



**RESEARCH ARTICLE**

**Antibiotic Production by *Streptomyces* Species Medium Supplied With Vitamins**

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**ABSTRACT**

Three antibiotic producing species of *Streptomyces* viz; *S. carcinomycicus*, *S. galbus* and *S. hygroscopicus* were isolated from soil of unusual habitats. The effect of 5 vitamins viz; ascorbic acid, nicotinic acid, biotin, thiamine and riboflavin on antibiotic substance production by these species was studied in culture medium. This study revealed that riboflavin at the concentration of 100  $\mu$  gm/litre supported maximum production of antibiotic substance by *Streptomyces carcinomycicus* and *S. hygroscopicus*. Next in order for these species were ascorbic acid, nicotinic acid, biotin and thiamine. On the other hand, ascorbic acid at the concentration of 100  $\mu$  gm/litre was found to be the best treatment for the maximum production of antibiotic substance by *Streptomyces galbus*. The next in order for this species were riboflavin, biotin, nicotine acid and thiamine. In all the species of *Streptomyces*, thiamine was found to be least effective in increasing antibiotic production.

**Keywords:** *Streptomyces*, antibiotic production, vitamins

**INTRODUCTION**

Out of all the metabolites of actinomycetes, the antibiotics are of utmost significance to mankind. Besides medicine, now a days they find extensive application in the control of plant diseases caused by bacterial and fungal pathogens. Several review articles indicate the wide spread interest in the field of agricultural antibiotics (Nandi *et al*; 1975; Iyengar, 1979). Besides environmental factors, the nutritional factors such as nitrogen and carbon sources, amino acids, organic acids, micronutrients profoundly influence the growth and antibiotic activity of the actinomycetes in culture media some workers have reported stimulating effect on antibiotic production by *Streptomyces* species in culture media supplied with different vitamins (Gupta 1967; Johri and Brodie 1971).

It was therefore, considered worthwhile to study the effect of 5 vitamins viz; ascorbic acid, nicotinic acid, Biotin, thiamine and riboflavin on the production of antibiotic substance by *Streptomyces carcinomycicus*, *S. galbus* and *S. hygroscopicus* in culture medium. The findings of this study are communicated in present paper.

**MATERIALS AND METHODS**

Three species of *Streptomyces* viz; *S. carcinomycicus*, *S. galbus* and *S. hygroscopicus*, isolated from soils of unusual habitat were found to be promising antibiotic producer during primary screening. These were maintained on tryptophan yeast extract agar medium. In the present study, these species were allowed to grow in glucose asparagine broth in 250 ml Erlenmeyer flasks in triplicate. The pH of the medium was adjusted at 6.5.

To study the effect of vitamins on antibiotic production, the medium was supplemented with vitamins viz; ascorbic acid, nicotinic acid, biotin, thiamine and riboflavin separately under aseptic conditions in four different concentrations at the rate of 0.1, 1, 10 and 100  $\mu$  gm/litre, keeping three

replicates for each treatment. Normal medium served as control in all these cases, the flasks were incubated at  $28 \pm 1^\circ\text{C}$  in B.O.D. incubator for 5, 10 and 15 days, Paliwal (1984).

In all the above sets, the antibiotic activity present in the culture filtrate was ascertained 5, 10 and 15 days of incubation following spore germination test using spores of *Colletotrichum gloeosporioides* (Brain, 1957). The data obtained for the percentage inhibition of spore germination of *Colletotrichum gloeosporioides* was subjected to the test of significance using "Analysis of variance" method. At the end of 15<sup>th</sup> days, the mycelia mat from the flasks was removed by filtration through previously weighed whatman No 1 filter paper and mycelial dry weight was determined for each treatment. Further, the initial pH of the medium and final pH of the culture filtrate obtained after the growth of organism was determined by Cambridge glass electrode pH meter. The results obtained are presented in Table 1, 2 and 3.

**Table 1:** Effect of different vitamins on the growth and production of antibiotic substance by the strain of *S. carcinomyicus* (isolate A-3)

Treatment	Conc. gm/lt.	Percentage inhibition of spore germination (Mean of 30 observations)			Mean of 3 Replicates	
		Incubation period in days			Mycelial dry weight in gms after 15 days of growth	Final pH
		5	10	15		
Ascorbic Acid	0.1	32.37	41.52	53.15	0.2416	6.2
	1.0	41.72	52.16	62.35	0.2695	6.4
	10.0	52.98	64.38	71.53	0.3852	6.8
	100.0	62.56	73.95	86.93	0.4257	6.9
Nicotinic Acid	0.1	31.82	40.56	52.83	0.2596	6.4
	1.0	41.25	51.76	61.82	0.2984	6.8
	10.0	50.56	62.83	70.63	0.3257	7.1
	100.0	60.12	70.52	82.53	0.3692	7.2
Biotin	0.1	34.53	42.85	54.56	0.2853	6.4
	1.0	42.13	53.62	64.51	0.3164	6.6
	10.0	51.32	62.56	69.11	0.3692	6.8
	100.0	60.23	70.86	81.59	0.3953	6.9
Thiamine	0.1	24.62	31.56	49.68	0.1426	6.4
	1.0	29.52	34.13	52.61	0.1995	6.6
	10.0	32.12	38.52	57.93	0.2358	6.8
	100.0	36.96	43.39	62.35	0.2634	7.0
Riboflavin	0.1	32.53	42.32	51.35	0.2698	6.8
	1.0	42.26	51.52	63.57	0.3548	6.8
	10.0	53.16	63.26	73.92	0.3985	7.1
	100.0	64.35	75.62	88.32	0.4453	7.2
Control	-	20	35	50	0.1485	6.8

**Table 2:** Effect of different vitamins on the growth and production of antibiotic substance by the strain of *S. galbus* (isolate S-9)

Treatment	Conc. gm/lt.	Percentage inhibition of spore germination (Mean of 30 observations) Incubation period in days			Mean of 3 Replicates	
		5	10	15	Mycelial dry weight in gms after 15 days of growth	Final pH
Ascorbic Acid	0.1	41.25	49.28	64.16	0.2538	6.6
	1.0	52.28	62.63	72.82	0.3295	6.7
	10.0	63.36	75.13	81.83	0.3645	6.8
	100.0	72.56	84.59	96.38	0.4268	7.2
Nicotinic Acid	0.1	41.26	48.53	62.68	0.2139	6.6
	1.0	50.18	58.12	70.92	0.2476	6.7
	10.0	58.62	63.29	86.82	0.2953	6.8
	100.0	70.15	75.28	90.26	0.3515	7.2
Biotin	0.1	42.28	51.62	62.85	0.2431	6.7
	1.0	52.68	61.85	74.21	0.2763	6.8
	10.0	67.59	74.81	81.08	0.3258	6.9
	100.0	73.26	81.15	90.83	0.4096	7
Thiamine	0.1	33.24	38.82	56.53	0.2045	6.7
	1.0	35.65	40.38	58.69	0.2437	6.8
	10.0	38.08	42.82	68.52	0.2856	6.9
	100.0	42.68	48.96	74.58	0.3168	7.3
Riboflavin	0.1	40.24	48.52	60.83	0.2859	6.7
	1.0	50.56	55.63	70.83	0.3154	6.9
	10.0	56.71	61.52	84.83	0.3572	7.0
	100.0	61.85	75.32	94.25	0.4276	7.2
Control	-	25	40	55	0.1046	6.8

## RESULTS AND DISCUSSION

It is quite evident from Tables 1, 2 and 3 that the different vitamins when added separately to the culture medium, stimulated the production of antibiotic substance to variable extent. Out of the five vitamins tested, riboflavin was found to be the most effective as it supported the maximum production of antibiotic substance by *Streptomyces carcinomycicus* and *S. hygrosopicus*. However, ascorbic acid was found to be most effective treatment for *S. galbus*. It appears that concentration of vitamin used in the main factor affecting antibiotic production as assayed in terms of percentage in hyperon of spore germination of *colletotrichum gloeosporioides*, which is directly proportional to the concentration of the vitamins in the basal medium. It is also clear that the production of antibiotic substance is significantly increased with the increase in incubation period from 5 to 15 days. The maximum inhibition is spore germination was noted after 15 days in all the cases.

Thus, it can be concluded that riboflavin 100µgm/litre was the best treatment for maximum antibiotic production by *S. carcinomycicus* and *S. hygrosopicus*. Next in order for these two species were ascorbic acid, nicotinic acid, biotin and thiamine. On the other hand, ascorbic acid at the

concentration of 100 µg/mlitre was the best treatment for maximum production of antibiotic by *S. galbus*. The next in order for this species were riboflavin, biotin, nicotinic acid and thiamine. In all the three species, thiamine was found to be least effective in increasing antibiotic production. The results are in conformity with the findings of Eiser and McFarlane (1948), Gupta (1967) and Jayant (1982) that vitamin effectively stimulated the antibiotic production by the *Streptomyces* species.

**Table 3:** Effect of different vitamins on the growth and production of antibiotic substance by the strain of *S. Hygroscopicus* (isolate F-14)

Treatment	Conc.g m/lt.	Percentage inhibition of spore germination (Mean of 30 observations) Incubation period in days			Mean of Replicates	
		5	10	15	Mycelial dry weight in gms after 15 days of growth	Final pH
Ascorbic Acid	0.1	26.52	36.92	47.34	0.2648	6.7
	1.0	36.32	46.97	54.39	0.2965	6.8
	10.0	48.16	60.32	67.93	0.3124	6.9
	100.0	54.34	64.15	80.42	0.3753	7.2
Nicotinic Acid	0.1	25.95	34.82	46.93	0.2395	6.7
	1.0	34.91	45.62	55.92	0.2746	6.8
	10.0	46.32	56.82	64.38	0.2985	6.8
	100.0	56.92	63.92	78.5	0.3162	6.9
Biotin	0.1	29.62	37.85	49.82	0.2148	6.7
	1.0	27.14	49.35	59.96	0.2452	6.8
	10.0	46.82	58.36	64.74	0.2693	6.8
	100.0	55.62	65.93	76.25	0.2975	7.0
Thiamine	0.1	21.53	27.52	45.18	0.2016	6.7
	1.0	25.25	30.82	48.08	0.2168	6.7
	10.0	28.14	34.49	52.63	0.2472	6.8
	100.0	32.95	40.83	60.25	0.2659	6.9
Riboflavin	0.1	28.19	38.42	50.12	0.2436	6.7
	1.0	38.34	48.75	58.92	0.2965	6.8
	10.0	49.13	59.04	69.42	0.3462	7.1
	100.0	60.92	71.53	84.18	0.3796	7.2
Control	-	20.00	30.00	40.00	0.1142	6.7

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#### REFERENCES

1. Brian P.W. (1957): Ecological significance of antibiotic production. In *Microbial Ecology*. Cambridge University, press, 168-188.
2. Eiser H.M. and McFarlane W.D. (1948): Metabolism of *Streptomyces griseus* in relation to the production of *Streptomycin* Can. J. Res., 26: 164-173.

3. Gupta S. (1967): Physiological studies on the production and action of an antifungal substance by an *Actinomycetes*. Ph.D. thesis, Agra University, Agra
4. Iyengar M.R.S. (1979): Antibiotics in plant disease control an overview of trends and needs. *Indian Phytopath* 32(2): 343-351.
5. Jayant M. (1982): Studies on the metabolism of a streptomycetes species and the biological control of *Alternaria* leaf spot of crucifers. Ph.D. thesis Agra University, Agra.
6. Johri B.N. and Brodei H.J. (1971): The physiology of production of the antibiotic cyathin in *Cyathus helena*. *Can. J. Microbiolo*, 17: 1243-1245
7. Nandi J., De B.K. and Bose S.K. (1975): Evaluation of mycobacillin and versicolin as agricultural fungicides. Antimicrobial spectrum and phytotoxicity. *J. Antibiotics* 28; 988-992
8. Palwal M. (1984): The antibiotic substances produced by *Streptomyces* sp., Ph.D. thesis, Agra University, Agra.