Isoenzymes of some Oxido-Reductases in the *Candida* Genus as a Basis of Species Identification after Electrophoresis

Studies on isoenzymes in microorganisms give ample opportunity for analysing biological peculiarities of their metabolism, reactivity, and properties. The identification of various types of multimolecular forms in the same enzyme would not only contribute to determining antigenic peculiarities of the *Candida* genus, but would also provide a basis for discerning each of the *Candida* species.

Material and methods. Extracts from *Candida albicans*, *C. tropicalis*, *C. pseudotropicalis*, *C. crusei*, and *C. utilis* were prepared from 25 mg of a 48 h culture on glucose agar. The cells were homogenized repeatedly at 4 °C with 2 ml of a 0.85% solution of sodium chloride, after freezing at – 15 °C. The mass so prepared was centrifuged for 1 h at 6000 rpm. The supernatant fluid was treated for 20 min with chloroform in equal parts, then centrifuged again for 30 min at 5000 rpm. The extracts thus obtained were tested for isoenzymes.

A 1% solution of Difco-Noble agar, sodium diethylbarbiturate barbital buffer (pH 8.6, ionic strength 0.04). was used. The electrophoretic separation of the enzymes was carried out for 60 min on glass slides and an agar layer of 1.5 mm thickness, at 150 V and 3–4 mA current strength, according to WIEME's method[1]. Subsequent to electrophoresis, incubation was carried out according to the method applied by VAN DER HELM[2] and others[3–6].

Results and discussion. (1) *Candida albicans*: (A) Isoenzymes of G-6-PDH: 2 fractions, the first one near the start, displaying an activity more strongly marked than that of the second fraction. (B) Isoenzymes of LDH: 2 fractions, the first one being less intense. (C) Isoenzymes of MDH: 3 fractions; the activity proved most strongly marked in the third fraction, and least of all in the first one.

(2) *Candida tropicalis*. (A) Isoenzymes of G-6-PDH: 2 fractions with a very clear-cut activity, the second one being at the start. (B) Isoenzymes of LDH: 2 fractions with an equally slightly marked activity, the second one being near the start. (C) Isoenzymes of MDH: 2 fractions with an equally clear-cut activity of medium intensity, the second one comprising both the anode and cathode sides of the start.

(3) *Candida pseudotropicalis*. (A) Isoenzymes of G-6-PDH: 2 fractions, the second one near the start and its activity less strongly marked. (B) Isoenzymes of LDH: 2 fractions with a slightly marked activity, the first one being near the start. (C) Isoenzymes of MDH: 3 fractions with a very slightly marked activity, the third one comprising simultaneously both the anode and cathode sides of the start.

(4) *Candida crusei*. (A) Isoenzymes of G-6-PDH: 3 fractions, the third one near the start, with an activity as equally clear-cut as that of the first one, but more strongly marked than that of the second fraction. (B) Isoenzymes of LDH: 2 fractions, the second one displaying an activity more strongly marked. (C) Isoenzymes of MDH: 2 fractions, the second one displaying an activity far more strongly marked.

(5) *Candida utilis*. (A) Isoenzymes of G-6-PDH: 2 fractions, the first one displaying an activity far more strongly marked. (B) Isoenzymes of LDH: 2 fractions, the first one with an intense activity, while the second one is at the start and displays an activity less intensely marked. (C) Isoenzymes of MDH: 2 fractions of equal activity.

Nearly all of the isoenzymes are expressed towards the anode and only some of them are localized around and at the start. The arrangement towards the anode is in keeping with the greater part of the fractions on the proteinogrammes.

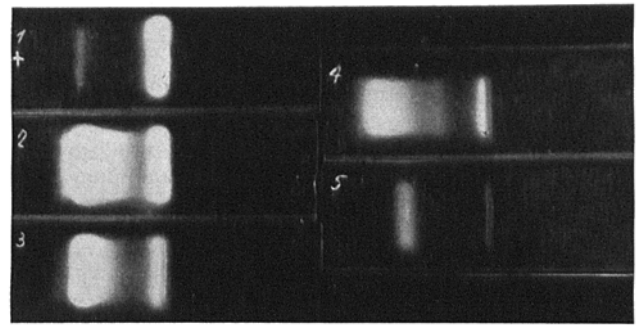

Fig. 1. Isoenzymes of G-6-PDH: (1) *Candida albicans*, (2) *C. tropicalis*, (3) *C. pseudotropicalis*, (4) *C. crusei*, (5) *C. utilis*.

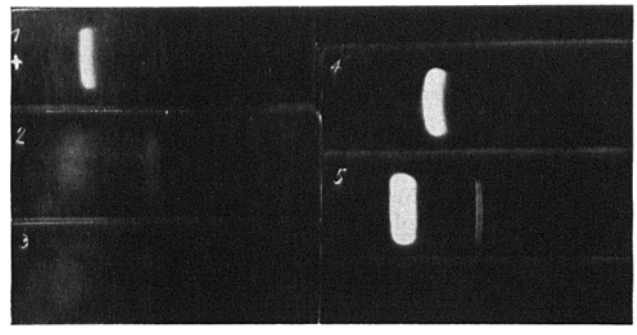

Fig. 2. Isoenzymes of LDH: (1) *Candida albicans*, (2) *C. tropicalis*, (3) *C. pseudotropicalis*, (4) *C. crusei*, (5) *C. utilis*.

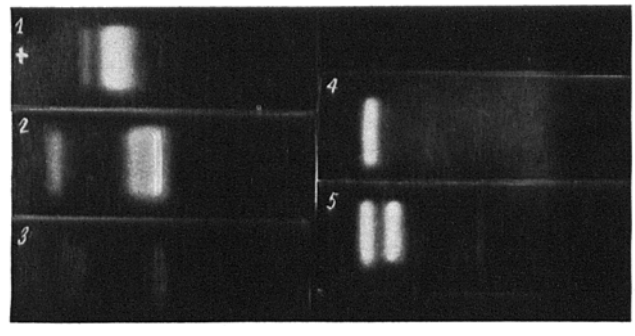

Fig. 3. Isoenzymes of MDH: (1) *Candida albicans*, (2) *C. tropicalis*, (3) *C. pseudotropicalis*, (4) *C. crusei*, (5) *C. utilis*.

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So far, our results on the isoenzymes of the enzymes tested give reason to suppose that the G-6-PDH isoenzyme activity (except in *C. utilis*) is pronounced most strongly (Figure 1), followed by the LDH and MDH isoenzyme activity respectively (Figures 2 and 3). On the other hand, our data show that each *Candida* species has its individual isoenzyme features. From the number, the degree of isoenzyme activity, and the arrangement of isoenzymes, conclusions can be drawn as to the *Candida* species actually observed[7].

Zusammenfassung. Nach Elektrophorese in Agargel wurden die Isoenzyme von G-6-PDH, LDH und MDH in Extrakten von *Candida albicans, C. tropicalis, C. pseudotropicalis, C. crusei* und *C. utilis* untersucht. Es liessen sich diverse Isoenzyme von unterschiedlichem Aktivitätsgrad und wohl auch verschiedener Lage in den Isozymogrammen nachweisen, was bestimmte Schlüsse auf betreffende *Candida*-Arten erlaubt.

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A Proposed Uniform Method of Reporting Response Values for Crustacean Chromatophorotropins: the Standard Integrated Response

Several different groups of investigators, including ourselves, are currently at work attempting to purify and characterize crustacean chromatophorotropins[1]. However, there is no standard, universally accepted method of reporting the potencies of these substances. Consequently, a great deal of difficulty exists when one group of investigators wishes to compare its findings with that of another group. Examples of some of the methods that have been used will demonstrate the difficulty one faces in trying to compare data from different laboratories. '*Leander* units'[2] have been defined as 'the smallest amount of the hormone which will cause within 20 min a distinct blanching of eye-stalkless specimens of *Leander adspersus*' (the name of this prawn has in the meanwhile been changed to *Palaemon squilla*). We also find[3] the use of '+' for 'a complete concentration which lasted for an hour or less' and '++++' for 'a complete concentration which lasted for longer than an hour'. Most recently, LOWE and HORN[4] designed a response value calculated from an equation involving the time interval between the observations and a mathematical factor such that 'the amount of hormone which will just effect complete concentration of the red pigment in 15–30 min has an activity value of approximately 100'. Herein, we propose a standardized system that will, if accepted by other workers in the field, minimize the problem. Unfortunately, no crustacean chromatophorotropin has been purified in sufficient quantity to enable definition of a unit based on weight of the hormone preparation. Instead the response is based on the amount of tissue or the number of organs from which the extract was prepared.

The schemes used with the most frequency for reporting effects of chromatophorotropins are various modifications of that described by SANDEEN[5]. Her procedure was to calculate the sum of the average HOGBEN and SLOME chromatophore stages[6] determined at each time of observation for the duration of the response for both the experimental and control groups. According to the HOGBEN and SLOME system, stage 1 represents maximal pigment concentration, stage 5 maximal dispersion, and stages 2, 3, and 4 the intermediate conditions. When pigment dispersion occurs the sum for the control group is subtracted from the sum for the experimental group. For pigment concentration the sum of the experimental group is subtracted from the control. The difference is the response value. The advantage of this measure of the response is that it encompasses both intensity and duration. However, even when investigators use the basic scheme outlined by SANDEEN they do not all use the same time intervals for the readings and consequently the response values are not comparable[7,8].

We, therefore, propose, for the sake of uniformity and ease of comparison of data from different laboratories that, in addition to whatever method of obtaining and presenting data the investigator feels is most beneficial to his particular set of experiments, he should also present response values calculated in the manner described by SANDEEN. For pigment dispersion the values should be based on observations of the chromatophores performed at the time of injection and 15, 30, and every 30 min thereafter for the duration of the response. For all pigment-concentrating hormones the same time intervals should be used plus an observation 5 min after injection because in some crustaceans, such as the prawn *Palaemonetes*[9], for example, its red pigment-concentrating hormone has maximal effect after only 5 min. We recommend that this value be given the name Standard Integrated Response, which can be conveniently abbreviated SIR[10].

Résumé. Les investigateurs des chromatophorotropins n'emploient pas une méthode uniforme pour présenter leurs résultats. Par conséquent, la comparaison des données fournies par des laboratoires différents est souvent difficile. Pour éliminer ce problème, une méthode uniforme et pratique est proposée.

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