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Effects of Some Amino Acids on Ammonia Secretion and Extracellular Protease Activity by Three Oomycetes in Synthetic Medium with or without Glucose

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The effects of different concentrations of three amino acids as carbon and or nitrogen sources on mycelial dry weights, changes in pH values of synthetic medium, ammonia secretion and extracellular protease activity by three zoosporic fungi, pathogens of fish and shellfish, were studied. As compared with the control, the addition of isoleucine and aspartic acid as nitrogen sources were generally stimulative for mycelial dry weight production whereas phenylalanine was inhibitory irrespective to the tested fungal species. When amino acids served as carbon and nitrogen sources, the mycelial dry weights of the three fungi were increased (mostly non-significantly) relative to untreated control but weights were decreased as the concentrations of the three amino acids raised. The addition of individual amino acids as carbon and nitrogen sources to the medium significantly increased pH values of the medium comparable to the control. The addition of each of the three amino acids as carbon and nitrogen sources to the medium significantly induced ammonia secretion by the three species of zoosporic fungi. Ammonia secretion in synthetic medium amended with amino acids as nitrogen source raised by the three zoosporic fungi relative to untreated control except in case of *Achlya racemosa* treated with isoleucine. Extracellular protease activity was almost promoted in case of *Achlya proliferoides* and *Saprolegnia furcata* cultures treated with isoleucine and aspartic acid individually in presence of glucose and vice versa in case of phenylalanine. However, extracellular protease activity of *A. racemosa* decreased compared with the control at various concentrations of isoleucine and both phenylalanine and aspartic acid assumed inconsistent effects. Extracellular protease activity of the three zoosporic fungi in the medium devoid of glucose varied depending upon zoosporic fungal species, the tested amino acid and the applied concentrations. The values of protease activity were approximately less two folds than that obtained in presence of glucose.

KEYWORDS: Ammonia secretion, Protease activity, Zoosporic fungi

Zoosporic fungi cause many serious fish and shellfish diseases through penetrating of their bodies as reported by Durborow *et al.* (1991), Bly *et al.* (1992), Hatai (1992), Dieguez-Uribeondo *et al.* (1996), and Bangyeekhun *et al.* (2001). Also, some species of freshwater zoosporic fungi were capable of infecting protein rich bodies such as mosquitoes (Sparrow, 1960; Dick, 1968; McInnis and Zattau, 1982), mushroom grubs (Couch, 1924) and nematodes (Nolan, 1983). Proteolytic enzymes are needed for insect pathogenic fungi for degrading the host cuticle, which, consists of chitin fibrils, embedded in a protein matrix (Neville, 1975). Peduzzi and Bizzozero (1977) demonstrated proteolytic chymotrypsin-like enzyme activity in the aquatic fungus *Saprolegnia*. Exoprotease activity leads to the breakdown of proteinaceous substances into small peptides and amino acids and these accumulated amino acids repressed exoprotease production as mentioned by Li *et al.* (1997). In synthetic media, amino acids were reported to be a suitable carbon sources for some genera of the class Oomycetes especially order Saprolegniales (Gleason, 1968; Gleason *et al.*, 1970a, b; Faro, 1971). On the other hand, some authors found their effects are inhibitory and attributed that due to ammonia accumulation in the medium, which followed by the rise of pH value (Coll and Leal, 1972; Nolan, 1976). Amino acids served as carbon sources by *S. megasperma* when glucose was present and must have functioned as such in the absence of glucose (Nolan, 1975). Deamination of amino acids occurred when glucose was present or absent from the medium also indicated by Nolan (1975). On the other hand, Nolan (1976) found that *S. ferax* utilized some amino acids more rapidly in the absence of glucose. However, no attention had been given for studying the effects of the accumulated amino acids on protease activity and ammonia secretion by zoosporic fungi. Hence, this investigation aimed to study the effects of three amino acids; isoleucine (aliphatic), aspartic (dicarboxylic) and phenylalanine (aromatic) on biomass mycelial dry weights, changes in pH values of the culture medium, ammonia secretions and protease activity of three zoosporic fungi (*Achlya proliferoides*, *A. racemosa* and *S. furcata*) which were the commonest fungi in the River Nile system in Egypt and also known as fish pathogens. The results were compared when amino acids were served only as nitrogen source (in presence of glucose in the synthetic basal medium) and when they functioned as carbon and nitrogen sources (when glucose

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was omitted from the medium).

Materials and Methods

Tested zoosporic fungal species. Three zoosporic fungal species related to the family Saprolegniaceae (Oomycetes) were tested during this investigation. These species namely; *A. proliferoides*, *A. racemosa* and *Saprolegnia furcata*. These zoosporic fungal species were found to be of common occurrence in various Egyptian water habitats (El-Hissy *et al.*, 1982, 1992; El-Hissy and Khallil, 1989) and were associated with seven fish species of the River Nile (El-Hissy *et al.*, 1989) and were also isolated from some fish species inhabiting some Egyptian lakes (unpublished data).

The synthetic medium. A modified glucose-peptone synthetic medium was used during this investigation. This medium was used in a liquid form for studying the effect of the selected amino acids on biomass mycelial dry weights, changes in pH values of culture medium, ammonia secretions and protease activity of zoosporic fungal species in presence of glucose and when glucose was omitted. This medium includes in g/*l*; glucose 3, $MgSO_4 \cdot 7H_2O$ 0.128, KH_2PO_4 0.0136 together with the trace micro-nutrient elements, mg/*l*; $CaCl_2$ 8, $FeCl_2$ 0.5, $MnCl_2$ 0.5, $CuSO_4$ 0.1 and $ZnSO_4$ 0.1. Directly, after autoclaving the medium and while the conical flasks were still hot, 0.5 mg/*l* sodium benzylpenicillin and 0.5 mg/*l* streptomycin sulphate were also added.

Inoculation and treatments. Mycelial inoculations of medium were made using 1.5-cm-diameter discs cut from the margins of 8-days-old solidified cultures of the three fungi with a sterile cork borer under aseptic conditions. These mycelial discs of the three zoosporic fungi were used as inoculum for 100 *ml* Erlenmeyer conical flasks, each containing 20 *ml* liquid synthetic basal medium which were adjusted to give concentrations of each of the three tested amino acid, 0 (control free from any amino acid addition), 400, 800, 1200 and 1600 $\mu g/ml$. Other inoculated conical flasks containing the same concentrations of the three amino acids and medium composition devoid of glucose were also prepared. The inoculated flasks were incubated at 20°C. Three replicates of conical flasks were used for each amino acid concentration and fungal species tested. At the end of incubation period (10 days) the mycelial mats were harvested and dry weight was determined while culture filtrates were used for estimation of ammonia secretion and exoprotease activity in addition to follow changes in pH by the tested zoosporic fungi.

Biomass dry weight of mycelium. The mycelial mats were removed from the conical flasks at the end of the incubation period (10 days) through Buchner funnel using a hardened filter paper Whatman No. 1. The biomass mycelia were then washed three times with distilled water and dried in an oven at 80°C until constant weight. Thereafter, the biomass-dried mycelia were calculated per 20 *ml* synthetic culture medium.

Changes in pH values. For following the changes in the pH values of the culture filtrate by the three tested zoosporic fungi as they affected by the applied amino acids, the pH values were measured using pH-meter (GTP NR: 9133).

Determination of ammonia. Ammonia was estimated in the culture filtrate by using Nessler's reagent as described by Vogel's (1968). This procedure based on the reaction of sodium hydroxide with Nessler's reagent. One *ml* of the sample (culture filtrate) was transferred into 20 *ml* volumetric flask containing some distilled water for dilution. Thereafter, two *ml* of Nessler's reagent were added followed by three *ml* of 3 N NaOH and then completed with distilled water to the mark of the flask. A yellow color soon develops which measured at wavelength 490 nm. A calibration curve was constructed using ammonium chloride and the data were calculated as mg NH_4^{+3} (as ammonium chloride) per 20 *ml* culture filtrate medium.

Extracellular protease activity assay. The assay procedure adopted by Kunitz (1946/47). A 1% casein solution was prepared in 0.05 M sodium phosphate buffer (pH 7.0), heat denatured at 100°C for 15 minutes in water bath, cooled and used as substrate. Add 0.5 *ml* of culture filtrate to 1 *ml* of casein solution, mixed thoroughly and incubated at 35°C for 3 hrs. The reaction was terminated by adding 3 *ml* of cold 10% trichloroacetic acid (TCA). The tubes allowed standing for one hour at 4°C to allow undigested protein to precipitate. Blanks were made in the same way using boiled filtrate. The tubes were then centrifuged at 1000 rpm for 15 minutes. The supernatant fluid was analyzed for digested un-precipitated protein as described by Lowry *et al.* (1951). Calibration curve was made using casein and the data were expressed as mg casein/hr × 20 *ml* culture filtrate.

Statistical analysis. The data obtained subjected to statistical analysis known as analysis of variance (ANOVA). Each value presented in tables represents the mean value of three estimated replicates. This type of statistical analysis aimed to elucidate the difference between the control and the different treatments of amino acids in relation to the evaluated parameters of the three species of zoosporic fungi. Means were compared in each case and the significance was obtained using LSD at 5% level.

Results and Discussion

Mycelial dry weight. The results in Table 1 reveal that the application of isoleucine to the medium in presence of glucose (amino acid served as nitrogen source) enhanced mycelial dry weight of *A. racemosa* compared with the control (difference was significant only at 800 *μg/ml*) and the weights were almost increased with the rise of the concentration. Also, the biomass dry weights of *S. furcata* increased comparable to control but the difference was not significant and weights were decreased with increase of concentration. However, the mycelial dry weight of *A. proliferoides* was increased with rising of isoleucine concentration and declined at the high dose (1600 *μg/ml*) but the values were still lower than that at control except at 1200 *μg/ml* (equal to the control value).

The addition of aspartic acid to the basal synthetic medium provided with glucose was generally stimulative (non-significantly) for the mycelial dry weights of the three investigated zoosporic fungi (Table 1) at all doses except in few cases (at 800 and 1200 *μg/ml* in case of *A. proliferoides* and 1600 *μg/ml* in case of *A. racemosa* were inhibitory). The dry weight increased with rising of the concentration in case of *A. proliferoides* and *A. racemosa* whereas it was decreased in case of *S. furcata.*

The data presented in Table 1 indicate that treatment of phenylalanine to the medium amended with glucose resulted in inhibition of dry weight mycelia of *A. proliferoides*, *A. racemosa* and *S. furcata* (except at 1200 *μg/ml*) and the difference was significant relative to untreated control in case of *A. proliferoides.*

Similarly, Herr (1973) harvested a highest yield of *Aphanomyces cochlioides* mycelium grown in a combination of low glucose, high asparagine and high methionine. The dry weight production of *Verticillium dahliae* varied greatly depending on the amino acid used as a nitrogen source in Czapek's-Dox medium (Ducan and Himelick, 1987). They found a similar variation when sugar maple (Acer saccharum) sap was amended with individual amino acids. Coll and Leal (1972) interpreted the high mycelium yield of the fungi *Aspergillus nidulans* and *Penicillium italicum* must be due to the utilization of the carbon skeleton of the amended amino acid (L-tryptophan) as additional carbon source. Moreover, Leal *et al.* (1971) using the dry weight mycelium, divided the amino acids into two groups, poor and good during their studies on the value of 21 amino acids as nitrogen sources for *Phytophthora cactorum* and *P. heveae.* In addition, Oladiran and Oso (1980) found that *Botryodiplodia theobromae* grew best on glutamic acid and *Pythium aphanidermatum* on glycine. Also, Abdel-Rehim *et al.* (1974) reported that asparagine stimulated the growth of *Geotrichum candidum* and *Alternaria alternata.* Other works were also

Table 1. Effects of different concentrations of the three tested amino acids on mycelial dry weights, pH values of the medium, ammonia secretion and extracellular protease activity by the three zoosporic fungal species at 20±2°C in presence of glucose in the basal synthetic medium

Fungi	Conc. *μg/ml*	Isoleucine				Aspartic acid				Phenylalanine			
		DW	pH	AS	PA	DW	pH	AS	PA	DW	pH	AS	PA
A. proliferoides	0	49.67	4.92	3.03	10.04	49.67	4.92	3.03	10.04	49.67	4.92	3.03	10.04
	400	31.33	7.54*	8.24	16.04	65.67	3.10*	1.74	18.93	33.00*	7.55*	6.34	5.43
	800	48.50	6.59*	13.80	15.09	25.33	4.03*	7.40	12.57	29.00*	7.34*	5.22	4.51
	1200	49.67	6.11*	7.16	12.78	34.00	5.95*	5.94	12.10	24.33*	7.24*	6.31	4.10
	1600	29.67	4.70	5.85	8.82	67.00	4.57	4.99	9.40	22.33*	6.53*	6.72	3.95
LSD5%		NS	0.99	NS	NS	0.57	0.57	NS	NS	16.51	0.61	NS	NS
A. racemosa	0	52.33	4.20	2.43	12.47	52.33	4.20	2.43	12.47	52.33	4.20	2.43	12.47
	400	76.50	7.52*	1.87	10.93	81.67	4.15	5.22	16.71	41.00	7.52*	12.23*	10.72
	800	174.50*	7.13*	2.84	10.05	89.33	4.82	6.23	13.56	42.67	7.57*	9.03*	13.11
	1200	88.00	6.66*	2.17	6.65	101.00	5.22	7.65	8.48	20.33	6.82*	6.47*	19.84
	1600	121.00	5.41*	1.57	5.37	51.00	5.81	14.46	8.27	15.00	4.15	4.69	1.83
LSD5%		70.00	0.86	NS	NS	NS	NS	NS	NS	NS	0.78	3.77	12.75
S. furcata	0	56.00	4.81	3.62	14.63	56.00	4.81	3.62	14.63	56.00	4.81	3.62	14.63
	400	143.33	7.70*	5.82	20.66	94.67	3.43*	2.43	21.26	46.00	7.60*	10.38	5.62
	800	63.67	7.12*	5.76	23.46	60.67	3.82	8.74	19.38	37.67	7.19*	7.35	5.60
	1200	82.33	5.52	4.12	18.09	75.00	6.19*	9.74*	19.26	68.33	7.12*	6.29	5.15
	1600	57.00	5.28	11.60	18.26	76.67	5.70	11.57*	14.46	40.67	5.36	5.10	1.63
LSD5%		NS	1.38	NS	NS	NS	1.29	5.62	NS	NS	1.33	NS	NS

LSD5%: least significant difference at 5% level.
NS: Non-significant compared with the control.
*: Significant difference compared with the control.

Table 2. Effects of different concentrations of three tested amino acids on mycelial dry weights, pH values of the medium, ammonia secretion and extracellular protease activity by the three zoosporic fungal species at 20±°C in absence of glucose from the basal synthetic medium

Fungi	Conc.μg/ml	Isoleucine				Aspartic acid				Phenylalanine			
		DW	pH value	AS	PA	DW	pH	AS	PA	DW	pH	AS	PA
A. proliferoides	0	27.00	5.19	1.90	10.47	27.00	5.19	1.90	10.47	27.00	5.19	1.90	10.47
	400	64.67	7.73*	16.59*	13.29	54.67*	8.00*	11.90*	7.15	71.33*	8.19*	8.79*	9.64
	800	58.00	8.19*	16.16*	10.40	35.00	9.83*	13.11*	7.32	36.33	9.02*	13.76*	8.52
	1200	58.00	8.89*	17.76*	8.46	31.67	10.74*	14.41*	8.95	26.66	10.53*	14.78*	1.98
	1600	33.00	10.24*	16.85*	6.19	15.67	11.33*	16.04*	4.58	18.00	11.32*	12.92*	1.85
LSD5%		NS	1.10	5.51	NS	21.92	1.32	5.89	NS	17.28	1.01	4.16	NS
A. racemosa	0	24.67	5.53	3.53	6.48	24.67	5.53	3.53	6.48	24.67	5.53	3.53	6.48
	400	95.67*	7.57*	13.11*	8.35	58.33	8.02*	11.02	9.85*	93.67*	8.12*	8.44*	14.48
	800	65.33	7.97*	18.07*	4.06	55.33	7.84*	23.24*	8.93*	80.33*	8.55*	10.31*	1.83
	1200	66.00	8.80*	22.63*	3.31	35.00	8.34*	20.21*	9.17*	64.67*	9.98*	15.00*	0.92
	1600	35.00	9.31*	25.17*	1.92	28.33	9.29*	24.69*	3.69*	33.67	10.97*	15.31*	0.96
LSD5%		43.95	0.54	9.18	NS	NS	0.63	8.96	2.12	19.47	0.90	3.24	NS
S. furcata	0	21.67	4.64	3.77	10.52	21.67	4.64	3.77	10.52	21.67	4.64	3.77	10.52
	400	79.50*	7.59*	7.13	15.26	25.67	8.01*	19.38*	11.77	43.33	8.19*	5.60	13.87
	800	42.00	8.46*	18.82*	20.39*	77.33*	8.30*	20.02*	8.74	32.00	9.25*	11.60*	7.96
	1200	21.00	9.27*	23.18*	16.23	35.67*	8.78*	23.34*	8.27	35.33	11.21*	10.97*	3.38*
	1600	24.33	9.99*	25.84*	5.65	14.00	10.62*	24.41*	1.89	18.67	12.04*	21.83*	2.46*
LSD5%		20.88	0.58	5.34	9.13	9.84	0.63	5.66	1.76	NS	0.53	5.49	3.58

L. S. D. 5%: least significant difference at 5% level.
NS: Non-significant compared with the control.
*: Significant difference compared with the control.

available on the utilization of amino acids as a source of carbon and energy by many of the fungi in the class Oomycetes (Gleason, 1968; Faro, 1971; Gleason *et al.*, 1970a, b).

The data obtained when glucose was omitted from the medium and amino acids acted as carbon and nitrogen sources showed that the dry weight mycelia of the three tested zoosporic fungi was generally increased compared with control but the difference was almost significant at the lowest treatment (400 μg/ml) of amended amino acids. In this case, the dry weight mycelia of the three tested zoosporic fungi were generally lowered with increasing the dose concentration of the applied three amino acids as shown in Table 2. In accordance with these results, Papavizas and Davey (1960) obtained a very limited growth of *Aphanomyces euteiches* in absence of glucose and they indicated that the amino nitrogen source of synthetic media was poorly utilized as carbon sources. Gleason *et al.* (1970a) indicated that *Saprolegnia* spp. can utilize the majority of the amino acids tested although in some cases very poorly. They attributed the poor utilization or non-utilization of certain amino acids by *Saprolegnia* spp., which, was observed, might be due to toxic effects of these substrates at high concentrations. On the other hand, Nolan (1976) reported that the isolates of *S. ferax* utilized alanine, leucine, tyrosine and phenylalanine more rapidly in the absence of glucose.

Changes in pH values. Nearly, the addition of different treatments of both isoleucine and phenylalanine individually to the synthetic medium in presence of glucose significantly increased the pH values of the medium by the three tested zoosporic fungal species compared with the control as illustrated in Table 1. The measured pH values were exponentially decreased with the rise of the concentration of these amino acids. On the other side, the application of aspartic acid to the medium in case of *A. proliferoides* lowered the pH value at all the applied doses except at 1200 μg/ml (increased) comparable to untreated control (Table 1) and the difference was significant at 400, 800 and 1200 μg/ml. With regards to *A. racemosa*, the treatments with aspartic acid raised (non-significantly) the pH value of the culture medium except at the low dose (slightly decreased) compared with the control. With respect to *S. furcata*, aspartic acid lowered the pH value of the medium at low concentrations (400 and 800 μg/ml) and raised it at high doses (1200 and 1600 μg/ml) comparable to the control and the difference was significant only at 400 and 1200 ppm. Generally, the pH values of the medium were increased by the three zoosporic fungal species as the concentration of aspartic increased.

The measured pH values of synthetic medium devoid of glucose and amended with different treatments (400, 800, 1200 and 1600 μg/ml) of each tested amino acid which used as carbon and nitrogen sources for growing of

zoosporic fungal species were significantly raised compared with the control. As the concentrations of the three tested amino acids (isoleucine, aspartic and phenylalanine) elevated, the pH values by the three zoosporic fungal species were gradually climbed from 7.57~12.04 (Table 2).

Ammonia secretion. Table 1 reveals that ammonia secretion in synthetic medium provided with isoleucine as nitrogen source and glucose as carbon source was non-significantly increased comparable to the control at all doses in case of both *A. proliferoides* and *S. furcata* whereas it was suppressed in case of *A. racemosa* (except at 800 *μg/ml*). In most cases, ammonia secretion was lowered as the concentration of isoleucine increased.

Application of aspartic acid to the medium in presence of glucose enhanced ammonia secretion by the three zoosporic fungi compared with the control (Table 1).

However, ammonia secretion was raised as the concentration of aspartic acid increase in case of *A. racemosa* and *S. furcata* but it was decreased in case of *A. proliferoides*. The difference was significant compared with the control only in case of *S. furcata* at the high doses (1200 and 1600 *μg/ml*).

With the supplements of phenylalanine, the results presented in Table 1 show that ammonia accumulation in synthetic medium provided with glucose was increased by the three investigated zoosporic fungi at all tested doses relative to untreated control and the difference was significant in case of *A. racemosa* at all doses except the highest one. The level of ammonia secretion by the three tested fungi was raised with rising the concentration of phenylalanine in case of *A. proliferoides* but it was gradually declined in case of both *A. racemosa* and *S. furcata*. Many authors (Gleason *et al.*, 1970; Gleason, 1973; Nolan, 1975, 1976) working on the effects of amino acids on zoosporic fungal species (*Saprolegnia* species, *S. megasperma*, *S. ferax*, *A. ambisexualis*, *Leptolegnia eccentrica* and *Dictyuchus sterilis*) reported that ammonia is released into the medium during catabolism of the amino acids. In contrast to these results, St Leger *et al.* (1999) found that ammonia production by *Metarhizium anisopliae*, *Neurospora crassa* and *A. fumigatus* declined or ceased altogether when amino acids were supplemented with glucose. They concluded that glucose might have repressed production of deaminases. It is also possible that the higher growth rate with glucose allowed more complete utilization of ammonia released from amino acid catabolism. In addition, the fungi may have switched from catabolizing amino acids to catabolizing sugars for the production of nitrogen non-containing polymers, thereby decreasing free ammonia production.

When glucose was omitted from the medium and amino acids acted as carbon and nitrogen sources, ammonia secretion by the three zoosporic fungi significantly increased comparable to control and the accumulation of ammonia was elevated with the rise of the concentration of each individually amended amino acid (Table 2). In this connection, Coll and Leal (1972) mentioned that ammonium nitrogen by *Fusarium culmorum* did not accumulate in the media until glucose was consumed.

Evaluated data during this investigation showed a clear direct interrelationship between the measured pH values of the culture filtrates of the three zoosporic fungi and the accumulated ammonia in the synthetic medium either in presence or absence of glucose. The values of either pH or ammonia secretion were nearly two folds in absence of glucose as compared with the medium containing glucose. In this respect, St Leger *et al.* (1999) found that increasing concentrations of ammonia correlated with increasing the pH of the medium in cultures of *Metarhizium anisopliae*, *Neurospora crassa* and *A. fumigatus*. Similar result was also obtained by Coll and Leal (1972).

Extracellular protease activity. The results in Table 1 reveal that the addition of isoleucine (as nitrogen source) to the medium promoted extracellular protease activity in presence of glucose compared with control in case of *S. furcata* and *A. proliferoides* (except at 1600 *μg/ml*, suppressed). The activity of extracellular protease was dropped with increase of the concentration of isoleucine. However, extracellular protease activity by *A. racemosa* was gradually repressed as the concentration of isoleucine increased.

Extracellular protease activity was lowered compared with the control in case of *A. proliferoides* and *S. furcata* grew in medium containing glucose as they affected by different treatments of phenylalanine as presented in Table 1. In case of *A. racemosa*, extracellular protease activity inhibited slightly at low concentration relative to that at control then increased gradually at 800 and 1200 *μg/ml* and finally sharply declined at 1600 *μg/ml*.

As shown in Table 1, in case of supplements with aspartic acid, in presence of glucose protease activity was promoted by *A. proliferoides* and *S. furcata* comparable to untreated control except at the high dose (slightly suppressed). However, this promotion was lowered with the rise of the concentration. Extracellular protease activity was also stimulated in case of *A. racemosa* at 400 and 800 *μg/ml* and it was dropped at the higher applied doses (1200 and 1600 *μg/ml*).

Proteolytic activity by the three tested zoosporic fungi over broad range of amino acids concentrations were came in agreement with the result obtained by Karup *et al.* (1993) who found that most of the tested isolates of Chytridiales and Spizellomycetales (Chytridiomycetes) were able to produce extracellular proteases hydrolyzing substrate over a broad pH range. The inhibitory effects of the

three amended amino acids on exoprotease activity by *A. racemosa* in most cases were in accordance with those obtained by Karup *et al.* (1993) and Li *et al.* (1997) who found a repression in proteolytic activity by fungi in response to amino acids additions. Moreover, Tsuboi *et al.* (1989) reported that extracellular proteinase activity by *Candida albicans* reduced after full fungal growth and subsequent neutralization of tested media to pH 7. In a similar manner, Markovits and Acevedo (1980) indicated that protease synthesis by *Aspergillus* sp. was sensitive to repression by rapidly assimilable carbon source such as glucose and they also concluded that the synthesis of this enzyme is controlled by induction, catabolite repression and ammonia repression.

Extracellular protease activity by *A. proliferoides* in synthetic medium treated with different concentrations of the three amino acids and lacking glucose gradually suppressed compared with the control but the difference was not significant. The same result was also obtained in case of *A. racemosa* treated with isoleucine and phenylalanine but the addition of aspartic acid activated protease activity at all doses except the highest one (1600 *µg/ml*). Extracellular protease activity by *S. furcata* almost significantly declined as the concentrations of the applied amino acids increased when compared with the control and the lowest concentration (400 *µg/ml*) of the tested amino acids was stimulative for the enzyme activity (Table 2). The values of extracellular protease activity by the three tested fungi were approximately lower than that found when glucose was added to the medium at each opposite treatment of each amino acid.

Parallel to these results, Al'Nuri *et al.* (1981) indicated that the rate of protease biosynthesis by *A. candidus* noticeably decrease if proline, alanine, glycine, valine, phenylalanine, serine, threonine, aspartic and glutamic acids are added. Tryptophane, leucine, asparagine and glutamine considerably decreased the rate of exoprotease biosynthesis. Arginine, histidine, lysine, ornithine and methionine added to the medium without a carbon source almost entirely inhibits biosynthesis of exoproteases.

General conclusions. Generally, when amino acids served as nitrogen source in presence of glucose in the synthetic medium the mycelial dry weights of the three zoosporic fungal species were increased compared with the control. The same result was also obtained when glucose was omitted from the medium and amino acids were the sole source of carbon and nitrogen but dry weights were decreased as the concentration of the employed amino acid increase. Supplements of synthetic medium with the three tested amino acids significantly raised the pH value of the culture medium by the three zoosporic fungal species either in presence or absence of glucose (with few exceptions in presence of glucose) but the values of pH were approximately two folds in absence of glucose. Ammonia secretion in synthetic medium amended with glucose as carbon source and amino acids as nitrogen source was activated (almost non-significant) by the three species of zoosporic fungi relative to control except in case of *A. racemosa* treated with isoleucine whereas in absence of glucose the accumulation of ammonia was significantly elevated and the content was nearly doubled in this case. Extracellular protease activity by species of zoosporic fungi in the synthetic medium treated with amino acids were varied either in the presence or absence of glucose depending upon species of zoosporic fungi, amino acid used and the concentration of the amino acid.

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