Production of antibacterial agent from Streptomyces griseus by using Semi Solid Fermentation

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Summary:

Backround: The Solid state fermentation has several advantage including absence of free water , reduced volume of production media utilized for high products and the relatively low costs of production.

Methods: Thirty local isolates of soil obtained from Genetic Engneering and Biotechnology Institute. Nutrient agar was used to growth strains examination to antibacterial agent and Wheat bran and fish meal were used in combination (0-100%of each)and divided in 10 gm lost /flask . Each flask is inoculated with different numbers of Streptomyces spores and incubated for 5 days at 28°C, then the supernet was extracted and were assayed as antibacterial

Results: The ability of 30 local isolates of Streptomyces and the standard strain *S.griseus* for antibacterial production were tested by grow in Muller Helton agar. The later isolate (standard isolates) showed highest antibacterial activity with inhibitory effect of (30 mm,24 mm ,20mm, 18mm) compared with local isolates (S_1 ,S_2 , S_3) resectively

Conclusion: only two soil isolates (among 30 isolates) and standard isolates of Streptomyces griseus were appears able to produce antibacterial agent under optimum condition . The optimum condition for the production of antibacterial is solid state fermentation included the use of wheat bran hydrate with distilled water pH=8 and the optimum hydration ratio was 1:3 (w:v) with an inoculum size of $6x10^9$ spores/flask (10 gm wheat bran) and incubation for 5 days at 28°C.

Key Words: Streptomyces Solid state fermentation, antibacterial

Introduction:

The genus Streptomvces belongs to order Actinomycetales. This bacteria is filamentous, aerobic, gram positive and spread mainly in soil and considered as a good source for more than half of all antibiotic(1). Also it is known to produce many other products like extra cellular enzymes and inhibitors (2 ,3 ,8 ,11 ,12).

Solid state fermentation was used earlier in many countries in Asia like Japan and China in the production of many kinds of food such as soy souse (4) This methods used in production many antibacterial from S. halstedii , *S.hydgrcophca and S. griseus (5)* .

The development of antibacterial

Production depended to use cheep raw material or neutral culture media , as solid state fermentation technical instead of liquid fermentation . This technique used for cephalosporin production from *S.clavuligerus* (6) and tetracycline from sweet potatoes (7) The Solid state fermentation has several advantage including a bsence of free water (6) , reduced volume of production media (7) utilizated for high products and the relatively low costs of production (8 ,9 ,10) .

The purpose of this study is to select the Streptomyces isolate that produces antibacterial and to determine the optimal conditions for production of this antibacterial by solid state fermentation.

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Material and methods:

Source of isolates:Thirty local isolates of soil obtained from Genetic Engneering and Biotechnology Institute for Postgraduate studies, University of Baghdad, were used in this study.

culturing media : Nutrient agar was used to growth strains examination to ntibacterial agent ,but gose media was used for Streptomyces growth

Production media :Wheat bran and fish meal were used in combination (0-100%of each)and divided in 10 gm lost /flask .These media were humidified at rate of 1:3 (W:V) pH 7.2 by 0.2M potassium phosphate buffer or Distilled water or minimal media ,and autoclaved .

Inoculum Size: Each flask is inoculated with different numbers of Streptomyces spores ($6x10^2$, $6x10^3$, $6x10^5$, $6x10^7$, $6x10^9$) and incubated for 5 days at 28°C, then the supernet was extracted and were assayed as antibacterial

Results and discussion:

The ability of 30 local isolates of Streptomyces and the standard strain S.griseus for antibacterial production were tested by grow in Muller Helton agar. Selected of isolates of heavy antibacterial production were compared with standard isolates .The later isolate (standard isolates) showed highest antibacterial activity with inhibitory effect of (30 mm,24 mm ,20mm, 18mm) compared with local isolates (S_1 ,S_2 , S_3) resectively .

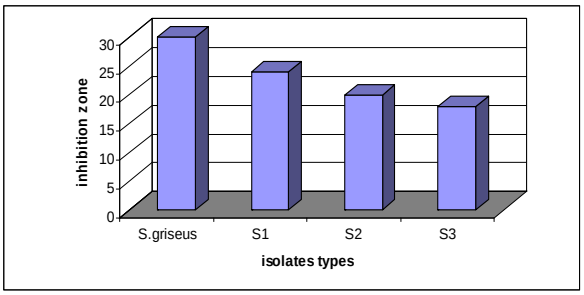

Fig 1:Production of antibacterial by different Streptomyces isolates

However standard streptomyces griseus isolates were tested for antibacterial production by usage of solid phase fermentation , antibacterial inhibitory effects of later isolates shows mild effect in comparison to the effect of gose isolates . (fig 2)

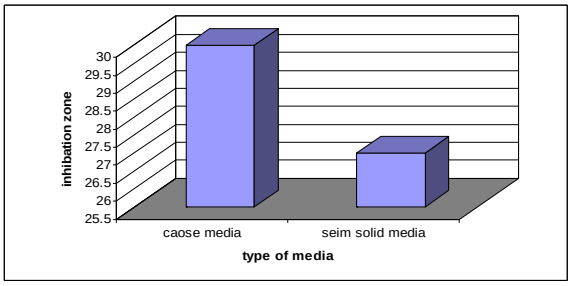

Fig 2: The effect of type media in antibacterial production from S.griseus

That might be due to complete nutrition compound of structural media in comparison to Wheat media (6)

Optimum environment of antibacterial production effect of solid phase fermentation components :-

Plant and animal by products were examined on antibacterial production medium for S. grises . plant media (Wheat bran) show highest antibacterial inhibitory effects(27 ,24 ,21 ,20 ,18 ,15 ,11.10 ,10 mm) respectively on E.coli culture in comparison to the animal media (fish media) (fig . 3) .

This is might justified as that, plant media of rich sources for carbon and nitrogen compound. However other research (6) mention to use wheat bran to produce highest cephalosporin quantity in comparison to rice granules from S . clavuligerus .

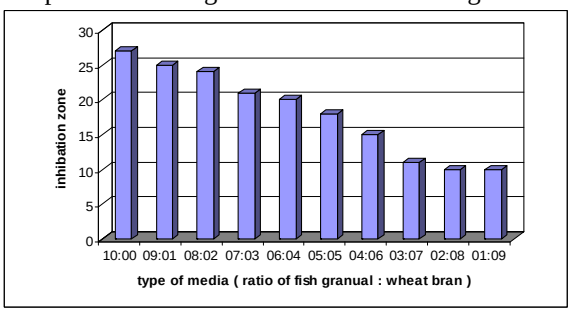

Fig3:The effect of solid media components (Wheat bran and Fish granule) on antibacterial production from S.griseus

Effect of hydration solution on antibacterial production

The best hydration solution for wheat bran was distilled water with pH adjustment of medium to yield pH8 before sterilization (27 mm). Antibacterial production was much lower (24 mm) when wheat bran was hydration with phosphate buffer (fig 4) . Previous studies (6) indicate that the significant role of water content of solid substrate in production of cephalosporin from S. clavuligerus .

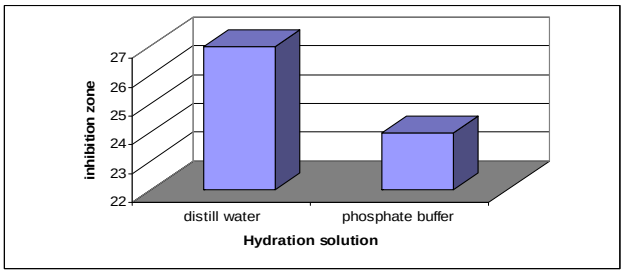

Fig 4:The effect of moisting solution in antibacterial production from S.griseus

Incubation period

The result showed gradual increase in antibacterial production with incubation time up to 120 hrs. (fig 5) Shows that protein and metabolite production was highest within vegetative phase than in case of spore phase.

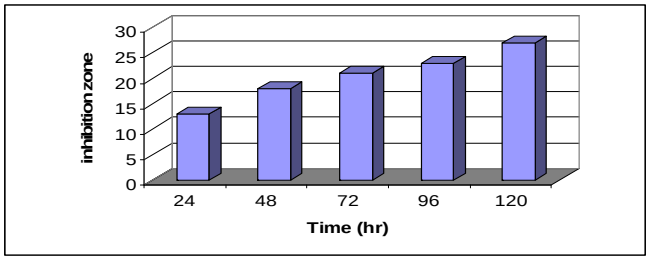

Fig5:The effect of incubation period on antibacterial production from S.griseus

Inoculums size

Antibacterial production was directly proportional with inoculums size (fig 6).The highest production was obtained when the inoculums size was 6×10^9 spore / flask ,Where the inhibitory effect was 27 mm . This increment due to the increase in cell numbers which produce more antibacterial compounds .(6,7)

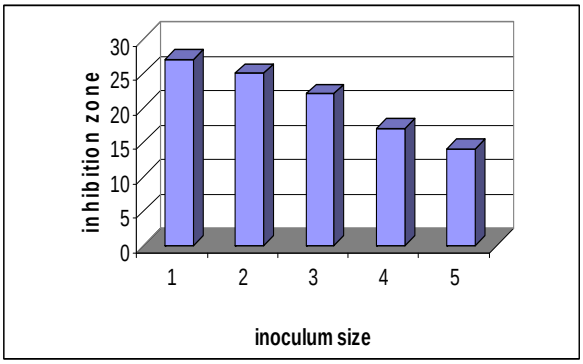

Fig 6The effect of inoculums size in antibacterial production from S.griseus

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