PHYSIOLOGICAL STUDY ON RIBOFLAVIN PRODUCTION BY A HYDROCARBON - UTILIZING CULTURE OF *CANDIDA GULLIERMONDII WICKERHAM*

S.A. SABRY*
A.H. EL-REFAI**
S.Y. GAMATI*

SUMMARY: The production of riboflavin (Vitamin B_2) by Candida guilliermondii Wickerham cultivated on solar-containing medium was stimulated in presence of corn steep liguor (2 g/l), corn oil (0.1 g %), arginine, phenylalanine (1m mole/l) or $COSO_4$. $7H_2O$ (1000 ug/l). On the other hand, emulsifying agents strongly inhibited yeast growth and vitamin production. Similarly, $FeSO_4$. $7H_2O$ and $MnSO_4$. $4H_2O$ at 50 and 200 ug/l levels respectively also showed inhibitory effect. Among the tested purines, xanthine enhanced the vitamin production.

Key Words: Riboflavin, Hydrocarbon utilization, Candida guillemondii Wickerham.

INTRODUCTION

The unsuitability of complete synthetic medium for the production of riboflavin by yeasts has been previously reported (Osman and Shaheen, 1966) III-defined natural substances such as yeast extract, corn steep liquor were known to be stimulative for both growth and flavinogenesis (Ragab, 1968; Teranishi *et al*, 1971). Emulsifying agents exerted variable effects on yeasts producing riboflavin from hydrocarbons (Nishio and Kamikubo, 1971). Similarly, metal ions, oils, fatty acids, amino acids and vitamins influenced the riboflavin productivity in different manners (Olczyk, 1978; Keller *et al*, 1983). On the other hand purines, were known to stimulate vitamin overproduction but the particular purine of choice appears to depend on the organism strain used (Demain, 1972).

In previous communications, it was announced that *Candida guilliermondii* Wickerham was the best riboflavin producer which would efficiently utilize solar as a sole carbon source. Some factors affecting growth and vitamin B_2 productivity were also studied (Sabry *et al*, under publications).

*From Botany Department, Faculty of Science, Alex. Univ.,Egypt.
**From Microbial and Natural Products Chemistry Laboratory, National Research Centre, Cairo.

The purpose of this work is to study the response of *C. guilliermondii* to different compounds (including surface active agents, salts, vitamins, amino acids and purines) in an attempt to optimize riboflavin production by the tested yeast.

MATERIALS AND METHODS

Organisms

Candida guilliermondii Wickerham was kindly provided by DSM, Germany. Solar of boiling range (248-270°C) is a fraction of El-Alameen crude oil. It was kindly provided by the refinery plant at Alexandria of Misr Petrol Company.

Culture media

Cultures of *C. guilliermondii* were maintained on solid agar media in slant tubes. The basal medium has the following composition (g/l): $(NH_4)_2SO_4$, 5.25; $NH_4H_2PO_4$, 5.25; KH_2PO_4 2.5; K_2HPO_4, 2.5; $MgSO_4$. $7H_2$ 0.1. The initial pH of the medium was adjusted to 6.0. Media were sterilized at 121°C in an autoclave before inoculation.

The organism was allowed to grow in 20 ml portions of the basal medium dispensed in 100 ml Erlenmeyer flasks. The carbon source (solur 5% v/v) was added to the autoclaved, cooled medium. A2% (v/v) inoculum was used and the inoculated media were incubated at 30°C ± for 14 days in the dark.

METHODS OF ASSAY

The cultures obtained at the end of the incubation period were heated for 30 min at 75°C in order to liberate the vitamin bound in the cells into the culture medium. The cells were separated by centrifugation, washed and dried at 70°C to constant weight to express it as dry weight. Riboflavin was determined in the filtrate by measuring light absorption at 450 nm.

Table 1: Growth as dry weight and riboflavin yield (both as mg/100ml) of *Candida guilliermondii* Wickerham as affected with the addition of some natural substances to the medium.

Additivies (2 g/L)	Dry weight	Riboflavin
No additve (basal medium)	630	10.45
Corn steep liquor (CSL)	659	10.52
Casein	516	8.28
Peptone	588	8.79
Yeast extract	597	10.44
Malt extract	447	7.48
Meat extract	558	6.96

Table 2: Growth as dry weight and riboflavin yield (both as mg/100ml) of Candida guilliermondii Wickerham in the presence of some surface active agents.

Additivies (20 μg/ml)	Dry weight	Riboflavin
No additve	631	10.41
Tween 20	320	3.84
Tween 40	122	0.00
Tween 80	228	0.00
Span 20	0.00	0.00
Span 80	0.00	0.00
Span 85	420	2.24

RESULTS AND DISCUSSION

Response to some natural additives

The response of the experimental yeast to some natural additives has been tested (Table 1). Evidence has been presented that the corn steep liquor-containing medium supported relatively high riboflavin production. The stimulatory effect of corn steep liquor on riboflavin production may be due to its content of amino acids, keto acids, certain vitamins (Teranishi *et al*, 1971).

Emulsifying agents

The data given in Table 2 indicate that the addition of the surface active agents exerted deleterious effects on cell biomass yield. The riboflavin formation was greatly retarded. On accordance with our findings, Olama (1985) reported the inhibitory effect of tweens 20, 40 and 80 on growth and lipid production by *C. parapsilosis*. On the contrary, Nishio and Kamikubo (1971) and Teranishi (1971) proved the stimulatory effect of tween 60 and 80 on flavinogenesis by *Pichia guilliermondi* and Candida tropicalis respectively.

Table 3: Growth as dry weight and riboflavin yield (both as mg/100ml) of *Candida guilliermondii* Wickerham as affected with the addition of some oils, or fatty acids to the medium.

Additive (0.1 g/100ml)	Dry weight	Riboflavin
No additive (control medium)	631	10.40
Corn oil	717	10.68
Cotton seed oil	424	3.77
Olive oil	397	6.14
Caproic acid	543	6.98
Lauric acid	309	2.04
Stearic acid	409	7.65
Oleic acid	688	10.40

Oil additives

The fermentation medium was individually supplemented with selected oils in an attempt to explore their role on the riboflavin production of the tested organism

Table 4: Growth as dry weight and riboflavin yield (both as mg/100ml) of *Candida guilliermondii* Wickerham as affected with the addition of different amino acids to the medium.

Amino acid (1 mmole/l)	Dry weight	Riboflavin
No additive (control medium)	6.59	10.50
Arginine-HCl	566	12.06
Phenyl alanine	557	11.97
Serine	540	9.86
Aspartic acid	486	8.36
Valine	352	7.88
Asparagine	335	6.86
Glycine	339	6.53
Isoleucine	467	4.53
Proline	476	5.75
Glutamic acid	566	4.98
Methionine	578	4.88
Alanine	619	4.73

Table 5: Growth as dry weight and riboflavin yield (both as mg/100ml) of *Candida guilliermondii* Wickerham as affected with the addition of different Vitamin to the medium.

Vitamin	Amount added mg/l00 ml	Dry weight	Riboflavin
No vitamin (control medium)	0	659	10.50
Nicotinic acid	10.0	505	10.43
Biotion	1.0	607	10.39
Ca pantothenate	47.6	585	9.25
Folic acid	5.0	618	8.26
Inositol	30.0	344	6.76
P. aminobenzoic acid	13.7	474	5.39
Thiamine HCl	33.7	435	5.39
Pyridoxine HCl	20.6	375	4.79

(Table 3). The growth yield was appreciably enhanced in the presence of corn oil (about 13% increase, as compared with the control treatment). Nothing was superior to corn oil which exhibited the maximum riboflavin out put. The stimulatory effect of corn oil might be due to its alteration of the surface tension, decrease in oxygen transfer in the liquid substrate or to permeability increase of the cell membrane. It may also provide the yeasts with additional acetyl Co A sources by undergoing B-oxidation and by sharing in the oxidative phosphorylation process, it may provide more ATP molecules (Schlee and Straube, 1984).

Table 6: Growth as dry weight and riboflavin yield (both as mg/100ml) of *Candida guilliermondii* Wickerham as affected with the addition of some trace elements to the medium.

Metal ion	Amount added μg/l	Dry weight	Riboflavin
Fe^{2+} ($FeSO_4.7H_2O$)	50 500 1000 5000	325 303 266 181	6.91 6.90 5.73 0.00
CO^{2+} ($COSO_47H_2O$)	10 100 1000 10.000	343 691 912 793	10.38 10.52 10.98 10.52
Mn^{+2} ($MnSO_4.4H_2O$)	20 200 2000 20.000	565 579 746 463	10.20 9.78 6.50 5.91
No metal ions (Basal medium)	Zero	631	10.40

Amino acids

The addition of L-arginine-HCI or DL-phenyl alanine (1 mmole/l) stimulated the production of riboflavin (Table 4). In contrast, the growth and vitamin productivity were inhibited by the addition of the other tested amino acids. Chenouda (1963) correlated this stimulatory effect of arginine to a probable capacity of furnishing precursor for flavinogenesis. The stimulatory effect of arginine on riboflavin production by *C. guilliermondii* from hydrocarbons has also been proved by Nishio and Kamikubo (1971).

Table 7: Growth yield as dry weight and riboflavin yield (both in mg/100ml) of *Candida guilliermondii* Wickerham in the presence of different purines and purine-related compound.

Supplement	Conc. mg/l.	Dry Weight	Riboflavin
None	-	650	10.32
Adenosine	5 10	525 513	8.74 7.22
Adenine	5 10	531 561	8.59 7.71
Xanthine	5 10	662 649	10.89 11.21
Xanthone	5 10	465 422	8.36 8.74

Vitamins

Under the tested conditions, the riboflavin formation seems to be unaffected by the addition of nicotinic acid or biotin (Table 5).

Moreover, the addition of any of the other tested vitamins inhibited both growth and riboflavin productivity. This led to the conclusion that the experimental yeast exhibited no special requirements for the tested vitamins. On the other hand, nicotinic acid and biotin were reported to stimulate riboflavinogenesis by *C. guilliermondii* (Nishio and Kamikubo, 1971; Olczyk, 1978).

Effect of some trace elements

Three trace elements namely, Fe, Co and Mn were tested for their effect on the riboflavin production by the experimental organism (Table 6). The data given clearly indicate that the addition of the lowest concentration (50 μg/l) of $FeSO_4.7H_2O$ to furnish iron ions into the medium exerted an inhibitory effect on both cell biomass and

riboflavin production. In harmony with our results, many investigators proved that flavinogenesis was adversely affected in the presence of iron salts, particularly when used at higher levels (Teranishi *et al*, 1971; Nishio and Kamikubo, 1971; Schlee and Straube, 1984).

On the contrary, the increase of $COSO_4$. $7H_2O$ level in the medium up to 1000 μg/l proved to be conductive for cell biomass yield, through riboflavin production was unaffected under these conditions. In accordance with these results, are those of Nishio and Kamikubo (1971).

The lowest concentration of $MnSO_4$, $4H_2O$ (20 μg/l) added to the culture medium did not affect the riboflavin production, while the vitamin yields were seriously decreased on using increased amount of this salt.

Purines and purine-related compounds

Purines are known to act as precursors of the isoalloxazine ring of the riboflavin molecule. Therefore, different purines were tested for their effect on the flavino genesity of *C. guilliermondii* Wickerham (Table 7). Only the presence of 10 mg/l xanthine slightly improved the vitamin productivity. The importance of xanthine as a precursor for riboflavin production was previously reported by Osman and Soliman (1963). However Demain (1972) showed that the particular purine of choice appeared to depend on the fermentation condition and the organism used.

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Correspondence:
S.A. Sabry
Botany Department,
Faculty of Science,
Alex. University
EGYPT.