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BIOCHEMICAL STUDIES ON THE RICE BLAST DISEASE (PART 10) BIOCHEMICAL CLASSIFICATION OF PIRICULARIA ORYZAE CAVARA (NO 7) REGARDING THE PRODUCTION OF VITAMIN B GROUP BY P.O.C.

- Japan -

[Following is a translation of an article by Otsuka, Hotoshi in the Japanese-language journal Agr. Chem. Soc. Japan Journ., Vol 33, 1959, pages 1013-1018.]

Biotine and thiamine are indispensable elements of the growth of Piricularia oryzae cavara (hereafter referred to as P.o.c.) in general. This has been the topic of our intensive study with respect to the 47 stock-cultures. To summarize the results, 2 stock-cultures were found to propagate fairly well on the biotine-free synthetic culture medium (1), and they were also found not to possess any biotine formation capacity (2). Of the 47 stock-cultures, 38 needed thiamine, while the remaining 9 stock-cultures propagated fairly well on the thiamine-free synthetic culture medium. In general, thiamine cannot be replaced by nicotinic acid, but 8 out of the 38 afore-mentioned stock-cultures propagated well on the nicotinic acid-added culture medium. Furthermore, both thiamine-dependent and non-dependent stock-cultures were found capable of producing nicotinic acid of the free type (3). The present study deals with the case of cultivating the thiamine non-dependent stock-cultures (hereafter to be called B group stock-culture) in the thiamine-free culture medium and with the case of cultivating the nicotinic acid-dependent stock-culture (hereafter to be called C group stock-culture) in the thiamine-free culture medium containing nicotinic acid. It was a plausible hypothesis that the

stock-cultures in these cases might produce thiamine. This, however, was not confirmed by the bioassay analysis. In view of the fact that the vitamin B group, in addition to biotine and thiamine, plays an important role in the metabolism of microorganisms, we have investigated the problem of whether the P.o.c. produces such vitamins. Results of our experiment indicate that the P.o.c. produces riboflavin, folic acid, pantothenic acid and the vitamin B_6 group. We have also conducted 12 experiments in order to study the differences in metabolism as a function of differential need of biotine and thiamine, and the results are reported below.

Experiment

1) The P.o.c. used in the experiment: the same stock-cultures that were used in the previous experiments.

2) Previous cultivation: As described in Pt. 10 (No 1).

3) Culture medium: Synthetic culture medium used as a basis.

4) Determination of thiamine quantity: The determination of the amount of thiamine (of or its absence) was performed by the bioassay technique of using Lactobacillus fermenti ATCC 9338. The operation of bioassay as usual depended on the method of nephelometry that measures the cultivating solution (2 ml) after 16 hours' cultivation at 37° by means of photo-electric colorimeter.

The composition of the culture medium used in the bioassay analysis and the reference standard line are presented in Table 1 and Fig. 1 respectively.

5) Examination of thiamine-formation capacity: 50 ml of each stock-culture was transferred as specified below into 100 ml flasks which were inoculated and cultivated for 14 days at 25°, and then subjected to the determination of thiamine quantity in the bacteria and the cultivating solution: thiamine-dependent stock-culture into a complete (non deficient) culture medium, thiamine-non-dependent stock-culture into thiamine-free culture medium, and thiamine-non-dependent but nicotinic acid-dependent stock-culture into thiamine-free but nicotinic acid-containing culture medium. Surviving bacteria were ground and added to 1/10 N-sulfuric acid of 15 times quantity, which was then decomposed for 30 min. on the boiling water (pH less than 1.5), and, upon

Table 1

The composition of basic culture medium used in thiamine bioassay

1) ビタミン free カゼイン加水分解物 (T.N 1.28%) 10 % 溶液 10 ml			
2) シスチン	20 mg	Folic acid	2 γ
3) トリプトファン	20 mg	9) アデニン-H_2SO_4	2 mg
4) リボフラビン	200 γ	10) グアニン-HCl	2 mg
5) ニコチン酸	200 γ	11) ウラシル	2 mg
6) ピリドキサール-HCl	200 γ	12) キサンチン	2 mg
7) ピリドキシン-HCl	200 γ	13) グルコース	2 g
P-Amino-bensoic acid	60 γ	14) K-アセタート	1 g
Ca-Pantothenate	200 γ	A 液* 15)	2 ml
8) ビオチン	2 γ	B 液**	2 ml

15) * A 液：KH_2PO_4 5g, K_2HPO_4 5g に蒸留水を加え 100 ml とす.
** B 液：$MgSO_4 \cdot 7H_2O$ 2g, $FeSO_4 \cdot 7H_2O$ 0.1g, $MnSO_4 \cdot 4H_2O$ 0.1g, KCl 0.1g に蒸留水を加え 100 ml とす.

[Legend:] 1) 10 ml of 10% solution of biotine free casein hydrolytic product (T.N. 1.28%); 2) Cystine; 3) Tryptophane; 4) Riboflavin; 5) nicotinic acid; 6) Pyridoxal-HCl; 7) Pyridoxin-HCl; 8) Biotine; 9) adenine-; 10) Guanine; 11) Uracil; 12) Xanthine; 13) Glucose; 14) K-acetate; 15) A solution: Add distilled water to make 100 ml to KH_2PO_4 5g and K_2HPO_4 5g; B solution: Add distilled water to make 100 ml into $MgSO_4 \cdot 7H_2O$ 2g, $FeSO_4 \cdot 7H_2O$ 0.1g, $MnSO_4 \cdot 4H_2O$ 0.1g, and KCl 0.1g.

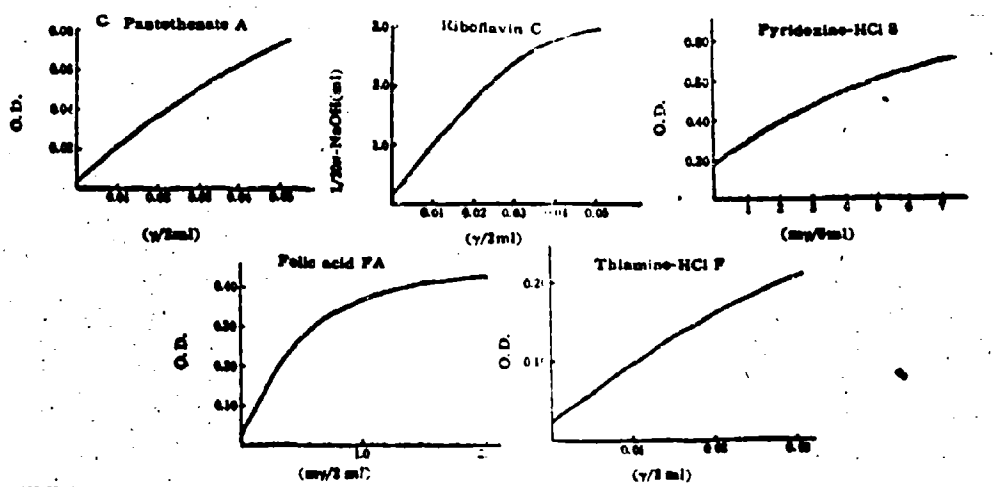

Fig. 1. Standard reference curve for Vitamin B Group

cooling, the pH level was raised to 4.5 by adding 4N-sodium acetate solution. To the resulting solution was added 10% takadiastase. The solution was left overnight at 37°. The pH level was than adjusted to 6.5 and the solution was filtered. It was now subjected to quantitative analysis upon being properly diluted (4). The cultivating solution was first diluted and examined as it was. The results are shown in Table 2.

Table 2

Bacteria weight and thiamine quantity on various cultivating soils

1)菌株	2)培地の種類	⑥乾燥菌体重量(g)	⑦培養濾液中のチアミン量(mγ/ml)	⑧培養液50 ml中のチアミン量(γ)	⑨菌体中のチアミン量(γ)	⑩培養液及び菌体中のチアミン総量	⑪損失又は生成チアミン1g量(γ)	⑫乾燥菌体1g中のチアミン量(γ)
No. 11 F8 h	3)完全合成培地**	0.2846	559.37	27.97	16.10	44.07	−5.93*	56.57
5528	〃	0.4155	393.75	19.68	22.88	42.56	−7.44	55.05
5425	〃	0.1222	707.80	35.39	10.28	45.67	−4.33	84.08
No. 1	4)チアミン欠合成培地	0.2487	0.00	0.0	0.63	0.63	+0.63*	2.51
5330	〃	0.2703	〃	〃	0.11	0.11	+0.11	0.39
5527	〃	0.1026	〃	〃	0.15	0.15	+0.15	1.44
5535	〃	0.0260	〃	〃	0.00	0.00	0.0	0.0
1	5)チアミン欠ニコチン酸添加合成培地	0.1384	〃	〃	0.35	0.35	+0.35	2.56
A. 36	〃	0.0909	〃	〃	0.34	0.34	+0.34	2.86
5415	〃	0.1293	〃	〃	0.14	0.14	+0.14	1.10
5529	〃	0.0507	〃	〃	0.25	0.25	+0.25	4.87

⑬ * −は培養中に損失又は破壊したチアミン，+は培養中に生成したチアミンを示す．** 完全合成培地はチアミン1γ/ml を含む．

(6) その他のビタミンB群の定量　生理化学的差異を示す代表的な6菌株を合成培地（グルコース2%）

[Legend:] 1) Stock-cultures; 2) Types of culture medium; 3) Complete synthetic culture medium; 4) thiamine-free synthetic culture medium; 5) thiamine-free, nicotinic acid-containing synthesized culture medium; 6) weight of dry bacteria; 7) thiamine quantity in the cultivating filtered solution; 8) thiamine quantity in 50 ml of cultivating solution; 9) thiamine quantity in the total bacteria; 10) the total thiamine quantity in both the cultivating solution and the bacteria; 11) thiamine quantity either lost or produced per 1 g; 12) thiamine quantity in 1 g of dry bacteria; 13) * " " indicates either loss or destruction of thiamine; " " indicates the thiamine produced during cultivation; ** the complete synthesized culture medium includes 1 γ/ml of thiamine.

6) Determination of the other vitamin B group: six representative stock-cultures showing biochemical differences were inoculated in the synthesized culture medium (2% glucose) and cultivated for 14 days at 25°. This was then filtered and, upon dilution, served as experimental material.

(a) Quantitative determination of riboflavin: We depended on the bioassay method that uses Lact. casei ATCC 7469. The bioassay technique was the usual acidimetric method that titrated 2 ml of the cultivating solution with 1/20 N-NaOH after 72 hours' cultivation at 37°. The composition of the culture medium was that of Table 1, except that vitamin-free casein hydrolytic products and riboflavin were replaced by Difco's casamino acids (vitamin free) 1 g, thiamine 200γ , and K-acetate 4 g. The standard reference curves are shown in Fig. 1.

(b) Pantothenic acid: We relied on the nephelometric bioassay technique that utilizing Lact. arabinosus ATCC 8014 measured 2 ml of the cultivating solution after 16 hours' cultivation at 37° by means of photo-electric colorimeter. The culture medium consisted of the riboflavin basic cultivating soil with Ca-pentothenate replaced by riboflavin 200γ and K-acetate 1 g. The reference curve is shown in Table 1.

(c) Quantitative determination of folic acid: We relied on the nephelometric bioassay technique which measured 2 ml of the cultivating solution after 16 hours' cultivation at 37° by means of photoelectric colorimeter (Coleman made) using Streptococcus faecalis AtCC 8043. The composition of the basic culture medium was as shown in Table 1 except that vitamin-free casein hydrolytic products, folic acid, K-acetate, A-solution and B-solution were removed and replaced by 1 g of Difco's casamino acid (vitamin free), thiamine-HCl 200γ, K-acetate 0.3 g, K-citrate 1 g, KH_2PO_4 1 g and inorganic salt solution* 2 ml. [See Note].

([Note]: Inorganic salts solution: Distilled water was added to make 100 ml to $MgSO_4 7H_2O$ 8 g, $FeSO_4$ $7H_2O$ o.4 g, $MnSO_4 \cdot 4H_2O$ 1.6 g and KCl 0.4 g.)

(d) Quantitative determination of Vitamin B_6 group: We relied on the bioassay technique that determined the total quantity of pyridoxine, pyridoxal, and pyridoxamine using Saccharomyces carlsbergensis ATCC 9080. That is, it was a nephelometric method of measuring 6 ml of the cultivating solution by means of photoelectric colorimeter after cultivating at 20° slope for 16 hours at 30° (5, 6).

Table 3 gives the composition of the basic culture medium, while the standard reference curve is given in Fig. 1. Quantitative determination of vitamin in the filtered cultivating solution gave the results that are shown in Table 4.

Table 3

Composition of the basic cultivating solution for vitamin B_6 group

Casamino acids vitamin free (Difco) 0.8 g

1) グルコース 10g, 2) イノシトール 5 mg, 3) ニコチン酸 500 γ, 4) チアミン 2 γ, 5) ビオチン 3 ml, 6) 無機塩溶液* 10 ml, 7) クエン酸緩衝液** 10 ml, 8) Ca-Pantothenate 500 γ, 蒸留水を加え 100ml とす, 9) リン酸緩衝液 (pH 6.0)

*) 無機塩溶液：KH_2PO_4 2.2g, KCl 1.7g, $CaCl_2 \cdot 2H_2O$ 0.5 g $MgSO_4 \cdot 7H_2O$ 0.5g, $FeCl_3$ 0.01g, $MnSO_4 \cdot 4H_2O$ 0.01 g 蒸留水を加え 100 ml とす.

**) クエン酸緩衝液：K-Citrate 10 g, クエン酸 2g 蒸留水を加え 100 ml とす.

[Legend:] 1) Glucose; 2) inositol; 3) nicotinic acid; 4) thiamine; 5) biotine; 6) inorganic salts solution; 7) citric acid buffer solution; 8) add distilled water to make 100 ml.; 9) inorganic salts solution: add distilled water to make 100 ml into KH_2PO_4 2.2 g, KCl 1.7 g, $CaCl_2 \cdot 2H_2O$ 0.5 g $MgSO_4 \cdot 7H_2O$ 0.5 g, $FeCl_3$ 0.01 g, $MnSO_4 \cdot 4H_2O$ 0.01 g; 10) citric acid buffer solution: add distilled water to make 100 ml into K-citrate 10 g and citric acid 2 g.

Table 4

Produced quantities of vitamin B_6

① 使用菌株	②パントテン酸 (γ/100 ml)	③ビタミンB_6群 (ピリドキシン, ピリドキサール, ピリドキサミン塩酸塩) (γ/100 ml)	④リボフラビン (γ/100 ml)	⑤菌体量 (乾燥重量 mg/100 ml)
No. 11F8-hetero	179.1	12.8	7.3	1.69
P_3	195.6	21.9	16.5	6.60
5532	194.2	10.0	6.8	9.35
5516	71.4	13.1	12.0	1.54
5536	87.9	11.9	13.8	3.70
No. 1	98.9	5.0	24.2	3.15

[Legend:] 1) Stock-cultures that were used; 2) pantothenic acid; 3) vitamin B_6 group

(pyridoxin, pyridoxal, and pyridoxamine all together); 4) riboflavin; 5) folic acid.

7) Other experiments: Three kinds of synthesized culture medium (2% glucose) were prepared by adding 1 γ/l, 5γ/l and 25γ/l respectively. Furthermore, 50γ/ml, 100γ/ml and 250γ/ml each of aspartic acid was added to the 1γ/l biotine-added cultivating soil. 50 ml each of these preparations was transferred each to a 100 ml triangular flask, which after sterilization was inoculated either by P_2 or No 1 and cultivated for 14 days at 25°. The quantity of nicotinic acid produced and the bacteria weight (after drying) were determined by the bioassay quantitative method of using Leuc. mesemteroides and Lact. arabinosus (3). The results are presented in Table 5.

Table 5

Bacteria weight and the quantity of nicotinic produced acid as a function of increasing amounts of biotine and aspartic acid addition

①	②	③	④ ニコチン酸生成量 (mγ/ml)	
			Leuc. mesenteroides による	*Lact. arabinosus* による
P_2	ビオチン 1γ/l	0.1010	250	230
	〃 5 〃	0.2070	250	230
	〃 25 〃	0.2290	460	340
	ビオチン 1γ/l +レーアスパラギン酸 50γ/ml	0.1320	250	220
	〃 + 〃 100 〃	0.1450	350	230
	〃 + 〃 250 〃	0.1500	300	270
No.1	ビオチン 1γ/l	0.1140	130	70
	〃 5 〃	0.2020	610	590
	〃 25 〃	0.2035	640	490
	ビオチン 1γ/l +レーアスパラギン酸 50γ/ml	0.2300	630	470
	〃 + 〃 100 〃	0.2320	500	550
	〃 + 〃 250 〃	0.1550	210	130

② * 完全合成培地(1)中よりビオチンを除いた培地に添加する量を示した。

[Legend:] 1) Stock-cultures; 2) composition of the culture medium; 3) biotine; 4) aspartic acid; 5) weight of dried bacteria; 6) the quantity of nicotinic acid produced; 7) *indicates the quantity to be added to the complete synthesized culture medium that is freed of biotine.

Glucose, L-glutamic acid and L-aspartic acid 2% each were added as carbon source to prepare a synthesized cultivating soil, 5 ml each of which was sterilized and inoculated

with No 1 of A group stock-culture, Co-homo of B group and No 188-hetero and P_2 of D group. They were then cultivated for 14 days at 25°, and the weight of dried bacteria and the amount of carbon source consumed were determined. The results are given in Table 6.

Table 6

Bacteria weight and the extent of carbon consumption

①炭素源	②菌株	③培養前の炭素源量 (mg/50 ml)	④培養後の炭素源量 (mg/50 ml)	⑤炭素源の消費量 (mg/50 ml)	⑥炭素源の消費率 (%)	⑦乾燥菌体重量 (g)	⑧菌体比量 (g) ⑨炭素源消費量 (g)
⑩グルコース	No. 1	960	245.8	714.2	74.40	0.0430	0.0602
	Co-homo	〃	386.3	573.7	59.76	0.0320	0.0558
	No. 188-hetero	〃	550.6	409.6	42.67	0.0260	0.0635
	P_2	〃	185.5	774.5	80.68	0.0375	0.0484
⑪L-グルタミン酸	No. 1	1090	544.0	546.0	50.09	0.0205	0.0375
	Co-homo	〃	676.0	414.0	37.98	0.0190	0.0459
	No. 188-hetero	〃	604.0	486.0	44.59	0.0150	0.0309
	P_2	〃	472.0	618.0	66.70	0.0170	0.0275
⑫L-アスパラギン酸	No. 1	1403	716.0	687.0	48.97	0.0128	0.0186
	Co-homo	〃	840.0	563.0	40.13	0.0113	0.0201
	No. 188-hetero	〃	787.0	616.0	43.91	0.0098	0.0159
	P_2	〃	664.0	739.0	52.67	0.0098	0.0133

[Legend:] 1) carbon sources; 2) glucose; 3) L-glutamic acid; 4) L-aspartic acid; 5) the amount of carbon sources before cultivation; 6) the amount of carbon sources after cultivation; 7) the amount of carbon source consumed; 8) the consumption rate of carbon sources; 9) the weight of dried bacteria; 10) bacteria weight; 11) amount of carbon source consumed; 12) stock-cultures.

The quantitative determination was carried out by the bioassay technique of acidity titration that used Lact. arabinosus ATCC 8014 for L-glutamic acid and Leuc. mesenteroides ATCC 8042 for L-aspartic acid (7). Glucose determination was by the method of Mioro-Bertrand.

Discussion

As is self-evident from Table 2, when D group stock-culture (biotine- and thiamine-dependent) is cultivated in the complete culture medium (thiamine 1γ/ml is contained) for 14 days at 25°, we notice 20.54 - 47.75% of the added thiamine to have been transferred into the bacteria, 39.36 - 70.78% to be remaining in the cultivating soil and 8.9-14.88%

to have been lost or decomposed during cultivation. Approximately 55.05 - 84.00 γ of thiamine per 1 g of surviving bacteria weight (dried) remains in the bacteria, which serves as a reminder to be cautious in deciding on the culture medium for P.o.c.

B group stock-culture (thiamine-non-dependent) and C group stock-culture (thiamine-non-dependent but nicotnic acid-dependent) were cultivated in the thiamine-free and the thiamine-free, nicotinic acid-added culture media, respectively, and they were found to grow, though the extent of growth was far less compared to the growth in the complete culture medium. In these culture media, they were found not to produce thiamine by themselves.

As shown in Table 4, the 6 stock-cultures used in the experiment produced such substances as riboflavin, pantothenic acid, vitamin B_6 group and folic acid. The roles these substances play in the metabolism of micro-organisms vary: riboflavin acts as a yellow enzyme in the process of oxidation and reduction, pantothenic acid acts as coenzyme A or some such other, vitamin B_6 group plays the role of a coenzyme in the metabolism of amino acid (transamination in particular), folic acid, on the other hand, also plays an important role in the metabolism of nucleic acid and tyrosin.

It is quite conceivable that the capacity for the vitamin B_6 group exists in the P.o.c. and is related in an important way to the metabolism is in microorganisms.

Thiamine is closely and significantly related to metabolism in the form of co-carboxylase; the foregoing results, however, seem to indicate a course of metabolic participation for B and C group stock-cultures that is different from that for D group.

It was for this reason that we examined the extent of consumption of carbon sources such as glucose, L-glutamic acid and L-aspartic acid in P_2 and No 188-hetero of D group, No 1 of A group (No 1 possesses some of the characteristics of B group) and in Co-homo of B group. As shown in Table 6, with L-glutamic acid and L-aspartic acid, No 1 of A group and Co-homo of B group show a greater value (ratio) for the bacteria weight/carbon consumption rate than No 188-hereto of D group. In other words, both A and B groups stock-cultures show a greater amount of bacteria weight increase than D group when L-glutamic acid and L-aspartic acid are used as carbon sources; thus A and B groups show a greater degree of efficiency.

However, the metabolic process based on L-glutamic acid and L-aspartic acid is far less efficient compared to the regular metabolic process of P.o.c. that is that based on glucose, as is clearly revealed by the examination of the column for bacteria weight/carbon consumption quantity.

As for the role of thiamine in the metabolism of P.o.c. further study seems to be in order.

In the metabolism of microorganisms thiamine is known to play various roles, including participation in the Wood-Werkman reaction. By experimenting with enzymes of various kinds, Rose and Nickerson (8) have shown that with the addition of increasing amounts of biotine to the culture medium some stock-cultures show reduction in nicotinic acid formation, some show no change at all, while other stock-cultures show an increase, from this they have concluded that different enzymes are involved in the production of nicotinic acid.

Taking into consideration another fact, that biotine is capable of transforming typrophane to formyl kynurenine (9, 10), it seems reasonable to suggest that biotine is involved in the production of nicotinic acid from tryptophane and also that with some enzymes, such as Neurospora (11), tyrptophane is connected with the production of nicotinic acid, while, in such enzymes as E. Coli and Bac. subtilis (12), tryptophane is unrelated. In one of previous reports we have confirmed the production of nicotinic acid in the P.o.c. In order to verify the role of biotine in nicotinic acid production, we have replicated the experiment of Rose and Nickerson. As the results shown in Table 5 indicate, P_2 of D group stock-culture propagate well with the increased addition of biotine, but shows no sign of sudden increase in the production amount of nicotinic acid.

In contrast, No 1 of A group shows favorable propagation and an increase in the production amount of nicotinic acid.

These facts together suggest that biotine is clearly related to nicotinic acid production in some P.o.c. stock-cultures, while it is not in others.

When the less-than-adequate amount of biotine (P.o.c. is known to need 5γ/l of biotine (13-15)) is compensated by L-aspartic acid of 50, 100, 250 γ/ml each. P_2 showed no sign of better growth compared to the case of ordinary amount of biotine addition, whereas No 1 showed better growth than with ordinary amount of biotine.

With the biotine-non dependent A group (ex. No 1), the effect of adding aspartic acid to the less than adequate biotine is enormous, while the same effect is almost absent with the biotine-dependent D group (ex. P_2). It thus appears plausible that in the stock-cultures of P.o.c. biotine plays an important role even in the metabolic processes other than the Wood-Werkman reaction.

The foregoing results are summarized in Table 7. There seems to exist a noticeable difference between the metabolism of the P.o.c. involving biotine and thiamine, respectively. Such difference seems to be related in an important way to the degree of resistivity of P.o.c.*, which is the focus of our investigation at present. [See note following page]

Table 7

Summary of experimental results

①群菌株	②ビオチン欠乏増地に於けるアスパラギン酸の効果	③ビオチン増加に伴うニコチン酸生成量	④グルタミン酸アスパラギン酸の利用度
A群⑤	大⑥	多⑦	⑧大
B群	—⑫	—⑫	大
D群	小⑨	少⑩	⑪小

⑫° 実験を行わなかった。

[Legend:] 1) Group stock-cultures; 2) Group; 3) The effect of aspartic acid in the biotine free culture medium; 4) great; 5) little; 6) The amount of production of nicotinic acid resulting from the increased addition of biotine; 7) large; 8) small; 9) The consumption extent of glutamic acid and aspartic acid; 10) large; 11) small; 12) "indicates that experiment has not been performed.

Summary

Following results have been obtained from the experiments with representative groups of P.o.c. on the formation of vitamin B group.

1) When D group stock-culture (biotine and thiamine-dependent) is cultivated in the complete culture medium, (containing thiamine) for 14 days at 25°, 39.36 - 70.78% of the added thiamine remains in the cultivating solution, and 20.54 - 47.75% transfers into the bacteria body, while 8.90 - 14.68% decomposes during cultivation.

([Note]: Findings at Agr. Res. Ctr. (16) and by Suzuki (17) suggest that our A and G groups are relatively harmless, while C and D groups are very toxic and harmful.

2) When B group stock-culture (thiamine-non-dependent) is cultivated in the thiamine-free culture medium and C group (thiamine-non-dependent, but nicotinic acid-dependent) in the thiamine-free but nicotinic acid-added culture medium, they show no sign of thiamine formation.

3) By cultivating the six representative stock-cultures in the synthesized culture medium, it was confirmed that riboflavin, pantothenic acid, folic acid and vitamin B_6 group were produced in the cultivating solution.

4) Addition of aspartic acid to A group stock-culture (biotine-non-dependent) in the biotine-free culture medium resulted in better propagation than in the complete culture medium, while such an effect of aspartic acid addition was almost absent in the D group stock-culture.

5) A group stock-culture shows a greater extent of consumption than B or D when glutamic acid and aspartic acid are provided as carbon sources.

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