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EFFECT OF NUTRITIONAL FACTORS ON GROWTH AND SPORULATION OF GEOPHILIC KERATINOPHILES

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Nutritional factors directly influence the growth of micro-organisms. The present study deals with the effect of nutritional factors viz. carbon, nitrogen, phosphorus on growth and sporulation of soil borne keratinophilic fungi. The effect of different nutritional factors, mannitol and sorbitol as carbon sources, glycine and ammonium sulphate as nitrogen sources, potassium dihydrogen phosphate and disodium hydrogen phosphate as phosphorus sources were studied on growth and sporulation of *Chrysosporium tropicum* and *Trichophyton mentagrophytes*. Among the carbon sources, mannitol showed maximum growth and sporulation in both the fungi. Glycine as a nitrogen source gave maximum growth and sporulation in both the fungi. Both fungi showed maximum growth and sporulation in the medium supplemented with disodium hydrogen phosphate as the phosphorus source.

Key words: Nutritional factors, keratinophilic fungi, *Chrysosporium tropicum, Trichophyton mentagrophytes*

Nutritional studies of the fungi or of any other organism are necessary for understanding their relationship with the substratum/host on which they grow. A fungus grows in a substratum when its nutritional requirements are fulfilled and external conditions are favourable for its development. This information is usually gathered by growing the fungus *in vitro* on various media, and exposing it to different environments. The nutritional factors comprise different sources of carbon, nitrogen, vitamins, trace elements and synthetic hormones. The studies of various nutritional factors will give some information concerning the general physiology of the fungi. It is well known that all substances are not equally suitable for the growth and sporulation of different fungi (Lilly and Barnett, 1951).

The effect of different nutritional factors on growth and sporulation of *Aspergillus tamarii* (Otutiola, 1976), *Trichophyton mentagrophytes* (Mosher *et al.*, 1936) and *Trichophyton tonsurans* (Verujsky, 1887) was reported.

The present investigation was done to analyze the effect of different nutritional factors on growth and sporulation of geophillic keratinophiles. The study of the growth requirements including nutritional factors will be promising in understanding the possible distribution of the fungi and its pathogenicity. The data may also be helpful in understanding the different limiting factors for the growth of these possible dermatophytes and hence will be valuable in their control.

MATERIALS AND METHODS

Two keratinophilic fungal species viz. *C. tropicum* and *T. mentagrophytes* were isolated from soil through To.Ka.Va. hair baiting technique (Vanbreuseghem, 1952). Garrett agar disc method (1936) was used to inoculate the fungi in nutrition medium. The degree of sporulation of fungi was determined using standard methods as recommended by Wilson and Knight (1952) and Tuite (1969).

Results are given as mean ± standard error of the mean of N observations. Data sets were examined by one-way analysis of variance (ANOVA). P-value of less than 0.05 was considered significant.

RESULTS

Effect of different nutritional factors on growth of the isolated fungi was determined from the dry

weight of mycelium and spore count, using Sabouraud's Dextrose broth (modified) medium.

Effect of carbon sources : In this study, different concentrations of two carbon sources viz. mannitol and sorbital (5000ppm, 7500ppm, 10000ppm, 12500ppm, 15000 ppm, 17500 ppm and 20000 ppm) were added in the medium. Among the carbon sources, both fungi showed best growth and sporulation in medium supplemented with mannitol. Dry weight of mycelium, sporulation and change in pH of the growth medium is shown in Table 1. The control treatment did not show any sporulation and found to be less suitable for growth of fungi.

Effect of nitrogen sources : To study the effect of different nitrogen sources on growth and sporulation of selected fungi, different concentrations of glycine and ammonium sulphate (2000 ppm, 2500 ppm, 3000 ppm, 3500 ppm, 4000 ppm, 4500 ppm and 5000 ppm) were used separately in the medium. Among the nitrogen sources glycine proved to be best for the growth and sporulation of both fungi. Dry weight of mycelium, sporulation and change in pH of the growth medium is shown in Table 2. The control treatment of nitrogen sources also found less suitable for growth and sporulation of fungi.

Effect of phosphorus sources : For evaluation of growth and sporulation of isolated fungi, different concentrations of potassium dihydrogen phosphate and disodium hydrogen phosphate (500ppm, 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm and 3000 ppm) were used separately in the medium. Among the two phosphorus sources, disodium hydrogen phosphate turned out to be the best for the growth and sporulation of both the fungi. Dry weight of mycelium, sporulation and change in pH of growth medium is shown in Table 3. The control test was completely negative for sporulation and showed less suitability for growth of both fungi.

DISCUSSION

Availability of nutritional factors is necessary for the growth and development of all living organisms. Fungi too, shows maximum growth in

Table 1: Effect of different concentrations of carbon sources on growth and sporulation of test fungi. (Initial pH = 7.5)

Carbon sources	Concen-tration	*Chrysosporium tropicum*			*Trichophyton mentagrophytes*		
		Final pH (ppm)	Dry weight of mycelium	Sporulation	Final pH	Dry weight of mycelium	Sporulation
Mannitol	Control*	8.2	0.029 ± 0.001	+	8.0	0.033 ± 0.006	+
	5000	8.2	0.078 ± 0.002	++	8.2	0.123 ± 0.061	++
	7500	8.3	0.134 ± 0.008	+++	8.2	0.129 ± 0.042	+++
	10000	8.1	0.149 ± 0.006	+++	8.1	0.160 ± 0.051	+++
	12500	6.9	0.152 ± 0.012	++++	7.3	0.164 ± 0.023	++++
	15000	7.3	0.172 ± 0.085	++++	6.4	0.165 ± 0.012	++++
	17500	6.6	0.187 ± 0.062	++++	6.6	0.215 ± 0.025	++++
	20000	6.6	0.189 ± 0.035	++++	6.6	0.211 ± 0.021	+++
Sorbitol	Control*	8.2	0.028 ± 0.001	+	8.0	0.033 ± 0.006	+
	5000	8.3	0.033 ± 0.004	++	8.2	0.039 ± 0.012	+
	7500	8.2	0.052 ± 0.005	+++	8.2	0.048 ± 0.013	+
	10000	8.3	0.061 ± 0.022	+++	8.1	0.095 ± 0.012	+++
	12500	6.4	0.098 ± 0.028	++++	8.2	0.085 ± 0.028	+++
	15000	6.8	0.079 ± 0.042	++++	8.2	0.092 ± 0.032	+++
	17500	8.2	0.066 ± 0.008	++++	8.3	0.083 ± 0.031	++
	20000	8.0	0.076 ± 0.003	++++	8.1	0.077 ± 0.021	++

Values are means ± standard errors (SE) of measurements taken in triplicates (n=3) and $P<0.05$
(*Control without carbon source); (- = No sporulation, + = Poor sporulation, ++ = Fair sporulation, +++ = Good sporulation, ++++ = Excellent sporulation)

Table 2: Effect of different concentrations of nitrogen sources on growth and sporulation of test fungi. (Initial pH = 7.5)

Carbon sources	Concen-tration	*Chrysosporium tropicum*			*Trichophyton mentagrophytes*		
		Final pH (ppm)	Dry weight of mycelium	Sporulation	Final pH	Dry weight of mycelium	Sporulation
Glycine	Control*	5.9	0.011 ± 0.002	-	5.3	0.017 ± 0.003	+
	2000	8.0	0.016 ± 0.003	+	6.9	0.023 ± 0.008	++
	2500	8.3	0.071 ± 0.021	++	6.8	0.066 ± 0.006	+++
	3000	7.9	0.098 ± 0.012	+++	7.4	0.087 ± 0.007	+++
	3500	8.0	0.126 ± 0.012	++++	7.9	0.108 ± 0.018	++++
	4000	7.6	0.132 ± 0.002	++++	7.8	0.103 ± 0.012	++++
	4500	8.2	0.138 ± 0.001	++++	7.7	0.101 ± 0.031	++++
	5000	8.0	0.113 ± 0.013	++	8.4	0.073 ± 0.007	+++
Ammonium sulphate	Control*	5.9	0.011 ± 0.002	-	5.3	0.017 ± 0.003	-
	2000	6.1	0.031 ± 0.012	+	5.8	0.023 ± 0.008	-
	2500	6.7	0.052 ± 0.026	+	6.6	0.036 ± 0.012	+
	3000	7.1	0.073 ± 0.013	++	7.1	0.043 ± 0.018	+
	3500	7.3	0.086 ± 0.021	++	7.0	0.069 ± 0.024	++
	4000	7.6	0.089 ± 0.032	++	7.4	0.071 ± 0.019	++
	4500	7.9	0.051 ± 0.011	+	7.5	0.078 ± 0.024	++

Values are means ± standard errors (SE) of measurements taken in triplicates (n=3) and P<0.05
(*Control without nitrogen source); (- = No sporulation, + = Poor sporulation, ++ = Fair sporulation, +++ = Good sporulation, ++++ = Excellent sporulation)

Table 3: Effect of different concentrations of phosphorus source on growth and sporulation of test fungi. (Initial pH = 7.5)

Phosphorus sources	Concen-tration	*Chrysosporium tropicum*			*Trichophyton mentagrophytes*		
		Final pH (ppm)	Dry weight of mycelium	Sporulation	Final pH	Dry weight of mycelium	Sporulation
Potassium dihydrogen phosphate	Control*	3.8	0.011 ± 0.002	-	5.2	0.021 ± 0.008	-
	500	7.2	0.014 ± 0.018	-	7.3	0.023 ± 0.009	-
	1000	7.6	0.032 ± 0.009	++	7.9	0.049 ± 0.001	+
	1500	7.6	0.058 ± 0.012	+++	7.6	0.063 ± 0.002	++
	2000	7.9	0.068 ± 0.006	++++	7.7	0.065 ± 0.006	+++
	2500	8.1	0.068 ± 0.017	++++	7.6	0.063 ± 0.012	++++
	3000	7.5	0.061 ± 0.011	++++	8.0	0.067 ± 0.018	++++
Di sodium hydrogen phosphate	Control*	3.8	0.011 ± 0.002	-	5.2	0.021 ± 0.008	-
	500	8.1	0.048 ± 0.028	+	8.3	0.023 ± 0.003	-
	1000	7.3	0.095 ± 0.019	+	7.9	0.057 ± 0.004	+
	1500	7.4	0.115 ± 0.011	+++	7.8	0.078 ± 0.012	+++
	2000	7.7	0.072 ± 0.031	+++	7.9	0.107 ± 0.041	+++
	2500	7.7	0.064 ± 0.005	+++	7.4	0.099 ± 0.011	+++
	3000	7.8	0.059 ± 0.012	++	7.7	0.051 ± 0.015	++

Values are means ± standard errors (SE) of measurements taken in triplicates (n=3) and P<0.05
(*Control without phosphorus source); (- = No sporulation, + = Poor sporulation, ++ = Fair sporulation, +++ = Good sporulation, ++++ = Excellent sporulation)

the medium when all its nutritional requirements are provided in optimum concentrations.

Carbohydrates, fats and proteins of different forms are the main components of cell, in which carbon, oxygen and nitrogen are the framework elements. Carbon, phosphorus and nitrogen are the building blocks of proteins and nucleic acids.

Different forms of carbon, phosphorus and nitrogen are available in nature, and are utilized by different micro-organisms.

Qualitatively, fungi are known to be very specific and selective in utilizing different carbon sources and can be attributed to the presence or absence of specific enzymes in the fungal hyphae. Das Gupta and Shome (1959) reported moderate growth of some Indian strains of *Trichophyton rubrum, T. mentragrophytes* and *Epidermophyton flocossum* on pentose sugars (Xylose and Arabinose). Hejtmanek (1960) and Schatik (1964) studied and reported the utilization of disaccharides by some keratinophilic fungal isolates.

In the present study, mannitol was found to be suitable for maximum growth and sporulation of both fungi. Increase in concentration of mannitol and sorbitol in the medium seems, not suitable, as growth and sporulation declined in both the test fungi.

Nitrogen is an essential element for fungal growth and sporulation. It is important in functional and structural process of the organism. Cochrane (1963) opined that nitrites are a poor source of nitrogen for many fungi due to their toxic effect. Mathison (1962) and Schatik (1964) reported the ability of keratinophilic fungi to assimilate ammonium nitrogen in both nitrate and nitrite form. Glycine, among the two source of nitrogen studied, showed maximum growth and sporulation of both fungi.

Phosphorus is a major constituent of DNA, RNA and cell membrane. Therefore, along with carbon and nitrogen it also plays an important role in the growth of fungi. Hube *et al.* (2001) studied the role and relevance of phospholipase D1 as an essential phosphorus source during growth and dimorphism of *Candida albicans.* In the present study disodium hydrogen phosphate gave maximum growth and sporulation.

The concentrations of nutritional factors were taken based on the data available from the previous reports. The initial pH of the medium was set at 7.5. A change in pH of the medium was observed after the experiment. This could be attributed to the production of fungal metabolites or to the breakdown of nutrient source. The strains of geophilic fungal isolates studied are also reported as dermatophytes. The results suggest that nutritional factors acts as limiting factors for the growth and sporulation of these fungi and can be utilized in controlling its growth.

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