Journal of Genetic and Environmental Resources Conservation, 2014, 2(2):198-200.
www.jgerc.com

Genotoxicity of *Bacillus subtilis* and *Bacillus circulaus* on human lymphocytes

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Abstract

The present study aimed to evaluate the genetic damage of internal toxin of *Bacillus subtilis, Bacillus circulaus* on the genetic material of human lymphocytes INVITRO. where isolation and diagnose two species of bacteria from contaminated water by hydrocarbon diagnosed laboratory followers (Bergey's Manual) and after cultivation on the Nutrient Broth has been broken using homogenized. Dilution per isolation, has been tested Geno-toxicity per dilute on human lymphocyte and using indicators which included cell division MI, coefficient Blast index BI and rate chromosomal aberration CA. The results showed decrease in the rate of cell division of lymphocyte exposed to Bacillus; 2.38 *subtilis* 2.42 *circulaus*, compared with control 8.27. Also have been observed decline coefficient Blast index BI both sexes, 20.96 and 21.13, respectively, compared with control 48. 43. It was also the study of the rate of rate chromosomal aberration where the results showed high rates of rate chromosomal aberration up to 4.15 and 3.92, respectively, unlike with the control solution which scored 0.08. All dilution were treated with Buffer solution PBS and analyzed by SPSS v.23.

Keywords: *Bacillus subtilis, Bacillus circulaus,* Genotoxicity, Human, Lymphocytes.

Introduction

Bacteria of the *Bacillus* group are widely used for the manufacture of important industrial enzymes, food products, pesticides, and insecticides. The many species of the genus *Bacillus* produce valuable metabolites with the potential for technical and scientific applications (Outtrup and Jörgensen, 2002). *Bacillus* species with a GRAS (generally regarded as safe) status are able to produce toxins. *Bacillus* species are able to produce physically and chemically resistant endospores if the conditions needed for the vegetative bacteria to survive are limited. These spores can survive extreme environmental conditions such as dryness, heat, radiations and chemical treatments. Also, The Species of Bacillus produce poisoning protein and peptide toxins: as many as 10 different heat-labile protein enterotoxins causing abdominal cramps, diarrhea, and nausea and the heat-stable peptide toxin cereulide which causes vomiting. The usefulness and application of many *Bacillus* species and, on the other hand, their ability to produce toxic compounds call for careful monitoring of their environmental occurrence (Schallmey *et al.*, 2004). *Bacillus subtilis* and use 4-7% of it genome for producing bioactive compounds. Most of the toxic non-protein substances from *Bacillus*, such as those listed in the Table (1) are well known but their role as contaminants in food or moisture-damaged buildings is incompletely understood. The reasons are historical, since most of these substances were found mainly before 1980, whereas the toxicity or poisoning related to such substances was established much later. The aim of this study was effect of bacteriocin extracted from two *Bacillus* isolated from waste hydrocarbon on normal cells of human *in vitro* (Raimo, 2006).

Table (1): Non-protein substances from Bacillus

Bacteria	Compound	Biological Effects on eukaryotic cells
B. subtilis	Rhizocticin A	Antifungal
B. amyloliquefaens,	Surfactin	Hemolytic
B. subtilis		Cytotoxic
B. cereus	Zwittermicin A	Fungicidal

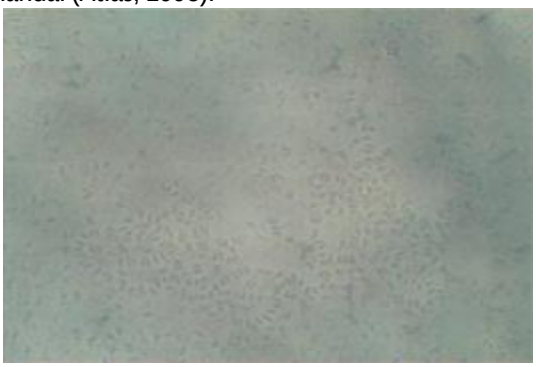
Materials and Methods

Isolation of bacteria: The bacteria were isolated from the collected samples water contaminated by waste hydrocarbon by spreading 1ml from the

sample on nutrient agar medium. After 48hrs. incubation of new passage from nutrient agar plates to differentiation the bacteria for 48 hrs. Also, used the bergey's manual to isolated species. The species was *Bacillus subtilius, Bacillus circulaus* (Fritz, 2004).
Extraction toxin from Bacillus: Transfer the colony bacteria from plate and suspend universal tube contain 5ml the sterilized NB, incubated 72hrs. in the incubator shaker. The cultured treated by homogenizer (Johnson *et al*., 1976).
Genotoxiccity test: Added 0.1 ml from PHA to the 5ml RPMI-1640 in the sterilized tube, with 0.5 ml blood. added0.3 from extraction bacteria that diluted from 0.1-0.4.ml after 24 hrs. then incubated 37c° through 72hrs 3times for each extraction. The process stop in metaphase when the chromosome appear by added 0.1ml colchicine for all tubes before half hour the end of time culture. The tube centrifuged 2000through 10min the serene discard. The pellet mix with Hypotonic solution 0.075µ with little shake. The tube transfer to the water bath at 37 C° for 1 hr. The fixation process of was achieved by centrifuged 2000rpm through 10min. and discard serene. The pellet shake with added fixative solution by dropping until the volume arrived to 5ml. then The process of centrifuged repeat in the same speed and time and discard serene. The fixative repeat for many times until the serene go to the fuzzy. The dropping of cells achieved by mix the stuck of cells while homogenized, and dropped on the cool slide by Pasteur pipette the distance 30cm, the slide was oblique. The slide let to dry. The slide staining by added 5ml from Giemsa stain through 2-2.5min. then washed by Sorenson's buffer solution. the slide let to dry. all slides examination by light microscope to detected the BI, MI and chromosome abbretiation (Verma and Babu, 1989) .

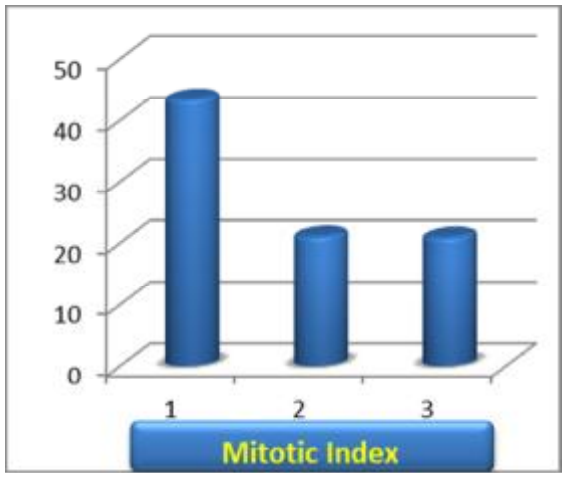
Results and Discussion

In order to isolate Bacillus, 10 heavily oil contaminated water samples were collected from fuel stations and Al- Dorah oil refineries in Baghdad Province, from which 6 bacterial isolates were isolated. Oil contaminated water were chosen for isolation of Bacillus since they create a selective niche to such microorganisms that have enhanced ability to utilize hydrocarbons as nutrition source. When these isolates were subjected to morphological and cultural characteristics on nutrient agar and microscopic examination with gram staining 6 isolates where suspected to be Bacillus isolates since they were gram positive, spore forming bacilli, some of them occur singly, others arranged in pairs but most of them form chain, their spores either ellipsoidal. The differentiation of isolated dependent on bregy's manual (Atlas, 1995).

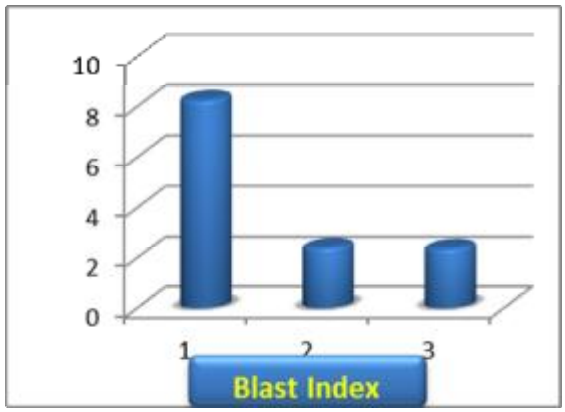


Figure(2): The Bacillus at light microscopic 100X
The parameters of Bacillus toxicity on the human Blood.

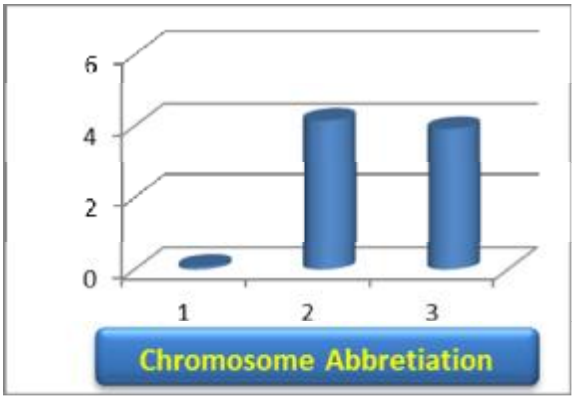
The results for this research aimed to show the Blastic index, Mitotic index and chromosome aberration for the normal human blood that treated with crude toxin of Bacillus. the below figures showed the measurements of all parameters like BI, MI and CA compared with control.



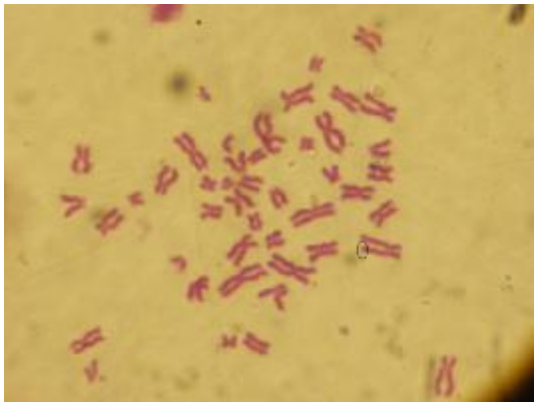
Figure(2): Measurements of MI



Figure(3): Measurements of BI



Figure(4): Measurements of C.A.


Figure(5): The Karyotype

From the results in the above tables showed the Decreased mitotic index and Blast index in the figure (2, 3), the Bacillus toxin induced lymphocytes culture showed the cytotoxic effect of drug. the Bacillus toxin inhibit DNA gyrase and Topoisomerase II, which is important for resolving the super-helical interwinded structure of DNA during replication. It also interfered in segregation of chromosome at anaphase in mitosis consequently resulting in the delay of cell cycle and prolongs metaphase stage. (Miller and Therman, 2001). while observed in the C.A. in the Fig(4) showed increase in the measurements of CA, the reason induce the chromosome aberration in human lymphocyte cells culture *in vitro*, by irreversible breakage in DNA and enhancing eukaryotic topoisomerase II mediated DNA cleavage *in vitro-were also reported* (Morah *et al.,* 2001; Talaro, 2005).

Conclusions

Through the research showed the high percentage Genus bacteria was Bacillus depend on the morphological and biochemical tests. Also, the side effect of this study discern the toxin of this genus have been effect on the genetic material. The toxin showed the decline in the MI and increasing of BI and CA. whereas, the workers in the fields of oil refinery always affected in this toxin.

References

Atlas, R. M.; Brown, A. E., and Parks, L. C. 1995. Laboratory Manual Experimental Microbiology. 1st ed., Mosby.

Fritz, D. 2004. Taxonomy of the genus *Bacillus* and related genera: The aerobic endospore –forming bacteria. Phytopathology. 94: 1245-1248.

Johnson, K. G.; Perry, M. B. 1976. Improved techniques for the preparation of bacterial lipopolysaccharides. Canadian J. Microbiol., 22: 29-34.

Miller, O.J. and Therman, E. 2001. Human Chromosome. The Mitotic Cell Cycle. Ch.2, 4th ed., Springer- Verlag, New York, 13 – 19pp.

Morah, S.; Geyer, A., and Hzrting, T. 2001. Structure- Function Relationship of Cytokine Induction by LTA from *Staphylococcus aureus*. JEM, 193(3): 393 – 398.

Outtrup H, Jorgensen ST. 2002 The importance of *Bacillus* species in the production of industrial enzymes. In: Berkeley, R.; Heyndrickx M.; Logan N. and De Vos P. ed., Applications and systematics of Bacillus and relatives. Blackwell publishing, UK, 206–218pp.

Raimo, M. 2006. Food and Indoor Air Isolated Bacillus Non-Protein Toxins: Structures, physico-chemical properties and mechanisms of effects on eukaryotic cells. ISSN 1795-7079. Helsinki, Finland.

Schallmey M.; Singh, A.; Ward, O.P. 2004. Developments in the use of *Bacillus* species for industrial production. Canadian J. Microbiol., 50: 1-17.

Talaro, K.P. 2005. Foundation in Microbiology, 5th ed., McGraw- Hill Companies, Inc., New York, 433 – 435pp.

Verma, R. and Babu, A. 1989. Human Chromosomes: Manual of Basic Techniques Pregramon Press New York.