Pak. J Bot, 5 (1) : 33-43, 1973.

SOME GROWTH PROPERTIES OF HAPLOID AND DIPLOID STRAINS OF *PROTOMYCES INUNDATUS.*

M. SALIH AHMAD

Nuclear Institute for Agriculture & Biology, Lyallpur

Abstract

The fungus *Protomyces inundatus* Dangeard grows readily in liquid culture, budding in a yeast like fashion. Glucose saturation constant is 0.45×10^{-3} M, while the maximum exponential growth rate is 0.136 divisions per hour. Thiamine is required for growth and it can not be replaced with pyrimidine or thiazole moieties when present in the medium alone or simultaneously. This fungus can utilize a number of nitrogen or carbon sources. No differences are found in the haploid and diploid strains for the growth properties studied. The differences in the behaviour of nucleic acids in haploid and diploid strains are discussed in the light of this new investigation.

Introduction

The life history and taxonomic position of *Protomyces inundatus* Dangeard have been reinvestigated (Valadon, Manners & Myers, 1962). This fungus is parasitic on *Apium nodiflorum*, and the infection is recognized by characteristic galls on the leaves of the host plant. It produces intercellular septate, multinucleate mycelium in the leaves and later large thick walled chalamydospores which occur in aggregates at the surface of leaf as galls. The chalmydospores have no dormancy period and on germination give rise to adnospores of opposite mating type, that fuse in pairs and escape through a split in the wall of chalmydospore. It is shown that fungus exhibits bipolar hetero-thallism and the mating type endospores are incapable of infection to the host plant. The endospores grow on synthetic medium by budding in yeast-like manner.

Nucleic acids behaviour in non-infectious mating type haploid and infectious diploid endospores of *P. inundatus* is reported by Valadon, Myers & Manners (1962). It is established that deoxyribo-nucleic acid (DNA) and ribonucleic acid (RNA) contents per cell decreased with age as the culture grew old. Also it is shown that the culture exhibited constancy of RNA to DNA ratio close to 12 for haploid cell and 6 for diploid cell in other words; the RNA produced per unit DNA in diploid cell was one half of that produced in haploid cell. These authors suggested that either DNA alone is injected from one of the conjugants (Male gamete) to the zygote and thus RNA remained at the level of haploid or both DNA and RNA are contributed from each of conjugants, and RNA undergoes an extra replication without concomitant increase in RNA before it settles down to the normal division cycle.

Venitt, Myers & Manners (1968), tested the above mentioned hypothesis by labelling RNA with 3H-uridine in a cell of mating type which was mated with unlabelled cell of opposite mating type, and *vice versa*, and the fate of radioactivity being traced with the help of stripping film autoradiography. The results of this experiment proved the hypothesis untenable because both the parents to the zygote contributed RNA and also radioactivity was present in cells just budding off the zygote.

Much evidence is available that the nucleic acids metabolism is affected appreciably by the composition of the growth medium and as well as the genetic constitution in bacteria (Neidhardt, 1964). What is true for bacteria, it can be true for fungi. Some

important growth characteristics, growth rate constant, total yield constant, saturation constant for growth limiting factor (Monod, 1942); for reasons of comparison in growth properties of haploid and diploid endospores of *P. inundatus*, are presented. Observations on the requirements of thiamine and biotin have been extended. Assimilation of various carbon and nitrogen sources have been investigated. No differences in the growth properties, that can be correlated to the differences in ploidy or nucleic acids metabolism of the cell, have been found.

Materials and Methods

(a) Chemicals and reagents

All the chemicals were purchased from British Drug Houses and these have the highest purity. Glass distilled water was used for preparing the medium. Sterilization was carried out in an autoclave at 10 lb/in.^{2} Heat labile chemicals were sterilized by passage through millipore filter.

(b) Strains

A wild growing plant of *A. nodiflorum*, while infected heavily with *P. inundatus*, was collected. One of the culture 2nB was established by the isolation of a single fused endospore, with the help of a micromanipulator, from a germinating chlamydospore taken out of an infectious gall. The mating type cultures 18+ and 2— were established by the isolation of unfused endospores produced in a germinating chlamydospore taken from the leaf of a host plant infected with 2nB culture under green house conditions; and these were considered haploid. Neither culture 18+ nor 2— was sexually compatible with 2nB. Culture F2, established by isolating a criss cross body resulted in a cross between 18+ and 2—, could infect *A. nodiflorum* and therefore it was considered diploid. Estimations for DNA, on per cell basis, supported the haploid nature of cultures 18+ and 2— and the diploid nature of cultures 2nB and F2. The cultures were maintained on 2% malt agar.

(c) Culture growth

The cultures were grown, for any given nutrient, in 100 ml medium using 350 ml Erlenmeyer flask on a reciprocating mechanical shaker. The incubation was at 20°C. After 15 days of incubation, when growth was complete, the cells were harvested. The cells were washed two times with ice cold water and dried to a constant weight at 85°C. Glass electrodes were used for recording pH of the medium before inoculation and also just before harvesting of cells.

(d) Growth rate determination

The growth rate was determined for basal medium glucose, 10g: l—asparagine, 2g; KH_2PO4, lg; $MgSO_4 . 7H_2O$, 0.5g; Fe^{++},0.2 mg; Zn^{++},0.2 mg; Mn^{++},0.1 mg; biotin, 5 μg; thiamine, 100 μg; and water to 1000 ml, (Valadon, Manners & Myers, 1962). Cells grown in basal medium were harvested in log phase, washed with fresh medium, and diluted to suitable cell concentration for inoculation. Growth was measured from the increase in optical density at 450 nm using Unicam SP 500 Spectrophotometer.

Optical density measurements were calibrated in terms of cell concentration. The growth rate during exponential phase was calculated using relationship;

$$r = \frac{\log_2 x_1 - \log_2 x_0}{t_1 - t_0}, \text{ Monod (1942).}$$

In exponential growth phase x_0 cells were present at time t_0 then after growing at time t_1 there would be x_1 cells.

(e) Growth yield constant for glucose

It was found that glucose was the growth limiting factor in the basal medium. Addition of glucose stimulated the growth of *P. inundatus* in stationary culture. Growth yield constant for glucose was calculated from a relationship arrived at by Monod (1942).

$$\text{Yield constant} = \frac{\text{quantity of the fungus formed}}{\text{quantity of the limiting factor utilized}}$$

(f) Growth saturation constant for glucose

Growth saturation constant for glucose was determined by an empirical relationship, $\mu = \mu m \left(\frac{S}{Ks + S}\right)$, Monod (1950). S = substrate concentration, μm = growth rate at saturation levels of substrate, μ = growth at low substrate concentration and remains proportional, reaching maximum value μm at saturation substrate concentration, Ks = saturation constant and it is numerically equal to the substrate concentration at which the growth rate is half the maximum.

(g) Vitamin requirement

Need for biotin or thiamine was assessed by elimination of these vitamins from the basal medium. The growth was recorded for successive subcultures on vitamin free medium. Also stimulation of growth was observed in vitamins deprived subcultures by addition of biotin and thiamine. Further experiments were conducted to replace thiamine for pyrimidine or thiazole moieties of thiamine.

(h) Assimilation of carbon and nitrogen sources

Utilization of different carbon sources was observed by replacing glucose in the basal medium. Similarly growth was measured for various nitrogen sources replacing l-asparagine.

Results

The growth rate was 0.136 divisions per hour on basal medium, glucose contents 1g per 100 ml; and thus *P. inundatus* cells were doubling with mean generation time of 7.35 hr. Haploid as well as diploid cultures revealed no differences in exponential growth rate (Table 2). It was noted that the diploid cell concentration was 72—75 x 10^7 cells per ml of medium, in stationary phase cultures, a number somewhat lower than that of haploid strains which contained 81—83 x 10^7 cells per ml, perhaps due to greater volume of diploid cells.

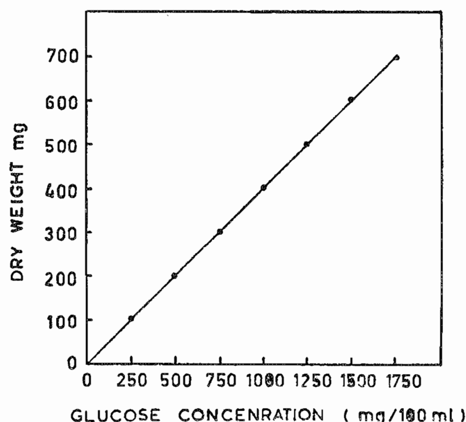

Fig. 1. Total growth of *P. inundatus* in basal medium with glucose as limiting factor.

Glucose was the growth limiting factor in the basal medium, however, it ceased to be a limiting factor at 1750 mg per 100 ml of medium (Fig. 1). The concentration of glucose beyond the limiting value at 2000 mg or above per ml of medium, reduced the total growth to some extent (Table 1). Presumably glucose exerted repression effect and inhibited the utilization of l-asparagine. It is likely that cells keep growing

TABLE I. Growth of *Protomyces inundatus* strains in basal medium at various glucose concentrations.

Glucose concentration (mg/100 ml)	pH (b.i)	18+ DW	18+ pH (b.h)	2— DW	2— pH (b.h)	2nB DW	2nB pH (b.h)	F2 DW	F2 pH (b.h)
250	4.51	101	8.62	105	8.70	99	8.81	100	8.68
500	4.51	200	8.43	190	8.51	198	8.60	199	8.55
750	4.51	296	8.21	301	8.09	293	8.13	304	8.00
1000	4.51	394	7.85	400	7.59	405	7.73	403	7.71
1250	4.51	502	7.35	503	7.45	496	7.41	507	7.41
1500	4.51	605	6.60	601	6.80	593	6.78	598	6.69
1750	4.51	705	6.20	689	5.93	700	6.00	696	6.01
2000	4.51	665	6.00	660	5.80	685	5.78	670	5.73
2500	4.51	660	6.05	670	5.78	670	6.01	665	5.84
3000	4.51	650	6.01	688	5.96	673	5.77	670	5.98

DW = dry weight (mg),

pH (b.i) = pH of the medium before inoculation.

pH (b.h) = pH of the medium before growth harvest.

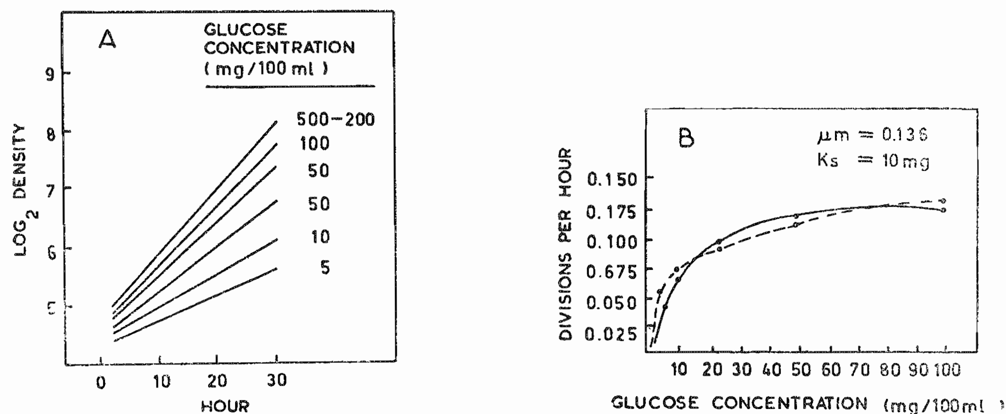

Fig. 2A. Exponential phase of the growth of *P. inundatus* in basal medium at various concentration of glucose. B. Growth rate as function of glucose concentration. Dotted curve was drawn through practical points and solid curve was drawn theoretically with μ_m = 0.136 divisions per hour and Ks=10 mg. per 100 ml. medium.

on l-asparagine under conditions where glucose was a limiting factor. Also glucose concentration, within the range of which it is limiting in the basal medium, seems to control the pH of the growing culture. The pH of grown culture was 8.7 when glucose concentration was 250 mg per ml and it decreased to a constant value of 5.9 when glucose ceased to be the limiting factor. The growth yield constant for glucose is 0.4 (Table 1). Regarding these properties, the haploid and diploid strains showed no differences.

At low concentration of glucose, the exponential growth rate remained proportional (Fig. 2A). The maximum growth rate (μm) was 0.136 divisions per hour and glucose saturation constant Ks equalled 0. 45 x 10^{-3} M (10 mg per 100 ml), (Table 1). No differences were present in haploid and diploid strains. The practical value of μm and Ks could fit the mathematical formulation (Fig. 2B). No differences were present in the values of μm and Ks found for haploid and diploid strains.

TABLE 2. Growth rate of *Protomyces inundatus* strains in basal medium at various concentration of glucose (Growth rate expressed as divisions per hour).

Glucose concentration (mg/100 ml)	STRAINS			
	18+	2—	2nB	F2
5	0.055	0.045	0.044	0.060
10	0.073	0.068	0.085	0.066
25	0.078	0.097	0.085	0.098
50	0.100	0.123	0.110	0.116
100	0.125	0.123	0.125	0.120
250	0.127	0.130	0.230	0.130
500	0.128	0.133	0.125	0.134
1000	0.133	0.134	0.133	0.135
1500	0.133	0.135	0.130	0.133
2000	0.136	0.135	0.136	0.134

It is shown that growth in the first subculture from basal medium to vitamin deprived medium (i.e. basal medium with biotin and thiamine omitted) was only 35% of that in basal medium and subsequent sub cultures in the vitamin deprived medium had only small growth. It appeared that *P. inundatus* had residual constant growth on vitamin deprived medium. The withdrawal of biotin from the basal medium had no effect on total growth so it cannot be considered essential for growth. Thiamine was essential and its deletion had marked effect on the growth (Table 3). When cultures maintained on vi amin deprived medium for three successive subcultures and then fortified with biotin there was no increase in growth, but addition of thiamine had marked effect and restored the growth at original level (Table 4). Further experiments showed that thiamine was not replaceable with the pyrimidine or thiazole moieties of thiamine when present alone or simultaneously, though there was some increase in growth (Table 5). The replacement of glucose with glycerol and l-asparagine with sodium nitrate in the vitamin deprived medium with addition of pyrimidine and thiazole did not induce the high growth rate obtained with thiamine. It appears that residual constant growth of *P. inundatus* might be due to the leakiness of thiamine genome rather than because of the repression due to glucose or l-asparagine. There were no differences as regard to the above observations between haploid and diploid strains.

TABLE 3. Effects of deletion of biotin or thiamine or both from the basal medium on the growth of *Protomyces inundatus* strains.

Medium	pH	STRAINS							
		18+		2−		2nB		F2	
		DW	pH	DW	pH	DW	pH	DW	pH
	(b.i)	(b.h)		(b.h)		(b.h)		(b.h)	
1. Basal (Control)	4.50	400	7.28	395	7.28	390	7.32	405	7.30
2. Biotin	4.50	400	7.28	395	7.30	400	7.29	400	7.18
3. Thiamine	4.50	140	3.58	140	3.71	140	3.60	138	3 69
4. Biotin, thiamine	4.50	140	3.55	135	3.50	135	3.51	145	3.57
5. Biotin, thiamine (Sub1)	4.50	18	5.81	17	5.50	15	5.70	17	5.80
6. Biotin, thiamine (Sub2)	4.50	9	5.61	12	5.91	11	5.50	11	5.50
7. Biotin, thiamine (Sub3)	4.50	8	5.50	11	5.50	10	5.72	9	5.72

Sub1, Sub2 & Sub3 are successive subcultures from medium 4.

TABLE 4. Effect of addition of biotin or thiamine or both to the vitamin deprived medium on the growth of *Protomyces inundatus*.

	STRAINS								
		18+		2—		2nB		F2	
Medium	pH (b.i)	DW	pH (b.h)	DW	pH (b.h)	DW	pH (b.h)	DW	pH (b.h)
1. +Biotin, +thiamine	4.50	405	6.98	395	6.79	395	6.85	400	6.81
2. +Thiamine	4.50	395	6.84	400	6.72	400	6.81	400	6.89
3. +Biotin	4.50	8	5.56	8	5.54	8	5.54	9	5.49

TABLE 5. Effect of addition of pyrimidine or thiazole or both to the vitamin deprived medium on the growth of *Protomyces inundatus* strains.

	STRAINS								
		18+		2—		2nB		F2	
Medium	pH (b.i)	DW	pH (b.h)	DW	pH (b.h)	DW	pH (b.h)	DW	pH (b.h)
1. +Pyrimidine	4.71	4	4.45	4	4.22	5	4.39	4	4.41
2. +Thiazole	4.71	14	4.35	12	4.25	14	4.40	16	4.12
3. +Pyrimidine, +thiazole	4.71	16	4.22	14	4.17	11	4.23	15	4.27
4. +Pyrimidine, +thiazole +glucose, +glycerol —l-asparagine, +sodium nitrate	4.71	20	3.25	20	3.28	24	3.35	19	3.29

Replacement of l-asparagine from the basal medium by nitrogen sources showed that *P. inundatus* can utilize nitrogen from NH_4^+, NO_3^-, urea, and other amino acids (Table 6). Nitrogen from ammonium nitrate and ammonium sulphate was not utilized as efficiently as from diammonium hydrogen phosphate or ammonium acetate. Harmful effects due to NO_3^- or SO_4^{--} ions could be ruled out because sodium nitrate and magnesium sulphate supported good growth. Measurements of the pH of the cultures before and after the growth period showed that the adverse pH set up in the cultures grown on ammonium nitrate and ammonium sulphate might be responsible for the decreased growth and the buffer capacity of ammonium acetate is likely important in supporting growth in that medium. The good growth on diammonium hydrogen phosphate is surprising in view of the low final pH but this may be attained less rapidly than the low pH values found in case of the other ammonium salts, alternatively the high concentration of phosphate ions may be counteracting the adverse effect of this pH. The growth on glutamic acid medium was very small and it might be due to initial high acid pH of the medium. There were no differences between haploid and diploid strains in the utilization of the nitrogen sources.

TABLE 6. Effect of various nitrogen sources on the growth of *Protomyces inundatus* strains.

(l-asparagine replaced with other nitrogen source)

		STRAINS							
		18+		2—		2nB		F2	
Nitrogen source	pH (b.i)	DW	pH (b.h)	DW	pH (b.h)	DW	pH (b.h)	DW	pH (b.h)
1. Ammonium acetate	6.05	365	5.92	372	6.78	369	6.15	383	6.72
2. di-Ammonium hydrogen phosphate	7.15	360	2.62	340	2.90	355	2.61	350	2.72
3. Ammonium nitrate	4.77	10	3.40	13	3.44	13	3.43	13	3.48
4. Ammonium sulphate	4.82	30	3.00	32	3.01	33	3.01	28	3.26
5. Potassium nitrate	4.78	360	8.20	367	7.89	369	8.00	351	7.80
6. Sodium nitrate	4.78	355	8.08	360	8.08	378	8.05	376	7.80
7. Urea	4.82	260	8.65	275	8.80	265	8.78	276	8.80
8. dl-Leucine	4.82	305	3.82	295	3.60	303	3.62	310	3.89
9. dl-Glutamic acid	2.45	5	2.35	5	2.37	6	2.35	5	2.41
10. Peptone	5.58	300	5.01	295	5.78	290	5.20	305	5.01

A number of carbon sources can be utilized by *P. inundatus*. Arabinose, rhamnose, galactose and lactose are poor carbon sources (Table 7). Metabolism of carbon sources set alkaline pH in the culture; and it is not certain whether the level of high alkaline pH is responsible to restrict the growth in the cultures where arabinose, rhamnose, galactose and lactose were present. However, there were no differences between haploid and diploid strains with regard to utilization of the carbon sources studied.

TABLE 7. Effect of various carbon sources on the growth of *Protomyces inundatus* strains.

(Glucose replaced with other carbon source in basal medium)

		STRAINS							
		18+		2—		2nB		F2	
Carbon source	pH (b.i)	DW	pH (b.h)	DW	pH (b.h)	DW	pH (b.h)	DW	pH (b.h)
1. Glycerol	4.60	366	6.75	352	6.80	364	6.80	354	7.01
2. Mannitol	4.95	397	7.15	400	7.11	408	7.02	405	7.21
3. Sorbitol	4.95	344	7.65	633	7.66	358	7.71	355	7.63
4. Arabinose	4.95	41	8.65	39	8.68	39	8.65	43	8.60
5. Xylose	4.95	348	7.65	355	7.50	345	7.62	341	7.48
6. Rhamnose	4.95	47	8.78	49	8.78	55	8.78	49	8.68
7. Fructose	5.15	378	7.81	384	7.88	383	7.75	387	7.80
8. Galactose	5.00	51	8.95	56	8.98	53	8.10	57	9.15
9. Sucrose	5.00	412	7.80	421	7.71	427	7.62	418	7.78
10. Maltose	5.01	326	8.19	332	8.15	325	7.95	325	7.98
11. Lactose	5.01	54	9.19	52	9.19	57	9.25	51	9.25

Discussion

Information on nucleic acids metabolism in fungi is rather scanty. Mostly the investigations are confined to the biosynthesis of nucleotides (Cochrane, 1958), and nucleic acid depolymerases (Egami & Nakamura, 1969). Studies on the contents of nucleic acids are reported from the polyploid series of *Saccharomyces* (Ogur *et al*, 1952). It is demonstrated that dry weight, RNA, metaphosphate; apart from DNA

contents, are ploidy dependent in yeast. The diploid yeast cells have DNA as well as RNA two times more in amount as compared to the haploid cells. Behaviour of nucleic acids and protein contents, on cell basis, in growing cultures of haploid and diploid endospores of *P. inundatus* has been reported (Valadon, Myers & Manners, 1962). It is shown that diploid cultures of *P. inundatus* have double the amount of DNA per cell than that found in the haploid cultures, but the amount of RNA per cell, in contrast to yeast cultures, in haplid and diploid cells was essentially equal. Further it is established that DNA and RNA contents per cell, in both the haploid and diploid cultures of *P. inundatus*, decreased with age, however, the RNA to DNA ratio remained remarkably constant at 6:1 for diploid and 12:1 for haploid cultures at all stages of growth irrespective of age.

Investigations on the total RNA contents in *Salmonella typhimurium* and in *Aerobacter aerogenes* show that RNA synthesis, over a wide range of growth rate, is a function of growth rate at a given temperature, and it is unimportant whether the growth rate set by the nature of the carbon, nitrogen or energy sources or by the presence of amino acids, nucleotides, or other growth stimulating vitamins, or by the rate of supply of a required nutrient in chemostat cultures. If two media support growth at the same rate, the cells grown in them will have equal amount of RNA (Neidhardt, 1964).

This study has shown that the haploid and diploid cultures of *P. inundatus* do not differ in growth rate, glucose yield constant and glucose saturation constant while grown in basal medium. Moreover, various carbon or nitrogen sources are metabolized with equal efficiency by the haploid and diploid cells. These r. sults, as it stands, suggest that nutritional requirements do not take part in adjusting the rate of RNA synthesis in diploid cells at the level of RNA contents present in haploid cells.

It was suggested (Valadon, Myers & Manners, 1962) that during conjugation of endospores in *P. inundatus* it may be that RNA is contributed by only one of the conjugants or that DNA undergoes an extra replication without the concomitant increase in RNA before it settles down to normal division cycle. The examination of the behaviour of the RNA of conjugant haploid cells of *P. inundatus* failed to support the above hypothesis (Venitt *et al*, 1968). Furthermore, the above mentioned hypothesis implied that RNA like DNA is self replicating and this appears trivial in light of new evidence which concludes that RNA is transcribed on DNA template and the RNA synthesis is under very subtle genetic control (Jacob & Monod, 1961).

Genetic experiments on *Xenopus laevis* and *Drosophila melanogaster* indicate that ribosomal RNA (rRNA) which is about 90% of the total RNA is under DNA control. Embryos of *X. laevis* that lack the ability to form nucleoli were incapable of synthesis of rRNA. Homozygotes of this mutant die at the tail-bud stage, possibly because the maternal ribosomes are no longer able to support the embryos demands for protein synthesis (Brown & Gurdon, 1964). Hybridization of rRNA to DNA provides evidence that DNA stretches complementary to ribosomal RNA are directly proportional to the number of nucleolar organizers (Wallace & Birnstiel, 1966). In stocks of *D. melanogaster* containing genomes possessing one, two, three and four nucleolar organizers, it is shown that the number of stretches of DNA complementary to rRNA is directly proportional to the number of nucleolar organizers (Ritossa and Spiegelman, 1965).

It can be concluded, in absence of any indication of nutritional factors adjusting the rate of RNA synthesis or growth, that one half of the DNA is not transcribed in diploid cells of *P. inundatus*. Several haploid and diploid strains of *P. inundatus* can prove suitable material to study the transcriptional control that regulates the RNA synthesis.

Acknowledgements

I express my sincere gratitude to Drs. A. Myers and J.G. Manners, of Department of Botany, University of Southampton, for suggesting the problem and for many discussions throughout this work. I am obliged to the Colombo Plan Authority for the Colombo Plan Overseas Training Award and to the British Council for the hospitality.

This work is a part of a thesis submitted for the degree of Doctor of Philosophy in the Faculty of Science, University of Southampton, England.

References

Brown, D.D. and J.B. Gurdon. 1964. Absence of ribosomal RNA synthesis in the anucleolate mutant of *Xenopus laevis*. Proc. Nat. Acad. Sci. U.S.A., **51**: 139-146.

Cochrane, V.W. 1958. Physiology of Fungi. John Wiley and Sons, Inc., New York.

Egami, F. and K. Nakamura. 1969. Microbial Ribonucleases. Springer-Verlag Berlin. Heidlberg. New York.

Jacob, F. and J. Monod. 1961. Genetics regulatory mechanism in the synthesis of proteins. J. Mol. Biol., **3**: 318-356.

Monod, J. 1942. Recherches sur la croissance des cultures bacteriennes. Hermann, Paris.

Monod, J. 1950. La technique de culture continue; theorie et applications. Ann. Inst. Pasteur, **79**: 390-410.

Neidhardt, F.C. 1964. The regulation of RNA synthesis in bacteria. In: Progress in Nucleic Acid Research and Molecular Biology, Vol. 3., pp. 145-181. J.N. Davidson and W.E. Cohn, eds., Academic Press, New York and London.

Ogur, M., S. Minckler, G. Lindegren and C.C. Lindegren. 1952. The nucleic acids in polyploid series of *Saccharomyces*. Arch. Biochem. Biophys., **40**: 175-184.

Ritossa, F.M. and S. Spiegelman. 1965. Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of *Drosophila melanogaster*. Proc. Nat. Acad. Sci. U.S.A., **53**: 737-745.

Valadon, L.R.G., J.G. Manners and A. Myers. 1962. Studies on the life-history and taxonomic position of *Protomyces inundatus* Dangeard. Trans. Brit. mycol. Soc., 45: 573-586.

Valadon, L.R.G., A. Myers and J.G. Manners. 1962. The behaviour of nucleic acids and other constituents in *Protomyces inundatus* Dangeard. J. Exp. Bot., **13**: 378-389.

Venitt, S., A. Myers and J.G. Manners. 1968. Studies of conjugation in *Protomyces inundatus*. Exp. Cell Res., **50**: 9-18.

Wallace, H. and M.L. Birnstiel. 1966. Ribosomal cistrons and the nucleolar organizer. Biochem. Biophys. Acta, **114**: 296-310.