CYST MEMBRANE PROTEIN COMPOSITION AS A DISCRIMINANT CHARACTER IN THE GENUS ARTEMIA. (INTERNATIONAL STUDY ON ARTEMIA LV)

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Isolated embryonic membranes (i.e. the outer cuticular membrane, the fibrous layer, the inner cuticular membrane and the hatching membrane) and homogenates of incubated, decapsulated cysts of 16 populations from five Artemia species (Crustacea, Anostraca) were examined by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Characteristic differences in major polypeptides between 45 and 205 kD were seen. These differences might be used as markers for identification of different Artemia species. The validity of this approach in characterizing Artemia populations is discussed.

Artemia is an anostracan crustacean found in coastal saltmarshes and inland salt lakes. The genus is a complex of sibling species and superspecies with cosmopolitan distribution. The study of allozyme polymorphisms, using horizontal starch gel electrophoresis has been used to characterize Artemia populations, estimate the genetic differentiation between them (Beardmore & Abreu-Grobois, 1983; Pilla & Beardmore, 1994) and verify the presence of mixed and/or contaminated material in a given sample of cysts (Triantaphyllidis et al., 1994b). However, the disadvantage of this method is that it requires Artemia cultures to be grown until the adult stage (three weeks at least) and is relatively expensive. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of total proteins has been used to characterize and identify the protein profiles (PP) of several species, populations, and clones, providing taxonomically useful descriptors with the potential to elucidate problems at higher taxonomic ranks (Ferguson & Grabe, 1986; Aiken & Gardiner, 1991; Bruce & Pennington, 1991; Triantaphyllidis et al., 1994a). As in other embryonic systems, Artemia embryos contain yolk granules that constitute the major cell component and contain at least 80% of the total embryonic protein (Vallejo et al., 1981). The embryo is surrounded by a complex shell and several membrane layers and the structure of these has been described by Morris & Afzelius (1967) and Anderson et al. (1970). Details of the control of emergence and hatching can be found in Clegg (1964).

This study attempts to test the applicability of SDS-PAGE protein profiles of cyst embryonic membranes in characterizing and discriminating Artemia species. This approach was chosen because it is rapid and inexpensive.

Artemia cysts used in this study belong to the following species: (1) A. parthenogenetica: Megalon Embolon, Greece (MEM), Citros, Greece (CIT), Kalloni, Greece (KAL), Tanggu, P.R. China (TGU) and Margherita di Savoia, Italy (MDS); (2) A. franciscana: San Francisco Bay, California, USA (SF-1 and SF-2), Ecuador (ECL), Macau, Brazil (MAC), Vin Chao, Vietnam (VIE), Great Salt Lake, Utah, USA (GSL-1, GSL-2 and GSL-3); (3) A. tunisiana: Larnaca, Cyprus (LAR); (4) A. urmiana: Lake Urmia, Iran (URM); and (5) A. persimilis: Argentina (PER).
The cysts were decapsulated using a NaOCl solution and stored in saturated NaCl solution at 4°C. For preparation of the embryonic membranes the method of Pilai & Clark (1990) was slightly modified: cysts were incubated in sterilized hatching medium for 4 h; and subsequently harvested on a 100-μm filter and transferred into a glass homogenizer. Cysts were homogenized with 20 times their volume of ice-cold dH2O containing 2.5mM phenylmethylsulphonyl fluoride (PMSF) to inhibit degradation. Homogenates were centrifuged at 300 rpm for 10 min using a Jouan centrifuge (model CJ1000 S5L). The supernatant was discarded and the remaining pellet was washed and centrifuged three more times in 4°C dH2O containing 2.5mM PMSF, for 10 min at 300, 400 and 1000 rpm. The pellets containing the embryonic membranes, i.e. the outer cuticular membrane, the fibrous layer, the inner cuticular membrane and the hatching membrane, were checked under the microscope for purity (absence of yolk granules and/or cells). Then, they were transferred to Eppendorf tubes for sample preparation according to Triantaphyllidis et al. (1994a); the period of incubation at 100°C was 15 min instead of 5 min. The PP of cysts harvested after 2 h incubation in hatching medium using SDS-PAGE were obtained following the method of Triantaphyllidis et al. (1994a). Equal amounts of protein, calculated following the technique of Bradford (1976), were applied to each well.

Repeated analyses of the embryonic membrane protein pattern of the same population resulted in the same banding pattern suggesting that the method is reproducible. Figure 1 illustrates the embryonic membrane PP of A. parthenogenetica, A. franciscana, A. tunisia, A. urmiana and A. persimilis. The most prominent differences in protein banding patterns that might discriminate the populations studied were observed between the molecular weights of 66 and 97.4 kD. The areas of 150–205 and 45–66 kD also have some diagnostic value.

Figure 1. Comparison of the protein pattern of embryonic membranes analysed by 15% SDS-PAGE. The polypeptides stained with Coomassie blue G-250. Lane 1, MEM; lane 2, CIT; lane 3, KAL; lane 4, TGU; lane 5, MDS; lane 6, SIB-1; lane 7, ECU; lane 8, LAR; lane 9, URM; lane 10, CSL-3; lane 11, PER (P, parthenogenetic; B, bisexual).

Close inspection of the PP of parthenogenetic populations revealed the following: (1) no or some slight quantitative differences were observed between the three Greek Artenia populations MEM, CIT and KAL (Figure 1, lanes 1–3). We must stress that these parthenogenetic populations are tetraploid (Abatzopoulos et al., 1986) and share closely-located similar environments i.e. coastal saltworks and have proved to be almost identical in many aspects (Abatzopoulos et al., 1989; Triantaphyllidis et al., 1994a). (2) The Greek populations show a double band in the area of 66 kD while in the TGU and MDS populations there is a single band (all these bands are denoted by arrows, Figure 1). (3) Great similarity between the MDS and TGU populations is seen, though some minor quantitative differences in bands exist (see Figure 1, lanes 4 & 8).
Figure 2. Comparison of the protein pattern of 2-h hydrated cysts from different strains of _Artemia franciscana_, after analysis by SDS-PAGE. Lane 1, GSL-3; lane 2, VIE; lane 3, MAC; lane 4, SFB-2; lane 5, GSL-1; lane 6, GSL-2.

Comparisons of the bisexual populations studied disclosed the following: (1) the most differentiated bisexual species is the LAR population which presents the most striking difference in the area of 66 kD, where a three-banded pattern is observed (see asterisks in Figure 1, lane 8); also, a unique band appears in the area of 150–205 kD (see square in Figure 1, lane 8). (2) The three _A. franciscana_ populations (SFB-1, ECU and GSL-3) show great similarities in prominent bands in the areas between 45–66 and 66–97.4 kD (see dots in Figure 1, lanes 6, 7 & 10); these are of less intensity in the other bisexual species (LAR, URM and PER, see Figure 1, lanes 8, 9 & 11). Although the _A. franciscana_ populations are located in geographic areas which are distant from each other, the embryonic membrane protein pattern appears to be quite conservative. The LAR Old World bisexual population, which belongs to _A. tamiaia_, is distinct from the New World bisexual species _A. franciscana_.

The PP of embryonic membranes of both _A. urmiana_ and _A. persimilis_ do not appear to be as sharp as those of most of the other populations (Figure 1, lanes 9 & 11), although many samples were analysed. _Artemia urmiana_ displays a single band in the area of 66 kD similar to TGU and MDS. It is important to note that the banding pattern of _A. urmiana_ is significantly different from the _A. franciscana_ group and resembles the parthenogenetic populations. According to Beardmore & Abreu-Grobois (1983) parthenogenetic populations and _A. urmiana_ have a common ancestor and the genetic distance (Nei’s D) between them is smaller than for other bisexual species. _Artemia persimilis_ seems to be well differentiated from the rest (i.e. SFB, URM and parthenogenetic populations), specifically in the area of 66 kD (Figure 1, lane 11, arrowhead). This is in agreement with the observations of Beardmore & Abreu-Grobois (1983) who pointed out its relatively large genetic distance from both Old and New World species.

Why are isolated/purified embryonic membranes more ‘suitable’ for characterizing _Artemia_ populations than whole homogenates of decapsulated cysts? This question is answered by close study of the results obtained with different populations and batches of _A. franciscana_. The three GSL populations (GSL-1, GSL-2 and GSL-3, Figure 2, lanes 1, 5 & 6) present significantly different PP compared to SFB populations (SFB-2, MAC and VIE) but also among themselves. It should be noted that MAC and VIE populations are the results of inoculation of SFB cysts in Macau, Brazil and Vich Quao, Vietnam. San Francisco Bay populations, even when they are geographically isolated, appear practically identical, in contrast to GSL populations/batches that show impressive differences in their PP. The differences (denoted by arrowheads in Figure 2) are found mainly in the area of 66–116 kD. Comparing the above results with the results obtained from the embryonic membranes of _A. franciscana_ (Figure 1, lanes 6, 7 & 10) we may conclude that it is more safe to look for the most conservative PP when discriminating species.
Thus, unlike whole cyst preparations, the PP of cyst embryonic membranes appear to be very similar across populations of a species while exhibiting main differences between species. This suggests that the proteins that constitute the membrane layers of the cysts are more highly conserved than those present within the embryo which come mainly from the yolk platelets and are, to some extent, maternally affected (Vallejo et al., 1981). Questions such as those concerning the efficiency of this method in diagnosing the composition of mixed Artemia populations (Triantaphyllidis et al., 1994b), require further experimentation.

REFERENCES


