Egypt. J. Chem. **Vol. 62,** No. 11, pp. 2033 - 45 (2019)

Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Retting and Degumming of Flax Using Biotechnology Eco-friendly Approach

Hassan Moawad[1], Wafaa M. Abd El-Rahim[*1], Mohamed M. Hashem[2], Gebreil M. Gebreil[1], Ali Sabbor[4] and Mohamed Zakaria[3]

[1]Department of Agricultural Microbiology, Agricultural and Biological Research Division, National Research Centre, Egypt.
[2]Department of Pretreatment and Finishing of Cellulosic Based Fibers, Textile Research Division, National Research Centre, Egypt.
[3]Department of Agricultural Microbiology, Faculty of Agriculture, Cairo University, Cairo, Egypt.
[4]Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Cairo, Egypt.

FLAX retting is a treatment process employed to dissolve or rot away much of the cellular tissues and pectins surrounding bast/fiber bundles thus facilitating separation of the fiber from the stem. The microbial retting is one of the most environmentally friendly process. The rich source of microbes contributing to flax retting is the retting water in commercial retting plants. The main objective of this work is to accelerate the flax retting using selected bacterial strains contributing to retting and degumming of flax in order to avoid chemicals causing environmental pollution. In this work, reuse of retting water for acceleration of flax retting was tested. The results show that, the reuse of retted water in new cycle of flax retting has accelerated the retting completion by 8.3-25% depending on the ratio of reused retted water in the retting liquor. Therefore, samples of flax straw retted water and soils from flax fields located in Nile Valley and Delta soils were collected for isolation of flax retting bacteria predominant in the retting effluents. Identification of the isolates was done using morphological, physiological and biochemical tests. Ten bacterial isolates were identified as *Lactococcus cremoris, Chryseobacterium culicis, Serratia marcescens, Capnocytophage granulose, Micrococcus luteus, Enterobacter homraechei, Klebsiella oxytoca, Paenibacillus polymyxa, Proteus mirabilis* and *Bacillus humi.* Three efficient strains in pectinase production necessary for flax retting were selected to test their role in enhancing the retting process. These strains are *Bacillus humi, Chryseobacterium culicis* and *Micrococcus luteus* which showed specific pectinase activity 37.17, 33.53 and 28 Umg^{1} respectively. The effect of these strains on retting duration and fiber quality were studied. The combinations of the three strains were tested in the lab scale cylindrical experimental bioreactor and each of the combinations was compared with the classical retting process of the industrial units. The combined treatment using strains: *Chryseobacterium culicis, Micrococcus luteus* and *Bacillus humi* resulted in the reduction of the retting duration by 30%, whereas, the mixed inocula containing *Chryseobacterium culicis* and *Micrococcusluteus* reduced the retting time by 25%. The mixture of *Micrococcus luteus* and *Bacillus humi* reduced the retting duration by 20%. The determination of weight loss, tensile strength, whiteness and yellowness of the fibers after microbial retting was monitored. These results indicate that the obtained specific bacterial strains enhanced the retting process.

Keywords: Flax, Microbial retting, Strength, Whiteness and yellowness.

*Corresponding author e-mail: wafaa10m@hotmail.com, wafaa10m@gmail.com
Received 12/2/2019; Accepted 14/5/2019
DOI: 110.21608/ejchem.2019.9571.1641

Introduction

Retting and degumming of flax straw is an important step in textile industry. The retting, is a process employing the action of micro-organisms and moisture on plants to dissolve or rot away much of the cellular tissues and pectins surrounding bast-fiber bundles, and so facilitating separation of the fiber from the stem [13, 14]. In the wet retting process of the flax, bundles of flax stems are to be immersed in water for fermentation by anaerobic bacteria to degrade pectins and matrix components in the plant cell wall [17]. When the retting is complete, the bundles of flax feel soft and slimy, and quite a few fibers are standing out from the stalks.

Flax has a long history as a double purpose crop cultivated in Egypt, China and India since long ago [1-3, 7]. The processed flax is used to make very fine linen fabrics [4-6, 8]. Flax on the other hand is grown in tropical as well as in temperate countries for its oil-bearing seeds [9, 10]. The seeds are crushed to make linseed oil, and the remaining cake is used for fodder. Additionally, dried flaxseed has been used in various medicinal preparations [11, 12]. Egyptian Silty clay soils in Delta and Nile valley regions are used for growing economical food crops [15, 16] and fiber and oil crops [1].

Several methods are used for retting of flax. Enzymatic retting methods use polymer degrading enzymes (pectinase, hemicellulase and cellulase) [19]. The chemical method uses harsh chemical agents. The biological methods use microorganisms with polymer degrading enzymes. The aim of this study is to accelerate the flax retting using selected bacterial strains contributing to retting and degumming of flax in order to avoid harsh chemicals causing environmental pollution.

Materials and Methods

Collection of samples and isolation of native microbes

Samples from retted water of flax straw were collected from Flax Tanta Company, El-Gharbia, Egypt. The retted water samples were kept in refrigerator at 5°C until analysis. Serial dilutions of each sample were prepared in distilled water and spread on nutrient agar (NA). The plates were incubated at 30°C for 24h. Colonies of bacteria were picked from each plate and streaked on the same media for further purification [20- 23].

Primary screening of bacterial isolates for pectinase activity on solid media

Isolates were tested on pectin mineral agar salt medium (PMAS) for primary selection of isolates. The medium composition is as follows; 0.2% pectin (the sole source of carbon), 0.3% KH_2PO_4, 0.6% Na_2HPO_4, 0.2% NH_4Cl, 0.5% NaCl, 0.01% $MgSO_4.7H_2O$ and 1.5% agar. Solution pH was adjusted at 7, and plates were incubated at 30°C for 24 hrs [24, 25].

Screening of pectinase producing isolates in broth media

Bacterial isolates were grown on pectin mineral salt medium (PMSM broth). Inoculated broth media with isolates were incubated at 30°C with shaking at 120 rpm. Optical density was estimated at 600 nm after 24 hours. The broth culture was centrifuged and supernatants were used for the screening of pectinase activity using well plate method. Petri dishes containing pectin mineral agar salt medium (PMAS) amended with 1.5% agar were used. The wells of 5 mm diameter were cut in the agar with the help of cork borer. Each well was filled with 25 µL of supernatant and incubated for 24 hrs at 30°C. The plates were flooded with iodine solution according to Martin et al. and Miller [25, 26]. The enzyme activity was judged by measuring the diameter of clear zone around the well in millimeters. The background of the agar containing pectin turns into black color, while the clear zones around the wells indicate the production of pectinase that breakdown the pectin to galactouronic acid.

Confirmatory analysis of pectinase producing bacteria

Quantitative production of pectinase enzyme by bacterial isolates in pectin mineral salt medium (PMSM broth) of pH 7 was tested. The culture was incubated for 24 hrs at 30°Con shaking incubator 120 rpm for measuring pectinase activity. The Pectinase was estimated using Dinitrosalicylic Acid (DNS) method according to Miller [26]. A standard curve of D-Galactouronic (1 $mgml^{-1}$) was developed under identical conditions to determine the reducing sugars formed. The enzymatic activity of supernatant was expressed as enzyme Units per ml (Uml^{-1}) which was defined as the amount of enzyme which liberates 1 µmole of reducing sugar per ml per min under assay conditions.

Determination of protein by Folin-Lowry's method

The total protein was estimated in supernatant of culture broth and after precipitation by Folin-

Lowry's [28]. Bacterial cultures were grown on pectin broth media on incubator shaker (120rpm) for 24 hours at 30^0C. The cultures were then centrifuged for 15 minutes at (8000rpm). The protein in the supernatant was estimated using Folin-Lowry's method. The estimation of pectinase activity was calculated according the following formula:

Pectinase specific activity (Umg^{-1}) = Pectinase Activity Uml^{-1}/ Total Protein $mgml^{-1}$

Biochemical characterization of bacterial isolates producing pectinase

The selected strains were identified to the species level using Biolog, Hayward, GenIIIMicroPlate™. The bacteria was released into the Inoculation Fluid (IF-A) by rubbing the swab tip against the bottom of the tube containing IF-A. Any cell clumps in the IF-A were crushed against the tube wall. Then, the IF-A suspension was gently tilted upside-down a few times to obtain a uniform cell suspension and the transmittance (% T) was read using Biolog Turbid meter (Biolog, Hayward, CA, USA). Then, the GENIII Micro plates were incubated at 33°C for 24 h. The resulting patterns were read using the Biolog automated Micro-Station instrument (Biolog, Hayward, CA, USA).

Retting of flax fiber using different ratios of retted water

Experiment was sit using cylindrical bioreactor for retting of flax fiber. The liquor flax ratio in each` unit of the bioreactor is 1:30. this experiment was done in July month 2016, whereas, the ambient air temperature fluctuated between 28-36ºC (Egyptian Meteorological Authority). The treatments were:

- Conventional retting underground water.
- Ground water with 25 % reused retted water.
- Ground water with 50 %reused retted water.
- Ground water with 75 % reused retted water.
- 100 % reused retted water.

Completion of the retting flax process in the bioreactor was evaluated using Fried test as described by Nayebyazdi and Norris [29, 30]. In this test, retting is scored based on visual standards from 0 (no fiber separated from stems) to 3 (extensive fiber separation), which is done through facilitating broken flax stalks with distinctive sound like break down stick and easing fibers separation from straws. After retting the flax stalks in the bioreactor, the stalks were left to dry in open air for a short period to allow "curing" to occur and facilitate fiber removal. Final separation of the fiber is accomplished by a breaking process in which the brittle woody portion of the straw is broken by the scutching to remove the broken woody pieces (shives). The acceleration of retting process % was calculated throughout the equation.

Acceleration of retting process was calculated using the following equation :

Commercial Retting Completion Time - Treatment Completion Time / Commercial Retting Completion Time X 100

Enhancing retting using three potential bacterial strains producing pectinase

Three selected potential pectinase producing bacterial strains were grown on nutrient broth in 5L conical flask at 28ºC for 24 h and the growth intensity was measured as O.D at 600 nm by JENWAY-6305 UV-VIS spectrophotometer. The strains were mixed equally to form inoculant mixtures containing double or triple and mixed inoculants were used in the retting of flax fiber in the experimental cylindrical bioreactor. The liquor ratio (LR) representing weight of fiber to weight of water was constant in all treatments being 1/30 at initial time. This experiment was conducted in January 2017, whereas, the ambient air temperature fluctuate between 18-25^0C (Egyptian Meteorological Authority).The bacterial treatments used for retting of flax straw were as follows:

- Conventional retting without addition external bacteria.
- Mixture of strains *Chryseobacterium culicis* and *Bacillus humi*.
- Mixture of strains *Micrococcus luteus* and *Bacillus humi*.
- Mixture of strains *Chryseobacterium culicis* and *Micrococcus luteus*.
- Mixture of strains *Chryseobacterium culicis*, *Micrococcus luteus* and *Bacillus humi*.

The completion of flax the retting process was evaluated as previously mentioned.

Retting indices measurements

The microbial load was determined by measuring the optical density at wave length 600 nm by JENWAY-6305 UV-VIS spectrophotometer. The retting liquor color was measured at wave length 300 nm after colorization scanning by JENWAY-6305 UV-VIS spectrophotometer. Determination of weight loss was done by oven-drying method of 5g straw at105ºC before and

after retting until constant weight. The loss (%) in weight was calculated from a mean of three replicates [19]. The tensile strength (g/den.) and elongation (%) of fibers were determined by the ASTM machine using test D5035 method [31, 19]. The whiteness index (WI) and yellowness index (YI) were determined on Ultra Scan Pro. Hunterlab [18, 19 and 32].

Results and Discussions

Isolation of bacterial colonies dominant in retted water

In this study, 75 bacterial isolates were obtained from retting water of flax at Tanta Company, Egypt. Serial dilutions of retted water and soil samples were diluted in serial distilled water and spread on Nutrient agar (NA) pH 7. The plates were incubated at 30°C for 24h [33, 34]. Representative of similar colonies were picked from each plate and streaked on the same media for purification. Table 1 shows the number of isolates obtained from four industrial retting basins located in the flax retting plant in Egypt and a soil sample taken from flax growing field.

Primary Screening of bacterial isolates for pectinolytic activity

The isolates from different basins were grown on agar media containing pectin as the sole source of carbon. The idea originated from the potential of finding certain bacteria actively producing pectinolytic enzymes produced by microbes in the retting fluid. Several authors isolated the bacteria performing flax and hemp retting from the retting fluid of the industrial plants [33-35]. Table 1 shows that only 20 out of 75 isolate were able to grow and utilize pectin as sole source of carbon.

Growth of pectinase producing bacteria in liquid media

The growth of the twenty bacterial isolates in broth media containing pectin as a sole source of carbon was studied. The culture fluids were centrifuged to obtain the supernatants containing the exo-pectinase enzyme. The supernatant were then tested in the well plate method to evaluate the intensity of pectinase enzyme activity. Table 2 shows that isolate B40, B32, B35 and B31 gave the significantly higher growth as measured by OD after incubation for 24 hours on incubator shaker as compared with the other 16 isolates.

TABLE 1. Source of indigenous bacterial isolates and their growth on pectin agar media.

Factory retting basins	Number of isolates	Growth	
		+	-
Basin1	16	5	11
Basin2	17	4	13
Basin3	14	5	9
Basin4	16	5	11
Soil	12	1	11
Total	75	20	55

TABLE 2. Optical density of twenty bacterial isolates on pectin broth media.

Serial number of isolate	Isolates	OD
1	B40	1.552 a
2	B32	1.491 a
3	B35	1.443 a
4	B31	1.406 a
5	B33	1.389 a
6	B38	1.211 b
7	B39	1.112 bc
8	B34	1.024 c
9	B36	0.978 cd
10	B37	0.962 cde
11	B12	0.845def
12	B22	0.801def
13	B45	0.784efg
14	B49	0.699fg
15	B50	0.613gh
16	B41	0.612gh
17	B9	0.459 hi
18	B11	0.387ij
19	B10	0.274 j
20	B51	0.269 j

Duncan's test: values followed by different letters are significant different.

This method was previously applied to assess the pectinase activity by bacteria isolated from flax retting fluids [26, 35- 37].

Phenotypic characterization of the highest indigenous pectinolytic bacterial isolates

The differentiation between promising ten pectinase producing isolates were examined using colony and cell morphology as well as gram staining. The results are presented in Table 3. Four isolates were G^{+ve} and 6 were G^{-ve} bacteria. Most of the isolates had rod shape cells.

Screening of potential pectinase production by ten promising isolates in broth media

The diameter of zone around wells amended with bacterial supernatant indicates pectin degradation. The results show that isolate B40, B32, B35 and B31 were the most effective isolates in pectinolytic activities with isolates B40 and B32 being the highest significant pectinase produces as compared with other isolates (Table 4). These results are in line with reports published by other authors [38-40].

Confirmatory analysis for pectinase production by bacterial isolates obtained from retting water

The pectinase activity of the isolates as measured by using dinitrosalicylic acid (DNS) colorimetric method is presented in Table 5. Table 5 shows different pectinase and pectinase specific activity by the different isolates. The maximum pectinase specific activity was recorded with isolate B40 (37.17Umg^{-1}). The pectinase specific activity of these results was significantly higher than any other isolate. Isolate B32 and B35 were next in pectinase specific activity being 33.53 and 28.00 Umg^{-1}respectively. All other isolates gave different pectinase specific activity significantly lower than the previous three isolates. Pectinase production by certain microbial strains was stated in several reports [26, 41- 44].

TABLE 3. Phenotypic characterization of ten pectinolytic bacterial isolates.

Isolates	Gram stain and cell shape
B31	Gram-positive, cocci shape occur singly, in pairs, or in chain
B32	Gram-negative, rods shape
B33	Gram-negative, straight rods cells
B34	Gram-negative, fusiform to rod shape
B35	Gram-positive, spherical, occurring in pairs, tetrads, or irregular clusters
B36	Gram-negative, straight rods shape
B37	Gram-negative, rod-shape; singly and in pairs or short chains.
B38	Gram-positive, rod shape
B39	Gram-negative, rod shape
B40	Gram-positive, rod shape

TABLE 4. Indicating diameter of pectin degradation.

Isolates numbers	Zone diameter (mm.)
B40	36 a
B32	35 a
B35	29 b
B31	19 c
B33	17 d
B38	16 d
B39	14 e
B34	12 f
B36	11 f
B37	8 g

Duncan's test: values followed by different letters are significant different.

TABLE 5. Pectinolytic activity of ten bacterial isolates possessing pectinase enzyme.

Isolates	Pectinase activity of crude extract (Uml^{-1})	Crude protein ($mgml^{-1}$)	Pectinase specific activity (Umg^{-1})
B31	0.916 d	0.037 ef	24.76 d
B32	1.274 b	0.038 e	33.53 b
B33	0.775 e	0.036 fg	21.53 e
B34	0.698 f	0.047 a	14.85 g
B35	1.232 c	0.040 d	28 c
B36	0.616 g	0.045 b	13.69 g
B37	0.338 h	0.036 fg	9.39 h
B38	0.74 e	0.035 g	21.14 e
B39	0.681 f	0.041 cd	16.61 f
B40	1.561 a	0.042 c	37.17 a

Duncan's test: values within the same parameters followed by different letters are significant by different.

Identification of bacterial isolates producing pectinases

Table 6 presents the biochemical identification of the ten pectinase producing bacterial isolates obtained from retting water using biolog GENIIIMicroPlates and automated software. The isolates were diverse in biochemical characterization defining the species to which the isolate belongs. The results of the isolates identification are presented in Table 6. The tested isolates belonged to ten different species of bacteria. The number of the isolates to which specific bacteria belonged are presented in Table 6. The biolog identification of bacteria was employed in other studies [21, 45, 46]. *Bacillus licheniformis* and *Bacillus cereus* were previously used for retting of flax fibers [50].

Testing of flax retting pectinase producing bacteria in experimental cylindrical bioreactor

The three bacterial strains active in pectinase production nearly; *Chryseobacterium culicis, Bacillus humi* and *Micrococcus luteus* were used in double or triple mixtures to assess the efficient in pectinase production and retting process. The retting was performed in 30 L retting fluid where 1 kg of flax straw was submerged. Each treatment was replicated 3 times.

Effect of retted water reuse as a starter for accelerating retting process

The acceleration of flax retting process and preserving fiber quality is of great importance for the growth of flax textile industry. Flax fibers are considered promising natural fibers with high economic value. The retting process starts with the degradation of pectin responsible for adhering the plant stem with fibers. The removal of pectin can be performed by chemical treatments or smooth enzymatic process [19, 48]. The enzymatic approach is an eco-friendly process and is preferable as compared with other approaches. Since the indigenous bacteria in the retting fluids are known to perform dewaxing and degumming of pectin from the plant stems, it is expected that the retting liquor must contain high density of bacterial consortium contributing to the process. In this study, the retting water was used in different portions to assess the acceleration of the retting completion due to the activity of the indigenous bacteria already existing in the retting liquor. The reuse of different proportions of retted water in new retting cycle was studied. The effect of reuse of retted water in different ratios (0, 25, 50, 75 and 100 %) in retting fluid supplemented with ground water routinely used in factory retting process was studied. The major changes in retting media were followed throughout 72 hours of retting process. The groundwater in used as a control treatment to compare its results with those obtained from

TABLE 6. Biochemical identification of pectinase producing bacteria using biolog GENIIIMicro Plates.

Isolates	Species
B31	*Lactococcus cremoris*
B32	*Chryseobacterium culicis*
B33	*Serratia marcescens*
B34	*Capnocytophage granulose*
B35	*Micrococcus luteus*
B36	*Enterobacter homraechei*
B37	*Klebsiella oxytoca*
B38	*Paenibacillus polymyxa*
B39	*Proteus mirabilis*
B40	*Bacillus humi*

different proportion reused retted water in media used for flax retting. The temperature of the retting fluid ranged between 29 to36^{o}C depending on the time of sampling (Table 7). The sampling at day time in all treatments showed higher temperature (35-36^{0}C) as compared with the sample taken at the night time being 28-29^{o}C (Table 7). The pH of retting fluid (Table 7) showed gradual decrease in all treatments and the control throughout the retting process (72 hours). The microbial load in the retting fluid increased gradually by as the time of the retting increased until 72 hours (Table 7). The highest microbial load was observed in the last three samples taken at 48, 60 and 72 hours particularly in the treatments receiving 75 and 100% of reused retted water. The Changing of retted liquor colour that indicates the bacterial degumming and de-waxing of the crude flax straw was also assessed (Table 7). While the change in that colour in the control receiving only ground water was increased significantly and reached 5.537 (ten times more than the starting value 0.541), the increase in all other treatments receiving 25-100% of retted water was almost doubled only (Table 7). This may be due to the already existing colour in the reused retting water which interferes with the additional color removal by the bacterial load. However, the fluid color released in the growth media was much higher in the treatment receiving reused water as compared with the control.

Effect of retted water reuse on flax retting and fiber quality was studied. The results in Table 8 show that the retted water reuse in proportions of 25, 50 and 75% accelerated the retting process whereas the use of 100% of retted water did not show significant effect on retting process completion. The reuse of retted water in proportion of 75% induced significant the highest effect on retting completion (54 hours), weight loss (7.92%), fiber strength (0.654 g/den) and fiber elongation (1.398%). The values of these parameters are in general significantly different

TABLE 7. Change of temperature, pH, microbial load and retting liquor color throughout the retting process using different proportion of retted water.

Parameters	Treatments	Sampling time (hours)						
		0	12	24	36	48	60	72
Temperature	GW1	29*	35**	28	36	29	35	29
	GW + 25% RW2	29	35	28	36	29	35	29
	GW + 50% RW	29	35	28	36	29	35	29
	GW + 75% RW	29	35	28	36	29	35	29
	100% RW	29	35	28	36	29	35	29
pH	GW1	6.68	6.18	5.87	5.68	5.46	5.35	5.26
	GW + 25% RW2	6.35	6.01	5.71	5.52	5.34	5.27	5.19
	GW + 50% RW	6.04	5.81	5.57	5.41	5.27	5.19	5.11
	GW + 75% RW	5.71	5.53	5.39	5.25	5.12	5.04	5.01
	100% RW	5.28	5.23	5.21	5.09	4.98	4.87	4.85
Microbial load	GW1	0.04	0.186	0.261	0.308	0.344	0.375	0.396 d
	GW + 25% RW2	0.206	0.423	0.578	0.649	0.699	0.743	0.776 c
	GW + 50% RW	0.337	0.565	0.732	0.813	0.859	0.904	0.932 bc
	GW + 75% RW	0.451	0.702	0.863	0.955	1.012	1.047	1.078 b
	100% RW	0.546	0.826	0.993	1.102	1.159	1.195	1.218 a3
Retting liquor color	GW1	0.541	3.387	3.993	4.202	4.412	4.729	5.537 e
	GW + 25% RW2	1.926	4.533	5.212	5.431	5.658	6.059	6.889 d
	GW + 50% RW	3.312	5.786	6.387	6.717	7.058	7.476	8.478 c
	GW + 75% RW	4.607	6.812	7.535	7.875	8.223	8.753	9.788 b
	100% RW	5.598	7.897	8.693	9.081	9.397	9.957	10.957 a3

1. GW, Groundwater, 2. RW, Retted water reuse,
3. Duncan's test: values followed by different letters are significantly different.
*ambient room temperature at night time.
** ambient room temperature during the day.

TABLE 8. Effect of retted water reuse on retting completion and fiber quality.

Indices	Retted water reuse				
	GW_1	GW +25% RW_2	GW+ 50% RW	GW +75% RW	100% RW
Retting completion (h)	72 (0%)$_4$ a_3	66 (8.3%) b	60 (16.6%) c	54 (25%) d	72 (0%) a
Straw weight loss (%)	10.87 a	9.79 b	8.52 c	7.92 c	10.31 ab
Fiber strength (g/den.)	0.439 d	0.513 c	0.579 b	0.654 a	0.464 cd
Fiber elongation (%)	1.529 ab	1.489 abc	1.438 bc	1.398 c	1.541 a
Whiteness index	-111.28 cd	-110.79 bc	-110.28 ab	-109.76 a	-111.56 d
Yellowness index	54.29 b	52.71 c	51.72 c	50.48 d	55.75 a

1. GW, Groundwater, 2. RW, Retted water reuse, 3. Duncan's test: values followed by different letters are significantly different.
4. Acceleration of retting process %= Completion Time - Treatment Completion Time / Control Retting Completion Time X 100 .

from those obtained in the control receiving only ground water and treatments receiving different proportions of reused retting water (Table 8). The treatment receiving 50% of retted water came next to the 75% treatment. The results indicate that the microbial consortium existing in the retted water can play significant role in acceleration of retting process. This may be due to the presence of active pectinolytic bacteria in the retting liquor [19 and 49].

Use of potential pectinolytic bacteria for acceleration of flax retting and fiber quality

The acceleration of retting process using specific bacterial strains, proven to have high pectinase specific activity, was tested in the laboratory scale cylindrical bioreactor. The quality of flax fibers obtained after retting process depends on many factors among which microbial fermentation play significant role. In this study the dominant bacterial strains isolated from commercial retting facilities in Flax Tanta Company, Egypt, were studied in relation to performing retting of the straw and the quality of fibers obtained after retting. Three bacterial strains were used in this study: *Chryseobacterium culicis, Bacillus humi* and *Micrococcus luteus.* The combination of pairs of these bacteria in equal amounts and consortium of the three strains were included. The change of retting liquor temperature, pH, microbial load and liquor color were followed throughout 120 hours. Control treatment simulating commercial retting condition with no addition bacterial amendments was used for comparison. The results are presented in Table 9. Table 9 shows that, the temperature of retting fluid fluctuated between 18-25^{o}C. All samples taken in the day hours were slightly higher in temperature than those taken at evening. The pH of retting liquor in all treatments including the control showed gradual decrease from 6.74-6.92 at zero time to 5.06- 5.33at 120 hours of inoculation as shown in Table 9. The sharp pH decrease in first 72 hours in all treatments may be due to the biodegradation of lignin, waxes and pectin which result in the formation of organic acids. The microbial load in the retting fermentation fluid after the end of the experiment (120h) showed that the consortium of the three strains *Chryseobacterium culicis*, *Micrococcus luteus* and *Bacillus humi* gave microbial load (OD) 0.736 and the pair of strains *Chryseobacterium culicis* and *Micrococcus luteus* gave 0.671. These treatments were significantly higher than other treatment. All strains combinations were significantly higher than the standard retting process (Table 9). The change of retting liquor color has increased markedly with time being the highest in the treatment receiving bacterial strains consortium *Chryseobacterium culicis*, *Micrococcus luteus* and *Bacillus humi,* followed by mixture of strain *Chryseobacterium culicis* and strain *Micrococcus luteus*. This indicates the release of alcohols and organic acid resulting from the degradation of lignin, waxes and pectin

TABLE 9. Change of temperature, pH, microbial load and retting liquor color throughout the retting process using three bacterial strains isolated from retted water.

Parameters	Treatments	Sampling time (hours						
		0	12	24	36	48	60	72
Temperature	Control	18*	24**	25	24	25	25	18
	1 + 3	18	24	25	24	25	25	18
	2 + 3	18	24	25	24	25	25	18
	1 + 2	18	24	25	24	25	25	18
	1 + 2 + 3	18	24	25	24	25	25	18
pH	Control	6.92	6.76	6.49	5.73	5.56	5.33	6.92
	1 + 3	6.83	6.62	6.28	5.57	5.41	5.19	6.83
	2 + 3	6.87	6.69	6.39	5.64	5.48	5.25	6.87
	1 + 2	6.79	6.54	6.18	5.49	5.34	5.12	6.79
	1 + 2 + 3	6.74	6.47	6.09	5.41	5.27	5.06	6.74
Microbial load	Control	0.036	0.102	0.162	0.216	0.387	0.555	0.036 e
	1 + 3	0.108	0.179	0.249	0.302	0.474	0.618	0.108 c
	2 + 3	0.095	0.16	0.228	0.278	0.453	0.598	0.095 d
	1 + 2	0.148	0.219	0.299	0.375	0.549	0.671	0.148 b
	1 + 2 + 3	0.203	0.275	0.359	0.459	0.631	0.736	0.203 a_3
Retting liquor color	Control	0.594	3.546	4.407	5.382	5.627	6.289	0.594 e
	1 + 3	0.728	3.767	4.753	5.779	6.068	6.678	0.728 c
	2 + 3	0.623	3.664	4.597	5.646	5.876	6.521	0.623 d
	1 + 2	0.845	4.011	5.155	6.098	6.438	6.996	0.845 b
	1 + 2 + 3	0.925	4.237	5.437	6.354	6.727	7.298	0.925 a_3

a-e: Duncan's test: values followed by different letters are significantly different. 1.*Chryseobacterium culicis*, 2. *Micrococcus luteus,* 3. *Bacillus humi*.
*ambient room temperature at night time. ** ambient room temperature during the day.

of flax straw (Table 9).

Effect of using selected bacterial inoculation retting processing quality of flax fibers after retting

The acceleration of retting process by using bacterial strain combinations showed that the consortium of the three bacterial strains resulted in early completion of flax retting process (Table 10). The combined treatment using three bacterial strains: *Chryseobacterium culicis, Micrococcus luteus* and *Bacillus humi* resulted in the reduction of the retting completion time by 30%, whereas, the mixed culture of two bacterial strains *Chryseobacterium culicis* and *Micrococcus luteus* reduced the retting completion time by 25%. The mixture of *Micrococcus luteus* and *Bacillus humi* also reduced the retting duration by 20%. The weight loss of the retted water flax straw differed from one bacterial combination to another (Table 10). While, the retting completion under commercial factory condition was reached at 120 hours, using the bacterial inoculates reduced the completion time significantly (84-108 hours). The weight loss of flax straw was measured at the end of retting completion in inoculant treatment. The results show that the weight loss was almost identical in all the treatments regardless the microbial combination and/or the time of retting completion. The fiber strength was the highest in the treatment receiving bacterial mixture of *Chryseobacterium culicis* and *Micrococcus luteus* being 0.546 g/den (Table 10). The elongation (%), whiteness index and yellowness index did not show significant differences among the different bacterial inoculates used for accelerating retting process. These results are in harmony with those obtained by other scientists [14, 47, 50 and 51]

TABLE 10. Effect of selected bacterial inoculants on flax fiber quality.

Indicators	Inocula				
	GW	***C. culicis* + *B. humi***	***M. luteus* + *B. humi***	***C. Culicis* +*M. luteus***	***C .culicis* + *M .luteus* + *B. humi***
Retting completion (h)	120 (0%)[a] a	96 (20%)[a] c	108 (10%)[a] b	96 (20%)[a] c	84 (30%)[a] d
Weight loss (%)	7.400 a	7.860 a	7.447 a	7.020 a	8.027 a
Strength (g/den.)	0.342 b	0.358 b	0.429 ab	0.546 a	0.516 ab
Elongation (%)	0.879 a	0.859 a	0.770 a	0.693 a	0.710 a
Whiteness Index	-85.84 a	-86.11 a	-87.40 a	-88.16 a	-81.91 a
Yellowness Index	49.89 a	51.12 a	50.06 a	49.08 a	48.13 a

a, Acceleration of retting process %= Completion Time - Treatment Completion Time / Control Retting Completion Time X 100
Duncan's test: values followed by different letters are significant different.

who stated that specific bacterial strains can enhance retting process.

Conclusion

Acceleration of flax straw retting and preservation of fiber quality represent important steps toward the promotion and revitalization of flax textile industry. In this study, the seventy-five indigenous bacterial isolates were obtained from retted water rich in native microbes and enzymes contributing to the degumming and dewaxing of flax straw. The results clearly show that the microbial consortium existing in the retted water play significant role in acceleration of retting process, when used as starter in the new retting cycle. The use of retted water in the proportion of 25-75% reduced the completion of retting by 8.3-25%. The reuse of retted water in proportion of 75% induced the highest significant effect on retting completion time (54 hours). The results indicate that the obtained specific bacterial strains enhanced the retting process. The potent bacterial strains in the pectinase enzyme production were used as double or triple inocula to accelerate the flax retting. The acceleration of retting process using the bacterial consortium ranged between 10 - 30%. In general, the results show that the use of the three bacterial strains combined was the best in reducing retting completion time by 30% and improved fiber quality as measured by fiber strength, elongation, whiteness and yellowness. It is evident that the use of pectinase producing bacteria for flax retting represents an eco-friendly approach to avoid harsh chemical uses causing environmental pollution.

Acknowledgement

The authors acknowledge the financial support granted by Egyptian Academy of Scientific Research and Technology to conduct research on the eco-friendly bio-processing of flax fibers and bioremediation of industry wastes. Thanks are due to Flax Tanta Company for providing the necessary information and materials needed for this study.

References

1. Akin, D. E., Foulk, J. A., Dodd, R. B., and McAlister III, D. D., Enzyme-retting of flax and characterization of processed fibers. *Journal of Biotechnology*, **89**(2-3), 193-203 (2001).
2. Al-Dhabaan, F. A. M., and Bakhali, A. H., Analysis of the bacterial strains using Biolog plates in the contaminated soil from Riyadh community. *Saudi Journal of Biological Sciences*, **24**(4), 901-906 (2017).
3. Billmeyer Jr, F. W., Kumar, R., and Saltzman, M., Identification of organic colorants in art objects by solution spectrophotometry: Pigments. *Journal of Chemical Education*, **58**(4), 307 (1981).
4. Bos, H., Van Den Oever, M., and Peters, O., Tensile and compressive properties of flax fibres for natural fibre reinforced composites. *Journal of Materials*

Science, **37**(8), 1683-1692 (2002).

5. Burns, J., The polygalacturonases and lyases. *The Chemistry and Technology of Pectin,* 165-188 (1991).

6. Charlet, K., Jernot, J., Eve, S., Gomina, M., and Bréard, J., Multi-scale morphological characterisation of flax: From the stem to the fibrils. *Carbohydrate Polymers*, **82**(1), 54-61 (2010).

7. Chiliveri, S. R., Koti, S., and Linga, V. R., Retting and degumming of natural fibers by pectinolytic enzymes produced from *Bacillus tequilensis* SV11-UV37 using solid state fermentation. *Springer Plus*, **5**(1), 559 (2016).

8. Danial Thomas, P., Ambikapathy, V., and Panneerselvam, A., Exploring pectinolytic activity of *Hormodendrum cladosporioides*, a less studied fungus. *Advances in Applied Science Research*, **4**(6), 1- 5 (2013).

9. Das, B., Chakraborty, A., Ghosh, S., and Chakrabarti, K., Studies on the effect of pH and carbon sources on enzyme activities of some pectinolytic bacteria isolated from jute retting water. *Turkish Journal of Biology*, **35**(6), 671-678 (2011).

10. Di Candilo, M., Bonatti, P., Guidetti, C., Focher, B., Grippo, C., Tamburini, E., and Mastromei, G., Effects of selected pectinolytic bacterial strains on water retting of hemp and fibre properties. *Journal of Applied Microbiology*, **108**(1), 194-203 (2010).

11. Fuller, D., Korisettar, R., Venkatasubbaiah, P., and Jones, M. K., Early plant domestications in southern India: some preliminary archaeobotanical results. *Vegetation History and Archaeobotany*, **13**(2), 115-129 (2004).

12. Gerhardt, P., *Methods for General and Molecular Bacteriology:* American Society for Microbiology (1994).

13. Ghazala, I., Haddar, A., Romdhane, M. B., and Ellouz-Chaanouni, S., Screening and Molecular Identification of New Microbial Strains for Production of Enzymes of Biotechnological Interest. *Brazilian Archives of Biology and Technology,* **59** (2016).

14. Goyal, A., Sharma, V., Upadhyay, N., Gill, S., and Sihag, M., Flax and flaxseed oil: an ancient medicine and modern functional food. *Journal of Food Science and Technology*, **51**(9), 1633-1653 (2014).

15. Hassan Moawad, Wafaa M. Abd El-Rahim, DossokiAbd El-Aleem., Performance of phaseolus bean rhizobia in soils from the major production sites in Nile Delta. *Journal of Comptesrendus - Biologies*. **327**(5), 445-453 (2004).

16. Hassan Moawad, Wafaa M. Abd El-Rahim, Dossoki Abd El-Aleem, S. Abo Sedera. Persistence of two *R. etli* inoculant strains in clay and silty loam soils. *Journal of Basic Microbiology* **45** (6), 438-446 (2005).

17. Hitha, P., and Girija, D., Isolation and screening of native microbial isolates for pectinase activity. *Int J Sci Res (Ahmedabad)*, **3**, 632-634 (2014).

18. Jankauskienė, Z., and Gruzdevienė, E., Physical parameters of dew refitted and water refitted hemp (Cannabis sativa L.) fibres. *Zemdirbyste-Agriculture,* **100** (1), 71-80 (2013).

19. Jhala, A.J., and Hall, L.M., Flax (*Linum usitatissimum* L.): current uses and future applications. *Aust. J. Basic Appl. Sci,* **4**(9), 4304-4312 (2010).

20. Karkun, A., Verma, S., and Patle**, K.,** Study of Pectinolytic Bacteria residing in Mandeepkhol cave, Rajnandgaon district of CG India. *Int. J. Curr. Microbiol. App. Sci*, **3**(8), 547-552 (2014).

21. Khan, I., and Barate, D., Effect of various parameters on activity of pectinase enzyme. *International Journal of Advanced Research,* **4**(1), 853-862 (2016).

22. Kumar, A., and Sharma ,R., Production of alkaline pectinase by bacteria (Cocci sps.) isolated from decomposing fruit materials. *Journal of Phytology*, **4** (1), (2012).

23. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193** (1), 265-275 (1951).

24. Lucas, A., and Harris, J. Ancient Egyptian materials and industries: Courier Corporation (2012).

25. Martin, N., Mouret, N., Davies, P., and Baley, C., Influence of the degree of retting of flax fibers on the tensile properties of single fibers and short fiber/polypropylene composites. *Industrial Crops and Products*, **49**, 755-767 (2013).

26. Miller, G. L., Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, **31**(3): 426-428 (1959).

27. Nag, S., Mitra, J., and Karmakar, P., An overview on flax (*Linum usitatissimum* L.) and its genetic diversity. *International Journal of Agriculture, Environment and Biotechnology*, **8**(4), 805 (2015).

28. Nair, G. R., Singh, A., Zimniewska, M ,. and Raghavan, V., Comparative Evaluation of Physical and Structural Properties of Water Retted and Non-retted Flax Fibers. *Fibers*, **1**(3), 59-69 (2013).

29. Nayebyazdi, N., Pectinase Activity of some Micromycetes isolated from agricultural soils. *Journal of Sciences, Islamic Republic of Iran*, **23** (4), 305-311 (2012).

30. Norris, P. K., *Cotton production in Egypt,* US Department of Agriculture (1934).

31. Oksman, K., Mathew, A. P., Långström, R., Nyström, B., and Joseph, K., The influence of fibre microstructure on fibre breakage and mechanical properties of natural fibre reinforced polypropylene. *Composites Science and Technology*, **69** (11-12), 1847-1853 (2009).

32. Oumer, O. J., and Abate, D., *Screening and Molecular Identification of Pectinase Producing Microbes from Coffee Pulp.* Bio Med research international, (2018).

33. Priya, V., and Sashi, V., *Pectinase Enzyme Producing Microorganisms* **1**(2014).

34. Rosemberg, J., Bacteria responsible for the retting of Brazilian flax. *Applied Microbiology,* **13**(6), 991-992 (1965).

35. Ruan, P., Raghavan, V., Gariepy, Y., and Du, J., Characterization of flax water retting of different durations in laboratory condition and evaluation of its fiber properties. *Bio Resources*, **10**(2), 3553-3563 (2015).

36. Sharma, H., The role of bacteria in retting of desiccated flax during damp weather. *Applied Microbiology and Biotechnology*, **24**(6), 463-467 (1986).

37. Sharma, H., Studies on chemical and enzyme retting of flax on a semi-industrial scale and analysis of the effluents for their physico-chemical components. *International Biodeterioration*, **23**(6), 329-342 (1987).

38. Sharma, P., and Sharma, N., Molecular Identification, Production and Optimization of Pectinase by using *Stenotrophomonas maltophilia* P9 Isolated from Algal Biomass of Himachal Pradesh, India. Int. *J. Curr. Microbiol. App. Sci,* **7**(1), 670-680 (2018).

39. Sharma, S. K., Ramesh, A., Sharma, M. P ,.Joshi, O. P., Govaerts, B., Steenwerth, K. L., and Karlen, D. L., Microbial community structure and diversity as indicators for evaluating soil quality Biodiversity, biofuels, agroforestry and conservation agriculture, 317-358 (2010).

40. Shea, A ,.Wolcott, M., Daefler, S., and Rozak, D. A., Biolog phenotype microarrays Microbial Systems Biology, Springer Science+Business Media, 331-373 (2012).

41. Soares, M. M., Silva, R. D., and Gomes, E., Screening of bacterial strains for pectinolytic activity: characterization of the polygalacturonase produced by Bacillus sp. *Revista de Microbiologia,* **30**(4), 299-303 (1999).

42. Sridevi, K., SivaRaagini, P., Sumanth, M., and Vijalakshmi, K., Isolation and screening of pectinolytic bacteria from the mango fruit yards. *World Journal of Pharmacy and Pharmaceutical Sciences,* **5**(1), 738-748 (2015).

43. Sundaresan, S ,.Ramesh, M., Sabitha, V., and Ramesh, V., A detailed analysis on physical and comfort properties of bed linen woven fabrics (2016).

44. Tamburini, E., León, A. G., Perito, B., and Mastromei, G., Characterization of bacterial pectinolytic strains involved in the water retting process. *Environmental Microbiology*, **5**(9), 730-736 (2003).

Tariq, A., and Latif, Z., Isolation and biochemical characterization of bacterial isolates producing different levels of polygalacturonases from various sources. *African Journal of Microbiology Research,* **6**(45), 7259-7264 (2012).

45. Vaisey-Genser, M., and Morris, D. H., *Introduction: History of The Cultivation and Uses of Flaxseed Flax,* CRC Press, 13-33 (2003).

46. Van Sumere, C., Retting of flax with special reference to enzyme-retting The biology and processing of flax, M publications, 153-193 (1992).

47. Wisseman, S. U., and Williams, W. S., Ancient technologies and archaeological materials: Routledge (2013).

48. Zhao, D., Liu, P., Pan, C., Du, R., Ping, W., and Ge, J., Flax retting by degumming composite enzyme produced by *Bacillus licheniformis* HDYM-04 and effect on fiber properties. *The Journal of The Textile*

Institute, **108**(4), 507-510 (2017).

49. Zhao, D., Pan, C., Ping, W., and Ge, J., Degumming Crude Enzyme Produced by *Bacillus cereus* HDYM-02 and its Application in Flax Retting. *Bio Resources*, **13**(3), 5213-5224 (2018).

50. Zohary, D., and Hopf, M., *Domestication of Plants in the Old World: The Origin and Spread of Cultivated Plants in West Asia. Europe, and the Nile Valley* Oxford University Press, New York (2000).

تعطين وإزالة اصماغ الياف الكتان باستخدام تفنية حيوية صديقة للبيئة

حسن معوض عبدالعال[١]، وفاء محمد عبد الرحيم[١]، محمد محمود هاشم[٢]، جبريل مصطفى جبريل[١]، علي صبور[٤] و محمد زكريا[٣]

[١]قسم قسم الميكروبيولوجيا الزراعية - شعبة البحوث الزراعية والبيولوجية - المركز القومي للبحوث - مصر.

[٢]قسم التحضيرات والتجهيزات - شعبه البحوث النسيجية - المركز القومي للبحوث- مصر.

[٣]قسم الميكروبيولوجيا الزراعية - كلية الزراعة - جامعة القاهرة - القاهرة - مصر.

[٤]قسم النبات الزراعي - كلية الزراعة - جامعة القاهرة - القاهرة - مصر.

أن عملية تعطين الكتان ميكروبيا تعتمد على قيام الكائنات الحيه الدقيقة في وجود الرطوبة باذابه و إبعاد الكثير من الأنسجة الخلوية والبكتينية المحيطة بحزم الياف الكتان، يعد التعطين الميكروبي أحد أكثر العمليات الصديقة للبيئة. مياه التعطين تعتبر مصدر غني بالميكروبات التي تساهم في تعطين الكتان حيث توجد في مصانع التعطين التجارية. في هذة الدراسة، تم اختبار إعادة استخدام مياه التعطين لتسريع عملية تعطين الكتان. حيث أظهرت النتائج أن إعادة استخدام المياه الناتجه من التعطين في دورة جديدة لتعطين الكتان قد ساعدت على اختزال وقت التعطين بنسبة ٣,٨-٥٢٪ على حسب نسبة المياه المعاد استخدامها في تعطين الكتان. لذلك، تم جمع عينات من سيقان الكتان الناضجة و مياه صرف الاحواض والتربة من حقول الكتان الموجود في وادي النيل والدلتا لعزل بكتريا تعطين الكتان السائدة في الفضلات السائلة. وتم التعرف على العزلات باستخدام الاختبارات المورفولوجية والفسيولوجية والكيميائية الحيوية حيث تم تعريف عشر عزلات بكتيرية وهى*Lactococcus crremoris* و *Chryseobacterium culicis* و *Serratia marcescens* و *Capnocytophage granulose* و *Micrococcus luteus* و *Enterobacter homraechei* و *Klebsiella oxytoca* و *Paenibacillus polymyxa* و *Proteus mirabilis* و *Bacillus humi*. وتم اختيار ثلاث سلالات فعالة في إنتاج انزيم البكتيناز اللازم لتعطين الكتان لاختبار دورها في الاسراع من عمليه التعطين وتنتمى تلك السلالات الى *Bacillus humi* و *Chryseobacterium culicis* و *Micrococcus luteus* والتي أظهرت نشاط نوعى للبكتيناز ٧١,٧٣ و ٣٥,٣٣ و ٨٢ وحده/ مجم على التوالى. و تمت دراسة تأثير تلك السلالات على مدة التعطين وجودة الألياف. وتم اجراء اختبار على مخاليط تلك السلالات الثلاث في مفاعل حيوي تجريبي أسطواني صغير في المركز القومى للبحوث وتمت مقارنة كل مخلوط مع ناتج عمليه التعطين التقليديه للوحدات الصناعية. وأدى استخدام مخلوط السلالات: *Chryseobacterium culicis* و*Micrococcus luteus* و *Bacillus humi* إلى تقليل مدة التعطين بنسبة ٣٠٪، في حين أن المجاميع المختلطة التي تحتوي على *Chryseobacterium culicis*و *Micrococcus luteus* خفضت زمن التعطين بنسبة ٥٢٪. وخفض خليط كلا *Micrococcus luteus* و *Bacillus humi* مدة التعطين بنسبة ٢٠٪. كما تم تحديد فقدان الوزن، وقوة الشد، والبياض والاصفرار للألياف بعد التعطين الميكروبى.