Identification and characterization of bacterial and fungal isolates in raw milk samples from different breeds

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Keywords:

Bacillus
Penicillium
Lactis
Lactobacillus
Aspergillus

Article history:

Received 27.09.2015
Received in revised form 11.12.2015
Accepted 24.12.2015

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Abstract

Introduction. This study was conducted to identify and name the fungal and bacterial isolates in raw milk samples from different breeds.

Materials and methods. Milk samples were collected from White Fulani breed, New Jersey breed and the breed mixture (White Fulani and New Jersey breed). The samples were further grouped into four and were pasteurized at 71◦C for 15seconds, 66◦C for 15minutes and 61◦C for 30minutes using pasteurizer made of aluminium, stainless steel and galvanized steel

Results and discussion. The raw samples were also identified and characterized for bacterial and fungal isolates; *Staphylococcus aureus, Bacillus substilis, Euterobacter aerogenes, Escheria coli, Streptococcuss lactis, Proteus vulgaris, Pseudomonas aeruginose, Serratia marcescens*, and *Lactobacillus ferment* for bacterial isolates and *Aspergillus flavus, Aspergillus niger, Penicillium citrinium, Sacchanomyces Cerevisae, Paecilomyces Varioti, Penicillium Chrysogenum* for fugal isolates. *Staphylococcus aureus* can be seen to be 78.79% present in the total samples before and after pasteurization. *S. auereus* was seen in the raw samples i.e. the White Fulani breed, New Jersey breed, the breed mixture and the locally fermented samples (nono), making them highest. The following percentage shows the distribution of the other bacterial present; *B. subtillis* (6.06%), *E. aerogenes* (42.42%), *E coli* (3.03%), *S. lactis* (48.48%), *P. vulgaris* (18.18%), *P. aeruginosa* (30.30%), *S.marcescens* (33.33%), *L. fermentum* (30.30%). Furthermore, the percentage distribution of the fungi present from table 4.4 are; *A. flavis* (54%), *A. niger* (18.18%), *P. citrinum* (21.21%), *S. cerevisae* (57.58%), *P. varioti* (15.15%), *P. chrysogenum* (6.06%). The best treatment combination within the scope of this research work in order to get low bacterial counts is pasteurizing at 71◦C using stainless steel for 15seconds, indicating high temperature short time pasteurization. Also, in order to get low fungi counts, the temperature of 61°C should be used for pasteurizing using a stainless steel for 30minutes.

Conclusion. Nine bacterial isolates and six fungi isolates were identified and characterized in raw milk samples from the different breeds

Introduction

Measurement of bacterial numbers in milk is of interest because they are indicator of poor milk hygiene production or ineffective pasteurization of milk. Some microbes such as gram negative Psychrotrophs, Coliforms and other pathogenic bacteria such as Escherichia Coli ,Staphylococcus aureus may also be found in milk.

The hygienic quality of milk at the point of production is also of importance from both public health and consumer perception points of view. For milk to be produced with a low bacterial count the temperature must be kept low until the point of processing. The milk is contaminated after pasteurization, usually through unsanitary handling of the milk. An example of post-pasteurization contamination involving a multi-drug resistant strain of *Salmonella Typhimurium* occurred in Pennsylvania and New Jersey in 2000 [1]

The microorganisms which cause spoilage in milk, which is intended to be sterilized (UHT-treatment) are either resistant types that have survived the heat treatment, or organisms that have contaminated the product after the sterilization process. Contamination spores are however, likely to be less heat resistant than those which might survive the heat treatment. The pasteurization equipment fails and there is raw milk in the product sold as pasteurized. This can happen if the temperature is not high enough, or if the milk is not heated long enough. For example, in 1984, an outbreak of *Salmonella Typhimurium* occurred in a convent in western Kentucky [2].

There were 16 illnesses and one patient developed a Guillain-Barretype illness. The convent had a steam pasteurizer and investigators believe that the temperature may not have been high enough and/or the holding time was too short. The convent had no timetemperature gauge to record and monitor the process. The milk is contaminated after pasteurization, usually through unsanitary handling of the milk. An example of postpasteurization contamination involving a multi-drug resistant strain of *Salmonella Typhimurium* occurred in Pennsylvania and New Jersey in 2000 [1]

UHT treatment of milk leads to a much larger production of small sized casein micelles compared to raw or pasteurized milk [3]**.**

Biochemical processes involved are "heat resistance" and reactivation of natural and bacterial proteases and survival of bacterial spores [3]**;** [4]**.**

Proteolysis of UHT milk during storage at room temperature is a major factor limiting the shelf life through changes in its flavor and texture [5]**.**

The problem of post treatment contamination of "in container" sterilized product can either be through "poor seal" or through "pin hole" in the container. Post treatment contaminants in UHT milk may be either by spores which would not be expected to be heat resistant enough to survive the heat treatment or non-heat resistance vegetative organisms. Organisms of the first type will probably have entered from the ineffective sterilization of plant downstream from the heat treatment stage of the process, which includes spores of *Bacillus cereus* [6] and [7]. Organisms of second type will probably have entered through poorly sealed container after aseptic filling. The types of spores, which have been investigated as of particular relevance in the UHT, are those of *Bacillus Stearothermophillus, Bacillus Subtilis* and *Clostridium Botulinum* has been studied. The high spore counts can occur at the dairy farm and that feed and milking equipment can act as reservoirs or entry points for potentially highly heat resistant spores into raw milk. Lowering this spore load by good hygienic measures could probably further reduce the contamination level of raw milk, in this way minimizing the aerobic spore forming bacteria that could lead to spoilage of milk and dairy products[8]

The purpose of this work is to identify and characterize the bacterial and fungal isolates in raw milk samples from different breeds

Objectives of research:

-to determine the pasteurizing temperature and time that will favour low fungal and bacterial counts in raw milk samples

-to determine the pasteurizing materials that will favour low fungal and bacterial counts

Materials and methods

The various material and devices used in this work and the basis of their selection as well as some of their standard properties are discussed as follow:

Aluminium Pot. This is one of the material used to hold milk during pasteurization. The basis for it selection is not unconnected to some of it fundamental properties that are relevant to this research work. Aside its properties, it is selected due to its availability. It is readily available in different capacities. In terms of its properties, aluminium is highly resistant to corrosion and has a thermal conductivity "k" of 99.99% for pure aluminium is 244 W/mK for the temperature range 0-100^{0}C. Since this work is base on heat treatment, use of material that can easily transfer heat is necessary. Milk is also made up of 95% water in it composition hence the need to select a material that effectively resist corrosion. Other properties of aluminium that is of interest include: density of 2700kg/m^3, and approximate specific heat capacity of 900 J/kgK [9]**.**

Stainless steel pot. This is another material used as medium for holding milk during the pasteurization process. It is also readily available. It selection was base on its high corrosion resistance capability, good thermal conductivity (average of 15w/m^{0}C for all grades). It has a specific heat capacity of 500J/kgK on the average and a density of about 8.03kg/m^3.

Galvanized Steel Pot. Steel on its own is corrosive when if contact with water. Due to the high water content of milk, the galvanized steel pot is coated with Zinc-Aluminium alloy to prevent reaction of the milk with the steel. Steel has a thermal conductivity of 58.9W/mK, specific heat capacity of 420J/kgK and a density of 7900kg/m^3.

Heating Medium. An electric stove with an AC voltage of 220V, a frequency of 50/60Hz and a thermal coil element rating of 1000w was selected for heating the milk at regulated temperatures. The cost of the stove is relatively cheap and it is available and can provide the desired power rating required.

K-type Thermocouple. The thermocouple is a sensor attached to the material and connected to a temperature regulator. It senses the temperature of the milk and conveys this information to the regulator which then adjust the temperature if necessary to a predetermined set point. The K-type (Chromel - Alumel) was selected because of unlike other types of thermocouples (B, C, E, J, N, R, S, T types), it is well suited for oxidizing atmospheres; that is, it resist corrosion and has a useable temperature range of 95^{0}C to 1260^{0}C. It is has a good degree of sensitivity of 39µV/ºC, durable and readily available [10]**.**

Temperature Controller. The temperature controller is a device used to maintain the desired temperature for the different pasteurization treatments of the milk. It was selected because of the need to maintain the different temperatures for specific periods. It works on the principles of a temperature control loop. The sensor (k-type thermocouples) measures the temperature of the milk to be controlled and converts the measured value into a travel

signal. The information is received by the regulator and compared to the set point (pasteurizing temperature) and make adjustment when necessary.

Preparation of culture Media. The media to be used for this analysis are Nutrient Agar (NA) for total bacteria, Mac Conkey agar for enumeration of coliform bacteria, Eosin Methylene Blue agar for fecal coliform enumeration, Demann Rogossa Sharpe agar for enumeration of lactobacillus, Yeast Extraction agar for enumeration of yeast and Potato Dextrose Agar (PDA) for enumeration of fungi. count. The said culture media were prepared in line with the manufacturer's instruction. The colonies were counted and associated microorganisms were isolated, characterized and identified according to the techniques described by [11] in the laboratory manual of microbiology.

Results and discussion

Identification and Characterization of Bacterrial Isolates. Table 4.1 and 4.2 showed the identification and characterization of bacterial and fungal isolates from raw milk samples respectively.

Table 1

Table showing the identification and characterization of bacterial isolates

Isolates	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Gram reaction	+	+	-		+	-	-
Catalase	+	+	+	+	+	+	+
Mobility Test	+	+	+	+	+	+	+
Methyl red Test	-	+	+	+	-	+	-
Voges Preskauer Test	-	+	+	+	-	-	-
Oxygen relationship	FA	FA	FA	FA	FA	AE	FA
Indole Test	+	-	-	+	-	+	-
Urease Test	-	-	-		-	+	+
Citrate Utilization Test	-	+	-	-	-	-	+
Coagulase Test	+	-	-	-	-	-	-
Oxidase Test	-	-	-	-	-	-	-
Starch Hydrolysis Test	+	+	-	-	+	-	-
Hydrogen sulphide test	-	-	-	-	-	+	-
Glucose	AG	A	AG	A	A	AG	-
Sucrose	A	A	AG	AG	A	AG	-
Lactose	A	A	AG	AG	A	-	-
Maltose	A	A	AG	AG	A	-	-
Fructose	A	A	AG	AG	A	-	-
Probable Organism	*Staphylococcus aureus*	*Bacillus subtillis*	*Enterobacter aerogenes*	*Escherichia coli*	*Streptococcus lactis*	*Proteus vulgaris*	*Pseudomonas Aerugeinose*

+ = Positive, AE= Aerobic, - = Negative, FA = Facultative Anaerobic, A= Acid Production, AG =Acid and Gas Production

Table 2

Table showing the fungal isolated from milk samples

	Surface Texture	Pigmentation	Under Surface	Tentative identification
AL_A	Powdery	Greenish yellow	Creamy	*Aspergillus flavus*
AL_B	Powdery	Black	Creamy	*Aspergillus niger*
AL_C	Powdery	Greenish Blue with Narrow margin	Creamy	*Penicillium citrinium*
AL_D	Smooth	Creamy	Creamy	*Sacchanomyces Cerevisae*
AL_E	Powdery	White	Creamy	*Paecilomyces Varioti*
AL_F	Powdery	Greenish Blue with Wide margin	Creamy	*Penicillium Chrysogenum*

+ = Positive, AE= Aerobic, - = Negative, FA = Facultative Anaerobic, A= Acid Production, AG =Acid and Gas Production

As shown in the table 4.1 and 4.2, the samples were subjected to gram reaction, catalase test, mobility test, methyl red test, voges presk auer test, oxygen relationship test, indole test, urease test, citrate utilization test, coagulase test, oxidase test, starch hydrolysis test, hydrogen sulphide test. Also, sugar fermentation test for glucose, sucrose, lactose maltose and fructose as well as the various identification and characterization of bacterial and fungi isolates were done on the samples according to the technique described by **[**11]. Organisms like *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Streptococcus lactis, Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens*, *Lactobacillus fermenum* and *Enterobacter aerogenes* were present as bacterial. Furthermore, organisms like Aspergillus flavus, Aspergillus niger, Penicillium citrium, Saccharomyces cerevisae, Paeccilomyces varioti and Penicillium chrysogenum were identified and characterized as fungi specie.

Distribution of bacterial species in the pasteurized milk samples. Table 3 and 4 showed the distribution of bacterial species and fungal species respectively in the milk samples.

Table 3

Table showing the distribution of bacterial species in the pasteurized milk samples

Sample Code	Bacterial species								
	Staphylococcus aureus	*Baccilus substilis*	*Enterobacter aerogenes*	*Escherichia coli*	*Streptococcus lactis*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Sematia marceseceus*	*Lactobacillus fermentum*
M1T1S1	+	-	-	-	-	+	-	+	-
M1T1S2	+	-	-	-	+	-	-	-	-
M1T1S3	+	-	-	-	+	-	-	+	-
M1T2S1	+	-	+	-	+	-	-	+	-
M1T2S2	+	-	-	-	+	-	+	-	-
M1T2S3	-	-	-	-	+	-	-	-	+
M1T3S1	-	-	+	+	-	-	+	+	-
M1T3S2	+	-	-	-	+	-	+	-	-
M1T3S3	+	-	-	-	-	-	+	-	-
M2T1S1	-	-	-	-	-	+	-	+	-
M2T1S2	+	-	+	-	-	+	-	-	-
M2T1S3	+	-	-	-	+	-	-	-	-
M2T2S1	-	-	-	-	-	+	-	+	+
M3T2S2	+	-	-	-	+	-	-	-	+
M2T2S3	+	-	+	-	-	-	-	-	+
M2T3S1	-	+	-	-	-	-	-	-	-
M2T3S2	+	-	-	-	-	-	-	-	-
M2T3S3	+	-	-	-	-	-	-	+	+
M3T1S1	+	-	+	-	+	-	+	-	-
M3T1S2	+	-	+	-	-	-	-	-	+
M3T1S3	+	-	+	-	-	-	-	-	-
M3T2S1	-	-	+	-	+	-	-	+	-
M3T2S2	+	-	-	-	-	-	-	+	-
M3T2S3	+	-	+	-	+	+	+	-	-
M3T3S1	-	-	+	-	-	-	-	-	+
M3T3S2	+	-	+	-	+	-	-	-	-
M3T3S3	+	-	-	-	-	+	-	+	+
RAW S1	+	-	+	-	+	-	-	-	-
RAW S2	+	+	-	-	+	-	+	-	-
RAW S3	+	-	-	-	+	-	+	+	-
FM S1	+	-	+	-	-	-	+	-	+
FM S2	+	-	+	-	+	-	-	-	+
FM S3	+	-	-	-	-	-	+	-	-

(+)= Present, (-)=Negative

Table 4

Table showing the distibution of fungal species in the pasteurized milk samples

Sample Code	Fungi species					
	Aspergillus flavus	*Aspergillus niger*	*Penicillium citrinum*	*Saccharomyces cerevisae*	*Paecilomyces varioti*	*Penicillium chrysogenum*
M1T1S1	+	-	+	-	-	-
M1T1S2	-	-	-	+	-	-
M1T1S3	+	-	-	-	-	-
M1T2S1	+	-	+	-	-	-
M1T2S2	-	-	-	+	-	-
M1T2S3	+	+	-	-	-	-
M1T3S1	+	+	+	-	-	-
M1T3S2	-	-	-	+	-	-
M1T3S3	+	-	-	-	-	-
M2T1S1	-	-	-	+	-	+
M2T1S2	+	-	-	+	-	-
M2T1S3	-	+	-	-	-	-
M2T2S1	+	-	+	-	-	-
M3T2S2	+	+	-	+	+	-
M2T2S3	+	-	-	+	-	-
M2T3S1	+	-	+	-	-	-
M2T3S2	+	-	-	-	+	-
M2T3S3	-	-	-	-	+	-
M3T1S1	-	-	-	+	-	-
M3T1S2	-	-	-	-	+	-
M3T1S3	+	-	+	+	-	-
M3T2S1	+	-	-	-	-	+
M3T2S2	+	+	-	-	-	-
M3T2S3	-	+	-	+	-	-
M3T3S1	+	-	-	+	-	-
M3T3S2	+	-	-	+	-	-
M3T3S3	+	-	+	+	-	-
RAW S1	-	-	-	+	+	-
RAW S2	-	-	-	+	-	-
RAW S3	-	-	-	+	-	-
FM S1	-	-	-	+	-	-
FM S2	-	-	-	+	-	-
FM S3	-	-	-	+	-	-

(+)= Present, (-)= Negative

M=Materials (**M1** = Aluminium, **M2**= Stainless steel, **M3**=Galvanized Steel), T=Temperature (T1=71°C, T2=66°C, T3=61°C) and S=Source (S1=White Fulani, S2= New Jersey, **S3**=Mixture), MT=Material and and Temperature Combination, MS=Material and Source Combination, TS=Temperature and Source Combination, MTS=Material, Temperature and Source Combination, **TVC**=Total Viable Counts, **CC**=Coliform Counts, **FCC**=Feacal Counts, **LBC**=Lactobacillus Counts, **FC**=Fungi Counts, df=Degree of Freedom

From table 3, *Staphylococcus aureus* can be seen to be 78.79% present in the total samples before and after pasteurization. *S. auereus* was seen in the raw samples i.e. the White Fulani breed, New Jersey breed, the breed mixture and the locally fermented samples (nono), making them highest. The following percentage shows the distribution of the other bacterial present; *B. subtillis* (6.06%), *E. aerogenes* (42.42%), *E coli* (3.03%), *S. lactis* (48.48%), *P. vulgaris* (18.18%), *P. aeruginosa* (30.30%), *S.marcescens* (33.33%), *L. fermentum* (30.30%). Furthermore, the percentage distribution of the fungi present from table 4.4 are; *A. flavis* (54%), *A. niger* (18.18%), *P. citrinum* (21.21%), *S. cerevisae* (57.58%), *P. varioti* (15.15%), *P. chrysogenum* (6.06%)

The presence of these large number of microflora suggests the extent to which the milk is contaminated by the animal, environment and the milking utensils [12]. The Fulani herdsmen do not disinfect the teats and udders prior to milking despite the fact that the cow lie in muddy barnyard and dirty environment which inevitably contaminate the milk and increase the microbial load [16]. [13] reported that organism associated with the beddings materials which contaminate the surface of teats and udders includes Staphylocci, Spore formers, coliforms, Streptococci and other Gram negative bacteria. The sampled raw milk has high microbial load probably due to the insanitary condition of the environment or post pasteurization contamination.

During this research, *E. coli* was found to be minimal indicating a very low fecal contamination showing a good milk hygiene. Coliform counts between 100 and 1000 are generally an indication of poor milking hygiene as the coli count less than 100 per ml of milk is considered acceptable for raw milk for pasteurization [14]. [15] also reported bacterial and fungi isolated from raw milk and pasteurized milk samples in his research done in Ilorin and it surroundings, in Kwara state, Nigeria. These isolates are similar to the isolates in this research work.

Conclusions

This study focused on the identification and characterization of bacterial and fungi isolates in raw milk samples from different breeds. It can be concluded that organisms like *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Streptococcus lactis, Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens*, *Lactobacillus fermenum* and *Enterobacter aerogenes* were present as bacterial. Furthermore, organisms like Aspergillus flavus, Aspergillus niger, Penicillium citrium, Saccharomyces cerevisae, Paeccilomyces varioti and Penicillium chrysogenum were identified and characterized as fungi specie.

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