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Production Of Pectinase By Saccharomyces Cerevisiae Using Sugar Beet Pulp

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Abstract:

Industrial waste management is a great matter of concern nowadays. Industrial waste is generated in the solid, liquid and in gaseous forms and discharged in the landfills. Residual organic compounds generated from raw material of many food industries are having adverse environmental impact. Waste material in the form of peels of fruits and vegetables, seeds of some fruits and some pulp are produce by the food processing industries in large amount. These waste material like sugar beet pulp and soybean powder contain Pectins, which can be used as the substrate for production of pectinolytic enzymes by microorganisms using solid state fermentation technique . In the Present study the yeast strains were isolated from active dry yeast powder and these potential yeast strains were screened for pectinolytic activity on selective media. The yeast strain S. cerevisiae was shown to possess polygalacturonase and pectin lyase activity in solid state fermentation.

This yeast strain was used for their ability to produce pectin's on sugar beet pulp and soybean powder medium in the ratio 1:9 and 9:1. The highest producer medium was identified as the medium medium containing soybean powder and sugar beet pulp in the ratio of 9:1.

The highest polygalacturonase and pectin lyase activity was observed in media containing ratio of 9:1 by growing the yeast on Soybean powder and sugar beet pulp and incubation for 10 days at 30^0C. Thus integrated approach towards utilization of waste from fruits and vegetable for pectin's production by SSF. This is step toward reducing waste and pollution.

Keyboard: polygalacturonase, lyase, pectinolytic

Introduction:

Enzymes are the biological catalyst, which have played an important role in many food industries for around hundreds of years. Enzymes play important role in various industries like Manufacturing of textiles, Pharmaceutical industry, Paper and pulp industry, enzymes are used as washing agents and in todays world of sustainable technology and green chemistry enzymes had attain the top most position.

Pectinases are enzymes which act on pectin and degrade it to smaller units. Depending on the pectin degradation they are classified into Pectinlyase, Polygalacturonase and pectines esterase Pectin substance consists of protopectins, pectin acids. The main chain of pectin is partially methyl esterifies 1, 4-D- galacturonan. Demethylated pectin is known as pectin acid or polygalacturonic acid. Pectinase enzyme is of great importance to fruit juice industry and wine industry. As the action of pectinase helps in clarification and reducing the viscosity of the fruit juices which is required factor for the industry (Kashyap et al. 2001). In view of industrial perspectives Pectinases play an important role as they are used in different processes like fermentation mashing treatment of fruits, baby food production treatment of industrial waste water etc (Alimardani-Theuil et al., 2011

Polygalacturonases:

Polygalacturonases(3.2.1.15) are the enzymes which catalyses the hydrolytic cleavage of polygalacturonic acid chain in presence of water. These are called pectinolytic enzymes (Kant et al 2013;Rebello et al 2017) The polygalacturonases are classified into two classes; endo-polygalacturonase (E.C. 3.2.1.15) and exo-polygalacturonase (E.C. 3.2.1.67). Endo-polygalacturonase hydrolyses polygalacturonic acids and liberates oligogalacturonic acids. Exo-polygalacturonase hydrolyzes pectic acids and liberates mono-galacturonate.

Pectin lyases:

Lyases can be classified into four types on the basis of the pattern of action namely Endo-polygalacturonate lyase (Endo PGL; E.C. 4.2.2.2), Exo-polygalacturonate lyase (ExoPGL, E.C. 4.2.2.9), Endo-polymethylgalacturonate lyase (Endo PMGL; E.C. 4.2.2.10) and Exo-polymethylgalacturonate lyase.These enzymes are responsible for non-hydrolytic cleavage of Pectinates and pectates(Jayani et al 2005) The lyases break the glycosidic linkages at fourth carbon and simultaneously eliminate hydrogen from fifth carbon, producing a unsaturated product. Polygalacturonate lyases (Pectate lyases) are produced by many bacteria and some pathogenic fungi with endo- polygalacturonate lyases being more abundant than exo-polygalacturonate lyases. Polygalacturonate lyases have been isolated from bacteria and fungi associated with food spoilage and soft rot.

Solid state fermentation has become a interesting process for production of pectinase using fungi as it has various advantages as it require less water, have high productivity, very less chances of contamination is also cost effective fermentation technology(Pandey et al. 2000; Viniegra-González et al. 2003). Using this technology Pollution problem is also solved in addition to utilization of agrowaste (Pandey 2003).

Several Microorganisms are used as a source of pectinase production. Solid state fermentation and submerged fermentation are the two methods used for the production of pectinase. In the present study Solid state production technique was used. Sugar beet pulp and soybean powder was used as a substrate for production of pectinase. From the past research it is evident that Biorefineries waste and agrowaste serve as novel low cost media for production. This is feasible because food processing industries and agricultural industry contains large amount of organic matter with higher level of nutrient

content and there it can be used in solid state fermentation for production of various enzymes for example pectin methylesterase enzyme .

Materials and Method:

Isolation of Microorganisms

The Yeast was isolated from locally available dry yeast powder by using yeast extract peptone dextrose agar. Dissolve 2gm of dry yeast powder dissolve in 10ml sterile distilled water. One loopful of this suspension was streaked on YEPD agar plate. After the incubation period of 2 days at 30^0C the contrasting colonies were purified by repeated streaking. Pure culture was subculture onto slant media

Collection of substrates

Sugar beet pulp and soybean powder was collected from vegetable vendors, dried under room temperature and then made powder.

Fermentation

The substrates of sugar beet pulp and soybean powder mixture in proportion of 1:9 and 9:1, respectively, was taken for fermentation.

Culture condition

Erlenmeyer Flask of capacity 250 ml was used for carrying out Solid state fermentation. Sterilized substrate 10g inoculated with 10 ml of S.cerevisiae suspension (10 ml of sterile distilled water + one loopful culture). 10 ml of nutrient solution composed of 0.1% NH_4NO_3; 0.1% $NH_4H_2PO_4$; 0.1% $MgSO_4$* $7H_20$, were added to each of the flasks after the inoculation. Aseptic condition were maintained throughout the inoculation procedure.

The inoculated flask were incubated for 10 days at 30^0C. After every 48h interval the solid fermented material; each was collected from both the flask of 1:9 and 9:1 proportion and was mixed with 30ml distilled water. This were stirred for 40 minutes, and filtered. The filtrates were subjected to centrifugation at 5000 rpm for 20 min at 4^0 C. The crude Enzyme was collected in the form of supernatant and was preserved in refrigerator for further use. Total five numbers of samples were collected at the interval of 48 hrs. From each flask.

Measurement of enzyme activity:-

Enzyme activity i.e. polygalacturonase and pectin lyase were measured by using standard citric pectin.

Preparation of citric pectin solution:-

2.5gm of citric pectin was dissolved in 40 gm of boiled distilled water.

Preparation of reaction mixture for polygalacturonase activity:-

Reaction mixture was prepared by using 0.8 ml citric pectin solution, 2 ml acetate buffer (pH 5), and 0.2 ml enzyme solution. The reaction started by adding the enzyme, and it was incubated at 40^0C for 10 min,

after the reaction, add 2ml dinitrosalicylic acid reagent (DNS). The solution was then boiled for 5min and absorbance measured at a wavelength of 520 nm.

Preparation of reaction mixture for pectin lyase activity:-

Reaction mixture was prepared by using 0.8 ml citric pectin solution, 2ml Ts HCL buffer (pH 8.5), 0.2 ml crude enzyme solution and absorbance measured at 235 nm.

Results:

Pectin lyase activity was observed with substrate ratio 9:1 (soybean powder: sugar beet pulp). It indicates that the medium with soybean powder and sugar beet pulp in ratio 9:1 shows the highest production of pectin's enzyme. It is observed that Pectin materials play an important role as an inducer, for production of pectinase in optimum condition

Time course study of fermentation cycle revealed that the enzyme production was obtained after 48h. Enzyme synthesis was associated with yeast growth. Enzyme production in SSF was analyzed during 10 days of course. The maximum **polygalacturonase** activity was detected at the 6th day and ac **pectin lyase ac**tivity at 4th day of incubation.

By using this SSF method, solid waste of sugar beet and soybean cake from food processing industries will be used. Apart from this the cost of production will be reduced due to raw material used. And the process of SSF will also increase the yield of the product. It will be a major step to minimize the pollution loud.

Polygacturonase Activity:

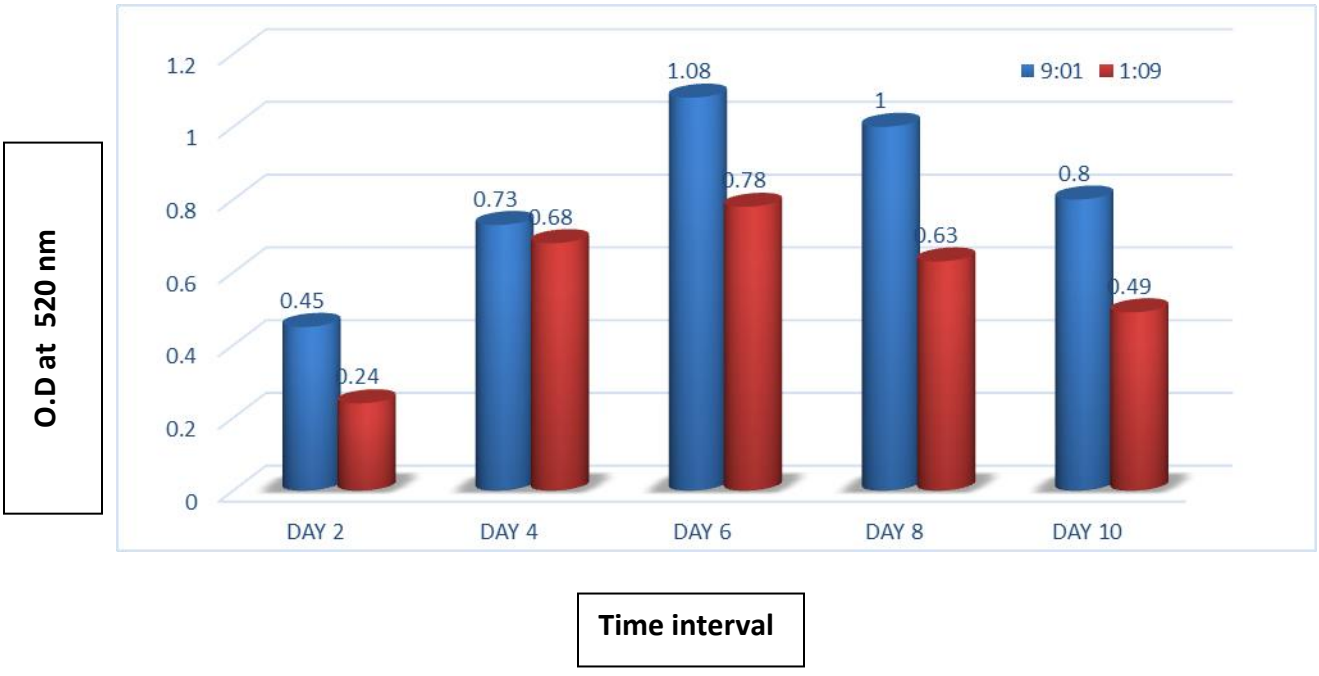

Fig no. 1; Effect of substrate concentration on production of polygalacturonase activity

Pectin Lyase Activity:

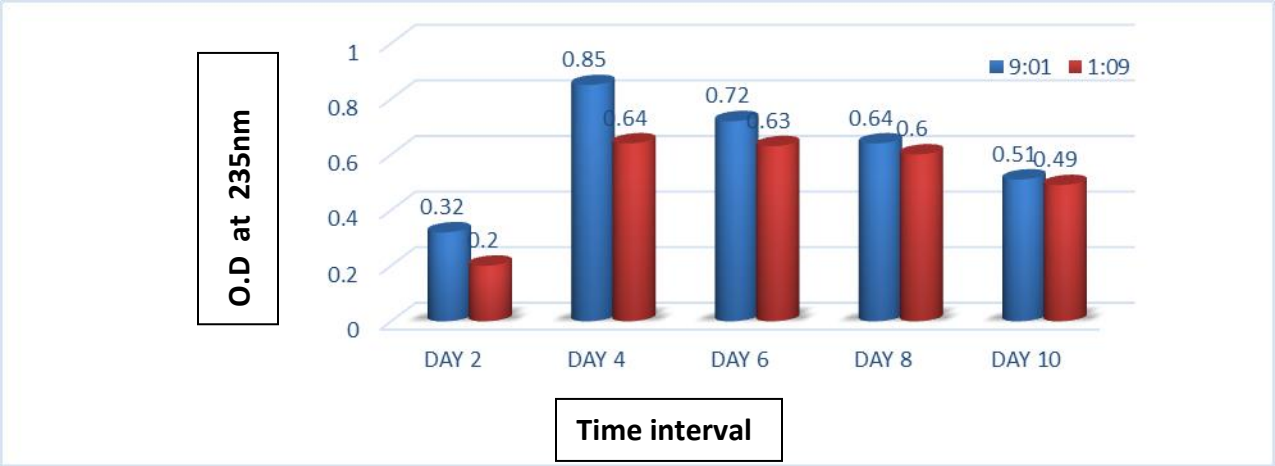

Fig no. 2 Effect of substrate concentration on pectin lyase activity

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