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Lab- scale production of extracellular pectinase by soil bacteria using fruit waste as substrate

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ABSTRACT

Pectinases are pectin-degrading enzymes predominantly used as biocatalyst in various industries for wine extraction, fruit juice extraction, making of paper pulp etc. The large-scale production of pectinase using biological systems (bacteria, fungi, plants etc.) is one of the common methods used in industry. In the current study, bacterial isolates obtained from various soil samples were used for pectinase production. An enrichment culture technique was used to isolate 04 bacterial strains named as P1, P2, P3 and P4 which were subsequently characterized by 16S rRNA gene sequencing and biochemical tests. Lab-scale submerged fermentation was carried out using these isolates in synthetic media containing pectin as substrate (extracted from peels of Lemon, Sweet lime, Orange) & Lab pectin. Fermentation conditions were optimized. Supernatant containing the extra-cellular crude pectinase enzyme was collected after fermentation. The maximum pectinase production was seen after 7 days of fermentation at R.T. (28±2^{0}C) and pH 7.0 in shaker conditions. Effect of environmental parameters (pH, temperature, enzyme and substrate concentration) was studied on the activity of crude pectinase enzyme. The maximum enzyme activity of crude pectinase enzyme was found to be at R.T. (28±2^{0}C) and 37˚C in the pH range of 6-8. Partial purification of pectinase was done using ammonium sulfate precipitation method followed by dialysis. The dialyzed enzymes obtained were subjected to DNSA assay (for enzyme activity) and Folin-Lowry assay (for protein estimation) for determining maximum pectinase activity and the amount of pectinase present in the supernatant respectively. By using SDS- PAGE, molecular weight of pectinase was determined. Thus, the current study may help in providing alternate and eco-friendly source of pectin that can be degraded by bacterial pectinase and used in food industry.

Keywords: Pectinase, Fermentation, SDS- PAGE.

INTRODUCTION

Pectinase is the heterogeneous enzyme that acts on pectin substances. It is present in higher plants & microorganisms. It is of prime importance for the plants as the enzyme helps in cell wall extension & softening of some plant tissues during maturation & storage. It maintains ecological balance by decomposing & recycling of waste plant materials.

The pectinolytic enzymes have great industrial importance and are required for food processing industries, especially for extraction and clarification of fruit juices, extraction of oils, flavours and pigments from plant materials, textile [2], pharmaceutical, leather, detergent and paper [4].

In order to extract pectinase, microbes are the best source as they allow an economical technology with low resource consumption. Such enzymes may be discovered by screening microorganisms sampled from diverse environments or developed by modification of known enzymes using modern methods of protein engineering or molecular evolution. New enzymes for commercial application with desirable biochemical and physico-chemical characteristics and a low cost production have become a focus of research [5], [1], [3].

The present study was concerned with the searching of novel bacterial cultures from different soil samples for the lab scale production of pectinase. Further the effect of bacterial pectinase was studied on pectin extracted from peels of Lemon, Sweet lime, Orange & Lab pectin. The bacterial isolates (sources of pectinase enzyme) were identified based on morphological and biochemical characteristics.

MATERIALS AND METHODS

Soil:
The soil sample used for the enrichment and isolation of pectinolytic bacteria was obtained from the local garden area of Kalyan, MS.

Media used and identification of bacterial isolates:
Dox-Pectin medium was used for enrichment and isolation of pectinolytic bacteria. The well isolated colonies were grown on sterile nutrient agar slants as pure cultures and maintained at 10^0C as stock cultures. The colony characteristics were identified based on the colony morphology and staining characters. Total 4 bacterial isolates were identified by 16S rRNA sequencing at NCCS, Pune.

Pectin Extraction:
By acid extraction method, jelly like pectin was extracted from Orange, Sweet-lime and Lemon.

Effect of environment on bacterial growth:
The optimum temperature, pH and NaCl concentration for every bacterial isolate was determined by inoculating the pure cultures in Nutrient broth. For effect of temperature, the inoculated tubes were incubated at 4^0C, R.T. (28 ± 2^0C), 37^0C and 55^0C for 24hours and checked for growth in the form of turbidity. The un-inoculated sterile nutrient broth tube was kept as negative control. For effect of pH, sterile nutrient broth with pH 3.0, 5.0, 7.0, 9.0 and 11.0 were used whereas for effect of NaCl, sterile nutrient broth with 0.5, 2,4,6,8, and 10% NaCl was used. All sets were performed in triplicates.

Fermentation and Extraction of Pectinase:
The pure cultures of 04 bacterial isolates were inoculated in the sterile fermentation medium containing pectin extracted from different sources (Orange, Sweet lime, Lemon and Lab pectin) and were incubated at R.T., for 7 days on rotary shaker at 120rpm. Fermentation medium used was Dox-pectin medium. After incubation, the fermentation medium was centrifuged at 5000rpm for 30 minutes. The supernatant was collected as crude enzyme source.

Enzyme Kinetics:
For determining the effect of pH, temperature, enzyme concentration and substrate concentration of the extracted pectinase enzymes, the enzyme kinetics was carried out using DNSA method.

SDS-PAGE:
Partial purification of pectinase was done using ammonium sulfate precipitation method followed by dialysis. By using SDS- PAGE, molecular weight of crude pectinase was found out.

RESULTS AND DISCUSSION

Identification and taxonomic characterization of pesticide tolerating bacteria
04 morphologically distinguishable bacterial colonies were observed on Dox Pectin agar plate which showed zone of clearance on adding Grams iodine indicating production and release of pectinase in the agar plate (Figure 1).

Morphological, cultural and biochemical studies were carried out and it was seen that all isolates were Gram negative in nature. The isolates were identified according to Bergey's Manual of Systematic Bacteriology (Vol I and II) and further characterized by partial sequencing of the 16S rRNA gene (Table 1).

Figure1: Iodine testing for detection of pectinolytic bacteria

Table 1: Identification of bacterial isolates

Sr. No.	Name of the bacterial isolate	Grams Nature
P1	*Pseudomonas plecoglossicida* strain P18	Gram negative
P2	*Klebsiella pneumoniae* strain VD	Gram negative
P3	*Stenotrophomonas maltophilia* strain 13635L	Gram negative
P4	*Pseudomonas* sp. CM2	Gram negative

Effect of environment on bacterial growth:
All 04 bacterial isolates showed maximum growth at the temperature of R.T. (28 ± 2^0C) and 37˚C. The optimum pH for all 04 bacterial isolates was in the range of 6.0-8.0. These bacterial isolates were able to grow in presence of 0.5% - 4% NaCl except P4 which could not grow in more than 2% concentration.

Enzyme Kinetics:
The maximum enzyme activity of crude pectinase enzyme was found to be at R.T. (28 ± 2^0C) and 37˚C in the pH range of 6-8 as per the source of pectin used. The effect of variation in enzyme concentration on reaction was studied by using different concentrations i.e. 0.1 – 1.0 mg/ml and it was found that rate of enzyme catalyzed reaction was directly proportional to the concentration of enzyme. Similarly by using different substrate concentrations *i.e.* 0.05 -0.35 mg/ml the effect of pectin (substrate) concentration on the activity of pectinase was determined.

SDS-PAGE:
Molecular weight of the crude pectinase samples was determined by SDS-PAGE (Bangalore Genei kit) and it was found to be approximately 29kD when compared with standard molecular weight marker.

CONCLUSION

The use of pectinase in food industries has been prevalent for many decades and a number of microbial sources exist for the efficient production of this enzyme, but only a few selected strains of fungi and bacteria meet the criteria for commercial production. However, with the advances in Biotechnology, the pectinase application has expanded in many fields such as industry, textile industry, waste treatment, fruit juice extraction *etc.* as well as widespread application for the preparation of jam, jelly, some medicinal formulations *etc.* Use of fruit peel wastes as the source for pectin (substrate) leads to cleaning of environment and an eco-friendly way of pectinase application. The use of pectinase produced at lab-scale can be further increased by immobilizing these enzymes that helps holding the enzyme in place throughout the reaction, following which they are easily separated from the products and may be used again - a far more efficient process and so is widely used in industry for enzyme catalyzed reaction.

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