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

M.A. Hamad¹, S.A. Hussein², E.N. Mahmmoud³ and A.M. Al-Aalim⁴

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

(Received May 3, 2019; Accepted July 3, 2019)

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 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 54x10⁷ and 52x10⁷ CFU/ml respectively at 1% EM1®, and 67x10⁷ and 86x10⁷ CFU/ml respectively at 0.5%, while the counting of the control group was 42x10⁹ and 67x10⁹ 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

Available online at http://www.vetmedmosul.com, © 2020, College of Veterinary Medicine, University of Mosul. This is an open access article under the CC BY 4.0 license (http://creativecommons.org/licenses/by/4.0/).
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 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 fish 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 °C for 24h, then 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 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 all dilutions, 0.1 ml from the 5th-8th 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°C 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 5th-7th dilutions for control tubes. The counting was done in the 8th dilution and the total count for S. aureus and E. coli was [42X 107(42X10X108) CFU/ml, 67X 107(67X10X108)] 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 54x107(54x10X108) CFU/ml, while the total number of E. coli bacteria was 52x107(52x10X108) 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 67x107 (67x10X108) CFU/ml, while the total number of E. coli was 86x107 (86x10X108) 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 47x108 (47x10X109) CFU/ml, while the total count of E. coli was 92x108 (92x10X109) 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 [23x108 (23x10X109) CFU/ml, 17x109 (17x10X109) 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  | 0.5%  | ---  | 52x10^7 |
|               | 0.25% | ---  | 52x10^7 |
|               | 0.125%| ---  | 17x10^6 |
| S. aureus     |       | ---  | 42x10^9 |
| without EM    |       | ---  | 17x10^6 |
| E. coli       |       | ---  | 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 8th dilution.

Figure 2: Bacterial count for E. coli in 1% concentration on EMB, the count was calculated in the 6th 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 *Lactohacilli* (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.

**Acknowledgements**

The authors very grateful to chair of Department of Microbiology and dean of College of Veterinary Medicine for their efforts and supporting us to finish the current research.

**References**

1. Spencer JFT, Ragout de Spencer AL. Public health microbiology: Methods and Protocols. New York: Humana Press Inc; 2004. 123-124p. ISBN-13: 978-1617373718
2. Cameron S, Crear S, Geue A,Cloud- Guest A. Foodborne disease Towards reducing foodborne illness in Australia. From the Foodborne Disease Working Party for the Communicable Diseases Network Australia and New Zealand. Technical Report Series No. 2: Commonwealth of Australia; 1997. ISBN 0 642 367434. https://www1.health.gov.au/internet/main/publishing.nsf/Content/cda-cdtech-foodborne.htm?SFILE/foodborne.pdf
3. Kelly L, Smith DL, Snary EL, Johnson JA, Harris AD, Wooldrige M, Morris JG. Animal growth promoters: To ban or not to ban? a risk assessment approach. Int J Antimicrobial Agents. 2004;24:205-212. DOI:10.1016/j.ijantimicag.2004.04.007
4. Huang TM, Lin TL, Wu CC. Antimicrobial susceptibility and resistance of chicken *Escherichia coli*, *Salmonella* spp., and *Pasteurella multocida* isolates. Avian Dis. 2009;53:89-93. DOI: 10.1637/8268-021608-Reg.1
5. Levy SB. The challenge of antibiotic resistance. New York: Scientific American; 1998. 46-53 p. DOI: 10.1083/scientificamerican0398-46
6. WU JR, Shieh HK, Shien JH, Gong SB, Chang PC. Molecular characterization of plasmids with antimicrobial resistant genes in avian isolates of *Pasteurella multocida*. Avian Dis. 2003;47:1384-1392. DOI: 10.1637/7035
7. Hegazy ESR. Effective microorganisms as an alternative to antibiotics [MSc thesis]. Cairo: Department of Biochemistry, Faculty of Agriculture, Al-Azhar University, Egypt; 2013. 1-126. https://inis.iaea.org/collection/NCLCollectionStore/_Public/46/066/46066325.pdf
8. Higa T. Kyusei nature farming and environmental management through effective microorganisms: The past, present and future [Internet]. 2012. http://www.infr.e.or.jp/english/KNF_Data_Base_Web/7th__Conf_KP_2.html
9. Condor AF, Gonzalez P, Lakre C. Effective microorganisms: myth or reality? Peruvian J Biol. 2007;14:315-319. http://agrionorge.se/wp-content/uploads/2019/06/2013-Effective-microorganisms-and-their-influence-on-vegetable-product...pdf
10. Wondmeneh E, Getachew T, Dessie T. Effect of effective microorganisms (EM®) on the growth parameters of fayoumi and horro chicken. Inter J Poult Sci. 2011;10(3):185-188. DOI: 10.7415/ijps.2011.185.188
11. Esatu W, Terefe G, Dessie T. Immunomodulatory effect of effective microorganisms (EM) in chickens. Res J Immunol. 2012;5(1):17-23. DOI: 10.3923/rji.2012.17.23
12. Jwher DM, Abd SK, Mohammad AG. The study of using effective microorganisms (EM) on health and performance of broiler chicks. Iraqi J Vet Sci. 2013;27(2):73-78. DOI: 10.33899/ijvs.2013.82784
13. Javaid A. Beneficial microorganisms for sustainable agriculture. Sus Agri Rev. 2010;4:347-369. DOI:10.1007/978-90-481-8741-6.12
14. Javaid A, Bajwa R. Field evaluation of effective microorganisms (EM) application for growth, nodulation, and nutrition of mungbean. Turkish J Agri Forest. 2011;35:443-452. DOI: 10.3906/tar-1001-599
15. Olle M, Williams HI. Effective microorganisms and their influence on vegetable production: A review. J Horticult Sci Biotechnol. 2011;88(4):380-386.DOI: 10.1080/14620316.2011.11512979
16. Namsivayam SKR, Narendrakumar G, Kumar JA. Evaluation of effective microorganism (EM) for treatment of domestic sewage. J Ex Sci. 2011;2(7):30-32. https://pdfs.semanticscholar.org/85b8/26988f2a6bd5804c1b90522290108f4f5221.pdf
17. Souza CF, Gates RS, Batista MD, Tinoco IFF. Effective microorganisms (EM) as biofeeders for anaerobic digestion. Eng Agricult. 2017;25(6):491-499. DOI: 10.13083/reveng.v25n6.687
18. Omar WA, Abdel Salam RG, Mahmoud HM. The use of effective microorganism (EM) as a probiotic on cultured Nile tilapia, oreochromisniloticus. Egyptian J Zool. 2017;67:67-90. DOI: 10.12816/0037795
19. Kim HN, Yim B, Kim ST. Effect of reducing the odor of food wastes using effective microorganism (EM). J Korean Soc Environ Eng. 2016;38(4):162-168.DOI:10.4491/KSEE.2016.38.4.162
20. AbMuttalib SA, Ismail SNS, Praveena SM. Application of effective microorganism (EM) in food waste composting: A review. Asia Pacific Environ Occu Health J. 2016;2(2):37- 47. http://www.apeohj.org/apeohj/ojs/index.php/apeohj/index
21. Bzdyk RM, Olchowik J, Studnicki M, Oszako T, Sikora K, Szmidla H, Hilszczanska D. The impact of effective microorganisms (EM) and organic and mineral fertilizers on the growth and mycorrhizal colonization of Fagus sylvatica and Quercus robur seedlings in a Bare-Root nursery experiment. Forests. 2018;9:597.DOI: 10.3390/f9100597.
22. Rhaymah MSH, Al-Obaidi QT, Hamad MA, Altalby MA. Mastitis in mare: case report. Iraqi J Vet Sci. 2018; 32(1):109-111. DOI: 10.33899/ivs.2018.153831.
23. Sadeq JN, Fahed KhH, Hassan HJ. Detection of Escherichia coli hlyA gene and Staphylococcus aureus sea gene in raw milk of buffaloes using RT-PCR technique in AL-Qadisiyah province. Iraqi J Vet Sci. 2018; 32(1):87-91. DOI: 10.33899/ivs.2018.153815.
24. Cappuccino JG, Welsh C. Microbiology a laboratory manual. 11th ed. England: Pearson Education Limited; 2018. 123 p. https://www.pearson.com/store/p/microbiology-a-laboratory-manual/P100002136161
25. Tille PM, Bailey S. Diagnostic microbiology.14th ed. New York: Elsevier Inc.; 2017. 217 p. https://evolve.elsevier.com/cs/product/9780323548207?role=student
26. Yusof NZ, Samsuddin NS, Hanif MF, Osman SB. Peat soils stabilization using effective microorganisms (EM). IOP Conf. Series: Earth and Environmental Science. 2018;140:1-8. doi :10.1088/1755-1315/140/1/012088
27. Jhner DM. Effects of feeding effective microorganisms on blood levels of antibodies to Newcastle disease virus vaccine and trace element in broiler chicks. Assiat Vet Med J. 2014;6(14):38-44. DOI:10.33899/ivs.2013.82784
28. Safwat SM, Rozaiq E. Growth inhibition of various pathogenic microorganisms using effective microorganisms (EM). Inter J Res Eng. 2018;4(12):283-286. DOI:10.21276/ijire.2017.4.12.2
29. Nuengjannong C, Luangtongkum T. Effects of effective microorganisms on growth performances, ammonia reduction, hematological changes and shedding of Salmonella enterica and Campylobacter spp. in broiler. Thai J Vet Med. 2014;44(1):15-22. http://www.thaiscience.info/Journals/Article/TJVM/10961874.pdf
30. Rahman S, Siddique M, Hussain T, Hussai S, Ansar M. An in vitro antibacterial activity of different effective microorganism cultures against pathogenic species. Pakistan J Biol Sci. 1999,2(1):214-216 DOI: 10.3923/pjbs.1999.214.216
31. Bothoko TD. Performance of Clostridium perfringens- challenged broilers inoculated with effective microorganisms [MSc Thesis]. South Africa: Department of Animal and Wildlife Sciences, University of Pretoria; 2009. 1-65. https://pdfs.semanticscholar.org/9d37/2bb6ba7ec1979eb812ed371a0f63e48f5a6.pdf
32. Vicente, JL, Avina L, Torres-Rodriguez A, Hargis B, Tellez G. Effect of a Lactobacillus sp based probiotic culture product on broiler chick’s performance under commercial conditions. Int J Poult Sci. 2007;6(3):154-156. DOI: 10.3923/ijps.2007.154.156
33. Panda AK, Reddy MR, Rao SVR, Raju MV, Prahara JK. Growth, carcase characteristics, immunocompetence and response to Escherichia coli of broilers fed diets with various levels of probiotic. Archiv Fur Geflugelkunde. 2000;64:152-156. https://www.european-poultry-science.com/artikel.dll/2000-64-152-156_NDK3MDk3MQ.PDF.