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A Peculiar Phenomenon of Cold-shocked Bacteria Recovered on Sano- Gam Media

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Authors' contributions

This work was carried out in collaboration between both authors. Author MEG designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Author SME managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

This study was conducted in order to display the peculiar appearance of bacteria which were recovered on Sano-Gam Media. The studied bacteria were *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Proteus vulgaris* and *Klebsiella pneumoniae*. Some of the tested bacteria recovered on SANO-GAM media reflected peculiar growth appearance which had been noticed as high carbon dioxide production by *Salmonella* spp., in Glucose medium. A huge mist growth of *P. vulgaris*, spider network and crystal manifestation of *E. coli* colonies, strange accumulation of *K. pneumoniae* on Cu Sano-Gam medium were observed as button-like colonies of *Salmonella* spp., and ferrum stained colonies of *E. coli* were seen. From the positive point of view the new invented Sano-Gam media were being able to recover all tested bacteria nicely irrespectively whether they have expressed their growth peculiarly or not.

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1. INTRODUCTION

World-wide colonial morphology is considered a distinctive criterion of most bacteria in medical, food and industrial microbiology, i.e., *S. aureus* on Mannitol salt agar medium has had obvious morphological appearance of their golden colonies which makes them so easy to distinguish from other staphylococcal colonies furthermore, *E. coli* on Eosin and methylen blue agar medium which has ability to produce metallic-green colonies or shiny greenish colonies on this medium and that could facilitate the differentiation process by own their colonial morphology [1].

Obviously, different mechanisms exist for bacteria to respond to temperature down-shifts and the time to re-adapt to the low temperature is not directly dependent on the range of growth of the bacterium [2,3,4], actually it connected directly with the degree of negative super coiling state of DNA which transiently increases after the temperature down-shift and that could open a door for a new phenomenon which will appear in a different morphological forms [5].

Throughout history of microbiology many phenomenon related to bacteria have been known as heat-shock, acid-shock and phoenix phenomenon and the latest one which had refuted by [6], who implemented the term of cold-shock phenomenon instead of phoenix phenomenon. In spite of many more scientists had insinuated to the term cold-shock such as [7,8,9,10] however, [6] is considered the first scientist to appoint the idiom of cold-shock phenomenon in modern microbiology.

As a result of re-adaptation with chemicals, physical and nucleic acid interferences so many foreign growth styles might be countered on some Gram-negative and/or Gram-positive bacteria [11,12].

All these strange patterns were thought to be due to chemical nature of bacteria because all bacteria have no distinctive nuclear membrane or could be man-made like biotechnology and genetic engineering or un intending as a side effects of industrial activities and laboratory manipulations, so the first thing we have to have sought to find out the exact cause/s of such phenomenon.

The aim of this study was to explain the peculiar appearance of some Gram-positive and Gram-negative bacteria which were recovered on different types of Sano-Gam media.

2. MATERIALS AND METHODS

2.1 Preparation of Sano-Gam Media

Twenty-eight grams of the dehydrated nutrient agar medium were suspended in one liter of distilled water, mixed well and then dissolved by boiling. The pH was adjusted to 7.2 and was sterilized at 121℃ for 15 minutes. After that different concentration of trace elements (copper (1%), zinc (1%), ferric (1%), magnesium (5%), catalase enzyme (10%) and pyruvic salt (1%) were added either separately or in combination before being dispensed onto Petri-dishes as 20 ml portions.

2.2 Preparation of Cold-shocked Bacteria

One milliliter of an overnight broth culture (Nutrient broth), of *E. coli*, *Salmonella*, *K. pneumoniae, P. vulgaris* and *S. aureus* were transferred to glass test tube containing 9ml of sterile normal saline and using ten glass test tubes, ten-fold dilutions were done. Two end dilutions of mentioned bacteria were taken and have been frozen in a deep freezer (-20℃), for one hour and left to thaw at the bench. 0.1 ml was removed using automatic pipette transferred to the surface of Sano-Gam media (surface inoculation method) and was well spread using sterile bent glass rods and incubated at 37℃ for 24 hours. The morphology of colonies was studied.

3. RESULTS

Table 1 showing cold-shocked bacteria recovered on different Sano-Gam media.

Fig. 1. showing *S. aureus* growth on Sano-Gam media where the colonies appeared ferrum stained like.

Fig. 2. *K. pneumoniae* growth on Sano-Gam media showing bacterial colonies accumulated around the Copper.

Fig. 3. showing *P. vulgaris* which appeared as huge mist swarming on Sano-Gam media.

Fig. 4. *E. coli* growth on Sano-Gam media showing crystal-like shaped colonies.

Fig. 5. *E. coli* growth on Sano-Gam media showing peculiar spider network like shape colonies.

Fig. 6. *K. pneumonia* growth on Sano-Gam media showing lysis of bacteria.

Fig. 7. *S. aureus* growth on Sano-Gam media showing button-like colonies.

Fig. 8. High production of CO_2 by shocked *Salmonella* spp., recovered on Sano-Gam media on the left, in comparison to un-shocked *Salmonella* spp., grown on ordinary Nutrient agar medium on the right which produced low percentage of CO_2 gas.

Fig. 1. *S. aureus* growth on Sano-Gam medium showing ferrum stained-like colonies

Fig. 2. *K. pneumoniae* growth on Sano-Gam media showing bacterial colonies surrounding the copper

Fig. 3. *P. vulgaris* showing huge mist swarming on Sano-Gam media

Fig. 4. *E. coli* growth on Sano-Gam media showing crystal-shaped colonies

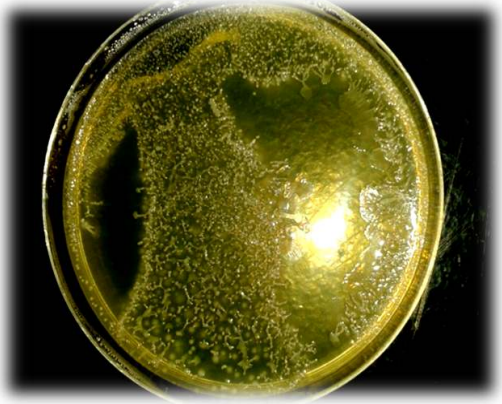

Fig. 5. *E. coli* growth on Sano-Gam media showing foreign spider network shaped colonies

Table 1. Viable count of some cold-shocked bacteria on different Sano-Gam media

Bacteria	Before freezing on nutrient agar	After freezing on nutrient agar	After recovering on Sano-Gam media with different concentrations and combination						
			Fe (10^{-5})	Zn (10^{-5})	Cu (10^{-5})	Mg (5/10000)	Catalase (10^{-3})	pyruvate (10^{-3})	Cu(10^{-5}) + Zn(10^{-5}) + Fe(10^{-5})+ Pyruvate (10^{-5}) + Mg(5/10000)
	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml	CFU/ml
Salmonella spp.	3.5×10^{9}	1×10^{7}	2.7×10^{8}	2.6×10^{8}	1.8×10^{9}	1.3×10^{12}	6×10^{13}	2.9×10^{8}	2.8×10^{14}
K. pneumoniae	3.2×10^{9}	1.8×10^{6}	5×10^{7}	2.4×10^{8}	2×10^{9}	1.2×10^{12}	1.2×10^{14}	2.2×10^{8}	6×10^{13}
S. aureus	2.8×10^{9}	3.5×10^{6}	2.8×10^{8}	2×10^{8}	1×10^{9}	1.8×10^{12}	2.8×10^{14}	2×10^{8}	1×10^{14}

Fig. 6. *K. pneumoniae* growth on Sano-Gam media showing lysis of bacteria

Fig. 7. *S. aureus* showing button-like colonies on Sano-Gam media

4. DISCUSSION

Boziaris and Adams [13] investigated the effect of thermal stresses on *Salmonella enterica* subsp. *enterica ser* enteritidis (PT4 and PT7) and *Pseudomonas aeruginosa* by heating at 55℃, rapid chilling to 0.5℃ or freezing at –20℃. They found that these thermal stresses produced transient sensitivity to niacin. They concluded that thermal shocks produce transient injury to the outer membrane allowing niacin access. This finding of [13] agreed with our results to some extend for *Salmonella* spp., where changes were observed in their colonial pattern which might give a remarkable indicator of internal injuries. Therefore, as undetermined response they also produced high amounts of carbon dioxide in Glucose medium.

Fig. 8. Increased production of CO_2 by shocked *Salmonella* spp., recovered on Sano-Gam media on the left, in comparison to un-shocked *Salmonella* spp., grown on ordinary Nutrient agar medium on the right which produced low percentage of CO_2 gas

Mesophilic *E. coli*, following cold-shock, exhibits a transient inhibition of DNA synthesis. Cold-shock also leads to increase in super-coiling of plasmid DNA in *E. coli* and *B. subtilis* [14], It has been proposed that low temperature induced negative super-coiling of chromosomal DNA which might facilitate DNA unwinding during replication and transcription at low temperature. This may give clear evidence to the cause/s of peculiar appearance of *E. coli* colonies and substantiated our finding in which *E. coli* could be grown in spite of having suffered from negative DNA super-coiling or genetic alterations.

Synthesis and/or transport of compatible solutes, such as betaine, choline and trehalose are also important for growth of bacteria at low temperature. The expression of trehalose synthetic genes (*otsA*, *otsB*, and *treC*) is induced at low temperature in *E. coli* [15]. The betaine transporting BetP is activated at low temperature. Our results may substantiate the results of [15], in that ferrum transport system has been enhanced to the maximum level and consequently delivered large amounts of ferrum so *S. aureus* colonies which appeared light brown. Antifreeze proteins (AFPs) are structurally diverse group of proteins that decrease the freezing point of cellular water (thermal hysteresis or TH activity) and possess ice re crystallization inhibition (RI) activity [16], These have extensively been studied in polar fish, insects, plants, and fungi but were thought to be absent in bacteria. This would support our result in which *K. pneumoniae* has given unusual colonial morphology appearance as lysis bacteria that might be lacking the ability to inseminate the anti-freezing protein. [17] reported that copper stimulates faster healing of the wound which emboldens skin regeneration that may connect with our result in which Cu enhanced the recondition of bacterial damage and may look like robust evidence as to why *K. pneumoniae* amassed around Cu.

Also these media would be able to improve the swarming of *P. vulgaris* that appeared as mist like. *S. aureus* colonies appeared very large and button-like. Thus these observations might be considered as evidence of excellent repairing of bacterial injures by Sano-Gam media, on which *P. vulgaris* and *S*. *aureus* returned to the state even better than their growing on normal media.

5. CONCLUSION

In conclusion of all, Sano-Gam media could successfully enhance the cold-shocked bacteria to return to their normal activities in spite of their manifestations of peculiar colonial morphology in a few cases. All these peculiar growth phenomena may be considered as a new findings because they were not reported before.

So to know the exact reason/s of these peculiar appearances more molecular research will be needed to determine the damage span of cold-shocked bacteria and how a recovery media had supported the repairing process.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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