SYNTHESIS OF THIAMINE BY MOULDS

by

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SCHEUNERT AND SCHIEBLICH[1] detected the formation of thiamine in *Aspergillus oryzae* grown on vitamin-free food used in rat experiments. The mycelium contained considerable quantities of vitamin B_1 (6 I.U. per gram) and much of vitamin B_2. GORCICA[2], PETERSON AND STEENBOCK found that 10% of the mycelium of Aspergillus Sydowi furnished enough vitamin B_1 to support good growth in rats. KYUYA SAKURAI[3] found that the Koji mould (*Aspergillus oryzae*) grown in sucrose medium containing iron salts and added nitrogen was able to synthesise vitamin B_1. ROSSI AND JACOLI[4] have observed that *Aspergillus niger* grown in a synthetic medium gives a thiamine content 12.9 γ/gram in the mat and the mat was used as a Beriberi preventive diet. RAMAKRISHNAN AND BANERJEE[5] while investigating moulds grown on oil seeds for their lipolytic activity to obtain good lipolytic strain, suspected the synthesis of certain water-soluble vitamins like ascorbic acid, riboflavin, thiamine, etc., in moulds isolated from oil seeds and found that these vitamins accelerate the lipolytic activity. Hence an investigation was undertaken to study the moulds grown on different oil seeds for their vitamin B_1 content with an idea to obtain a strain of mould which can synthesise large quantities of thiamine. The different factors which influence the synthesis of thiamine were also studied and the results recorded.

EXPERIMENTAL PART

The moulds grown on oil seeds were subcultured in Czapek agar medium in petri dishes and the pure strains prepared. The pure strains of different moulds obtained were inoculated in culture flasks containing 200 ml of Czapek medium and the pH was adjusted to 6.6. The culture flasks were incubated at 26° C for different periods. After the period of incubation the mycelial mat was removed from each flask and the thiamine content of the mat as well as the culture medium was estimated according to CLAUSEN AND BROWN'S[6] method. From Table I it may be seen that *Aspergillus Flavus* AF_1 synthesises a considerable amount of thiamine. It is found that in all cases the maximum synthesis takes place on the 15th day. Hence the optimum time of ageing is 15 days for the maximum synthesis of thiamine. The different factors which influence the synthesis of thiamine by this strain AF_1 were studied in detail.

Effect of pH of the medium

Aspergillus flavus AF_1 was inoculated in culture flasks containing 200 ml of Czapek liquid medium whose pH was adjusted to different levels in different flasks using dilute hydrochloric acid. The flasks were incubated at 28° C for different days. After the period of incubation the mycelial mat was removed, air-dried, and the thiamine content of the mat and the culture medium was estimated. The results are given in Table II.

From Table II, it can be seen that the maximum synthesis of thiamine takes place at pH 6.6. For further experiments the pH was adjusted to 6.6.

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TABLE I

MICROGRAMS OF THIAMINE PER GRAM OF SAMPLE

Name of the Mould	*Mycelial mat*						*Culture medium*					
	3rd day	*5th day*	*10th day*	*15th day*	*20th day*	*25th day*	*3rd day*	*5th day*	*10th day*	*15th day*	*20th day*	*25th day*
1. *Aspergillus flavus* AF_1	5.2	6.8	10.3	15.1	9.2	7.6	0.5	0.8	1.23	2.18	1.09	0.64
2. *Aspergillus fumigatus*	1.2	2.9	5.3	7.5	3.1	1.2	0.02	0.2	0.91	0.98	0.72	0.08
3. *Penicillium* SP.	0.5	0.6	0.9	1.3	1.0	0.4	0.01	0.68	0.09	0.13	0.08	0.02
4. *Aspergillus flavus*$_2$	3.1	4.6	7.8	11.3	8.1	6.4	0.41	0.62	1.08	1.13	0.94	0.51
5. *Aspergillus flavus*$_3$	2.5	3.2	5.9	9.8	7.2	5.8	0.32	0.54	1.01	1.09	0.89	0.48
6. *Aspergillus flavus*$_4$	1.3	2.1	4.2	8.3	6.9	5.2	0.29	0.51	1.01	0.07	0.82	0.44
7. *Aspergillus flavus*$_5$	0.9	2.3	4.8	6.9	4.1	1.8	0.06	0.12	0.86	0.89	0.76	0.09

TABLE II

MICROGRAMS OF THIAMINE PER GRAM OF SAMPLE

Name of the Mould: Aspergillus Flavus AF_1

pH	*Mycelial mat*						*Culture medium*					
	3rd day	*5th day*	*10th day*	*15th day*	*20th day*	*25th day*	*3rd day*	*5th day*	*10th day*	*15th day*	*20th day*	*25th day*
1.8	0.8	0.9	1.7	3.8	1.9	1.7	0.03	0.09	0.18	0.96	0.07	0.09
2.7	0.9	0.9	2.9	6.4	2.3	1.9	0.06	0.11	0.26	1.08	1.17	0.16
3.1	1.3	1.2	3.1	7.2	3.1	2.2	0.09	0.16	0.31	1.11	0.26	0.21
4.6	1.7	1.7	4.2	8.3	4.7	2.6	0.11	0.27	0.52	1.26	0.39	0.28
5.1	2.1	2.9	5.8	9.9	5.2	3.2	0.15	0.31	0.76	1.31	0.52	0.34
6.5	3.7	4.2	7.2	11.5	7.3	4.9	0.29	0.52	0.74	1.52	0.78	0.52
6.6	**5.2**	**6.8**	**10.3**	**15.1**	**9.2**	**7.6**	**0.52**	**0.78**	**1.23**	**2.18**	**1.09**	**0.64**
6.8	2.1	3.1	7.4	10.2	7.1	5.8	0.15	0.35	0.91	1.12	0.85	0.39
7.0	0.8	0.7	1.3	2.8	1.7	1.2	0.04	0.12	0.34	0.14	0.32	0.14

TABLE III

MICROGRAMS OF THIAMINE PER GRAM OF SAMPLE

Name of mould: Aspergillus flavus AF_1 — Effect of temperature

Temp. in °C	*Mycelial mat*						*Culture medium*					
	3rd day	*5th day*	*10th day*	*15th day*	*20th day*	*25th day*	*3rd day*	*5th day*	*10th day*	*15th day*	*20th day*	*25th day*
21° C	—	—	—	—	—	—	—	—	—	—	—	—
23° C	2.6	2.9	4.2	6.2	5.1	3.1	0.19	0.23	0.46	0.72	0.45	0.25
24° C	4.1	4.7	8.5	10.3	8.3	5.2	0.31	0.47	0.85	1.24	0.82	0.32
26° C	**5.2**	**6.8**	**10.3**	**15.1**	**9.2**	**7.6**	**0.52**	**0.78**	**1.23**	**2.18**	**1.09**	**0.64**
28° C	4.8	5.4	7.8	5.1	7.3	4.8	0.40	4.51	0.91	1.34	0.87	0.43
32° C	3.2	4.1	5.2	9.8	5.4	3.9	0.26	0.29	0.63	0.75	0.59	0.37
37° C	1.9	2.6	3.4	5.6	3.2	2.7	0.18	0.17	0.32	0.29	0.32	0.29

TABLE IV

MICROGRAMS OF THIAMINE PER GRAM OF SAMPLE

Name of mould: Aspergillus Flavus AF_1 — Effect of nitrogen salts

Concentration of the salt	*Mycelial mat*				*Culture medium*			
	Ammonium chloride	*Ammonium nitrate*	*Ammonium phosphate*	*Ammonium sulphate*	*Ammonium chloride*	*Ammonium nitrate*	*Ammonium phosphate*	*Ammonium sulphate*
0.05%	17.2	22.6	21.9	22.3	2.18	2.18	2.18	2.18
0.10%	20.5	31.9	27.6	28.9	2.21	2.19	2.18	2.29
0.2%	25.6	42.6	35.2	36.1	2.26	2.31	2.20	2.41
0.4%	29.32	59.3	41.3	40.4	2.32	2.42	2.52	2.83
0.6%	10.2	15.6	12.7	13.2	1.52	1.58	2.12	2.13
0.8%	3.8	5.9	4.4	5.1	0.49	0.59	0.98	0.97

TABLE V

MICROGRAMS OF THIAMINE PER GRAM OF SAMPLE

Name of mould: Aspergillus Flavus AF_1

Days	*Mycelial mat*				*Culture medium*			
	Czapek medium	*Groundnut-cake medium*	*Sesamum-cake medium*	*Coconut-cake medium*	*Czapek medium*	*Groundnut-cake medium*	*Sesamum-cake medium*	*Coconut-cake medium*
3rd day	5.2	15.6	13.2	11.1	0.52	0.87	0.54	0.18
5th day	6.8	22.8	19.8	15.3	0.78	1.96	0.82	0.32
10th day	10.3	35.9	30.6	21.7	1.23	2.32	1.21	0.46
15th day	15.1	62.3	54.2	35.9	2.18	3.15	2.19	0.92
20th day	9.2	46.1	39.6	26.8	1.09	1.72	1.76	0.65
25th day	7.6	30.5	20.1	21.9	0.64	0.98	0.89	0.52

Effect of temperature

Aspergillus Flavus AF_1 was inoculated in culture flasks containing 200 ml Czapek liquid medium. The pH was adjusted to 6.6. The flasks were incubated at different temperatures for different periods and after the period of incubation the mycelial mats were removed and the thiamine content of the mycelial mat and the culture medium was estimated.

From the Table III, it can be seen that the optimum temperature for the maximum synthesis of thiamine is 26° C. For further experiments the temperature was adjusted to 26° C.

Effect of light

Synthesis of thiamine by moulds is slightly more if the culture flasks containing the medium inoculated with the mould is kept in darkness than in day light. The experiments are being continued in this line.

Effect of nitrogen salts

The effects of different nitrogen salts at different concentrations on the synthesis of thiamine by *Aspergillus flavus* AF_1 were studied. AF_1 was inoculated in different culture flasks containing 200 ml liquid Czapek medium and different amounts of nitrogen salts and adjusted to pH 6.6 and incubated at 26° C for 15 days. After the period of incubation the mycelial mat was removed and the thiamine content of the mat and the culture medium was estimated. Always a control accompanied each experiment. The results are given in Table IV.

From Table IV, it can be seen that, in general, nitrogen salts added to the medium at the concentration of 0.4% accelerate the synthesis of thiamine by moulds.

Effect of different media on the synthesis of thiamine by Aspergillus flavus AF_1

As it is found that *Aspergillus flavus* AF_1 synthesises a considerable amount of thiamine, an attempt was made to find out a cheap medium to grow this strain on a large scale. Different oil-seed cake media, groundnut-cake medium, coconut-cake medium, and sesamum-cake medium each containing 20% oil-free cake were tried. *Aspergillus flavus* AF_1 was inoculated in culture flasks containing 200 ml of different cake solutions. The flasks were incubated at 26° C for different periods. After the period of incubation, the thiamine content of the mat and the culture medium was estimated and the results are given in Table V. From Table V, it may be seen that groundnut-cake medium is very suitable to grow this mould. The experiments are being carried out to study the effect of different amount of oil in the cake medium, addition of sterols, vitamins, unsaponifiable matter etc., to the media, on the synthesis of thiamine, in order to find out the optimum conditions for the maximum growth of the mould which can synthesised maximum amount of thiamine. The results will be communicated as the work proceeds.

CONCLUSION

It has been found that *Aspergillus flavus* AF_1, isolated from the moulds grown on oil seeds synthesises an appreciable quantity of thiamine and grows well in groundnut cake medium. The work is under progress to prepare best mutants of this strain by using different carcinogens, hormones and ultraviolet treatment, etc.

SUMMARY

Certain strains of moulds grown on oil seeds synthesise thiamine when grown in Czapek liquid medium. The synthesis of thiamine by *Aspergillus flavus* AF_1 was studied in detail.

1. The optimum pH for the maximum synthesis of thiamine by *Aspergillus flavus* AF_1 is 6.6. The synthesis is maximum on the 15th day. The optimum temperature is 26° C.
2. Synthesis of thiamine is more in the case of culture flasks kept in darkness than those kept in daylight.
3. Nitrogen salts at 0.4% concentration accelerate the synthesis of thiamine by *Aspergillus flavus* AF_1 to a considerable extent.
4. Groundnut-cake medium seems to be a good medium to grow on a large scale.

RÉSUMÉ

Certaines souches de moisissures cultivées sur des graines oléagéneuses synthétisent de la

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thiamine lorsqu'on les cultive dans du milieu liquide de Czapek. La synthèse de thiamine par *Aspergillus flavus* AF_1 a été étudiée en détail.

1. Le pH optimum de la synthèse maximale de thiamine par *Aspergillus flavus* AF_1 est de 6.6. Le maximum de synthèse est atteint le quinzième jour. La température optimale est de 26° C.
2. La quantité de thiamine synthétisée est plus importante lorsque l'on garde les bouteilles à l'obscurité que lorsqu'on les expose à la lumière du jour.
3. Les sels azotés à la concentration de 0.4% accélèrent considérablement la synthèse de thiamine par *Aspergillus flavus* AF_1.
4. Le tourteau d'arachides semble être un bon milieu pour des cultures en grand.

ZUSAMMENFASSUNG

Gewisse Stämme von Schimmelpilzen, welche auf Ölsamen gezüchtet sind, synthetisieren Thiamin wenn sie in Czapek's Nährlösung gezüchtet werden. Die Synthese von Thiamin durch *Aspergillus flavus* AF_1 wurde eingehend untersucht.

1. Das pH-Optimum für die Maximalsynthese von Thiamin durch *Aspergillus flavus* AF_1 ist 6.6. Die Synthese ist am 15. Tage maximal. Die optimale Temperatur beträgt 26° C.
2. Wenn die Kulturflaschen im Dunkeln bewahrt werden, wird mehr Thiamin synthetisiert als wenn man sie dem Tageslicht aussetzt.
3. Stickstoffsalze in einer Konzentration von 0.4% beschleunigen die Thiaminsynthese durch *Aspergillus flavus* AF_1 bedeutend.
4. Erdnusskuchen scheint ein gutes Medium zum Züchten im Grossen zu sein.

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Received October 9th, 1951