STUDIES ON PECTIC ENZYMES

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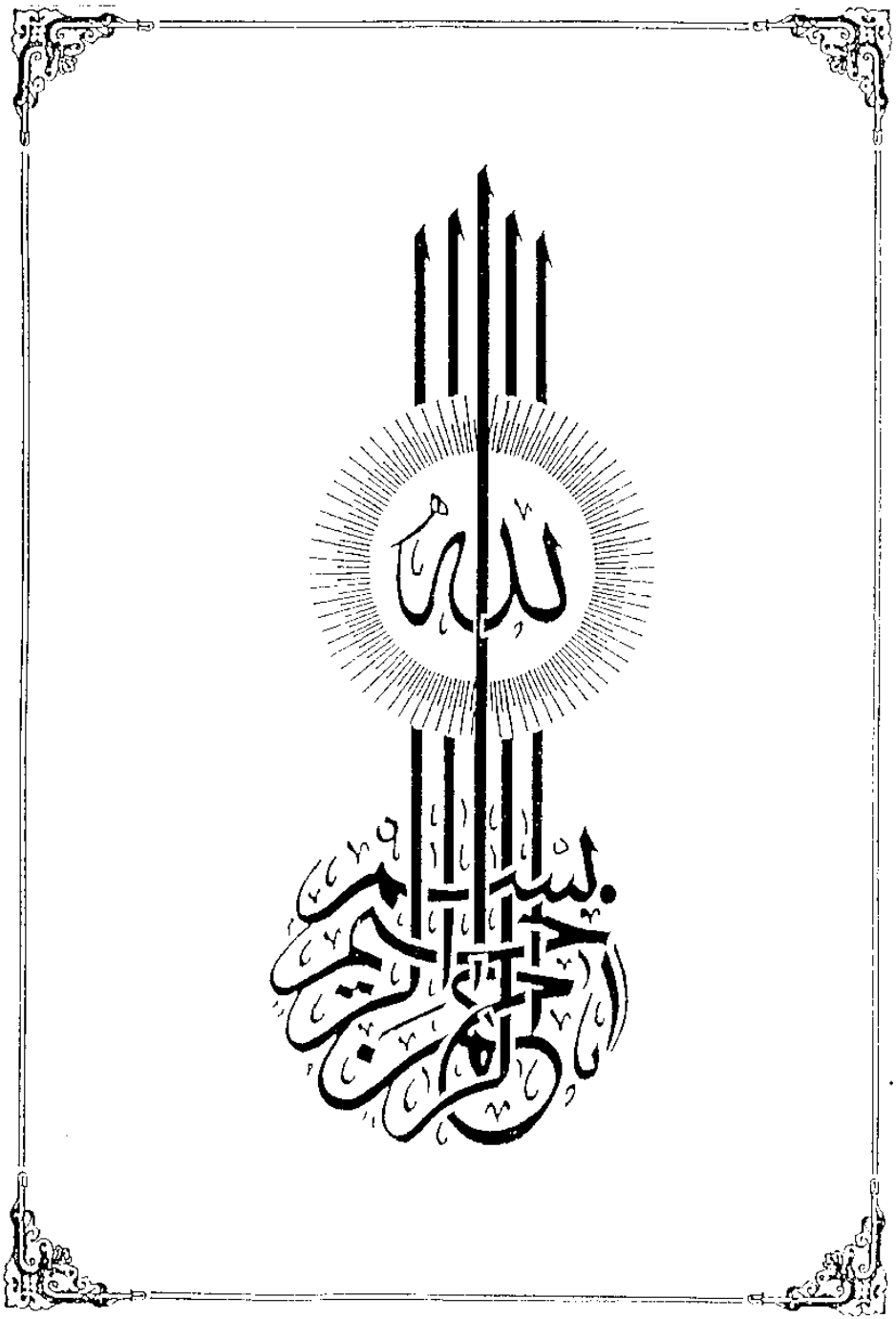

A C K N O W L E D G M E N T

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Contents

I N T R O D U C T I O N

Pectic substances are complex colloidal carbohydrate derivatives. Their basic structures are polymers of anhydro-D-galacturonic acid in the pyranose form, connected by α-1,4-glycosidic linkages. They are classified as (i) protopectin, (ii) pectinic acids, (iii) pectins, and (iv) pectic acid by the Amer. Chem. Soc. Only two types of enzymes have been thought to be involved in the degradation of pectin. They are hydrolases and transeliminases. The former includes pectin methyl esterase (PME), polygalacturonase (PG), and polymethylgalacturonase (PMG). The latter group contains pectin transeliminase (PTE) and polygalacturonic-transeliminase (PATE). The following categories are a modification of the classification of pectic enzymes proposed by Demain and Phaff (1957). They recognized two different types of enzymatic attack on the pectin and the pectic acid molecule; a random or endo-attack, and a terminal or exo-attack. With each individual type of attach there are separate enzymes for the pectin molecule (endo- or exo-polymethygalacturonase or PMG) and for the pectic acid molecule (endo- or exo-polygalacturonase or PG).

The classification can be summarized as follow:

I. Hydrolases

A. Substrate : Pectin

1- Pectinesterase (PE).

2- Endo-polymethyl galacturonase (Endo PMG)

3- Exo-polymethylgalacturonase (Exo PMG)

B. Substrate: Pectic acid.

1- Endo-polygalacturonase I (Endo-PG I)

2- Endo-polygalacturonase II (Endo-PG II)

II. Trans-eliminases.

A. Substrate : Pectin

1. Endo-pectin trans-eliminase (Endo-PTE)

2. Exo-pectin trans-eliminase (Exo-PTE)

B. Substrate : Pectic acid

1- Endo-polygalacturonic acid trans-eliminase (Endo-PATE)

2- Exo-polygalacturonic acid trans-eliminase (Exo-PATE)

Various studies have shown that polygalacturonase are induced by certain compounds, whereas others seems to be constitutive.

While PG is established as distinct from PATE in fungi, Edstrom and Phaff, (1964); accurate and definite data

are not yet available which clarify the situation of the bacterial pectinases. After a discovery of bacterial PATE, most of the bacterial PG reported in the earlier papers could be considered to be PATE on the basis of optimum pH that was around 8.5 to 9.3 and calcium ion requirement. The differentiation of PG from PATE was assured through the analysis of the changes in optical density at 235 mu, thiobarbituric-acid reacting materials, reducing values, viscosity, and paper chromatographic behavior.

Pectolytic enzymes, beside their occurance in tissues of several plants and vegetables; i.e. Tomato (Patel & Phaff., of 1960), Avocado (Dominique & Phaff.,1965) and Garlic (Uisekew et al., 1953), and secreted by many species of microorganisms; i.e. A.niger, Botrytis cinerea and Sclerotinia liberian (Verbina et al., 1969), A.Soyae No. 48 (Ishii, Shigetaka et al., 1970) , Penicillium digetatum and Penicillium italicum (Bush 1970), Penicillium chrysogenium (Phaff 1946),Saccharomyces fragilis and Candida pseudo tropicalis (Phaff et al., 1951 and 1966), Erwinia caratovova (Kraght 1953), Bacillus polymyxa (Nagel and Vaughn 1961), Pseudomonas marginalis (Seiichi Nasuno et al., 1966) and Clostridium multifermentans (Macmillan et al., 1964).

Preparations of fungal pectolytic enzymes are used extensively as a phase for filtration and clarification of wines and fruit juices. Pectic enzymes have been also useful in the production of mixtures of oligouronides, being succeeded in the study of plant anatomy, and used in other processes; i.e. agar clarification (Philip Morse, 1965).

The practical application and uses of pectin-degrading enzymes in fruit processing industry, for the production and clarification of fruit juice, dates back about forty years. Advantages of enzymic treatment of fruit pulps and juices, for extraction and clarification have been reported by Reid, 1961, Dressler and Joslyn (1960), Charley (1961), Sreekantiah et.al.,1963, Joseph et al. 1963 and Yamasaki et al., (1967).

Several variety of strains of bacteria, yeast, and molds are capable of producing pectolytic enzymes as described by Deuel and Stutz (1958). Although considerable studies have been made with the pectin-degrading enzymes from different sources, enzyme preparations for use in the food industry are of a fungal origin because the optimum pH of mold enzymes lies approximately to that ofmany fruits, which ranges from 3.0 to 5.5 , (Mukherjee, 1971).

Extensive review by Demain and Phaff (1957) and Endo (1961) showed that members of the genera Penicillium, and Aspergillus, and other fungi, like Rizopus tritici, Solerotinia libertiana, Botrytis cinerea, Fusarium moniliforme, and Coniothyrium diplodiella produced pectolytic enzymes. In addition, many of the plant-pathogenic fungi and air-borne spores of Aspergilus species are capable of producing pectic enzymes as reported by Puvgi et al., (1951), and Deuel and Stutz (1958). Details for the commercial production of pectolytic enzymes are rather scanty.

The production of pectic enzymes by microorganisms is dependent to a large extent on the metabolic characteristics of the cultures. According to Kertez (1928), a variation in the composition of the medium will cause a shift in the metabolic products produced by the organisms, which have a profound effect on the rate of the enzymes.

With these facts in mind it is worthy to mention that there was lack in the study of bacterial pectic enzymes, since the mold pectinases have received the most interest.

Scope of Research

This work was carried out to study the possibilities of producing pectic enzymes from bacteria belonging to the genus Bacillus. The study is devided into two parts:

1- Environmental conditions affecting the production of pectic enzymes by some organisms of the genus Bacillus. To achieve this, screening test was conducted for the production of pectic enzymes by different species of the genus Bacillus, in addition, the effect of culture conditions on its production, in terms of poly galacturonase, by the selected strain was studied.

2- Polygalacturonase activity as a function of environmental conditions of the reaction mixture was concerned within two specific factors, i.e. pH and temperature.

REVIEW OF LITERATURE

Review of Literature

I. Organisms and media used for pectic enzymes production.

In 1952, Potter et al. used both Clostridium felsineum strain 195 , and Cl. felsineum, strain 41, to ferment pectin and pectic acid. The two strains caused a rapid fermentation, and the rate of pectin fermentation was greatest during the first 12-18 hours.

This means, that the two Clostridium strains produced pectic enzymes.

Salssac et al. (1953) noticed that, Clostridium aurantibutyricum displayed an intense pectinolytic activity with demonstrable protopectinase, pectinesterase and polygalactuonase. Plactridium pectinovorum PC, Clostridium butyricum C8 , Cl. acetobutylicum By significantly lowered viscosity of pectin solutions. P. virens and C. Saccharolyticum 860 had less activity while C. roseum A 39.x, C. felsineum and P. pectinovorum 3723, previously grouped with the pectolytics, had low or negligeable activity. Thus, the latter authors reported that the pectinolytic

bacteria did not constitute a natural group.

Kra ht et al. (1953) noticed that the culture of Erwinia carotovora, isolated from rotted Mexican bell peppers, liberates methanol quantitatively from pectin during growth on a pectin medium. This phenomenon is considered to be positive evidence of pectinesterase activity. Polygalacturonase cannot be demonstrated directly in the culture medium containing actively growing cells because the rapid utilization of galacturonic acid made positive identification of the enzyme uncertain. E. carotovora polygalacturonase production or secretion into the medium was greatly increased by the addition of pectic substances to the growth medium.

Nagel and Vaughn (1961) reported that maximal cell yield and polygalacturonase production could be obtained with mineral medium containing biotin and 3% pectin. They further added that the growth and Bacillus polygalacturonase (BPG) production were greatest when the culture was aerated on a rotary shaker.

The same authors showed that, pectinesterase production was dependent upon pectin concentration up to 4%, and EPG production was inducable. Pectin was the best inducer, followed by pectic acid and galacturonic acid in that order.

Agate et al., 1962, demonstrated that pectin transeliminase was detected in a pectolytic culture of Streptomyces viridochromogens, and pectin was preferred to polygalacturonic acid as an inducer and substrate.

Bilimoria and Bhat (1963) studied the medium comprising $(NH_4)SO_4$ 0.05 , KH_2PO_4 0.02, $MgSO_4$ 0.02, $CaCl_2$ 0.01, $MnSO_4$ $5x10^{-4}$, H_3BO_3 $5x10^{-6}$, $CuSO_4$ $7x10^{-7}$, Pectin 0.5, Na_2HPO_4 0.08, NaCl 0.005, $FeCl_4$ 1 x 10^{-4}, $ZnSO_4$ 0.0011, $CoSO_4$ $4x10^{-6}$, Na_2MoO_4 , $2x10^{-4}$ % , and H_2O was adjusted to pH 7. and autoclaved. The medium was inoculated with Strept. virido., the culture allowed to grow at 25-30 for 7 days.

The culture filtrate has both pectinmethyl esterase and pectin glycosidase.