

## EFFECTS OF CARBON AND NITROGEN SOURCES ON PRODUCTION OF PROTEASES BY *BACILLUS SUBTILIS* IC-5

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### Abstract

The effects of various carbon and nitrogen sources were evaluated on production of proteases by *Bacillus subtilis* IC-5. Both type and concentration of carbon and nitrogen sources influenced the production of proteases. Among the carbon sources glucose was found to be the most effective. It gave maximum production at 2% w/v concentration i.e., 1875 and 950 U/ml, alkaline and neutral protease, respectively. The response of *Bacillus subtilis* IC-5 towards synthesis and excretion of enzymes varied with the type of nitrogen sources. The addition of organic nitrogen sources to basal medium repressed the synthesis of proteases while the addition of inorganic nitrogen source such as sodium nitrate was found to be the best stimulating for alkaline and neutral protease synthesis. Sodium nitrate enhanced the production up to 62.40 and 10.52% of alkaline and neutral protease, respectively against w.r.t. control.

### Introduction

Industrial enzymes are obtained from three major sources i.e., plant, animal and microorganisms. The extraction of enzymes from plant or animal source is limited and seasonal in case of plant source. However, production of enzymes by fermentation process is unlimited and can be produced round the year. Proteolytic enzymes are ubiquitous in occurrence, being one of the largest and most important groups of enzymes which accounts for about 30% of total worldwide production (Horikoshi 1996) and about 60% of sale (Beg *et al.* 2003). The extracellular proteases have commercial value and find multiple applications in various industrial sectors. The detergent industry has emerged as one of the major consumers of hydrolytic enzymes working at higher pH (Adinarayana *et al.* 2003). It is used as cleansing additives in detergents to facilitate the release of proteinacious materials in stains due to grime, blood, milk, etc (Olajuyigbe and Ajele 2005). Proteolytic enzymes applied in other industries are food, pharmaceutical, leather, silk and recovery of silver from used X-ray films (Prakasham *et al.* 2002). Proteases are produced by a wide range of microbes i.e., bacteria, mould and yeast (Gupta *et al.* 2002). Among bacterial strains of *Bacillus* sp. dominate the industrial quarters.

The extracellular protease production by microorganisms is greatly influenced by media components especially carbon and nitrogen sources being the main energy and growth promoting factors (Kole *et al.* 1988). The selection of cost effective nutrients (carbon and nitrogen) is of utmost desire for optimum production of enzymes (Varela *et al.* 1996). In present study, the effects of carbon and nitrogen sources and their concentrations on production of proteolytic enzymes by *Bacillus subtilis* were investigated.

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## Materials and Methods

The bacterial strain *Bacillus subtilis* IC-5, inoculum preparation and fermentation procedure was adopted as by Gul *et al.* (2008).

The effects of carbon sources arabinose, fructose, maltose, mannose, starch, sucrose and xylose on protease production were investigated. The basal medium devoid of carbon source served as control. The effects of different concentrations (10, 20 and 30 g/l) of the carbon sources in the medium were also investigated.

The effects of nitrogen sources on protease production were studied by adding in basal medium at the concentration of 10 g/l (W/V), individually. Effects of concentrations studied up to 30 g/l of these nitrogen sources were also studied. Organic nitrogen sources were casein, casein hydrolysate, meat extract, peptone, tryptone, and yeast extract, Whereas, Inorganic nitrogen sources were potassium nitrate, sodium nitrate and calcium nitrate and ammonical nitrogen sources were ammonium carbonate, ammonium chloride, ammonium molybdate, ammonium oxalate, ammonium sulphate and ammonium phosphate.

The activity of proteolytic enzymes was assayed by the method of Horikoshi (1971). One unit of proteolytic activity was defined as the amount of enzyme required to liberate TCA soluble material equivalent to 1.0 µg of tyrosine from casein per min at pH 7.0 for neutral protease and at pH 11.5 for alkaline protease at 37°C.

## Results and Discussion

Results presented in Fig. 1 showed that glucose was the best carbon source for *Bacillus subtilis* IC-5 for producing proteolytic enzymes. In presence of glucose this *Bacillus subtilis* IC-5 produced 1875 U/ml alkaline and 950 U/ml neutral protease. Xylose showed the lowest production. Our results are in conformity with previous studies that protease production reached to maximum when glucose is added to the medium (Andrade *et al.* 2002) and it induces high protease activity in stationary phase (Deane *et al.* 1986). The presence of carbon sources in the

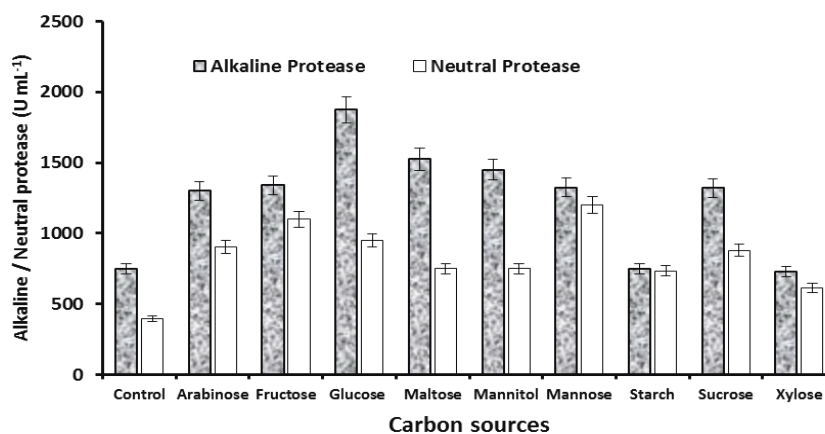


Fig.1. Effects of various carbon sources on production of proteases by *Bacillus subtilis* IS-5.

medium is reported to be essential for enzyme production in some microorganisms (Homma *et al.* 1993) but inhibitory to others (Joo *et al.* 2002). The results depicted in Fig. 2 showed that the carbon sources favor the production of enzymes up to certain concentration only. The response of the increase in concentration of all carbon sources in fermentation medium towards production of

alkaline and neutral proteases was more or less same. Maximum increase in alkaline protease (1875 U/ml) and neutral protease (950 U/ml) were observed at 2% of glucose. In presence of xylose the increasing production trend of both the enzymes was observed only up to 1% concentration. The present study also revealed that the production of proteolytic enzymes by *Bacillus subtilis* IC-5 not only depends upon the kind of carbon source but also the concentration of carbon source in the medium. Catabolite repression in response to carbon source availability (Magasanik 1961) is one of the regulatory mechanisms which govern the synthesis of extracellular enzymes. The catabolite repression of enzymes has been reported by Heineken and O'Conner (1972) in *Bacillus subtilis*. The proteolytic enzymes synthesis is controlled by availability of carbon sources in the fermentation medium (Lambert *et al.* 1997). The regulation of extracellular protease secretion by readily absorbable carbohydrates is well documented for several bacterial species (Litchfield and Prescott 1970).

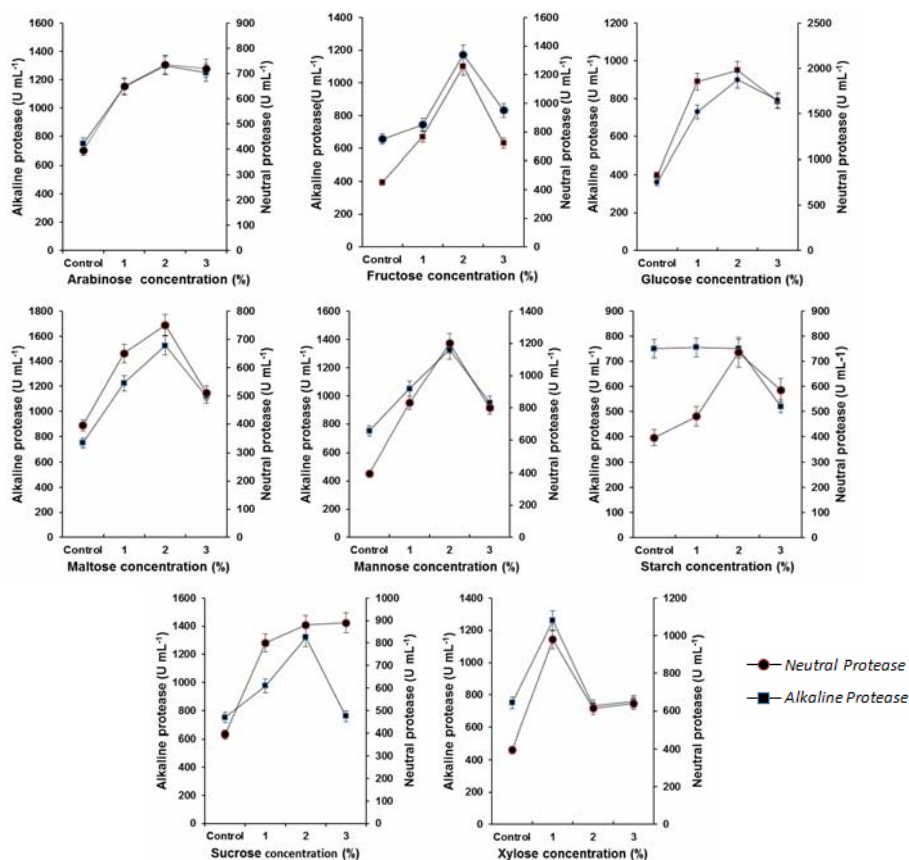


Fig. 2. Effects of different concentrations of various carbon sources on production of proteases by *Bacillus subtilis* IS-5.

The effects of organic nitrogen sources were evaluated by adding 1% w/v to the basal medium. All the organic nitrogen sources inhibited/reduced the production of proteases (Fig. 3). The overall reduction in the production of alkaline and neutral proteases against to control on addition of organic nitrogen source was in the range of 10.13 to 40.00 and 0.0 to 13.65%, respectively.

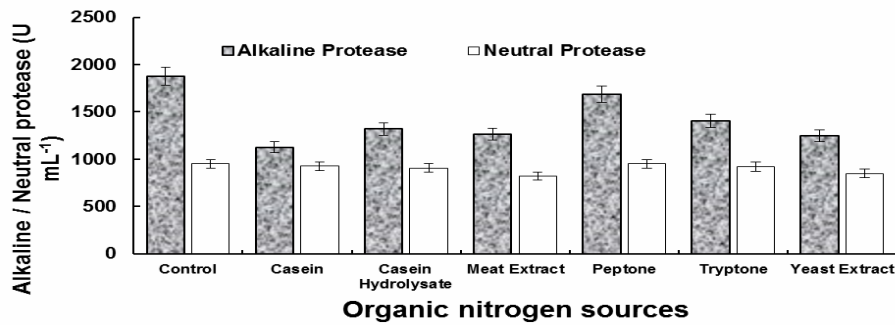


Fig. 3. Effects of various organic nitrogen sources on production of proteases by *Bacillus subtilis* IS-5.

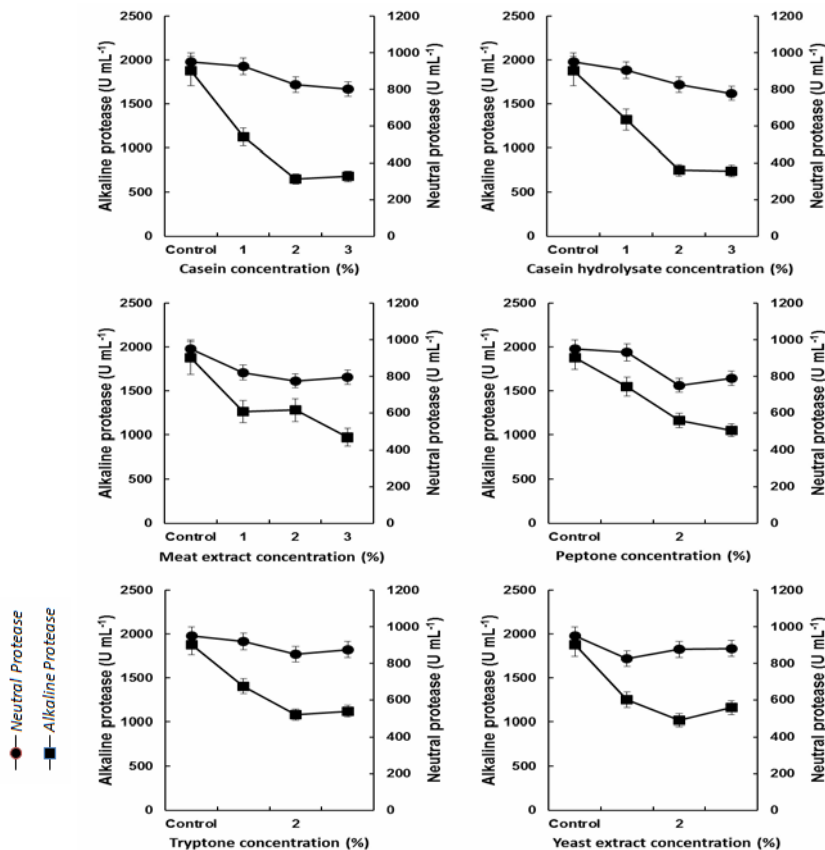


Fig. 4. Effects of different concentrations of various organic nitrogen sources on production of proteases by *Bacillus subtilis* IS-5.

The effects of increase in concentration of organic nitrogen sources in the fermentation medium on production of proteolytic enzymes were presented in Fig. 4. It revealed that decrease in production was more pronounced in case of alkaline protease than neutral protease on increase in concentration of all organic nitrogen sources in the basal medium. As the bacteria used in the present studies is a proteolytic one, probably, it hydrolyzed the added proteins and produced such

amino acids which inhibited the synthesis of proteolytic enzymes. An increase in concentration of amino acids decreased the synthesis of proteases and could be restored by their removal (Moracova and Chaloupka 1984).

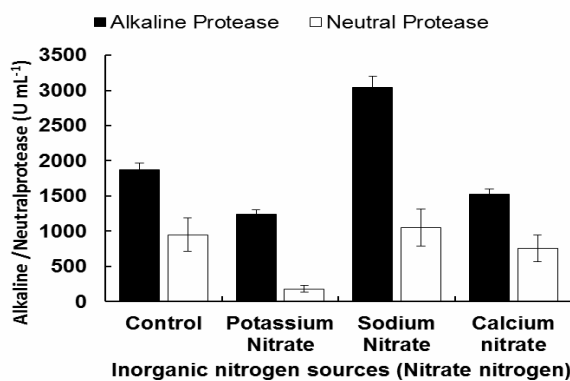


Fig. 5. Effects of various inorganic nitrate nitrogen sources on production of proteases by *Bacillus subtilis* IS-5.

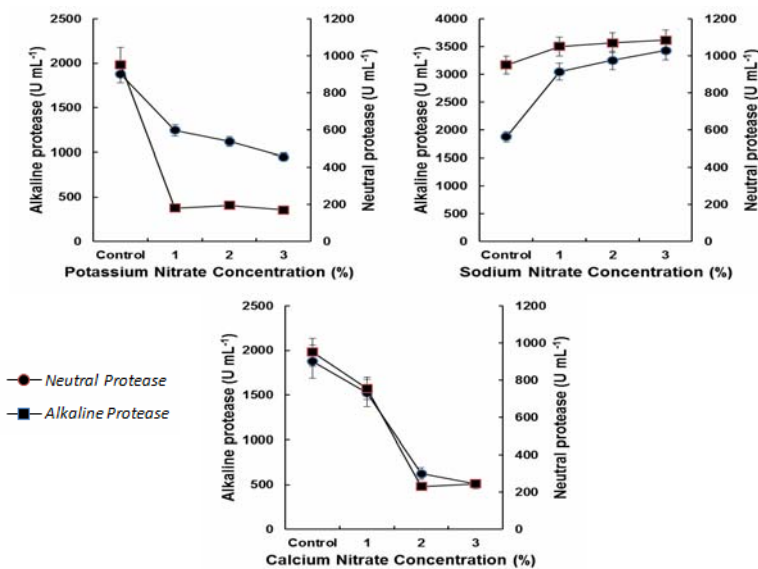


Fig. 6. Effects of different concentrations of various inorganic nitrate nitrogen sources on production of proteases by *Bacillus subtilis* IS-5.

The Fig. 5 showed that only sodium nitrate as inorganic nitrogen source had stimulatory effect on the production of both the proteases. It has increased the production 62.40 and 10.52% against control, of alkaline and neutral protease, respectively. The other sources exerted negative effect on production of both the proteases. Maximum reduction in synthesis of proteases was recorded on addition of potassium nitrate i.e. 33.6 and 81.05% against control for alkaline and neutral protease, respectively. The increase in concentration of sodium nitrate further increased the production of alkaline and neutral protease up to 3320 and 1085 U/ml at 3% w/v concentration. The increase concentration of other nitrogen sources did not have considerable effect (Fig. 6). The alkalophilic

*Bacillus* sp. JB99 produced high amount of protease by utilizing  $\text{NaNO}_3$  as nitrogen source (Johnvesly and Naik 2001). Sodium nitrate induces, stimulates and prevents the decrease in protease activity caused by the addition of glucose (Takii *et al.* 1990, Tsuchiya and Kimura 1984).

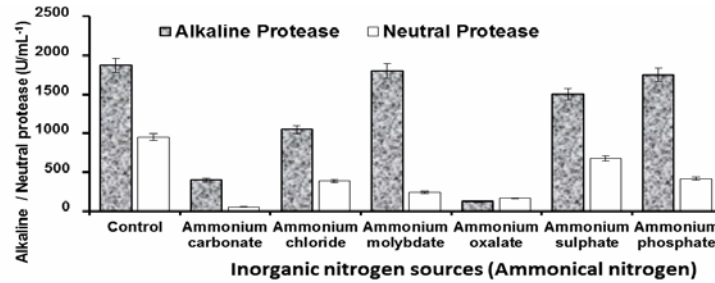


Fig. 7. Effects of various inorganic ammonical nitrogen sources on production of proteases by *Bacillus subtilis* IS-5.

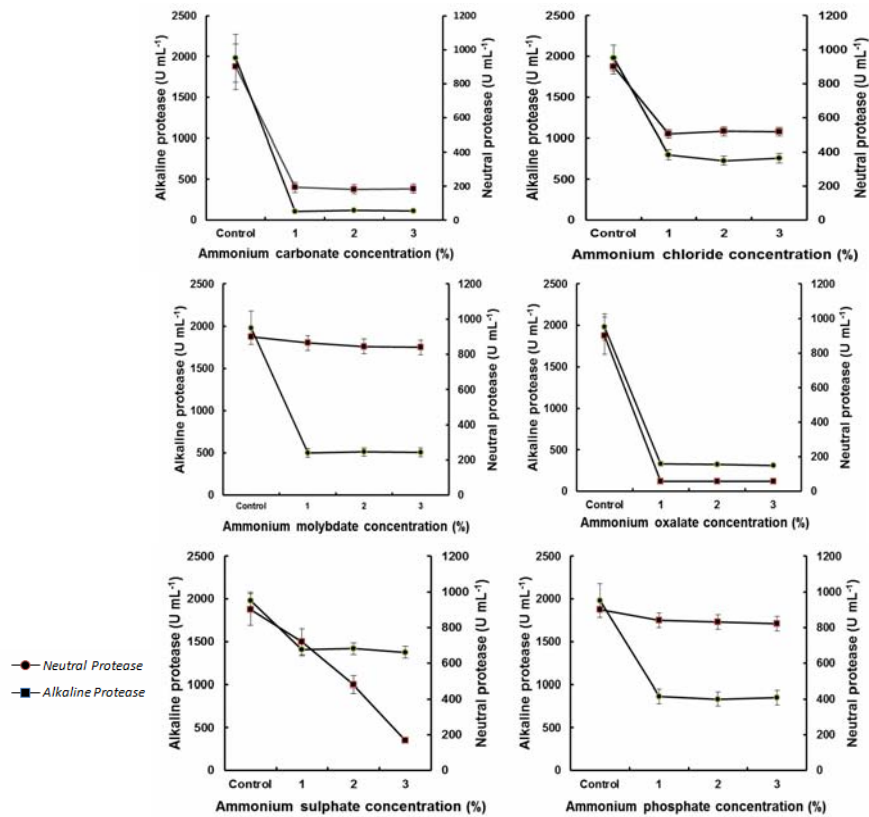


Fig. 8. Effects of different concentrations of various inorganic ammonical nitrogen sources on production of proteases by *Bacillus subtilis* IS-5.

The Fig. 7 showed that ammonical nitrogen sources had inhibitory effect on synthesis of proteolytic enzymes. This was perhaps due to the inability of bacterium to utilize ammonia in the medium. Such effect of ammonical nitrogen has also been observed for *Bacillus cereus* (Norazizah *et al.* 2005). The reduction in synthesis of proteases was up to 94% with respect to control. These

results also revealed that ammonium molybdate was the least inhibitor for alkaline and ammonium sulphate for neutral protease. The increase in concentration of ammonium salts in the medium did not have beneficial effect (Fig. 8). Ammonium ion control and repressed the proteolytic enzymes by checking the formation of spores and their effectiveness was higher on enzymes synthesized at the end of bacterial growth phase (Farias *et al.* 1996).

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