The inhibitory role of effective microorganisms on the growth of pathogenic bacteria

M.A. Hamad[1], S.A. Hussein[2], E.N. Mahmmoud[3] and A.M. Al-Aalim[4]

Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq,
[1] mahmah1073@gmail.com, [2] sabaabdulraheem9@gmail.com, [3] ebtehalnm22@gmail.com, [4] ammarmahmmod@yahoo.com

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Abstract

This study was conducted to evaluate the efficacy of Effective Microorganisms (EM1®) for inhibiting the growth of some pathogenic bacteria *Staphylococcus aureus* and *E. coli* were used in this study and isolated from pathological conditions. These bacteria were diagnosed in laboratory of microbiology, College of Veterinary Medicine, University of Mosul. The colonies that taken from blood agar were 5-7 and cultured in the nutrient broth and incubated at 37 ºC for 24 hours. Bacterial growth was calibrated with the second tube of the McFarland tubes 0.5%. Several concentrations of EM product were prepared 1, 0.5, 0.25 and 0.125%. Decimal dilutions were done for each concentration of EM product with bacterial suspension, except control group was done for bacterial suspension with nutrient broth. The bacterial count was done on nutrient agar, milk agar and EMB agar. The results of this study showed that the product of EM1® within concentrations 0.5-1% was highly efficient in inhibiting the growth of pathogenic bacteria under study. The bacterial count of both *S. aureus* and *E. coli* was $54x10^7$ and $52x10^7$ CFU/ ml respectively at 1% EM1®, and $67x10^7$ and $86x10^7$ CFU/ ml respectively at 0.5%, while the counting of the control group was $42x10^9$ and $67x10^9$ CFU/ ml respectively. This study concluded that EM1® at low concentrations have a clear role in inhibiting the growth of pathogenic bacteria, particularly *S. aureus* and *E. coli*.

***Keywords*:** Bacterial count, *S. aureus*, *E. coli*
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الدور التثبيطي لمنتج المتعضيات الفعالة على نمو الجراثيم المرضية

محمد علي حمد، صبا عبد الرحيم حسين، ابتهال نوفل محمود و عمار محمود العالم

فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

أجريت هذه الدراسة لتقيم كفاءة منتج المتعضيات الفعالة على تثبيط نمو الجراثيم المرضية، استخدمت في هذه الدراسة عزلتين جرثومية وهما المكورات العنقودية والإيشريكيا القولونية معزولتين من حالات مرضية ومشخصة في مختبر الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل. أخذت ٥-٧ مستعمرات نامية على أكار الدم وزرعت في المرق المغذي وحضنت بدرجة حرارة ٣٧ ºم لمدة ٢٤ ساعة. تم معايرة النمو مع الأنبوب الثاني من مجموعة أنابيب ماكفرلاند ٠,٥%. حضرت عدة تراكيز من منتج المتعضيات الفعالة ١، ٠,٥، ٠,٢٥ و ٠,١٢٥%. أجريت التخافيف العشرية لكل تركيز من التراكيز السابقة مع المعلق الجرثومي، باستثناء مجموعة السيطرة حيث أجريت التخافيف العشرية مع المرق المغذي. اجري العد الجرثومي على ثلاثة أوساط هي الأكار المغذي، وأكار الحليب، ووسط الايوزين المثيلين الزارق. أظهرت نتائج الدراسة إن منتج المتعضيات الفعالة ضمن التراكيز ٠,٥ - ١% كان ذو كفاءة عالية في تثبيط نمو الجراثيم المرضية قيد الدراسة وكان العد الجرثومي للنوعين المكورات العنقودية والإيشريكيا القولونية هو ٥٤×١٠^٧ و ٥٢×١٠^٧ وحدة تكوين مستعمرة/مل على التوالي عند التركيز ١% وفي تركيز ٠,٥% كان العد الجرثومي للنوعين ٦٧×١٠^٧ و ٨٦×١٠^٧ وحدة تكوين مستعمرة/مل على التوالي مقارنة بمجموعة السيطرة ٤٢×١٠^٩ و ٦٧×١٠^٩ وحدة تكوين مستعمرة/مل على التوالي. نستنتج من هذه

الدراسة أن المتعضيات الفعالة وبتراكيز قليلة لها دور واضح في تثبيط نمو الجراثيم المرضية عموماً وبشكل خاص المكورات العنقودية الذهبية والإيشريكيا القولونية.

Introduction

The population explosion that has taken place in the world in recent decades has led to an increase in the demand for plant and animal protein sources. As a result, various means have been used to develop and increase the production of food, including genetic selection methods for fast and high-yield assets (1). The high production of animal protein in short of time led to exposure to stress factors, which were the main cause of increased susceptibility to diseases and spread of their various causes, which include viruses, bacteria, fungi and fungal toxins, parasites, and protozoa in addition to chemical elements and toxins (2). The occurrence and spread of diseases led to intensive use of antibiotics and similar treatments, as well as the preventive use of these antibiotics and chemicals in feed. Treatment with antibiotics has many disadvantages, including transmission to humans by eating animal products (3,4), in addition to the spread of bacterial resistance factors for antibiotics (5-6). As a result, the world began to look for alternatives to antibiotics, like effective microorganisms (7), which was invented by the Japanese scientist Teruo Higa in the 1980s (8). EM are a mixture of beneficial microorganisms composed of photosynthetic bacteria (phototrophic bacteria) and their source of soil, lactic acid bacteria *Lactobacilli*, yeast used in the preparation of bread and beer, *Actinomycetes*, fermenting fungi *Aspergilli* and *Penicilliums*. Effective microorganisms are a mixture of microorganisms that are harmless, non-pathogenic, non-genetically modified, and finally chemically non processed (9). The technology of EM has been used in wide zones, it has been used in the poultry industry (10-12), improved agricultural crops (13-15), sewage treatment (16,17), increased production efficiency and removal of toxic substances especially in fishes farms (18), disposal of food waste and modified it for composting (19,20), and improvement of forest soils (21).

Therefore, the aim of this study was to evaluate the efficacy of this biological product as an inhibitor for the growth of pathogenic bacteria.

Materials and methods

Bacterial isolates

Bacterial isolates that used in this study were isolated from pathological conditions. *The Staphylococcus aureus* (Gram positive) was isolated from mastitis in cows, while *E. coli* (Gram negative) was isolated from chronic respiratory disease (CRD) in broiler. These isolates were diagnosed in laboratory of microbiology (Department of Microbiology, College of Veterinary Medicine, University of Mosul) according to the conventional techniques (22-23).

Culture Media

The Brain heart infusion broth (BHI) and Blood agar (BA) were used for cultivation of bacterial isolates, Nutrient broth (NB) for decimal dilutions, nutrient agar; Milk agar and Eosin Methylene Blue medium (EMB for *E. coli* only) for bacterial count.

Bacterial suspension

The two bacterial species were cultivated in BHI and incubated at 37 ºC for 24h, then cultured on BA in same conditions. Five to seven growing colonies from each bacterial species were taken and cultured in NB in same previous conditions, followed by calibrated of growth with the second tube of the McFarland tubes 0.5% (24).

Effective Microorganisms (EM1®)

Supplied by Al-Annam company for natural agriculture, Tortuous-Syria under the supervision of EMRO Japanese institute, Okinawa, Japan. Four concentrations of EM1® were prepared: i) Concentration 1%: (1 ml of EM+ 99 ml NB), ii) Concentration 0.5%:(5 ml of EM 1%+ 5 ml NB), iii) Concentration 0.25%:(2.5 ml of EM 1%+ 7.5 ml NB) and iv) Concentration 0.125%:(1.25 ml of EM 1%+ 8.75 ml NB).

Method of testing

Ten groups of ten test tubes containing 9 ml of NB were prepared. Also, ten tubes were used and added to each of them 1 ml of bacterial suspension [5 tubes for *S. aureus* suspension (one of them for control) and 5 tubes for *E. coli* suspension (also one of them for control)]. Then added 1 ml of each one of the EM previous concentrations to one of the 8 tubes, while added 1 ml of NB to each one of the control tubes (2 tubes). Then decimal dilutions were done for all tubes as following (24): Tube 1: contained 1 ml bacterial suspension + 1 ml (EM1®1%)**.** Then transported 1 ml to the first tube of one of the ten groups of test tubes (containing 9 ml of NB) which mean that this tube is 10^{-1} concentration, and completed the remaining dilutions. Tube 2: 1 ml bacterial suspension + 1 ml (EM1® 0.5%)**,** and the dilutions carried out as previous. Tube 3: 1 ml bacterial suspension + 1 ml (EM1® 0.25%), and the dilutions carried out as previous. Tube 4: 1 ml bacterial suspension + 1 ml (EM1® 0.125%), and the dilutions carried out as previous. Tube 5: 1 ml bacterial suspension + 1 ml NB, and the dilutions carried out as previous.

The same procedure was done for the second bacterial suspension. After completed the all dilutions, 0.1 ml from the 5^{th}-8^{th} dilution were spread on 3 agar plates (Nutrient agar and Milk agar for *S. aureus*, Nutrient agar and EMB for *E. coli*) for each dilution. The plates were remained in room temperature for 15 minutes for absorption, then all plates were incubated at 37 ºC for 24 hours. The bacterial count was calculated for each dilution as in below equation (25): Total Bacterial Count (TBC): medium of the counting of 3 plates x 10 x inverted dilution.

Results

Bacterial counting of control tubes

The current study showed no possibility of bacterial counting in the 5^{th}-7^{th} dilutions for control tubes. The counting was done in the 8^{th} dilution and the total count for *S. aureus* and *E. coli* was [42X 10^9($42X10X10^8$) CFU/ml, 67X 10^9($67X10X10^8$) CFU/ml, respectively (Table 1, Figure 1).

Bacterial counting of treated tubes (EM-Bacterial suspension tubes)

The results of the bacterial count were not reckoning on the nutrient agar of both types of bacteria, because the colonies of the tested bacteria could not be distinguished from the growing bacterial colonies of the content of EM product. While the bacterial counting on milk agar and EMB were performed as follows: The bacterial count in concentration 1%: The results showed that the bacterial count in the fifth dilution was not possible because of the heavy growth of the colonies. The sixth dilution was the ideal dilution for counting of both types of bacteria. So, the total number of *S. aureus* bacteria was $54x10^7$($54x10x10^6$) CFU/ml, while the total number of *E. coli* bacteria was $52x10^7$($52x10x10^6$) CFU/ml (Table 1, Figure 2). The bacterial count in concentration 0.5%: The Sixth dilution was the quantifiable dilution of both types of bacteria. The total number of *S. aureus* was $67x10^7$ ($67x10x10^6$) CFU/ ml, while the total number of *E. coli* was $86x10^7$ ($86x10x10^6$) CFU/ ml (Table 1, Figure 3). The bacterial count in concentration 0.25%: The bacterial count was done in the seventh dilutions for both bacterial types. In which the total count of *S. aureus* was $47x10^8$ ($47x10x10^7$) CFU/ml, while the total count of E. coli was $92x10^8$ ($92x10x10^7$) CFU/ml (Table 1, Figure 4). The bacterial count in concentration 0.125%: The total count for both bacterial types for this concentration was approximated to the control group, where the count was evident in the eighth dilution. So, the total counts of *S. aureus* and *E. coli* were [$23x10^9$ ($23x10x10^8$) CFU/ml, $17x10^9$ ($17x10x10^8$) CFU/ml] respectively (Table 1, Figure 5).

Table 1: The Bacterial count for EM Pathogenic bacteria in different concentrations

Bacterial Type	Conc.	10^{-5}	10^{-6}	10^{-7}	10^{-8}
S. aureus + EM	1%	---	$54x10^7$		
	0.5%	---	$67x10^7$		
	0.25%	---	---	$47x10^8$	
	0.125%	---	---	---	$23x10^9$
E. coli + *EM*	1%	---	$52x10^7$		
	0.5%	---	$86x10^7$		
	0.25%	---	---	$92x10^8$	
	0.125%	---	---	---	$17x10^9$
S. aureus without EM	---	---	---	---	$42x10^9$
E. coli without EM	---	---	---	---	$67x10^9$

--- Means that it is not possible to count in this dilution.

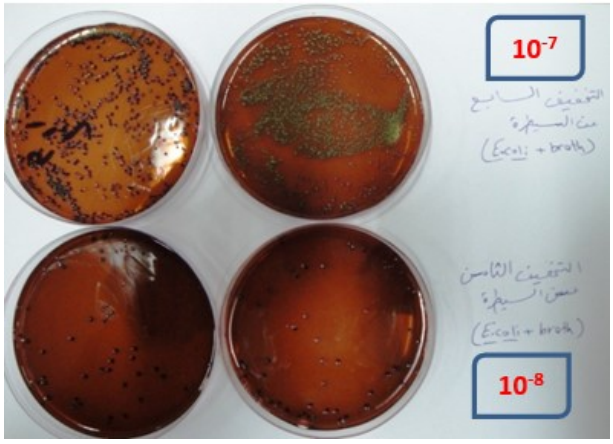

Figure 1: Bacterial count for *E. coli* in control group on EMB, the count was calculated in the 8^{th} dilution.

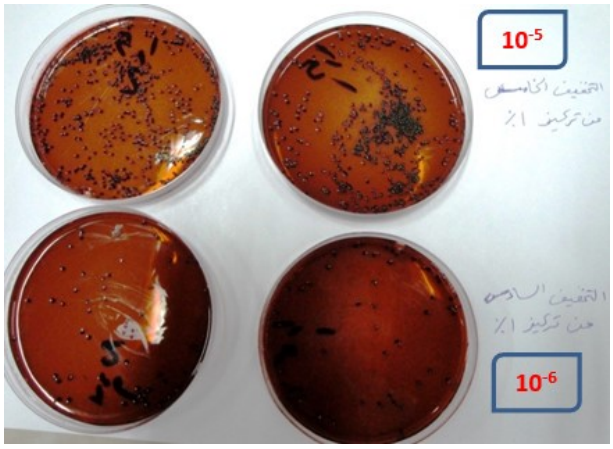

Figure 2: Bacterial count for *E. coli* in 1% concentration on EMB, the count was calculated in the 6^{th} dilution.

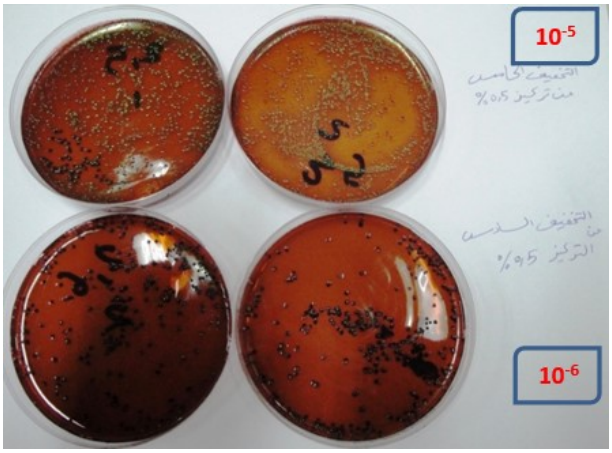

Figure 3: Bacterial count for *E. coli* in 0.5% concentration on EMB, the count was calculated in the 6th dilution.

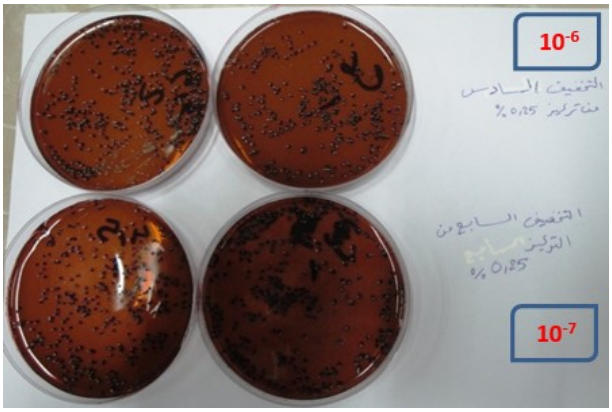

Figure 4: Bacterial count for E. coli in 0.25% concentration on EMB, the count was calculated in the 7th dilution.

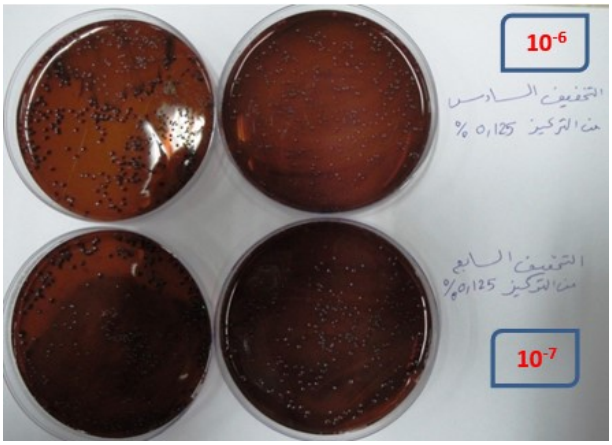

Figure 5: Bacterial count for *E. coli* in 0.125% concentration on EMB, the count was not possible in the 7th dilution.

Discussion

The effective microorganisms have been used in many fields including agriculture and crop improvement, animal production especially fishes, poultry industry and increasing of food conversion, and wastewater treatment (10-18). As well as their using in soil dredging and improvement (26), and there are local studies have used effective micro-organisms to improve weight; average consumption of the feed and increase body immunity of broiler (12, 27). So that the present study aimed to evaluate the efficacy of this product in inhibiting or reducing the growth of pathogenic bacteria. The results showed that the use of EM in low concentration 1% had the best effect in reducing the growth of pathogenic tested bacteria *S. aureus* and *E. coli*, where the bacterial count was done in the sixth dilution compared with the control group in which the bacterial count was performed at the eighth dilution. This indicates a high reduction in the number of pathogenic bacteria treated with a concentration of 1% up to 10^2 CFU / ml. The product using at 0.5% concentration had a close effect to concentration of 1%, whereas the bacterial count was also possible in the sixth dilution. This finding inferred that the possibility of using the product within the concentration 1 - 0.5% in curbing the growth of pathogenic bacteria. While the product using at less than 0.5% concentration had slight effect and did not meet the required level, which supports our reasoning about the use of the product within concentrations 1 - 0.5%.

The current results support the results of other studies, which also indicated the efficacy of this product in inhibiting the growth of pathogenic bacteria within the same concentrations. Where an Egyptian study (28) pointed that the use of this product and within the previously mentioned concentrations 1% had a significant effect on the growth of nine types of pathogenic bacteria, including *S. aureus* and *E. coli*, even they pointed to use of this product as a disinfectant and also the possibility of using it as a cleaner for sewage, while a study in Thailand (29) indicated that the EM did not have a significant effect in reducing the numbers of *Salmonella enterica* and *Campylobacter spp.* when used in the fields of broiler. The cause of different results between Thailand study and both of the current study and the Egyptian study, may be due to the difference in the bacterial species that used in the experiments; where *Salmonella spp.* and *Campylobacter spp* were not used by current study and also by the Egyptian study. But what supports the results of the current study on the efficacy of the product are the results of study of Rahman *et al* (30), They were tested the product on four bacterial species; *S. aureus*, *Pasteurella* spp, *Salmonella* spp and *E. coli*, which proved highly efficient in inhibiting and reducing the growth of the four bacterial species respectively. Also, an expanded study (31) conducted at the University of Pretoria

confirmed that the use of this product has inhibited the growth of *Clostridium perfringens* and the absence of necrotic enteritis in broiler with increased metabolism, feed consumption and improved intestinal mucosa.

The results of our study by using the product within the approved concentrations were consistent with the results of a local study (27) which indicated that the use of the product in drinking water for broiler and within the same concentration 1% had a positive effect on the immune response to the vaccination against Newcastle, where the researcher proved a significant increase in the titer of antibodies to the virus of Newcastle disease, also another study (11) indicated that the use of this product and by the same concentration in chicken via water or feed increases the level of immune response and activates the immune system as there was a significant increase in levels of IgG and IgM antibodies in general. Our results also agreed with the results of another local study (12), which used the product to improve food conversion and weight gain, they also used the product at 1% and noted that the use of the product and within this concentration had significant results in improving food conversion and weight gain and increasing the length of the villi in the jejunum and increasing the number of goblet cells. Also, a recent study (17) indicated that the use of this product with a concentration of 1% may be useful in anaerobic fermentation for the disposal of organic matter and waste.

The main role of the effective microorganisms in inhibiting the growth of pathogenic bacteria inside the host body is not definitively determined, but it is thought to compete with pathogenic bacteria on food inside the host body (8, 9). In addition, its metabolic products are harmful to the growth of other bacteria, especially pathogens, due to contain *Lactobacilli* (Lactic acid bacteria) bacteria that produce lactic acid, which is considered a powerful sterilizer that inhibit the harmful bacteria (28, 32). It also maintains or supports bacterial balance in the intestines (33), and also stimulates the specific and non-specific immune system (11).

Conclusion

The current study concluded that the effective microorganisms at low concentrations have a clear role in growth inhibition of pathogenic bacteria in general and *S. aureus* and *E. coli* particularly, and we recommend using it extensively in the field of animal health, especially in poultry and ruminants.

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