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Effect of Different Factors on Production of Bacterial Antibiotics

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Abstract

A number of antibiotics are known to be produced commercially with the help of different microorganisms. In the present study some soil bacteria with the antibiotic activities were screened and studied, especially for some important growth factors like Temperature, pH and source of Carbon and Nitrogen that affects production of antibiotics. The antibiotic producing microorganisms Micrococcus varians, Micrococcus luteus, Bacillus polymyxa and Bacillus alvei were tested against a most crucial pathogen Staphylococcus aureus. The study revealed that the optimum temperature was 30°C and pH 7 for the prodication of antibiotics. While the best Carbon source is Glucose and most suitable Nitrogen source is Peptone followed by Tryptone.

Introduction

Most bacteria produce antimicrobial compounds such as broad spectrum classical antibiotics, metabolic products *viz.* organic acids and lytic agents such as lysozyme. In addition, several types of protein exotoxin and bacteriotoxins, which are biologically active peptide moieties with bactericidal action, are also released. Members of the Bacillus genus are generally found in soil and most of these bacteria have the ability to disintegrate protein.

Materials and Methods

The microorganisms under study were isolated by dilution method Ahmed *et al* (2013) and purified after identification. All components of culture media are weighed and added to distilled water and is made up to 50ml. Now this 40ml broth is sterilized in autoclave at 120ºC at 15 lbs for 15 minutes. Sterilized broth is transferred to 4 test tubes with 10ml each and each tube is inoculated with the test cultures under aseptic conditions and is incubated for 2 days. After two days, the maximum amount of antibiotic produced as the secondary metabolites are released into the medium during their stationary phase and the extraction is done by centrifuging them at 10000 rpm for 10 min. Chandrashekhara *et al* (2010) The supernatant of each bacteria was collected as it contains secondary metabolite which is containing antibiotic and is stored at 4ºC. A pure colony of *Staphylococcus aureus* (standard pathogenic bacterium) is swabbed on the petriplates with a sterile swab which containing culture media with variable pH. These petriplates were incubated overnight without inverting at optimum temperature of 37ºC for 24 hrs. After that 1 ml of supernatant was placed in to petriplates and again incubated for 48 hrs different temperatures. The clear Zone of Inhibition around the pathogen was measured.

For Carbon Source optimization each flask of 20ml of customized production broth was prepared in which different carbon source of 1.0% i.e. wheat flour, rice flour, corn flour, glucose and starch was added. Similarly for Nitrogen Source optimization each flask of 20ml of customized production broth was prepared in which different Nitrogen source of 1.0% i.e. yeast extract, beef extract, peptone, tryptone, and urea was added. The pH was adjusted to 7 and media autoclaved and were incubated at 30°C (Optimized temperature) for 24 hrs. Espeso *et al.* (1993) and Kalyani *et al* (2012).

Results and Discussions

Effect of Temperature on Antibiotic Production

For optimization of temperature five sets of 20ml of production broth were inoculated with 2% of actively growing cultures. All flasks were incubated at different temperatures viz, 25°C, 30°C, 33°C, 38°C and 41°C, for 24 hrs. The observations were made and shown in table-1 which revealed that temperature below 30°C and above reduces the predication of antibiotics and optimum temperature for their production by microorganisms under study was 30°C. Datta, and Kothary (1993).

Effect of pH on Antibiotic Production

For pH optimization each flask of 20ml of production broth with different pH *viz*, 5, 6, 7, 8, 9 was inoculated with 2% of actively growing culture. The observations were made and shown in table-2 showing that the optimum pH

is near nutral. The high and low pH decreases the predication of antibiotics. Datta, and Kothary (1993).

Effect of Carbon and Nitrogen Source

Carbon and Nitrogen Source are also important factors that effect the production of antibiotic. Wheat flour, Rice flour, Corn flour, Glucose and Starch were used as Carbon Source and the results are presented in table-3 out of all carbon sources, Glucose (monosaccharide) showed highest potential in producing antibiotics and may be attributed to the most simple and easily digestible substrate for microorganisms. Datta, and Kothary, (1993). Five Nitrogen Sources Yeast extract, Beef extract, Peptone, Tryptone, and Urea was used and the results are presented in table-4 which indicates that best source of nitrogen in culture media is Peptone followed by Tryptone because of the presence of desired amino acids in abundance. Singh, *et al*. (2012).

Table 1: Effect of Temperature on Antibiotic Production.

Sr No.	Incubation temperature (°C)	Diameter of zone of inhibition (cm)
1.	25	1.5
2.	30	**1.9**
3.	33	1.8
4.	38	1.5
5.	41	1.1

Table 2: Effect of pH on Antibiotic Production.

S. No.	**pH Value**	**Diameter of zone of inhibition (cm)**
1	5	1.3
2	6	1.7
3	7	**2.0**
4	8	1.5
5	9	1.2

Table 3: Effect of Carbon Source on Production of Antibiotics

S. No.	**Carbon Source**	**Diameter of zone of inhibition (cm)**
1.	Wheat flour	1.7
2.	Rice flour	1.8
3.	Corn flour	1.7
4.	Starch	1.4
5.	Glucose	**2.1**

Table 4: Effect of Nitrogen Source on Production of Antibiotics

S. No.	Nitrogen Source	Diameter of zone of inhibition (cm)
1.	Beef extract	1.8
2.	Yeast extract	2.0
3.	Peptone	**2.9**
4.	Tryptone	2.3
5.	Urea	1.9

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