Establishing the proof of principle for functional eukaryotic ligninase expression

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The global threat of climate change and an ever growing human population necessitate the reduction of carbon footprints" of livestock production and reducing the competition for quality cereals between humans and livestock. This can be achieved by utilizing straw for feeding livestock, but the major limitation is the presence of high lignin content that binds to holocelluloses thereby reducing their bioavailability for ruminants. Considering the importance of delignification both in livestock and bioenergy sector, the potential of genetic engineering can be exploited for the generation of cattle that express salivary ligninase to digest the lignocellulosic fodders. A synthetic ligninase gene was designed and was cloned into Sleeping Beauty entry vector. The vector was transfected into bovine embryonic fibroblasts and immortalized rat ParC10 salivary cells. 24 h after electroporation, the cells were checked for reporter expression. The ligninase positive cells were sorted using co-expressed Venus reporter and pure populations of cells was maintained. The culture supernatant was harvested to check for the expression of ligninase using anti-his tag western blot. Secreted ligninase was purified using Ni-NTA columns to undertake functional characterization. We conclude that eukaryotic salivary cells can be genetically programmed to secrete recombinant ligninase.

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