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PROCESS FOR THE DEPILATION AND BATING OF PELTS AND HIDES

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This invention relates to a process for the depilation and bating of pelts or hides intended for the manufacture of leather.

It is known that in taming, at present use is made of certain enzymatic preparations for the bating of pelts, an operation which has the object, among others, of dissolving the undesirable proteins, such as the keratine residue coming from the roots of the hair and various glands, etc.

The proteolytic enzymes in question, among which are, mainly pancreatic enzymes, require, before bating proper, depilation of the pelts which is generally done by treatment in baths containing lime and alkali sulphide. The depilation itself is a rather long operation often accompanied by putrifaction and attack on the collagen by the microbic flora normally found on the skins, which in either case results in a loss of leather.

The present invention relates to the use, both for the depilation and bathing of pelts, of a thermolabile complex, probably of enzymatic nature, secreted by certain species of Streptomyces. It is known that the Streptomyces can secrete substances in their culture medium, whose lytic activity is manifested "in vitro" by clarification of suspensions of living gram-positive bacteria such as *Micrococcus pyogenes aureus,* giving rise to staphylolytic activity, *Streptococcus pyogenes,* giving rise to streptolytic activity and *Streptococcus pneumoniae,* giving rise to pneumolytic activity.

Now, in addition to the antibiotic activities of the actinomycetin type, which have been demonstrated particularly in the filtrate of active culture of *Streptomyces albus* G, we have now discovered in addition to an already known caseinolytic principle, two other distinct lytic principles, manifesting themselves respectively by hydrolysis of keratin and by massive desquamation of the epidermis with its annexes. These three latter principles, give rise to caseinolytic keratinolytic and depilatory activities.

These various principles can be obtained in regular and reproducible manner by the culture of various Streptomyces at 28° C. in submerged and agitated medium, for example in 1 liter flasks containing 250 to 500 ml. of an aqueous medium composed of 1% peptone, 0.2% $NaNO_3$, 0.05% KCl, 0.1% K_2HPO_4, 0.08% $MgSO_4.7H_2O$, 0.003% $Co(NO_3)_26H_2O$, with the agitation kept up for 60 to 70 hours at a speed of rotation of the vessel of 100 to 120 r.p.m., and the cultures, whose activities are then at maximum, subsequently filtered.

Since these tests showed that strains of Streptomyces of different origin could be distinguished from one another as much by the bacteriolytic principles as by the caseinolytic, keratinolytic and depilatory principles they secreted, we first of all set about evaluating the frequency with which these various types of activity occurred in the culture filtrates of a certain number of strains.

By thus studying a series of 88 strains of Streptomyces isolated from various natural substrata, we found 63 yielding at least one of the three bacteriolytic activities and among the 25 strains lacking bacteriolytic activity, 6 were actinomycin producers. As for the three lytic activities, which could exert an action in the depilation and/or bating of the pelts, we found, among these same 88 strains, 59 strains secreting the caseinolytic principle, 75 strains secreting the keratinolytic principle, 65 strains secreting the depilatory principle and 3 strains secreting none of the latter three principles.

Under the conditions described, 11 strains only developed one of the three principles, while 34 strains secreted two principles and 40 strains the three principles simultaneously. As the table below shows, the greater part of the 88 strains studied secreted at least one and even two or three of the principles capable of application in depilation and/or bating of the pelts.

Principle	Only One	Two			Three	Total	None
		Ker.+ depil.	Cas.+ depil.	Cas.+ ker.	Cas.+ ker.+ depil.		
Activity:							
Caseinolyt	2	-------	5	12	40	59	------
Keratinolyt	6	17	-------	12	40	75	------
Depilatory	3	17	5	-------	40	65	------
	11	34			40	------	3
	88						

Although in a general way we can now envisage the use of the majority of the strains of the Streptomyces for the treatment of pelts, previous to tanning, it is nevertheless more advantageous to select those showing a maximum of caseinolytic, keratinolytic and depilatory activity which can be evaluated, for example using the following methods:

For determination of the caseinolytic activity 0.75 ml. of Streptomyces culture filtrate is heated to 37° C. 0.5 ml. of a solution of K_2HPO_4 at 0.2 M and 1.25 ml. of a 0.6% aqueous solution of casein also heated to 37° C. is added thereto, the casein solution being prepared by dissolving in alkaline medium followed by neutralization. After five minutes of incubation at 37° C. 0.5 m. of the mixture is removed and added to 4.5 ml. of a 1% solution of trichoracetic acid. After 30 minutes' rest at room temperature the cloudiness which is proportional to the amount of non-hydrolyzed casein is evaluated to obtain direct measurement of the caseinolytic activity.

To establish the keratinolytic activity, we add to 1 ml. of filtrate of Streptomyces culture, brought to 37° C. 4 ml. of an aqueous suspension of keratin, also brought to 37° C. at such concentration that the resulting cloudiness is close to an absolute cloudiness of 0.142 and we determine the percentage of cloudiness that has disappeared after 90 minutes of incubation at 37° C.

The keratin used for this test comes from chicken feathers and is prepared by the method of H. P. Lundgreen, A. M. Stein, V. M. Koorn and R. A. O'Connel, described in J. of Phys. and Coll. Chem., 1948, vol. 52, No. 1, p. 180.

The caseinolytic and keratinolytic activities of the strains can be classified as follows:

Activity zero: disappearance of less than 5% of cloudiness

Activity moderate: disappearance of 5 to 20% of cloudiness

Activity strong: disappearance of 21 to 40% of cloudiness

Activity very strong: disappearance of more than 40% of cloudiness

For determination of the depilatory activity, we introduce, at room temperature, 1 ml. of filtrate of Streptomyces culture in a thick glass test tube about 12 mm. in diameter and 180 mm. high and then close the tube with a piece of fresh pelt (cut from a butt of a calf weighing 5 to 6 kg.), putting the flesh side towards the inside of the tube and stretching the pelt over the opening of the tube on which it is fastened, for example, by means of a metal collar. The tube is then turned over and kept in a chamber kept at 37° C. and after 15 hours we evaluate the size of the zone where the epidermis can be lifted off the derma by simple traction on the hair, thereby exposing the derma intact.

The depilatory activity of the strains can be classified in the following manner:

Activity: Depilation of the surface of the pelt in contact with the culture filtrate
Zero: None
Moderate: Partial
Strong: Total
Very strong total, extending even below the metal collar

The strains of Streptomyces which are thereby shown to be most suitable, both in the number of their activities useful for treatment of pelts and in the intensity of these activities, can be produced on an industrial scale by culture of these Streptomyces in a suitable culture medium as indicated above treated in a vat of for example 100 or 500 liters at a suitable rate of aeration. This can be obtained, for example, by passing at a constant super-pressure of the order of 0.5 kg./sq. ccm. of a volume of air equal to ⅛ of the volume of culture per minute.

According to the present invention, the filtrates of this treatment, when they have reached the desired rate of activity and possibly concentrtated by vacuum distillation are used to effect depilation and bating in one operation. This treatment, operated at a neutral or slightly alkaline pH takes only a few hours, which means not only a very considerable saving in time but also reduces the danger of collagen losses by putrefaction and, in addition, makes it possible to recover in an industrially usable condition, the hair obtained by this new method of depilation.

The depilatory and bating activity of the filtrates in question has proved effective on all sorts of skins of freshly slaughtered animals. Samples taken from the shoulder, butt or flanks of goats, sheep, calves, cows, bulls and horses have been thus treated at 37° C. with filtrates, sometimes concentrated, of cultures of *Sterptomyces albus* G, buffered to a pH of about 8.0, according to the various techniques as follows:

Painting or spraying the flesh side of the pelts, with an active solution,

Complete immersion of the pelts in an active solution,

Fulling of the pelts in a bath composed of a volume of active solution equal in weight to the weight of the pelts to be depilated.

We have thus observed that, for a given quantity of active product, the fulling process makes for a much more rapid depilation than the other two processes. At 5 g. of active product per kg. of pelt, we succeeded for example, in the depilation of a sheepskin in 39 hours by simple painting and in 15 hours by fulling, or, again the depilation of a butt of a heifer in 29 hours by complete immersion and in 9 hours by fulling. In these tests the depilation was complete for all pelts examined.

According, on the one hand, to the nature of the pelts treated, i.e. the species and the age of the animals, the part of the skin selected and according, on the other hand, to the process used, the amount of active product used, the temperature of the baths etc., the speed of depilation can vary somewhat.

As indicated above, the technique of depilation by fulling is the quickest. Among the pelts most rapidly depilated are those of goats, calves, and heifers.

Tests of treatment of pelts by painting the flesh side with filtrates of culture of *Streptomyces albus* G which underwent, by means of vacuum distillation, a preliminary concentration to 1/10 of the original volume, have shown that a quantity of 500 liters allows simultaneous depilation and bating of about 4 tons of heifer pelts in 15 hours of incubation, although this figure does not represent the best possible result which could be obtained under different operating conditions. The industrial tests of combined depilation-bating discussed above, were actually run with strains of *Streptomyces albus* G cultivated more particularly with a view to the utilization of the bacteriolytic activities of the actinomycetin thus produced.

Now, among the 40 strains which, in the tests described above, secreted the three non-bacteriolytic principles, 17 strains manifesting the latter in a particularly intense manner, were not producers of actinomycin and only 14 of the 17 strains secreted all or a part of the bacteriolytic complex of acinomycetin, while the three others were even completely without it.

Likewise, among the 6 strains producing actinomycin, 5 secreted a depilatory principle, 4 a keratinolytic principle and 3 a caseinolytic principle but only two of these strains secreted the three principles together. This absence of correlation between the various lytic and depilatory activities of the streptomyces shows that they are due to distinct principles which moreovere it is possible to separate by conjugation of different methods of fractioning by means of adsorption, desorption, fractional extraction, dialysis, etc. Certain strains, other than *Straptomyces albus* G or even completely without antibiotic activities, thus may prove to be still more interesting from the industrial point of view, for the treatment of pelts. By means of methods of determination of the lytic and depilatory activities, described in the present patent application, the specialist can search for and choose, among the innumerable strains of streptomyces, those which are most suitable for a given treatment of depilation and/or bating of pelts.

What we claim and desire to protect by Letters Patent is:

1. A process for the treatment of skins and hides for the manufacture of leather by tanning which comprises contacting said skins and hides in a neutral to slightly alkaline reaction medium with cultures of Streptomyces of the *Streptomyces albus* species.

2. A process for the treatment of skins and hides for the manufacture of leather by tanning which comprises contacting said skins and hides in a neutral to slightly alkaline reaction medium with cultures of Streptomyces strains of the *Streptomyces albus* G species.

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