Isolation and characterisation of bacteria isolate from gin trash and its use in cotton wax degradation

Article *in* Indian Journal of Biotechnology · February 2015

CITATION
1

READS
168

3 authors:

Ann Wairimu Mburu
Kenya Industrial Research and Development Institute
8 PUBLICATIONS **7** CITATIONS
SEE PROFILE

Josphat Igadwa Mwasiagi
Moi University
73 PUBLICATIONS **303** CITATIONS
SEE PROFILE

Edward Odhiambo Anino
Rongo University College
6 PUBLICATIONS **80** CITATIONS
SEE PROFILE

ISSN : 0974 - 7435 Volume 11 Issue 6

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 11(6), 2015 [225-232]

Isolation and characterisation of bacteria isolate from gin trash and its use in cotton wax degradation

Ann Wairimu Mburu[1,2]*, Josphat Igadwa Mwasiagi[1,3], Edward Anino[4]

[1]School of Engineering, Moi University, Po Box 3900(30100), Eldoret, (KENYA)

[2]Kenya Industrial Research and Development Institute (KIRDI), Po Box 30650(00100), Nairobi, (KENYA)

[3]iOTEX, Bahir Dar University, Po box 26, Bahir Dar, (ETHIOPIA)

[4]University of Eldoret, Po Box 1125 (30100), Eldoret (KENYA)

E-mail : Wairimuann012@gmail.com

ABSTRACT

Bio scouring of cotton, which is an eco-friendly alternative to the expensive and polluting conventional caustic scouring, has not been successfully adopted mainly due to its inability to remove substrate wax. However, with surfactants, substrate's absorbency can be boosted during the enzymatic scouring. An investigation of possibility of integrating cotton wax degrading bacteria with pectinase at mutually favorable temperature, pH and time during bio scouring was hence undertaken. Hydrocarbon degrading bacteria were isolated from cotton gin trash collected from Kibos and Kitui ginneries in Kenya, characterized and tested for the cotton fibre wax removal. The isolates were successfully pre inducted on paraffin wax then used to treat cotton fibres at 45^0C and pH 8 for one hour. Wax removal efficiency was assessed by the percentage weight loss of the fibres after soxhlet solvent extraction. An average cotton fibre wax degradationof 0.765% out of 1.2% present in the fibres was recorded. This amount was comparable to that removed by caustic and pectinase with surfactants pre-scours. The isolates would hence enhance wax removal during pectinase bio scouring of cotton.

KEYWORDS

Gin trash;
Bacteria isolates;
Characterization;
Isolates scour;
Bio scouring

INTRODUCTION

Enzymatic scouring has been evaluated as an alternative to the expensive, environmental unfriendly and fibre damaging conventional caustic scouring. Use of enzymes during scouring allows the development of environmentally friendly technologies in fibre processing and strategies to improve final product quality. Various enzymes have been proposed by various researchers to provide effective scouring responses. As reported by Etters *et al*.[1], Emre and Merih[2], Qiang*et al*.[3], Vigneswaran*et al*.[4] and Vigneswaran*et al*.[5] alkaline pectinase enzymes have been found to be the most effective and suitable for cotton bio scouring even in the presence of chelating agents. The mechanism of pectinase scouring reportedly assumes degradation and elimination of pectins and makes the loosened waxes more easily accessible with the help of a mechanical agita-

tion. Pectinase, however, is not generally active at temperatures over 60°C. These properties limits its application to bio-scouring of textiles, since the textiles must be pre-boiled to attenuate the waxy cuticle overlaying the pectin layer[6,7]. According to Li and Hardin[8], the cotton fabrics bio-scoured with pectinase show improved softness and wet-ability but much less wax is removed as compared to the conventional alkaline scouring. Added surfactants have a big influence on removing non cellulose impurities. The cuticle of the cotton fibre which is cross linked to the primary cell wall by esterified pectic substances hinders pectinase action on the back bone. Cutin forms three dimensional network structures in which other amorphous waxy materials are embedded[6,9]. Pre-rinsing in hot water (≥90^{0}C) with a surfactant or extraction with boiled n-hexane helps to reduce wax impurities and, subsequently results in better destabilization of the primary cell wall, as reported by Qiang*et al*.[3], Agrawal *et al*.[6] and Kiromojsov[10]. However such a high temperature treatment on the other hand would compromise the desirable low temperature treatments associated by enzyme scouring hence increase on the process costs.

The Alternative possibility of integrating wax degrading bacterial isolates with the pectinase scouring bath conditions was therefore evaluated by this research, to alleviate the high process costs and reduce environmental pollution. This was studied by culturing and isolating wax degrading bacterial isolates from sampled cotton gin trashes. Bacillus, a Gram-positive, rod-shaped, non-pathogenic and endospore-forming aerobic bacterium found in soil and rotting plant material has been reported to have hydrocarbons emulsification capacity. Such emulsifiers produced by a variety of microorganisms such as yeast, bacteria and filamentous fungi are referred to as bio surfactants[11]. According to ZoBell[12] hydrocarbons degrading bacteria exists in cotton gin trash, and the bacteria may last even for three years even if the gin trash has been in dry storage for over three years. These bacteria could have come from cotton field soil and feed on the cottonseed protein, along with the many carbohydrates present in the cotton gin trash plant debris. According toLeuschner[11], Endospores of bacilli represent a metabolically inactive survival form. They are characterized by high resistance against heat, dryness, irradiation and other unfavorable environmental conditions. The success of the endospore for the survival of the species depends on an effective mechanism to resuscitate and enter the vegetative cell cycle to multiply. Therefore long dumped cotton gin trash can be used for culturing wax degrading bacteria, hence the essence of this study to investigate the feasibility of the bacteria in cotton wax removal.

MATERIALS AND METHODS

Bacteria cultures, isolation and characterization

Cotton gin trash was collected from two different ginneries. The trash samples were macerated and about 10 g put into a beaker containing 200 mls of water. The beakers were then covered and incubated at 30^{0}C for 24 hrs to allow for microbial growth.Bacteria cultures were then grown using pour plate dilution technique as described by Ian and Charles[13]. Each of the original samples was diluted five times to dilute the microbial population so as to obtain separate colonies when plating. Small volumes (about 0.1 to 1 mls) of the diluted samples were mixed with about 15 mls liquid nutrient agar (45^{o}C) in sterile culture dishes which were then incubated at 37^{0}C for 24 hrs.

The different colonies obtained were then isolated by the streak and spread methods. In the streak methods, small amount of colour and morphologically distinct colony was picked by the tip of an inoculation loop and streaked across the surface of the agar medium. For the spread plate method on the other hand, a small amount of distinct colony was picked, suspended in distilled water and then mixed with about 15 mls of sterile nutrient agar (45^{o}C). All the streaked and spread plates were then incubated at 37^{0}C for 24 hrs to allow for isolates growth. The two isolation techniques gave three pure isolates.

The pure isolates obtained were then subjected to simple and gram staining for respective morphological identification and differentiation. Crystal violet was used for the simple staining.

Gram staining is essential for differentiating bacteria isolates in two broad groups; Gram-negative and Gram-positive. Gram-positive bacteria retain the primary dye giving a purple to blue-black appearance. Gram-negative bacteria take-up the colour of the

counter stain (i.e red or pale red colour). Thus, gram-positive bacteria stain violet, and gram-negative bacteria stain pink. Crystal violet, Gram's iodine, 95% alcohol and safranin stains were used.

Preliminary hydrocarbon activity induction of the isolates

Each of the three isolates was tested for hydrocarbon activity by streaking on 10mls pre melted and solidified paraffin wax in sterile petri dishes. The petri dishes were then incubated at 37^{0}C, 45^{0}C and 50^{0}C for 24 hours to test for optimal temperature for the bacteria growth.

Preliminary caustic and pectinase scouring

Five samples of cotton fibres collected at the carding stage in a spinning factory were first treated with *n*-hexane (30 min, 75^{0}C), as a control for the evaluation of wax removal efficiency by the caustic and pectinase procedures. The solvent extracts all the wax contained in the fibre[14]. The fibres were also scoured using differently constituted baths; Caustic (0.25M, LR 20:1, 1 hr, 10 cotton fibre samples) and commercial Pectinase(*A. Niger*) (4g/l, LR 50:1, pH8, 1hr, 10 cotton fibre samples). Both procedures were done with and without 1% non-ionic surfactant (Sodium Lauryl sulphate). Each of the scoured samples was then extracted using trichloroethylene by a soxhlet apparatus as specified in ASTM D2257-04 at the rate of six extractions per hour for a total duration of 2 hours. The weight difference between the scoured and the solvent extracted fibres gave each of the procedure's wax removal efficacy. The weight loss calculation after each scour procedure was done by the following equation:

$$Y = Wf - [(Wp - WS/Wp)] * 100 \quad (1)$$

Where Y is the wax removed (%), W_f is the total wax content in the cotton fibres, W_p and W_s are the wax contents (%) in specified scour procedure and solvent extracted fibres respectively.

Isolates and isolates/pectinase cotton wax removal

Five tests for each of the three isolates were conducted on the isolates fibres scour procedure. About 2 mls of Tris-HCL Buffer, pH 8 put into each of the fifteen test tubes and a flame sterilized wire loop was used to pick each of the isolate and introduce them into the test tubes. Fibre samples weighing 1.00±0.01 g were prepared and each put into each test tube's contents and incubated at 45^{0}C for one hour. The contents were then subjected to boiling to deactivate the enzyme and the isolates. The Percent wax removed was calculated using the following formula as adapted from Vigneswaran *et al*.[4]:

$$Y = 1.2 - [(WI - WS/WI)] * 100 \quad (2)$$

Where Y is the wax removed (%), W_I and W_s are the content of swaxes (%) in the isolates scoured fibres and solvent-extracted fibres respectively. Average wax removed from each isolate was obtained from which the overall average was calculated. The same procedure of isolate scour was then repeated but with addition of about 2mls of 4g/l of pectinase at LR 50:1.

Investigation for enzyme presence

The isolates bacterial cells rupturing tests were conducted to determine if the bacteria contained an enzyme that might have had the activity on the wax. Each pure isolate was Sub-divided into 10 eppodorf tubes, labeled and put into a centrifuge. The bacterial cells/ isolates were then pelleted by centrifuging at 6000 rpm for 5 minutes, supernatants aspirated and the pellets frozen at -30^{0}C for 30 minutes. Five test tubes of pellets of the bacterial cells from each isolate were then re-suspended in 0.75 mls of lysis buffer, and then incubated at 30^{0}C for 15 minutes. The other five pellets from each pure culture were re-suspended with nutrient agar as controls. All the tubes were then vortexed for 1 minute until the cell suspension was homogeneous. The pellet tubes were then centrifuged at 12,000 rpm for 20 minutes at 4^{0}C, supernatants from the lysis buffer-suspended tubes collected to new tubes and pellets re-suspended in 0.75 mls of lysis buffer as described in[15]. Each of the supernatants, nutrient agar and lysis buffer suspended pellets, were then used to test for their activity on wax, by streaking the contents onto solidified pre-melted wax in petri dishes which were then incubated at 37^{0}C for 24 hours to allow for growth.

RESULTS AND DISCUSSION

Bacteria cultures, isolation and characterization

Each of the two gin trash samples that were cultured through the plate dilution technique gave a composition three distinct colonies: Large serrated yellow

FULL PAPER

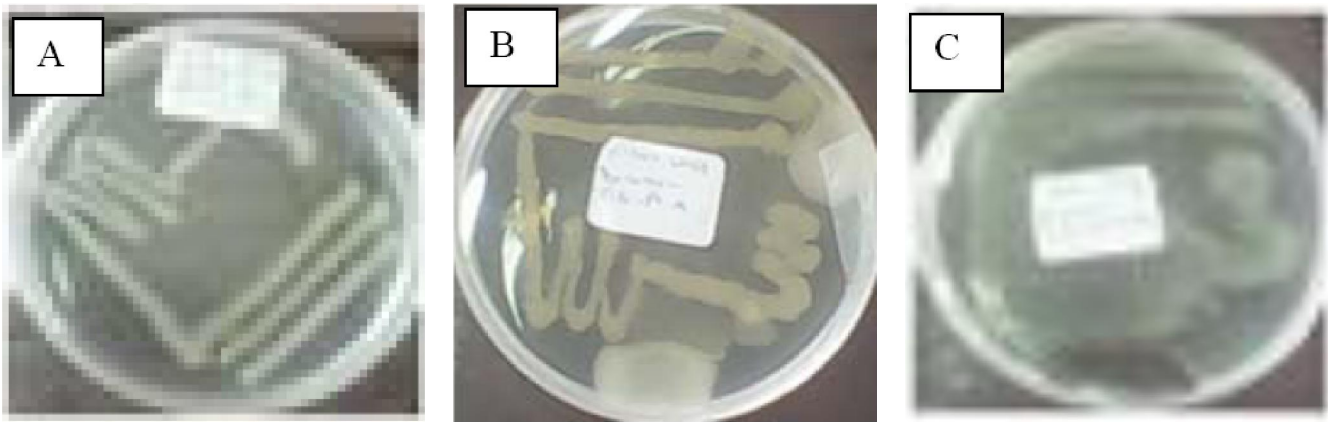

Figure 1 : White, yellow and bluish greenish isolates plates from streak method

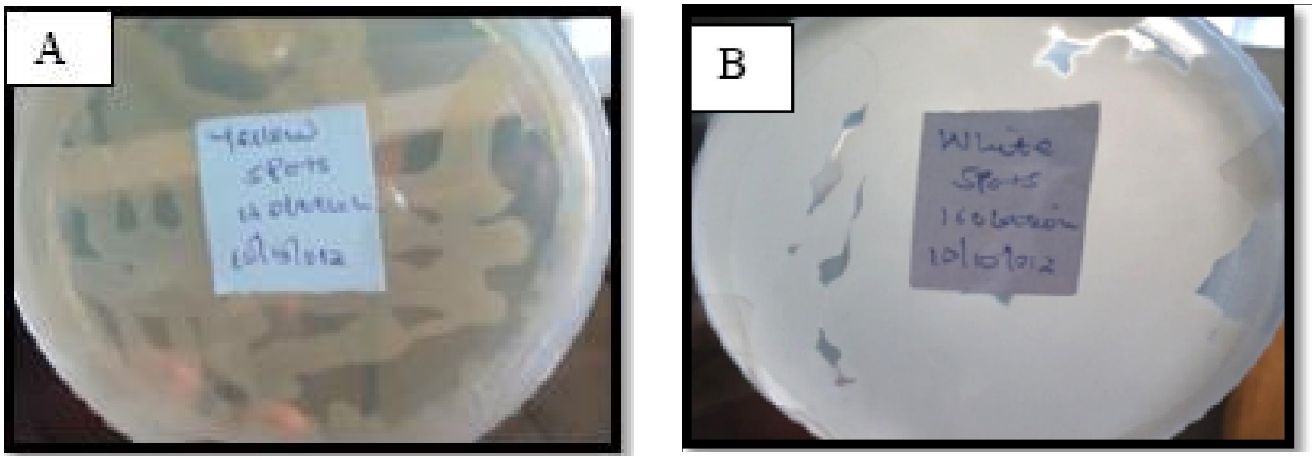

Figure 2 : Yellow and white isolates from spread plate method

colonies, small round white colonies and oval shaped transparent bluish greenish colonies. The distinctive colonies which were then isolated through the spread and streak plate methods gave three pure isolates. The different isolates obtained are as shown in the photo illustrations in Figure 1 A, B and C and Figure 2 A and B.

Simple staining of all the isolates revealed purple stained rod shapes indicating that the bacteria were bacilli. The observation under the microscope also showed chained rods occurrence for all the isolates meaning that the bacteria were streptobacilli in nature. Under the Gram staining test, all the isolates stained purple, the primary colour used, indicating that the bacteria isolates were Gram-positive.Respective illustrations are as shown in Figure 3 A-C.

Preliminary hydrocarbon activity induction of the isolates

The three pure isolates from the two sample gin trashes streaked on the wax and incubated at 37^{0}C gave a thick bacterial growth along the streak lines. The plates incubated at 45^{0}C gave a relatively thick growth along the streak lines while the plates incubated at 50^{0}C gave the least growth along the streak lines, meaning the isolates survival at that temperature was declining as respectively illustrated in Figure 4 A, B and C.

Preliminary caustic and pectinase scouring

The *n*-hexane solvent extraction at 75^{0}C for 30 min on the five fibre samples gave an average 1.2% fibre wax content. The caustic with surfactant scour that was conducted on 10 cotton fibre samples at 0.25M NaOH, 1% Sodium Lauryl sulphate surfactant, LR 20:1 for 1 hour gave 7.2% weight loss (general impurities contained in the fibres). This amount (7.2%) was found to be comparable with that reported by Bahrum[16]; 6.87-7.27% and Tzanko*et al*.[17]; 6.3%. It is on this basis that the effectiveness of each of the scouring procedures in wax removal was evaluated.

The subsequent solvent extraction of the caustic with

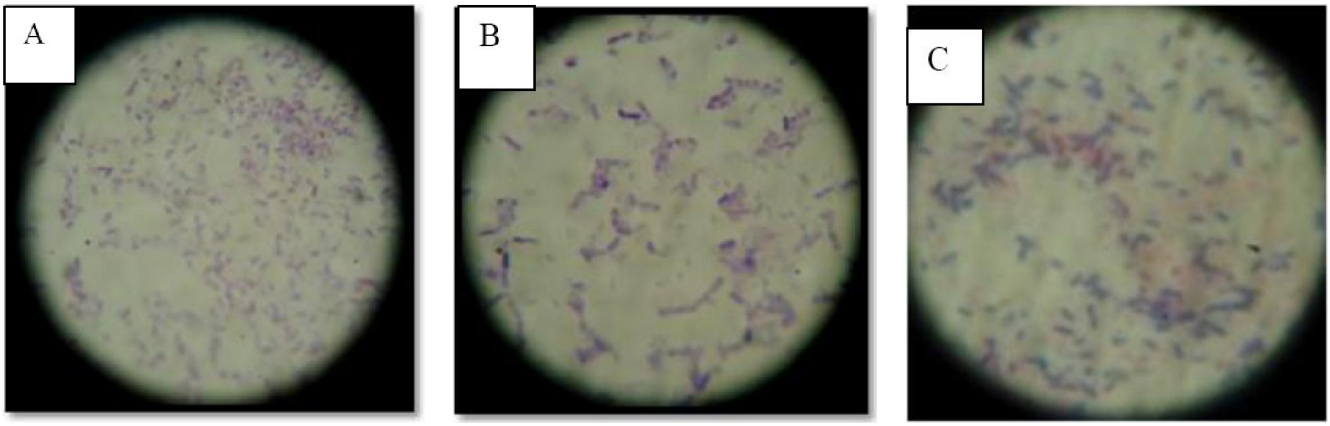

Figure 3 : Isolates simple and gram staining results plates; (A) The isolates basic rod shapes; (B) The isolates chained rods arrangements; (C) Gram staining results plates

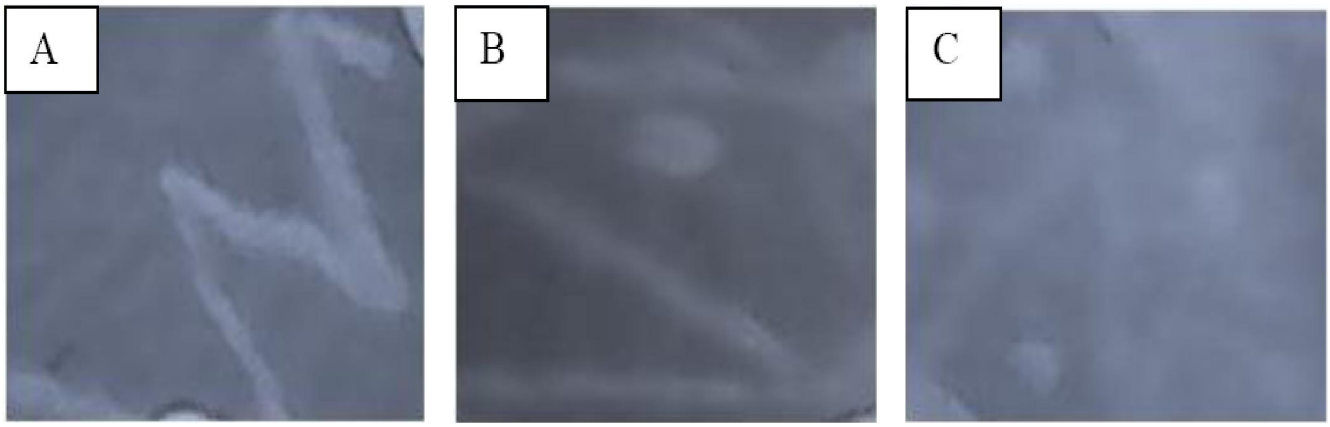

Figure 4 : Respective isolates wax activity induction at 37^{0}C, 45^{0}C and 50^{0}C plates

surfactant scoured fibres gave percentage average values wax removed of 0.72%. Caustic without a surfactant scour on the contrary gave respectively low values of 2.65% and 0.3% of general and wax impurities removed. The low values be explained by the ability of the hot caustic soda solution with the aid of the surfactant to remove more impurities by breaking, releasing and emulsifying them effectively as argued by Tzanko*et al*.[17]. Pectinase with surfactant procedure gave a percentage average general impurities removal value of 4.14% and a wax removal value of 0.844% while Pectinase without a surfactant on the other hand, gave respective percentage average impurities and wax removal values of 2.4% and 0.267%. These results can be explained by the substrate specific nature of the enzyme in impurities removal and hence the inability of the enzyme to destabilize the cuticle layer containing the wax. Comparatively, under pectinase cotton scour process conditions of 45^{0}C, reaction time of 60 minutes and a pH of 8.5, Vigneswaran*et al*.[18] obtained the best opted test results of overall impurity removal efficiency of 4.80% which can be related to the 4.14% obtained.

Isolates and isolates/pectinase cotton wax removal

All the fifteen tests of the isolates scour gave an average of 0.765% wax removed which is comparable to that degraded by the caustic and pectinase in the presence of a surfactant; 0.72% and 0.844%. This

TABLE 1: Total fibre impurities and wax removal efficiency by different scour procedures

Procedure	Total fibre Impurities Removal (%)	Total fibre wax Removal (%)
n-Hexane	-	1.2
Caustic with surfactant	7.2	0.72
Caustic without surfactant	2.65	0.3
Pectinase with surfactant	4.14	0.844
Pectinase without surfactant	2.4	0.267
Isolates	-	0.765
Isolates and pectinase	-	0.729

FULL PAPER

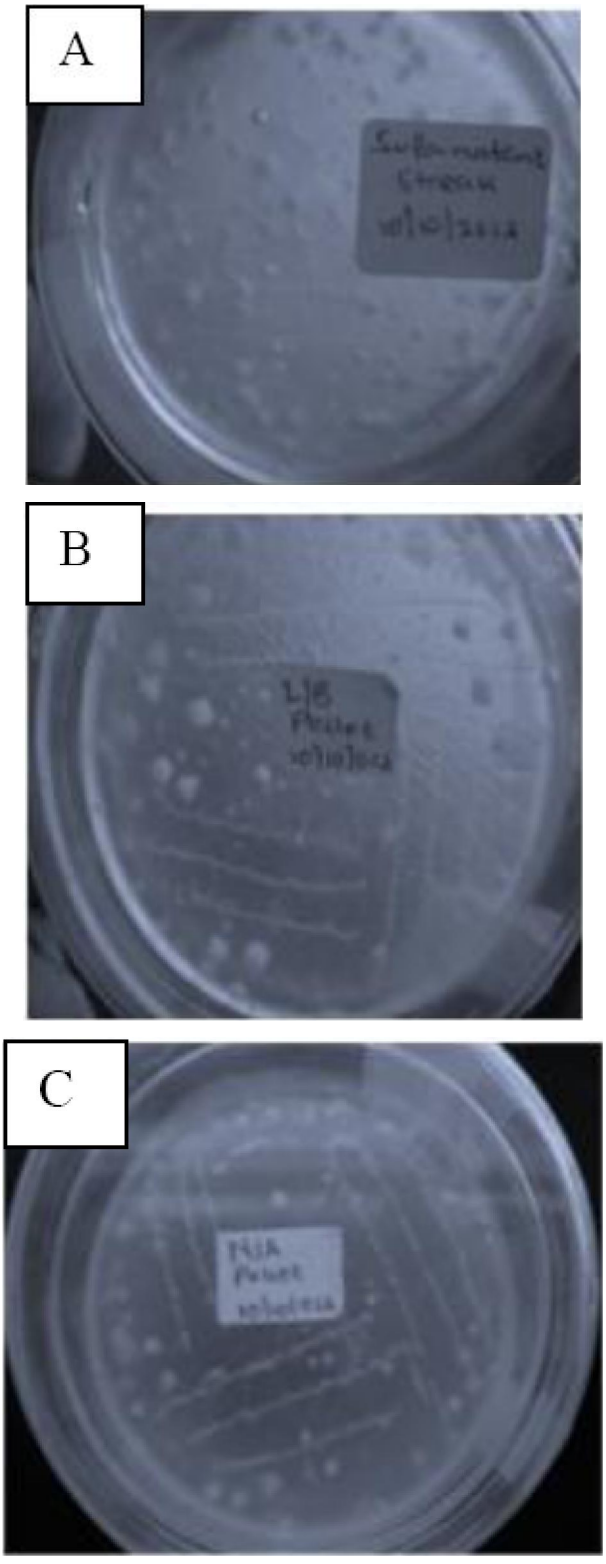

Figure 5 : Respective supernatant, Nutrient agar and Lysis buffer suspended pellets wax induction streak plates; (A) Supernatant wax activity induction streak growth; (B) Lysis buffer suspended bacteria pellet wax activity induction streak growth; (C) Nutrient Agar suspended bacteria pellet wax activity induction streak growth.

amount is also notably higher than that reported by Vigneswaran*et al.*[18] of an average loss in wax content of 0.525% at 60^0C, pH 8.5-9.0 and treatment time of 60 minutes using 6% pectinase enzyme concentration. Isolates and pectinase combination scour gave an average of 0.729% wax removed. This value is also comparable those recorded under the respective caustic and pectinase with surfactant procedures (0.72% and 0.844%). These results can therefore explain that the isolates played a similar role as the chemical surfactant as explained by Tzanko*et al.*[17]. TABLE 1 gives the summary of all the scour procedures described above.

Investigation for enzyme presence

All the plates streaked with the supernatants from all the isolates did not show any growth along the streak lines. All the plates streaked with lysis buffer- suspended pellets from all the isolates gave comparatively distinctive growths along the streak lines. The plates streaked with nutrient -suspended pellets from all the pure cultures gave relatively thick and clear growths along the streak lines as shown in Figure 5 A-C. This indicates that there was no bacterial enzyme contained in the bacteria and it is the bacterial cells that had the activity on the wax.

The bacteria cells lysis tests showed that the nutrient agar suspended bacteria pellets control streaks, gave relatively thick lines of action on the paraffin wax indicating that it is the bacteria that acted on the wax as a supplement and not as a source of food for the bacteria. The lysis buffer suspended bacteria pellets however gave comparatively thin lines of action on the paraffin wax, meaning that the bacteria cells relied solely on the wax for their nutrients hence the slow rate of action. The supernatants from all the isolates did not exhibit any action on the wax, explaining the absence of any protein/enzyme in the bacteria isolates.

CONCLUSION

It can be concluded that, three different bacteria isolates (white, yellow and bluish greenish) can be isolated from cotton gin trash. All the isolates have activity on paraffin wax and on the cotton fibre wax as a sources of hydrocarbon. It was also demonstrated that the isolates can feasibly be integrated into the pectinase, which would enhance the enzyme's capacity to destabilize the

cuticle waxy layer from 0.267% (pectinase without surfactant scour) to 0.729% of the percentage average wax degraded (isolates/pectinase scour). It was also established that the bacterial isolates did not contain any enzyme, indicating that it was the bacteria that degraded the cotton fibre wax.

Environmental consciousness being one of the major concerns for the textile industry, there is increasing need of replacing the chemical processes with biopreparation techniques. As it has been demonstrated, chemical surfactants, which have been found to be very important in the cotton fibre impurities/wax removal can potentially be replaced with hydrocarbon degrading bacteria isolates from cotton gin trashes.

Optimum temperature, pH and incubation time for the isolates cotton fibre wax removal should therefore be established.

ACKNOWLEDGEMENT

The financial assistance received from VLIRUOS enabling this research is hereby acknowledged.

REFERENCES

[1] J.N.Etters, B.D.Condon, P.A.Husain, N.K.Lange; Alkaline pectinase: Key to Cost- Effective Environmentally Friendly preparation, American Dyestuff Reporter, **88(6)**, 19-23 **(1999)**.

[2] K.Emre, O.S.Merih; Scouring of cotton with cellulases, pectinases and proteases, Fibres and Textiles in Eastern Europe, **12(47)**, **(2004)**.

[3] W.Qiang, F.Xuerong, G.Weidong, C.Jian; Characterization of bioscoured cotton fabrics using FT-IR ATR spectroscopy and microscopy techniques, CarbohydrateResearch, **341**, 2170–2175 **(2006)**.

[4] C.Vigneswaran, N.M.Anbuman, M.Ananthasubramanian; Neural Network Approach for optimizing the bio scouring performance of organic cotton fabric through aerodynamic system,Journal of textile and apparel, technology and management, **7(3)**, **(2012)**.

[5] C.Vigneswaran, N.M.Anbuman, M.Ananthasubramanian; Effect of sonication on bioscouring of organic cotton through mixed enzymatic system-Neural Network Approach, Indian Journal of Fibre and Textile Research, **38**, 44-56 **(2013)**.

[6] P.B.Agrawal, V.A.Nierstrasz, M.M.C.G.Warmoeskerken; Role of mechanical action in low-temperature cotton scouring with *F. solanipisi*cutinase and pectatelyase, Enzyme and Microbial Technology, **42(6)**, 473-482 **(2008)**.

[7] F.T.Petra; Biotechnology in Textiles – an Opportunity of Saving Water, Waste Water -Treatment and Reutilization In Tech, Fernando SebastiánGarcíaEinschlag, (Ed); CC BY-NC-SA, 2011. Information on http://www.intechopen.com/books/waste-water-treatment-andreutilization/ biotechnology-in-textiles-an-opportunity-of-saving-water (Retrieved July 2012), May,2014;Engineering » Environmental Engineering » "Waste Water - Treatment and Reutilization", **(2011)**.

[8] Y.Li, I.R.Hardin; Enzymatic scouring of cotton: effects on structure and properties, Textile Chemist and Colorist, **29(8)**, 71-76 **(1997)**.

[9] O.Degani, S.Gepstein, C.G.Dosoretz; Potential use of cutinase in enzymatic scouring of cotton fibre cuticle, Applied Biochemistry and Biotechnology, Part A, Enzyme Engineering and Biotechnology, **102(14)**, 277-290 **(2002)**.

[10] Kiromojsov; Biotechnological Applications of Pectinases in Textile Processing and Bioscouring of Cotton Fibres: A paper presented at the II International Conference on "Industrial Engineering and Environmental Protection 2012 (IIZS 2012), Zrenjanin, Serbia, October 31st, **(2012)**.

[11] R. Leuschner; Occurrence, Detection and Food Poisoning in BACILLUS, Elsevier Science Ltd., Central Science Laboratory, York, UK, 355-358 **(2003)**.

[12] C.ZoBell; Assimilation of hydrocarbons by microorganisms, scipps institute, New Series No., **438**, 479-481**(2005)**.

[13] L.P.Ian, P.G.Charles; Environmental Microbiology. A Laboratory Manual, 2nd Edition, Elsevier Science/ Academic Press. San Diego, 173-189 **(2004)**.

[14] P.B.Agrawal, V.A.Nierstrasz, G.H.Bouwhuisand, M.M.C.G.Warmoeskerken, Cutinase, pectinase; in cotton bioscouring: an innovative and fast bioscouring process, Biocatalysis and Biotransformation, **26(5)**, 412-421 **(2008)**.

[15] Lysis of bacterial cells, information on*http://*http://wolfson.huji.ac.il/expression/procedures/cell_lysis/Lysis_of_bacterial_cells *ml,* (Retrieved October 2012), May, **(2014)**.

FULL PAPER

[16] A.M.K.Bahrum; Comparison of Effectiveness between Conventional Scouring &Bio- Scouring on Cotton Fabrics, International Journal of Scientific &Engineering Research, **3(8)**, **(2012)**.

[17] T.Tzanko, T.M.Calafell, G.M.Guebtiz, P.A.Cavaco; Biopreparation of cotton fabrics, Enzyme and Microbial Technology, **29(6)**, 357-362 **(2001)**.

[18] C.Vigneswaran, N.M.Anbuman, M.Ananthasubramanian, R.Rajandran; Prediction and processs optimization of pectinolytic reaction on organic cotton fabrics for bio scouring with alkaline pectinse, Indian Journal of Fibre and Textile Research, **37**,183-190 **(2012)**.