Fruit Body Formation of Red Bread Mold *Neurospora crassa* III. Effect of Nitrogen Sources with Special Reference to the Ionic Ratio of Ammonium and Nitrate in the Medium

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伊藤太郎：アカパンカビの子実体形成 III. 窒素源の影響，とくに培地中におけるアンモニウム塩と硝酸塩のイオン比について

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In order to analyze the physiological mechanism of sexual reproduction of heterothallic fungi, *Neurospora crassa*, as an endogenous factor, the hormonal effects of the culture filtrates of both mating type strains on the formation of fruit body should be studied.

In the vegetative stage the mycelial and conidial development of the vegetative thallus is achieved by hyphal tip elongation and somatic hyphal cell division. In the reproductive stage, the fruit body formation is initiated by gamentangial copulation of vegetative hyphae accompanying nuclear fusion. Consequently, it is assumed that there is a differential metabolic process in both the asexual and sexual growth phase. Thus it seems convenient for studying this mechanism also to examine nourishment differentiation inducing a qualitative change in the metabolic status of the vegetative and reproductive growth phase. N. Fries¹⁾ determined a nourishment constitution to be given to the minimal medium which would be favourable to the vegetative growth of this fungus. It contains ammonium tartrate and ammonium nitrate as the nitrogen source in the medium. Westergaard and Mitchell have recommended potassium nitrate (1g./l.) to be the best nitrogen source applied in the synthetic medium to favour the perithecial formation²⁾. It became clear that the presence of both ammonium and nitrate is necessary for vegetative growth, and also that the application of nitrate and elimination of ammonium as a nitrogen source in the medium for reproductive growth are required.

Material and Method

The strains used in the present experiment are A(+) and a(−) isolate from the progeny obtained in a crossing of the wild type of *N. crassa* 4A(+) and 8a(−). These strains are normal in every external feature, i. e. in mycelial, macro- and micro-conidial feature. They form fruit bodies after about a 2 week period when cultured on a synthetic basal medium at 25°.

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The medium used for mating in this experiment is the synthetic medium supplemented with various ammonium type nitrogen which contains the same gradients with the synthetic medium* devised by Westergaard and Mitchell¹³). Various ammonium type nitrogen compounds to be added to the synthetic medium are $(\text{NH}_4)_2\text{C}_4\text{H}_4\text{O}_6$, $(\text{NH}_4)_2\text{C}_2\text{O}_4\text{H}_2\text{O}$, $(\text{NH}_4)_2\text{SO}_4$, $(\text{NH}_4)_2\text{CO}_3$, NH_4Br , NH_4I , NH_4NO_3 and NH_4Cl . Eight series of agar cultures in which the amounts of these nitrogens varied from $1 \times 10^{-4}\text{M}$ to $1 \times 10^{-2}\text{M}$ to give the ionic concentration ratio of NH_4^+ and NO_3^- varied from 10:10 to 1:10 were set up. As NH_4NO_3 has a nitrate ion group, the amount of KNO_3 contained in the synthetic medium is varied to keep the above ionic concentration ratio. The method devised for studying the effect is as follows: the examination of the direct effect of the minute amount of ammonium is carried out by the perithecial formation test on a slant medium containing various ammonium types of nitrogen in the ionic concentration ratio of NH_4^+ and NO_3^- varied from 10:10 to 1:10. The examination of the indirect effect is done by using a filtrate agent. The process is as follows: A loop of each spore suspension (ca. $28 \times 10^6/\text{ml}$.) of the A(+), a(-) single strain, and A(+) and a(-) mixed strain is planted in a liquid medium 100 ml. in a flask, and incubated at 26° for 3 to 6 days in a dark chamber. The liquid medium is then filtered through a Seitz Filter (Toyo Asbestos Sterilizing Film No. 85) to remove completely free mycelia and conidia which are measured after drying completely. The culture filtrate or the CF**, contains the filtrate agent. The pH of the medium was adjusted at 6 to 6.5 by Na_2HPO_4 before sterilization. The perithecial formation test as a measure of the effect is conducted by counting the protoperithecia formed in the test tube containing the slant medium.

Experimental results

In order to examine the effect on protoperithecial and perithecial formation of various ionic concentrations of ammonium, a test for the formation and counting of protoperithecia and perithecia was conducted. The influence of the $[\text{NH}_4^+]:[\text{NO}_3^-]$ ratio in the medium on protoperithecial and perithecial formation was tested in the first experiment. A loop of spore suspension was placed on all synthetic agar media to which had been added various gradient amounts of each of the ammonium type nitrogen sources containing either $(\text{NH}_4)_2$ or (NH_4) group. The number of protoperithecia and perithecia formed after 10 to 14 days incubation at 25° in a dark chamber was counted. The result are shown in Table 1. The formation of protoperithecia was normally initiated after a lag period of 5 to 7 days and became stationary within 10 to 14 days after shedding large number of perithecia. Mycelial growth was not so abundant. The formation rates in two cases where either $(\text{NH}_4)_2\text{C}_4\text{H}_4\text{O}_6$ or $(\text{NH}_4)_2\text{C}_2\text{O}_4\text{H}_2\text{O}$ had been added were more remarkable than the

* The gradients are KNO_3 1g, KH_2PO_4 1g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g, NaCl 0.1 g, CaCl_2 0.1 g, sucrose 20 g, trace elements, biotin 5×10^{-6} g, agar 20 g, in 1 liter distilled water.

** The term is referred to the culture filtrate by Dr. N. Tanaka (1957).

others. In addition, the number of perithecia increased remarkably at a particular ionic concentration ratio of ammonium and nitrate ions among all ratios tested from 0:10 to 10:10.

In the case of (1) the ratios at which peak of perithecial formation was revealed, were 8:10, 5:10 and 1:10; in the case of (2) they were 9:10 and 6:10; in the case of (3) they were 7:10, 5:10 and 2:10; in the case of (4) they were 8:10, 6:10 and 2:10; in the case of (5) they were 9:10, 6:10 and 1:10; in the case of (6) they were 9:10, 6:10 and 2:10; in the case of (7) they were 9:10, 5:10 and 3:10; in the total they were 8:10, 6:10, and 1:10. At a ratio of 10:10, the case in which the peak appeared was (2); at 9:10, it being in (2), (5), (6) and (7); at 8:10, it being in (1), (4); at 7:10, it being in (3); at 6:10, it being in (2), (4), (5) and (6); at 5:10, it being in (1), (3) and (7); at 4:10, it being in (2); at 3:10, it being in (1) and (7); at 2:10, it being in (3), (4) (5) and (6); at 1:10, it being in (1), (5) and (6).

Table 1. Perithecial formation resulting from variation in the amounts of ammonium type nitrogen sources.

Ammonium type nitrogen added to KNO ₃	Conc. ratio [NH ₄ ⁺]: [NO ₃ ⁻]	Number of perithecia formed on agar-agar medium supplemented with various concentration ratios of either ammonium or nitrate ion											
		10/10	9/10	8/10	7/10	6/10	5/10	4/10	3/10	2/10	1/10	0/10	Total
1. (NH ₄) ₂ C ₄ H ₄ O ₆		218* ±47**	250 ±22	420 ±17	382 ±22	362 ±23	460 ±36	303 ±15	327 ±30	210 ±23	470 ±27	110 ±23	3512
2. (NH ₄) ₂ C ₂ O ₄ H ₂ O		243 ±17	330 ±17	306 ±24	238 ±17	380 ±12	215 ±18	274 ±18	125 ±9	155 ±14	172 ±9	"	2548
3. (NH ₄) ₂ SO ₄		40 ±7	60 ±22	140 ±16	173 ±31	145 ±25	190 ±22	167 ±25	144 ±32	182 ±16	118 ±22	"	1469
4. (NH ₄) ₂ CO ₃		71 ±8	50 ±8	69 ±14	31 ±5	108 ±22	0	45 ±7	83 ±8	117 ±12	20 ±8	"	704
5. NH ₄ Br		26 ±10	200 ±12	137 ±15	72 ±12	156 ±10	80 ±17	44 ±7	92 ±9	130 ±12	140 ±11	"	1187
6. NH ₄ I		0	105 ±25	4 ±1	51 ±11	132 ±17	17 ±5	65 ±20	10 ±7	100 ±47	98 ±10	"	692
7. NH ₄ NO ₃		81 ±28	84 ±17	38 ±8	0	45 ±6	100 ±20	0	105 ±19	0	50 ±11	"	613
8. NH ₄ Cl		0	0	0	0	3 ±2	10 ±5	12 ±2	2 ±1	0	0	"	137
Total		679	1079	1114	947	1331	1072	910	888	894	1067	880	10822

* The average number of ten tubes under culture duration 5-11 days after planting at 26°.

** Standard error $\left(\sqrt{\frac{\Sigma d^2}{n(n-1)}}\right)$.

Furthermore, the second experiment was conducted to investigate the influences of the CFs produced in the synthetic media in which the ratio of [NH₄⁺]:[NO₃⁻]

Table 2. A relationship between various ammonium type nitrogen source combined with potassium nitrate in ten grades of $[\text{NH}_4^+]:[\text{NO}_3^-]$ and the CFs ($\pm\text{CF}$, $+\text{CF}$ and $-\text{CF}$) obtained from synthetic medium supplemented with these nitrogen sources for protoperithecial and perithecial formation. In the $\pm\text{CF}$ series A(+) and a(-) were inoculated, in the $+\text{CF}$ and $-\text{CF}$ series either a(-) or A(+) was inoculated respectively. Ion ratio of $[\text{NH}_4^+]$ and $[\text{NO}_3^-]$ was varied in the range of 10:10 to 1:10. Measures were accompanied by the standard error.

CF agent Nitrogen source		RATIO	Number of protoperithecial formation													Total	Exp. ratio of CF.
			10/10	9/10	8/10	7/10	6/10	5/10	4/10	3/10	2/10	1/10					
1. $(\text{NH}_4)_2\text{C}_4\text{H}_4\text{O}_6$	±		28 ±6*	55 ±8	110 ±14	65 ±11	85 ±11	164 ±24	105 ±15	64 ±13	140 ±22	85 ±12	901	1.5:2:1**			
	+		7 ±3	23 ±6	58 ±9	58 ±9	92 ±8	240 ±27	183 ±21	118 ±20	133 ±24	165 ±23	1137				
	-		53 ±6	77 ±11	161 ±20	79 ±7	67 ±8	64 ±13	26 ±7	4 ±3	133 ±21	4 ±2	668				
2. $(\text{NH}_4)_2\text{C}_2\text{H}_1\text{H}_2\text{O}$	±		24 ±6	45 ±14	26 ±16	25 ±5	26 ±4	55 ±12	20 ±9	13 ±4	48 ±13	12 ±3	294	1:1:1***			
	+		0 ±18	74 ±11	0 ±12	53 ±13	14 ±16	79 ±5	— ±13	— ±13	2 ±4	11 ±4	233				
	-		26 ±4	37 ±11	0 ±12	40 ±13	40 ±13	23 ±5	29 ±5	45 ±13	16 ±4	15 ±4	271				
3. $(\text{NH}_4)_2\text{SO}_4$	±		23 ±11	37 ±12	14 ±5	36 ±10	58 ±16	41 ±6	45 ±5	24 ±6	69 ±17	17 ±5	364	1:1:1***			
	+		85 ±11	48 ±15	2 ±2	0 ±14	56 ±11	36 ±11	0 ±1	1 ±1	41 ±12	64 ±15	333				
	-		119 ±18	40 ±11	46 ±12	13 ±4	46 ±11	72 ±11	0 ±6	16 ±4	12 ±4	10 ±1	374				
4. $(\text{NH}_4)_2\text{CO}_3$	±		0 ±11	0 ±11	0 ±7	0 ±1	0 ±12	0 ±9	10 ±8	0 ±12	0 ±9	0 ±8	10	0.5:11:1			
	+		18 ±11	16 ±11	15 ±7	2 ±1	38 ±12	11 ±9	18 ±8	70 ±12	33 ±9	21 ±8	242				
	-		0 ±1	0 ±3	0 ±7	3 ±3	6 ±7	12 ±3	4 ±3	0 ±3	0 ±3	0 ±3	22				
5. NH_4Br	±		89 ±7	109 ±6	88 ±7	105 ±5	99 ±4	45 ±4	59 ±3	58 ±5	85 ±6	50 ±4	787	2:1			
	+		84 ±6	34 ±5	84 ±8	8 ±3	5 ±2	30 ±3	96 ±6	18 ±4	6 ±2	12 ±3	377				
	-		0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0	0				
6. NH_4I	±		0 ±1	0 ±2	1 ±0	3 ±0	3 ±4	5 ±1	7 ±1	7 ±0	3 ±0	3 ±1	31	0.5:0.5:1			
	+		2 ±1	5 ±2	0 ±0	0 ±0	10 ±4	3 ±1	3 ±1	1 ±0	0 ±17	6 ±8	27				
	-		0 ±4	0 ±4	0 ±4	0 ±4	13 ±4	0 ±4	31 ±4	0 ±3	17 ±3	8 ±3	69				
7. NH_4NO_3	±		29 ±6	34 ±2	13 ±3	33 ±4	6 ±4	26 ±5	42 ±6	22 ±3	0 ±3	3 ±2	208	1:1:1			
	+		30 ±12	15 ±3	46 ±14	16 ±3	16 ±3	0 ±2	16 ±2	0 ±1	0 ±7	8 ±11	137				
	-		26 ±7	41 ±6	60 ±9	3 ±2	18 ±3	0 ±3	24 ±4	1 ±4	7 ±2	11 ±3	191				
8. NH_4Cl	±		54 ±9	72 ±12	70 ±13	85 ±14	50 ±7	60 ±9	35 ±6	36 ±6	32 ±7	40 ±7	534	3:1.4:1			
	+		16 ±5	66 ±11	50 ±8	45 ±9	24 ±7	18 ±4	16 ±6	16 ±6	6 ±3	0 ±3	257				
	-		35 ±6	17 ±4	12 ±3	19 ±4	30 ±6	20 ±6	7 ±3	9 ±4	7 ±3	30 ±3	186				

* Standard error
** Highly significant χ^2 ($P < 0.01$)
*** Significant χ^2 ($0.05 < P < 0.2$).

was changed in 10 grades for every combined ammonium salt. The protoperithecial formation test was conducted by inoculating two sexual mating types, A(+) and a(-), on the agar medium supplemented with either the +CF or -CF prepared from the liquid synthetic media in which single culture of two mating type hyphae was grown. The results were shown in Table 2. In general, in the case of (1), (2), (3), (7) and (8) the effective formation was revealed. The concentration ratio of the ions in question in the medium was certainly related to the perithecial yield. In order to see whether the significant difference does exist or not in the experimental lots for perithecial yield, the standard error $\left(\sqrt{\frac{\sum d^2}{n(n-1)}}\right)$ was calculated and described in Table 2 as well as in Fig. 1. In the first experimental case (1) the ratios at which the peak of perithecial formation was revealed, were 8:10, 5:10 and 2:10 in the $\pm$ CF series; they were 5:10 and 1:10 in the +CF series; they were 8:10 and 2:10 in the -CF series. As a whole, the developmental stimulation in the experimental lots (1), (2) and (3) supplemented with the $\pm$ CF was seemed to be intermediate of those observed in the +CF and -CF series, and in others it was predominant.

Fig. 1. The influence of CFs from the synthetic medium containing ammonium and nitrate in 10 grades ionic concentration ratio on the protoperithecial and perithecial formation. The signs used in the figure show the following notes:

- ▲—: perithecial formation of A(+) and a(-) by $\pm$ CF,
- : protoperithecial formation of a(-) by +CF,
- : protoperithecial formation of A(+) by -CF,
- I —: range of standard error.

The numerals in abscissa indicate the ratio of $[\text{NH}_4^+]:[\text{NO}_3^-]$ in the synthetic media for preparation of the CF.

Consideration

In the sexual reproductive process, the physiological sequence concerned with

the fruit body formation in bipolar fungi, *N. crassa*, should be pursued primarily by analyzing conditions which seem to be responsible for creating differentiation in the physiological process which may work in developing a reproductive phase from a vegetative one. This will partly be achieved through an examination of the nourishment differentiation among the minimal media and of a lot of synthetic media that were recommended by several investigators in the past. The minimal medium used heretofore for the vegetative growth of *N. crassa*, contains $(\text{NH}_4)_2\text{C}_4\text{H}_4\text{O}_6$ 5 g. ($5/2 \times 10^{-2}\text{M}$) and NH_4NO_3 1 g. ($4/3.2 \times 10^{-2}\text{M}$) as the nitrogen sources²⁾. Meanwhile the synthetic medium recommended for the reproductive growth contains only KNO_3 1 g. ($1 \times 10^{-2}\text{M}$) without ammonium compound³⁾. In the minimal medium, if all nitrogen sources had dissociated completely into the ionic condition in the medium, the ionic concentration ratio of $[\text{NH}_4^+]:[\text{NO}_3^-]$ should be $5+4/3.2:4/3.2=5:1$, and in the latter this ratio will be 0:1. This fact, namely, seems to indicate that the excess amount of NH_4^+ in the medium may be responsible for reduction in the perithecial formation in reproductive growth. At the same time, less frequent formation of the perithecia to be produced when this fungus cultured in the synthetic medium which contains only NO_3^- as a nitrogen source might be due to lack of NH_4^+ in the medium. It should be expected, at the same time, that the activity of NH_4^+ in the synthetic medium might have an analogous inhibiting activity for perithecial growth as in the case when the amount of some metal ion is too great.

On the activity of NO_3^- in the perithecial formation Hirsh³⁾ stated that protoperithecial formation increased with an increasing of the amount of KNO_3 until it reaches a maximum at a concentration of 1 g. per litre after which the number fell off. Furthermore, he examined precisely the nitrate effect and concluded that the presence of NO_3^- did not affect fertilization and fruit body development but rather some stage in the differentiation of the protoperithecium, and that there should be a radically differential mode of utilization of nitrate in conjunction with the appearance of the two different "physiological states" at two temperatures.

As shown in Table 1, the distribution frequency in the number of perithecia is not randomised but shows clotting somewhat; roughly speaking, around the three different ionic concentration ratios of $[\text{NH}_4^+]$ and $[\text{NO}_3^-]$ 8:10, 6:10 and 1:10. It is presumably considered that there are three peaks represented by the three concentration ratios of $[\text{NH}_4^+]$ and $[\text{NO}_3^-]$. It may possibly indicate the presence of a specific feature in the utilization of a minute amount of both NH_4^+ and NO_3^- for perithecial formation by two sexual mating type strains. This may depend also upon the differential utilization of both NH_4^+ and NO_3^- based upon the physiological differentiation of either sexual mating type strains, because, as shown in Fig. 2 in the previous report⁴⁾, in the column 1, 2 and 3 in the Table 2 and Fig. 1, either the +CF or -CF effectively acts on perithecial formation for the opposite sexual mating type strain respectively, especially, at two definite ionic concentration ratio of NH_4^+ and NO_3^- . The +CF has a stimulative activity for protoperithecial formation at the

ionic concentration ratios $[\text{NH}_4^+]:[\text{NO}_3^-]=5:10$ and $1:10$, on the other hand the $-CF$ at $8:10$ and $2:10$. That is, it is assumed that $A(+)$ strain would produce more stimulative substances at certain lower concentration of NH_4^+ than the $a(-)$ strain does, and at certain higher concentration of NH_4^+ *vice versa*. The ionic concentration ratios $[\text{NH}_4^+]:[\text{NO}_3^-]$ at which either the $+CF$ or $-CF$ reveals discriminative effects on protoperithecial formation are different from each other. As shown in Table 2 (column 1, 2 and 3) and Fig. 1, either $A(+)$ or $a(-)$ produces more markedly stimulative substance at two concentration ratios, viz. $5:10$ and $1:10$, $8:10$ and $2:10$, of NH_4^+ and NO_3^- on the protoperithecial formation. Thus, the remarkable perithecial formation, as shown in Table 1, at ratios $8:10$, $5:10$ and $1:10$ in the case of (1), at ratios $9:10$, $6:10$ and $4:10$ in the case of (2) and at ratios $7:10$, $5:10$ and $2:10$ in the case of (3) etc. could be considered as a combined effect of the $+CF$ and $-CF$ on the perithecial formation which is initiated by protoperithecial formation of single sexual strain mycelia occurred by stimulative substances which are produced abundantly at a particular ratio of these ions in the CFs produced from the complementary strain culture. Furthermore, this interpretation could be justified by comparing the perithecial yields in the $\pm CF$ series with those in the $+CF$ series and $-CF$ series in the Table 2. If the formation effect of the $\pm CF$ is a sum of that of both the $+CF$ and $-CF$ as presumed in the previous report⁵⁾, that is;

$$\begin{array}{l} \text{The formation effect} \\ \text{of the } \pm CF \end{array} = \begin{array}{l} \text{The formation effect} \\ \text{of the } +CF \end{array} + \begin{array}{l} \text{The formation effect} \\ \text{of the } -CF, \end{array}$$

the perithecial yield in the $\pm CF$ should be one half of the sum of the yield in the $+CF$ and $-CF$ when approximately equal number of spore were inoculated. In the case of (1), (2), (3) and (7) in the Table 2, the ratios of the formation yield among the three CF series were approximately estimated $1.5:2:1$, $1:1:1$, $1:1:1$ and $1:1:1$, respectively. However, in the case of (8) it was $3:1.4:1$. Thus, it can be concluded that the formation effect of the $\pm CF$ series seen at the three concentration ratios would be composed of (a) the effect of the $+CF$ and (b) that of the $-CF$ as shown in the case of the (1), (2), (3) and (7), and of the either effect and the effect of unknown factor in the case of the (8).

The problem of the stimulative substances in *Neurospora* is interesting to be solved concerning the nature of the mating type factor and the biochemical mechanisms in the sexual reproduction in this organism as well as in the other fungi. E. R. Sansome⁶⁾ also stated that although the sexual reproduction depends upon the fusion of two nuclei of opposite mating types, there is no evidence that heterokaryon formation is necessarily required for perithecial formation, and it would be caused by the stimulation of the mycelium of one mating type by hormone-like substance produced by the other mating type. In this experiment the existence of the sexual hormone-like substances has been clearly recognized as well as in the other species.

If the developmental perithecial formation seen at the several ionic concentration

ratio is initiated by several sexual hormone-like substances, it seems that either sexual mating type factors (mt^+ and mt^-) act to produce these substances at these ionic concentration ratios and they work independently to produce at least two kinds of the stimulative substances. At the same time, it seems to be likely that the production process of the substance by the both strains is composed of at least two processes in relating to nitrate reductase system. This explanation described above is supported by the following facts reported by Hirsh³⁾ and W. S. Silver and W. D. McElroy⁷⁾.

According to Hirsh, in *Neurospora crassa*, a lot of protoperithecia and perithecia were formed only when the organism was grown at 25° and kept continuously at the same temperature. When the mold was grown first at 35° and transferred to 25° after conidiation produced also a lot of mature perithecia. While those cultured continuously at 30° produced incipient protoperithecia which do not develop further. However, when the organism was grown at 35° and kept on at the same temperature after conidiation, neither protoperithecia nor perithecia were formed. When the culture was kept at 25° before conidiation and transferred to 35° after conidiation, only protoperithecia were formed, but they did not develop into mature perithecia. Thus, it was considered that some stimulative substances were produced at the temperature range between 25° and 35° before conidiation. But this prediction was not proved substantially, for the extract of any culture before conidiation exerted neither stimulative effect nor inhibitory effect for fructification in the culture after conidiation.

On the other hand, W. S. Silver and W. D. McElroy showed that there were several enzymatic steps in the reduction process of nitrate to nitrite and they were controlled by several different genetic factors which affected either directly or indirectly the synthesis of nitrate reductase.

Consequently, it is suggested that the induction and initiation of the perithecial and protoperithecial formation will be presented differentially by trace agents contained in the filtrate agent of either the +CF or -CF, in other words, the whole sexual reproduction will be consisted at least of two different physiological processes of either the A(+) or a(-) differentiated in the utilization of both ammonium and nitrate. In order to make evident this problem, it seems necessary that (i) to analyze quantitatively the amount of turnover of the NH_4^+ and NO_3^- with the lapse of culture time under some temperature range, and that (ii) to demonstrate chemically as well as physically the potential activity of the specific substances in the CFs for the protoperithecial formation.

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Summary

The present report deals with the effect of different ammonium type nitrogens in gradient amounts on the perithecial formation in *Neurospora crassa*.

The perithecial induction is activated in media containing either ammonium tartrate or ammonium oxalate rather than the other ammonium type compounds as nitrogen source. It is made evident that the perithecial formation is conspicuously activated in the media supplemented with both potassium nitrate and ammonium tartrate to make up the ionic concentration ratio of ammonium and nitrate to be either 8:10, 6:10 or 1:10 (cf. Table 1).

This activity is found to be related to some substance produced in the media under special ionic conditions; the filtrate of the A(+) culture (+CF) produces substance which stimulates the perithecial formation of the opposite a(−) mycelia and the filtrate of the a(−) culture (−CF) produces substance that activates the A(+) perithecial formation.

There is a compensating declination in the effect of the single filtrate on the perithecial formation; in the A(+) strain culture, the stimulative substance increases in the lower ammonium concentrations and in the a(−) strain culture it increases in the higher. At the same time, the filtrate of the mixed culture (±CF) has a combined effect of both single filtrate (+CF and −CF) on the perithecial formation.

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摘 要

本報は *Neurospora crassa* の被子器形成におよぼす種々のアムモニウム態窒素源の量的影響についての研究報告である。被子器形成は、用いられた種々のアムモニウム態窒素源のうち、酒石酸アムモニウムを微量 ($0.5 \times 10^{-3}M$ 以下) 添加の合成子実体形成用培養基上でいちじるしい。特に前者の場合 NH_4^+ と NO_3^- の濃度比が 8:10, 6:10 および 1:10 の時に顕著である。

この添加窒素源の特定量添加の際にみられる形成作用は、 NH_4^+ と NO_3^- の特定濃度比を有する合成培養基中で、A(+) 系および a(−) 系がそれぞれその反対性型系すなわち a(−) 系および A(+) 系菌糸に原被子器 (protoperithecium) 形成を誘起する性ホルモン様物質のはたらきによることが明らかとなった。そして A(+) 系は NH_4^+ 濃度が低い場合、a(−) 系は NH_4^+ 濃度が高い場合に著るしい原被子器形成の誘起作用をあらわし、A(+) および a(−) 混合系では両者の中間的效果がみられる。