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Enrichment and Identification of Ceramide Synthase 2 in Subcellular Components: Novel Insights from Porcine Pancreatic Tissue

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Summary

Ceramide Synthase 2 (CerS2), a pivotal enzyme within the sphingolipid metabolic pathway, plays a critical role in various cellular processes. Although CerS2 is widely expressed in several mammalian tissues, such as the kidney, liver, lung, and intestine, its concentration in pancreatic tissue remains relatively low, setting challenges to its isolation and identification. Here, we present a novel, robust, and easy methodology for enriching and localizing CerS2 within pancreatic tissues.

Our approach involves the utilization of an automated homogenization technique coupled with a special buffer composition adjusted for pancreatic tissue homogenization. Subsequently, low and high-speed centrifugation steps were utilized to enrich nuclear, mitochondrial, and membrane proteins, assisting the identification of CerS2 within subcellular fractions. Immunodetection following 1D electrophoresis confirmed the presence of CerS2 in these fractions. Furthermore, CerS2 identification was performed by MALDI-MS analysis after protein separation by 1D and 2D electrophoresis, followed in gel digestion. This comprehensive approach provides valuable insights into the isolation, subcellular localization, and molecular characteristics of CerS2 within pancreatic tissue.

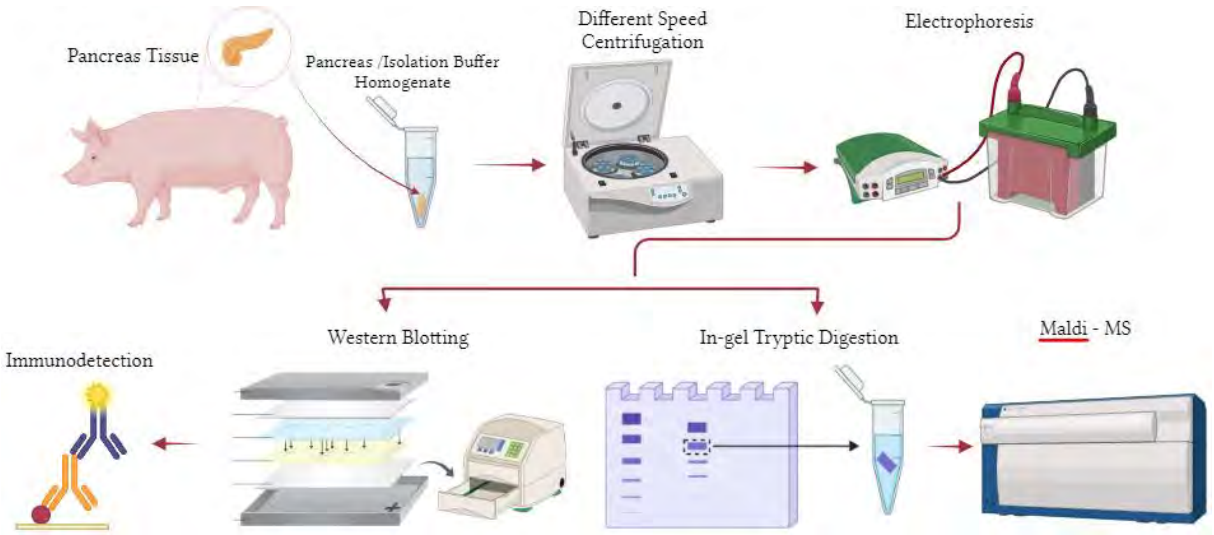

Fig 1. Graphical abstract

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