Optimization of protease and lipase production by Bacillus pumilus SG 2 isolated from an industrial effluent

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Citation

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Abstract

This investigation was aimed at isolating a potent bacterial strain which can produce both protease and lipase. Effluents of meat processing industry, dairy industry, food processing industry and oil industry were collected. Among the four positive strains screened, a bacterial strain identified as Bacillus pumilus SG2 isolated from food processing industry effluent was found to be a comparatively potent producer of both protease and lipase (52U/ml and 38U/ml respectively). The process parameters for the optimal production of both enzymes using this strain were studied. The optimum pH for protease production was 8.0 and that of lipase was 9.0. The optimum temperature was 37ºC for production of both enzymes. The optimum incubation time was found to be 36 hours for protease and 63 hours for lipase. The best carbon source for enzyme production was glucose and the best nitrogen sources were yeast extract and casein. Addition of additives like SDS, Triton X-100 and Tween-20 influenced enzyme production. Whey was the best crude substrate that could be used for protease production and castor oil, gingelly oil and olive oil were the best substrates for lipase production. This study has indicated the possibility of producing both protease and lipase simultaneously on a common production medium by a single bacterial strain, Bacillus pumilus.

INTRODUCTION

Enzymes have a great industrial potential and are widely found in various sources like plants, animals and microbes. Microbes have undermined plants and animals as sources of enzymes due to their broad biochemical diversity, ease of mass culture and also due to the ease with which they can be genetically modified. Of all the industrially important enzymes, proteases and lipases are exploited maximally due to their various applications. Proteases are hydrolytic enzymes and find their utmost application in laundry detergents (Banerjee et al., 1999). They also find applications in food industry for cheese making and baking, in pharmaceutical industry in combination with antibiotics to treat ulcers and wounds (Mala Rao et al., 1998). Proteases are used effectively for dehairing in leather processing industry (Nilegoankar et al., 2006). Lipases too have considerable industrial potential and find promising applications as additives in detergent (Gerritse et al., 1998) and food additives for flavour enhancement in cheese ripening, baking etc (Kauzlauskas and Bornscheuer, 1998). Lipases are used to synthesise chiral building blocks for pharmaceuticals (Patel, 2000) and as component of personal care products (Maugard et al., 2002).

Protease and lipase have many common applications and are best used in a mixture in various industries like tanneries and also in detergents. Hence organisms which can produce both these enzymes simultaneously can be best exploited. There are very few reports on the concomitant production of lipase and protease by Pseudomonas species but there are no such reports with Bacillus species. This present report has described the isolation of a Bacillus species which produces both protease and lipase and optimization of the production parameters for both the enzymes.

MATERIALS AND METHODS

ISOLATION AND SCREENING

Effluents from various industries like oil industry, food processing industry, dairy industry and meat processing industry were collected, serially diluted and plated on sterile nutrient agar (0.5% peptone, 0.3% yeast extract. 0.5% sodium chloride and 2% agar) plates. The isolated pure colonies were then screened for extracellular protease production using casein agar (0.5% casein in nutrient agar) and nutrient gelatin (0.5% gelatin in nutrient agar) and lipase production was screened using tween agar (0.5% tween in nutrient agar). The strains which produced both the enzymes were then analysed for their enzyme production potential. The most potent strain was considered for future study.

ENZYME PRODUCTION

The production medium for protease consisted of (w/v) 0.04% $CaCl_2$, 0.02% $MgCl_2$, 1% glucose, 0.5% peptone, 0.5% NaCl, 0.3% yeast extract (pH 7.0). The production medium for lipase comprised of (w/v) 0.01% $MgSO_4$, 0.1% KH_2PO_4, 1.0% tween 20, 0.2% glucose, 0.5% peptone (pH 7.0). Fermentation was carried out with 100ml medium inoculated with 5% overnight culture and incubated on a rotary shaker (180rpm) for desired period.

ENZYME ASSAY

The enzyme assays were performed with the cell free supernatant of the fermented broth as the crude enzyme source.

ASSAY OF PROTEASE

Caseinolytic activity was measured by the photometric method of Rahman et al., (2005). One unit (U) of protease activity is equivalent to 0.5µg of tyrosine liberated by 1.0ml of enzyme solution under the assay conditions. The amount of tyrosine was determined from the tyrosine standard curve.

ASSAY OF LIPASE

Lipase activity was assayed by the photometric method of Benjamin and Pandey (1995). One unit (U) of lipase activity is equal to 1micromole of free fatty acid liberated/min/ml under the assay conditions. The amount of free fatty acid was determined from the oleic acid standard curve.

PARAMETER OPTIMIZATION STUDIES

Various parameters were studied in order to achieve maximum enzyme production.

1. Effect of incubation temperature: the production medium with the inoculum was incubated at different temperatures ranging from 28 to 52°C with 5°C interval and enzyme activities were determined.

2. Effect of pH: pH of the production medium was adjusted between 4 and 9 to study the effect of pH on enzyme production.

3. Effect of incubation time: the production medium with the inoculum was incubated and the enzyme activities were analysed at regular time intervals

4. Effect of various carbon sources: the effect of various sugars at a concentration of 0.1% w/v fructose, maltose, galactose, lactose, sucrose and xylose on enzyme production was studied.

5. Effect of various nitrogen sources: the effect of organic nitrogen sources (0.5% w/v) – beef extract, gelatin, casein and inorganic sources (0.1%) like urea, potassium nitrate, ammonium nitrate was studied

6. Effect of additives: additives like Sodium dodecyl sulphate (SDS), Triton X-100, Tween-20 at a concentration of 0.1% w/v were used to study their effect on enzyme production.

7. Production of enzymes from various crude substrates: efficiency of substrates like (2.0%) corn, whey, soybean, cotton seed for protease production and castor oil, gingelly oil, olive oil, coconut oil, groundnut oil for lipase production were studied.

RESULTS

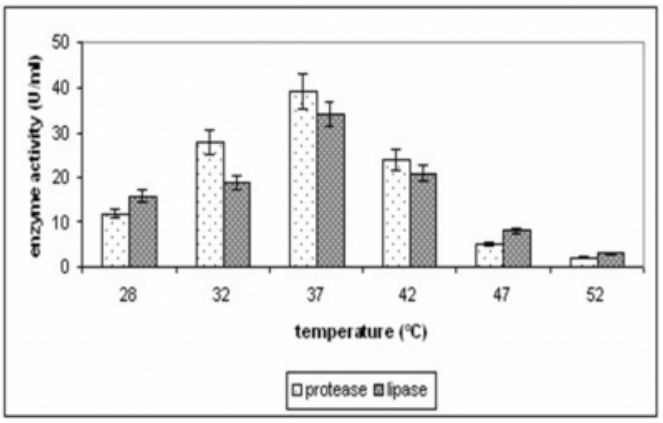
Sixteen bacterial isolates were obtained from the different industrial effluents, of which four were found to be promising producers of both protease and lipase and were coded as SG1, SG2, SG3 and SG4. Of these four isolates, SG2 isolated from food processing industrial effluent exhibited a large zone of hydrolysis which was later confirmed by enzyme assay (protease activity 52U/ml, lipase activity 38U/ml) and was selected for further studies. Based on Bergey's manual of systematic microbiology, SG2 was identified as Bacillus pumilus (Bergey et al., 1984)

EFFECT OF INCUBATION TEMPERATURE ON ENZYME PRODUCTION:

Temperature is one of the critical parameter that has to be optimized. The optimum growth temperature was found to be 37ºC for both protease and lipase production. But considerable enzyme production was observed between 32-42 ºC (Fig.1).

Figure 1

Figure 1: Effect of temperature on enzyme production



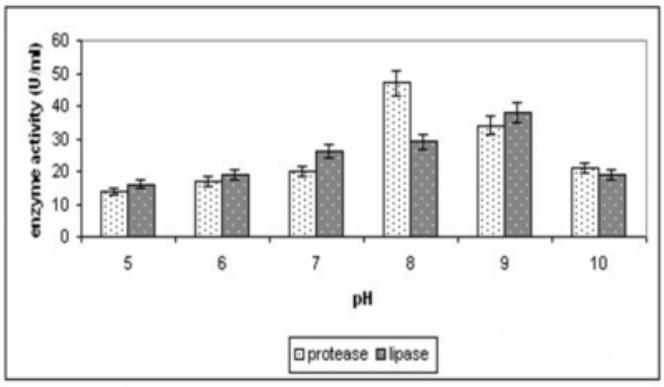
Enzyme activity is the mean of four independent experiments.

EFFECT OF PH ON ENZYME PRODUCTION:

The initial pH of the fermentation medium needs to be controlled. The enzyme production was maximum when the pH was 8.0 for protease and 9.0 for lipase. The production decreased significantly above and below these values (Fig 2).

Figure 2

Figure 2: Effect of pH on enzyme production



Enzyme production carried out at 37°C.

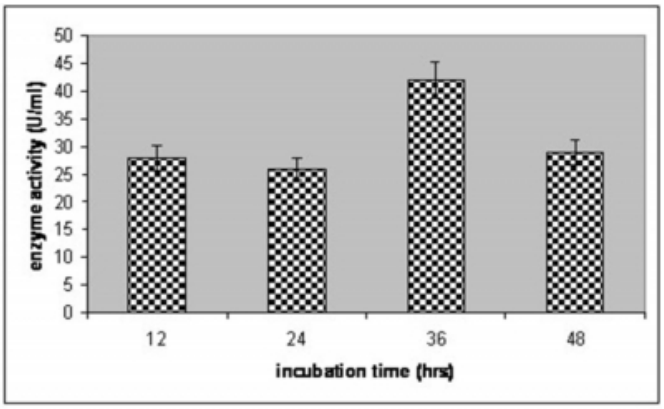
Enzyme activity is the mean of four independent experiments.

EFFECT OF INCUBATION TIME FOR ENZYME PRODUCTION:

Protease and lipase activities were determined at different incubation periods. Maximum enzyme activity for protease and lipase were obtained at different incubation periods. Maximum protease activity was obtained at 36hrs of incubation (Fig 3) while maximum lipase activity was obtained at 63hours of incubation (Fig 4). Any prolongation in incubation period decreased enzyme production.

Figure 3

Figure 3: Protease production during log phase

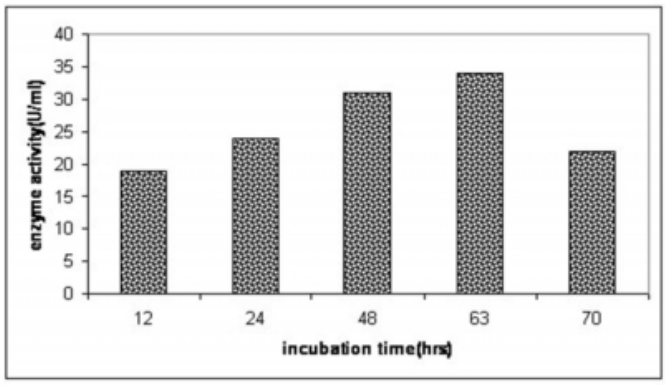


Enzyme production at 37°C, pH 8.0

Enzyme activity is the mean of four independent experiments.

Figure 4

Figure 4: Lipase production during log phase



Enzyme production at 37°C, pH 9.0

Enzyme activity is the mean of four independent experiments.

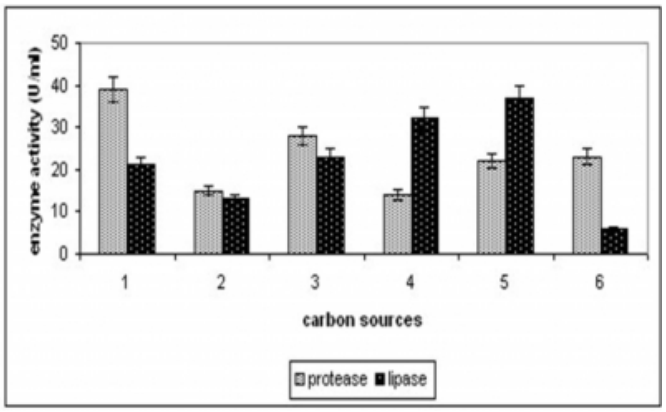
EFFECT OF CARBON SOURCES ON ENZYME PRODUCTION:

The basal production medium for protease and lipase had glucose as carbon source. When glucose was replaced by various sugars, fructose was found to be an effective carbon source for protease production and sucrose was the effective source for lipase production. When glucose in the basal medium was replaced with fructose, the protease yield was

decreased by 20% while sucrose was effective as glucose for lipase production. (Fig 5).

Figure 5

Figure 5: Effect of different carbon sources on the production of lipase and protease



1 – fructose 2- galactose 3- lactose 4- maltose 5- sucrose 6- xylose

Glucose in the basal production medium is replaced by these sugars

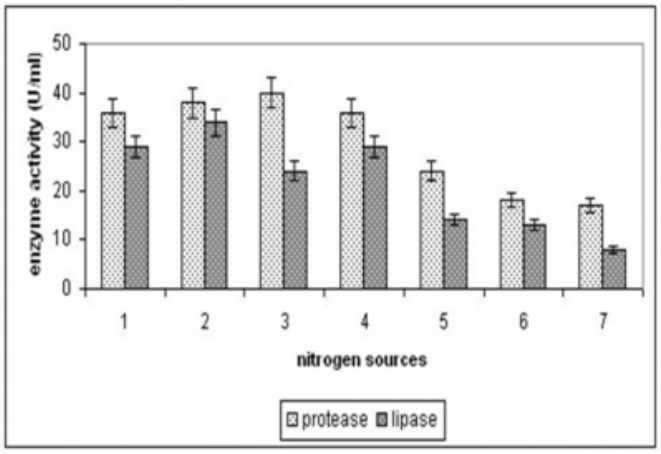
Enzyme activity is the mean of four independent experiments.

EFFECT OF NITROGEN SOURCES ON ENZYME PRODUCTION:

Peptone was the nitrogen source in the production medium for the enzymes. Peptone was replaced by various organic and inorganic nitrogen sources. Among the organic nitrogen sources tested, casein and gelatin exhibited a prominent effect on the yield of protease, while casein and yeast extract gave better yields of lipase. Beef extract and yeast extract produced a small decrease in enzyme yield, approximately 10%. Though inorganic nitrogen sources were not as effective as organic sources, urea was found to be better among them for the production of both the enzymes (Fig 6).

Figure 6

Figure 6: Effect of nitrogen sources on the production of protease and lipase



1-yeast extract 2- casein 3- gelatin 4- beef extract 5- urea 6- potassium nitrate

7- ammonium nitrate

The basal production medium has peptone as the nitrogen source

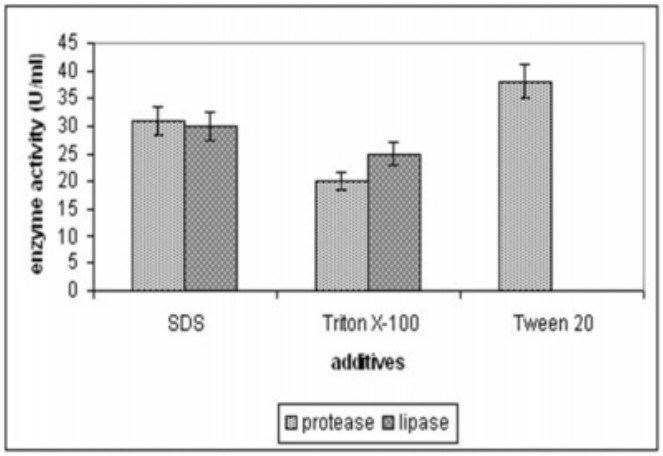
Enzyme activity is the mean of four independent experiments.

EFFECT OF ADDITIVES ON ENZYME PRODUCTION

The effect of additives like SDS, Triton X-100, Tween 20 on enzyme production was investigated. SDS did not have a pronounced effect on the production of protease and lipase. Addition of Triton X-100 to the production medium decreased the production of protease by 40% and lipase by 30%. Addition of Tween-20 to the production medium of protease decreased the enzyme production by 5% (Fig 7).

Figure 7

Figure 7: Effect of additives on the production of protease and lipase



Tween-20 is present in the lipase production medium as an inducer

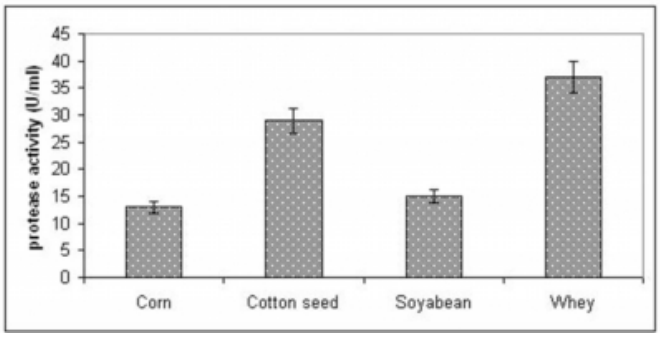
Enzyme activity is the mean of four independent experiments.

EFFICIENCY OF CRUDE SUBSTRATES FOR ENZYME PRODUCTION:

Among the various crude substrates used for enzyme production, whey was found to be the best substrate for protease production followed by cotton seed (Fig 8). Efficiency of different oils as substrate for lipase was also investigated. Coconut oil, olive oil and castor oil have shown best enzyme production among all those oils used (Fig 9).

Figure 8

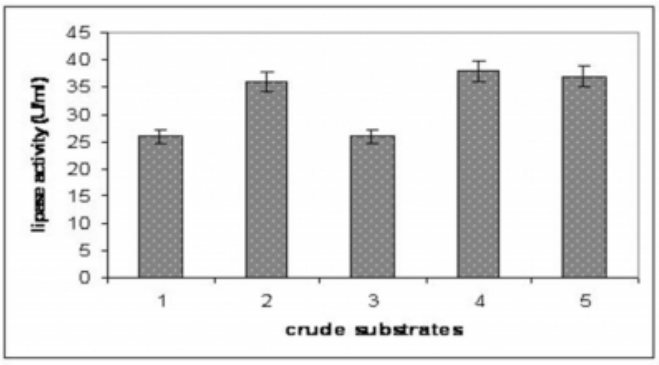
Figure 8: Protease production with crude substrates



Enzyme activity is the mean of four independent experiments

Figure 9

Figure 9: Lipase production with crude substrates



1-Gingelly oil, 2- Coconut oil, 3- Groundnut oil, 4- Olive oil, 5- Castor oil

Enzyme activity is the mean of four independent experiments

DISCUSSION

Among the four strains (SG_1, SG_2, SG_3 and SG_4) which produced both protease and lipase, Bacillus pumilus SG2 isolated from food industry effluent was identified as the potent producer of both the enzymes. This difference could be attributed to the genetic makeup of the organisms and the sources of isolation, where SG_1 was from oil industry effluent, SG_3 was from dairy industry effluent and SG_4 was from meat processing industry.

Investigations on the effect of temperature on enzyme production revealed that 37ºC was optimum for both protease and lipase production and hence SG_2 was mesophilic. Supportively, Genckal and Tari, 2006 also have reported 37 ºC as the optimum temperature for protease production by two Bacillus species. The optimum temperature for lipase production by Bacillus mycoides sp was reported to be 28 ºC by Thomas et al., 2003. The isolate used in our study showed increase in production of protease with increase in pH of the fermentation media. Protease production was maximum when the initial pH of the production medium was 8.0. Production of lipase was maximum at pH 9.0. Further increase in pH reduced enzyme production significantly. Patel et al., 2005 has reported a pH of 9.0 to be optimum for protease production by Bacillus sp. An earlier report has optimized a pH 8.5 for lipase production by Bacillus coagulans. (Satyendra Kumar, 2005).

Protease was produced almost throughout the course of fermentation and maximum production was observed at 36

hours, i.e., during the mid log phase of our isolate. Mehrotra et al., 1998 has observed maximum production of protease during the late exponential phase of growth of Bacillus sp. Lipase was also produced throughout the course of fermentation with maximum production at 63hours. This corresponded to the late log phase of bacterial growth. Thus the organism had consumed the protein primarily and after the exhaustion of the protein, the lipid was used. Lipase production by a Bacillus sp was detected from 4 hrs to 30 hrs and reached a maximum (6.9 U/ml) after 20 hrs in a study by Limpon Bora and Kalita, 2007.

The effect of replacement of glucose in the basal medium by various sugars was studied. Fructose proved to be the best source for protease production and sucrose the best carbon source for the production of lipase. Lactose reduced the production of both protease and lipase by 40% when compared to control. Maltose also gave considerable yield of lipase. Protease production was optimal when 0.5% glucose was used and 2% glucose was found to repress enzyme production (Mehta et al., 2005). Carbon sources were found to have no effect on lipase production by a Bacillus sp strain 42 as reported by Eltaweel et al, 2005.

Organic nitrogen sources were used efficiently by Pseudomonas sp for production of both protease and lipase. Production media with casein, gelatin and beef extract yielded better production of protease than the control media which had yeast extract as nitrogen source whereas casein was the best nitrogen source for lipase production. Inorganic nitrogen sources were not as efficient as organic nitrogen sources. Abdel-Naby et al., 1997 have identified a mixed nitrogen source containing peptone and ammonium sulphate as the best nitrogen source for protease production. High specific activity of lipase was reported with beef extract for production by a Bacillus sp LBN4 (Limpon Bora., Kalita 2007).

Additives like SDS and Triton X-100 did not completely inhibit enzyme production. Protease production did not exhibit any significant difference with SDS when compared to control but Triton X-100 reduced it by 40%and Tween20 reduced it by 5%. Also, lipase production did not suffer any major reduction with Triton X-100 and SDS. A supportive result was reported by Mabrouk et al., 1999, who found that addition of SLS to the medium produced the same amount of protease. Incorporation of surfactants like Triton X-100 during solid state fermentation by Aspergillus niger increased lipase yield three fold (Mahadik et al., 2002).

Among the crude substrates tested for enzyme production, whey was found to give excellent yields of protease, followed by cotton seed. A study by Uyar and Baysal, (2004) showed wheat bran as a better source for protease production by Bacillus sp. Mehta et al., 2006 reported molasses as the best substrate for protease production by Actinomycete. Castor oil, coconut oil and olive oil were found to be the best crude substrates for lipase production and gave yields similar to that of basal medium. Thomas et al, 2003 have reported olive oil as the carbon source for lipase production by Bacillus mycoides.

CONCLUSION

Bacillus pumilus SG2 isolated from food industry effluent was identified as the potent and promising producer of both protease and lipase. The process parameters for the production of both enzymes were optimized. Thus a promising producer of both protease and lipase was isolated from industrial effluent and study for commercialization of these can be considered in future.

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