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Production of Riboflavin by Local Isolates of *Aspergillus terreus*

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Riboflavin is a yellow, water soluble solid which plays an important role in growth and normal health of many living organisms as well as man. *Aspergillus terreus* was used for the production of riboflavin which was markedly affected by the composition of the culture medium, incubation period and agitation speed. The growth and production rate of vitamin was high by using shaked flask method. Maximal productivity (1044mg/L) was achieved when fungus was allowed to grow on synthetic medium fortified with glycine, oleic acid and ferric chloride. The used media at pH 6 and agitation speed at 250 rpm showed a good productivity rate for riboflavin in supplemented medium rather than medium containing no additives.

Key words: Riboflavin, *Aspergillus terreus*, human health.

Riboflavin, the so called vitamin B2, is widely distributed in plants and animals and plays an important role in organism. It is yellow crystalline compound C17 H20 N4 O6, is precursor of flavin mononucleotide and flavin adenine dinucleotide which acts as coenzymes for wide variety of enzymes in the intermediate metabolism (Lim, S.H. *et al,* 2001). It plays an important role in growth and normal health of many living organism as well as amn. Lack of riboflavin causes ariboflavinosis (Cooperman and Lopaz, 1984). Many *Aspergillus spp*. had been used for riboflavin production using a variety of cheap by products containing media (Sabry, S.A. *et al,* 1993). Three kinds of microorganisms are currently in use for industrial riboflavin production are *Ashbya gossypii, Candida formata, Bacillus subtilis*(Stahmann, K.P., *et al,* 1999). Riboflavin is an important vitamin as it plays an important role in producing antioxidants agent by scavenging damaging particles in the body known as free radicles (Lim, S.H. *et al,* 2001). *Aspergillus terreus* is used as riboflavin production in a medium containing beet molasses as the sole carbon source (Sabry, S.A. *et al,* 1995).

The present study involves the production of riboflavin by local isolates of *Aspergillus terreus*, cultivated on different culture media at different incubation periods.

MATERIAL AND METHODS

Micro-organism

Aspergillus terreus was used in present study procured from NCIM, National Chemical Laboratory, Pune as NCIM-660.

Maintenance of the micro-organism

The tested fungus was maintained on agar

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slants at 4°C using potato dextrose agar medium. Subcultures were almost carried out after every 4 weeks.

Substrate

Corn steep liquor was used for riboflavin production which is obtained form ANIL Products Ltd. Ahmadabad.

Media

Medium 1

1 gm of corn steep liquor in100ml of distilled H_2O. Add 1 gm of glucose to it and then add 0.5 gm of peptone.

Medium 2

1 gm of corn steep liquor in100ml of distilled H_2O. Add 1 gm of glucose to it and then add 0.5 gm of peptone. Then medium id fortified with 0.2 gm of yeast extract. Also 0.15 gm of KH_2PO_4 and 0.025 gm of $MgSO_4.7H_2O$.

Medium 3

It has the same composition of medium 2, but supplemented with FeCl3, 0.005 mg/l, oleic acid 1.5 g/l and glycine 0.2 g/l (Heba A. *et al,* 2009).

Material for riboflavin assay

Sodium hydroxide (2ml) and Potassium phosphate buffer pH 6.5.

Preparation of spores

The spore suspension was prepared by dispersing the spores from potato dextrose agar slant in saline suspension (which is prepared by taking 0.085 gm NaCl in 100 ml of distilled water) with sterile inoculating loop.

Cultivation

The tested medium was initially adjusted to pH 6.5. 50 ml of portions of the medium were dispensed in 250 ml Erlenmeyer flasks. The flasks were sterilized by autoclaving at 121°C for 15 minutes. And inoculated with 2 ml spore suspension of 72h old culture and incubated at 30°C on a rotary shaker.

Dry weight estimation

The fungus growth was estimated by centrifugation technique. The fungus was washed, dried at 60°C until constant weight. The culture filtrate was then analyzed for their contents of riboflavin.

Estimation of riboflavin

0.8 ml of the above culture filtrate is taken into the flasks and to this add 0.2 ml of sodium hydroxide. Now take 0.4ml of resulting solution in flasks which was then neutralized with 1 ml (0.1M) potassium phosphate buffer and the absorbance of the produced color was measured at λ 445 nm. The amount of riboflavin produced was calculated from a previously prepared standard curve (Lim, S.H. *et al,* 2003).

RESULTS AND DISCUSSION

Effect of cultivation technique and Medium Composition

The present work was started by finding the ability of the tested fungus to grow and produce riboflavin on different tested media using shaking fermentation conditions. In present study, three different media were used with different concentration of some constituents.

The result of the study shows that better production of riboflavin was observed under shaked culture techniques. The results in Table 1 and 2 showed the fungus growth and riboflavin production during the cultivation of *Aspergillus terreus* on medium (1) and medium (2) at different agitation speeds using shaked flasks on different incubation period. The tested fungus survived well on all cultivation medium but the medium (3) proved to be most suitable for both growth and riboflavin production. Similar findings were observed by Heba, A. *et al,* 2009 and Sabry and Ghozlan, 1994. The good productivity was recorded with the formulation of medium 3 as compared with those attained with medium 2 was due to addition of Fecl3, oleic acid and glycine.. Therefore, it is clear that the additives in medium support the fungus growth and vitamin production. In support to the finding, Schlee and Straube also reported that such supplements stimulate the falvogenesis process (Heba, A. *et al,* 2009).

Effect of Agitation speed on riboflavin production

The agitation speed has a great effect on the production rate of vitamin as well as fungus. The result given in table 2 and table 3 containing medium 2 and 3 revealed that the vitamin production rate increases with the increase of agitation speed. The maximum vitamin production (1044) mg/l was obtained after 7 days at 250 rpm.

K.M. Ghanem and H.A. Ghozlan have given that production of Riboflavin by *Aspergillus terreus* from beet molasses. Similar finding were observed by Sabry and Ghozlan, 1994. Foaad and Affif, 2000 proved that *Asp. terreus* could utilize

glucose as carbon source and yield 0.6 g/L riboflavin. The *Asp. terreus* during Riboflavin fermentation using corn steep liquor as a substrate utilizes some organic nutrient in substrate. In certain cases, the glucose may be replaced by a lipid such as corn oil or low level of corn oil may be added to glucose to stimulate Riboflavin production rate or yield.. Hence maximum production observed was 1044 mg/L and corn steep liquor supported maximum production of riboflavin by using the respective fungus.

Table 1. Fungus growth and riboflavin production during the cultivation of *Aspergillus terreus* on medium 1 at different agitation speed using shaked flasks

Incubation	Agitation rate 150 rpm		Agitation rate 250 rpm	
period(day)	Dry weight (mg/l)	Riboflavin (mg/50ml)	Dry weight (mg/50ml)	Riboflavin (mg/l)
5	0.45	354	0.35	347
6	0.48	562	0.42	420
7	0.53	596	0.45	562

Table 2. Fungus growth and riboflavin production during the cultivation of *Aspergillus terreus* on medium 2 at different agitation speed using shaked flasks

Incubation	Agitation rate 150 rpm		Agitation rate 250 rpm	
period(day)	Dry weight (mg/l)	Riboflavin (mg/50ml)	Dry weight (mg/50ml)	Riboflavin (mg/l)
5	0.63	665	0.64	578
6	0.65	782	0.67	682
7	0.68	780	0.69	790

Table 3. Effect of agitation speeds on the growth and riboflavin production by *A. terreus* grown on medium 3 at different incubation periods using shaked flasks

Incubation	Agitation rate 150 rpm		Agitation rate 250 rpm	
period(day)	Dry weight (mg/l)	Riboflavin (mg/50ml)	Dry weight (mg/50ml)	Riboflavin (mg/l)
5	0550	855	615	800
6	680	900	643	1044
7	659	780	600	980

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