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Digestion of Solid Tannery Wastes by Strains of *Bacillus Sp* Isolated From Compost in Morocco

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ABSTRACT

Eight strains of *Bacillus* were isolated from non treated soil, characterized and used for the digestion of crude leather wastes in the laboratory. Non-protein nitrogen (NPN) and total protein (TP) were determined during the incubation time and the microbial counts of the different strains during leather hydrolysis were also monitored. Results of the screening tests showed that the solid pieces of leather wastes were completely digested by all the strains. The most efficient isolated strain (*Bacillus subtilis* strains 11) was compared with *Bacillus subtilis* ATCC 6633. Results showed that the total nitrogen was decreased from 1032.1 to 93.5 mg/100 g by our strains and from 1064.4 to 385 mg/100 g by the standard strains *B. subtilis* ATCC 6633. The NPN reached the concentration of 30 mg/100 g for our strains and only 10.8 mg/10 g by the standard strains. This may show a suitable biotechnological process for the treatment of solid wastes from the tannery industry. The obtained product may also be used as fertilizer or as a source of nitrogen for microbial populations.

Keys Words: Leather; Tannery; Waste; Collagen; *Bacillus*

INTRODUCTION

The tannery industry is one of the most representative strategic industries in Morocco and may constitute an important economic component besides other activities. The environmental aspects in this industry are generally ignored and the processes used for leather production are still more traditional than modern. Huge amounts of solid leather wastes are discarded directly with the urban wastes.

Several works in the field of bioengineering were carried out on enzymatic hydrolysis of collagen which is the most representative protein of leather wastes (Kawahara *et al.,* 1993; Matsushita *et al.,* 1994; Asdormithee *et al.,* 1994). This hydrolysis was carried out by collagensases which are enzymes that can hydrolyze both native and denatured collagens. The enzymes are widely used not only in chemical and medical industries but also in food and basic biological science (Hisano *et al.,* 1989; Raju *et al.,* 1996; Rao *et al.,* 1998; Tran & Nagano, 2002). However, most of the microorganisms isolated from soil presented low collagenase activity and their applications were limited, therefore the strains most efficient in tannery wastes hydrolysis are needed for more efficient yield and application because these organic wastes are hard to treat and have potential recycling into useful products (Chen *et al.,* 2001; Taylor *et al.,* 2002). The objective of the present investigation is to accelerate leather digestion process by inoculating with collagenolytic species of Bacillus isolated from soil.

MATERIALS AND METHODS

Chemical determination. The pH of medium was checked by the use of a pH-meter type Crison MicropH 2000. The dry matter of the crude tannery waste was determined by oven drying a weighed amount of the product at 105°C until constant weight. Ash was determined by incineration in a furnace at 550°C for 6 h. Total nitrogen (TN) was determined by the Kjeldhal method described by the APHA (1989). Non Protein Nitrogen (NPN) was determined by the Kjeldhal method on the filtrate after precipitating with a 10% trichloracetic acid solution. Minerals (Ca, P, K, Fe, Mg, Zn & Cu) were determined by an atomic absorption spectrophotometer apparatus (Type JENWAY PFP 7).

Isolation and characterization of bacterial strains. Samples of natural composted materials were taken from the soil of the dumping-ground unit near the city of Casablanca (Morocco). All the samples (500 g each) were transported in sterile plastic bags to the laboratory.

Initial dilution from each sample was prepared by adding 10 g of the compost to 90 mL of sterilized saline water. This dilution was heat activated at 70°C during 15 min and dilutions up to 10^6 in sterilized saline water were prepared in tubes. All the dilutions were plated on trypticase soy agar (TSA, Difco, USA) and incubated at 30°C for 24 h. The appeared colonies were checked for spore presence and streaked on agar slants for further characterization.

Characterization. All the collected strains were grown on TSA to obtain fresh cultures. Spore production and localization were examined by microscopic observations.

Table I. Chemical composition of tannery solid waste used in experiment

Chemical	% in dry matter (µg/g of ash)
Protein	79
Fat	7.57
P	147.6
K	0.47
Ca	0.59
Iron	2.24
Mg	1.84
Zn	0.116
Cu	0.002

The identification was done according to the method described by Larpent and Larpent (1985). Spore shape, growth at different temperatures, growth in anaerobic conditions, lecithinase, gelatinase, caseinase, amylase, indole formation, and nitrate and carbohydrates utilization were checked.

Screening test. Eight strains were chosen among the 10 isolates to be screened for the degradation of tannery wastes. Growth was evaluated by optical density (OD) at 600 nm in a spectrophotometer type Hitachi. The strains were grown on a minimal medium containing 0.1 g/L Mg SO_4, 2 g/L KH_2 PO_4, and 2 g/L glucose. 2g of the crude tannery waste cut in 2cm wide slices were added to 100 mL of minimal medium in 250 mL flasks. The flasks were sterilized at 121°C for 25 min and inoculated. The inoculum was prepared by picking a loop from cultures on slants and suspending in saline water to have an OD_{600n} nm of about 0.4 in the medium.

Batch assays. One of the isolated strains identified as *Bacillus subtilis* and the reference strain, *Bacillus subtilis* ATCC 6633 were compared for degradation of leather waste. Total nitrogen, Non Protein Nitrogen and growth were monitored for 16 days.

RESULTS AND DISCUSSION

Tannery solid wastes (TSW) were first characterized for their chemical composition (Table I). Major compounds including proteins and minerals were determined. The protein content is high compared to other animal wastes, with average value of 79%. Also, the fat content is high because of the presence of fat parts in the intern side of the hide, the value was 7.75%. Among the minerals, the phosphorus content is the most important measure which approaches 147.6 µg/100 g of the ash. The content of iron and manganese are represented by 2.24 µg/100 g and 1.84 µg/100 g. The chemical composition of the TSW indicates a very balanced medium, which may not need other nutrients for culturing the degrading microorganisms.

Eight isolates identified as *Bacillus* strains were first screened on the tannery solid waste. Growth and protein digestion were determined to compare the activity of these isolates with a reference strains *(B. subtillis* ATCC 6633). Results showed that *B. subtilis* and *B. licheniformis* were the most efficient strains by reaching the high level in OD_{600nm}, the values were 4.87 and 3.86, respectively the reference strain showed only 2.64 (Fig. 1). Collagen hydrolysis expressed as protein in g/L was determined before incubation and after 10 days. Results of residual protein contents in the medium are shown in Table II. As it could be pointed out, *B. subtilis* strains11 and *B. licheniformis* strains12 gave the lowest values respectively i.e. 1.44 and 1.71 g/L, respectively, while the other strains including the standard strains did not reach this level. The strain ATCC 6633 gave 2.2 g/L residual protein content.

The final dry matter was 0.11 and 0.31g, respectively for the two strains while the strains ATCC 6633 reached only 0.6 g (Table II). *B. subtilis* strains 11 (the most efficient strains in tannery waste hydrolysis) was selected for further uses in a controlled system for both hydrolysis and growth.

In the second step, assays of TSW digestion were carried out in flasks (150 g of TSW in 1 liter), and they were inoculated with *B. subtilis* isolated in our laboratory as well as with the reference strains. Growth of the two strains is plotted in Fig. 2. As can be seen in this figure, the strain isolated in our laboratory showed a growth pattern higher than the strains ATCC 6633. The difference is significant between the two strains. The OD at 600 nm reached 6.8 for our strain and only 2.8 for the strains ATCC 6633 after 13 days incubation at 50°C. Growth was determined to assess the nutritional need for the strains and their capacities to use the nitrogen and other nutrients from the leather waste by hydrolyzing the proteins. *Bacillus* strains are ubiquitous microorganisms, which can grow on natural media without any special requirements for nutrients (Henner, 1990; Raju *et al.,* 1996; Rao *et al.,* 1998; Hyyryläinen *et al.,* 2001). These proprieties can be exploited in the field of waste degradation such as the TSW, which are produced in huge amounts in Morocco.

Results relative to the TSW hydrolysis by the two strains are reported in (Fig. 3) which showed the decrease of the total nitrogen from 1032.1 to 93.5 mg/100 g for the *B. subtilis* and from 1064.4 to 385 mg/100g for the strain ATCC 6633. The decrease pattern of our strains was higher than that of the ATCC. The total

Table II. Protein contents and dry matter in the assays of degradation of 8 g/L of tannery solid waste by different strains of *Bacillus*

Bacterial strain	11	12	15	8	Bacillus subtilis ATCC6633	13	1	X	6
Amount of protein g/L	1.44	1.71	1.73	1.88	2.2	2.23	2.63	2.33	2.84
Final dry matter (g)	0.11	0.31	0.42	0.47	0.6	0.52	0.86	0.58	0.9

Fig. 1. Growth patterns of different strains on tannery waste (8g/L)

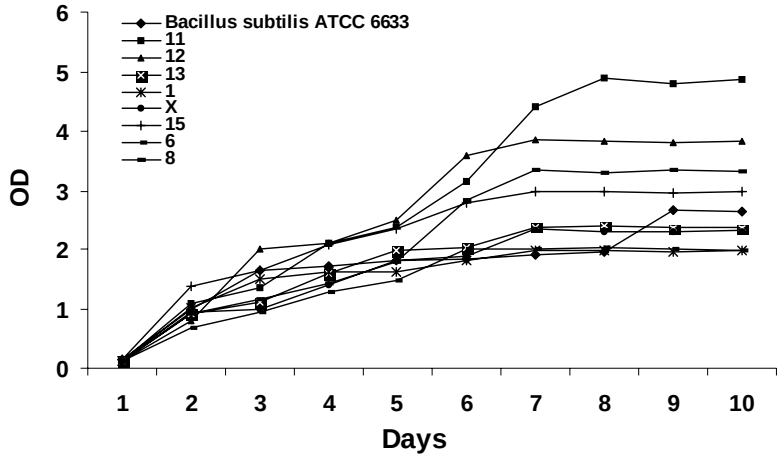


Fig. 2. Growth pattern of the two selected strains on tannery waste (150 g/L)

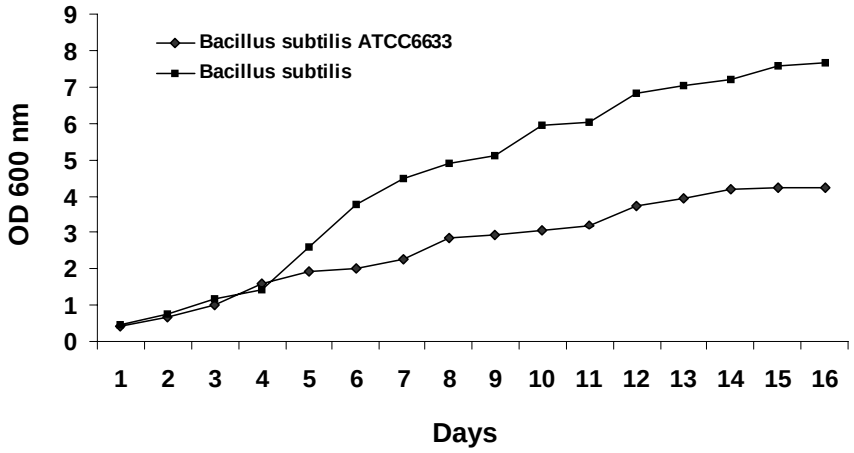
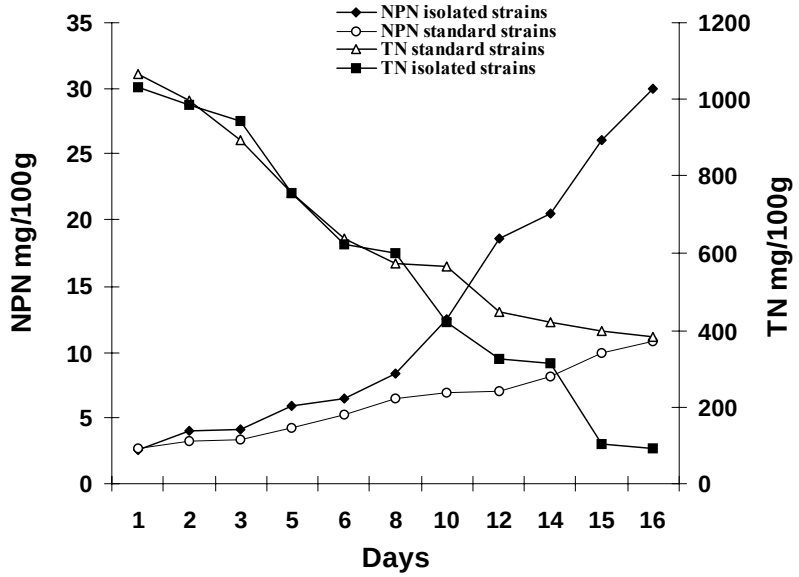


Fig. 3. Total nitrogen and no protein nitrogen profiles in the assays of degradation of tannery waste (150 g/L) by Bacillus subtilis isolated and standard strains Bacillus subtilis ATCC 6633



nitrogen decrease is due to the hydrolyzing activity by the strains and probably its use by the cells for growth. Results of NPN analysis confirmed that the hydrolyzing activity of isolated strains is higher (30 mg/100 g) than the standard strains (10.8 mg/100 g).

The strain growth rate may indicate the collagenase production by the strains and consequently the proteins hydrolysis. The enzymatic activity was broadly and indirectly evaluated by the hydrolysis of insoluble material, which may be mainly constituted by collagen and other connective tissues in the skin. Other bacterial strains are known by their collagenolytic activity include, *Clostridium histolyticum* (Bond & ward, 1984; French, 1987), *Clostridium perfringens* (Matsushita *et al.,* 1994), *Pseudomonas sp*. (Hisano *et al.,* 1989) *Bacillus licheniformis* (Asdormithee *et al.,* 1994), *Bacillus alvei* (Kawahara *et al.,* 1993). *B. subtilis* is, however, very convenient for the collagen degradation and several studies (Christner, 1994; Nagano & To, 2000; Nakayama *et al.,* 2000; Tran & Nagano 2002) confirmed the high activity of collagenase produced. Moreover, *B. subtilis* are thermophile microorganisms with ability to grow at high temperature (50-65°C) and this property can be used in controlled process for efficient and fast degradation of collagen.

CONCLUSION

Tannery wastes can be treated biologically by collagenase producing microorganisms, this enzymatic hydrolysis could be a safe method of recycling these organic materials. Our isolates were better adapted to the digestion of tannery wastes compared to the standard strain of *B. subtilis* ATCC 6633. These encouraging results may be continued, and the isolated bacilli may used for biocompost elaboration. Future work may also be focused on optimizing the environmental parameters.

REFERENCES

APHA (American Public Health Association), 1989. *Standard Methods for Examination of Waste Water*. 19th Ed. APHA Pub, Washington DC, USA

Asdormithee, S., K. Akiyama, T. Sasaki and R. Takata, 1994. Isolation and characterization of a collagenolytic enzyme from *Bacillus Licheniformis N22. J. Fermen. Bioengi.,* 78: 283–7

Bond, M.D. and H.E. Van Ward, 1984. Characterization of the individual collagenase from *Clostridium histolyticum*. *Biochem.*, 2: 3085–91

Chen, W., P.H. Cooke, G.L. DiMaio, M.M. Taylor and E.M. Browm, 2001. Modified collagen hydrolysate, potential for use as a filler for leather. *J. American Leather Chemists Assoc.,* 96: 262–7

Christner, J., 1994. The evaluation of modern bating agents. *Leather Magazine*, 3: 10-4

French, M.K., 1987. Limited proteolysis of type I collagen at hyperreactive sites by class I and II *Clostridium histolyticum* collagenases: Complementary digestion patterns. *Biochem*., 26: 681–710

Henner, D.J., 1990. Expression in *Bacillus subtilis*. *Meth. Enzym.,* 185: 199–223

Hisano, T., S. Abe, M. Wakashiro, A. Kimura and K. Murata, 1989. Isolation and properties of a collagenase with caseinolytic activity from a *Pseudomonas sp. J. Fermen. Bioeng.,* 68: 399–403

Hyyryläinen H.L., A. Bolhuis, E. Darmon, *et al.,* 2001. A novel tow-component regulatory system in *Bacillus subtilis* for the survival of severe secretion stress. *Mol. Microbiol.,* 41: 1159–72

Kawahara, H., M. Kusumoto and H. Obata, 1993. Isolation and characterization of a new type of collagenase producing bacterium, Bacillus alvei DC-1. *Biosci. Biotech. Biochem.,* 57: 1372–3

Larpent, J.P. and M. Larpent-Gourgaud, 1985. *Eléments de Microbiologie,* pp: 247–50. Hermann, Paris

Matsushita, O., K.Yoshihara, S. Katayama, J. Minami and A. Okabe, 1994. Purification and characterization of *Clostridium perfringens* 120-kilodalton collagenase and nucleotide sequence of the corresponding gene. *J. Bacteriol.,* 176: 149–56

Nagano, H. and K.A. To., 2000. Purification of collgenase and specificity of its related enzyme from *Bacillus subtilis* FS-2. *Biosci. Biotechnol. Biochem.*, 64: 181–3

Nakayama T., N. Tsuruoka, M. Akai and T. Nishino, 2000. Thermostable collagenolytic activity of a novel thermophilic isolate, Bacillus sp. strain NTAP-1. *J. Biosci Bioeng*., 89: 612–4

Raju, A.A., N.K. Chandrababu, N. Samivelu, C. Rose and N.M. Rao, 1996. Eco-friendly enzymatic dehairing using extracellular proteases from a Bacillus species isolate. *J. American Leather Chemists Assoc.,* 91: 115–9

Rao, N.M., A.A. Raju and C. Rose, 1998. Enzymatic hydrolysis of tannery fleshing using chiken intestine proteases. *Anim. Feed Sci. Technol.,* 66: 139–47

Taylor, M.M., C. K. Liu, N. Latona, W.N. Marmer and E.M. Brown, 2002. Enzymatic modification of hyrolysis products from collagen using a microbial transglutaminase. II. Preparation of films. *J. American Leather Chemists Assoc.,* 97: 225–34

Tran, L.H. and H. Nagano, 2002. Isolation and characteristics of *Bacillus subtilis* CN2 and its collagenase production. *J. Food Sci.,* 67: 1184–7

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