

Biochemical variation among three isolates of *Alternaria brassicae*

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Taxonomy of fungi based on morphology has been universally accepted as a sound approach. However, in some genera/species, it becomes difficult because morphological characteristics are not distinct enough to differentiate. In such cases, differences in biomolecule composition, lipids, carbohydrates, proteins, aminoacids and nucleic acids among different species of the genus have been used as supplementary information to differentiate among races or distinct isolates of fungal pathogen.

A. brassicae (Berk) Sacc. comprises a diverse group at physiological level with overlapping characters (Awasthi and Kolte, 1989). The present study was undertaken to study the biomolecule composition among three distinct isolates of *A. brassicae* designated as isolates A, C and D producing three distinct spots on leaves of *Brassica carinata* cv ppcs - 1 (Ethiopean mustard) as reported by Kolte *et al.* (1991). Isolate A (IMI 303099) produced larger brown concentric spots with dark grey centre and light grey margin; isolate C (IMI 303100) produced smaller spots with dark black margin; and isolate D (IMI 303101) produced black solid dot-like spots without necrosis in the centre. Based on host response to conidial inoculation of the three isolates, isolate A was rated to be highly virulent, C as moderately virulent and D as avirulent. Fresh isolations from the

above respective category of isolate-specific lesions from *B. carinata* were obtained separately as generation of single conidial culture on radish root mannitol agar (radish root extract 200g, agar 20g/l and mannitol 20g/l) Supplemented with Rose Bengal (50 µg/l). The liquid culture of each of the above isolate was further obtained on modified synthetic Fries medium for determining the biomolecule composition. For this purpose, one hundred ml medium was poured in 250 ml Erlenmayer conical flasks which were inoculated and incubated at $24 \pm 2^\circ\text{C}$ for 21 days. The mycellal growth of the fungus was filtered through Whatman No. 42, washed with distilled water, dried at $60 \pm 1^\circ\text{C}$ in an oven, ground and fractionated by the method of Gottlieb and Van Etten (1966), for biochemical analysis of the cell constituents. The following biomolecules were analysed.

Carbohydrates : The anthrone - positive carbohydrates were determined by the method of Morris (7) using glucose standard; (ii) **Amino acids :** The ninhydrin - positive aminoacids were determined by the method of Moore and Stein (6) with aminoacid L leucine as the standard and (iii) **Nucleic acids :** RNA was estimated by orcinol procedure of Aswell (1) and yeast RNA was used as standard. DNA was measured by the diphenyl amine test (8) with calf thymus DNA as standard;

Table 1 : The contents of various cell biomolecules in different isolates of *A. brassicae*

Sl. No.	<i>A. brassicae</i> isolates	Contents of cell biomolecules dry mycelial weight (mg)*					
		Carbohydrates	Lipids	Proteins	Amino acids	Nucleic acids	
						RNA	DNA
1.	A	95.56	121.07	146.53	125.93	28.71	6.26
2.	C	87.67	158.33	149.59	129.49	30.90	7.08
3.	D	77.31	155.54	148.28	120.09	27.51	5.73
	CD at 5%	2.53	0.39	0.14	0.43	0.43	0.57
	CV	1.89	1.81	3.10	0.39	0.51	0.89

* Based on dry mycelial weight in 1000 mg.

(iv) *Proteins* : Proteins were estimated with Folin and Ciocaeteu reagent by the method of Lowry *et al.* (5); Bovine plasma serum albumin was used as standard and (v) *Lipids* : Total lipids were estimated by extracting separate portions of the dried mycelium twice with chloroform : methanol (3 : 1 V/V). The extract filtered through Whatman filter paper No. 41 and evaporated to dryness after adding a few crystal of β -hydroxy toluene and antioxidant. The residue was dissolved in 1 ml of chloroform : methanol (2:1 V/V).

Highly virulent isolate A showed maximum amount of total carbohydrates content and the least virulent D isolate showed least amount of total carbohydrate content (Table 1). It therefore appears that the maximum content of carbohydrate is indicative of maximum virulent nature of pathogen. However, isolate C of the fungus, which was moderately virulent showed significantly higher content of total lipids, proteins, RNA and DNA in comparison to isolate A and D.

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