African Journal of Advanced Pure and Applied Sciences (AJAPAS)

Online ISSN: 2957-644X

Volume 3, Issue 2, April - June 2024, Page No: 180-187

Website: https://aaasjournals.com/index.php/ajapas/index

معامل التأثير العربي 2023: (1.55) **SJIFactor 2023: 5.689** **ISI 2022-2023: 0.557**

Isolation of *Bacillus Cereus* from Meat Ready to Eat Using Antimicrobial Resistance Pattern and Polymerase Chain Reaction - Deoxyribonucleic Acid (DNA-PCR)

Ahlam Abdaslam M. Ali *

Assistant Lecturer Department of Microbiology, Faculty of Sciences, University of Zintan, Libya

*Corresponding Author: ahlam.ali@uoz.edu.ly

Received: April 05, 2024	Accepted: June 10, 2024	Published: June 24, 2024

Abstract:

In September 2022, a total of 50 samples of ready-to-eat mutton meat were purchased from 10 different restaurants in Tripoli, Libya. Twelve isolates were identified as B. cereus through biochemical analysis. These isolates exhibited positive results for lecithinase production, starch hydrolysis, nitrate reduction, catalase production, Gram stain, and glucose fermentation, Nacl. No positive results were obtained for indole, sucrose, gas production , and lactose fermentation in any of the samples, blood hydrolysis To determine the relatedness and diversity of the B. cereus strains from RTE mutton meat, polymerase chain reaction was performed using primer S16. The antimicrobial pattern Erythromycin 88 %, Chloramphenicol 81.4 %, Trimothprim 93 % Clindamycin 90.7 %, Pencillin 100 % , Amikacin 76.4 % By combining the phenotype and genotype methods, a total of nine different types of B. cereus were identified. These findings provide evidence for the existence of both relatedness and diversity among the B. cereus strains isolated from mutton meat.

Keywords: *Bacillus Cereus*, Antimicrobial, RTE, Deoxyribonucleic, PCR, Isolation - MYP.

Cite this article as: A. A. M. Ali, "Isolation of Bacillus Cereus from Meat Ready to Eat Using Antimicrobial Resistance Pattern and Polymerase Chain Reaction - Deoxyribonucleic Acid (DNA-PCR)," *African Journal of Advanced Pure and Applied Sciences (AJAPAS)*, vol. 3, no. 2, pp. 180–187, April-June 2024.

عزل بكتيريا *Bacillus cereus* من اللحوم الجاهزة للأكل باستخدام نمط مقاومة مضادات الميكروبات وتفاعل البوليميراز المتسلسل - حمض الديوكسي ريبونوكلييك (DNA-PCR)

أحلام عبد السلام محمد علي *

قسم الأحياء الدقيقة، كلية العلوم، جامعة الزنتان، ليبيا

الملخص

في سبتمبر 2022، تم شراء ما مجموعه 50 عينة من لحوم الضأن الجاهزة للأكل من 10 مطاعم مختلفة في طرابلس، ليبيا. تم التعرف على اثني عشر عزلة على أنها B. cereus من خلال التحليل الكيميائي الحيوي. أظهرت هذه العزلات نتائج إيجابية لإنتاج الليسيثيناز، وتحلل النشا ، واختزال النترات وإنتاج الكاتالاز، وصبغة جرام ، وتخمير الجلوكوز، وNacl. لم يتم الحصول على نتائج إيجابية للإندول ، والسكروز، وإنتاج الغاز، وتخمير اللاكتوزفي أي من العينات، وتحلل الدم . لتحديد مدى ارتباط وتنوع سلالات B. cereus من لحوم الضأن الجاهزة للأكل، تم إجراء تفاعل البوليميراز المتسلسل باستخدام البادئ S16. النمط المضاد للميكروبات إريثروميسين 88٪، كلورامفينيكول 81.4٪، تريموثبريم 93٪ كليندامايسين 90.7٪، بنسيلين 100٪، أميكاسين 76.4٪ من خلال الجمع بين أساليب النمط

الظاهري والنمط الجيني، تم تحديد ما مجموعه تسعة أنواع مختلفة من B. cereus. توفر هذه النتائج دليلاً على وجود كل من القرابة والتنوع بين سلالات B. cereus المعزولة من اللحوم.

الكلمات المفتاحية: البكتيريا العصوية الشمعية، مضادات الميكروبات، PCR، عزل البكتريا.

Introduction

Bacillus cereus strains secrete extracellular toxins and enzymes that are important factors in diseases such as diarrheal toxins and hemolytic toxins. The toxicity of the disease begins after eating food contaminated with the bacteria and is sometimes fatal. There are also other diseases associated with the toxicity of these bacteria, such as conjunctivitis, dermatitis, endocarditis, and urinary tract infections [47],[22]. Histopathological changes occur with bleeding in the intestinal lining, which means the onset of food poisoning, as food poisoning causes skin necrosis and oozing of white blood cells due to internal bleeding in the intestine. Bacillus cereus is frequently found in a range of foods as well as the environment, such as dirt. Even at typical cooking temperatures, the spores can survive hostile conditions [10], [23] [12], [48], [37]. Unless preservation techniques are used, meat is a highly perishable food with a short shelf life because it relies on the presence of protein that is rapidly degraded at high temperatures to degrade [5]. Many people have become aware of foodborne diseases. The majority of hospital admissions and deaths from foodborne infections are caused by bacterial agents including Bacillus cereus [12]. Endospore-forming bacteria, such as B. cereus, due to the thermotolerance of endospores and the wide temperature range at which certain strains can develop, represent one of the greatest risks and the ability to produce botulism toxins in low-acid foods[46], [36]. Therefore, food safety in Libya still needs to be assessed on the prevalence of B. cereus poisoning. In an attempt to shed more light on the potential risks associated with such foods, the present study was conducted to assess the microbiological quality of ready-to-eat meat foods supplied in Tripoli, Libya.

Material and methods

Ten different restaurants in Tripoli provided Table 1 with a total of fifty lamb samples. After eight hours of cooking, all samples were collected and delivered to the laboratory at subzero temperatures to stop any decomposition that might occur after the specified eight hours[21]. In addition, the lamb sample was cut into small pieces. A homogenizer was used to homogenize ten grams of food samples in 100 ml of peptone water for one minute and then 1 ml of the solution was transferred to 5 ml of sterile peptone water[8],[1], [45], 24].

Table 1 Samples collection of meat.

Region	Restaurant
Tripoli	Multiple restaurants
Total	**50**

Confirmation and identification of Bacillus cereus

Using a glass diffusion rod, spread 0.1 ml evenly over the surface of each plate on selective medium and incubate for 24 h at 37°C. The B. cereus count is given as cfu/g, or component units, divided by the relative proportion of the tested colonies that tested positive for B. cereus. The final units are then measured in grams (cfu/g) and milliliters (cfu/ml) [20]. A ready-to-use unit for the counts is a microbe grown in a petri dish; if there is only one bacterium, all of the necessary units will be colonies.) Therefore, the cfu/g for the sample is as follows. $N = C/V(n) \times D$, where V is the volume applied to each layer, n is an infinite number, D is the incomplete number acquired, and C is the total colony data on all reliable information [8], [25],[19]. Starch hydrolysis production, nitrate reduction, catalase production, Gram stain, glucose fermentation, NaCl, indole, sucrose, gas production, and lactose fermentation were recorded in any of the samples, blood hydrolysis was determined by identification and followed by the real physical identification methods [4] and [13].

Antibiotic susceptibility testing

Antibiotic susceptibility was used. Single colonies of diluted solutions were placed on sterile antimicrobial test discs containing erythromycin 30 µg, chloramphenicol 25 µg, trimethoprim 20 µg, clindamycin 30 µg, penicillin 20 µg, and amikacin 15 µg and incubated at 37°C for 24 h[3],[2], [41],[18]. the zones were measured as follows: ≥15 susceptible and ≤14 resistant.

DNA isolation Boiling technique

Genomic DNA purification kit [7], [44],[17] and boiling technique [9] were used to extract DNA. Cells for gene identification were extracted during 24 h of growth at 37 °C, and centrifuged for 15 min at 10,000 rpm. 1 ml of sterile distilled water was used to wash and mix the cells. After that, they were boiled for ten min at 98 °C, and then frozen for ten min at -15 °C. After that, the tube was centrifuged for five min at 10,000 rpm. The gene template was generated [9] , [43] ,[40],[16].

DNA purification before extraction

1 ml of the solution was centrifuged at 13,000-16,000 rpm for 5 min. After adding 600 µl of nuclei lysis solution to the tube, it was incubated at 90 °C for 5 min before cooling to room temperature 37 °C. After adding 200 µl of protein precipitation solution, vortexing, and leaving on ice for 5 min, the mixture was centrifuged for 3 min at 13,000-16,000 rpm. The mixture was mixed with 600 µl of isopropanol at room temperature in a clean tube, and the upper liquid was centrifuged for 2 min at 13,000-16,000 rpm. After discarding the upper liquid, 600 µl of 70% ethanol at room temperature was added, mixed, and centrifuged for 2 min at 13,000-16,000 rpm. The pellets were then left to air dry for 15 minutes, according to the instructions in the technical manual [7], [42], [38],[28].

For quantitative and qualitative aspects of primer quality and DNA

extraction, DNA quantification required a total of two microliter volumes. A MaestroNano spectrometer was used to assess concentration by dividing the absorbance at 280 nm by the absorbance at 300 nm. The quantity is represented in ng/ml by the DNA concentration. S16, a random primer containing 60% of the gene sequence, was chosen for investigation because it provides a repetitive pattern [6], [27], [14], [34], [29].

Separation of DNA fragments by agar gel electrophoresis and amplification using polymerase chain reaction

PCR was performed in a volume of 30 µl per tube containing a mixture of potassium chloride and magnesium to accelerate the separation and replication process and a set of nitrogen solution and two primers to initiate the separation of DNA fragments and the solution was aided by the addition of buffer which acted as a catalyst in the replication process and millimolar enzyme [9],[25], [35], [26]. The amplification process was performed using a temperature program that included an initial denaturation for 5 min at 94 °C, 30 cycles of denaturation at 94 °C for 5 min, 30 s of annealing at 62 °C, and 30 s of polymerization at 72 °C [6], [15], [7],[30].

Results and discussion

The colonies of the presumptive Bacillus cereus appeared as rough purple surfaces surrounded by irregular white margins. Table 2 shows the standard deviation and the overall mean (cfu/g) of the presumptive Bacillus cereus for all samples taken from ten different restaurants in Tripoli. To interpret the result with a 90% confidence level, the comparison statistic was used. The samples that recorded the highest mean of the presumptive B. cereus were 1.55×108±26.20 cfu/g, 1.44×108±73.61 cfu/g, 1.33×108±61.37 cfu/g, and 1.22×108±26.33 cfu/g, respectively. This was followed by samples with scores of 6.11×107±27.73 cfu/g, 5.22×107±41.88 cfu/g, 4.22×107±33.8 cfu/g, and 3.1×107±11.44 cfu/g, respectively. The samples with the lowest means were 2.77×107±3.88 cfu/g, 2.66×107±6.21 cfu/g, 2.33×107±3.22 cfu/g, and 2.63×107±4.88 cfu/g, respectively.

Table 2: *Bacillus cereus* total mean cfu/g and standard deviation.

Region	B. cereus
Different restaurants at Tripoli	$1.55\times10^{8}\pm26.20$ cfu/g
	$1.44\times10^{8}\pm73.61$ cfu/g
	$1.33\times10^{8}\pm61.37$ cfu/g
	$1.22\times10^{8}\pm26.33$ cfu/g
	$6.11\times10^{7}\pm27.73$ cfu/g
	$5.22\times10^{7}\pm41.88$ cfu/g
	$4.22\times10^{7}\pm33.8$ cfu/g
	$3.1\times10^{7}\pm11.44$ cfu/g
	$2.77\times10^{7}\pm3.88$ cfu/g
	$2.66\times10^{7}\pm6.21$ cfu/g
	$2.33\times10^{7}\pm3.22$ cfu/g
	$2.63\times10^{7}\pm4.88$ cfu/g

Sample No: (cfu/g) (±), standard deviation and mean

Figure 1: At 37 °C, this bacterium displayed a noticeably high amount of contamination, confirming its elevated risk to humans. There could be a bad processing method or inadequate cleanliness practices to blame for this unexpected increase in the number of germs in Tripoli.

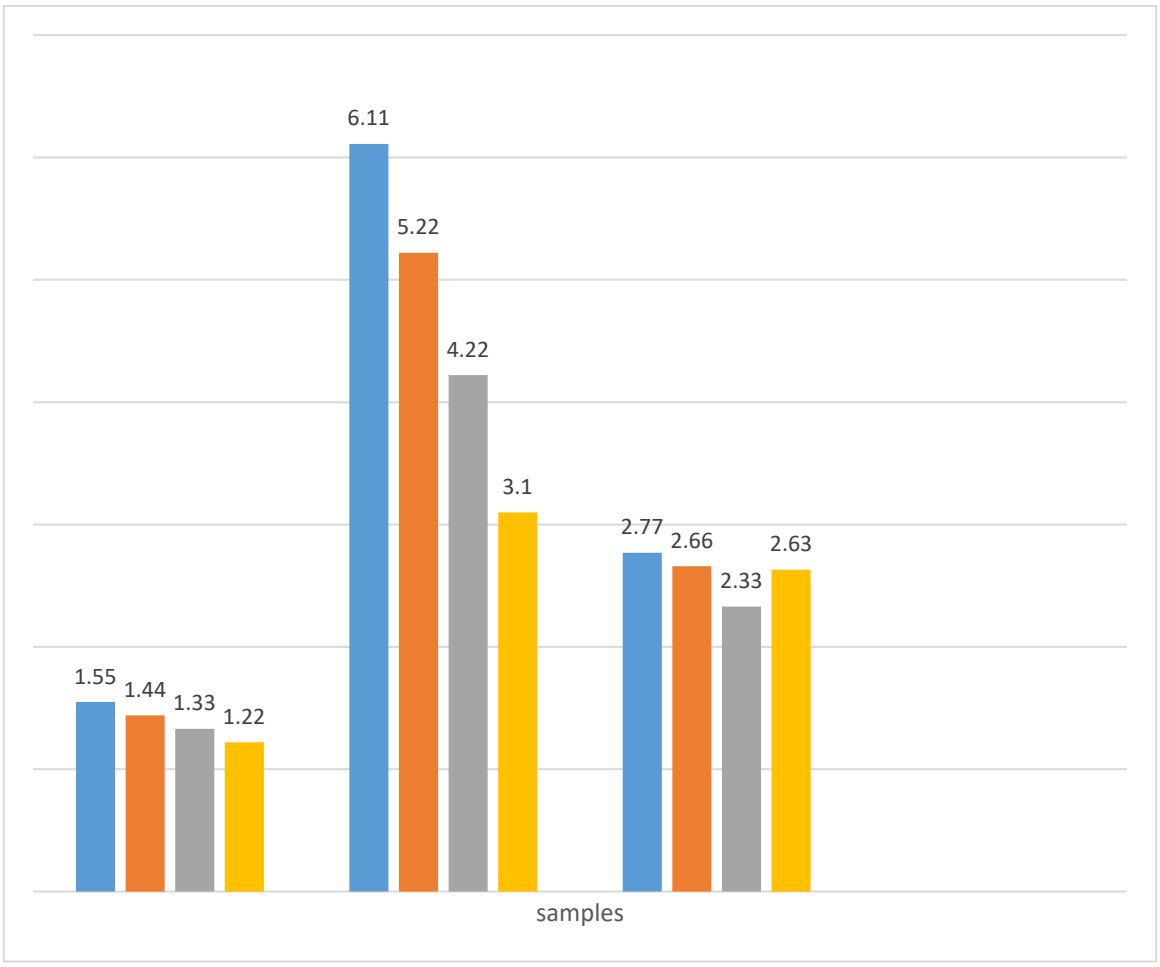

Figure 1 Total mean cfu/g and standard deviation of presumptive *Bacillus cereus*.

Authenticity of *Bacillus cereus* Presumptive B. cereus colonies' biochemical traits were identified and contrasted with those reported in [9],[33]. Twelve isolates were identified as *B. cereus* after displaying consistent phenotypic characteristics (Table 3).

Table 3: *Bacillus cereus* isolation and biochemical characterisation

Sample	SH	NR	CP	GS	GF	NCAL	IN	SU	GP	IF	BH
S1	+	+	+	+	+	+	–	–	–	–	–
S2	+	+	+	+	+	+	–	–	–	–	–
S3	+	+	+	+	+	+	–	–	–	–	–
S4	+	+	+	+	+	+	–	–	–	–	–
S5	+	+	+	+	+	+	–	–	–	–	–
S6	+	+	+	+	+	+	–	–	–	–	–
S7	+	+	+	+	+	+	–	–	–	–	–
S8	+	+	+	+	+	+	–	–	–	–	–
S9	+	+	+	+	+	+	–	–	–	–	–
S10	+	+	+	+	+	+	–	–	–	–	–
S11	+	+	+	+	+	+	–	–	–	–	–
S12	+	+	+	+	+	+	–	–	–	–	–

starch hydrolysis (sh), nitrate reduction (nr), catalase production (cp), Gram stain (gs), and glucose fermentation (gf), Nacl, indole (in), sucrose (su), gas production (gp) , and lactose fermentation(lf) , blood hydrolysis (bh)

Antimicrobial resistance pattern Antimicrobial drugs have been widely used. Six distinct antibiotics were evaluated on B. cereus isolates using the disk diffusion agar method [6], [32]. The inhibition zone for each of the different antibiotics used, as well as the antimicrobial resistance pattern of the B. cereus isolates, are shown in Tables 4 and 5.

The B. cereus isolates showed resistance to erythromycin in 88%, chloramphenicol in 81.4%, trimethoprim in 93%, clindamycin in 90.7%, penicillin in 100%, and amikacin in 76.4%. The results of the investigation are consistent with those of previous research projects conducted by [6], [31], [39]. Foodborne pathogens resistant to antibiotics may pose challenges in the treatment of diseases or illnesses, while those susceptible to antibiotics recover from the disease caused by the organisms.

Table 4: Inhibition zone of several administered antibiotics.

Restaurant	ER	CH	TR	CL	PE	AM
S1	R	S	S	R	R	R
S2	R	S	S	S	S	R
S3	R	S	S	S	S	R
S4	R	S	S	R	S	R
S5	R	S	S	R	S	R
S6	R	S	S	R	R	R
S7	R	S	S	R	S	R
S8	R	S	S	R	S	R
S9	R	S	S	R	S	R
S10	R	S	S	R	S	R
S11	R	S	S	R	S	R
S12	R	S	S	R	S	R

R: 14 mm of resistance
S: susceptible to 15 mm
Twelve strains from Tripoli were examined in all.

Tested for the following: Erythromycin (ER), Chloramphenicol (CH), Trimothprim (TR), Clindamycin (CL), Pencillin (PE), and Amikacin (AM).

Table 5: *Bacillus cereus's* pattern of antibiotic resistance

Antimicrobial tested	Total no. (%) of strains resistance
Erythromycin	88 % (12)
Chloramphenicol	81.4 % (12)
Trimothprim	93 % (12)
Clindamycin	90.7 % (12)
Pencillin	100 % (12)
Amikacin	76.4 % (12)

Twelve strains from Tripoli were evaluated in all.

Random PCR-based polymorphic DNA amplification

The DNA concentrations of all isolates were ≥100 ng/µl, as measured by spectrophotometry. Twelve B. cereus isolates were subjected to random PCR using S16 primer. A large number of polymorphic bands were produced. However, genetic mutations in the primer sequences in these genes could explain these weak interactions. Random PCR with S16 primer pattern shows that B. cereus strains are related and diverse. S16 primer was used which produced nine distinct profiles (P1, P2, P3, P4 and P5) consisting of a number of bands with the same molecular weight. On the other hand, antibiotic resistance produced four patterns (R1, R2, R3 and R4), where R stands for a class of antibiotics to which the strains are resistant. The results show that the antibiotic resistance patterns were more diverse than the PCR patterns. Nine distinct species of B. cereus were identified in this investigation using a combination of two methods (Table 6).

Table 6: Using RAPD-PCR profiles and antibiotic resistance patterns, typing *B. cereus* isolates from RTE meat

Strains no.	Antibiotic resistance patterns and their groups		RAPD-PCR profiles
S1	CroCDaNaSVa	R1	N
S2	CroNaVa	R2	N
S3	CroDaNaVa	R3	P1
S4	CroDaNaSVa	R4	P2
S5	CroCNaVa	R4	N
S6	CroCDaNaSVa	R1	P3
S7	CroDaNaVa	R3	P4
S8	CroDaNaSVa	R4	P5
S9	CroDaNaVa	R3	N
S10	CroDaNaVa	R3	N
S11	CroDaNaVa	R3	N
S12	CroDaNaVa	R3	N
Total		**4**	**5**

R: Antibiotic group to which strains have developed resistance.
P: The quantity of molecular weight profiles; N: The absence of patterns

Conclusion

In this study, Bacillus cereus was successfully isolated and identified from lamb meat. It was found that Bacillus cereus was present in all restaurants to some extent. Since these meat isolates have the potential to produce toxins and other body-related diseases such as skin, eye, and small intestine lining inflammation, it is impossible to attribute the low incidence of food poisoning to the consumption of meat containing high concentrations of Bacillus cereus. Five distinct patterns were produced by PCR using primer S16, indicating the relatedness and diversity of Bacillus cereus strains present in lamb meat. It is also reported that the four patterns provided by the antibiotic resistance pattern were effective in helping to distinguish between distinct Bacillus cereus strains. The results of the present study revealed that among ready-to-eat lamb meat isolates, a combination of phenotypic and genetic methods revealed nine distinct Bacillus cereus forms. Food contamination can occur at any point in the cooking process, from the slaughterhouse to the end user. Since improper temperature management during storage of cooked foods is often associated with B. cereus food poisoning, appropriate measures should be implemented to control this pathogen.

References

[1]. Agata, N., Mori, M., Ohta, M., Suwan, S., Ohtani, I. & Isobe, M. 1994. A novel dodecadepsipeptide, cereulide, isolated from Bacillus cereus causes vacuole formation in HEp-2 cells. FEMS Microbiology Letters. 121: 31–34

[2]. Aly, M.M.; Albureikan , M.O.; El Rabey, H. and Kabia, S.A. (2013) Effects of culture conditions on growth and Poly-β-hydroxybutyric acid by Bacillus cereus MM7isolated from soil samples from Saudi Arabia. Life Science Journal, 10(4): 1884-1891.

[3]. Anderson, A.J. and Dawes, E.A. (1990) Occurrence, metabolism, metabolic role, and industrial uses of bacterial polyhydroxyalkanoates. Microbiological Reviews, 54 (4): 450-472

[4]. Anto, H.; Trivedi, U. and Patel, K. (2006) Alpha amylase production by Bacillus cereus MTCC 1305 using solid-state fermentation . Food Technology Biotechnology, 44 (2) 241–245.

[5]. Austin, B. and Allen- Austin, D. (1985). Microbial quality of water in intensive fish rearing. J. Appl. Bacteriol. Symp. Suppl., 14, 2075-2265.

[6]. Beecher, D.J. and MacMillan, J.D. (1991). Characterization of the components of hemolysin BL from Bacillus cereus. Infect. Immun. 59:1778-1784.

[7]. Beecher, D.J.and Wong, A.C.L. (2000). Tripartite Haemolysin BL: isolated and characterization of two Distinct homologous sets of component from a single Bacillus cereus isolate.Microbiology 146: 1371-1380.

[8]. Bernhard, K.; Schrempf, H. and Goebel, W. (1978). Bacteriocin and antibiotic resistance plasmids in Bacillus cereus and Bacillus Subtilis.J. Bacteriol. 133, 897-903.

[9]. Bonofiglio, L., Gardella, N. & Mollerach, M. 2012. Application of Molecular Typing Methods to the Study of Medically Relevant Gram-Positive Cocci, Gel Electrophoresis - Advanced Techniques. Buenos Aires: Department of Microbiology, Immunology and Biotechnology, University of Buenos Aires Argentina.

[10]. Chen, B. Y.; Shiau, T. J. ; Wei, Y. H.; Chen, W. M.; Yu , B. H.; Yen, C.Y. and Hsueh, C. C. (2012) Feasibility study of polyhydroxyalkanote production for materials recycling using

[11]. Chen, G. Q.; Konig, K. H. and Lafferty, R. M (1991) Occurrence of poly D-3-hydroxyalkanoates in the genus Bacillus. FEMS Microbiology Letters, 84: 173-176.
[12]. Clinical Laboratory Standards Institute. 2006. Performance standards for antimicrobial disk susceptibility tests, 9th ed. Wayne, PA: CLSI.
[13]. FDA. 1992. Bacteriological analytical manual Ed. 529. AOAC International.
[20]. Granum, P.E. (2003). Food poisoning. The Norwegian School of veterinary Science, Oslo, Norway.
[21]. Hablot, E. Bordes, P. Pollet, E. and Ave´rous, L. (2008) Thermal and thermo-mechanical degradation of poly(3-hydroxybutyrate)-based multiphase systems. Polymer Degradation and Stability, 93 : 413-421.
[22].Hahn, S. K.; Chang, Y. K. ; Kim, B. S. and Chang, H. N. (1994) Optimization of microbial poly(3-hydroxybutyrate) recovery using dispersions of sodium hypochlorite solution and chloroform. Biotechnology and Bioengineering, 44: 256-261.
[23]. Harwood, C.R. (1989) Bacillus. Biotechnology Handbooks 2. Plenum Press. New York.
[24]. Hassan, E.S., Ferage, M. & Nahla, T. 2006. Lactic acid & pH as indication for bacterial spoilage of meat and some meat products. Journal Applied Science Research. 2: 522–528.
[25]. Holt, J.G.; Kreig, N.R.; Sneath, P.H.A.; Staley, J.T. and Williams, S.T. (1994). Bergy,s Manual of Determinative Bacteriology."9th ed.". Williams and Wilkins. Co.Baltimore, London.
[26]. Mourey, A. and Kilbertus,G. (1979) Simple media containing stabilized Tributyrin for demonstrating lipolytic bacteria in foods and soils. Journal of Applied Bacteriology, 40(1): 47-51.
[27].Mousavioum, P. (2001) properties of lignin and Polyhydroxybutyrate blends Ph.D. thesis, Queensland university , Australia. 239p.
[28]. Muhamad, I.I.; Joon , L. K. and Noor, M.A.M. (2006) Comparing the degradation of poly-β-(hydroxybutyrate),poly-β-(hydroxybutyrate-co-valerate)(PHBV) and PHBV/cellulose triacetate blend. Malaysian Polymer Journal,1:39-46.
[29]. naturally occurring pollutant degraders. Journal of the Taiwan Institute of Chemical Engineers, 43(3): 455-458.
[30]. Okwuobi, P.N. and Ogunjobi, A.A. (2012) Characterization of polyhydroxyalkanoate (PHA) produced by Bacillus species isolated from garden soil. New York Science Journal, 5(12):159-163.
[31].Okwuobi, P.N. and Ogunjobi, A.A. (2013) Production and analysis of polyhydroxyalkanoate (PHA) by Bacillus megaterium using pure carbon substrates. World Applied Sciences Journal, 28 (9): 1336-1340.
[32]. Pielaat, A., Fricker, M., Nauta, M.J. & van Leusden, F.M. (2005). Biodiversity in Bacillus cereus. The Netherlands: National Institute for Public Health and the Environment
[33]. Pradhan, S. (2014) Optimization and characterization of bioplastic produced by Bacillus cereus SE1.MSc. thesis, national Institute of technology Rourkela, Odisha, India. 26p.
[34]. Pramanik, A. ;Mitra , A.; Arumugam , M.; Bhattacharyya , A.; Sadhukhan, S. ; Ray, A ; Haldar , S. ; Mukhopadhyay, U.K. and Mukherjee, J. (2012) Utilization of vinasse for the production of polyhydroxybutyrate by Haloarcula marismortui. Folia Microbiologica, 57:71–79.
[35]. Rangasamy, P.N.; Lyer, M. and Raginski, H. (1993). Isolation and characterization of Bacillus cereus in milk and dairy products manufactured in Victoria.Australian. Dairy Technol. 48: 93-95.
[36]. Roberts, D. & M. Greenwood. 2003. Practical food microbiology Ed: Wiley Online Library.
Sahilah, A.M., Audrey, L.Y.Y., Ong, S.L., Wan Sakeenah, W.N., Safiyyah, S., Norrakiah, A.S., Aminah, A. &
[37]. Ahmad. 2010. DNA profiling among egg and beef meat isolates of Escherichia coli by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) and random amplified polymorphic DNA-PCR (RAPD-PCR). International Food Research Journal. 17: 853-866
[38]. Senesi, S. & Ghelardi, E. 2010. Production, secretion and biological activity of Bacillus cereus enterotoxins. Toxins. 2: 1690–1703
[39]. Sutherland, A.D. & Limond, A.M. 1993. Influence of pH and sugars on the growth and production of diarrhoeagenic toxin by Bacillus cereus. Journal of Dairy Research. 60: 575–580
[40]. Teplitski, M., Wrigh, A.C. & Lorca, G. 2009. Biological approaches for controlling shellfish-associated pathogens. Current Opinion in Biotechnology. 20: 185-190.
[41].Turnbull, P.C.B.; Nottingham, J.F. and Glosh, A.C. (1977). Asevere necrotic enterotoxin produced by certain food, food poisoning and other clinical isolates of Bacillus cereus. Br. J. Exp. Pathol. 58, 273-280.
[42]. Vilain, S., Luo, Y., Hildreth, M. & Brözel, V. 2006. Analysis of the life cycle of the soil saprophyte Bacillus cereus in liquid soil extract and in soil. Applied and Environmental Microbiology. 72: 4970–4977
[43]. Wang, J. and Yu, J. (2000) Kinetic analysis on inhibited growth and poly(3-hydroxybutyrate) formation of Alcaligenes eutrophus on acetate under nutrient-rich conditions. Process Biochemistry, 36:201–207.
[44]. Wang, L.; Armbruster, W. and Jendrossek, D. (2007) Production of medium-chain-length hydroxyalkanoic acids from Pseudomonas putida in pH stat. Applied Microbiology and Biotechnology, 75:1047-1053.
[45]. Wang, Y.; Li F.; Wang, Z. ; Liu, D.; Xia, H.; Liu, L. and Chen, S.(2012) Purification and properties of an extracellular Polyhydroxybutyrate depolymerase from Pseudomonas mendocina DSWY0601. Chemical Research Chinese Universities, 28(3): 459-464.

[46]. Weisse, M.E.; Bass, J.W; Jarrett, R.V. and Vincent, M.J. (1991). Non- Anthrax Bacillus infections of central nervous system. Pediatr. J. infect. Dis.10: 243-246.
[47]. Wijnands, L.M., Pielaat, A., Dufrenne, J.B., Zwietering, M.H. & van Leusden, F.M. 2009. Modelling the number of viable vegetative cells of Bacillus cereus passing through the stomach. Journal of Applied Microbiology. 106: 258–267
[48].Zigha, A., Roesnfield, E., Schmitt, P. & Duport, C. 2006. Anaerobic cells of Bacillus cereus F4430/73 respond to low oxidoreduction potential by metabolic readjustments and activation of enterotoxin expression. Archives of Microbiology. 185(222): 23