme hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism substrate admixtures. Each card has a pre-inserted transfer tube used for inoculation (described below). Cards have bar codes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading the card onto the system. Figure (2-2) shows the GN card.

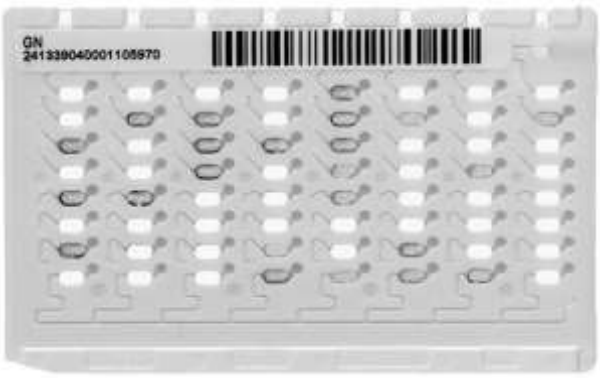

Figure 2: VITEK 2 GN Colorimetric Identification Card

There are currently four reagent cards available for the identification of different organism classes as follows:

1. GN - Gram-negative fermenting and non-fermenting bacilli
2. GP - Gram-positive cocci and non-spore-forming bacilli
3. YST - yeasts and yeast-like organisms

BCL - Gram-positive spore-forming bacilli[(10)].

Preparation 10% of TCA solution

Ten percent of TCA was prepared by dissolving 10 g of TCA powder in 100 ml of distil water at room temperature.

Precipitation of Whey protein

Whey protein of Iraqi Kurdistani local yogurt was precipitated by centrifugation using 3000 rpm for 10 minute, and then 3 ml of TCA solution was added to 10 ml of curd whey.

The protein precipitation was achieved after 24 hr then it was separated from the supernatant.

Preservation and maintenance of bacterial isolates: The bacterial isolates were preserved on nutrient agar at 37°C. The isolates were maintained monthly by culturing on new culture media. Nutrient broth supplemented with 15% glycerol was used for long preservation and the isolates were maintained frozen for several months (long term maintenance) (Su, S.C., et al. (1996), Collee *et al*., 1996).

III RESULTS AND DISCUSSION

Table (3-1) shows the isolated bacteria from oral Infection patients.

Table 1: Diagnostic test to isolate bacteria

No.	**Test**	***Proteus mirabilis***
1	Gram-stain	-

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2	Oxidase test	-
3	Catalase test	+
4	Microscope	Rod

(-): no growth

(+): growth

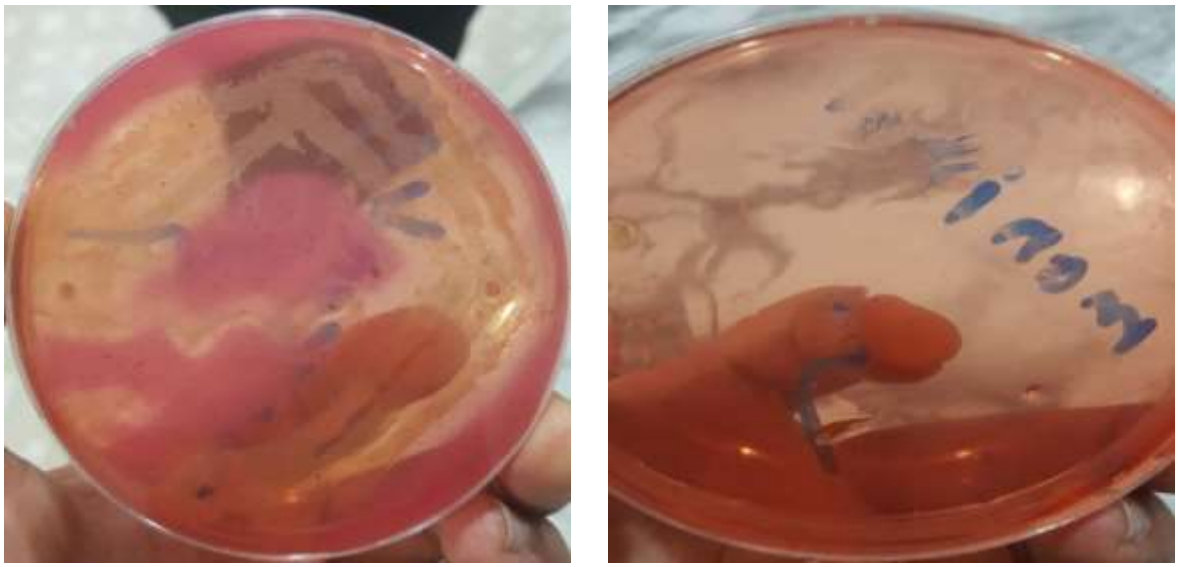

Figure 3: Shows Macconkey Agar on Left While Manital Agar on Right

A physiological test of the separated protein was investigated as antimicrobial agent against the isolated bacteria. Figure (3-2) shows precipitation of whey protein.

Figure 4: shows precipitation of protein

100 µl Volume of distilled water was added to 0.151 gm (represent g/ml concentration) of the separated whey protein were added to the isolated bacteria with a pinch off in the center. Figure (3-2) shows the inhibitory effect on bacteria growth. The result reflects a significant inhibitory effect on bacteria growth.

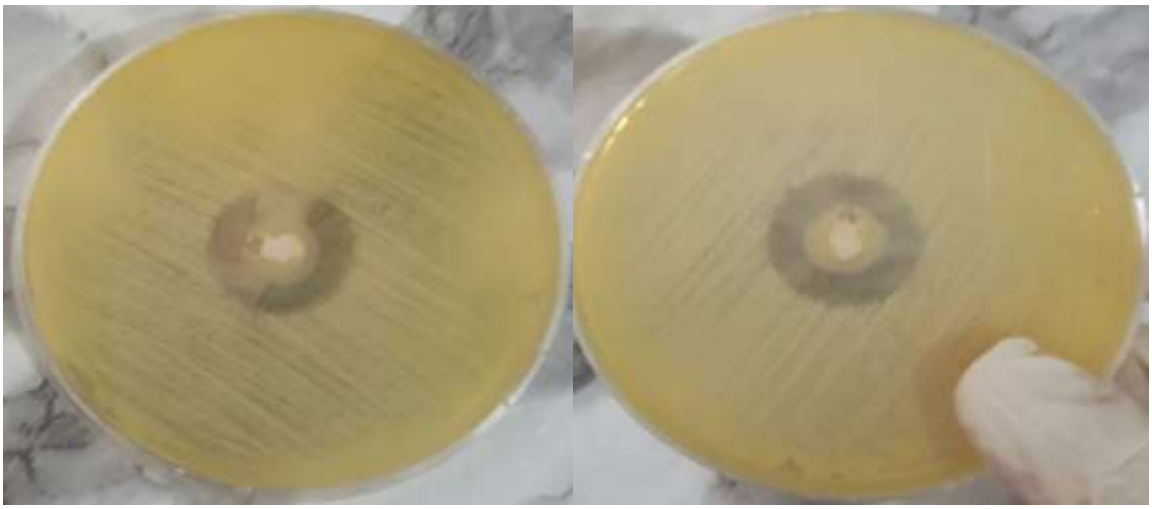

Figure 5: Antibacterial activity of natural proteins on Proteus mirabilis bacteria

The result of this study indicates that the bacteria strain was highly sensitive for whey protein.

The sensitivity of proteus species against the isolated protein obviously seen thus it was suggested to be act as a treatment for oral diseases, ***but*** it required comprehensive trial using volunteers and a specific identification of the isolated protein. Such suggestion has been improved by (Robin A McGregor 2013, Poppitt etal, 2013).

The diversity of whey proteins make it strong inhibitor to pathogen. Research was proved that the major proteins found in dairy whey are β-lactoglobulin and α-lactalbumin which make up 80% of the proteins, as well as a small amount of immunoglobulins, bovine serum albumin and α-caseins (Shawna Marie Simpson, 2012)., as shown in below table:

Table 2: Shows major components of dairy whey

No.	**Protein**	**Concentration (g/L)**
1	β-Lactoglobulin (monomer)	2.7
2	α-Lactalbumin	1.2
3	Immunoglobulin	0.65
4	Bovine serum albumin	0.4
5	Lactoferrin	0.1
6	Lactoperoxidase	0.02

IV CONCLUSION

From the results obtained in this study:

1. One species of bacteria were isolated from oral infection of patient is *Proteus mirabilis.*
2. It was found that the isolated protein whey have strong inhibitory effect on *Proteus mirabilis*.
3. Depending on the above hypothesis, the authors suggested that this protein can be used after purification using column chromatography as a treatment for oral infection.

REFERENCES

1. Guentzel MN (1996). Baron S; et al., eds. Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter, and Proteus. In: Barron's Medical Microbiology (4th ed.). Univ of Texas Medical Branch.
2. Bacteria of the species *Proteus mirabilis* are widely distributed in soil and water in the natural environment. In humans, *Proteus* is found as part of the normal flora of the gut....from BioMedHTC
3. *Proteus mirabilis* and Urinary Tract Infection.
4. O'hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. Clin Microbiol Rev. 2000;13(4):534-46.
5. *Gonzalez, Gus; et al. "Proteus Infections Medication". Medscape. Retrieved 30 October 2015.*
6. Marshall, K (2004). "Therapeutic applications of whey protein". *Alternative Medicine Review.* **9** (2): 136–156.
7. Jay R. Hoffman & Michael J. Falvo (2004). "Protein - Which is best?" . *Journal of Sports Science and Medicine* (3): 118–130.
8. *Luhovyy BL, Akhavan T, Anderson GH (2007). "Whey proteins in the regulation of food intake and satiety". Journal of the American College of Nutrition.* ***26*** *(6): 704S–712S.*
9. Haug A, Høstmark AT, Harstad OM, A; Høstmark, AT; Harstad, OM (2007). "Bovine milk in human nutrition – a review". *Lipids Health Dis*. **6**: 25.
10. David H. Pincus. Microbial Identification using the Biomerieux Vitek 2 system, USA (WWW.pda.org/bookstore).
11. Atlas RA. Handbook of Microbiological Media. CRC Press, Ann Arbor. 1993.
12. McFaddin,J.F. (2000) Biochemical test for identification of medical Bacteria.3rd edition.Lippincott Williams & Wilkins,Co. London. 1
13. Rasheed H.R, , Alwan S.S, , Salman D 2017, "Detection of aminoglycoside resistance gene *aph(2")Ic* in" clinical isolates of *Enterococcus faecalis* Volume: 13, Issue: 3, Part: 3 : 255-264
14. Chelikani P, Fita I, Loewen PC. 2004, 'Diversity of structures and properties among catalases. Cell Mol Life Sci. 61(2):192-208.
15. Boon EM, Downs A, Marcey D. 2007, "Catalase: H_2O_2 Oxidoreductase. Catalase structural Tutorial Text, pp 02-11.

16. Poppitt Sd, Strike CM, McArdle BH, McGill A, 2013, " Evidence of enhanced serum amino acid profile but not appetite suppression by dietary glycomacropeptide (GMP): a comparison of dairy whey proteins". J Am Coll Nutr, 32(3):177-86.
17. Su, S.C., et al. (1996). Temperature variations in upright mechanical freezers. Cancer Epidemiol Biomarkers 5(2):139-40.
18. Finegold S.M, and Martin W.J, 1982 "Diagnostic Microbiology," 6th Edition, C.V. Mosby Co. St. Louis, Toronto, London.
19. Etablering af et globalt salmonella-overvågningsnetværk: WHO global salm-surv.
20. Wegener, Henrik Caspar ; Petersen, A. ; Evans, M. C. ; Hendriksen, Rene S. ; Wong, D. M. A. Lo Fo ; Aarestrup, Frank Møller in: Alimenta, vol: 2, pages: 10-15, 2002
21. Shawna Marie Simpson," Fate of pathogenic organisms in a whey beverage treated with high pressure processing", 2012, An MSC, Thesis, Iowa State University Capstones, USA.