A practical and fast adaptation of CHO cells expressing human prolactin to grow in suspension and its application to laboratory production

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Human prolactin (hPRL) is a polypeptide with 199 amino acids and a molecular mass of 23 kDa. Previously, an eukaryotic hPRL expression vector based on a selectable dehydrofolate reductase (dhfr) marker was used to transfect anchorage-dependent dhfr⁻ Chinese hamster ovary (CHO) cells. The present work describes a fast and practical laboratory adaptation of these transfected cells, in ~40 days, to grow in suspension in serum-free and protein-free medium. High cell densities of up to 4.0×10^6 cell/ml were obtained from spinner flask cultures and a stable and continuous production process with a duration of at least 30 days was developed. Two harvesting strategies were set up, 50 or 100% of the total conditioned medium being collected daily and replaced by fresh culture medium. The volumetric productivity was 5-7 μg hPRL/ml, as determined directly in the collected medium via reversed-phase HPLC (RP-HPLC). A two-step process based on a cationic exchanger followed by size exclusion chromatography was applied to obtain purified hPRL from the conditioned medium. Two hPRL isoforms, non-glycosylated (NG-hPRL) and glycosylated (G-hPRL) could also be separated by high-performance size-exclusion chromatography (HPSEC) and, when analysed by RP-HPLC, HPSEC, Western blotting, and bioassay, were found to be comparable to the WHO International Reference Reagents of hPRL. This laboratory scale production was used as a model to compare different culture media and effects of drugs such as cycloheximide and sodium butyrate on hPRL isoforms synthesis. These results provide important subsidies for the practical scale-up to the pilot and industrial scale of a bioprocess based on CHO cell culture.