

Two new media earthworm powder agar for enumeration of pathogenic bacteria and to identify *Serratia marcescens*

Manal Kahlid Mohammad,

Collage of science, Department of Biology , mustansiriyah university /Iraq

Abstract :

The study aimed to prepare new enriched media for enumeration and identification of some human pathogen .Two media were prepared earth worm agar medium and enriched earth worm media .The first medium was inoculated with some human pathogenic bacteria isolated from Al-Kindy Educational Hospital ,Baghdad, Iraq including *Staphylococcus aureus* ,*Acinetobacter baumannii* ,*Klebsiella pneumonia* ,*Serratia marcescens* ,*Escherichia coli*,*Salmonella typhi* and *Pseudomonas aeruginosa* .While enriched earth worm medium was inoculated with *Serratia marcescens* isolate only ,after incubation at 37C⁰ for 48hrs results showed that all pathogens were grown successfully on earth worm agar medium but it was noticed that *Serratia marcescens* failed to produce its red pigment (prodigiosin) while *Serratia marcescens* produce the pigment when inoculated on modified earth worm agar .It was concluded that earth worm agar medium considered a useful medium for enumeration to some human pathogens and modified earth worm agar medium is considered a useful medium for rapid identification and enumeration of *Serratia marcescens*.

Key Words : Earth worm powder, *Serratia marcescens*, prodigiosin, modified

INTRODUCTION :

Pathogenic bacteria causes diseases in health individuals and immunocompromised patients and have wide range for antibiotics resistance because of the presence of R-factor ,which are a type of plasmid that carry one or more genes that encode resistance (1).While most species of bacteria are harmless can contribute to many worldwide disease including tuberculosis , pneumonia, and food born illnesses . *Serratia marcescens* secreted serratiopeptidase used in medical for treatment pain and inflammation like arthritis , sinusitis, surgery, bronchitis and another disease (2,3,4) .Also prodigiosin is the red pigment produce by many strains of the bacterium by regulated gene product ,its wide range of biological activities as antimalarial , antifungal , immunosuppressant and antibiotic agent ,its also require a special condition to produce the pigment (5,6) Earthworm return to Annelida phylum and to class Clitellata they play a major role in Environment (7) . They support water infiltration maintain soil structure and contain many elements like nitrogen ,potassium,calcium,iron ,magnesium and zinc ,also regulate of nutrient to assimilated of plant(8).

MATERIALS AND METHODS :

Preparation of earthworm powder : Earthworms orchard and house garden been collected from different region of Diyala by digging soil washing to remove dirt from the body surface of earthworm were soaked in distilled water for 6-8 hours to allow the soil in its tract to be removed ,later earthworm have been washed with distilled water and collected in Petri dish then were dried in an-oven for 24h. at 55C⁰ after drying the earth worm crushed into powder (9,10)

Earthworms powder agar : agar-agar (20g /l) (Difco) was Prepared autoclave at 121C for 15 min and cool to(45-55)C⁰ then earth worm powder (50g/l) was added the pH was adjusted to 7.0 mixed well and dispended into sterile petri dishes.(1)

Microbial isolates: Seven isolates *Staphylococcus aureus* ,*Acinetobacter baumannii* ,*Klebsiella pneumonia* ,*Serratia marcescens* ,*Escherichia coli*,*Salmonella typhi* and *Pseudomonas aeruginosa* were belonged to samples originated from clinical specimens that had been submitted to the bacteriology laboratory in Al-Kindy Educational Hospital ,Baghdad, Iraq. All isolates identified using the Vitek 2 system (Biomerieux) .

Modified earth worm agar : Was prepared by adding soy bean meal (65g/l) ,NaCl (5.0 g/l),and agar-agar (20g /l) after autoclaving at 121C⁰ for 15 min and cooling to (45-55) C⁰ then earth worm powder (50g/l) was added the pH was adjusted to 7.0 the prepared media was dispended into steril petri dishes.(1,2)

RESULTS AND DISCUSSION :

As shown in(fig 1) all isolates shows a heavy growth on Earthworm powder agar incubated in 37C at 24 hours because *its contain many elements like nitrogen ,potassium , calcium, iron ,magnesium and zinc as enrichment media that use of certain growth media to favor the growth of a particular microorganism over others, this allows for the detection and identification of microorganisms with a variety of nutritional needs Fig(1) (7,8) . Earthworm powder agar considered as antimicrobial activity in high concentration while in low concentration become growth media (10). As shown in(fig 2,3)Serratia marcescens grow on Earthworm powder agar without producing its red pigment (prodigiosin) because the production of pigment required a special condition including the presence of soy bean meal, NaCl , other salts , PH ,temperature and other condition (2,3). so when modified Earthworm powder agar, Serratia marcescens produced prodigiosin(PG) in the media . prodigiosin have a biological activity as antioxidants and anticancer agents ,its produced by bacteria gram negative and positive bacteria as sources of pharmaceutically important, and bioactive compound.(5,6).*



Fig1: Pathogenic bacteria growth on Earthworm powder agar in 37C⁰ at 24hours



Fig2 : *Serratia marcescens* growth on Earthworm powder agar in 37C⁰ at 24hours.without produce a pigment



Fig3 : *Serratia marcescens* growth on modified Earthworm powder agar in 37C⁰ at 24hours.produce apigment

REFERENCES:

1. Hamzia,A.A. 2014 . Two new media apple leaves agar and eggplant leaves agar for identification of *Creptococcus newformance*: J. of Biology Agricultural and Healthcare, 4:126-131 .
2. Manal,K. 2016 .Production, partially purification and estimation of serratiopeptidase from *Serratia marcescens* : Al-Mustansiriyah Journal of Science,27: 33-35 .
3. Manal,K.M. 2015 .Effect of temperature and mutation on serratiopeptidase secreted from *Serratia marcescens*: J. of Genetic and Environment Resources Conservation ,3:35-37.
4. Chaudhari ,A ; Mali ,K.,2013 : Production characteristic and optimization of potent protease (*serratiopeptidase*) from *Serratia marcescens* E15.Int.Res.J.Pharm.App.Sci ;3(4):95-98.
5. Salih ,A. 2016 : Optimal condition measured for production of prodigiosin from Environmental research center *Serratia marcescens* bacteria isolated from different source ,(3):116-128.
6. Schlegel,Hanc G,1995 .General microbiology(7ed).Cambridge Univ.Press.pp.204-205.
7. Nyle C. Brady ; Ray R Weil 2009 .Element of the Nature and Properties of soil (3rd Edition).Prentice Hall.
8. Tuan ,N.,N. and et al ,2015 .Earthworm powder as an alternative protein source in diets for common carp (*Cyprinus carpiol*).47:2917-2927.
9. A.J.Bhorgin, andK.U.ma 2014 .Antimicrobial activity of Earthworm powder (*Lampito mauritii*).Int.J.Curr.Microbiol.App.Sci3(1):437-443.
10. Yegnanaryan ,R., P.P.Sethi,P.A..Rajhan,K.Pulandiran and Ismail,S.A. ,1987. Anti-inflammatory activity of total Earthworm extracts in rats .Indian J.Pharmacol.19:221-224.