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RESEARCH ARTICLE

THE ROLE OF MAGNESIUM, COPPER, ZINC AND FERRUM IN RECOVERY OF COLD-SHOCKED BACTERIA

[1,*]Gamer, M. E. and [2]Elsanosi, S. M.

[1]Blue Nile National Institute for Communicable Diseases, Gezira University, P.O. Box 101, UAE
[2]Faculty of Veterinary Medicine, University of Khartoum, P.O. Box 32 Khartoum North, Sudan

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ABSTRACT

This work was conducted to study the role of Mg, Cu, Fe and Zn, on the recovery of cold-shocked bacteria in different combinations. Isolation of bacteria contaminating the frozen foods was performed and a design of a proper medium that recover almost all injured cells was achieved. The studied bacteria were Escherichia coli, Salmonella spp. Staphylococcus aureus and Klebsiellapneumoniae. The organisms were isolated from frozen foods (Sausages, Hamburgers, Meat ball and Ice creams) and identified to the species level. The isolated bacteria were sub cultured in nutrient broth medium and incubated at 37˚C for 24 hours and ten-fold dilutions were prepared for each species of bacteria using normal saline as a diluent. The bacteria were shocked at -20˚C for one hour. The frozen bacteria were left to thaw at room temperature for 30min and from the last two end dilutions, 0.1ml was transferred to the surface of recovery media and incubated at 37˚C for 24 hours. Using Miles and Misra (1938) method was applied for counting the bacteria.

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INTRODUCTION

Food may contain a variety of bacteria such as *Salmonella, E. coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The existence of bacteria in food stuffs can affect the safety and quality of the product and may harm the consumer's health. The injured cells are those bacteria that are most difficult to notice and count even by up-to-date microbiological approaches. Therefore, suitable test methods to detect injured bacteria within foodstuffs should be advocated (Linda, 2001). Organisms and *microorganisms* require minute quantities of chemical elements (Goldhaber, 2003). Such chemicals were reported by Mertz (1998) were chromium, cobalt, copper, iodine, iron, manganese, molybdenum, nickel, magnesium, selenium, tungsten, vanadium, zinc and perhaps several other elements. The aims of this study were: Isolation of the bacteria incriminated in frozen food adulteration and study the effect of Mg, Cu, Fe and Zn in different combinations for the recovery of cold-shocked bacteria and improve a medium which could recover almost all cold-shocked cells.

Corresponding author: Gamer, M. E.
Blue Nile National Institute for Communicable Diseases,
Gezira University, P.O. Box 101, UAE.

MATERIALS AND METHODS

Recovery medium

Preparation: Nutrient agar was prepared according to Oxoid and different combinations of Mg, Cu, Fe and Zn were prepared before being dispensed onto Petri-dishes as 15-20 ml portions.

Trace elements concentrations: Copper (0.01g), zinc (0.01g), ferric (0.1g), and magnesium (0.05g).

RESULTS AND DISCUSSION

The biogas production increased by 26.6% within 11 days when Fe^{2+} concentration was 25mg/L with VS concentration of 50g/L. With the increase of Fe^{2+} concentration, the biogas production was improved significantly. The biogas production increased by 48.7%, 52.1% and 54.8% at Fe^{2+} concentration of 100, 250, 500mg/L (Demirel and Scherer, 2011). However, the biogas production was inhibited by 57.9% when Fe^{2+} concentration was as high as 5000mg/L. Here our result may substantiate that of Demirel and Scherer (2011) because low concentration of Fe^{+2} was found to be very useful in recovery of shocked cells but dissimilar to its high concentration which diminished the recovering process.

Table 1. Viable count of the four model bacteria before freezing, after freezing and after recovery

Bacteria	Before freezing	After freezing	Fe+Cu+Zn+Mg	Cu+ Zn+ Fe (10^{-5})	Cu (10^{-5}) +Fe (10^{-5})	Fe (10^{-4})	Cu+Fe (10^{-5})+Mg (5/100000)	Zn+ Fe (10^{-5})	Zn+ Fe (10^{-5}) + Mg (5/100000)
			CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml
E. coli	2.9×10^{9}	1.5×10^{6}	1.2×10^{13}	2×10^{13}	1.5×10^{14}	1.5×10^{13}	1×10^{14}	7×10^{13}	2.5×10^{14}
Salmonella spp.	3.5×10^{9}	1×10^{7}	3×10^{14}	2.5×10^{14}	6×10^{13}	1.8×10^{13}	2.8×10^{14}	2.5×10^{14}	2×10^{14}
K. pneumoniae	3.2×10^{9}	M	1.2×10^{13}	1×10^{14}	1×10^{14}	5×10^{12}	1.5×10^{14}	$3 \ 10^{14}$	2×10^{14}
S. aureus	2.8×10^{9}	3.5×10^{6}	1×10^{14}	1.5×10^{14}	2.8×10^{14}	2.4×10^{13}	1.2×10^{14}	5×10^{13}	N

M= No growth, N= Growth inhibited

The addition of Zn at a rate of 256 moll kg ^{1} to a high pH (pH 7.8) agricultural soil initially resulted in almost complete inhibition of thymidine incorporation, but bacterial growth recovered rapidly, resulting in activity levels similar to or higher than those in the non-contaminated soil after 16 days (Diaz-Ravina and Baath, 1996a). Also the addition of Cu to the same soil resulted in bacterial activity that recovered to levels more than twice that in the control soil after 1 month (Diaz-Ravina and Baath, 1996b). However, bacterial activity only recovered slowly in the most-contaminated soils during the first month. Our results substantiated these findings in which Zn^{2+} and Cu^{2+} play a vital role in recovery of bacterial injures while Cu^{2+} showed surrounded by *Klebseilla* colonies. Lusk *et al*., (1968) described the limitation of bacterial *Escherichia coli*, growth on Mg^{2+}-poor media, suggesting that bacteria required Mg^{2+} and were likely to activities as mediated division and recovery their nucleic acids after environmental stresses, take this ion from the environment. However, a preliminary report by Marnocha *et al.,* 2011 suggests that certain bacteria can survive or grow at $MgSo_4$, $FeSo_4$, and $Fe_2 (So_4)_3$ media. This observation is in agreement with our results in which we found that Mg^{2+} and Fe played remarkable role in recovering of shocked bacteria. The observation of Gyang *et al.* (1984) in which selenium injection resulted in increased bacterial activity in cattle that may support our result in the vital role which plays by trace elements in enhancing the bacterial growth. Hiraishi *et al*., (1991) reported that Zn and Cu are known to be essential for cellular defense against peroxidative damage, likely to our finding in which Zn alone or with Cu refreshed the injured cells. Also closely related situation reported by Borkow *et al*. (2008) in the human, Cu plays a major role in wound healing, thought that introducing copper into wound dressings would not only reduce the risk of wound and dressing contamination, but also stimulate faster healing and releasing Cu from the dressings directly onto the wound promotes skin regeneration that may connect with our result in which Cu promoted the repairing process of bacterial damage and may represent strong evidence in why *Klebsiellapneumonia* accumulated around Cu, while this is so is that Cu improves regeneration of damaged tissues in wounds, also it may help recovering damaged bacterial cells. We do not agree to this since this may aggravate the wound conditions.

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