

THE IMPACT OF HALOPHILIC ORGANISMS ON THE GRAIN QUALITY OF BRINE CURED HIDES

ABSTRACT

Halophilic organisms have long been thought to be associated with reduced grain quality of brine cured cattle hides. However, a direct correlation of the presence of halophiles and damage to surface of the grain has not been previously reported. In this research, a fresh cattle hide was cured in saturated brine to which an inoculum of several isolates of extremely halophilic organisms were added. These halophilic organisms were previously isolated from commercially brine-cured hides. After curing, samples prepared from the hide were stored at three different temperatures, 39°F, 70°F and 106°F. Half of the samples were tanned after four weeks of storage and the remainder of the samples were tanned after seven weeks. Physical testing showed that there was no difference in physical strength between the inoculated and uninoculated samples. Grain damage was observed visually in the samples held for seven weeks at 106°F. Scanning electron micrographs clearly show the nature of this damage.

INTRODUCTION

The presence of halophiles on brine cured hides has been well known for centuries. Halophilic organisms produce a pigment that gives a red color to the flesh side of the hide. Prior to understanding the actual cause of this condition, it was referred to as "red heat" because it only occurred during the warm summer months. The presence of "red heat" on hides was generally considered to be a sign that other organisms were also active on the hide and this could result

in hide damage if the hides were not tanned soon after the condition appeared.¹⁻⁷ Actual damage to the hide as a result of this condition was never proven experimentally.

Earlier work done in this laboratory⁸ on extremely halophilic (salt loving) bacteria demonstrated that these organisms are almost universally present on brine cured hides processed in the United States. More than 150 hides from over twenty curing sources were tested for halophiles and only three were found to be negative. Typical morphology of pure cultures of one of these extremely halophilic bacteria can be seen in Figure 1. Temperature-dependent growth studies demonstrated that under ideal culture conditions these organisms are capable of rapid growth at 106°F. The experiments reported in this paper were designed to demonstrate whether or not the growth of halophilic organisms on brine-cured cattle hides could be directly related to grain damage.

EXPERIMENTAL

CURING

A fresh hide was sided and fleshed. Each side was cut into six to eight inch wide segments perpendicular to the backbone, from backbone to belly. Most samples were approximately thirty inches long. All of the segments were placed in a Canbar[†] drum containing a 400% float of freshly prepared saturated brine (400 gm of brine/100 gm of hide). The drum was run at 4 rpm for 4 minutes each hour for eighteen hours. Additional dry salt was added to the drum at the beginning of the experiment to compensate for the moisture removed from the fresh hide samples and to ensure a saturated level of salt in the float.

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INOCULATION WITH HALOPHILES

Several halophilic isolates obtained from commercially brine cured hides were cultured in usual Norberg medium.⁸ Ten ml of culture medium from each of three extremely halophilic isolates was added to the drum along with the hide samples at the beginning of the cure. This was to ensure that a sufficient population of halophiles was present on the brine cured hide samples at the end of curing. Fresh hides were cured in saturated salt brine that had been inoculated with cultures of extremely halophilic organisms previously isolated from commercially brine cured hides. After curing, the hide was segmented and the cured hide samples were divided into three groups to be stored at three different temperatures until organoleptic observations (odor and visual condition) suggested that there was considerable growth of the halophiles on the samples.

STORAGE CONDITIONS

After draining the freshly cured segments overnight, the brine-cured samples were separated into three groups. One dozen samples were stored at each of the temperatures, 39°F, 70°F and 106°F.

STORAGE TIME

The first set of samples, six from each incubation temperature, were taken out of storage after four weeks. These samples were immediately put into lime to stop the growth of the halophiles and to begin the tanning process. The remaining brine-cured hide samples were tanned after seven weeks.

TANNING

All of the samples were processed into crust leather using the standard USDA procedure carried out in the pilot plant tannery at the Eastern Regional Research Center in Philadelphia.⁹ All of the samples were then tanned and examined for grain damage. Where damage was observed the samples were further processed for observation under the scanning electron microscope.

PREPARATION OF SAMPLES FOR THE SCANNING ELECTRON MICROSCOPE

Dry leather samples, 10 mm in diameter, were cut from the areas of visible damage on the crust hide samples held for seven weeks at 106°F. Similar areas were taken randomly

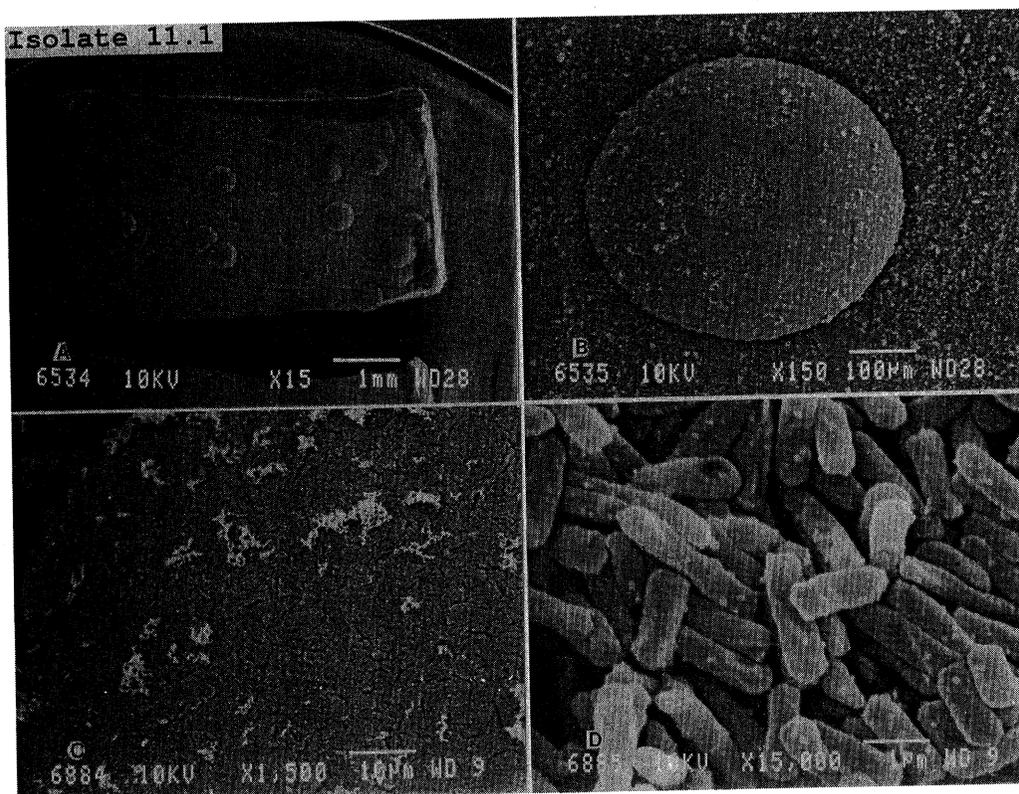


FIGURE 1. — Scanning electron microscopy of the colony morphology of a typical halophilic bacteria isolated from commercially brine cured cattle hides. A. Colonies on agar plate. B. Single colony with surface coating on edges and opening in center. C. Surface coating on edge of colony. D. Rod-shaped halophilic bacteria in the interior of the colony.

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from leather prepared from the hide segments held for four weeks at 39°F for the control samples. These samples were mounted on aluminum specimen stubs using colloidal silver adhesive, then coated with a thin layer of gold by DC sputtering. Frame-averaged secondary electron images at a magnification of from 100 to 500 X were digitized using an Imix-1 workstation, (Princeton Gamma-tech, Princeton, NJ) integrated to the scanning electron microscope. In addition to the surface of the sample, cross sections were also prepared for observation under the scanning electron microscope.

RESULTS

The grain surface of all of the leather samples was examined using a binocular light microscope (Bausch & Lomb, StereoZoom 7). The only samples that appeared to have more than the usual amount of grain damage associated with crust leather were from the hide samples that were held for seven weeks at 106°F. All six of the segments held at this temperature had some degree of damage that was clearly visible without the aid of the microscope (Figure 2).

Samples were removed from the damaged area and prepared for observation in a model JSM840A scanning electron microscope (Joel USA, Peabody, MA). Scanning electron photomicrographs clearly showed the suedeing effect caused on these samples by the halophilic bacteria. The top surface of the grain has been partially removed and the underneath collagen fibers had been loosened up and are visible on the surface (Figure 3). This disruption of the fibers is even more clearly seen in the cross-section through the damaged area, which shows the disruption of the fiber structure under the grain.

DISCUSSION

The type of suedeing we observed in the halophile damaged samples lowers the value of the leather and can represent a huge annual loss to the leather tanning industry. One of the reasons that the connection between grain damage and red heat has not been more closely associated with the presence of halophiles, is that hides exhibiting "red heat" are generally processed very quickly and little or no grain damage is observed when this is done.

This result is understandable in light of the growth characteristics of the extreme halophiles. The growth of extremely halophilic bacteria is very slow at room temperature.^{2,6,8,10} In these experiments, even though all of the samples were inoculated with halophiles, it was only after seven weeks of storage at high temperature that visible damage was observed. Even at four weeks there was no damage visible on the leather stored at the higher temperature. This suggests that although it is possible for sufficient halophiles to grow to the point of producing a visible red pigment it takes much more rigorous conditions (high temperatures and length of storage) for damage to occur. These extreme conditions are probably not reached often when hides are shipped within the United States.

On the other hand, it is much easier to envision such damage during the transportation of hides from the West Coast of North America to Southeast Asia, during which the necessary and adverse combinations of long storage periods coupled with high temperature conditions could occur. This means that even when the curing of hides is done properly, in a period of seven weeks under extreme temperature conditions, extremely halophilic bacteria can do severe damage to cattle hides.

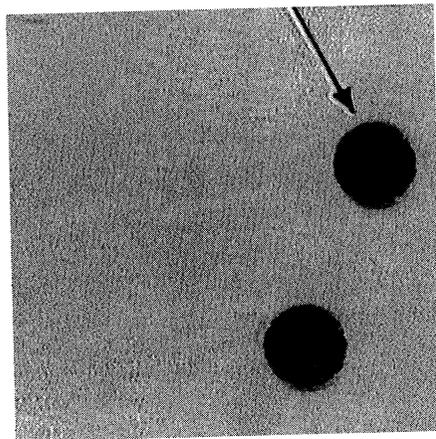


FIGURE 2. — Light microscopy of damage observed on the grain of crust leather by halophilic bacteria held for seven weeks at 106°F. Arrow denoted sample removed for scanning electron microscopy.

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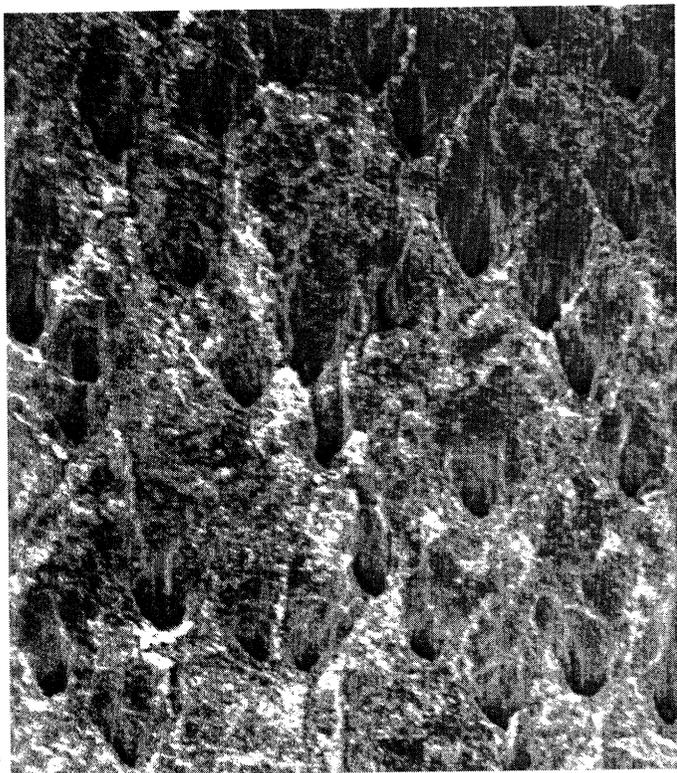


FIGURE 3. — Scanning electron micrograph of the grain surface of crust leather prepared from halophilic bacteria treated cattle hide of samples held for seven weeks at 39°F.



FIGURE 4. — Scanning electron micrograph of the cross section of crust leather prepared from halophilic bacteria treated cattle hide samples held for seven weeks at 39°F.

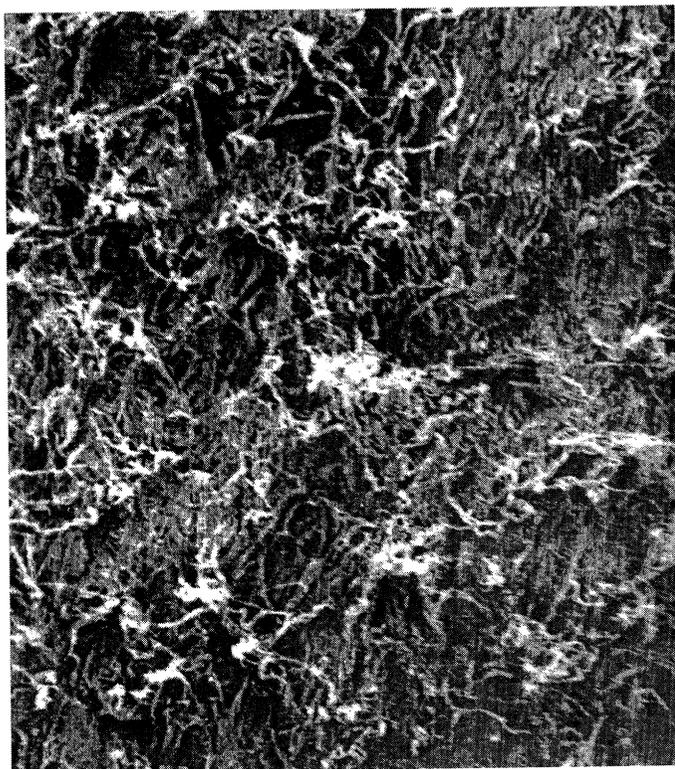


FIGURE 5. — Scanning electron micrograph of grain surface of crust leather prepared from halophilic bacteria treated cattle hide samples held for seven weeks at 106°F.

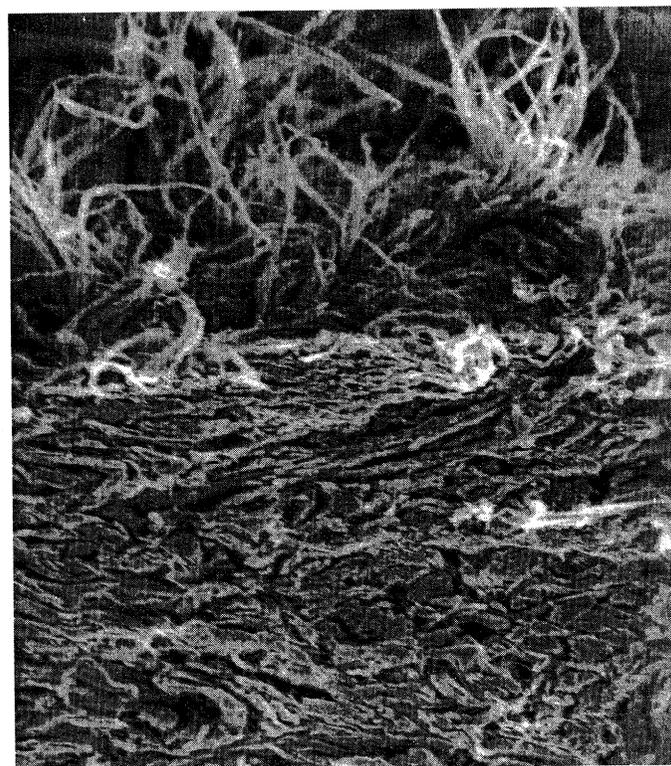


FIGURE 6. — Scanning electron micrograph of the cross section of crust leather prepared from halophilic bacteria treated cattle hide samples held for seven weeks at 106°F.

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CONCLUSIONS

Brine-cured hides have always been known to maintain their quality longer under cool conditions in a hide cellar. This knowledge acquired through long years of experience is only reaffirmed by this work. In these experiments we showed that within seven weeks at a temperature of 106°F, extremely halophilic organisms can damage the grain on a brine-cured hide. This damage is easily observed by the naked eye and scanning electron microscopy clearly shows that the damage done by halophilic organisms resembles sueded grain. These results clearly demonstrate the need to eliminate "red heat," not just because it may be an indicator of general microbial activity on the hide, but because extremely halophilic bacteria themselves are potential causes of serious grain damage. The results also suggest that even moderate temperature control during storage can control the growth of halophilic organisms.

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