Scanning electron microscopy of wool fibre degradation by *Streptomyces* bacteria

D. Brady* and J.R. Duncan
Department of Chemistry and Biochemistry, Rhodes University, Grahamstown 6140, Republic of South Africa

R.H.M. Cross
Electron Microscopy Unit, Rhodes University, Grahamstown 6140, Republic of South Africa

A.E. Russell
Leather Industries Research Institute, Rhodes University, Grahamstown 6140, Republic of South Africa

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Various strains of *Streptomyces* bacteria were found to be capable of degrading Merino wool when incubated together in a basal salts medium for extended periods. There was a decrease in filterable solids in the cultures as a result of bacterial action. Scanning electron micrographs of the process of wool fibre degradation are presented.

Daar is bevind dat verskeie stamme van *Streptomyces* bakterieë die wol van Merinoskape kan degradeer wanneer dit oor 'n lang tydperk saam met dié bakterieë in 'n basale soutmedium geïnkubeer word. Filtreerbare deeltjies in die kulture het as gevolg van bakteriële uitwerking afgeneem. Mikrograwe van die wolveseldegraderingsproses, geneem deur 'n skandeerelektronmikroskoop, word aangebied.

Keywords: Degradation, *Streptomyces*, wool fibre.

* To whom correspondence should be addressed.

The epidermis of the skin and associated wool are composed mainly of keratinized cells. It has previously been found that, in basal salt cultures containing wool inoculated with the bacterium *Streptomyces fradiae* 3739, the wool was decomposed and the enzymes responsible were therefore considered to be keratinases (Noval & Nickerson, 1959). An extracellular cell-free filtrate of *S. fradiae* 3739 caused the degradation of the wool, but did not cause the release of soluble sulphydryl compounds such as those that had been detected in the cell cultures. No conclusions could therefore be drawn as to whether there was an extracellular disulphide reductase associated with the bacterium (Cino, 1976). An extracellular protease was isolated from the culture fluid, crystallized and characterized and found to have strong keratinolytic activity which was maximal at pH 9,0 (Nickerson, Noval & Robison, 1963; Nickerson & Durand, 1963). A patent was taken out on processes of keratin breakdown using this bacterium and enzyme (Nickerson & Noval, 1961). Other organisms with keratinous activity were also reported (Noval & Nickerson, 1959), one of which was *Streptomyces griseus* which produces the extracellular protease mixture sold commercially under the trade name Pronase (Narahashi, Shibuya & Yanagita, 1968).

Another group of workers (Morihara, Tatsushi & Tsuzuki, 1967) proved that more than one extracellular protease was released by *S. fradiae* and that these proteases could be induced in the presence of protein substrates other than wool. Five proteases and two exopeptidases were separated, isolated, and characterized according to their properties and specificities. The enzymes as a group had high activity not only against keratin, but also against elastin and casein, indicating that they were general proteases and not specifically keratinases.

In later publications, the enzymes were shown to have very broad specificity of proteolytic activity (Morihara & Tsuzuki, 1968; Morihara & Tsuzuki, 1969; Morihara, Oka & Tsuzuki, 1971; Morihara, Oka & Tsuzuki, 1974). Further research was carried out on these enzymes and their ability to degrade wool and modified wools (Purvathingal & Robson, 1975), feather keratin (Young & Smith, 1975), and to promote enhanced degradation of wool by inclusion of reducing agents (Everett, Cordon & Windus, 1962).

In the present study, a number of *Streptomyces* species were screened for their wool degradation activity, as this would be of relevance to studies of enzymic depilation of skins being conducted in this laboratory.

Materials and Methods

A freeze-dried culture of *Streptomyces fradiae* I.M.R.U. 3739 was obtained from the Rutgers State University's Waksman Institute of Microbiology. *Streptomyces rimosus* and *Streptomyces* TK 64 were obtained from the John Innes Institute, Norwich. *Streptomyces griseus* was obtained from the NCIB (Aberdeen) collection 8056. *Streptomyces S.* 5058 was obtained from Liverpool University. Cultures of these strains of *Streptomyces* bacteria were grown on YEME (Hopwood *et al.*, 1985, p. 239) agar slants. After five days of growth at 35°C, these slants were used to produce spore suspensions (Hopwood *et al.*, 1985). Aliquots (1 cm^3) of these spore suspensions were inoculated into 100 cm^3 of wool / basal salt media (Noval & Nickerson, 1959) (see Table 1) in a 300 cm^3 conical flask. The pH of the medium after autoclaving was 8,3.

Merino wool was clipped from salt-cured skins. The discoloured tips were removed and the white wool was washed repeatedly in distilled water. After drying in an oven

Table 1 Formulation for wool/basal salts medium

Composition	Concentration (g/dm^3)
K_2HPO_4	1,50
$MgSO_4.7H_2O$	0,05
$CaCl_2$	0,05
$FeSO_4.7H_2O$	0,015
$ZnSO_4.7H_2O$	0,005
Sterile Merino wool[a]	0,85

[a] Added after the medium was autoclaved.

at 40°C for 20 h to remove the moisture, the wool was washed repeatedly in chloroform to remove the wool grease (lanolin). The wool was then kept in fresh chloroform for one week to ensure sterility. Residual chloroform associated with the wool after decantation was removed under vacuum in a UV-light sterilized vacuum desiccator. This method of sterilization has no apparent effect on the integrity of the wool structure (Noval & Nickerson, 1959).

The cultures were grown aerobically at 30 ±2°C for 30 days on a rotary shaker at 150 r.p.m. After incubation was completed, the wool remains were separated from the culture broth by vacuum filtration through preweighed Whatman filter paper (no. 1). The wool and accompanying bacterial mycelia were dried in an oven at 40°C for 20 h and then weighed. The extent of wool degradation was expressed as the percentage loss of mass from the original 85 mg of wool. No microbial growth was observed in the control flask (with no inoculum included) and less than 1% of the wool in this flask was solubilized.

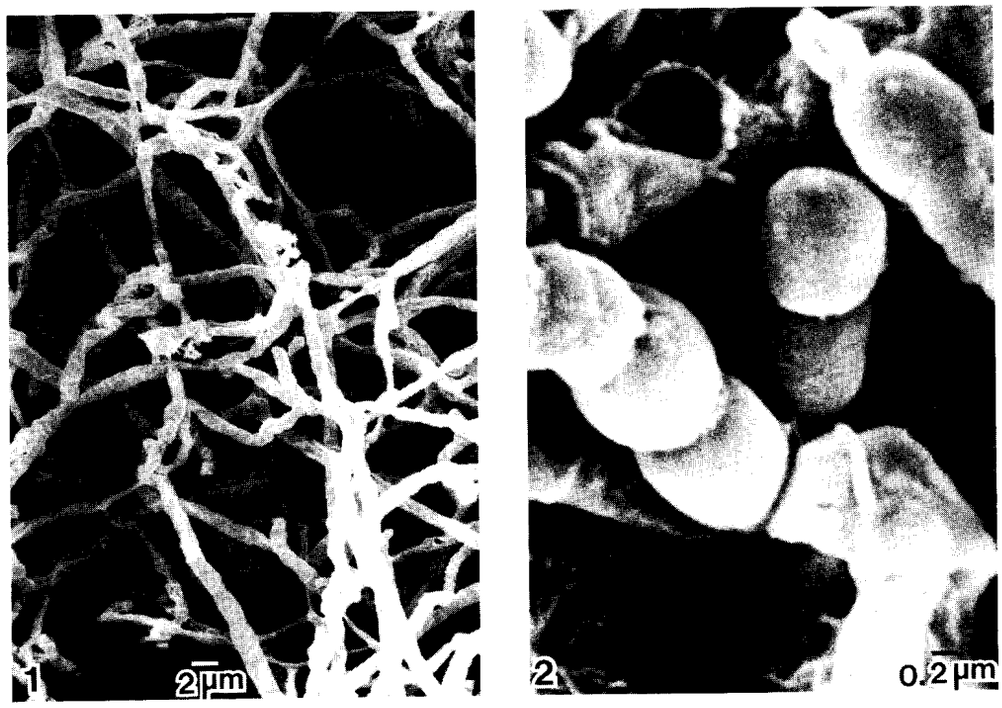
The wool fibres were visualized by both light microscopy and scanning electron microscopy (SEM). The wool required no preserving procedure for SEM. Dry wool fibres were attached with adhesive to SEM stubs and sputter-coated with approximately 5 nm gold in a Polaron E5100 sputter coater prior to observation in the JEOL JSM 840 SEM.

Colonies of *S. fradiae* 3739 were grown on nutrient agar plates until sporulation occurred (usually within 3—5 days) with consequent formation of an opaque white colony and, if the colonies were separate, by the formation of a dark lavender pigment in the centre of the colony. For SEM observation portions of the colony were quench-frozen in sub-cooled liquid nitrogen and transferred to the cryo stage of the SEM via a Hexland CT 1000 cryo-SEM accessory.

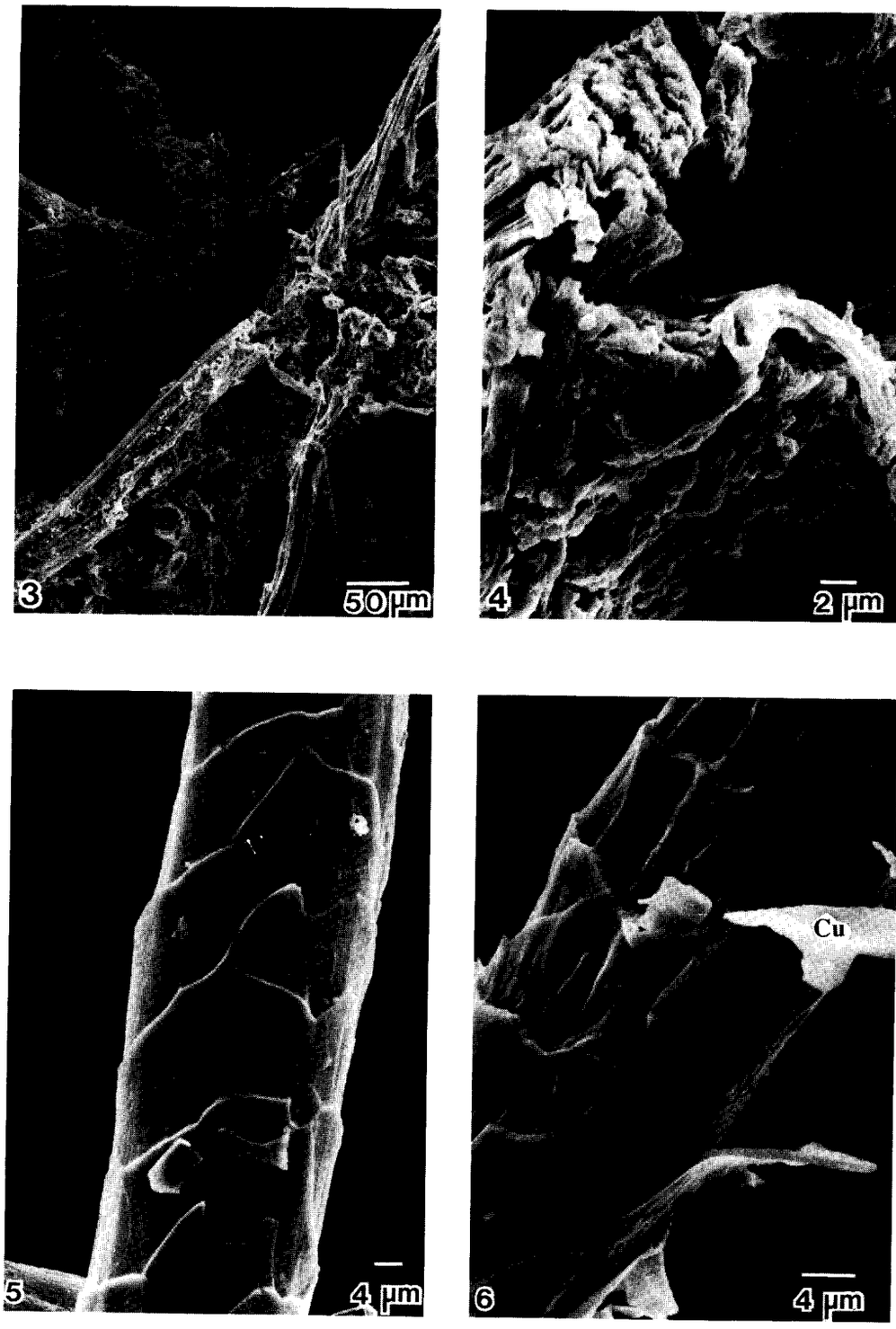
Results

The SEM observations of *S. fradiae* 3739 confirmed that the aerial hyphae had formed spores at their terminals which were smooth-walled (Figures 1 & 2) as is characteristic of this species (Kutzner, 1981).

During the incubation period, wool degradation in the presence of some *Streptomyces* strains became evident. When separated from the basal salts medium and dried, the partially degraded wool could be seen to have lost its lustre and, compared to the wool from the control flask, it looked dull when viewed under low magnification light microscopy. The improved resolution afforded by scanning electron microscopy (Figures 3—8) showed the reason for this. The wool fibres had been extensively degraded by the bacteria (Figures 3 & 4). Although the original wool fibre (Figure 5) was intact, initial enzyme attack on the wool fibre caused loosening of the cuticle cells (Figure 6) which leads to dulling of the wool and felting as the raised cuticle cells interlock. Eventually the cuticle cells were completely lost,

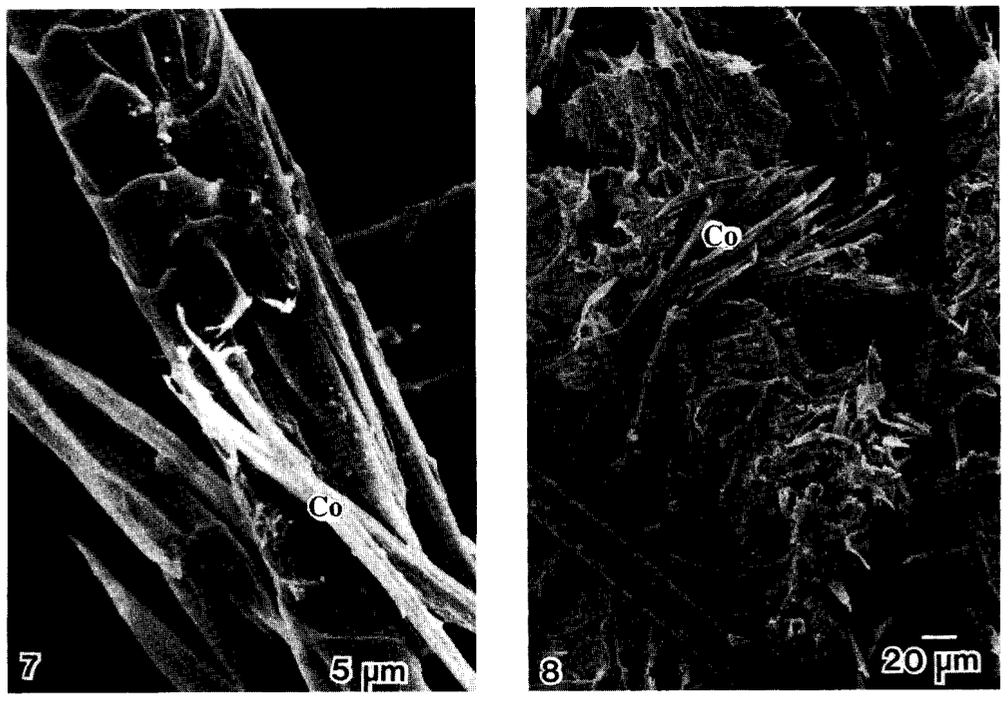


Figures 1 & 2: **1,** A SEM micrograph of aerial mycelia of *Streptomyces fradiae* 3739, (× 2500). **2,** The aerial mycelia of *Streptomyces fradiae* terminate in chains of smooth-walled spores of *ca.* 1 µm diameter, (× 20000).



Figures 3—6: **3,** Breakdown of the Merino wool fibre structure caused by the action of *Streptomyces fradiae* 3739, (× 250). **4,** With increased magnification (× 3500) the central wool fibre in Figure 3 can be seen to be degraded at specific positions, indicating that some regions of the wool fibre are more resistant to the enzyme action than others. **5,** A Merino wool fibre was included as a control into some of the basal solution without an inoculation of bacteria. There is no obvious structural damage to the fibre, (× 1200). **6,** The cuticle cells (Cu) can be seen to lift away from the wool fibre after incubation with *Streptomyces griseus*, (× 2500).

the cortex of the wool was exposed, and the corticle cells were released (Figure 7). The enzymes appeared to degrade the cementing material binding the corticle cells together until finally only individual cells remained (Figure 8). A similar process was observed by Carter, Best & Seal (1988), with incubation of human hair and a strain of *S. fradiae*. Degradation of the wool fibre, as measured by a decrease in dry mass of filterable solids, was 49% (*S. rimosus*), 42% (*S. griseus*), 25% (*S. fradiae* 3739), 22% (*Streptomyces S.* 5058), and 17% (*Streptomyces* TK 64), respectively. It was noted that considerable variation (10—30% degradation) occurred within duplicates utilizing the same strain (*S. fradiae* 3739). Although the possible variation between cultures means that the degree of degradation caused by each



Figures 7 & 8: **7,** Incubation of the wool fibre with *Streptomyces rimosus* resulted in the complete loss of the scale cells to expose the cortex which was itself degraded to yield individual corticle cells (Co), (× 1 000). **8,** A SEM micrograph depicting varying degrees of wool fibre degradation caused by *Streptomyces rimosus*, (× 300).

strain reported here may not be absolute, these figures did parallel the SEM observations in that loss of wool mass was highest in those examples where wool degradation was most obvious. The figures also include the dry mass of the bacterial mycelia; however, this mass was found to be insignificant when compared to the mass of the wool residue and therefore contributed only a small percentage to the final figure.

Discussion and Conclusions

The extracellular enzymes of some *Streptomyces* strains are able, according to the SEM studies, to degrade the binding proteins which hold the corticle cells together, but not the cells themselves. This is in agreement with the results of other workers who pointed out that keratin structures such as mature human *stratum corneum* were resistant to a commercial preparation of keratinase from *S. fradiae* (Dobson & Bosely, 1963).

Non-keratinous proteins of the wool are found in the cell membrane complex, the epicuticle, the endocuticle and probably in the intercellular cementing substance (Bradbury, 1973). At these sites, enzymes with little or no keratinase activity are able to break down the proteins that bind the keratinous cells together, and thereby degrade the wool fibre. This results in the release of cuticle as well as cortical cells as observed by SEM.

The strain of *S. rimosus* used in these experiments degraded 20% more of the wool fibre than the strain used by Noval & Nickerson (1959). The strain of *S. griseus* degraded a similar amount of wool compared to the strain they used, whereas *S. fradiae* was less efficious (25% compared to 90%) even though the strain used was identical. This reversal of wool degrading ability may reflect strain affinity for certain types of wool.

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