微 生 物 學 報
第3卷 第1期 1955年6月

鋅和碘游子對於 *Pichia membranaefaciens* 培養中氨基酸形成的研究

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Pichia membranaefaciens 是一種酵母菌，能在祇含有銨鹽作爲唯一氮源的人工綜合培基內生長。由於這種微生物在上述培基中的生長與發酵，培養產物中有幾種氨基酸的形成。正如 Goodlow, Braun 和 Mika [1] 觀察到布氏桿菌在培基內產生 L-氨丙酸一樣。氏等發現由於這種氨丙酸在培養基中的蓄積，限制了該菌的光滑型的生長，而利於粗糙的變異型的繁殖。

作者等發現於上述人工培基中，如加以小量的鋅與碘，能影響其形成的氨基酸的種類。

實　　驗

1. 培基

氯化鈉	0.025 克
硫酸鉀	0.025 克
碳酸鈣	0.025 克
碳酸鎂	0.030 克
磷酸氫二鈉	0.025 克
加稀鹽酸使其澄清，再加	
硫酸銨	0.5 克
葡萄糖	5 克

加蒸餾水使總量成爲 100 毫升，調節 pH 至 45

製備上述培基 3 份，裝於 150 毫升容量的平底瓶中，於 1 份培基內加 0.0125 克硫酸鋅，於另 1 份中加碘化鉀 0.0125 克，第 3 份培基作爲對照。每個平底瓶中裝 100 毫升培基，在高壓蒸氣滅菌器內於 10 磅壓力下滅菌 30 分鐘。

2. 微生物的接種與氨基酸的測定

每瓶內各加入1滴 *Pichia membranaefaciens* 活菌液，培養於20°C 60天後，以已知重量的乾濾紙過濾，用Giri[2]氏的紙層析法測定濾過液中的氨基酸。

3. 結果

加入的化合物	酵母產量（克）	培基內剩餘的葡萄糖（克）	以糖消耗量爲基礎算出的酵母量的%	氨基酸的形成
硫酸鋅	0.8012	2.2	28.53	苯丙氨酸、組氨酸、亮氨酸
碘化鉀	0.7640	0.00	15.28	色氨酸、酪氨酸、組氨酸、氨丙酸
對照	0.7836	0.00	15.79	苯丙氨酸、色氨酸、酪氨酸、軟氨酸

討論

使 *P. membranaefaciens* 生長於含有葡萄糖爲炭源與硫酸銨爲氮源的培基中，則生長的酵母菌的濾過液中有苯丙氨酸、色氨酸、酪氨酸與軟氨酸的形成。如在培基中加入少許硫酸鋅，則有不同的氨基酸出現，其產物中有苯丙氨酸、組氨酸和亮氨酸。同時，在培基中產生較大量的酵母菌，如在培基內加入小量的碘化鉀，則於培養物中有色氨酸、酪氨酸、組氨酸、氨丙酸存在，但酵母的產量約與對照瓶相同。

一般說來，培養物中存在的氨基酸多半是芳香或環狀的氨基酸。

總結

P. membranaefaciens 生長於含葡萄糖與硫酸銨的人工綜合培基中，於培養過程中有數種氨基酸的形成。如果在培基中有鋅或碘游子的存在，則形成的氨基酸的種類受到影響。對照培基中有苯丙氨酸、色氨酸、酪氨酸與軟氨酸的存在。如培基中加有鋅游子，則產物中有苯丙氨酸、組氨酸和亮氨酸；又如培基中加有碘游子，則培養液中有色氨酸、酪氨酸、組氨酸以及氨丙酸。

（陳華粹 譯）

參考文獻

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A STUDY OF THE INFLUENCE OF ZINC AND IODIDE IONS ON THE AMINO ACID FORMATION IN THE CULTURE OF *PICHIA MEMBRANAEFACIENS*

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Introduction

Pichia membranaefaciens is an organism which can grow on absolutely synthetic media containing only ammonium salts as the sole source of nitrogen. It is seen that when ammonium sulphate is used as the source of nitrogen in the culture of this organism a few amino acids are found to be present in the culture at the end of the fermentation. A similar accumulation of *l*. alanine in cultures of *Brucella abortus* has also been observed by Goodlow, Braun and Mika who found that this accumulation limits the growth of smooth type cells and favours that of non-smooth mutants.

It has been seen that in the case of *Pichia membranaefaciens*, the presence of small amounts of zinc or iodide profoundly influences the nature of amino acids which are formed in the culture.

Experimental

Three cultures, each containing 0.025 gm. of sodium chloride, 0.025 gm. of potassium sulphate, 0.025 gm. of calcium carbonate, 0.030 gm. of magnesium carbonate and 0.025 gm. of disodium hydrogen phosphate, were prepared. These mineral nutrients were digested with dilute hydrochloric acid and to the clear solution were added, 0.5 gm. of ammonium sulphate and 5 gm. of glucose. The total volume of each culture was made up to 100 c.c. and the pH adjusted to be 4.5.

To one culture 0.0125 gm. of zinc sulphate was added to another 0.0125 gm. of potassium iodide and the third one was kept as such as the control.

These cultures were prepared in 150 c.c. flat bottom pyrex flasks which were cotton-plugged with surgical cotton and then were sterilized by heating in an autoclave at 10 pounds pressure for 30 minutes. After cooling, they were seeded with one drop of an activated sample of *Pichia membranaefaciens*.

These cultures were kept at 20°C for 60 days. Then the cultures were filtered in dried filter papers of known weight. The filtrates were tested for amino acids by Round paper chromatographic method by Giri.

Compound added	Yeast produced (in g)	Glucose left in the cultures(in g)	% yield of yeast on the basis of sugar consumed	Amino acids formed
Control	0.7836	0.00	15.79	Phenylalanine, tryptophan, tyrosine lysine
Zinc sulphate	0.8012	2.2	28.53	Phenylalanine, histidine leuecine
Potassium iodide	0.7640	0.00	15.28	Tryptophan, tyrosine, histidine, alanine

Discussion

When *P. membranaefaciens* grows in a culture containing glucose as source of carbon and ammonium sulphate as the source of nitrogen, phenylalanine, tryptophan, tyrosine and lysine are found in the solution which is obtained by filtering off the yeast grown in it. Different amino acids are formed when zinc ions are present in the cultures. Thus, if the culture contains a little of zinc sulphate, phenylalanine, histidine and leucine are found to be present in the culture. This is followed by a high yield of yeast in this culture. If potassium iodide is present in the culture tryptophan, tyrosine, histidine and alanine are seen in the culture and the yield of yeast is almost equal to the yield of yeast in the control culture.

In general, the amino acids left in the culture are mostly aromatic or cyclic.

Summary

When *P. membranaefaciens* grows in glucose cultures containing ammonium sulpate as the source of nitrogen, a few amino acids are found to be present in the culture. The nature of these amino acids is profoundly influenced by the presence of zinc or iodide ions in the culture. Thus whereas the control cultures shows the presence of phenylalanine, tryptophan, tyrosine and lysine, the culture containing zinc ions shows the presence of phenylalanine, histidine and leucine and the culture containing iodide ions indicate the presence of tryptophan, tyrosine histidine and alanine.