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Riboflavin production by *Candida Guilliermondia*: Alternative modes of improving vitamin yield

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ABSTRACT

The importance of nutrition for human health and its influence on the onset and course of many diseases are now a days considered as proven. Vitamins trace organic substances are required in the diet because they are of great value in the growth and metabolism of living cells. The present study focuses on the riboflavin production by *Candida guillermondia* NCIM 3126 and effect of β cyclodextrin on the production of riboflavin. Sodium lauryl sulphate shows higher yield of riboflavin as compare to Triton 100X. β cyclodextrin shows the highest yield of riboflavin than surfactant. Therefore, use of β cyclodextrin as an alternative mode for improving the riboflavin production from *Candida guillermondia* NCIM 3126.

Keywords; Riboflavin, *Candida guillermondia*, sodium lauryl sulphate, β cyclodextrin

INTRODUCTION

Vitamins, a trace organic substance is required in the diet because they are of great value in the growth and metabolism of the living cells. Riboflavin, the so called vitamin B2, is yellow green fluorescent watersoluble pigment widely distributed in plants and animal cells. It was discovered in 1920 and first isolated in egg albumin [1]. It has molecular weight of 376.37. It is a vital precursor to the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Both these compounds are co-factors of a variety of enzymes in energy metabolism, particularly the dehydrogenases and oxidases [2].

Riboflavin is especially used in the pharmaceutical industry, food enhancement and animal food industry. Thus, vitamins have become one of the important fermentation products in biotechnology industry. Riboflavin is commercially produced by chemical or by biochemical synthesis, the latter being the preferred route because it is cheaper [3]. Approximately 4000 tones of this vitamin are consumed annually, and chemical and biological synthesis is both employed for its production [4]. Biological synthesis is preferred because it has the merits of saving half the cost, reducing waste and energy requirements, and using renewable resources like sugar or plant oil [5].

Riboflavin is produced by many microorganisms, including bacteria, yeasts, and molds [6]. The first microorganism reported to be capable of producing riboflavin was *Clostridium acetobutylicum* and subsequently two other microorganisms, *ascomycetes*, namely, *Eremothecium ashbyii* and *Ashbya gossypii* have been reported by Prabhakar et.al. (1993)[7].

In the present work, riboflavin production by *Candida guillermondia* NCIM 3126 was studied and the effect of surfactants- sodium lauryl sulfate (SLS) and Triton- 100X was observed. Finally, the effect of β-cyclodextrin on the production of riboflavin was observed.

MATERIALS AND METHOD

During experimentation research grade media components made from high media and Loba chemicals were used.

1. **Organism** – *Candida guillermondia* NCIM – 3126
2. **Components for fully defined media** – Glucose 40 gm/L, urea 1.25 gm/L, KH_2PO_4 0.50gm, $MgSo_4$ 0.20gm, biotin

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0.001gm, trace elements --, agar 31gm, distilled water 1000ml.

3. **Components for fermentation media**- Glucose 40gm/L, urea 1.24gm KH_2PO_4 0.50gm, $MgSo_4$ 0.20gm, biotin 0.001gm, trace elements --,pH 5.0, distilled water 1000ml [8].
4. **Other chemicals** –
   a. Concentrated ammonium sulphate solution
   b. Phenol crystals
   c. Diethyl ether
   d. Surfactants – SLS and Triton 100X

Method for the production of riboflavin by *Candida guillermondia* NCIM – 3126 was inoculated. It was incubated at 28^0c, 200rpm on orbital shaking incubator (Remi instrument) for 6-7 days.

Extraction of riboflavin: After completion of fermentation, the broth was subjected to extraction. The broth was heated at 70-80^0c for 30 min then add concentrated ammonium sulfate and centrifuge the broth at 5000-7000 rpm to separate precipitation. Supernatant was separated add 2ml phenol centrifuge the broth at 1000-2000 rpm. 2 ml phenol was added with constant shaking and centrifuge and adds 0.3 to 0.5 ml of water and 15 ml of ether shake and centrifuge. Separated water layer from ether layer was subjected for chromatographic separation for detection of riboflavin [9].

Qualitative detection of riboflavin: For qualitative detection of riboflavin, thin layer chromatography (TLC) was used. For this technique the saturated solvent system consisted of n-butanol: acetic acid: water (40:10:50). TLC plate was prepared and then it was loaded with standard riboflavin as well as water layer, containing riboflavin. The TLC plate was run for 6-8 hrs, in above solvent system. The plate was then dried and observed for fluroscence at UV short 254 nm and UV long 365 nm in the ultraviolet fluroscence chamber (Associate technical Bombay). [10].

Quantitative detection of riboflavin: This technique of quantitative detection of riboflavin was the Beer and Lambert's law [11]. In this technique solution containing different concentrations of riboflavin was prepared. Optical densities of each solution at specified wavelength (450 nm) were measured. Optical density verses concentration of riboflavin curve was prepared and this curve was known as standard curve. By plotting optical density of unknown on standard curve, the concentration of unknown in the fermented broth was determined [12].

Determination of effect of surfactants on the riboflavin production: Fermentation media having same composition was prepared. Different concentrations of surfactants such as 0.4 %, 0.8 %, 1.2 %, 1.6 %, 2.0 % were taken. Before it was added in to the fermentation medium, each concentration sterilized separately. Then medium inoculated with organism and incubated for 6-7 days at 28^0c, 200 rpm. Extraction procedure was similar mentioned as above. After extraction, water layer was obtained, used for quantitative determination of riboflavin. Along with these five sets, a control i.e. without surfactant was also prepared. Results are then compared with control [13] (Table 2 &3).

Determination of effect of β-cyclodextrin on the production of riboflavin: β cyclodextrin was separately sterilized at 10 pounds pressure. Then medium plus β cyclodextrin together kept on fermentation broth was incubated for 6-7 days at 28^0c and 200 rpm. Method for extraction was similar like mentioned above. Control set without β- cyclodextrin was also prepared and results were compared with control set [14].

RESULT AND DISCUSSION

Qualitative Detection of Riboflavin: After completion of fermentation and extraction process, the water layer was separated from ether layer, water layer contains riboflavin. This water layer subjected for qualitative detection by TLC and yellowish green fluroscence in the test sample was observed. In qualitative determination of riboflavin by TLC RF value of test was compared with RF value of standard riboflavin (500 mg/ml) (Table 1).

$$RF = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

$$RF\ (test) = \frac{5.5}{7.5}$$

$$= 0.73$$

$$RF\ (standard) = \frac{6}{7.5}$$

$$= 0.80$$

Quantitative Detection of Riboflavin

Standard stock = 0.5 mg/ml = 500 mg/ml
Filter = 450 nm

Concentration of riboflavin from the standard graph was 75 ug / ml.

The concentration of riboflavin in the fermentation broth (100ml) was 7500 ug/100 ml.

Consequences for surfactants SLS

Two surfactants were used during experimentation (Fig.1)

1. Sodium lauryl sulfate
2. Triton 100X

Effect of β Cyclodextrin: For the determination of effect of β cyclodextrin on riboflavin production various concentrations of β cyclodextrin were added into fermentation broth and results were shown in (Table 4).

DISCUSSION

The knowledge of vitamin B_2 i.e. lactoflavin began with the isolation from biological materials of yellowish green fluorescence in the years around 1879. Around 1910, Hopkins in U.K.[15] and Mendel in the USA initiated modern vitamin research and develop a theory, stating that diseases such as scurvey, pellagra, Rickett's, Beriberi are the result of a lack of certain essential food components i.e. riboflavin. In 1912, the polish chemist Casmir Funk [16] isolated from rice bran a beriberi preventing compound. He gave the name vitamin for this type of compound. In 1935 the structure of riboflavin was established as a derivative of isoalloxazine by European chemist R. Kuhn and P. Karrer.

It was finally demonstrated that these flavins are identical in chemical composition. In 1974, a competitive fermentation process was introduced at Merck employing the ascomycete, *Ashbya gossypii.* Riboflavin production by *Candida guillermondia* was first proved by Burkholder in 1943 [17] and Jenner et al. in 1945 [18]. Surfactants are surface active agents decreases the surface tension and increases fluid wet ability. Surface tension is the force acting on the surface of the liquid, tending to minimize the area of the surface. Surfactants interfere with the normal interaction between cells, surface, and its aqueous environment. During experimentation two surfactants were used, sodium lauryl sulfate and Triton 100X. In last few years β cyclodextrin in many ways fascinated the world of biotechnologist. Now it is very clear that β cyclodextrin can minimic a number of enzyme catalyzed reactions because they possess a hydrophilic outside and hydrophobic central cavity which enables them to form inclusion complex with various guest molecules of suitable sizes. Formations of such inclusion complexes make it easier to study hydrophobic interaction of biological importance.

After performing experiment, it was showed that riboflavin was produced by the cells of *Candida guillermondia* NCIM 3126. Sodium lauryl sulfate shows higher yield of riboflavin as compare to Triton 100X. The higher yield was obtained at concentration 1.2 % and was 280.0 ug/ml.Triton 100X shows negative effect on riboflavin production. β cyclodextrin shows the highest yield of riboflavin than surfactants. The highest yield was obtained by using 5.28 x 10 - 4M of β cyclodextrin and was 360ug/ml. Therefore, β cyclodextrin can be used as an alternative mode for improving the riboflavin production.

CONCLUSION

This study shows that riboflavin was produced by the cells of *Candida guilllermondia* NCIM 3126. Sodium lauryl sulfate shows higher yield of riboflavin compared to Triton 100X. The higher yield 280 ug/ml was obtained at 1.2 % sodium lauryl sulfate concentration whereas use of Triton 100X shows no significant effect on riboflavin production. β cyclodextrin shows the highest yield of riboflavin than surfactants. The highest yield 360 ug/ml was obtained by using 5.28 x 10-4 M of β cyclodextrin. Thus it can be concluded that β cyclodextrin can be used as an alternative mode for improving the riboflavin production.

Table 1: Observation Table for standard graph of Riboflavin

Sr. No.	Distilled water	Stock in ml	Concentration mg/ml	Optical density
1	10 ml	10 ml	10 ml	10 ml
2	8 ml	8 ml	8 ml	8 ml
3	6 ml	6 ml	6 ml	6 ml
4	4 ml	4 ml	4 ml	4 ml
5	2 ml	2 ml	2 ml	2 ml
6	-	-	-	-
7	-	-	-	-

Table 2 observation table for surfactant- Sodium laury lsulfate

Sr. No.	Concentration	Optical density	Conc. of VB2 ug/ml
1	0.4 %	0.095	122.5
2	0.8 %	0.08	105.0
3	1.2 %	0.23	280.0
4	1.6 %	0.20	247.5
5	2.0 %	0.063	85.0
6	control	0.060	75.0

Table 3 observation table for surfactant- Triton 100X

Sr. No.	Concentration%	Optical density	Conc. of VB2 ug/ml
1	0.4 %	0.20	30 ug/ml
2	0.8 %	0.05	60 ug/ml
3	1.2 %	0.03	40 ug/ml
4	1.6 %	0,03	40 ug/ml
5	2.0 %	0.04	50 ug/ml
6	control	0.06	75 ug/ml

Fig.1 Effect of surfactants on Riboflavin production

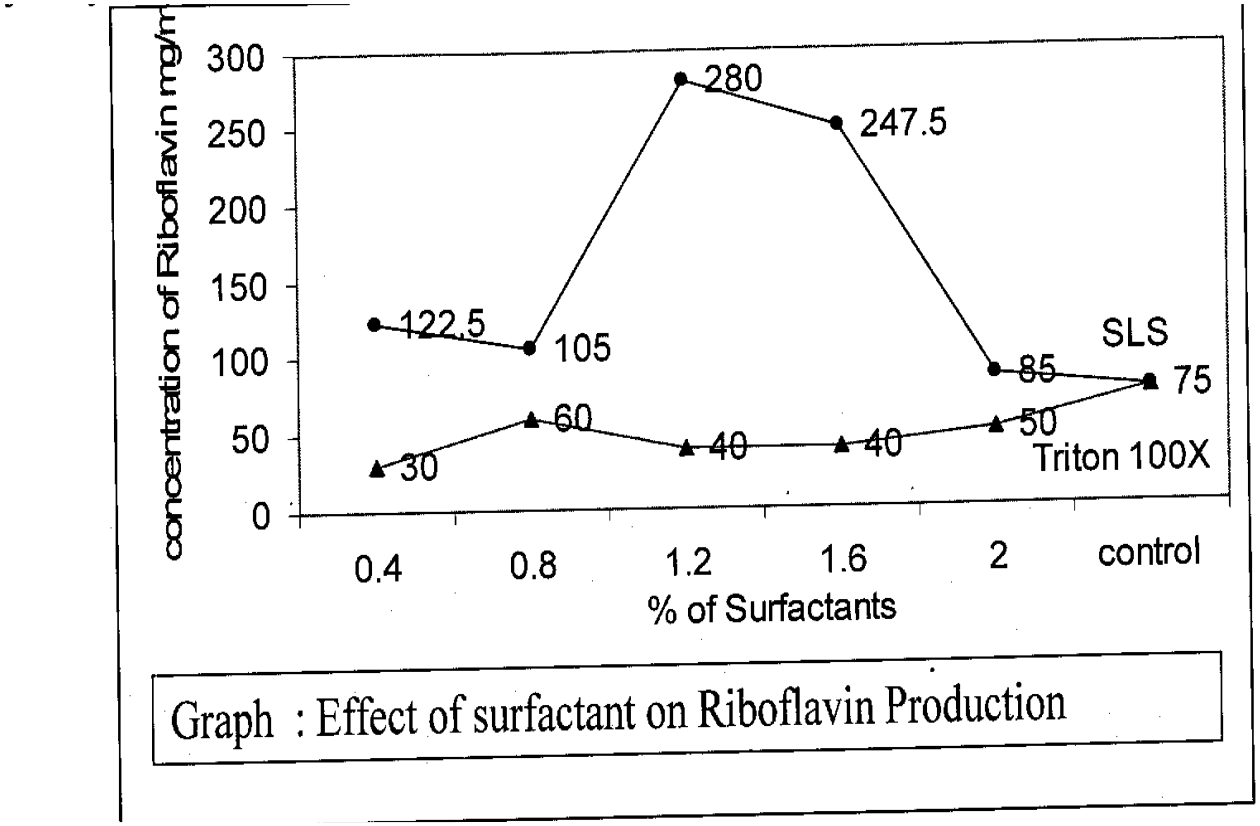

Table 4 Effect of cyclodextrin on Riboflavin production

No.	Concentration of B cyclodextrin	Optical Density	Concentration of Vit.B_2ug/ml
1	$5.28x10^{-1}$M	0.30	360
2	$7.04x10^{-1}$M	0.05	60
3	$3.25x10^{-1}$M	-	No Production
4	control	0.06	75

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