University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 13, Issue 1 (2024) PP 152-158

https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/utjagr.v13i1.314

Effect of watery extract of Curcuma longa powder (Turmeric) on multidrug- resistant Bacillus cereus recovered from fresh soft cheeses in Kirkuk

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Abstract

The frequency and distribution pattern of Bacillus cereus in cow soft cheese were investigated indifferent regions in Kirkuk. 50 Pooled soft cheeses samples for five months in Kirkuk were collected during February until June (2020), ten samples collected from different regions for five months were contaminated with B. cereus Based on cultural, morphological, and biochemical characteristics, Susceptiple to resistant genotypes 31 isolates **15(48)%** were resistant to Ampicillin ,ClX, AZM and VA but mostly sensitive to VA; versus 8(**4.50)%** in the 5 month were isolates 10 and then 7 **(42.8)%**) resistance to antibiotics from the 4 months and isolates 6(**50)%**) from 3 months. Total bacterial Count were a significant effect between of soft cheese samples ranges from 5.2 x 10^{4} to 5.68 x 10^{11} CFU/g. These results are higher than Iraqi Quality Standards (Microbiological Limits in Food IQS(ICOSQC ,1988)especially in June 5^{th} months were mean 6.63 CFU/g. The microbiological results indicated that showed the highest total bacteria counts were indicate of adequate preventive measures and personnel hygiene in dairy processing lines to minimize B. cereus load for better hygienic and keeping quality of soft cheese.

Keywords : foodborne bacteria Bacillus cereus , biofilm, antibiotics resistance,

I. INTRODUCTION

Curcumin Is extensively used as an aromatic medicinal cosmetic .It has been in traditional use and it is mentioned as a remedy for various diseases. These findings indicated that action of curcumin had significant inactivation effect on foodborne bacteria, the present study investigate action of curcumin on foodborne bacteria Bacillus cereus (B. cereus) (Roberts and Tyler, Blumenthal et al., 1998;Mukherjee, 2002; Bodeker et al., 2005; Bandaranayake, 2006; Calapai, 2008; Braun et al., 2010).Soft cheese is a popular food all over the world. It is typically consumed within 3-4 weeks of manufacturing in Iraq. In rural areas and remote villages, traditional un ripened soft cheese is made from unpasteurized milk. Since raw milk contains around 30% of the total microbial count of undesirable microorganisms, this issue means that strict hygienic precautions must be followed in cheese production (Abdulghani and Kareem, 2019).

Food products are subject to spoilage by undesirable microbes through out harvest, production, storage, and distribution. Dairy products, because of their nutritional value, particularly their high protein and fat content, provide an ideal development environment for a wide range of microorganisms (Lasloand Gyorgy, 2018: Abdul aali and Alobaidi 2020). Many entero pathogenic species have been found in milk and cheese that has been refrigerated and eaten without being heated. High levels of pathogenic microorganisms in cheese may be caused by post pasteurization contamination, the manufacturing and handling process, equipment, and temperature abuse during transportation, and storage conditions (Araújo et al. 2002; Pal and Shukla, 2003; WHO, 2005a Ismail,et.al.2016;). Food spoilage endangers human health and causes enormous economic losses . The type of spoilage microorganism is primarily determined by the type of dairy product(Abdulla and al Alobaidi 2020). The microbiological content of a product is correlated to the manufacturing steps. Fungi and spore-forming bacteria are linked to cream cheese and

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processed cheese. Soft, fresh cheese spoilage is the associated with lactic acid bacteria, psychrophilic , coliforms, and their enzymatic degradation (Bandaranayake, 2006). While traditional soft white cheese is considered healthy, it could be a good medium for infectious microbes. The risk of contamination is a problem internationally and not restricted to one area.

Although some herbal medicines have promising potential and are widely used, many of them remain untested and their use not monitored (WHO, 2002a; WHO, 2002b; WHO, 2004; Kong et al., 2003). It is also common knowledge that the safety of most herbal products is further compromised by lack of suitable quality controls, inadequate labeling, and the absence of appropriate patient information (Blumenthal, Goldberg, and Brinckmann 2000;Raynor et.al,2011).

many of the plants from which Medicines derived from plants have played a pivotal role in the health care of many cultures, both ancient and modern (Newman, Cragg, and Sander 2003; Butler 2004; Balunas and Kinghorn 2005; Gurib-Fakim 2006; Newman and Cragg 2007).

Turmeric is a product of Curcuma longa, a rhizomatous herbaceous perennial plant belonging to the ginger family Zingiberaceae, which is native to tropical South Asia (Mills and Bone 2000). It lends curry its distinctive yellow color and flavor. (Bundy et. al, 2004), for treatment of diseases such as familial adenomatous polyposis in the bowels, for treatment of inflammatory bowel disease (Hanai and Sugimoto 2009); and in the colon, for treatment of colon cancer (Cruz-Correa et al. 2006; Naganuma et al. 2006). For arthritis, dosages of 8–60 g of fresh turmeric root three times daily have been recommended (Fetrow and Avila 1999).

Four extracts of Curcuma longa rhizomes were evaluated for their anti- bacterial action against pathogenic bacteria of gram-negative (Escherichia coli, Salmonella typhimurium) and gram- positive (Staphylococcus aureus, Bacillus cereus) comparing with antibiotics (gentamycin, ampicillin and erythromycin).(Kasetsart,2010).

Therefore the aim of the study In conclusions. cheese production in Kirkuk are encountered by the problems of bacterial contamination and presence of multidrug resist strain of Bacillus cereus that considered a risk to public health, may be due both insufficient and misuse treatment with antibiotics or in somewhat due to bad quality and/or poor hygiene of processing involved in milk production. was to undertake to evaluate the microbiological quality and of traditional soft cheese of Kirkuk in Iraq.

II. MATERIALS AND METHOD

Collection and Processing of Samples: All chemicals and media were purchased form Oxoid, UK . Fifty (50) Locally produced fresh-sweet cheese (home-made from raw unheated milk) with their risky whey collected for five months (10 samples in one month). They pooled aseptically in sterile non-permeable & non-durable plastic bags (500-1000) ml and transported in icebox to a milk hygiene laboratory as soon as possible within two hours. Then refrigerated at 4 °C for (2) days

Soft Cheese Samples were emulsified by 2% Buffered Sodium Citrate inside a Stomacher for 3 minutes ,then inoculated on Tryptone Soya agar- Yeast Extract(TSB-YE) as one part sample (20 ml) to 9 parts (180 ml) broth, then mixed well for two minutes and incubated at 37 °C for 24-48 hours for resuscitation of stressed cells, then streaked on Bacillus cereus chrome agar.

Then Counting and Confirmation of isolates by Gram stain, Catalase, Oxidase, Capsule. Then gram positive, rod shaped features ,spore-forming bacterium that preserved in slant bottles inside a refrigerator as pure seeds for further identification .

Biofilm Formation Assays by Freeman et al., 1989 had described modified method of screening biofilm formation , A modification was done by replacing BHI agar with double-strengthen TSA-YE (8 g Tryptone Soya Agar + 1 g Yeast Extract/100 ml d. w.) supplemented with 5% sucrose (5 gm/100 ml) and Congo red (10 gm/L) for better results. Plates were inoculated and incubated for 24 to 48 hr. at 37°C. Positive result was indicated by black colonies with a dry crystalline consistency. Weak slime producers usually remained pink, though occasional darkening at the centers of colonies observed. A darkening of the colonies with the absence of a dry crystalline colonial morphology indicated an intermediate result.

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Antiprogram Assay :A Kirby-Bauer technique or disk diffusion method was dependent according to instructions of Clinical Laboratory Standards Institute (CLSI) or National Committee for Clinical Laboratory(NCCLS) by using a Muller Hinton agar and McFarland opacity tubes(Bauer et al.,1966; CLSI. 2009).

Mueller-Hinton agar should be prepared from a commercially available dehydrated base according to the manufacturer's instructions (Oxoid-UK). In standard method five well-isolated colonies of the same morphological type are selected from a TSA-YE agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, TSB-YE. The broth culture is incubated at 37°C until it achieves or exceeds the turbidity of the 0.5 McFarland standards (usually 2 to 8 hours) and for (18-24 hours) for Bacillus cereus. The turbidity of the actively growing broth culture is adjusted with sterile phosphate buffered saline (PBS) or broth to obtain turbidity optically comparable to that of the 0.5 McFarland standard. a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab. The dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking three times,. As a final step, the rim of the agar is swabbed. The lid may be left ajar for (3-5) minutes, but no more than fifteen minutes . Then applicate 5-6 selected discs to inoculated agar plates. The plates are inverted and placed in an incubator set to 37°C within fifteen minutes after the discs are applied After (16-24) hours of incubation .

Extracts assay: Preparation of watery crude extracts of curcum in final concentrations: 0.5%. prepare **Mannitol salt egg yolk agar**:Suspend 12g in 100 ml of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Then mixing one egg yolk with 25 ml warmed phosphate buffer saline then add half amount to the media. Extracts (curcum):Boiling 0.5 liter of water the added the herb (curcum) 105 grams lets to boiling for half hour then filtrated with tea strainer.

III. Conclusions

Statistics:

The results were analyzed statistically, determining using completely randomized design (CRD) . The significance of differences between groups was verified by the Duncan multiple range test; Levels of significance: $p < 0.05$ = non-significant (ns), using SAS program(SAS,2010)

IV. Results and dissection :

Logarithmic scale for the means of the microbial counts during five months of the study (Table 1) Total bacterial Count were a significant effect between of soft cheese samples ranges from 5.2 x 10^4 to 5.68 x 10^{11} CFU/g. These results are higher than Iraqi Quality Standards (Microbiological Limits in Food IQS(ICOSQC ,1988)especially in June 5th months were mean 6.63 CFU/g and agreement with previous studies 9×10^5 CFU/g. Alper and Nesrin (2013) Al-Manhal (2013) demonstrated that the5.2 x 10^4 to 5.68 x 10^{11} CFU/g.

Table (1): Means of total bacterial counts(TBAC) in soft cheese in supermarket in kirkuk.

months	Samples NO: means	Means of total bacterial counts Log TBAC cfu /grams
1	10	5.06
2	10	5.37
3	10	5.62
4	10	5.89
5	10	6.63
P< 0.05		

Depending on guidelines of disc diffusion method and instructions of CLSI, including standrized reference table of susceptibility of Bacillus cereus to selected antibiotics ;the recovered colonies were categorized into versatile groups: Susceptiple to resistant genotypes 31 isolates **15(48)%** were resistant to Ampicillin ,ClX, AZM and VA but mostly sensitive to VA; versus 8(**4.50)%** in the 5 month were isolates 10 and then 7 **(42.8)%**) resistance to antibiotics from the 4 months and isolates 6(**50)%**) from 3 months .table (2)

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Table(2): Resistant (R) Intermediate (I) and susceptible (S) antibiotics segregation chain.

month	Recoverd B.cereus		AMP (10µ)			CLX (5µ)			AZM (5µ)			VA (30µ)		
			R	I	S	R	I	S	R	I	S	R	I	S
1	10	5	3	0	2	2	2	1	0	3	2	0	3	2
2	10	5	2	2	1	3	1	1	1	2	2	1	1	3
3	10	6	3	2	1	3	3	0	3	2	1	2	2	2
4	10	7	3	3	1	3	2	1	5	1	1	2	4	1
5	10	8	4	4	0	3	4	1	4	2	2	3	2	2
Total	50	31			15			14			13			8

Development of resistance in these recovered isolates might occurs due to accumulative sub lethal stress or selective pressure of antibiotics or heat processing or acidic brined environment controlled by regulatory genes. abuse or missing treatments of mastitis hidden un prophylaxes of subclinical mastitis recurrent infections (alyais and al-shammary2019).beta lactems such as ampicillin inhibit bacterial growth by altering a group of enzymes called penicillin –binding proteins on cell wall synthesis cross link side chains of peptidoglycan peptides.

Table(3): Resistance profile index of recovered B.cereus.

.	Recoverd B.Cereus strains	Resistance Profile Index%
1	**5**	**3(60)%^{C}**
2	**5**	**2(40)%^{D}**
3	**6**	**3(50)%^{C}**
4	**7**	**3(42.8)%^{B}**
5	**8**	**4(50)%^{A*}**
Total	**31**	**15(48)%**

*indicate highest resistance profile index in the last month (5)

Most experimentally processed as in table (3) thirty one multidrug resistant isolates verified as intermediate to resistant and acquired immune barriers especially neutrophils .biofilm recovered clones were more thick and recalcitrant from soured yogurt and brined soft cheese. Data reveled that presence of resistance behavior in recovered B. cereus isolates .modified sensitivity –susceptibility assay segregate bacteria into susceptible ,intermediate and resistant phenotypes. Selected antibiotics in the current study reflect flexibility and complexity of the test procedure in which five types of different ordinary to new generations of antibiotics were selected and used in veterinary and human medicine(figer1).

The results confirmed recovery of In conclusions. cheese production in Kirkuk are encountered by the problems of bacterial contamination and presence of multidrug resist strain of Bacillus cereus that considered a risk to public health, may be due both insufficient and misuse treatment with antibiotics or in somewhat due to bad quality and/or poor hygiene of processing involved in milk production. Thus recommended monitoring these products for better hygienic status.

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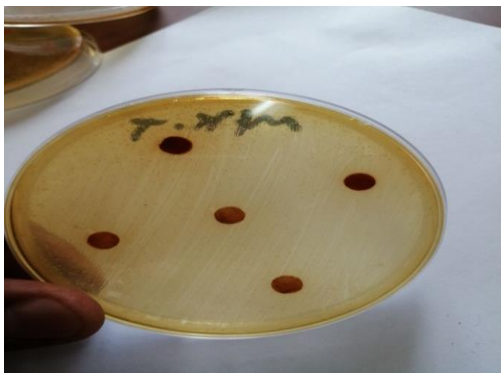

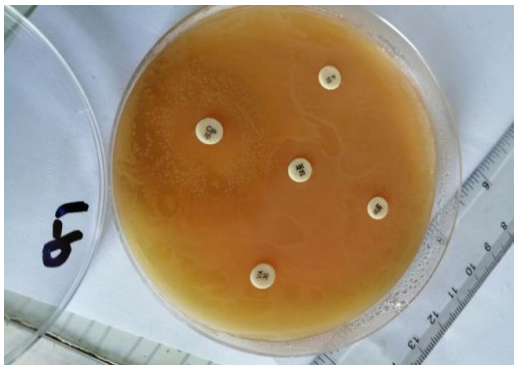

Figer(1): antibiotics reflect flexibility and complexity of the test procedure in which five types of different ordinary to new generations of antibiotics were selected and used in veterinary and human medicine.

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