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IDENTIFICATION OF THE AMINO-ACID COMPOSITION OF CELLULOLYTIC ENZYMES BY A PHENYLTHIOCARBAMOYL METHOD

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The fungus *Aspergillus terreus* is known to produce highly active cellulolytic and xylanolytic enzymes depending on the cultivation conditions [1–3]. The enzymes can be used for biodegradation of ligno-cellulose wastes [4] and to destroy plant cell walls [5]. Knowledge of the physicochemical properties of the enzymes is needed in order to apply them successfully in various economic sectors.

Our goal was to study the physicochemical properties of cellulase-II, xylanase-I, xylanase-II, and pectinase isolated from *A. terreus* by determining the amino-acid composition, the *N*-terminus amino acids, and the isoelectric points (pI).

The aforementioned enzymes were isolated and purified by the literature methods [1, 6]. The classical method for determining the amino-acid composition of proteins and peptides consist of separating their acid hydrolysates using ion-exchange chromatography with subsequent identification as ninhydrin derivatives [7]. We used a highly sensitive phenylthiocarbamoyl (PTC) method [8] because the classical determination method has rather low sensitivity.

For this, the studied enzymes were hydrolyzed by the literature method [7]. A mixture of 18 standard amino acids (control) and the hydrolysates of the studied enzymes were converted to their PTC derivatives and analyzed using reversed-phase chromatography. Complete analysis required 50 min. The retention times of the PTC amino-acid derivatives were: Asp, 2.72; Glu, 3.45; Ser, 6.90; Gly, 7.85; Thr, 14.85; Ala, 16.20; Pro, 17.69; His, 20.29; Arg, 24.71; Tyr, 25.04; Val + Met, 25.99; Cys, 28.15; Ile, 29.36; Leu, 30.20; Phe, 31.64; Trp, 34.23; and Lys, 36.60 min. Table 1 presents the amino-acid compositions of the studied enzymes.

A total of 18 amino acids was detected in the studied enzymes by analyzing their acid hydrolysates as the PTC derivatives. Asparagine (Asn) and glutamine (Gln) are known to be converted during acid hydrolysis into the corresponding acids (Asp and Glu). Therefore, accurate quantitative ratios Asp/Asn and Glu/Gln could be determined only after establishing the primary structure of the studied enzymes. Considering that tryptophan (Trp) decomposes completely during hydrolysis by HCl (5.7N), the enzymes were hydrolyzed by methanesulfonic acid (4N) and then modified with FITC-reagent for its quantitative analysis.

The total content of hydrophobic amino acids was 43% in cellulase-II; 44, xylanase-I; 42, xylanase-II; and 41, pectinase. The content of basic (Lys, Arg, His) and acidic (Asp, Glu) amino acids was 18.93 and 9.6% in cellulase-II; 18.28 and 12.77, xylanase-I; 20.45 and 12.47, xylanase-II; and 17.35 and 11.12, pectinase, respectively.

A theoretical calculation of the isoelectric points of the studied enzymes showed that they were located at the following pH values: cellulase-II, 7.1; xylanase-I, 6.8; xylanase-II, 6.9; and pectinase, 6.7.

The *N*-terminus amino-acid units of the enzymes were also determined. For this, the PTC derivatives were first synthesized and then hydrolyzed and identified by the literature method [8].

Thus, we established the qualitative and quantitative amino-acid composition of cellulase-II, xylanase-I, xylanase-II, and pectinase from *A. terreus* and determined their compositions of 235, 379, 326, and 517 amino acids, respectively. Results from a theoretical calculation of the isoelectric points showed that they fell between pH values 6.7–7.1. The *N*-terminus amino acids in cellulase-II, xylanase-I, xylanase-II, and pectinase are Ala, Phe, Lys, and Leu, respectively.

Acid-hydrolysis Conditions. Acid hydrolysis was carried out in HCl (5.7N) at 110°C for 24, 48, 72, and 96 h as described previously [7]. PTC derivatives of free amino acids were synthesized and the *N*-terminuses of the enzymes were bound using the literature method [8].

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TABLE 1. Amino-Acid Composition of Enzymes (Number of Units)

Amino acid	Cellulase-II	Xylanase-I	Xylanase-II	Pectinase
Met	10.34	26.03	11.37	24.44
Glu	9.87	21.23	16.78	27.27
Ile	15.05	8.97	17.79	37.97
Thr	12.69	10.05	18.72	31.31
Ser	11.75	23.89	11.68	34.80
Gly	25.85	28.40	17.87	46.77
Phe	26.79	18.96	21.67	18.97
Leu	7.05	35.48	23.35	13.23
Val	10,34	26.03	11.37	24.44
Ala	14.13	33.52	16.87	50.69
Arg	14.73	20.76	24.32	19.47
Pro	7.99	13.04	22.55	21.99
Cys	5.17	9.12	16.15	14.10
Asp	12.69	27.17	23.86	30.25
His	16.92	19.02	22.44	35.76
Lys	12.83	29.50	19.90	34.50
Tyr	11.75	23.81	17.52	31.87
Trp	8.93	3.81	12.09	19.83
Σ	≈ 235	≈ 379	≈ 326	≈ 517

HPLC Conditions for Analyzing PTC Derivatives. Amino-acid PTC derivatives were identified on a Du Pont 8800 chromatograph using a Nucleosil C_{18} column (4 × 250 mm).

Samples (0.05 mL) were injected onto the column. Solution A consisted of CH_3COONa (0.14M) + TEA (0.05%) at pH 6.4; solution B, CH_3CN. A four-step linear gradient of CH_3CN concentration was used as follows: 1–6%/5 min; 6–30%/5.1–40 min; 30–60%/40.1–45 min; 60–1%/45.1–50 min. The flow rate was 1.2 mL/min. The column was rinsed for 3 min before injecting the next sample. Amino acids that eluted from the column were detected at 269 nm. The detection limit of the PTC derivatives was 1 pmol. Qualitative analysis and quantitative calculations of the concentration of free amino-acid PTC derivatives were carried out by comparison with the elution times and peak areas of corresponding standard amino-acid PTC derivatives.

Isoelectric points of enzymes were calculated using the formula:

$$pI = (N_{Glu} \times 3.22 + N_{Asp} \times 2.77 + N_{Lys} \times 9.74 + N_{Arg} \times 10.76 + N_{His} \times 7.59)/\Sigma paa.$$

where N is the total amount of amino acids; Σpaa, the total polar amino acids; 3.22 = pI Glu; 2.77 = pI Asp; 9.74 = pI Lys; 10.76 = pI Arg; and 7.59 = pI His.

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