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Effect of Nutritional Broth on the Actinomycetes and their Antibiotic Activity

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The study of nutritional broth for the production of antibiotic substance was obtained by selection on growing the strain *Streptomyces carcinomycicus* on 9 different nutrients broths including both natural & synthetic *viz*. Beef extract broth, yeast extract broth, Oat meal broth, Glycerol asparagine broth, soluble starch broth, soyabean meal broths, Glucose broth, Glucose asparagine broth & Modified glucose asparagine broth. The strain was found to grow both in natural & synthetic media. Beef extract, Glucose broth, Oat meal broth supported the maximum production of antibiotic substance giving 98.28%, 97.62%, 94.00% inhibition respectively. Modified Glucose asparagine was next best giving 86.91% inhibition. Modified glucose asparagine medium was selected as the production medium for further experimentation because the constituents of the medium are chemically defined. The assaying was done by spore germination test in all the experiment and the antibiotic potency was calculated in term of percentage inhibition of spore germination. The growth of actinomycetes was determined in terms of mycelial dry weight in grams.

Key Words: Nutritional broths, antibiotic activity, actinomycetes, Hanging drop method.

Nutritional requirement vary from one organism to another in respect to their growth & antibiotic production. Flamming (1929) used nutritional broth for the production of penicillin. Abrahim *et al.* (1941) concluded that higher yields of penicillin could be obtained by supplementing Czapek-dox medium with yeast extract. Moyer & Coghill (146) tested a number of media & concluded that higher antibiotic yield could be obtained when fungi were grown in media containing corn steep liquor or seed cake meals. Banerjee *et al.* 1974 reported that the addition of paddy soak water in the medium instead of corn steep liquor increased the maximum growth of the mold & wortinamin production. Sinha & Chaudhary (1979) reported that the production of antibiotic substance was higher in broths containing natural product *viz* Soyabean meal extract, Beef extract, Yeast extract. Demain & Jnamine (1970) suggested that soyabean meal which is a natural product stimulates the production of *Streptomycin* because of its higher amino acid contracts several other media containing substance derived from natural sources were used by Waksman *et al*. 1946, Tsao *et al*. (1960), Thirumalchar (1971, 1972) Jayaram *et al.* (1970), Sambamurti & Elliah (1974), Bhemauerova *et. al* (1970), Kamatsu *et. al* (1980), Sharma & Sinha (1982) Jayant and Sinha (1982) Sharma *et al.* (1983), Sinha & Sharma (1984), Gupta & Singh (1992).

But on the other hand Rank & Donovick (1946), obtained higher yields of *Streptomyces* in the media containing higher beef extract nor corn steep liquor by *Streptomyces griseus* indicating there by that the natural sources were not essential for higher antibiotic production Emerson *et al.* (1946), Florey *et al.* (1946), Perlman (1966), Soni & Viyas (1973), Mukerjee and Chandra (1975), also

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employed synthetic media & obtained the antibiotic yield approaching or exceeding that in natural media.

MATERIAL AND METHODS

The actinomycetes isolate (*S. carcinomycicus*) was cultured on 9 different broths both natural and synthetic. The composition of different broths were as (1) Soyabean Meal Broth (Glucose 10.0 gm, Soyabean meal 10.0 gm, Calcium Carbonate 0.75 gm, Distilled Water 1.0 litre), (2) Yeast Extract (Glucose 10.0 gm, Dipotassium Phosphate 0.5 gm, Asparagine 0.5 gm, Magnesium Sulphate 0.25 gm, Yeast Extract 0.5 gm, Distilled Water 1.0 Litre), (3) Glucose Broth (Glucose 10.0 gm, Peptone 5.0 gm, Beef Extract 5.0 gm, Sodium Chloride 5.0 gm, Distilled Water 1.0 litre), (4) Glycerol Asparagine (Glucose 10.0 gm, Asparagine 1.0 gm, Dipotassium Hydrogen Phosphate 1.0 gm, Distilled Water 1.0 litre) (5) Glucose Asparagine (Glucose 10.00 gm, Asparagine 0.5 gm, Dipotassium Hydrogen Phosphate 0.5 gm, Distilled Water 1.0 litre), (6) Modified Glucose Asparagine (Glucose 10.0 gm, Dipotassium Hydrogen Phosphate 0.5 gm, Magnesium Sulphate 0.25 gm, Asparagine 0.5 gm, Distilled Water 1.0 litre) (7) Soluble Starch (Soluble Starch 20.0 gm, Dipotassium Hydrogen Phosphate 1.0 gm, Magnesium Sulphate 0.5 gm, Potassium Chloride 0.5 gm, Sodium Nitrate 2.0 gm, Calcium Carbonate 2.0 gm, Distilled Water 1.0 litre. (8) Beef Extract (Glucose 10.0 gm, Peptone 0.4 gm, Beef Extract 0.5 gm, Sodium Chloride 0.5 gm, Distilled Water 1.0 litre (9) Oat Meal Broth (Agar-Agar 17.0 gm, Oatmal 15.0 gm, Yeast extract (if desired) 1.0 gm, Distilled Water 1.0 Litre).

30 ml of each broth was taken in 250 ml Erlenmeyer flasks in triplicate and sterilized at 10 lbs pressure for half an hour. Each flask was inoculated with spore suspension of *S. carcinomycicus* and incubated for 15 days at $28^0C(\pm2^0C)$. The antibiotic activity of the culture filtrate was assayed in terms of percentage inhibition of spore germination of *Alternaria solani* after 5, 10 and 15 days incubation adopting the "Hanging drop method" of Brain and his co-workers (1960) spore suspension of *A.solani* was prepared in sterilized distilled water from a 10 days old culture grown on oat-meal agar medium. The dilution of spores in the suspension was adjusted to 10-15 spores per microscopic field in low power. 0.1 ml of spore suspension was added to 5 ml of the cultural filtrate to be assayed to ensure uniform distribution of spore and throughout the filtrate. Drops of the filtrate containing spores of *A. solani* were placed on slides which were inverted and kept in moist chamber on 'V' tubes aseptically and incubated for 8 hours at $30^0C(\pm2^0C)$. The drops were then examined under the microscope and number of ungerminated spores were counted. The percentage inhibition of spore germination was calculated. 30 observations each from three replicate were taken. After 15 days of growth of *S. carcinomycicus* in different culture media mycelial mat was harvested by filtration through previously weighed whatman no. 1 filter papers. The mycelial mat was thoroughly washed with distilled water and the dry weight was recorded for each treatment.

RESULTS AND DISCUSSION

The actinomycetes isolate under study (*S. carcinomycicus)* produces antibiotic substance in liquid culture media. The culture filtrate inhibits the spore germination of *Alternaria solani*. The calculated value (F) is more than the tabulated value at 5% level. Therefore, the data are significant. The Streptomyces isolate (*S. carcinomycicus*) produced antibiotic substance in all media tested but the amount synthesized varies from one medium to another. A significant rise in production and antibiotic substance was observed with the increase of duration and it was maximum after 15 days of fermentation in all the media tested. The production on antibiotic substance occurred in beef extract broth 98.28% inhibition of spore germination of the test organism. Glucose broth and oat meal broth were equally good permitting 97.62% and 94.00% inhibition. In descending order of activity are modified glucose asparagine yeast extract, Soyabean meal and Glucose asparagine media 86.91%, 75.10%, 72.54% and 62.70%, inhibition of spore germination respectively considering C.D. Value at 5% probability (C.D.=4.33%) and the antibiotic production as measured in terms of percentage inhibition of spore germination of *A. solani* various broth can be grouped as under:

The maximum production of antibiotic substance occurred in breef extract broth giving (98.28%) inhibition of spore germination of the test

Table 1. Effect of different broths on the actinomycetes isolate (S. carcinomycecus) & its growth

S. No.	Nutritional Broths	Percentage inhibition of spore germination *Alternaria solani* (Mean of 3 observations for 3 replicates			Mean of 3 replicates (mycelial dry wt. in gm 15 days of growth)	Initial pH	Final pH
		5 days	10 days	15 days			
1.	Beef Extract broth	52.56	82.25	98.28	0.9864	5.8	6.9
2.	Yeast Extract broth	56.40	71.01	75.10	0.9734	5.6	5.8
3.	Oat meal broth	71.55	80.01	94.00	0.95711	6.0	6.8
4.	Glyceral asparagine broth	25.05	53.00	52.12	0.1584	6.4	6.7
5.	Soluble starch broth	15.76	35.76	50.14	0.7150	5.1	6.2
6.	Soyabean meal broth	35.12	68.12	72.54	0.9718	6.0	7.0
7.	Glycose asparagine broth	16.76	29.18	62.70	0.1619	6.7	7.2
8.	Glucose broth	45.52	92.42	97.62	0.9709	6.5	7.0
9.	Modified glucose asparagine broth	52.62	72.10	86.91	0.9412	5.6	6.2

Table 1(a). Analysis of Variance

S. No.	Source of variance	Degree of Freedom DF	Sum of Squares SS	Mean Squares MSS	Variance Ratio (F)		
					Calculated Value	Table Value	
						1%	5%
1.	Duration	2	5858.230	2929.115	466.568	6.23	3.63
2.	Treatment	8	8671.302	1083.913	172.652	3.86	2.59
3.	Residual	16	100.450	6.278			
	Total	26	14629.982				

CD at 5% probability = 4.33

fungus by descending order by glucose broth (97.62%) oat meal broth (94.10%). All these constitute a group of media which are quite promising and the same standard with regards to antibiotic production as the assay valves obtained were not significantly different (C.D.=4.33) next in order are modified glucose asparagine broth, yeast extract broth, soyabean meal broth, Glucose asparagine broth which support moderate production of antibiotic substance giving only 86.91%, 75.10%, 72.54%, 62.70% inhibition of spore germination. Glycerol asparagine broth and soluble starch supported the least production of the antibiotic substance giving only 52.12%, 50.14% inhibition hence not considered promising any further. The maximum growth was recorded in Beef extract broth, Yeast extract broth, Soyabean meal broth followed in order by mycelial dry weight being 0.9864 gm, 0.9734 gm, 0.9718 gm respectively.

Data presented in Table 1 indicate that are production of antibiotic substance & the growth of *Streptomyces carcinomycicus* occurs both in natural and synthetic media. This Corroborates the finding of some of earlier workers that actinomycetes are capable of producing the antibiotic substance in the both type of media.

In the present investigation beef extract medium supported the greater rate of production of antibiotic substance by *S. carcinomycicus*. On the other hand modified glucose asparagine medium, a chemically defined medium was found to support 86.91% production of antibiotic substance. Ainsworth *et al*.. (1947), Sawnder and Sylvester (1947), Dulaney (1948) Eiser and Mefartane (1948), Tharnibery and Anderson (1948), Woodruff and Ruger (1948), Flaconer *et al*.. (1949) devised chemically defined media. In same instances the yield obtained with culture grown in these chemically defined media exceeded those obtained when the same culture were grown in meat extract, soyabean meal or yeast containing media. Gottliets *et al*.. (1951), 1954) and Matsuoka (1953) formulated a defined medium that supported growth and chloramphemical production by selected cultures of *Streptomyces venezuelae*.

The other synthetic media tested in the present investigation (Glycerol asparagine and soluble starch) gave low assay values, being 52.12%, 50.14% respectively. This may be because they lack glucose from their composition. This shows that Glucose is an important constituent for the production of active antibiotic substance by *S. carcinomycicus*, Glucose was also shown to be an important constituent for the production of active antibiotic substance by *S. griseus* Agra strain (Basu Chaudhary 1961)

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