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A Study of Tannic Acid Degradation by Soil Bacteria

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Abstract: A tannin-degrading strain of *Bacillus* sp. AB1 was isolated from a garden soil by enrichment. This organism was able to utilize 1% (w/v) tannic acid-a gallotannin at 30°C and pH below 4.5 in a defined mineral medium where the acid was the sole source of carbon and energy under 96 h. Growth resulted in increase in OD concomitant with gradual decrease in pH of the culture medium. Analysis of the culture fluid by paper chromatography revealed glucose and gallic acid as major metabolites of tannic acid degradative pathway. Mineralization of tannic acid was informed when none of the metabolites was recovered after 96 h of incubation. The degradation potential of this isolate could be exploited for the production of tannase, improvement of livestock production and also detoxification of tannery effluents at extreme acidic conditions.

Key words: Tannic acid, degradation, metabolite, paper chromatography

INTRODUCTION

Biological degradation is adjudged as an important mechanism of organic chemical removal in natural systems, owing to its environmental compatibility and plasticity. Consequently, microbial metabolic potentials are widely exploited for a number of industrial applications including decommissioning of environmental pollutants. An important group of contaminants from which efficient treatment methods are needed are aromatic compounds. Aromatic compounds such as polyphenols comprise the second largest group of natural products, in addition to a variety of xenobiotics that are man-made aromatic pollutants.

Tannins are water-soluble polyphenolic secondary metabolites of higher plants which are either galloyl esters or their derivatives (Khanbabae and Ree, 2001). They are the fourth most abundant plant constituents following cellulose, hemicellulose and lignins; and together with the latter are the most abundant and widely distributed phenolic polymers of higher plants (Brooker *et al*., 1999; Mingshu *et al*., 2006). They widely occur in common foodstuffs such as tea, strawberry, grape, mango, walnut, cashew nut etc., (Clifford and Scalbert, 2000). On the basis of their structural characteristics, tannins are divided into four major groups namely, gallotannins, ellagitannins, complex tannins and condensed tannins (Mingshu *et al*., 2006). The most famous source of gallotannins is tannic acid, which is in the form of a yellowish-white or pale brown powder usually obtained from twig galls of *Rhus semialata*.

In animals, especially ruminants, tannins have toxic or nutritional effects, which can reduce feed intake and lower nutrient digestibility and protein availability (Lowry *et al*., 1996; Van Soest, 1994). The reasons being that tannins can form complexes with protein, starch and digestive enzymes and reduce the nutritional qualities of feeds (Chang *et al*., 1998). Tannins have long been considered toxic to microorganisms and this activity is mainly due to enzyme inhibition and substrate deprivation, action on membranes and metal ion deprivation (Reed, 1995). Nevertheless, some fungi yeasts and bacteria are quite resistant to tannins and can also degrade them (Scalbert, 1991; Mingshu *et al*., 2006). Amongst bacterial species with ability to degrade these polyphenolics are *Citrobacter*, *Streptococcus* and *Corynebacterium* (Mingshu *et al*., 2006).

Owing to encouraging results recorded from various investigations, tannin degradation by microorganisms are now exploited at industrial scale for the production of tannase, detanification of foods, melioration of fodder, upgrading of beverages and decontamination of tannery effluents. Although, degradation of tannins is widely reported in the literature, but reports describing isolation and characterization of tannin degradation from African tropical environments are very scarce in view of the fact that tannery effluents emanating from industries are indiscriminately discharged into the environment. The purpose of the present study therefore was to isolate tannin degrading bacterial strain from soil and to ascertain its growth characteristic on tannic acid (a major constituent of tannery effluent) when used as the sole

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source of carbon and energy. In addition to its environmental importance, the organism may also be useful as a microbial cell for effective conversion of wastes in tannery effluent to wealth.

MATERIALS AND METHODS

Enrichment, isolation and characterization of bacterial cultures: Initial enrichment culture was established by a soil sample collected from the Biological garden, University of Lagos, Nigeria in a Minimal Salts (MS) medium previously described by Mondal *et al.* (2001). An antibiotic (amphoteric B) was added to inhibit fungal growth and the pH was adjusted to 4.5. The medium was dispensed into 500 mL Erlenmeyer flask and sterilized by autoclaving. One gram of the soil was used to inoculate the medium and 1% (w/v) tannic acid was added as the sole carbon source. Incubation was performed at room temperature (30°C) for 96 h. Following intense turbidity of the medium, the enrichment culture was transferred into a fresh MS medium using 2% inoculum and incubation continued for another 96 h. The culture was serially diluted up to 10^{-4} and aliquots of appropriate samples were spread on MS agar already supplemented with 1% (w/v) tannic acid. The plates were incubated at 30°C for 96 h. Developed colonies were purified on nutrient agar and classified on the basis of their cultural and biochemical properties.

Growth of isolates on tannic acid: The biodegradation experiments were performed in 250 mL flasks which contained 50 mL of MS medium supplemented with 1% (w/v) tannic acid. Tannic acid was filter-sterilized before it was added to the autoclaved medium. Optical density (wavelength 540 nm) and pH readings were determined periodically as biodegradation indices. The data reported are the average of the values obtained from triplicate experiments.

Detection of degradation products: The degradation products were detected by paper chromatography. The method used was ascending chromatography. The solvent system was butanol/acetic acid/water at 4:1:1 (Mondal *et al.*, 2001). The components on the paper plate were visualized by heating after spraying with ferric chloride in 30% methanol.

RESULTS AND DISCUSSION

The use of microorganisms or their bioactive compounds for the transformation of tannins is gaining worldwide attention. This has resulted in unprecedented investigation into isolation of competent microbial strains and the end product distribution of tannin metabolic pathways. In the present study, the method of batch enrichment on tannic acid was used to isolate 2 bacterial cultures namely, AB1 and AB2. However, only the former was use for further studies on the basis of rapid and extensive growth on tannic acid. Typical cultural morphologies of AB1 on nutrient agar are fast spreading creamy white large colony, on tannic acid agar, the colony was tiny, round, irregular and dry-surfaced. Cellular characteristics revealed Gram-positive, spore-forming, motile long rods. Additionally, the isolate was catalase, oxidase, H_2S and indole-positive. Gelatin and starch were hydrolyzed and most sugars were fermented. The isolate was subsequently classified as *Bacillus* sp. AB1 following the taxonomic scheme of Buchanan and Gibbons (Holt *et al.*, 1994).

Figure 1 shows the potential of *Bacillus* sp. AB1 in degrading tannic acid under batch conditions. The utilization of this acid as the sole carbon and energy source resulted in increase in OD typified by intense visual turbidity with a concomitant production of acidic metabolites as indicated by further decrease in the pH of the culture fluid. The ability of the organism to grow effectively on 1% tannic acid was consistent with earlier studies (Deschamps *et al.*, 1980; Gandhi, 1990). These authors reported that *Bacillus pumilus*, *B. polymyxa*,

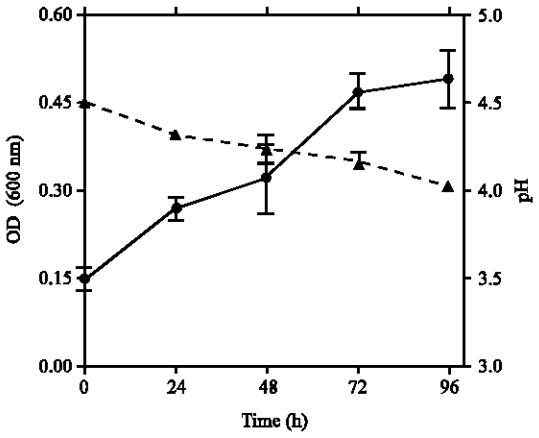

Fig. 1: Time course for degradation of tannic acid. (•), OD; (▲), pH. In the controls without cells, no appreciable increase in OD was observed. Data represent the averages and standard deviations of triplicate determinations. Large error bars were due to differential response of cells to substrate in replicate tubes

Corynebacterium sp. and *Klebsiella pneumonia* were able to grow on 1% (w/v) gallotannins. In contrast, growth of the more common ruminal *Streptococcus* such as *S. bovis*, was inhibited by gallotannins at concentration lower than 0.5%.

The ability of microorganisms to degrade tannins has been attributed to production of tannase, an important enzyme capable of catalyzing gallotannins to gallic acid and glucose. Interestingly, analysis of the culture fluid of AB1 revealed these two compounds as the only metabolites of tannic acid degradation (Fig. 2). Recently, Mondal and Pati (2000) using paper chromatography identified gallic acid as the major metabolite in the culture fluid of *Bacillus licheniformis* KBR6 obtained from laterite soil. Detection of this metabolite in this study does not only suggest tannase production by strain AB1, but also indicates, to some extent, the existence of similar metabolic pathway in our isolate and *B. licheniformis* KBR6.

Since extensive growth was observed on tannic acid, it is most unlikely that both glucose and gallic acid metabolites accumulated in the culture medium. Analysis of the growth medium at 24, 48 and 72 h, indicates the presence of these metabolites. However, at 96 h, none of the metabolites was detected. This trend suggests to us further metabolism of gallic acid and glucose intermediates and therefore potential mineralization of tannic acid by this organism.

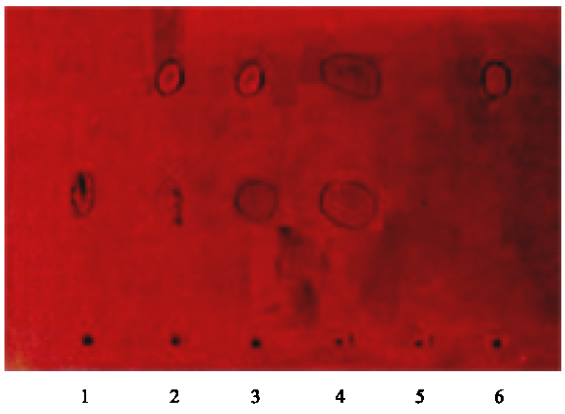

Fig. 2: Paper chromatogram of tannic acid biodegradation metabolites. Lane 1, gallic acid sample; lanes 2, 3, 4 and 6, culture fluids at 24, 48, 72 and 96 h; lane 6, glucose sample. Gallic acid eluted earlier than glucose as shown in the first layer. Note that no metabolites were recovered from culture fluid after 96 h of incubation

CONCLUSIONS

Our findings together with the fact this strain is able to grow on tannic acid at 1% concentration at pH 4.5 or below, confer to this bacterium a remarkable potential for its application in bioremediation and waste water treatment especially in the stabilization of tannery effluents. It could also be very valuable in industry for the production of tannase and also of the detoxification of the bark content of animals feed. Furthermore, tannase producers may find application in food and pharmaceutical industries since tannase is used in the manufacture of instant tea and in production of gallic acid, a substance for chemical synthesis of propyl gallate and trimethoprim. Other potential uses of tannase are stabilization of malt polyphenols, clarification of beer and fruit juices and reduction of antinutritional effects of tannins in animal feeds.

REFERENCES

Brooker, J.D., L. O'Donovan, I. Skene, L. Blackall and P. Muslera, 1994. *Streptococcus caprinus* sp. Nov., a tannin resistant ruminal bacterium from Fera Goats. Lett. Applied Microbiol., 18: 313-318.

Brooker, J.D., L. O'Donovan, I. Skene and G. Sellick, 1999. Mechanism of Tannin Resistance and Detoxificationin the Rumen Microbial Biosystems. In: New Fronties Proceedings of the 8th International Symposium of Microbial Ecology. Bell, C.R., M. Brylinsky and G. Johnson (Eds.) , Atlantic Canada Society for Microbial Ecology Halifax, Canada.

Chang, K.T., C.I. Wei and M.G. Johnson, 1998. Are tannins a double-edged sword in biology and health? Trends Food Sci. Technol., 9: 168-175.

Clifford, M.N. and A. Scalbert, 2000. Ellagitannins-nature, occurrence and dietary burden. J. Sci. Food Agric., 80: 1118-1125.

Deschamps, A.M., G. Otuk and J.M. Lebeault, 1983. Production of tannase and degradation of chestnut tannins by bacteria. J. Ferment. Technol., 61: 55-59.

Gandhi, P., 1990. Microbial degradation with special reference to tannery. Ph.D Thesis, Madurai Kamaraj University, Madurai, India.

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Stanley and S.T. William, 1994. Bergey's Manual of Determinative Bacteriology. William and Wilkins, Baltimore.

Khanbabae, K. and T.V. Ree, 2001. Tannins: Classification and definition. Nat. Prod. Rep., 18: 640-649.

Lowry, J.B., C.S. McSweeney and B. Palmer, 1996. Changing perceptions of the effect of plant phenolics on nutrient supply in ruminant. Aust. J. Agric. Res., 47: 829-842.

Mingshu, L., Y. Kai, H. Qiang and J. Dongying, 2006. Biodegradation of gallotannins and ellagitannins. J. Basic Microbiol., 46: 68-84.

Mondal, K.C. and B.R. Pati, 2000. Studies on the extracellular tannase from newly isolated *Bacillus licheniformis* KBR6. J. Basic Microbiol., 40: 223-232.

Mondal, K.C., D. Banerjee, R. Banerjee and B.R. Pati, 2001. Production and characterization of tannase from *Bacillus cereus* KBR9. J. Gen. Applied Microbiol., 47: 263-267.

Reed, J.D., 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci., 73: 1516-1528.

Scalbert, A., 1991. Antimicrobial properties of tannins. Phytochemistry, 30: 3875-3883.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, New York.