A new medium for the detection and production of bacterial alkaline phosphatase

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Abstract

Different types of culture media are used for culturing bacteria isolates to having a pure colonies, a lot of bacterial types can be growing in the research laboratory condition on a express medium, some bacterial isolates have need of a complex culture medium and because production in all in over the world, especiallyin the development country efforts be there made to synthesis a cultural medium as naturally to the investigation for alkaline phosphatase production and detection from different bacterial genus. In the new natural culture medium used reed plant abstract (phragmites austuralis). The component of the medium was available locally and found quite efficient in enriched and detection of the enzyme. The results displayed that the difference amongst prepared new culture medium and the controller culture medium in growth of bacterial isolates. the natural culture medium was inexpensive, available, easy preparation in addition to capable to detect the production of alkaline phosphatase enzyme.

Keywords: bacterial alkaline phosphatase, culture media, Klebsiella spp, Phragmites spp

Introduction

All microbes require appropriate culture medium which provision their nutritional needs in addition, in vitro when culturing the microbes their needing of various environmental factors, such as optimal (temperature and pH, level of oxygen. a typical culturing medium generally contains a basis of nitrogen, inorganic ions for instance phosphorus, magnesium. When the culturing a heterotrophic microbes for example E.coli, Pseudomonas spp., Klebsiella spp. need requirement for support their growth such as carbohydrates compound (glucose, starch) are contained within the culture medium as a substrate to run into the carbon and energy (3). Phragmites spp. The communal reed ,is a large perpetual greensward plant originate in wetlands all through temperature and tropical regions of the world . P.australis is former regarded as the sole species of the genus Phragmites . this plant may be used for artificial purposes such as phytoremediation water treatment or used for made various things such as baskets ,mats ,pencils and a rough form of paper (4,5).

Alkaline phosphatase (EC3.1.3.1) exist extensively in natural , as well as air originate in many organisms from the bacteria to the man .the work of this enzyme catalyze the hydrolysis of monoesters of phosphoric acid and also catalyze a transphosphorylation reaction in the presence of high concentration of phosphate acceptors (6,14).

Alkaline phosphatase play more important roles in a wide range of applications such as human beings in addition various industries products :this enzyme takes serves a useful

instrument in molecular biology laboratories ,meanwhile generally have phosphate groups on the 5´ of the end . keeping DNA molecules linear until the next step of the process for which they are being prepared was the function of this enzyme by removing these phosphates prevents the DNA from ligating (7,16) . the organic molecules like ribonucleotides, deoxy-ribonucleotides , proteins ,alkaline phosphatase esters and anhydrides acid can be hydrolyzes by the phosphomonoesterase enzyme which is present in many types of bacteria, like :**Escherichia coli, Pseudomonas aeruginosa ,Staphylococcus spp. And Bacillus spp**.(15)

As well as commonly alkaline phosphatase is formed at mercantile level from E.coli or calf intestine (8).the alkaline phosphatase bacteria usually is consumed in research purpose , for the reason that it is comparatively resistant to inactivation , denaturation , degradation and higher rate of activity due to its so many industrial uses , it is necessary to produce it on large scale for commercial and research purpose(9,10).

Materials &Methods

1-prepared of culture medium: matured leaves of **Phragmites australis** and garden-fresh which far away named a reed ,the place of collected the leaves was Baghdad city .leaves sample washed in a row tap water and take place at room temperature (25-28c^0) for 2-3 day for dried after that converted to powder by using a sterilized electric blender followed used air tight container for store .

The twenty-gram of leaves powder quantity was absorbed in hot sterilized water (100C°) the volume was 100 ml after that take place in water path for 5 minutes . different weights of leaves powder were expired (8,16,20 grams) in 100 ml of distilled water ,but 20 g is the best concentration, and all these concentration were filtered by a sterilized filter paper No.1 (whatman).

2-composition of the medium: this medium is consisted 100 ml of plant extract containing : **Glucose= 1g ,$MgSo_4$.$7H_2O$=0.2g , KH_2PO_4= 0.02g,NaCl =0.5g , $CaCl_2$=0.01g , Agar=0.17g**

All component were dissolved in 100 ml of plant extract ,pH was adjusted to 8.5 and then autoclaved .

3-preparation of growth medium : this medium is consisted 100 ml of plant extract containing :Glucose =1g , NaCl =0.5 g pH was adjusted to 7 , then sterilized at autoclaved .

4- Alkaline phosphatase : it was consisting of the following :Disodium phenyl phosphate 5mmol/L , 4-Amino antipyrine : 60mmol/L , Sodium arsenate : 240mmol/L, Potassium ferricyanide : 150mmol/L.

5-Collection of isolates : A total of 5 bacterial species (10) isolates were tested for growth and production of alkaline phosphatase on the new medium . these isolated included : **Escherichia coli , Staphylococcus aureus , Klebsiella sp. , Serratia marcesence, Pseudomonase aeruginosa .**these isolated originally isolated from clinical specimens . they identified using standard biochemical tests , cultural and microscopical examination(14) .

Results and Discussion

All ten bacterial isolates initially identified the morphology properties in addition biochemical tests, also were identified again with VITEK^{R}2 GP ID card ,and GN ID card .

From the ten types of bacteria ,the bacterial suspensions for four devers gram negative and gram positive types cultured in the new medium indicated the results that the cultured bacterial in the new medium showed significant growth for **E.coli , Klebsiella , P. aeruginosa , S. aureus and S.marscence** (table 1-1) .

A culture medium which locally prepared have available basis of carbon source , nitrogen , mineral salts and other growth promoting ingredients (11). **Phragmites austuralis** have considerable amount of protein (nitrogen source) and carbohydrate so it gives dense growth of bacterial isolates (12,13) . the ability to isolated many types of bacteria from **Phragmites austuralis** from all parts of the plant (leaves and stems) then point out increase metabolic activity for types of bacterial by this plant.

The results of alkaline phosphatase production and detection showed that the bacterial growth and production of alkaline phosphatase enzyme on the new medium , when the color of the colonies changed to pink color comparing with colorless colonies on the control medium (table 1-2).the modification of the medium to be suitable for detection of alkaline phosphatase enzyme by addition some component .

All isolates of **E.coli** (2 isolates) , **P.aeruginosa** (2isolates) **, S.aureus** (2isolates)and **S.marscence**(2isolates) exhibit alkaline phosphatase production except the **klebsiella spp**.(2isolates), which gave negative results (colorless colonies) .

The study concluded that the two new mediums were found to encourage the growth of different bacterial species and stimulate the production of alkaline phosphatase enzyme ,and the medium also is easy to prepare and inexpensive also presented in addition to efficient to others and could uses as enrichment medium as well as for production and detection of alkaline phosphatase enzyme .

Table (1-1) Characteristics of bacterial species growth on the new medium

Bacterial species	**Characteristics of colonies**
Escherichia coli	**Circular , smooth, convex**
Klebsiella spp.	**Circular , smooth , convex ,muses**
Pseudomonase aeruginosa	**Smooth , round colonies , convex**
Serratia marsence	**Circular, smooth , convex**
Staphylococcus aureus	**Circular , smooth , convex ,gold colonies**

Table (1-2) production and detection of alkaline phosphatase enzyme on the new medium

Bacterial species	Production of enzyme	No. of isolates
Escherichia coli	+ pink colonies	2
Klebsiella spp.	-no change	2
Psudomonase aeruginosa	+ pink colonies	2
Serratia marsence	+ pink colonies	2
Staphylococcus aureus	+ pink colonies	2
Total		10

Compliance with ethical standards:

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Disclosure of conflict of interest

No conflict of interest to be declared.

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