RESEARCH ARTICLE

Characterization of Proteases Production by Varying Nitrogen Sources from *Bacillus subtilis* Isolated from Agriculture Soil of Lalitpur Dist. UP

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ABSTRACT- Proteases is among the largest groups of industrial enzymes that also has the potential to contribute in the development of high value added products due to their characteristic nature that aids in digestion. Protease account for about 60% of the total worldwide sale of enzymes and is widely used in several industries ranging from silk industry, leather tanning, meat processing, organic fertilizers, diary and bioleaching. The bacteria produce a variety of proteolytic enzymes. Among them a major contributor of proteases producers is *Bacillus subtilis*. An attempt was made to formulate media using varied nitrogen sources to optimize media for maximum production of proteases. It was observed that media supplemented with soya meal as a nitrogen source had maximum biomass yield of 135 mg/ml while Tryptone supplemented media yielded 115.6 mg/ml and peptone supplemented media yielded only 101 mg/ml which was comparatively less than soya meal while the other nitrogen sources supplemented media were found to be poor in comparison to that supplemented by soya meal extract.

Key-words- Proteases, *Bacillus subtilis*, Optimize media, Soya meal extract

INTRODUCTION

Bacillus subtilis is a gram negative rod shaped bacteria found commonly in soil. *B. subtillus* is endospore forming bacteria and thus resistant to extreme physical conditions. It is an extremely valuable microbe as it produces a variety of proteolytic enzyme that is sable at varied physical conditions and is in high demand for commercial use. [1-3]

Proteases due to their huge application spectrum of various biotechnological processes have been the focus of intense research for many decades. Proteolytic enzymes are essential in various industrial sectors.[4,5] Proteases are quite interesting as they can act on insoluble keratin substrates and on a variety of proteinacious substrates. [6] Although there are many microbial sources available for producing proteases, only a few are recognized as a commercial producers as they lack desired properties. [7] Proteases are one of the most important group of industrial enzymes and account for nearly 60% of the total enzyme sale The major uses of free proteases occur in leather industry [8], dry cleaning, detergents, meat processing, cheese making [9], silver recovery from photographic film, production of digestive and certain medical treatments of inflammation and virulent wounds. Due to the various demand and challenges faced search for better and efficient proteases, is a continuous practice. High cost of enzyme production is another major challenge for wide range of industrial applications of proteases and about 30–40% of the production cost of industrial enzymes accounts for the cost of substrate/growth medium. [10] Exploring various cheap nitrogen sources for bulk production of industrial enzymes may play a pivotal role in reducing costs of proteases production through media optimization for culturing *B. subtilis*.

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MATERIALS AND METHODS

Screening and Isolation of proteases producing *B. subtilis*

Soil samples were collected in December, 2015 from the agricultural fields of Lalitpur district of Uttar Pradesh,

India. Pure culture was obtained using serial dilution method on NAM plates.[11] Six strains of *B. subtilis* were identified namely BS-1, BS-2, BS-3, BS-4, BS-5 and BS-6 were isolated and identified based on cellular morphology, growth condition, and biochemical analysis. [12]

For protease screening, casein agar medium (g/l) (peptic digest of animal tissue, 5.0; beef extract, 1.5; yeast extract, 1.5; sodium chloride, 5.0, agar, 15, casein, 10 and 0. 0015% (w/v) BCG-Bromocresol Green Dye) was prepared and streaked with bacterial isolate (*B. subtilis*) and incubated at 37°C for 48 hrs.[13] A zone of proteolysis was detected only on the casein agar plates of BS-5. Further analysis was conducted on BS-5 strain alone.

Optimization of media for maximum production of protease using different Nitrogen sources

The broth containing *B. subtilis* was used for optimized production of protease enzyme consisted of varying nitrogen sources (urea, peptone, soya meal extract, ammonium sulphate, casein, KNO_3, tryptone) 0.5 %, Glucose, 1% (w/v), yeast extract 0.55, KH_2PO_4 0.2%, Na_2CO_3 1%, $MgSO_4.7H_2O$, 0.2%, and pH 8.0 at 140 rpm. [14,15] Optical density (OD) was taken using UV-Visible spectroscopy at 660 nm at different time intervals and a graph was plotted (Fig. 1).

Calculation of dry weight of *B. subtilis* (BS-5)

Dry weight was calculated for each broth (having different Nitrogen sources) containing the cultures after 96 hrs.[3] One ml of cultures from each broth was transferred to centrifuge tubes of 1.5ml followed by centrifugation at 10,000 rpm for 15 minutes. The supernatant was discarded and the tubes containing the pellet were kept in air drying for overnight, then the weight of the cells was measured (Table 1).

RESULTS

The effect of different nitrogen sources (0.5 g/l) on protease production by *B. subtilis* has been depicted in Fig. 1. On taking Optical density (OD) using UV-visible spectroscopy at 660 nm it was observed that the growth rate of *B. subtilis* decreased with time in case of urease (OD 0.261), casein (OD 0.330) and ammonia (OD 0.233) as nitrogen source after 72 hours with urease (OD 0.221), casein (OD 0.262) and ammonia (OD 0.233) after 96 hrs of incubation. While in case of Soya meal (OD 0.576), peptone (OD 0.555), tryptone (OD 0.383) and KNO_3 (OD 0.231) the growth rate has still increased from 72 hrs to Soya meal (OD 0.592), peptone (OD 0.562), tryptone (OD 0.492) and KNO_3 (OD 0.231) after 96 hours of incubation (Table 1).

Depicts the dry cell weight of *B. subtilis* after 96 hrs of incubation in the media described above in material and method having various nitrogen supplements. It is observed that maximum dry weight is of soyameal extract and is organic in origin. It was also observed that the organic derivatives of nitrogen sources are more readily used by *B. subtilis* as compared to inorganic source of nitrogen.

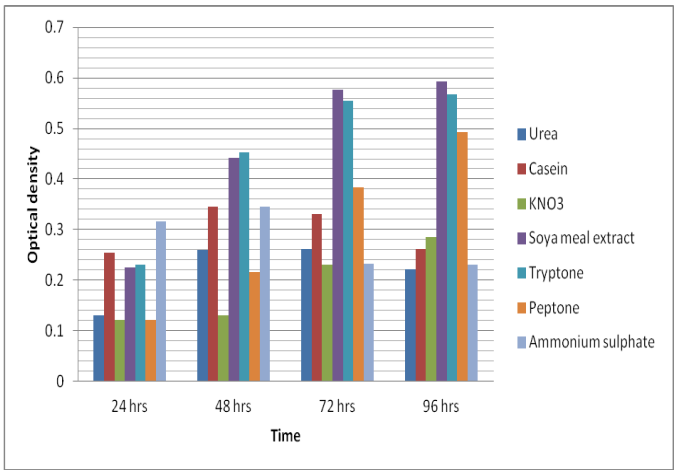

Fig. 1: Depicting optical density of BS-5 using different Nitrogen sources in broth

Table 1: Dry weight of *Bacillus subtilis* (BS-5)

Broth inoculated with BS-5 containing different Nitrogen sources	Dry Weight after 48 hrs of incubation 96˚C mg/ml
Urea	42
Casein	65
KNO_3	67
Soya meal extract	135
Tryptone	115.6
Peptone	101
Ammonium sulphate	50

DISCUSSION

Of the six strains of *B. subtilis*, namely BS-1, BS-2, BS-3, BS-4, BS-5 and BS-6 were isolated and identified BS-5 was selected on the basis of its proteolytic activity. *B. subtilis* is known to utilize crude substrates for protease production observed by Murab *et al.* [12]. The type and availability of nitrogenous precursor in the medium play a crucial role in metabolism of extracellular enzyme as suggested by Kumar *et al.* [5] and Reddy *et al.* [17]. Although complex nitrogen sources were usually needed for protease production, the requirement for a specific organic nitrogen supplement differs from organism to organism as observed by Lakshmi *et al.* [18]. The present study was undertaken with the objective to find out the effect of the different nitrogen supplement for this various organic and inorganic nitrogen sources were investigated (urea, casein, $KMNO_3$, soya meal extract, tryptone, peptone and ammonium sulphate) for growth of *B. subtilis* (BS-5). Broth supplemented with various nitrogen sources were inoculated with *B. subtilis* (BS-5) and incubated it was observed that soya meal extract yielded a significant increase in the growth rate as seen in (Fig. 1) followed by tryptone and peptone while other three supplements were found to be poor. On further analysis of the dry weight it was confirmed that

soya meal extract is the best supplement for *B. subtilis* BS-5 followed by supplements like tryptone and peptone. Urea, casein, $KMNO_3$ and ammonium sulphate was found to be least yielding (Table 1).

CONCLUSIONS

The bacterial strain of *B. subtilis* BS-5 in this study was previously isolated from soil samples. It was determined that soya meal extract is a nitrogen supplement most productive for culturing *B. subtilis* for large scale production in industries for extraction of proteases. Soya meal is the major by-product is produced by soya bean industry, while producing tofu, soya sauce and soya nuggets and thus a cheap source of nitrogen supplement for media formulation for *B. subtilis*. Nitrogen source is one of the components i.e. needed to be optimized for further formulation of low cost media for growth of *B. subtilis* without compromising the production capabilities of proteases. Further, investigations are required to optimize media keeping in mind the variability of strain can change the outcome of the experiment.

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