Detection of proteolytic activity of *Bacillus spp*. isolated from cooked and raw food in Khartoum State, Sudan.

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Abstract

The present study was carried out to detect the proteolytic activity of *Bacillus* species isolated from some sources (beef, milk, chicken, egg and rice). Period of sampling extended from 22/11/2016 to 15/1/2017. A total of fifty isolated samples were collected randomly from public restaurants in Khartoum state, 10 samples from each source, 5 were freshly cooked (10-30 minute before sampling) and 5 were raw. According to the primary and secondary biochemical tests, the total isolated Bacillus from 50 samples was 20 isolates which comprised 40% of total samples. They were *Bacillus circulan* 5%, *Bacillus cereus* 5%, *Bacillus megaterium* 10%, *Bacillus macerans* 10%, *Bacillus licheniformis* 5%, *Bacillus pamilus* 5%, *Bacillus subtilis* 20%, *Bacillus coagulans* 15%, *Bacillus laterosporus* 5%, and *Bacillus amyloliquefaciens* 20%. After isolation of *Bacillus spp*. the investigation was continued to detect Protease production using milk agar medium, the most productive organism was *Bacillus macerans* and the lowest one was found to be *Bacillus amyloliquefaciens* whereas there was no production by *Bacillus circulans*. The study concluded that Bacillus species were found in all food sources so bacillus genera consider a major cause of food contamination. As well as Cooked food is most contaminant by bacillus than raw food.

Keywords: Protease, Food safety, *Bacillus*, Food contamination.

Introduction

Food safety has been so important nowadays due to increasing in numbers of consumers of manufactured food; also there are huge numbers of food industries, restaurants and super markets which sell canned food in entire world. So there are concerns about contamination rates, which affect firstly the quality of food, secondly the contaminant organisms mostly responsible for many food borne diseases [1].

According to (WHO) report which estimate the rate of food borne diseases as many as 600 million, or almost 1 in 10 people in the world, fall ill after consuming contaminated food, also the same report says approximately 420,000 people die including 125,000 children under the age of 5 years.

Proteases are degradative enzymes which catalyze the specific and selective modifications of proteins which were characterized for some gram positive species often playing a role as important virulence factors.

Recently due to specificity of their action Proteases has attracted worldwide attention in attempts to exploit their physiological and biotechnological applications [2]. Protease represents one of the largest groups of industrial enzymes and account 60% of the total worldwide sale of enzymes [3]. Generally proteases produced by Bacillus spp. are most important due to the stability of their enzymes under different environmental conditions.

Bacteria species represent the large source of contamination which leads to food borne disease [4]. Effect and degree of disease ranging from slight, moderate and severe illness, including (enterotoxaemia, vomiting, nausea and diarrhea) [5]. Bacillus species are playing an important role in food borne illness specially *Bacillus cereus*, which causing enterotoxaemia beside that, this species and others also responsible for ruin and degrade protein content in foods using their protease enzyme [6]. The objectives of this study were isolation and identification of *Bacillus spp*. from food sources (meat, milk, chicken, egg and rice), study the morphological properties and biochemical variation among species of Bacillus, and detection of the ability of isolated *Bacillus spp*. to produce protease enzyme.

Materials and Methods

Collection of samples

Samples of (beef, chicken, egg, rice and milk) cooked and raw were collected as swabs (10) samples from each source, five were raw and five were freshly cooked to give a total of fifty (50) samples, then the swabs were transferred in ice box to the Microbiology laboratory (Tables 1-5).

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***Received:** 01-June-2022, Manuscript No. AAFMY-22-59226 ;**Editor assigned:** 07-June-2022, PreQC No. AAFMY-22-59226 (PQ); **Reviewed:** 21-June-2022, QC No AAFMY-22-59226; **Revised:** 27-June-2022, AAFMY-22-59226 (R); **Published:** 04-July-2022, DOI:10.35841/aafmy-6.4.116*

***Citation:** Towor Eldow AB. Detection of proteolytic activity of Bacillus spp. isolated from cooked and raw food in Khartoum State, Sudan. J Food Microbiol. 2022; 6(4):116*

Table 1.*Bacterial isolates from egg samples.*

Number	Boiled egg	raw egg
1	*B. amyloliquefaciens*	No growth
2	*B. coagulans*	No growth
3	*B. amyloliquefaciens*	No growth
4	*B.macerans*	No growth
5	gram +ve cocci	No growth

Table 2. *Bacterial isolates from meat samples.*

Number	Cooked meat	raw meat
1	*B. megaterium*	gram+ve cocci
2	*B.licheniformis*	gram+ve cocci
3	No growth	gram+ve cocci
4	No growth	gram+ve cocci
5	*B.subtilis*	gram+ve cocci

Table 3. *Bacterial isolates from milk samples.*

Number	boiled milk	Raw milk
1	No growth	*B.pamilus*
2	No growth	*B. megaterium*
3	No growth	*B.cereus*
4	No growth	*gram+ve cocci*
5	No growth	*Pseudomonas*

Table 4.*Bacterial isolates from chicken samples.*

Number	Cooked chicken	Raw chicken
1	*B.laterosporus*	gram+ve cocci
2	*B.macerans*	gram+ve cocci
3	*B.coagulans*	gram+ve cocci
4	*B.subtilis*	gram+ve cocci
5	*B.subtilis*	gram+ve cocci

Table 5. *Bacterial isolates from rice samples.*

Number	Cooked rice	raw rice
1	*B. amyloliquefaciens*	*B.circulans*
2	No growth	No growth
3	*B. coagulans*	No growth
4	*B. subtilis*	No growth
5	*B. amyloliquefaciens*	No growth

Table 6. *Biochemical reactions of isolated bacillus.*

Test \isolate	1	2	3	4	5	6	7	8	9	10
Oxidase	-	2	-	-	-	-	+	-	+	-
Citrate	-	-	+	+	-	+	-	+	-	-
Urease	-	-	-	W	-	-	-	+	-	-
Growth in Starch	+	W	-	+	+	+	+	+	+	+
Carbohydrates,acid from ASS	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Glucose	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	-	W+	+	+
Glactose	+	+	+	+	+	+	-	+	+	+
Salicin	+	+	+	W+	+	+	+	+	+	+
Xylose	+	W	+	W+	+	+	-	+	+	+
Growth in 50c	+	W	+	-	-	+	-	+	+	+
Growth in 10% NaCl	+	+	+	-	-	+	+	-	-	+
Motility	+	-	+	+	+	+	+	+	+	+
VP	+	+	+	-	-	+	+	-	+	-
Milk agar	+	-	+	+	-	+	+	S+	+vs	+s

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Table 7. *Size of zones around colonies on skim milk agar medium.*

Organism	Hydrolysis zones (mm)
B. amyloliquefaciens	13
B. subtilis	25
B. coagulans	22
B. megaterium	29
B. macerans	39
B. licheniformis	33
B. cereus	26
B. pamilus	30
B. laterosporus	32
B. circulans	No hydrolysis

Table 8. *Distribution of isolated bacillus between raw and cooked sample.*

Source	No. of raw	No. of cooked	Percentage (%)
Meat	Zero	3	15%
Milk	3	Zero	15%
Chicken	Zero	5	25%
Egg	Zero	4	20%
Rice	1	4	25%
Total	4=20%	16=80%	

Methods

The identification was conducted using motility test as well as biochemical tests were performed such as catalase, oxidase, citrate utilization, V. P reaction, urease activity, starch hydrolysis, and sugar fermentation. Protease production was observed by culturing of all isolated species on milk agar medium for 24 hours at 37°C, and then the protease production was observed by formation of halo zone around colonies.

Results

The total isolated *Bacillus* from 50 samples was 20 isolates which comprised 40% of total samples. They were *Bacillus circulan* 5%, *Bacillus cereus* 5%, *Bacillus megaterium* 10%, *Bacillus macerans* 10%, *Bacillus licheniformis* 5%, *Bacillus pamilus* 5%, *Bacillus subtilis* 20%, *Bacillus coagulans* 15%, *Bacillus laterosporus* 5%, and *Bacillus amyloliquefaciens* 20%. According to the primary and secondary investigation there were no growths of *Bacillus* genus in raw egg, raw meat, raw chicken, and boiled milk. All isolates were motile, ferment sugar except *B.cereus*, and morphologically identical but there were some biochemical variations among species, as showed in tables 6-8.

Discussion

According to our findings this study agreed with Allaf (2010) and Narhi (1986) who studied presence of bacillus in (meat, milk, egg in addition to cheese and air) in which results showed that all the sources were contaminated with Bacillus spp but here the sources were categorized into cooked and raw so results shows differences in numbers of isolates in both cooked and raw putting into consideration process in which sources undergo [7-10].

Conclusion

The current results did not agree completely because researchers found that the heat treated milk going to be contaminant with *B. cereus* isolates it is recovered based on spore counts, while here there is no existence of bacillus at all perhaps because they collect large sample size in different seasons and their emphases by using high molecular techniques like PCR.

References

1. Chen Y, Jackson KM, Chea FP, et al. Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. J Food Prot. 2001;64(1):72-80. `
2. Sana B, Ghosh D, Saha M, et al. Purification and characterization of a salt, solvent, detergent and bleach tolerant protease from a new gamma-Proteobacterium isolated from the marine environment of the Sundarbans. Process Biochemis. 2006;41(1):208-15.
3. Kazan D, Denizci AA, Öner MN, et al. Purification and characterization of a serine alkaline protease from Bacillus clausii GMBAE 42. J Ind Microbiol Biotechnol. 2005;32(8):335-44.
4. Paranthaman R, Alagusundaram K, Indhumathi J. Production of protease from rice mill wastes by Aspergillus niger in solid state fermentation. World J Agri Sci. 2009;5(3):308-12.
5. Cheesbrough M. District laboratory practice in tropical countries, part 2. Cambridge university press. 2005.
6. Chouyyok W, Wongmongkol N, Siwarungson N, et al. Extraction of alkaline protease using an aqueous two-phase system from cell free Bacillus subtilis TISTR 25 fermentation broth. Process Biochemistry. 2005;40(11):3514-8.
7. Al-Allaf MA. Isolation of Bacillus spp. from some sources and study of its proteolytic activity. Tikrit j pure sci. 2011;16(4):1813-662.

Citation: *Towor Eldow AB. Detection of proteolytic activity of Bacillus spp. isolated from cooked and raw food in Khartoum State, Sudan. J Food Microbiol. 2022; 6(4):116*

8. Narhi LO, Fulco AJ. Characterization of a catalytically self-sufficient 119,000-dalton cytochrome P-450 monooxygenase induced by barbiturates in Bacillus megaterium. J Biol Chem. 1986;261(16):7160-9.
9. Doyle MP, Diez-Gonzalez F, Hill C, editors. Food microbiology: fundamentals and frontiers. John Wiley & Sons; 2020.
10. Bartoszewicz M, Hansen BM, Swiecicka I. The members of the Bacillus cereus group are commonly present contaminants of fresh and heat-treated milk. Food Microbiol. 2008;25(4):588-96.