CHAPTER 2

SCREENING OF SKIN MICROORGANISMS FOR THEIR COLD ACTIVITY

2.1  INTRODUCTION

In order to design alternative preservation methods for skins and hides, it is necessary to understand the process of skin degradation and also identify the microorganisms responsible for skin degradation on the whole and the microorganisms active at different stages of putrefaction. Though, chilling method is the most successful short term preservation followed in many Western countries historically, the microbiological aspects related to the method has not been studied and reported so far. It is necessary to study the growth profile of the microorganisms at different temperatures so that an effective method of chilling can be standardized. Such an approach is important especially in the case of tropical countries like India, where the ambient temperature is always above 30°C. Moreover, the microorganisms encountered in one country may be different from those responsible for the skin degradation in another country on account of difference in the climatic conditions.

2.1.1  The Course of Skin Putrefaction

Hides and skins are associated with a variety of bacteria. In living animals, these bacteria can not do harm due to physiological reasons. Hides and skins are subjected to spoilage if left untreated after death or flaying.
Although, it may not be immediately obvious if a hide or a skin is spoilt, the general characteristics of putrefaction are clear when contrasted with fresh material. Some of these include offensive smell, discoloration, slippery or slimy texture and hair slip.

The epidermis of the hide provides a barrier against penetration of bacteria during the lifetime of the animal. Bacteria usually enter the hides that have been damaged by abrasion or disease (Cooper et al 1972). After the death of the animal, the bacteria slowly migrate through the dermis and the underlying layers of the epidermis degrading the delicate junction between the two layers. At that stage, the hair or wool detaches from the dermis causing hair slip. So if the hide is exhibiting hair slip, it indicates the bacterial damage.

### 2.1.2 Identification of Putrefying Microorganisms - A Review

A wide variety of microorganisms are associated with the freshly flayed hide including yeasts, moulds and protozoa apart from bacteria (Mclaughlin et al 1921, 1925). They were found to be both proteolytic and non-proteolytic. The greatest damage to hide comes from the proteolytic type of bacteria. Numerous attempts have been made to identify and isolate microorganisms from putrefying skins and hides and study their growth profile (Woods et al 1970, Birbir and Ilgaz 1996, Orlita 2004). In one of studies, it was reported that more than 80 bacterial species have been identified on flayed hides and among the proteolytic bacteria, *Proteus vulgaris, Bacillus putrefaciens, Bacillus subtilis* and *Bacillus mesentericus* have been found to be common (Bienkiewicz 1983). In a recent study on network mode conducted in India, more than 130 aero and skin micro flora have been collected and specific organisms involved in skin spoilage have been recognized (Ramasami 2004). It has also been reported that five
microorganisms play important role in the onset of degradation as evidenced by the growth of these microorganisms in the freshly flayed skins and hides. Important conclusion in the study was that the *Bacillus* species are the dominant bacteria involved in the skin spoilage. *Bacillus* species identified in these studies include *Bacillus subtilis* and *Bacillus pumilus*. *Staphylococcus* and *Micrococcus* are the other bacteria reported. The recently concluded study in Indian goat skins also confirms that the *Bacillus* are the most dominant family of cultivable genus from fresh skins and account for more than 70% of the microorganisms present (Ganesh Babu 2007). Among the *Bacillus*, *Bacillus subtilis* was identified to be the dominant cultivable species and it represented 53.3% in the genus *Bacillus* and 38% in the total cultivable bacterial isolates in fresh goat skin. *Bacillus pumilus* was the second dominant bacterial species. *Bacillus sphaericus*, *Bacillus firmus* and *Macrococcus caseolyticus* are the other bacteria isolated and identified in that study.

### 2.1.3 Scope of the Proposed Study with Skin Bacteria

Hence, in the present investigation, it was proposed to select these five important bacteria present in the putrefying skins and study their growth profile at different temperatures. Another aspect, which would be addressed to in this investigation was whether these five bacteria do really have any collagen degrading ability or simply they feast on other non-collagenous proteins and grow on them. Collagen is the main fibrous protein present in the skin, which is of importance to the leather industry. The degradation of collagen was studied by following the hydroxyproline content in the degradation products. Hydroxyproline is the unique amino acid present in collagen.

It was also proposed to study their growth profile at low temperatures in the presence of some selected anti-microbial agents to check
if the duration of storage can be extended beyond that reported in earlier studies. Use of biocides for extending the storage period for the chilled hides has already been attempted and some of the biocides studied include biguanide, sodium chlorite and dithiocarbomate and they were used in solution used for ice making (BLC 1992). However, their inhibition against individual skin bacterial isolates at cold temperature has not been studied and hence, the scope of the present study has been extended to study this aspect as well.

On the whole, this study was expected to lead to a well-designed and scientifically optimized chilling method suitable for Indian conditions.

2.2 MATERIALS AND METHODS

2.2.1 Materials

2.2.1.1 Microorganisms

The microorganisms codenamed as AU1, AU2, AU3, AU4 and AU5 (Figure 2.1) were obtained from Alagappa University, Karaikudi, India and were originally isolated from raw goat skin and identified there (Table 2.1). These cultures were maintained in nutrient agar and used in the study.

<table>
<thead>
<tr>
<th>Code Name</th>
<th>Microorganism</th>
<th>GenBank Accession Number</th>
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<tbody>
<tr>
<td>AU 1</td>
<td><em>Bacillus sphaericus</em></td>
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<td>AU 3</td>
<td><em>Bacillus subtilis</em></td>
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<td>AU 4</td>
<td><em>Macrococcus caseolyticus</em></td>
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</tr>
<tr>
<td>AU 5</td>
<td><em>Bacillus firmus</em></td>
<td>EF032672</td>
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