An effective placental cotyledons proteins extraction method for 2D gel electrophoresis

ABSTRACT

Effective protein extraction is essential especially in producing a well-resolved proteome on 2D gels. A well-resolved placental cotyledon proteome, with good reproducibility, have allowed researchers to study the proteins underlying the physiology and pathophysiology of pregnancy. The aim of this study is to determine the best protein extraction protocol for the extraction of protein from placental cotyledons tissues for a two-dimensional gel electrophoresis (2D-GE). Based on widely used protein extraction strategies, 12 different extraction methodologies were carefully selected, which included one chemical extraction, two mechanical extraction coupled protein precipitations, and nine chemical extraction coupled protein precipitations. Extracted proteins were resolved in a one-dimensional gel electrophoresis and 2D-GE; then, it was compared with set criteria: extraction efficacy, protein resolution, reproducibility, and recovery efficiency. Our results revealed that a better profile was obtained by chemical extraction in comparison to mechanical extraction. We further compared chemical extraction coupled protein precipitation methodologies, where the DNase/lithium chloride-dense sucrose homogenization coupled dichloromethane-methanol precipitation (DNase/LiCl-DSH-D/MPE) method showed good protein extraction efficiency. This, however, was carried out with the best protein resolution and proteome reproducibility on 2D-gels. DNase/LiCl-DSH-D/MPE was efficient in the extraction of proteins from placental cotyledons tissues. In addition, this methodology could hypothetically allow the protein extraction of any tissue that contains highly abundant lipid and glycogen.

Keyword: 1D-gel electrophoresis; 2D-gel electrophoresis; Placental cotyledons tissues; Protein extraction; Proteomics