MICROBIAL DECOMPOSITION OF PECTIC SUBSTANCES
V. Evidence for the role of *Micrococcus* sp. in the retting of sisal and coconut husk

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Received on August 27, 1966

Abstract

Micrococcus sp. isolated from the rets of sisal and coir were shown to be pectinolytic in nature. Besides differing in their morphological and physiological traits, the differences in the pectinolytic enzymes of the isolates from the two ecosystems have been brought out. The interesting influence of NaCl on the glycosidase enzyme has been demonstrated.

Introduction

The ability of rod-shaped bacteria, yeasts and fungi to degrade pectic substances and their possible role in the retting of fibrous materials has been reviewed comprehensively.[1] Recently, it has also been demonstrated that ret-liquors of plant straws of *Calotropis* and *Hibiscus* carry protozoa possessing pectinolytic enzymes[2]; but, notwithstanding their presence in the rets of flax[3], hemp[4] and jute[5], the *Micrococcus* species have not been incriminated in the retting process. In a short note from this laboratory[6], however, the elaboration of pectinolytic enzymes in gram positive cocci was reported. The purpose of the present communication is to show that certain micrococci are not only associated with the retting of two different fibre-yielding plant materials but to demonstrate their ability to degrade pectin by more than one mode of attack by virtue of possessing different pectinolytic enzymes.

Materials, Methods and Results

Bacterial isolates were made by streaking sisal and coconut husk (coir) ret-effluents on nutrient agar plates fortified with 0.01% yeast extract. The medium contained 1% NaCl when used for the isolation of organisms from the coir rets All the pectinolytic cultures, after their purification, were examined for their nature and characteristics according to the methods outlined by the Society of American Bacteriologists[7] and identified by reference to the Bergey's Manual.[8] The differences in the morphological, cultural and physiological characteristics of the micrococcal isolates made from the two ecosystems were

TABLE I

Comparison of the characteristics of *Micrococcus* sp isolated from sisal and coir rets.

Characters	Coir rets (34)*	Sisal rets (16)
Morphological	Gm positive cocci, occurring singly, in pairs, short chains. Motile single flagellum.	Gm positive to variable cocci occurring singly, in pairs, short chains and clusters. Non motile, No flagella.
Cultural	Round, slightly raised, glistening, smooth, opaque pale orange colonies with entire margin on nutrient agar. In nutrient broth clear with somewhat orange sediment. No surface growth.	Small to pin point, round to oval, slightly raised, glistening, smooth, opaque yellowish white colonies with entire margin on nutrient agar. In nutrient broth with more of sediment than turbidity. No surface growth.
Physiological	No detectable change in BCP milk. Indole not produced. Acid from glucose, sucrose and glycerol, no acid from lactose. Gelatin liquefied. Starch hydrolysed. Nitrate not reduced. Chitin not utilised. Utilises Ammonium hydrogen phosphate as sole source of nitrogen. Aerobic.	Acid reaction in BCP milk. Indole not produced. Acid from glucose, lactose, sucrose and glycerol. Gelatin liquefied. Starch not hydrolysed. Nitrate reduced to nitrite and then to ammonia. Chitin not utilised. No utilisation of Ammonium hydrogen phosphate as sole source of nitrogen. Aerobic.
Source	Natural retting yards of coir.	Sisal rets.
Habitat	Unknown	Unknown.
Special features	All are pectinolytic.	All are pectinolytic.
Identity	The above characters do not tally with any of the described species.	The above characters do not tally with any of the described species.

* Numbers in parenthesis indicate number of strains (studied).

TABLE II

Qualitative Screening of pectinolysis of *Micrococcus* sp.

Source	No. of strains	No. of strains attacking pectin by	
		Deesterification	Glycosidase activity
Sisal rets	6	6	6
Coir rets	34	34	34

TABLE III

Pectinolytic activity of *Micrococcus* sp

Source	Number of Strains	PG *		Enzymatic activity**			% pectin decomposed
		P †	PA	*PE*	*PTE*	*PATE*	
Sisal rets	6	0.1	1.0	1.8	0.0	0.0	40.7
Coir rets	22	0.2	0.8	0.0	0 0	0.0	31 8
Do	12	0.4	0.9	0 2	0.18	0 26	39.9

* measured afrer 24 hr.

† P=pectin; PA=polygalacturonic acid

** *PG* measured as increase in reducing power in terms of 0.05 N $Na_2S_2O_3$

PE as ml of 0.02 N NaOH required

PTE and *PATE* as units of OD at 230–235 mμ

so striking (Table I) as to consider the two groups as different from each other. Besides, they differed distinctly in their behaviour in media containing NaCl, a factor which normally should not bring out such wide differences. Whereas the *Micrococcus* sp isolated from the sisal rets could not tolerate even 2% of NaCl, those from the coir rets exhibited maximal growth response only at concentration of 5% and the limit of halotolerance was of the order of 20%. What was more interesting was the inability of coconut husk strains to grow in the absence of NaCl at a temperature of 36°C. A detailed account of their behaviour in relation to the concentration of salt will be presented elsewhere.

The pectinolytic activity of the micrococcal strains isolated was tested qualitatively in a medium containing 0·5 % pectin according to the method of Bilimoria and Bhat [9] and the results are presented in Table II. All pectinolytic cultures were further tested quantitatively for their enzymic activity. For this purpose, the cultures were grown as submerged cultures in media containing 0 5% pectin or polygalacturonic acid for 7 days at room temperature (22-28°C). The action of polygalacturonase (*PG*) on pectin or polygalacturonic acid was

determined by estimating the increase in reducing power by a modification of Willstater-Schudel method.[10] Pectinmethylesterase (*PE*) activity was assayed by employing a method similar to that of Smith's modification[11]. Pectin / polygalacturonic acid *trans*-eliminase (*PTE/PATE*) was detected by recording the peak at 232-235 mμ in the reaction mixture and confirmation thereof was sought by the thiobarbiturate reaction.[12] The enzyme was assyed by the method of Nagel and Vaughn.[13]

The percentage decomposition of pectin was determined by using the method of Kaiser.[14] A glance at table III would indicate the enzymatic make up of the isolates along with their ability to degrade pectin in the media. The variation in pectinolytic enzymes elaborated by the different strains is apparent. The isolates from sisal rets contained a strong *PE* with a very weak *PG* which facilitated the determination of the *PE* activity with clarity. The enzyme *PTE/PATE* was absent in these strains. On the other hand, as many as 22 strains out of a total of 34 positive strains encountered from coir rets were found to elaborate *PG* while the remaining 12 strains possessed both *PE* and *PG* along with the enzyme *trans*-eliminase. However, isolates from both the systems behaved in an identical manner in so far as their preferenee to poly-alacturonic acid to pectin was concerned.

The products of degradation of pectin and polygalacturonic acid were examined by paper chromatographic techniques[15]. Chromatograms revealed the presence of various spots, below the galacturonic acid spot, and these presumably were of the polymers of galacturonic acid such as the dimer, trimer. etc , in as much as their Rm values, according to Hathway and Seakins[16] (Fig. 1) were in agreement with the polymers. Besides, the spots corresponding to the dimer were present only in those strains giving positive tests for the *trans*-eliminase. The conspicuous absence of galacturonic acid in sisal strains cultured in pectin medium was of interest in this context.

In order to gain further information on the differences, if any, in the pectinolytic enzymes of the organisms from the two ecosystems, these cultures were centrifuged off and acetone powder preparations were made from their supernatants.[17] These were dialysed for 24 hr against distilled water in the cold and the preparations so obtained were assayed for the different enzymes. The *PG* activity of *Micrococcus* sp isolated from sisal rets was somewhat peculiar in that it not only had a low pH optimum (4·4 – 4·6) but was activated by NaCl. (Fig. 2 and 3). The enzyme activity was slower with pectin than with polygalacturonic acid. On the other hand, NaCl surprisingly had no beneficial effect on the *PG* activity of the dialysed enzyme preparations of the isolates from the coir rets. In so far as the enzyme *trans*-eliminase was concerned, both *PTE* and *PATE* were present in the culture filtrates of the isolates from the coir rets and polygalacturonic acid served as the better substrate. The *PATE* appeared to be calcium dependent with an optimum pH around pH 8.0 (Fig. 4 and 5).

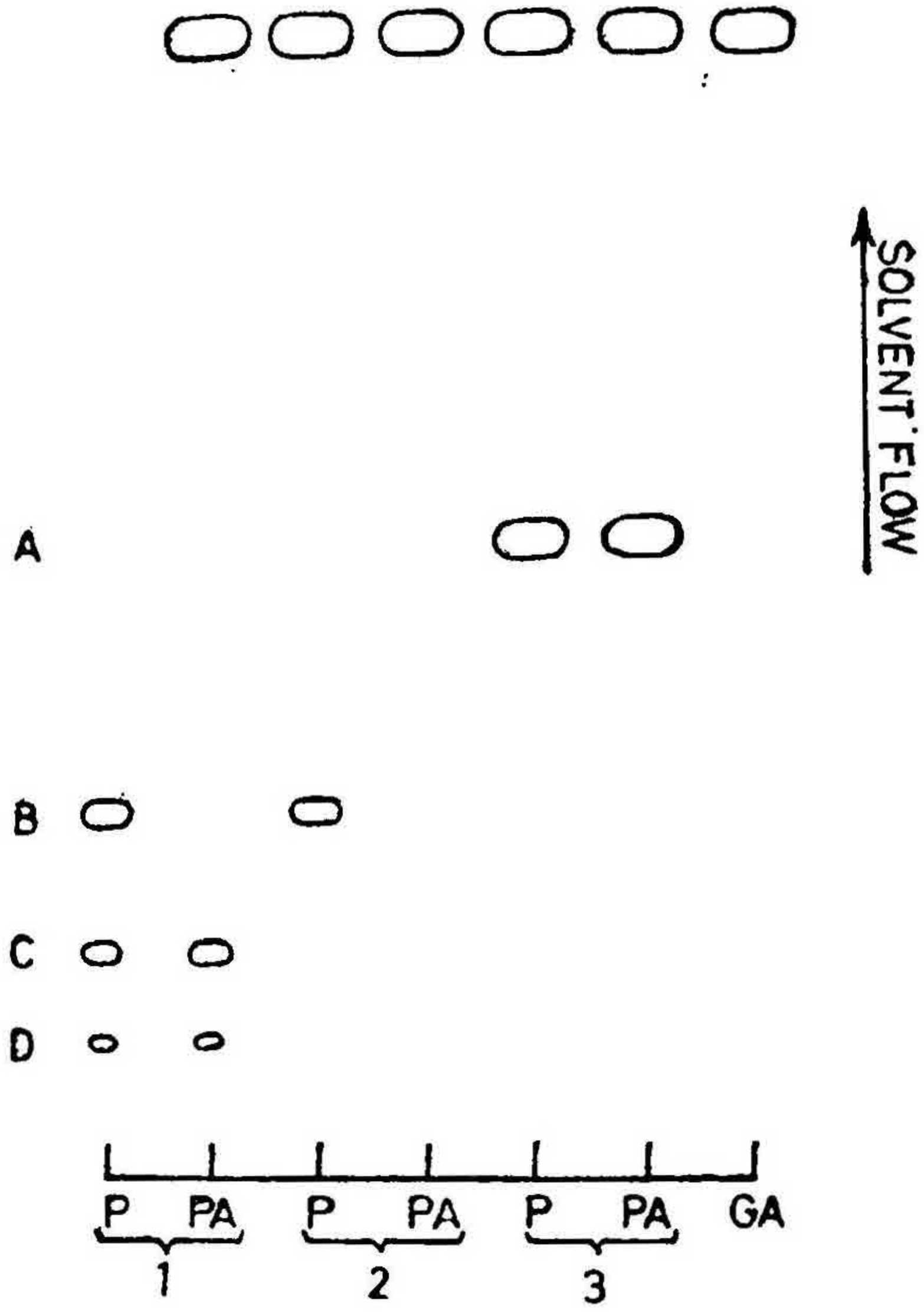

FIG. I

Chromatographic sketch of degradation products of pectinolysis by *Micrococcus* isolates

P = Pectin PA = Polygalacturonic acid

GA = Galacturonic acid (Marker) — R_f = 0.53

A,B,C,D = Galacturonic acid polymers (R_f = 0.28, 0.16, 0.085, 0.06 respectively)

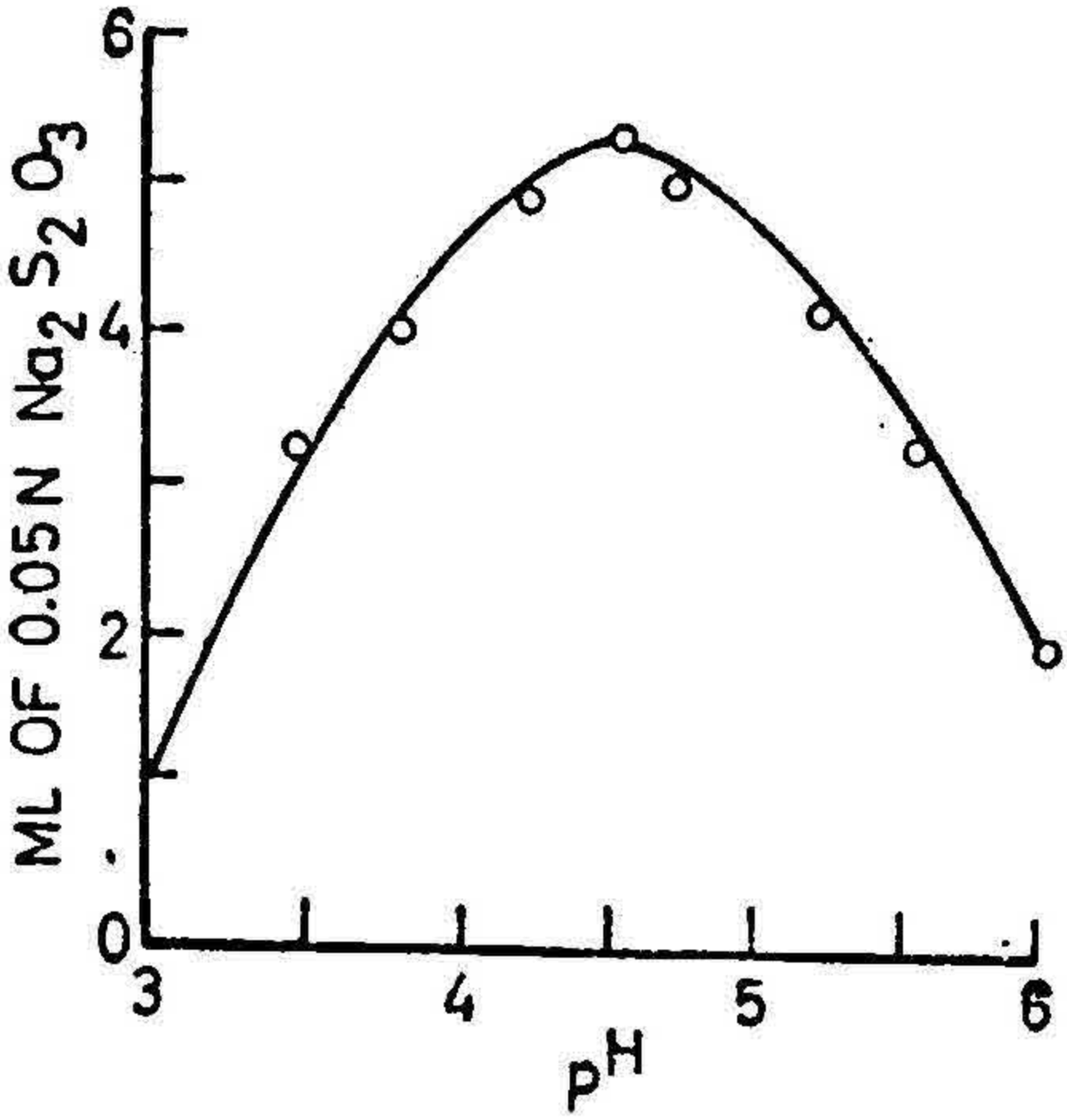

FIG. II

Effect of $_p$H on *PG* of *Micrococcus* isolates

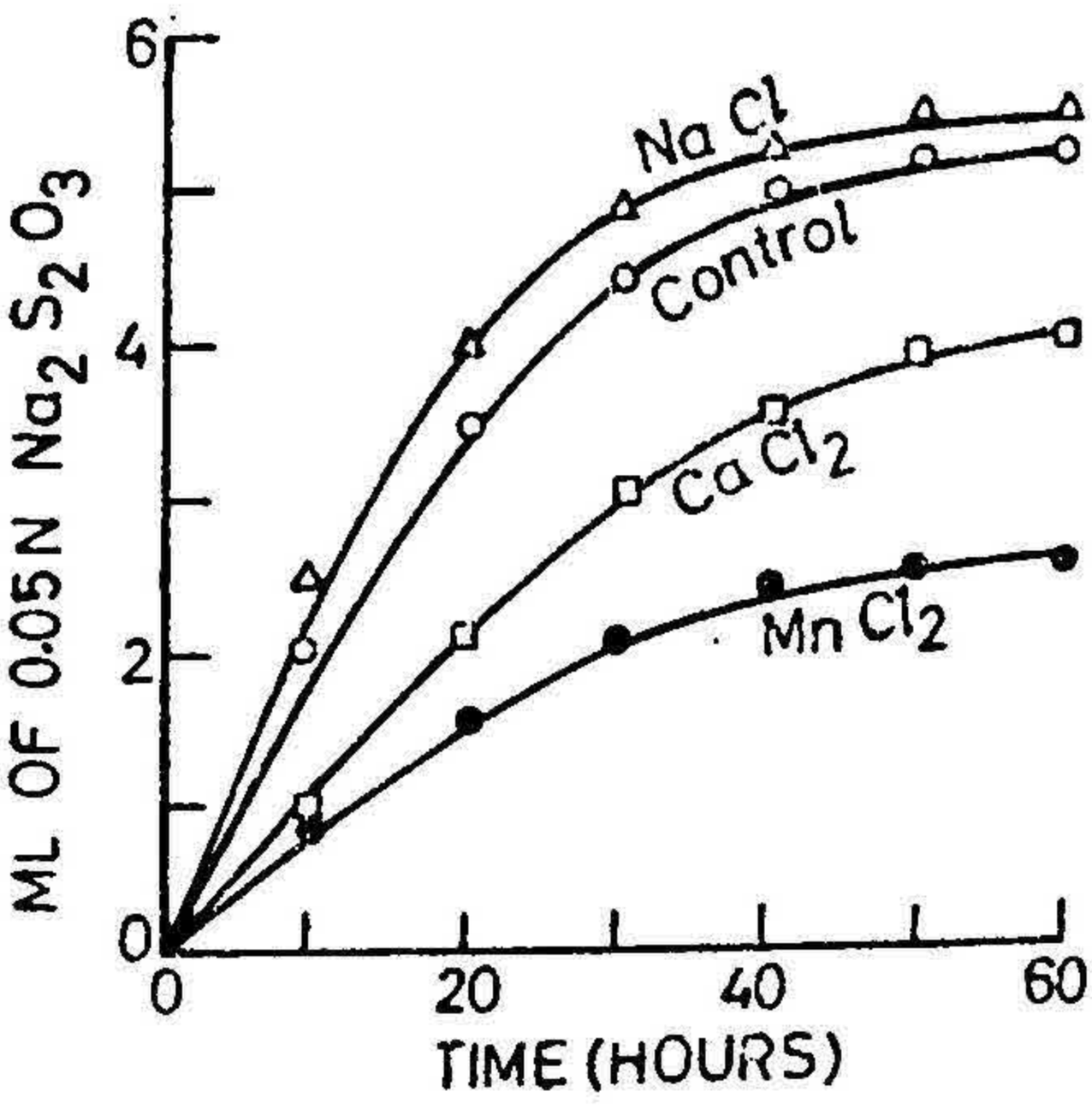

FIG. III

Activation of *PG* of *Micrococcus* isolates

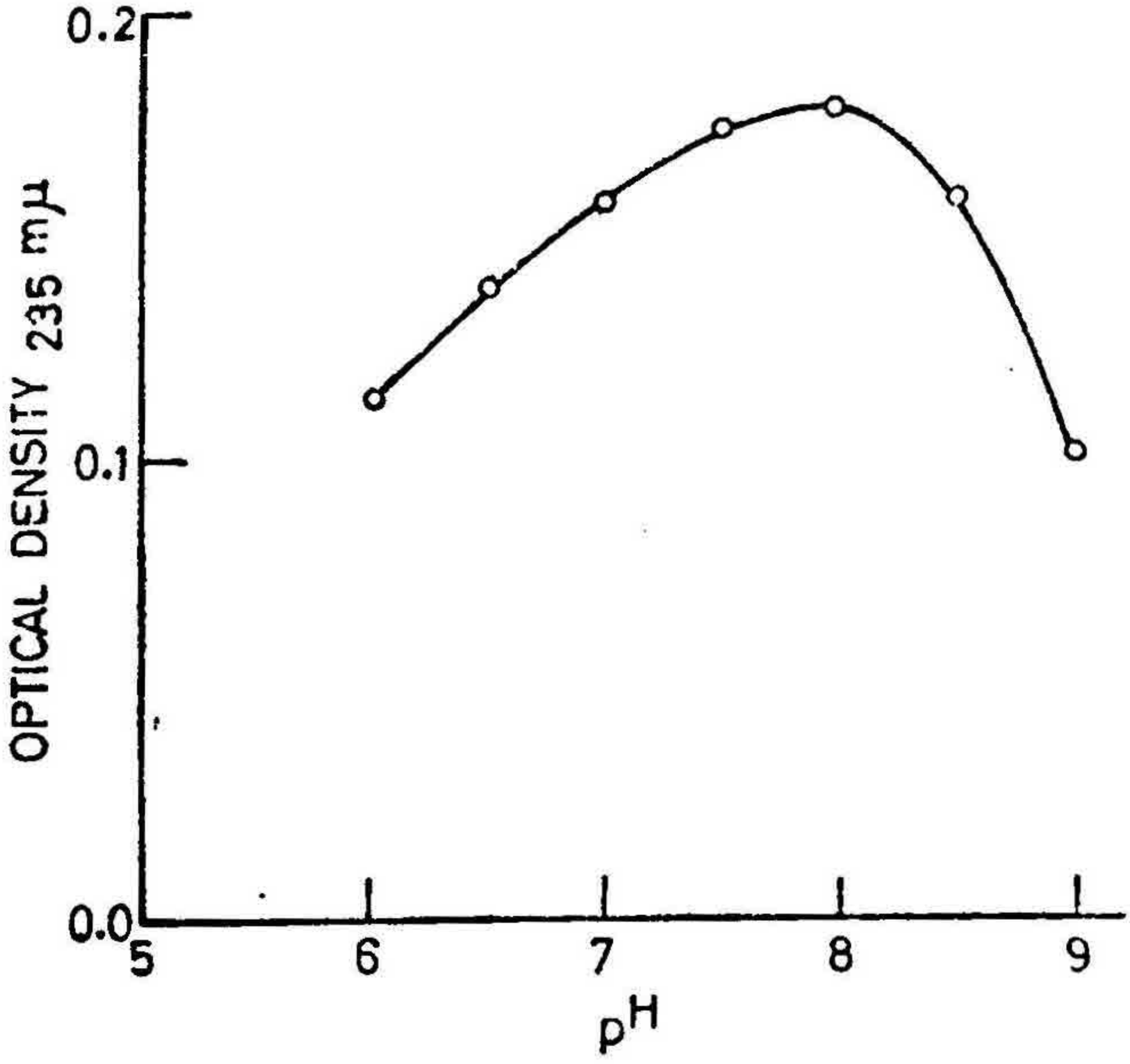

FIG. IV

Effect of pH on *Pate* of *Micrococcus* isolates

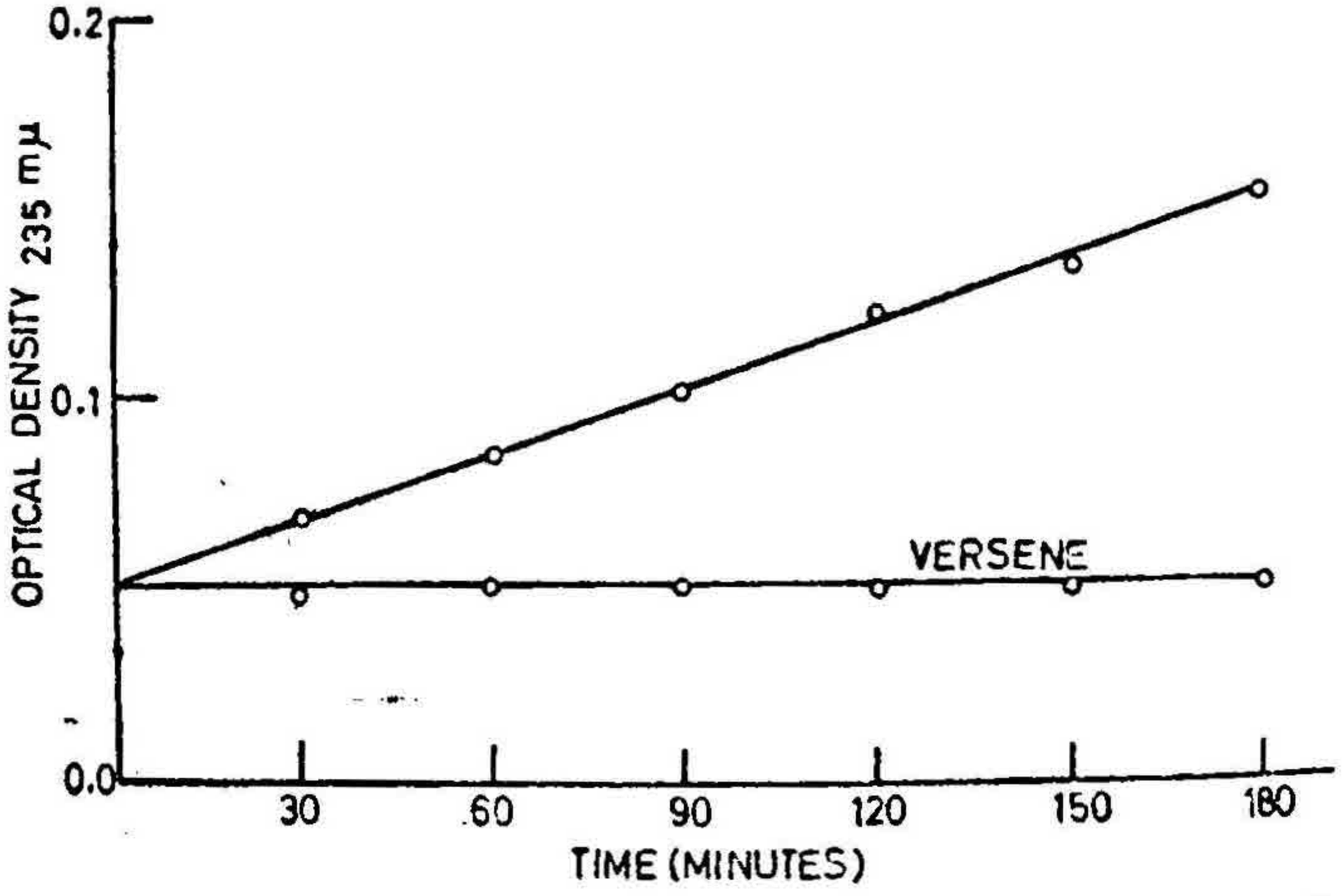

FIG. V

Calcium dependence of *Pate* of *Micrococcus* isolates

Discussion

The results presented here in addition to providing evidence in favour of the pectinolytic nature in general of *Micrococcus* species have revealed valuable information on the diverse enzymatic make up of the strains in the genus. The occurrence of *Micrococcus* sp in both the ecosystems, *viz* , sisal and coir rets, was in itself interesting ; the difference observed in the isolates obtained from the two systems was even of more sustained interest in this connection in that only 12 out of a total of 34 strains were found to elaborate the enzyme *trans*-eliminase comparable in properties with those of other bacterial enzymes[18,19] but differing considerably from those of fungal origin[20]. Yet another striking difference between the isolates from the two ecosystems was with respect to the enzyme *PG* and the inability of NaCl to activate the enzyme of the isolates of the coir rets despite the fact that the bacteria were highly halotolerant. Strangely, the glycosidase enzyme of the sisal isolates was activated by NaCl although the strains themselves were unable to thrive in media containing even 2% NaCl. Activation in the presence of NaCl and the extremely narrow range of pH optima, *viz.*, 4.5-4.6 has nevertheless been recorded for the enzyme pectic acid depolymerase (*DP*), of tomato[21] and the situation is reminiscent of the " widening effect " of salt on the widening of pH range for the growth of *E. coli.*[22] The lag in attacking pectin as compared to polygalacturonic acid could perhaps be attributed to the methyl ester groups of pectin which have to be initially hydrolysed to pectic acid before the *DP* can catalyse the subsequent changes. The enzyme itself has been shown to occur in yeasts[23] and its presence was suspected in the fungus *Byssochlamis fulva.*[24] So far as bacteria are concerned, this is perhaps the first time this enzyme has been demonstrated.

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