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Faculty of Agronomy
Mendel University in Brno

Czech Ministry of Agriculture
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Plant Nutrition Commission of the Plant Production division, Czech Academy of Agricultural Sciences
Czech Society of Soil Science
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Dept. of Agroenvironmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague
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Dept. of Agricultural Chemistry, University of Agriculture in Krakow
Dept. of Applied Plant Biotechnologies, University of South Bohemia in České Budějovice

cordially invite you to the international conference

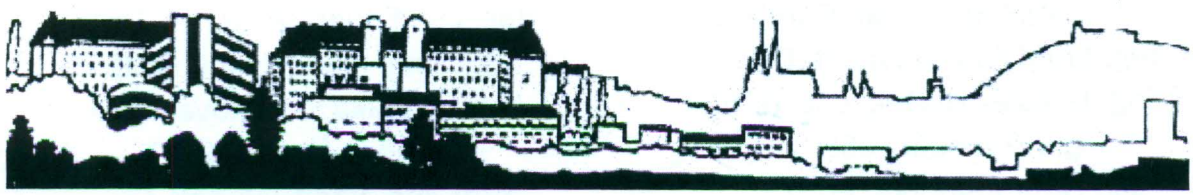
SOIL, PLANT AND FOOD INTERACTIONS

Mendel University in Brno

to be held

Faculty of Agronomy

6 – 8 September 2011



FIRST ANNOUNCEMENT

The international conference focuses comprehensively on mutual relations between soil, plant and fertilizers, directed at the production of quality consumables and consumables' raw materials. This fundamental function of agriculture (the production of sufficient quantities of quality, affordable and healthy food) must not be underestimated, namely with regard to the need for the production of healthy, high nutrition value consumables in the future. Nevertheless, it is necessary to respect principles of sustainability, with an emphasis on extra-production, and the social and environmental dimensions of agriculture. In order to sustain soil fertility, as soil's primary property, the immediate impact comprises particularly microbiological, physical and chemical properties, which can be significantly influenced by human activity. Therefore, the basic and applied research in these fields must focus on gaining new knowledge that will contribute to the higher competitive strength and economy of agricultural farms.

A number of new findings, especially in fields of plant nutrition, soil science and soil, and food microbiology will be presented at the conference by means of oral presentations and posters.

Main topics of the conference

Agrochemistry and plant nutrition

Soil Health

Soil and food microbiology

Preparatory Committee

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PRESENCE OF MICROORGANISMS IN SHEEP MILK PRODUCTS

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ABSTRACT

The aim of this study was isolation and identification of *Enterobacteriaceae* genera isolated from sheep milk products from conventional farm of Slovakia. Total of 45 food samples including cheese (n = 15), sheep cheese (n = 15) and parenica (n = 15) were obtained. *Enterobacteriaceae* genera isolation was performed by classical plates method. For more detailed biochemical identification we used ENTEROtest 24. Among 45 samples we isolated and identified 30 isolates of *Enterobacteriaceae* genera. 18 isolates were identified in fifteen of sheep cheese samples. From cheese samples were isolated six isolates of *Klebsiella oxytoca*, six isolates of *Serratia odorifera* bv. 1 and six isolates of *Serratia odorifera* bv. 2. In ship cheese (bryndza) three isolates of *Citrobacter braakii* and from parenica nine isolates of *Escherichia coli* were found and identified.

Key words: pathogens, microorganisms, sheep milk products, farm

Acknowledgements: This work was funded by Grant Agency VEGA 1/0372/09 and KEGA. 430-014SPU-4/2010.

INTRODUCTION

Milk is a suitable substrate for the growth of many pathogenic and toxicogenic microorganisms which may be the cause of foodborne diseases that can endanger the health of consumers (Bobková et al., 2008). Coliforms bacteria can not survive during pasteurization. If coliforms bacteria survive during pasteurization, it is indicative to the lack of pasteurization (Havlová et al., 1993). However, Görner and Valík (2004) distinguish the contamination of raw milk to primary and secondary. The primary source of bacteria contamination including tanks udder and teat channels, microorganisms from the surface of udder, from body and excrement of animal, from feed and dust, as well as hand and dairyman clothing, microorganisms with which to be in contact with milk, during milking, transport in the pipelines, when is filtering, cooling and under storage. Secondary contamination depends on the initiation of cooling after milking, milk temperature and time in which microorganisms can metabolize in milk.

The conditions during storage and transport in refrigerated tanks cause the raw milk microbiota to change from predominantly Gram-positive to predominantly Gram-negative organisms as they grow. Gram-negative bacteria usually account for more than 90% of the microbial population in cold raw milk that has been stored. The Gram-negative flora is composed mainly of psychrotrophic species of *Pseudomonas, Achromobacter, Aeromonas, Serratia, Alcaligenes, Chromobacterium, Flavobacterium* and *Enterobacter* (Maurilio et al., 2006).

Most of these bacteria produce extracellular proteolytic and lipolytic enzymes that are secreted into the milk. Many of these enzymes are not inactivated by pasteurizing at 72°C for 15 s or by Ultra-High Temperature (UHT) treatment. The activities of these enzymes can reduce the organoleptic quality and shelf life of processed milk products. Although many different conventional testing methods have been developed for the detection and enumeration of bacteria in food, these have relied almost exclusively on the use of specific culture media followed by a series of tests for confirmation of isolates. Conventional plate count methods are laborious, time-consuming and sometimes underestimate the numbers of bacteria. To overcome these limitations, molecular biological, biochemical and immunological techniques have been applied for the rapid and specific detection of microorganisms in food (Maurilio et al., 2006).

Members of the genus *Klebsiella* are frequently incriminated in different infections. *Klebsiella pneumonie* was isolated from mastitic cows especially from those kept in wood products bedding (Carter, 1995). In humans, *K. pneumoniae* is an important cause of nosocomial infections like pneumonia, septicaemia, urinary tract and soft-tissue infections (Maslow et al., 1993; Podschun and Ullmann, 1998). The indole producing *Klebsiella oxytoca* (Maslow et al., 1993) was reported as an enterotoxigenic micro-organism and causes an antibiotic-associated haemorrhagic colitis (Minami et al., 1994).

The aim of this study was isolation and identification of *Enterobacteriaceae* genera isolated from sheep milk products from conventional farm of Slovakia.

MATERIAL AND METHODS

The samples were obtained from sheep milk products from once conventional sheep farm from Slovakia. Total of 45 food samples including cheese (n = 15), bryndza (n = 15) and parenica (n = 15) were obtained. This samples were collected by sterile cotton swabs (Copan Inovation, Brescia) and transported to laboratory (SUA in Nitra, Department of Microbiology).

Enterobacteriaceae genera isolation was performed by classical plates method. The first the MacConkey agar (Biomark, Pune) for *Enterobacteriaceae* genera isolation was used. Incubation was conducted for 24 hours at 37°C. After incubation on the MacConkey agar, we used Chromogenic coliform agar (Biolife, Italiana), XLD agar (Biolife, Italiana) and SS agar (MkB test, Rosina) and we choose the method of linear insulating. Incubation was conducted during 24 hours at 37°C. We repeated this step until we received completely clean culture of strains from *Enterobacteriaceae* genera.

Biochemical identification of Enterobacteriaceae genera

For more detailed biochemical identification we used ENTEROtest 24 (Pliva-Lachema, Brno), that includes TNW Lite 7.0 identification software (Pliva-Lachema, Brno). Preparation of indentification plates of ENTEROtest 24 was done inside the Laminaire box (ADS Laminaire, Le Pre-Saint Gervais) to ensure a high sterility, less risk of contaminations from the air and precise results. Working procedure of ENTEROtest 24 is described in the competent manual.

RESULTS AND DISCUSSION

The presence of pathogenic bacteria poses a serious problem in safety sustaining of dairy products. Microbiological routine controls of these products make use of selective culture techniques. The aim of this study was isolation and identification of *Enterobacteriaceae* genera isolated from sheep milk products from conventional farm of Slovakia. In our study fifteen samples of sheep cheese, fifteen samples of bryndza and fifteen samples of parenica from conventional farm in Slovakia were used. From 45 samples we isolated and identified 30 isolates of *Enterobacteriaceae* genera (tab. 1). The most isolates (18) were identified in fifteen sheep cheese samples. From cheese samples were isolated six isolates of *Klebsiella oxytoca*, six samples of *Serratia odorifera* bv. 1 and six samples of *Serratia odorifera* bv. 2. From bryndza three isolates of *Citrobacter braakii* and from parenica nine isolates of *Escherichia coli* were isolated and identified.

Most of microbial contaminants including human pathogens are members of the family *Enterobacteriaceae*. The most frequently diarrhoeal diseases are caused by *Shigella*, *Escherichia coli* and *Salmonella*. *Klebsiella pneumoniae* is a frequent cause of respiratory disease, and *Yersinia pseudotuberculosis* is associated with enterocolitis and peritonitis. Common sources of food contamination by this group of bacteria, especially coliforms that

include the genera *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobater*, are feces (of animal and human origin), personnel, water and containers (Omore et al., 2001).

Table 1 Percentage of strains identification and number of isolates from sheep products

Strains	Percentage of identification by TNW software	Isolated from	Number of isolates
Klebsiella oxytoca	98.96 %	cheese	6
Serratia odorifera bv. 1	96.92 %	cheese	6
Serratia odorifera bv. 2	100.00%	cheese	6
Citrobacter braakii	97.42 %	bryndza	3
Escherichia coli	100.00%	parenica	9

El-Sukhon (2003) in his work found three *Klebsiella* species, namely: *K. pneumoniae, K. oxytoca* and *K. rhinoscleromatis* which were evident in all examined dairy products, except in the imported samples. *K. pneumonie* showed the highest prevalence followed by *K. oxytoca* and *K. rhinoscleromatis*. In milk the highest prevalence of *Klebsiella* (59.7%) was detected compared to other products.

Total of 90 raw milk samples and dairy products made from raw milk were screened by PCR method for the presence of *Listeria monocytogenes, Escherichia coli*, enterotoxigenic E. *coli, Campylobacter jejuni* and *C. coli*. Detection rates were 12/90 (13%) for *L. monocytogenes*. 41/90 (46%) for *E coli*, 18/90 (20%) for enterotoxigenic *E. coli* producing heat-labile toxin type I or heat-stable toxin type I, and 6/90 (7%) for *C. jejuni* or *C. coli* (Allmann et al., 1995).

Total of 534 isolates distributed among 10 genera and 20 species were identified in the work of Yilma et al., (2007). *Klebsiella*, *Escherichia* and *Enterobacter* were dominant genera in their order of abundance with *E. coli*, the most isolated species. *Erwinia*, *Klyuvera* and *Providentia* were the least isolated genera. Most of the genera/species identified were isolated predominantly from milk samples followed by butter and buttermilk during the dry season and from milk, buttermilk and butter during the wet season in order of their abundance. This high level of contamination of milk might be due to initial contamination originating from the udder surface, wash water, milking utensils and materials used for filtering of the milk. Further contacts with other possible sources of contamination such as the churn, container and warm water added during churning coupled with time elapsed since milking might attribute to the high level of contamination of butter and buttermilk. The genera identified from samples of cleaning water and udder swabs include: *Enterobacter*, *Erwinia*, *Escherichia*, *Klebsiella*, *Salmonella* and *Serratia* the most isolated genus being *Escherichia* (*coli*) followed by *Klebsiella*. These genera are also isolated from samples of milk and milk products indicating a similar source of contamination (Yilma et al., 2007).

CONCLUSION

Provision of milk and milk products of good hygienic quality is desirable from consumer health point of view. Bacteria identified in the dairy products assessed represent not only spoilage of the products and indicate presence of potential human pathogens of similar growth characteristics but some of them can also cause infections.

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