ABSTRACT

*Xanthomonas axonopodis* pv. *citri* is well known as they cause important plant diseases specifically named as citrus canker. The strains of *Xanthomonas axonopodis* pv. *citri* produce various types of pectinolytic enzymes Polygalacturonases (PG), Polymethyl galacturonases (PMG), Pectin lyases (PnL) and Pectate lyases (PgL) as well as extracellular polysaccharides in the form of xanthan gum during infection of plants. Species of viz. *Xanthomonas axonopodis* pv. *citri* and *Xanthomonas campestris* pv. *campestris* synthesizes cell wall degrading enzymes. Pectinases are extensively used in fruit juice processing (extraction and clarification), vegetable oil extraction, processing of alcoholic beverages and a variety of applications in food industries. Xanthan gum is used in food industries, the gum exhibits many advantages as a thickener, stabilizer, gelling agent and suspending agent, as creams, artificial juices, sauces for salads, meat, chicken or fish, as well as for syrups and coverings for ice creams and desserts as it has high degree of stability and solubility. Owing to the potential and wide applications of pectinases and xanthan gum, it was thought proper to study various aspects affecting their production. Considering all these physiological aspects of the *Xanthomonas* species, this study was undertaken with the following objectives:

1. Isolation of *Xanthomonas sps.* from plant and soil.
2. Screening of *Xanthomonas sps.* for production of cell wall degrading enzymes and xanthan gum.
3. Standardization and optimization of process for the production of cell wall degrading enzymes and xanthan gum.
For this study, infected samples of citrus plants were collected from Marathwada region as Nanded, Aurangabad, Parbhani and Latur from 2009 to 2011. Total fifty strains of *Xanthomonas axonopodis* pv. *citri* were isolated from these infected samples. Among all fifty strains screened in this study, four strains were used for optimization study as they were efficient strains and these were as *Xan* 4, *Xan* 13, *Xan* 28 and *Xan* 43. The optimization of pectinolytic enzymes production was studied, the earlier work showed that the production of pectinolytic enzymes was affected by various parameters like incubation time, temperature, pH, carbon sources and nitrogen sources. The ranges of parameters were used in this study as time (24 hrs, 48 hrs, 72 hrs, 96 hrs, 120 hrs and 144 hrs), temperature (25°C, 30°C, 35°C, 40°C and 45°C), pH (6.5, 7, 7.5, 8 and 8.5) and different sources of carbon (glucose, sucrose, starch, maltose and pectin) and for nitrogen (yeast extract, ammonium nitrate, ammonium sulphate, potassium nitrate, peptone).

Incubation time from 24 hrs to 144 hrs was implicated for the optimization of pectinolytic enzyme production synthesized by *Xanthomonas axonopodis* pv. *citri*. Pectinolytic enzyme production by strain *Xan* 4, *Xan* 13, *Xan* 28 and *Xan* 43 was maximum at 48 hrs of incubation and thus incubation time of 48 hrs was reported as the optimum incubation period for production of pectinolytic enzymes. At 48 hrs of incubation time the maximum pectinolytic enzymes isolated by all four strains were ranges from PG (0.6-0.8 U/ml), PMG (0.5-0.7 U/ml), PnL (1-1.5 U/ml), and PgL (0.7-1 U/ml). Maximum activity of pectinolytic enzymes was observed at 30°C and it was recorded that further increase lead to decrease enzyme production. Hence 30°C temperature was chosen as an optimum temperature for all enzymes except PnL, as the maximum production was observed at 35°C, hence optimum temperature for PnL production was 35°C. For all four strains the maximum
production of three different types of pectinolytic enzymes as PG (0.7-0.9 U/ml), PMG (0.5-0.8 U/ml) and PgL (0.8-1 U/ml) was at 30°C whereas of PnL (1-1.5 U/ml) was at 35°C.

The maximum pectinolytic activity of Xan 4, Xan 13, Xan 28 and Xan 43 was recorded at pH 7. Thus the highest production was at pH 7 indicated the optimum pH for pectinolytic enzyme production. Although there was variation among the production of enzymes but the similarity observed in the optimization showed by different types of pectinolytic enzymes. The maximum production of these four types of pectinolytic enzymes isolated by above mentioned strains were as PG (0.7-0.9 U/ml), PMG (0.5-0.8 U/ml), PnL (1-1.5 U/ml), and PgL (0.8-1 U/ml).

It is clear that PG, PMG, PnL and PgL these pectinolytic enzymes production synthesized by strain Xan 4, strain Xan 13, strain Xan 28 and strain Xan 43 was maximum in media contained pectin as a carbon-source. Hence pectin is considered as most suitable carbon-source for production of pectinolytic enzymes. Maximum enzyme production of all four strains was, as PG (0.7-0.9 U/ml), PMG (0.5-0.8 U/ml), PnL (1-1.5 U/ml), and PgL (0.8-1 U/ml) was recorded in the pectin containing medium.

Among yeast extract, ammonium nitrate, ammonium sulphate, potassium nitrate, peptone as nitrogen-sources, the maximum pectinolytic enzyme production was recorded when yeast extract was used in the medium. All these (PG, PMG, PnL and PgL) pectinolytic enzymes synthesized by strain Xan 4, strain Xan 13, strain Xan 28 and strain Xan 43 showed maximum production in the media containing yeast extract as PG (0.6-0.8 U/ml), PMG (0.5-0.7 U/ml), PnL (1-1.5 U/ml), and PgL (0.7-1 U/ml).
For the optimization of production of xanthan gum various parameters were studied such as incubation time (24 hrs, 48 hrs, 72 hrs, 96 hrs, 120 hrs and 144 hrs), temperature (25°C, 30°C, 35°C, 40°C and 45°C), pH (5, 5.5, 6, 6.5, 7, 7.5 and 8), c sources (starch, sucrose, glucose, lactose, and maltose) and nitrogen sources (peptone, yeast extract, ammonium sulphate, ammonium nitrate, potassium nitrate). The study of all these parameters during optimization was thought necessary for the xanthan gum production as these factors affected the production. Although, slight variation was observed among all strains of *Xanthomonas axopodis pv. citri* for xanthan gum production, four different strains were found to be more effective of total fifty strains isolated and screened.

In the study of optimization of incubation time for xanthan gum production it was found that the 72 hrs of incubation time was optimum time for production of xanthan gum. From the result, it was cleared that the maximum production of xanthan gum as 15.18 g/L, 14.97 g/L, 15.21 g/L and 14.51 g/L was observed by strain Xan 7, Xan 18, Xan 22 and Xan 36 respectively.

Temperature plays an important role in xanthan gum production. It was observed that the production of xanthan gum increased from 25°C up to 35°C and then further increase in temperature decrease the xanthan gum production. Thus, 35°C temperature was the optimum temperature for the production of xanthan gum. All four strains showed maximum production at 35°C. The strain Xan 7, Xan 18, Xan 22 and Xan 36 showed the maximum xanthan gum production as 15.12 g/L, 14.89 g/L, 15.19 g/L and 15.01 g/L respectively.

All four strains namely Xan 7, Xan 18, Xan 22 and Xan 36 used in this study of optimization of xanthan gum production showed maximum production of xanthan gum at pH 6. Lower or higher pH than pH 6 gave declined production. Strain Xan 7 showed maximum
production of 15.20 g/L whereas strain Xan 18 showed 14.81 g/L of xanthan gum. Xanthan gum production by the strain Xan 22 was found to be 15.22 g/L and 15.17 g/L was recorded by strain Xan 36.

The media containing sucrose as carbon source showed maximum production of xanthan gum. Four strains were used for the study of effect of carbon source on the xanthan gum production Xan 7, Xan 18, Xan 22 and Xan 36. Strain Xan 7 showed highest production as 15.23 g/L with sucrose whereas the maximum production of xanthan gum was 14.88 g/L by strain Xan 18. The maximum production of xanthan gum was 15.11 g/L and 14.16 g/L by strain Xan 22 and Xan 36 respectively.

The effect of nitrogen sources on the production of xanthan gum was studied. From the obtained results it is clear that yeast extract was the most suitable nitrogen source for the production of xanthan gum. The strains used in this study – Xan 7, Xan 18, Xan 22 and Xan 36, showed maximum production in the media containing yeast extract as a nitrogen source. Strain Xan 7, Xan 18, Xan 22 and Xan 36 showed maximum production of xanthan gum as 15.19 g/L, 14.92 g/L, 15.17 g/L and 14.82 g/L respectively.

The functional group of synthesized product were compared with the commercial xanthan by FT-IR spectra and NMR spectra. Thus from results of the spectra obtained from FT-IR and $^{13}$C NMR, it was concluded that the xanthan gum synthesized in this study was same with the spectra of FT-IR and $^{13}$C NMR with spectra obtained by commercially produced xanthan gum.

The overall study indicates that the production of pectinolytic enzymes and xanthan gum is affected by various parameters like incubation time, temperature, pH, carbon sources and nitrogen sources is important from their application point of view. The incubation time of 48 hrs at 30°C (for PnL -35°C) temperature with pH 7 were found to be optimum
environmental conditions for production of pectinolytic enzymes. Pectin was found to be best carbon source while yeast extract as a best nitrogen source for maximum production of pectinolytic enzymes. Xanthan gum production was maximum at temperature 35°C and pH 6.5 after 96 hrs of incubation was optimum and nutritional conditions such as pectin as a carbon source and yeast extract as nitrogen source were suitable for maximum production.