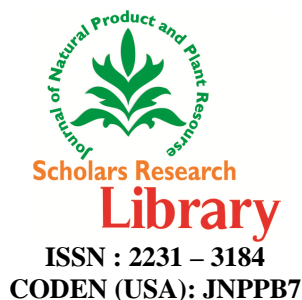




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Influence of Inoculum age and Inoculum ratio on the protease production from *Bacillus subtilis* isolated from soil sample

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ABSTRACT

Bacillus sp being industrially important organisms produces a wide variety of extra-cellular enzymes including proteases. *Bacillus* sp isolated from local from soil samples collected from Ghadi soap industry, Chanatoria, Sagar, M.P., India and identified as *Bacillus subtilis*. The isolated strain produced alkaline protease at the best inoculums age and inoculums ratio of 30 hr and 1ml^{-1} respectively.

Keywords: Protease, *Bacillus subtilis*, soap industry, industrial applications

INTRODUCTION

Proteases have many functions and have important biotechnological applications. They represent one of the three largest groups of industrial enzymes and involved in application in detergents, leather industry, food industry, pharmaceutical industry and bioremediation processes (1). Proteases are enzymes that hydrolyse proteins via the addition of water across peptide bonds and catalyse peptide synthesis in organic solvents and in solvents with low water content (2). Proteolytic enzymes are ubiquitous in occurrence, found in all living organisms but proteases obtained from microbes serve as a preferred source of these enzymes because of their fast growth, the limited space requirement for their cultivation (3). *Bacillus* produces a wide variety of extra-cellular enzymes, including proteases. Several *Bacillus* species involved in protease production are e.g. *Bacillus macerans*, *Bacillus licheniformis*, *Bacillus subtilis*, *B. cereus*, *B. halodurans*, *B.coagulans*, and *B. Subtilis*, *B.cereus* (4,5,6,7,8). In detergent industry, alkaline proteases are widely used as detergent additive to facilitate the removal of proteinaceous stains from fabrics during washing. (9,10). In textile industries proteases have been used in the hide dehairing process, where dehairing is carried out at pH values between 8 and 10 (11). Optimization of inoculums ratio and inoculum age is one of the most important tasks included for the production of protease enzyme. Maximum enzyme production were observed at 48 hours of growth and 1 ml of inoculums ratio (12,13). Maximum enzyme production was also found at inoculum age of 24 hrs (13). In this paper we aimed to study the effect of inoculums ratio and inoculums age factors affecting the activity to present potential application of the proteases for industrial applications.

MATERIALS AND METHODS

Isolation of Microorganisms producing Proteases

The culture used in this study was *Bacillus subtilis*, which was isolated from soil sample collected from Ghadi soap industry, Chanatoria, Sagar, M.P., India. The culture media used was Alkaline agar media consisting (g/L^{-1}) of Glucose, 10.0; Peptone, 5; Yeast Extract, 5; KH_2PO_4 , 10; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; Na_2CO_3 , 10; Agar, 20; Distilled water, 1 of pH 10.0. *Bacillus*

subtilis strain was poured in culture media and incubated at 55°C on rotatory shaker at 120 rpm for 30h. The culture media was separated by centrifugation and the supernatant was used for assaying enzyme activity.

Determination of Protease Activity

Protease activity was determined by a modified procedure based on the method of (14). One protease unit is defined as the amount of enzyme that releases 1µg of tyrosine per ml per minute under the standard conditions.

Effect of Inoculum age and Inoculum ratio on Protease from *Bacillus Sp*:

Effect of inoculums age was studied from age of 6 to 60 h. Effect of inoculums ratio was studied from 0.1, 0.2, 0.5, 1, 1.5, 2, 2.5, and 3 ml⁻¹. The procedure mentioned earlier was followed for estimating protease activity.

RESULTS AND DISCUSSION

To study the effect of various inoculums ratio and inoculums age on the alkaline protease production, different inoculums ratio (0.1, 0.2, 0.5, 1, 1.5, 2, 2.5, and 3 ml⁻¹) and inoculums age from 6-60 h were used. The enzyme assay was carried out as described earlier. Protease activity was found increasing from 0.1 to 1 ml⁻¹ but found decreasing from 1.5 to 3 ml⁻¹. The best inoculum age was found at 30 h (Fig I) and the best inoculums ratio was 1.0 ml⁻¹ (Fig II).

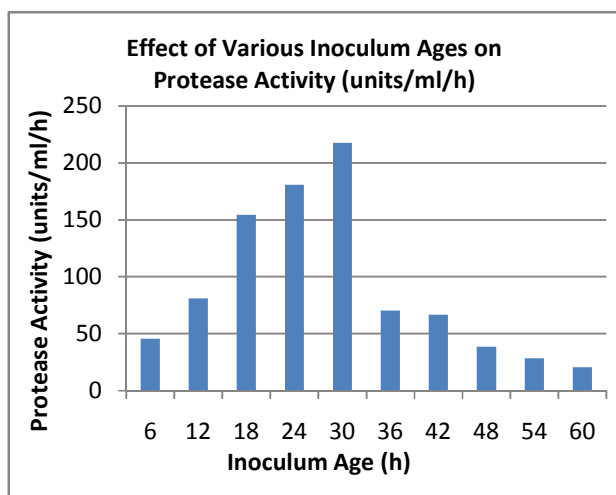


Fig I

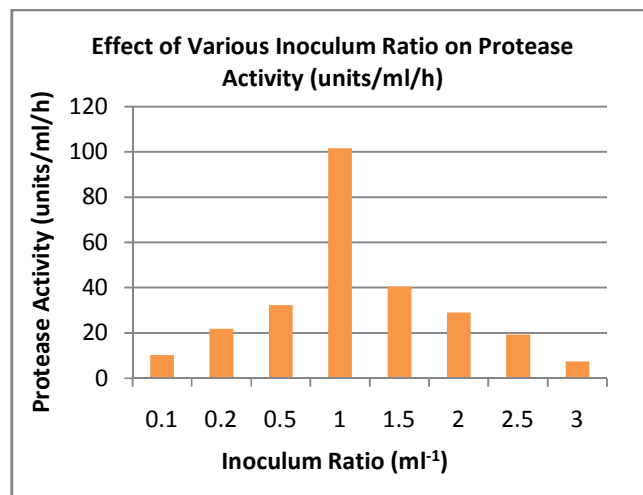


Fig II

CONCLUSION

Although many strains are in the market for enzyme production, researchers prefer studying on new strains because they could be alternative for industrial or commercial use. The isolated new source of protease producing bacteria from the local soil sample, having best inoculums age of 30 h and best inoculums ratio of 1.0 ml⁻¹ may be alternative source for the potential industrial applications.

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