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Thermoduric Bacteria in Milk- A Review

Amitha Thomas[1], Prasad V[2]

Department of Dairy Science, College of Veterinary and Animal Sciences, Mannuthy
Kerala, India

Veterinary and Animal Sciences University, Kerala, India

Abstract: ***Thermoduric bacteria are organisms capable of surviving the industrial pasteurization process of milk, and can carry over into product causing quality defects, or creating health hazards. The various species of genus Bacillus, Microbacterium, Micrococcus, Enterococcus, Lactobacillus and Corynebacterium are described as heat resistant species (Marth and Steele, 2001). A thermoduric bacterium in milk is significant due to their ability to survive the pasteurization temperature and subsequently carried to pasteurized milk. The organisms may cause loss of economy to the farmers by spoilage of the milk or may cause serious health hazards to the consumers.***

Keywords: thermoduric bacteria, milk, dairy plant

1. Introduction

Thermoduric bacteria in milk are the microorganisms which are able to withstand the pasteurization temperature. The various species of genus *Bacillus, Microbacterium, Micrococcus, Enterococcus, Lactobacillus and Corynebacterium* are described as heat resistant species (Marth and Steele, 2001). Sharma and Anand (2002) studied on the distribution pattern of microflora in dairy plants. The study revealed that the predominant organism was genus *Bacillus* in both Commercial Plant (CP) and Experimental Dairy Plant (EDP) with a distribution pattern of 37 per cent and 44 per cent, respectively. The other microflora of biofilms of CP included species of *Lactobacillus, Streptococcus, Lactococcus and Staphylococcus.* While in EDP in addition to *Bacillus*, additionally had *Micrococcus.*

2. Bacillus

Bacillus species are rod-shaped, endospore-forming aerobic or facultatively anaerobic, Gram-positive bacteria; in some species cultures may turn Gram-negative with age. The many species of the genus exhibit a wide range of physiologic abilities that allow them to live in every natural environment. Only one endospore is formed per cell. The spores are resistant to heat, cold, radiation, desiccation, and disinfectants. The studies of Kerala Varma (1949) on the organisms surviving the boiling of milk revealed the Bacillus as the only species withstanding boiling and the major species were *B. subtilis*, *B. cereus*, and *B. megaterium*. *Bacillus cereus was* first described by Frankland *et al*. (1887) following its isolation from air in a cowshed. Davies and Wilkinson (1973) found the best heat activation temperature for *B. cereus* to be 90 - 94^{0}C for 15 second. Also a higher percentage of *B. cereus* spores germinated in dialysate of pasteurized milk than in dialysate of raw milk. Thus, it seems that the growth of *B. cereus* in milk is dependant on, not only the individual strain, but also the degree of heat treatment. Mukunadan (1978) carried out isolation of organisms surviving the boiling of milk in Thrissur area and reported the heat resistant spore former present in the milk is various species of *Bacillus*. The frequency of distribution was *B. subtilis* (48 percent), *B. cereus* (14 percent) and *B. megaterium* (13 percent).

Due to the ability to cause 'bitty' or 'broken' cream and 'sweet curdling', *B. cereus* has become a significant limiting factor for the shelf life of pasteurized milk products (Crielly *et al.*, 1994). *Bacillus cereus* is a widely distributed organism and one of the most important endospore forming species found in pasteurized milk .Thermoduric, spore forming *Bacillus* species, which are frequently associated with spoilage of pasteurized milk and milk products, can survive pasteurization in the spore form (Frank, 1997). Spores of *B. cereus* are reported to possess a pronounced ability to adhere to the surface of stainless-steel equipment commonly used in food processing. Giffel *et al.* (1997) showed that spores of *B. cereus* adhered, germinated and multiplied on stainless-steel surfaces in a tube heat exchanger.The prevalence of *B. cereus* in pasteurized milk was higher during the summer (May-October) than during the winter period (November-April) also the prevalence of *B. cereus* increased gradually during spring (Larsen and Jorgensen, 1997).The results of Lin *et al.* (1998) showed that the raw milk is the majorsource of *B. cereus* in pasteurized milk and that post pasteurization contamination along the milk processing line is possibly a minor source. Lindsaya *et al.* (2000) isolated *Bacillus* species from alkaline wash solutions used for CIP in South Africa dairy factories, have been suggested to contaminate surfaces of dairy processing equipment and result in post pasteurization spoilage of milk and milk products. The pasteurized milk may be spoiled by spore formers, e.g. *Bacillus spp*., which have survived heat treatment or entered the milk process after heat treatment, re contaminating the milk (Faille *et al*, 2001). Heat stable spores of *B. cereus* in milk are a source of contamination for milk derived products, such as milk powder and infant food formulas (Shaheen *et al.*, 2006). Bartoszewicz *et al*. (2008) suggested that the fluctuation in contamination rates during different seasons could be dependent on the relative occurrence of *Bacilli* in the farm and industrial environments, and in particular, seasonal variations of microbial populations on teats and milking equipment.

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16S rDNA sequencing is one of the most commonly used methods to identify and determine the phylogenetic relationship of bacterial species. The use of 16S rDNA for studying phylogenetic relationships discovered during the mid seventies and, with the advent of sequencing, it became an important tool for bacterial identification. Many identification techniques are now based on PCR amplification and sequence analysis of the 16S ribosomal genes (Woese *et al.*, 1975). One disadvantage of using 16S rDNA for identification and phylogenetic studies is that it cannot differentiate between closely related species. Radhika *et al*. (2002) isolated strains of *B. cereus* from different food items from local shops of Mysore city and the total DNA was extracted from 1.5 ml aliquots of culture broth. The PCR has performed and a 0.9 Kb product was obtained, which then sequenced as *B. cereus*. A total of 65 of 83 *Bacillus* type strains were used for the study and the partial 16S rRNA gene sequence was determined using standard 16S rRNA gene primers for PCR and sequencing. To determine the current validity of a sequence-based method for identification and provide contemporary data, PCR and sequencing of a 500-bp of the 16S rRNA gene were undertaken using 65 of the 83 type strains of this genus (Blackwood *et al.*, 2004). Joo *et al.* (2007), isolated 45 *Bacillus* strains from the traditional Korean soybean-fermented food, Chungkookjang and seven strains were selected and identified based on morphological, physiological, and biochemical characteristics. To confirm the identities of the isolates, PCR amplification and sequencing of the 16S rRNA gene were performed using the bacterial universal primer set of 27f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492r (5'-GGC TAC CTT GTT ACG ACT T-3'. The 1.4 kb sequences obtained from the strains were aligned with all the presently available 16S rDNA sequences in the GenBank database.

B. cereus can cause two types of food borne illness, a diarrhoea syndrome and an emetic syndrome. Both syndromes have occasionally been associated with dairy products. Additionally, cytotoxin K (CytK), a 34 kDa protein with hemolytic and necrotic activities, also contributes to *B. cereus* intoxication (Lund *et al.*, 2000).*B. cereus* and *B. weihenstephanensis* strains that synthesize cereulide are responsible for emetic syndromes in humans. Though usually mild, with durations of 6–24 h (Lund *et al.* 2000), cereulide-induced emesis has been implicated in at least one case of child mortality (Dierick *et al.*, 2005). Apart from the presence of enterotoxin genes in all the *B. cereus* group members, little is known about the potential contributions of *B. thuringiensis* and *B. mycoides* to human diseases (Swiecicka and Mahillon, 2006). For milk consumers, the presence of toxigenic determinants in bacterial isolates is of obvious significance. Several studies have demonstrated the occurrence of enterotoxigenic *B. cereus* in the natural environments, in hospitals and in food products (Hendriksen and Hansen, 2006.) Not only horizontal gene transfers are known to occur for the cereulide gene among *B. cereus* isolates in various substrates like food, but also the potential for toxigenic gene transfers may exists (Auwera *et al.*, 2007).

3. Micrococci

Thermoduric bacteria most commonly found in pasteurized milk are generally some species of *Micrococci.* These thermoduric or heat-resistant *Micrococci* usually find their way into a milk supply from improperly cleaned and sterilized utensils at the producing farm. Milking machines have been found to be a very common source of these organisms (Hileman, 1940). Cowan and Steel (1964) classified *Staphylococci* and *Micrococci* on the ability to produce acid from carbohydrate under strict anaerobic conditions and in the semi anaerobic conditions of the sealed tube in Hugh and Leifson's medium. The catalase-positive gram positive, cocci that ferment glucose under anaerobic conditions can be regarded as *Staphylococci*, and those that fail to do so were *Micrococci.* Phenotypic characters can also be very useful in the classification and identification of the species of *Micrococcaceae.* Furthermore, numerical taxonomy has been used successfully to examine strains of *Micrococci* and *Staphylococci* isolated from different habitats (Sierra *et al.*, 1995). *Micrococci* possess no public health risk and are not mentioned in the regulations. However, spoilage of milk and its products were reported by these organisms isolated from goat's milk (Rozanda *et al.*, 1996).

4. Enterococcus

Enterococci are potential pathogens because some strains of the species *E. faecalis var. zymogenes* and even *E. faecium* can produce enterotoxins and may be the causative agents of food borne illness (Gracia *et al.*, 2002). Ahmad *et al.* (2002) observed the heat adaptation of *Enterococcus faecalis* and *E. faecium* at different temperatures, 65, 67.5 and 70^{0}C. The results shown that *E. faecium* was found more resistant to heat at 65, than 67.5 and 70^{0}C . Cells of *E. faecalis* survived at 65^{0}C for 20 min and at 67.5^{0}C for 10 min, while at 70^{0}C cells survived for five minutes . *E. faecium* isolate was found more heat tolerant as compared with the *E. faecalis.* McAuley *et al.* (2012) observed that two isolates each of *Enterococcus faecalis, Enterococcus faecium, Enterococcus durans* and *Enterococcus hirae*, exhibiting the median and the greatest heat resistance. McAuley (2012) tested 12 E*nterococci* isolates from six factories for their relative ability to survive laboratory pasteurisation. *E. faecalis, E. faecium, E. durans* and *E. hirae* were represented in all of the factories, except Factory D, in which *E. faecalis* was not isolated from the laboratory pasteurized milk.

5. Lactobacillus

Lactic acid bacteria (LAB) used in food fermentations are able to inhibit or kill food-borne and other pathogenic microorganisms as well as simple spoilage bacteria by producing a variety of antimicrobial agents. Acidity is probably in most cases the primary factor in the preservation of lactic acid-fermented foods (Adams and Nicolaides, 1997). *Enterococci* and *Lactobacilli* are diverse groups of bacteria, many of which are ubiquitously found in the intestinal microflora of humans and animals For this reason, they are common in environments contaminated by human and animal faecal material as well as in food products

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derived from animals (Kagkli *et al*., 2007). Ortu *et al.* (2007) identified 16 non-clonal *Lactobacilli* that are Gram-positive, catalase negative, non-motile isolates were analyzed to the species level by 16S rRNA gene sequence analysis and biochemical profiles.

6. Corynebacterium

The lipophilic species *Corynebacterium bovis* is frequently isolated from milk samples in many dairy farms. It is associated with very mild forms of mammary inflammation. Slightly increased somatic cell counts in the milk is usually the only manifestation of these infections. A new lipophilic oxidative species *C. mastitidis*, and a new fermentative species *C. camporealensis*, have been recognized from the milk of sheep and these bacteria are also associated with subclinical mastitis (Hommez *et al*., 1999). Aaku *et al.* (2002) collected raw and bottled commercial pasteurized milk from two processing plants in Gaborone, Botswana for assessing for mesophilic, psychrotrophic, proteolytic and lipolytic bacteria. The isolates of *Corynebaterium* degraded urea and fermented glucose, maltose, mannose and starch but not mannitol, salicin, sucrose, teharalose and xylose. The bacteria were positive for catalase, aesculin hydrolysis, methyl red, gelatin liquefaction, arginine hydrolysis and citrate, but negative for oxidase. *C. diphtheriae* is a gram positive, fermentative pleomorphic rod, catalase positive, urease negative, and nitrate reduction positive, haemolytic on blood agar and produce acid from glucose, sucrose maltose and xylose (Ahmed *et al*., 2012). Handling of infected dairy animals and consumption of contaminated milk have been associated with respiratory diphtheria like disease caused by *C. ulcerans*. Furthermore *C. ulcerans* causes alimentary intoxications and cutaneous lesions in humans. In routine work *C. ulcerans* could be misidentified with *S. intermedius*, because of cultural similarity, positive plasma coagulation tube test and absence of manitol fermentation of both species. (Suvajdzic *et al.*, 2012). The milk can be contaminated with *Corynebacterium* species by direct passage from the blood into the milk (systemic infection), mastitis (udder Infection), faecal contamination, external contamination from skin, environmental contamination of the milk during or after milking (Vandenplas, and Herman, 2012).

7. Streptococcus

The genus *Streptococcus* contains gram positive cocci, non motile, non spore forming, arranged mostly in chains or in pairs. Most species are facultative anaerobes and some of S*treptococci* are encapsulated. Aysrs and Johnson (1914) studied the survival of Streptococci at high temperatures and the survival rates were 99.28 per cent at 54.5^{0}C, 84.89 per cent at 57.20^{0}C, 64.03 per cent at 57.20^{0}C, 33.07 per cent at 62.80^{0}C , 2.58 per cent at 71.10^{0}C and 0 per cent at 73.9^{0}C, when maintained at constant temperature for 30 minutes. Devriesea *et al.* (1999) isolated Gram-positive, catalase-negative, aesculin degrading cocci from clinical and subclinical mastitis cases in dairy cows and characterized them to species level and the data obtained are, *Streptococcus* (52 per cent), *Enterococci* (26 per cent), *Aerococci* (15 per cent) and the remaining strains were *Lactococci*. Thirty four per cent of the strains belonged to the species *Streptococcus uberis*. *Enterococcus faecalis* (20 per cent) and *Aerococcus viridans* (15 per cent) were the second and third most frequently isolated species. According to Michaylova *et al*. (2002) *S. thermophilus* is frequently isolated from dairy environments, and they isolated different strains from plant samples in Bulgaria and it is a “Generally Recognized As Safe” (GRAS) species in food. (Facklam, 2002). *S. agalactia* is an obligate intramammary pathogen that causes mainly subclinical mastitis with corresponding economic losses to the dairy industry. But it is found that the isolation frequency of *S. agalactiae* from cows with clinical mastitis was 0.7 per cent, which was the lowest among mastitis pathogens (Maroney, 2005). *S. thermophilus* is the dominant species in starters used for producing fermented milk products and a large variety of cheeses. Despite of their low frequency of occurrence of spontaneous phage resistant mutants, it was possible to isolate a total of 100 spontaneous phage-resistant variants from 12 commercial phage-sensitive *S. thermophilus* strains under the selective pressure of specific phages (Binetti *et al.*, 2007). *Streptococcus* spp. cause a variety of diseases in humans including streptococcal pharyngitis, pyoderma, abscesses, cellulitis, endocarditis, polyarthritis, pneumonia and septicemia. Most human infections are associated with group A *Streptococci*, which are usually *S. pyogenes*. Many human infections by *Streptococci* are linked to the consumption of unpasteurized dairy products. Bacteria with the group D Lancefield antigen have been associated with an acute, self-limiting gastrointestinal illness characterized by diarrhoea, abdominal cramps, nausea, vomiting, fever, chills and dizziness. The illness is food borne and usually occurs after an incubation period of 2 to 36 hours (USFDA). Zotto *et al*. (2009) studied the growth and survival of three different *S. thermophilus* strains ctsR, hrcA and Sfi39 on different stress responses like heat, acid and oxidative stress. The data obtained have shown that numbers of survivors after heat stress for strains ctsR and hrcA were significantly higher than those of Sfi39. Acid adaptation resulted in a slight increase in heat tolerance for strain hrcA but not for ctsR and acid adaptation of ctsR was similar to Sfi39. Strain ctsR was slightly less tolerant of osmotic stress compared to Sfi39 and hrcA.

8. Conclusion

The occurance of thermoduric organisms in milk and milk products should me minimized as it cause many zoonotic diseases to the consumer. It is also important as it may cause severe economic loss to the producers by causing spoilage of the dairy products.

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Author Profile

Dr. Amitha Thomas: Presently working as Research Assistant in Dept. Of Dairy Science, Kerala Veterinary and Animal Sciences University (KVASU). Completed Masters in Veterinary Science From the same University. Areas of Interest include Dairy microbiology, probiotic dairy products, Quality control of milk and milk products etc.

Dr. V. Prasad is former Professor and Head, Department of Dairy Science, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University.

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