Rnase Purification from Isolate Rns

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Abstract— Bacteria are the most preferred sources of RNase due to their broad biochemical diversity. The present study involved optimization and partial purification of RNase from a bacterial isolate from soil sample. Out of 12 bacterial isolates one isolate (RNS 3) was selected based on relatively higher RNase activity in the culture broth. The various physico-chemical parameters are consecutively optimized for maximum enzyme production. The optimized parameters are at temperature of 34°C, incubation period (20 h) and inoculum size (10% v/v). Among different carbon and nitrogen sources supplementation of glucose and peptone, respectively to the broth showed enhanced RNase production. An alkaline pH (8.5) was suitable for maximum enzyme production by the bacterial isolate RNS3.

Index Terms—Bacterial isolates RNS, Extracellular RNase, Optimization of physico-chemical parameters

I. INTRODUCTION

Around 60% of total world supply of industrial enzymes is produced in Europe. Proteases dominant the market accounting for approximately 40% of all enzymes sales [1].RNase (commonly abbreviated RNase) is a type of nuclease that catalyzes the degradation of RNA into smaller components (Figure 1). RNases can be divided into end-RNases and exo-RNases, and they comprised several sub-classes within the EC 2.7 (for the phosphorolytic enzymes) and EC 3.1 (for the hydrolytic enzymes) classes of enzymes. A bacterium may contains many RNases of different classes, indicating that the RNA degradation is a very ancient and important process for microbes as well as cleaning of cellular RNA that is no longer required. In addition, RNases play key roles in the maturation of all RNA molecules, both messenger RNAs (mRNAs) that carry genetic material for making proteins and non-coding RNAs that function in varied cellular processes and their decay.

Initially, mRNA decay would be consequences of a random recycling pathway in which salvaged nucleotides could be recycled. However, it was believed that mRNA turnover was rapid, non-specific and inevitable end point for all transcripts regardless of their length and structure [2,3]. Since that time, much progress has been made regarding the understanding of RNA decay, and it is now believed to be a series of specific, temporally controlled events in which specific enzymes target the RNA species including mRNA [2,3] Consequently, our understanding of RNA decay and the enzymes responsible for this metabolic process has broadened greatly over the last couple of decades. The majority of RNA decay studies have predominantly used *E. coli* as a model organism. In sharp contrast, RNA decay has not been well characterized in Gram-positive bacteria with the exception of preliminary studies characterizing this process in *Bacillus subtilis* [4,5] *Streptococcus pyogenes* [6]*S. pneumoniae* [7] and *Staphylococcus aureus* [8] Common to both Gram-positive and Gram-negative organisms, RNases are enzymes that degrade ribonucleotides; and two broad types exist, endo- and exo-RNases. Endo-RNases cleave RNA molecules endo-ribonucleolytically at times in a 5′ end-dependent manner, while exo-RNases degrade RNA molecules in a 3′-5′ direction.

II. MATERIALS AND METHODS

A. *Microorganism*

The strain used for RNase production was a bacterial isolate RNS 3. Which were screened on the basis of zone of hydrolysis on nutrient agar petriplates containing 0.1% RNA The bacterial isolate was maintainedon agar slants containing (g l−1): Yeast extract, 5; peptone, 1.0; glucose, 20; agar, 20, cultured for 24 h at 250C, then stored at 4 0C and subculture every 2 months.

B. *Media and cultivation*

The inoculum medium was the same as above slant medium without agar. The fermentation medium used for optimization studies contained (g l−1): glucose, 30; NH_4SO_4, 2.0; KCl, 0.6; RNA, 1.0; pH 8.0. All growth experiments were carried out in 250 ml Erlenmeyer flasks each with 40 ml medium sterilized at 121 0C for 30 min. The flasks were placed on a rotary shaker at 180 rpm with 5 cm amplitude at 25 0C for a period. After incubation for 24 h, the medium was used as the inoculum. The production medium inoculated with 10% (v/v) of 24 h inoculum was incubated for 24 h to produce RNase. After incubation, the bacterial biomass were separated from the culture fluid by centrifugation and then the pallet and supernatant was used to determine the enzyme activity.

F. Partial purification of ribonuclease from bacterial isolate RNS 3

All steps were carried out at 4^0C. The cell free broth was subjected to various saturation or concentration of ammonium sulphate (0-90%). The ammonium sulphate precipitation [9] were followed to calculate the required amount of ammonium sulphate to be added into cell fee extract. It added with continuous stirring and kept at 4^0C for 45 minutes. The precipitated proteins recovered by centrifugation at 10,000 g for 10 minutes and suspended in Tris buffer. Protein concentration and ribonuclease activity were estimated in each fraction .Ribonuclease active fractions were subjected to SDS and native-acrylamide gel electrophoresis (PAGE) after dialysis. The fractions exhibiting maximum activity of ribonuclease were processed for further purification.

III. RESULTS AND DISCUSSION

A. Primary screening of extracellular ribonuclease producing bacterial isolates

Soil samples were collected from different sites of Summer Hill District: Shimla. The ribonuclease producing bacterial isolates grown in nutrient broth having pH 8. The culture broth(s) appropriately diluted were aseptically spread on nutrient agar plates enriched with RNA to obtain pure line cultures. In this level of screening, many colonies of different morphological types were obtained and same were replicated on fresh nutrient agar plates to check their ability to produce extracellular ribonuclease.

B. Ribonuclease production

Nutrient agar containing 0.1% (w/v) yeast RNA was used for primary screening of extracellular RNase producing bacterial isolates. The inoculated medium was incubated at 30°C for 24 h. The direct isolations of cfu(s) producing RNase as indicated by clear zone formation around cfu(s) following flooding with 1N perchloric acid were done.

The acid reacts with nucleate salts in the medium yielding the free nucleic acid and consequently clear zones were formed. The bacterial isolates/ cfu(s) producing RNase activity were carefully picked with a sterile inoculation loop and plated on fresh Nutrient agar plate(s) containing RNA (Table 1). A bacterial isolate namely RNS 3 that produced a clear zone of ~2.6 cm was selected for further studies. The bacterial isolates RNS 8 (2.55 cm), RNS 1 (2.33 cm) and RNS 5 (2.22 cm) also produced marked zones of clearance on the NA petriplates

Table 1 Diameter of zone of hydrolysis shown by different bacterial isolates on Nutrient Agar plates containing RNA.

Bacterial isolate	Diameter of zone of hydrolysis (cm)
RNS 1	2.33
RNS 2	2.03
RNS 3	2.60
RNS 4	2.10
RNS 5	2.22
RNS 6	2.11
RNS 7	1.33
RNS 8	2.55
RNS 9	0.33
RNS 10	2.10
RNS 11	2.13

C. Cultural characteristics of bacterial isolate RNS 3

The bacterial isolate RNS 3 with maximum zone of halos on agar plate was selected for further characterization (Fig.1) The extracellular RNase producing isolateRNS 3 showed filamentous growth, wavy margin and creamish rough colonies on RNAenriched NA plate.

Fig. 1. Plate showing holos

D. Effect of initial pH on extracellular RNase production of the broth

The broth containing RNA (0.1%; v/v), glucose (1%; w/v), sodium chloride (5 mM), and 0.1%(v/v) peptone was set to varying pH (6 to 10) to determine the optimum pH that could yield the maximal ribonuclease in the production broth. The maximal ribonuclease activity (0.26 U/ml) was recorded at 30°C at pH 9 under shaking of 160 rpm after 24 h incubation (Fig. 2).

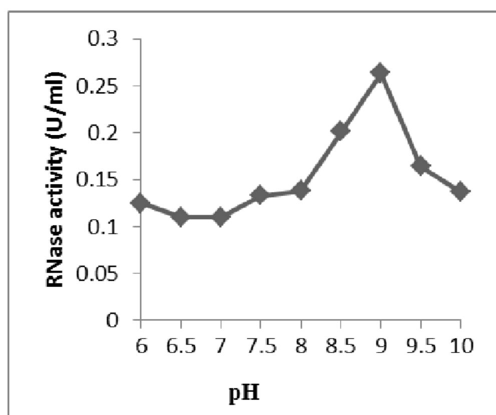

Fig. 2. Effect of initial pH on extracellular RNase production of the broth

E. Effect of organic carbon sources on the production of extracellular ribonuclease

The Nutrient broths (50 ml taken in 250 ml Erlenmeyer flask) supplemented with 0.1% (v/v) of the RNA were inoculated with 4% (v/v) of 24 h old seed culture (Fig. 3). Among all the commercially available carbon sources supplementation of glucose to the production broth relatively higher production of ribonuclease (0.29 U/ml) by bacterial isolate RNS 3.

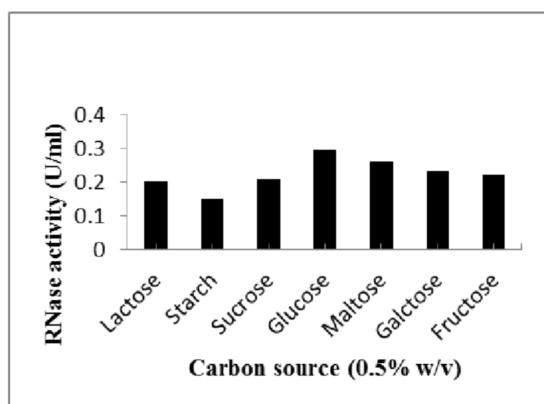

Fig. 3. Effect of different carbon sources on the production of ribonuclease by bacterial isolate RNS 3.

There are only few reports of extra-cellular ribonuclease production by bacterial isolates, including *Bizoonia* species [10], *Campylobacter jajunum* [11] and *Pseudomonas* species [12].Relation between effect of temperature, pH and salt concentration on growth of RNase producing bacterium and fungi like halophillic bacterium *Bizionia* species [10] *Asperzillus niger* [8] *Compylobacter jejunum* (Haddad *et al.,* 2013) and thermophilic fungus [14] have been reported.. From the observation, it can be seen, while the growth of bacterial isolate RNS 3 was promoted in the presence of 2.5 mM NaCl at an alkaline pH (9.0) and temperature 35°C, respectively.

IV. CONCLUSION

Production broth optimization of culture conditions using different Physico-chemical parameter and partial purification appears to be a valuable tool for the production of RNase by bacterial isolate RNS 3. RNase production increased with the increase of initial pH and with the decrease of NH_3SO_4concentration. The optimization enhanced the RNase production by 6.57 fold.

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