SHORT COMMUNICATIONS

PROSPECT OF DEW-RETTING OF JUTE IN PAKISTAN

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Introduction

According to Thaysen and Bunker,[1] a method of aerobic retting in which fibre plants are spread in thin layers on the surface of healthlands or on any other poor land containing few microorganisms and providing a firm and porous substratum and in which mostly fungi accomplish the retting is called "dew retting" and is known in many countries for centuries particularly in the Soviet Union. Hauman[2] isolated *Penicillium glaveum*, *Mucor muceds* and *Cladosporium herbarum* from the dew retted fibres of hemp and regarded *C. herbarum* as the chief retting agent. Behren[3] reported that the fungi, *Rhizopus nigricans* and *Mucor hiemalis*, were involved in the retting of hemp. Rusehmann[4] subjected both flax and hemp to dew retting and found that *Cladosporium herbarum* was the chief retting agent although *Mucor plumbeus*, *Aspergillus* and *Penicillium* spp. as well as some yeasts (*Oidium*) sp. were also partially involved.

According to the 1953-54 report of the Indian Central Jute Committee certain cultures of *Aspergillus* and *Penicillium* isolated from the decomposing fruit of *Guazuma tomentosa* hastened the retting of jute.[5] T.E. Summers[6] reported that there was no discernible difference in the appearance of the yarns from the water-retted and mist-retted fibres.

This investigation was undertaken in order to isolate certain fungi from decomposed materials, to test their ability to ret jute plants under mist condition and to prepare a foundation for exploring the possibility as regards carrying out dew retting in Pakistan.

Materials and Methods

Certain species of *Penicillium* and *Aspergillus* were isolated from decaying filter papers and jute fibres, and a strain of *Thieloviopsis pyrodoxa* was procured from Dr. Q.A. Ahmed, Plant Pathologist of this Institute. Spores and bits of mycelia of these fungi were applied around the surface of pieces of sterile jute stems in test tubes. The surroundings of the pieces of jute stems inside the test tubes were kept moist by keeping a little water at the bottom of the test tubes. The test tubes were then incubated at 34°C. and the stems periodically checked for retting.

Experimental Results

Although 10 isolates were obtained from decomposing filter paper and jute fibre, only 5 accomplished retting of jute stems. However, the nature of retting during this period varied. Among these five cultures of the fungi, one strain of *Aspergillus* and one strain of *Penicillium* retted faster and gave better fibres. *T. pyrodoxa* performed partial retting only.

Table 1.—Effect of Certain Fungi on the Retting of Jute in 7 Days.

Fungus	Culture No.	Effect on jute stem *
Aspergillus	F1	Partially retted
Penicillium	F2	,,
Penicillium	F3	Completely retted (best fibres)
Aspergillus	F4	,,
Thieloviopsis pyrodoxa	F5	Partially retted

* Average of three replications.

Discussion

Since the strains of *Penicillium* and *Aspergillus* did perform retting of jute, it would seem quite probable that they would also perform dew retting. Whether or not this is a fact would call for further research in this line. In Pakistan, there are many areas where farmers are confronted with difficulties in finding retting water. In some areas due to constant and frequent use of limited quantity of retting water in ponds and others, the same become unsuitable for further rets being overcharged with previous retting by-products. The dew retting, if found success-

ful, can easily circumvent these difficulties. Rusehmann[4] reported that dew retting of flax and hemp was completed in 7 days in summer and 14 days in winter. This would indicate that temperature is an important factor in the process of dew retting. It is possible that by studying temperature effect, an optimum temperature for this process of retting can be determined and the period of the common method of retting shortened to advantage.

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References

1. A. C. Thaysen and H. J. Bunker, *The Microbiology of Cellulose and Hemicelluloses, Pectins and Gums* (Oxford University Press, London, 1927) p. 363.
2. L. Hauman, Ann. Inst. Pasteur, **16**, 379 (1902).
3. J. Behrens, Zentrbl. f. Bakl., Abt. II, **8**, 264, 295, (1902).
4. G. Rusehmann, Faserforschung, **2**, 282 (1922).
5. T. E. Summers et al., *Biological Retting of Kenaf, The soil and crop science society Proc.*, **18**, 383 (1958).
6. *Annual Report of the Indian Central Jute Committee*, (Calcutta 1953-54), pp. 300.

PECTIC ENZYMES IN JUTE RETTING

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Introduction

It is now generally accepted that the loosening of jute fibres in the jute stems is accomplished by a process known as "retting". During this process the fibre bundles from the cortex and wood are separated, and a partial digestion of the cementing material (mostly of pectic nature) between the fibres in the bundles is effected. Mitscherlich[1] first attributed these changes in the process of retting to the pectolytic enzymes, produced by microorganisms. Comparatively little is known, however, concerning the enzymes which are involved in retting. While there are many organisms capable of attacking pectin and performing retting, only a few have been used for the production of enzymes, which are, in the absence of the living organism, capable of retting. Katagiri and Nakahama[2] compared the pectin-decomposing enzymes produced by retting bacteria and found that crude preparations showed remarkable specificity in their actions toward various fibre plants. Baruah and Baruah[3] reported that an enzyme mixture named Hiparol, secreted by *Thielaviopsis paradoxa* (De Seynes) V. Hohn was more active than bacterial enzymes in the retting of jute and coconut husk fibres. Kentesz[4] commented that a thorough reconsideration of the role, as well as the use, of pectolytic enzymes in the retting of plant fibres would be most desirable.

This investigation was carried out to determine the distinct and combined effects of some pectolytic enzymes such as pectin methyl esterase (PME) and pectin polygalacturonase (PPG) extracted from certain green plants and molds on the retting of jute plants.

Materials and Methods

PME was prepared using Willaman and Hills' method[5] from different green plants such as brinjal, carrot and potato leaves. The plant parts were macerated. The extract, after adjusting to pH values of 4.0-4.3, was filtered, and the filtrate discarded. The residual pulp was suspended in a small quantity of water. The pH value of the suspension was raised to 6.0, and the suspension was allowed to stand for a few minutes. The extract was pressed out of the suspension, and tested for PME activity. One c.c. of the extract was added to 2 c.c. of the slightly acid pectic solution. The mixture was then titrated with 0.1 N sodium hydroxide in presence of methyl red indicator until the mixture lost its last pink tint from one drop of the alkali. This mixture was incubated at 30°C. for 10 minutes. If the enzyme is present, red coloration appears. The plant parts giving relatively more PME were used for PME extraction.

PPG was extracted in a crude form from certain molds of *Penicillium* and *Aspergillus* group, following Ehrlich's method.[6] Molds were allowed to grow on a mixture of yeast and malt extract containing 2% pectin for about 3 weeks. Mycelia formed were ground and autolyzed at room temperature in the presence of toluene for 24 hrs. The extract was filtered off. Very fine white precipitates were formed when a portion of the extract was treated with a 4-fold volume of ethanol indicating the presence of pectin polygalacturonase.

Since the precipitate could not be separated from the extract, the enzymes could not be further purified. As such the filtrate was used as the PPG for testing for retting ability.

The retting ability of the enzymes was tested in test tubes. Portions of jute stem were put in the tubes and distilled water was added to just cover the jute stems. The tubes were then plugged with cotton and sterilized in the autoclave. The enzyme extracts were added separately and in combination to the tubes in duplicates after the distilled water in the tubes was poured off. The tubes were then incubated at 35°C. and periodically checked for retting.

Results

The PME and the PPG completed the retting in 7 days and the nature of retting varied with whether they were applied singly or in combination. Although the PME from brinjal and the PPG from *Penicillium* completed retting separately, retting was better when they were in combination.

TABLE 1.—EFFECT OF PME AND PPG FROM DIFFERENT SOURCES ON THE RETTING OF JUTE IN 7 DAYS.

Source of enzymes	Nature of retting
PME from brinjal	Completely retted
PPG from *Penicillium*	,,
PPG from *Aspergillus*	Partially retted
PMC from brinjal plus PPG from *Penicillium*	Completely retted and the fibre favourably softened
Control (No enzyme)	Not retted

Discussion

It is quite interesting to notice that the PME and PPG can accomplish retting singly as well as jointly. Further attempts should be made to determine if enzymes from other sources can accomplish better retting when combined together. With the increased knowledge in the nature of enzymes in an enzymatic mixture, the ideal combination for best retting can be obtained and much improvement in the quality of fibres effected. Baruah's Hiparol that accomplished retting in the shortest time was a mixture of enzymes. If the nature and character of all the enzymes in a combination like Hiparol can be known then the desired combination of enzymes can be prepared and used for the best retting. More research work is necessary so as to gather more information on the specific enzymes involved in best retting.

Acknowledgement.—The authors express thanks to Mr. Amirul Islam, Research Assistant in this Institute, for help in this work.

References

1. H. Mitscherlich, Ann. Chem. Pharm., **75**, 305 (1850).
2. H. Katagiri and T. Nakahama, J. Agr. Chem. Sec. Japan, **16**, 1151 (1940).
3. P. Baruah and H.K. Baruah, Science and Culture, **11**, 369 (1946).
4. Z.I. Kentesz, *The pectic substances*, (Interscience Publishers, Inc., New York, 1951) p. 628.
5. Willaman and Hills, U.S. Patent, 2, 358, 429 (1944).
6. F. Ehrlich, Biochem. Z., **250**, 525 (1932).

SYNERGISTIC EFFECT OF PENICILLIN IN THE PRESENCE OF CYANOCOBALTAMINE

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It has been observed[1] that certain elements enhance the action of antibiotics against microbes sensitive to them. Yet it remains to be seen as to why and how these elements play an important role in the enzyme systems of microörganisms or on the mode of their action on antibiotics. It is interesting to note that cobalt ions potentiate the activity of penicillin in *vitro* as well as in *vivo*.[2-5] This enhancement of activity was tried with other elements such as Ni, Mn, Pt, Ir, Fe, Zn, Cd, Li, Cu, Ag, Au and Bi by these workers but there was no such increase in activity.

Since vitamin B_{12} (cyanocobaltamine) contains cobalt ions, it is probable that the activity of penicillin is likely to be affected in the same way, and the present investigation was carried out to see if any enhancement can be brought about in the antibiotic properties of penicillin when used in conjunction with Vitamin B_{12}.

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Experimental

Pure culture of *Streptococcus viridans* was innoculated on solid media comprising nutrient broth and agar in petri dishes and a known quantity of penicillin solution was placed in standard glass cylinders (of 10 mm. height and 7 mm. diameter) and were allowed to incubate at 37°C. for 24 hours. Areas and the restriction of growth were measured. The exact procedure for the determination of sensitivity is given below:

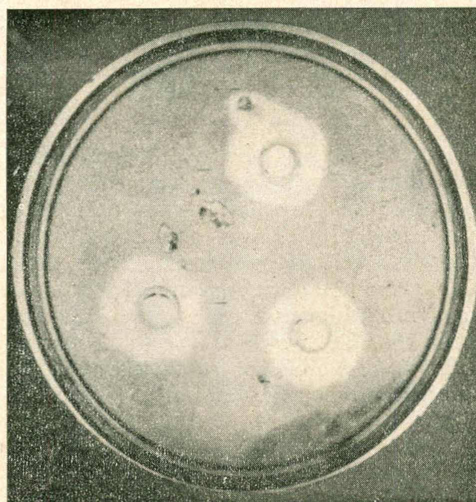
The sensitivity tests were performed by culturing bacteria first in liquid media and then reculturing on a solid medium in petri dishes. The small glass cylinders with their smooth edges were placed on the solid medium and the antibiotic, i.e. a known volume of penicillin solution was poured in cylinders and petri dishes incubated at 37°C. for 24 hours. The solution of penicillin diffused into the medium giving growth free zones were proportional to its concentration. Thus standard readings between the zone of inhibition and different concentrations of penicillin were obtained with base cultures of *Streptococcus viridans* and *Staphylococcus aureus*. It was observed that the zone of inhibition increased as the concentration of penicillin solution was increased. In another experiment it was found that for the same concentration of penicillin the growth of free zone was less in *streptococci* as compared to *staphylococci*. This indicates that the more sensitive is the microbe, the wider is the zone of inhibition. The experiments were further conducted in conjunction with vitamin B_{12}, and a known concentration of penicillin (0.5 unit) in cylinders on seeded agar and incubated at 37°C. for 24 hours. There was an increased zone of inhibiton, showing that in the presence of vitamin B_{12} the effect of penicillin was enhanced (*Staphylococcus aureus* was employed as the test organism).

The data is summarised in Tables 1 and 2.

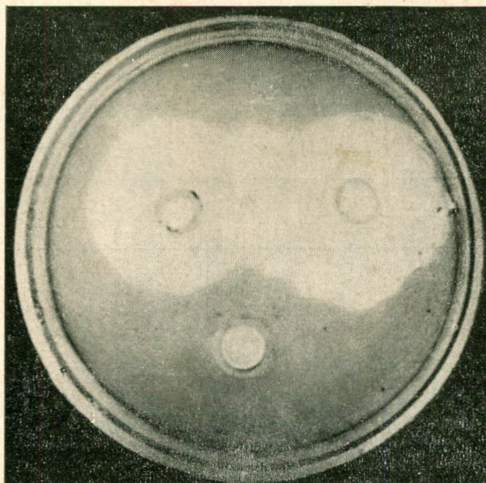
Cobalt nitrate was employed as a source of cobalt ions and its effect was studied on the activity of penicillin. After 24 hours of incubation at 37°C. the growth free areas were measured (Table 3).

TABLE 1.

Concentration of penicillin	Diameter of the zone of inhibition
1. 0.5 unit in 0.05 ml. of solution	11 mm.
2. 1.0 unit „ „	15 mm.
3. 1.5 units „ „	20 mm.



Sensitivity test showing zones of inhibition for fixed concentration of penicillin (0.5 unit) employing *S. aureus* as the test organism.



Sensitivity test with penicillin 0.5 unit in conjunction with optimum concentration of Vit. B_{12} (15 μg) for maximum inhibition. The third cylinder is a blank, containing Vit. B_{12} only and shows no growth-free area. employing S. aureus as the test organism).

A control experiment was also conducted with vitamin B_{12} alone using different concentrations and it was observed that there was profuse growth round the cylinder after 24 hours of incubation at 37°C. But when it was further incubated for another 24 hours, the growth stopped and lysis of bacteria was observed. This shows that vitamin B_{12} has a growth-promoting effect on the bacteria during the first 24 hours.

TABLE 2.

	Concentration of vitamin B_{12} in 0.1 ml. of solution and penicillin (0.5 unit in 0.05 ml. of solution)	Diameter of the zone of inhibition
1.	2.5 μg. of vitamin B_{12} + penicillin.	16 mm.
2.	5.0 μg. of vitamin B_{12} + penicillin.	25 mm.
3.	7.5 μg. of vitamin B_{12} + penicillin.	36 mm.
4.	10 μg. of vitamin B_{12} + penicillin.	40 mm.
5.	12.5 μg. of vitamin B_{12} + penicillin.	46 mm.
6.	15 μg. of vitamin B_{12} + penicillin.	48 mm.
7.	20 μg. of vitamin B_{12} + penicillin.	48 mm.

TABLE 3.

	Concentration of cobalt nitrate solution (in 0.1 ml. of solution)	Diameter of the zone of inhibition
1.	1 μg. of cobalt nitrate + 0.5 unit of penicillin in 0.05 ml. of solution.	14 mm.
2.	2 μg. of cobalt nitrate + 0.5 unit of penicillin in 0.05 ml. of solution.	21 mm.
3.	3 μg. of cobalt nitrate + 0.05 unit of penicillin in 0.05 ml. of solution.	29 mm.
4.	4 μg. of cobalt nitrate + 0.05 unit of penicillin in 0.05 ml. of solution.	37 mm.

Discussion

In experiments with penicillin alone, the growth-free area, is directly proportional to its concentration, but when vitamin B_{12} was used in conjunction with penicillin, the zone of inhibition was further increased.

It was further observed that as the concentration increased the zone of inhibition also increased up to 15 μg. of cyanocobaltamine, but above this range it became constant indicating the saturation point of B_{12} required for maximum inhibition. Similar experiments were repeated with cobalt nitrate which indicated the same results. These investigations show that increase in the activity of penicillin in the presence of vitamin B_{12} is probably due to the cobalt ion in the molecule.

Since the persons needing penicillin in toxaemia also require vitamin B_{12} due to the anaemia as secondary effect, combined therapy in such cases should lead to better results.

References

1. A. Albert, Pharm J., **158,** 275 (1947).
2. L.A. Strait, J. Dufrency and R. Pratt, J. Am. Ph. Assoc. Sci. Ed., (1947).
3. J. Dufrency and R. Pratt, J. Bact., **53,** 657 (1947).
4. R. Pratt and J. Dufrency, J. Bact., **54,** 127, 719 (1947).
5. R. Pratt, J. Dufrency and L.A. Strait, J. Bact., **55,** 75 (1948).

A NOTE ON EXCHANGE FORCES IN NUCLEI

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A short note seems to be called for on the exchange forces within the nucleus in the light of the latest picture of the structure of neutron (negative core) determined at the University of Stanford, U.S.A. Do we have to change Yukawa's theory? Or does some other mode of exchange also take place?

Generally speaking, if the potential between two nucleons is a function of space and spin co-ordinates, it is well known that there are three possible types of exchange interactions between two particles:—

$$\psi(\tau_1, \sigma_1)\ (\tau_2, \sigma_2) \rightarrow \begin{cases} \psi(\tau_2, \sigma_1)\ (\sigma_2, \tau_1) & \text{Majorana} \\ \psi(\tau_1, \sigma_2)\ (\tau_2, \sigma_1) & \text{Bartlett} \\ \psi(\tau_2, \sigma_2)\ (\tau_1, \sigma_1) & \text{Heisenberg} \end{cases}$$

In Majorana forces there is exchange of position co-ordinates but not of spin, which is visualised physically in terms of exchange of pi-mesons on the Yukawa theory and plays a very important role in the nuclei. These forces are attractive for two particles with even angular momentum. While Yukawa's theory is one of

the most useful theories evolved in physics, it suffers from several defects, particularly the following:—

(*a*) the mass of proton and pi-meson do not compute to that of neutron, and

(*b*) the nuclear forces calculate to be about 3-4 times stronger on the basis of the theory than they actually are. Yet if we treat the interacting nucleons as rotating round the centre of the coupling axis (as pointed out by the author in his paper on 'Nuclear Forces' published in the Pakistan Journal of Science, Vol. 9, No. 1, January 1957) then these forces come close to the normal figure even with Yukawa mode of exchange.

Further, there is now the added possibility of N-P forces being established by an exchange of electron-neutrino pair, which is rendered plausible by the fact that the pi-meson sometimes breaks directly into an electron, a neutrino and a photon, without passing through the mu-meson stage.* The author's view is that the type of exchange which actually takes place is a combination of the Yukawa type and the electron—neutrino pair (masses being equal) type in suitable proportions to establish the observed N—P forces.

When the Yukawa exchange takes place, the system would rotate as suggested round the centre of the coupling axis, but it remains without such rotation when the other mode of exchange occurs. From the practical angle, the mode of decay of mesons, especially the two alternate modes of breaking up of pi-meson suggest that these are the only significant types of exchange, and it would be worthwhile to make numerical comparisons with experiment.

*The objection that electron-neutrino exchange cannot establish nuclear forces because their observed interaction with matter is very limited can of course be countered by the statement that it is not yet possible to create laboratory conditions parallel to the inside of the nuclei.

CORRIGENDA

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Page 179, Table 2—line 3 : *For* 89.855 *read* 98.855.

Page 179, Table 2—last line : *For* 100.033, 99.378 and 99.502 *read* 100.028, 99.397 and 99.501, respectively.