Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

Identifying Microorganisms Able to Perform Biodegradation of Leather Industry Waste

Daniela Simina Stefan [a], Romulus Dima [a], Mirela Pantazi [b], Mariana Ferdes [a] & Aurelia Meghea [a]

[a] Faculty of Applied Chemistry and Material Science, University Politehnica of Bucharest, No 1-7, Polizu Street, Sector 1, 011061, Bucharest, Romania

[b] National Research and Development Institute for Textile and Leather-Division Leather and Footwear Research Institute, 93, Ion Minulescu Street, Sector 3, 74259, Bucharest, Romania

Available online: 02 Mar 2012

To cite this article: Daniela Simina Stefan, Romulus Dima, Mirela Pantazi, Mariana Ferdes & Aurelia Meghea (2012): Identifying Microorganisms Able to Perform Biodegradation of Leather Industry Waste, Molecular Crystals and Liquid Crystals, 556:1, 301-308

To link to this article: http://dx.doi.org/10.1080/15421406.2012.635983

Mol. Cryst. Liq. Cryst., Vol. 556: pp. 301–308, 2012

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421406.2012.635983

Taylor & Francis
Taylor & Francis Group

Identifying Microorganisms Able to Perform Biodegradation of Leather Industry Waste

DANIELA SIMINA STEFAN,[1] ROMULUS DIMA,[1] MIRELA PANTAZI,[2] MARIANA FERDES,[1] AND AURELIA MEGHEA[1,*]

[1]Faculty of Applied Chemistry and Material Science, University Politehnica of Bucharest, No 1-7, Polizu Street, Sector 1, 011061, Bucharest, Romania
[2]National Research and Development Institute for Textile and Leather-Division Leather and Footwear Research Institute, 93, Ion Minulescu Street, Sector 3, 74259, Bucharest, Romania

Among the waste generated in leather processing, proteic and lipidic compounds are particularly dominant. Enzymes, like proteases and lipases, have an important role in biodegradation of collagen, keratin and fats in leather waste. Present investigation deals with isolation, selection and characterization of proteolytic and lipolytic microorganisms in order to perform biodegradation of waste from leather industry. The inoculum of microorganisms was sampled from old waste storage dump leather. The proteolytic microorganisms were isolated using screening method in presence of casein and gelatin substrate type, respectively. The microorganisms that secrete the lipolytic enzymes were isolated using medium that contained the Tween 80. These microorganisms identified by microscopic analysis can be used for improvement of waste leather biodegradation process.

Keywords Biodegradation; enzymes; leather; lipase; protease

Introduction

High amounts of liquid and solid wastes result from leather industry, and finally are disposed in the environment. This could be the reason for listing leather industry among the most polluting industries worldwide [1]. One possibility to capitalize the solid leathery waste with low cost is biodegradation [2].

The cow hide has the following composition: 60–70% water, 30–35% proteins, 0.5–2% lipids and 0.35–0.5% mineral compounds. The fibril proteins are represented as collagen in proportion of 90%, elastine over 9% and keratins less than 1%. After tanning process the water content in the leather decreases, while the content of proteins increases at 70% (mostly as collagen and small amounts of elastine) and fats, while at the same time new compounds are introduced like Cr_2O_3, dyes, synthetical tanneries, pesticides (biocide) etc [3–5].

In accordance to the composition of waste, specific populations of microorganisms can be isolated, developed and identified in order to produce specific enzymes that sustain the

*Address correspondence to Aurelia Meghea, Faculty of Applied Chemistry and Material Science, University Politehnica of Bucharest, No 1-7, Polizu Street, Sector 1, 011061, Bucharest, Romania. E-mail: a.meghea@gmail.com

biodegradation. Enzymes like proteases and lipases play an important role in biodegradation of collagen, keratin and fats, respectively, in leather waste [6]. Proteases are complex multienzyme systems which catalyze the hydrolysis of amide bonds in the protein molecule [7].

Collagen is a highly organized biopolymer, consisting of a large number of individual collagen molecules linked together in a periodic manner. The collagenase is a class of metalloproteases, which are broadly defined as enzymes that catalyze the hydrolysis of the native collagen at pH around 7 and at a temperature of 37°C [8].

The microorganisms that can synthesis the proteases are the bacteria like ***Clostridium species*****:** *Cl. histolyticum, Cl. perfringens and Cl. capitovale* [8]; ***Bacillus species*****:** *B. subtilis*, *B. megaterium*, *B. anthracoides*, *B. pumilus* [9–11]; ***Pseudomonas species*****:** *Pseudomonas aeruginosa*, [12–15]; fungi like ***Paecilomyces species*****:** *Paecilomyces ehrlichii*, *Penicillium klebanii*, *P. aculeatum*, *P. purpurogenum* and *P. roseopurpureum, P. chrysogenum*, *P. luteum*, *P. brevicompactum*, *P. decumbens*, *P. rugulosum*, *P. aculeatum*, *P. funiculosum*, [16,17]; ***Aspergillus*** *species*: *Aspergillus niger*, *A. fumigatus*, *A. ochraceus*, *A. wentii*, *A. flavus-oryzae* (group), *Mucor mucedo*, *Rhizopus nigricans*, etc, [18–20].

The microorganisms that can synthesis the lipase are bacteria like *Pseudomonas fragi* [21], *Aspergilius sp.* [22], *Bacillus subtilis, Proteus, Acinetobacter, Aeromonas, Escherichia, Myroides, Brevibacterium, Vagococcus, Staphylococcus, Mycoplana etc*. [23–25].

Present investigation deals with isolation, selection and characterization of proteolytic and lipolytic microorganisms in order to perform biodegradation of waste from leather industry. The inoculum of microorganisms was sampled from old waste storage dump leather. The proteolytic microorganisms were isolated using screening method in presence of casein and gelatin substrate type, respectively. The microorganisms that secrete the lipolytic enzymes were isolated using medium that contained $CaCl_2$ and Tween 80. These microorganisms are used for improvement of waste leather biodegradation process.

Materials and Methods

Microorganism Isolation

Inoculums of microorganisms were sampled from leather waste storage dump. Amounts of 1 g of soil sample were suspended in 100 mL of physiological solution and vigorously mixed. Serial dilutions to 10^{-4} M were made and aliquots were flooded on nutritive agar plates containing the following components, (g/L): peptone, 5; meat extract, 3; agar, 15 [26]. Agar plates were then incubated for 48 h, one part at 37°C and another part a 17°C.

Visible morphological types of single colonies were isolated by transfer in tubes containing nutrient agar, by the method of exhaustion loop (scratching the surface), and maintained on the above medium at 4°C.

Enzymatic Activities

The developed colonies were tested for protease production on casein clearing zone technique; colonies with clear zones formed by the hydrolysis of casein were evaluated as producers, [27]. The culture medium contained the following components (g/L): casein 2.5, agar 15, $Ca(OH)_2$, 0.15, $CaCl_2$, 0.05. The samples were incubated for 48 h at 17 and 37°C, respectively. Depending on the zone diameter and clearance the optimum

microorganism as protease producer has been selected and the proteolytic index, I_p, has been calculated as the ratio between lyses zone diameter and colony diameter ($I_p = \Phi_{lysis}/\Phi_{colony}$).

The selected microorganisms were used for testing the gelatin hydrolysis from medium that contained (g/L): meat peptone, 5, meat extract, 3, gelatin, 120 and it was incubated for 48 h at 17°C and 37°C, respectively, [28].

Similarly the colonies have been tested for lipase production by hydrolysis of Tween 80 (sorbitol esters with the fat acids); colonies hazing the zones were evaluated as producers. The lipolitic index, I_l, was calculated as the ratio between lyses zone diameter and colony diameter, [26]. The microorganisms isolated from colonies were identified based on colonies morphology and microscopic analysis [29].

Results and Discussion

The images with proteolytic activity of selected microorganisms are illustrated in Fig. 1. Out of 57 colonies of microorganisms differently developed on nutritive agar, only 10 were able to produce efficiently clearing zones in medium containing casein, respective have a great I_p index.

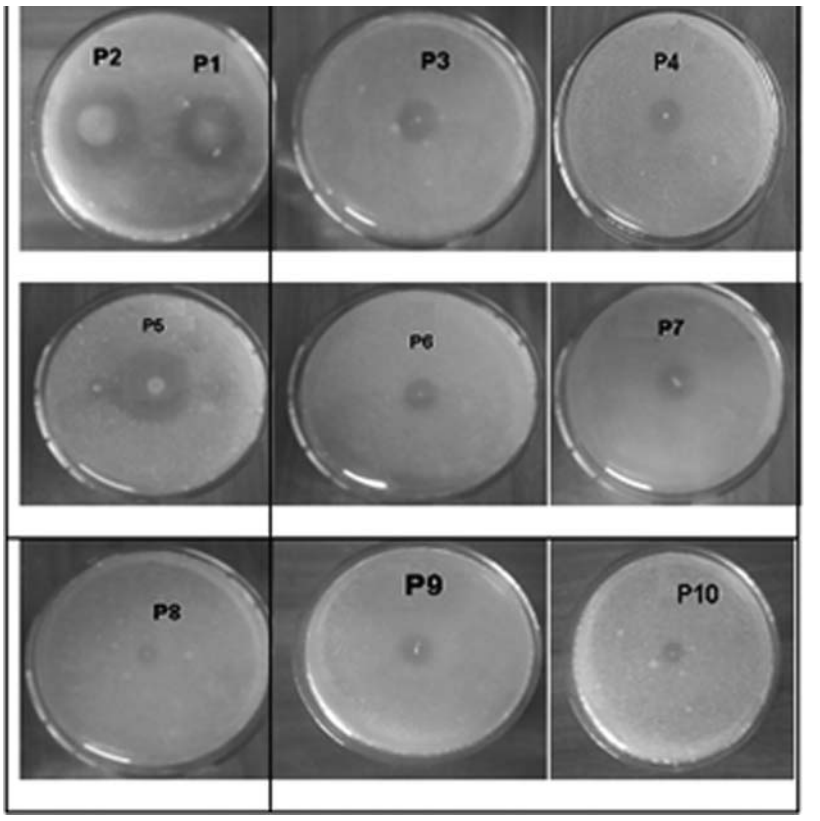

Figure 1. Images with proteolytic activity of selected microorganisms.

Table 1. Enzymatic activity for selected colonies

Colony number	Temperature of incubation, °C	Proteolytic activity	Gelatin hydrolysis	Lipolytic activity
P1	17	+	−	+
P2	17	+	−	+
P3	37	+	+	+
P4	17	+	+	+
P5	17	+	+	−
P6	17	+	−	−
P7	37	+	+	+
P8	37	+	−	+
P9	37	+	+	+
P10	37	+	+	+

The enzymatic activity for selected colonies is presented in Table 1, and the values of I_p and I_l indices for ten colonies selected as good protease producers for 24, 48 and 72 hours are collected in the Table 2.

The selected microorganisms showed good growth and protein synthesis with a varying level of proteolytic activity (see Table 2). The proteolytic activity was developed at different temperatures as follows: at 17°C for samples P1, P2, P4, P5, P6 and P7 of cryophilic microorganisms, and at 37°C for samples P3, P8, P9, P10 of mesophilic-thermophilic microorganisms.

From analysis of enzymatic activity results that the microorganisms from samples P3, P4, P7, P9 and P10 produce protease and lipase enzymes in the same time, proteases can hydrolyze the gelatin and thus can be used for leather biodegradation.

The analysis of data presented in Table 2 reveals that the proteolytic and lipolytic indicators are greater after 48 hours, the proteolytic indices decrease in order: P3 > P6 > P8 > P9 > P67 > P4 > P10 > P5 > P1 > P2 and the lipolytic indices decrease in order: P9 > P8 > P7 > P3 > P2 > P1 > P10 > P4. It is expected that microorganisms that were

Table 2. Values of I_p and I_l for colonies good protease producers

Colony Number	Proteolytic index, I_p			Lipolytic index, I_l		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
P1	1.0	1.8	2.8	2	2.5	2.25
P2	0.2	0.6	1.4	2	3	2.75
P3	10	18	14.5	3.34	3.25	3.00
P4	7	6.75	6.67	1.25	1.25	1.25
P5	2.75	4	5.6	—	—	—
P6	8	11.5	10.7	—	—	—
P7	10	7	6.3	0	4	4.5
P8	8	8	6.7	6.25	6.25	6.25
P9	8	7.5	7.2	14	14.3	14.7
P10	6	6	6	1.7	1.3	1.1

Table 3. Characterization of colonies

Colony number	Size	Color	Surface	Form	Visual Characteristics	Consistence	Height
P1	Great	White	Roughness	Circular edges lobed	Opaque with floccose white mycelium	Dry	Raised-convex
P2	Great	Grey-White	Roughness	Circular centrally umbonate	Opaque with floccose white mycelium	Dry	Raised-convex
P3	Great	White-cream	Roughness	Irregular	Translucent	Viscous	Raised-convex
P4	Small	Yellow	Smooth	Circular	Translucent	Mucous	Raised-convex
P5	Medium	Yellow-white	Smooth	Circular	Translucent	Mucous	Raised-convex
P6	Small	Yellow	Smooth	Circular	Translucent	Mucous	Raised-convex
P7	Medium	White- yellow	Smooth	Circular	Translucent	Mucous	Raised-convex
P8	Great	White-	Roughness	Irregular	Opaque	Mucous	Plate
P9	Great	White- yellow	Roughness	Irregular	Opaque	Mucous	Plate
P10	Medium	White	Roughness	Irregular	Opaque	Mucous	Plate

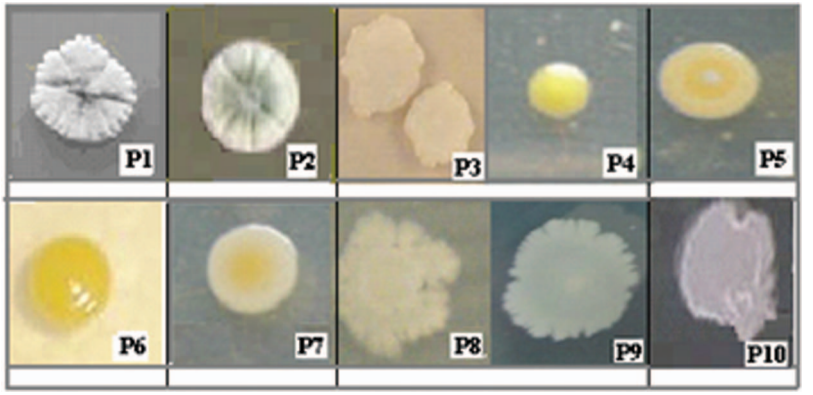

Figure 2. Microscopic images of microorganisms colonies: P1 - Penicillium sp., P2 - Aspergillum sp., P3, P8, P9, P10 - Bacillus sp., P4, P5, P6, P7 - Micrococcus sp.

developed in colonies P8, P9, P3 and P7 to have the best performance in the degradation of leather waste.

In Table 3 and Fig. 2 the characterization and images of microorganism colonies are presented.

After microscopic analysis of microorganisms in colonies and cells the following species have been identified: P1 - Penicillium sp., P2 - Aspergillum sp, P3, P8, P9, P10 - Bacillus sp., P4, P5, P6, P7 - Micrococcus sp.

Conclusion

The methodology used for isolation, selection and characterization of microorganisms sampled from leather waste storage dump results in obtaining of microorganisms able to produce the extracellular protease and lipase.

The Bacillus sp (samples P3, P9 and P8) showed higher extracellular proteolytic and lipolytic activity, all these being mesophilic-thermophilic microorganisms.

The maximum enzymatic activity obtained is function of cultivation time, the maximum level of protease production being obtained after 48 hours by Bacillus sp (P3).

Selected bacteria strains have biotechnological potential for degradation and utilization for leather waste biodegradation. Microorganisms selected will be also used for the synthesis of enzymes required for biodegradation of leather waste.

Acknowledgments

"This work has benefited from financial support through the 2010 POSDRU/89/1.5/S/52432 project, "ORGANIZING THE NATIONAL INTEREST POSTDOCTORAL SCHOOL OF "APPLIED BIOTECHNOLOGIES" WITH IMPACT ON ROMANIAN BIOECONOMY", project co-financed by the European Social Fund through the Sectoral Operational Programme Human Resources Development 2007–2013."

References

[1] Shede, P. N., Kanekar, P. P., Polkade, A. V., Dhakephalkar, P. K., & Sarnaik, S. S. (2008). *Intern. Biodet. & Biodegr.*, *62*, 65–74.

[2] Ruggieri, L., Artola, A. Gea, & Sanchez, T. A. (2008). *Intern. Biodet. & Biodegr.*, *62*, 297–303.

[3] Albu, L., & Deselnicu, V. (2007). *Raw Materials and Auxiliary Materials for Leather Industry*, Certex: Bucuresti.

[4] Chirita, G., & Chirita, M. (1999). *Treaty of Chemistry and Technology of Leather and Fur*, vol. 1, "Gheorghe Asachi", Iasi.

[5] Teles, F. R. R., & Santos, J. A. L. (2001). *Biotech. Let.*, *23*, 1159–1163.

[6] Bienkiewicz, K. (1993). *Fundamentals of Leather Manufacture*, Eduard Roether: KG.

[7] Kalaiarasi, K., & Sunitha, P. U. (2009). *Afr. J. of Biotech.*, *8*(24), 7035–7041.

[8] Paige, M. F., Lin, A. C., & Goh, M. C. (2002). Real-time enzymatic biodegradation of collagen fibrils monitored by atomic force microscopy. *Int. Biodet. & Biodegr.*, *50*, 1–10.

[9] Das, G., & Prasad, M. P. (2010). Isolation, purification & mass production of protease enzyme from *Bacillus Subtilis*. *Int. Res. J. Microb.*, *1*(2), 026–031.

[10] Zerdani, I., Faidi, M., & Malki, A. (2004). Digestion of solid tannery wastes by strains of *Bacillus Sp.* isolated from compost in morocco. *Int. J. of Agr. & Biol.*, *6*(5), 758–761.

[11] Olajuyigbe, F. M., & Ajele, J. O. (2005). Production dynamics of extracellular protease from *Bacillus* species. *Afri. J. Biotech.*, *4*(8), 776–779.

[12] Gambello, M. J., Kaye, S., & Iglewski, B. H. (1993). LasR of *Pseudonas auriginosa* is a transcriptional activator of alkaline protease gene (apr) and an enhancer of exotoxin A expression. *Infection and Immunity*, *61*(4), 1180–1184.

[13] Bach, H.-J., & Munch, J. C. (2004). Identification of bacterial sources of soil peptidases. *Biol. Fertil. Soils*, *31*, 219–224.

[14] Bach, H.-J., Tomanova, J., Schloter, M., & Munch, J. C. (2002). Enumeration of total bacteria and bacteria with genes for proteolytic activity in pure culture and environmental samples by quantative PCR mediated amplification. *J of Microbiol. Met.*, *49*, 235–245.

[15] Gupta, R., Beg, Q. K., & Lorenz, P. (2002). Bacterial alkaline proteases: molecular approaches and industrial applications. *Appl. Microbiol. and Biotech.*, *59*(1), 15–32.

[16] Orlita, A. (1968). Biodeterioration in leather industry. In: A. H. Walters & J. J. Elphick (Eds.), *Biodeterioration of Materials, Proceedings of the First International Biodeterioration Symposium*, Elsevier: London, pp. 297–301.

[17] Orlita, A. (1998). *Microorganisms in the tannery, Public Seminar on Biological Aspects of Leather Manufacture*, Central Leather Research Institute: Adyar, Madras, India, pp. 1–18.

[18] Fassatiova, O. (1979). Mould and Fungi in Technical Microbiology, SNTL, Prague, Czechoslovakia, 101–124.

[19] Sivaparvathi, M., Suseela, K., & Nanda, S. C. (1986). Purification and properties of pseudomonas aeruginosa protease causing hydrolysis of chrome shaving. *J. Leath. Sci.*, *33*(11), 303–307.

[20] Taylor, M. M., Diefendorf, E. J., & Na, G. C. (1992). Enzymatic Processing of Materials Containing Chromium and Proteins [P]. US Pat.:US5094946.

[21] Alford, J. A., & Pierce, D. A. (1963). Production of lipase by Pseudomonas fragi in synthetic medium. *J. Bacteorol.*, *86*, 24–29.

[22] Kennedy, B. W. (1968). Prevalence and detection of lipolytic microorganisms in Spybean Seeda. *Sci. J. Series*, 70–74.

[23] Ko, W. H., Wang, I. T., & Ann, P. J. (2005). A simple method for detection of lipolytic microorganisms in soil. *Soil Biol. and Biochem.*, *37*(3), 597–599.

[24] Guzman, M. N., Vargas, V. A., Antezana, H., & Svoboda, M. (2008). Lipolytic enzime production by halophilic/halotolerant microorganisms isolated from Laguna Verde, Bolivia. *Rev. Boliviana de Quim.*, *25*(1), 14–23.

[25] Arpigny, J. L., & Jaeger, K.-E. (1999). Bacterial lipolytic enzimes: clasification and properties. *Biochem. J.*, *343*, 177–183.

[26] Ferdes, M., & Ungureanu, C. (2009). *Industrial Microbiology*, Ed Printech: Bucharest.

[27] Ammar, M. S., Yassin, M. El-Saway, & Sherif, Y. M. (1998). Hydrolytic enzymes of fungi isolated from certain Egyptian antiquities objects while utilizing the industrial waste of sugar and integrated industries company (SIIC). *Egypt. J. Biotechn.*, *3*, 60–90.

[28] Harrigan, W. F., & Mc-Cance, M. E. (1966). *Laboratory Methods in Microbiology*, Academic Press: New York.

[29] Ülhan, S., Demurel, R., Asan, A., Bayu, C., & Kinaci, E. (2006). Colonial and morphological characteristics of some microfungal species isolated from agricultural soils in Eskißehir province (Turkey). *Turk J. Botanic*, *30*, 95–104.