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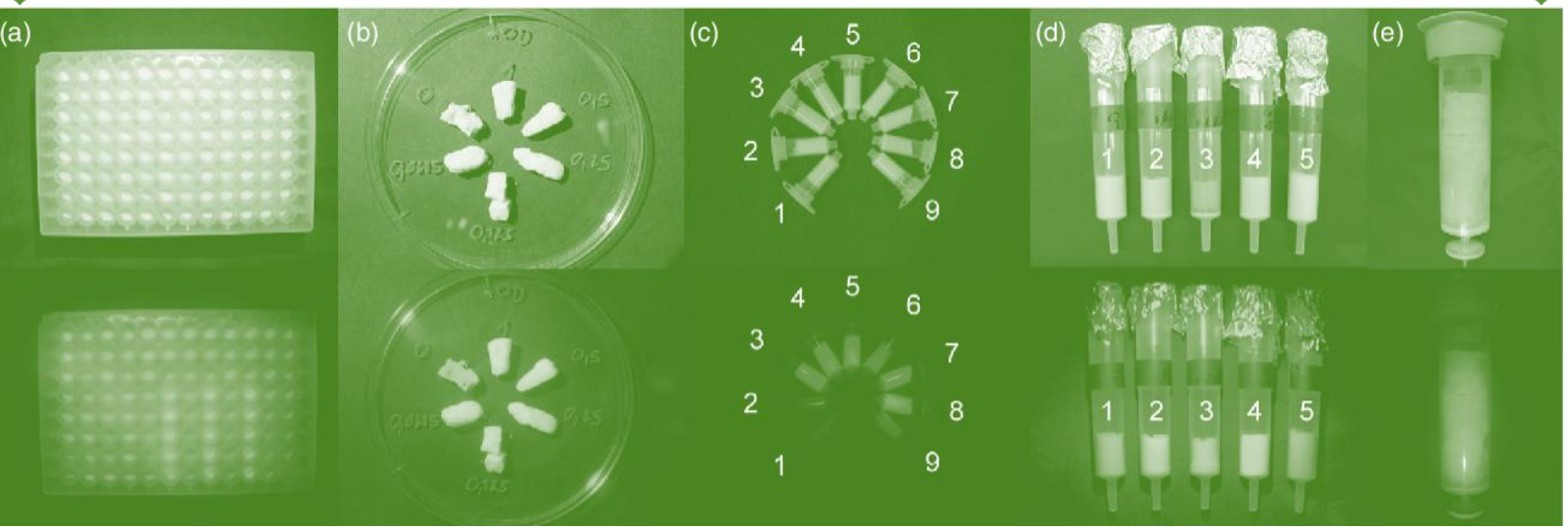

Plant cell packs

a new reliable method for screening and scaling the recombinant protein production and metabolic engineering

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Introduction

The application of recombinant DNA technology provides an invaluable opportunity for the production of different proteins or metabolites with human benefit. These methods involve the production of chimeric DNA by joining two DNA molecules from different sources and consecutive introduction and expression in a suitable host organism. The choice of expression host depends on a combination of host properties (growth rate/productivity, scope for genetic manipulation, the presence of cognate endogenous metabolic pathways, and constraints associated with intellectual property) and product attributes (conscious application, structural complexity, and degree of purification required). Further, the optimization of

production in terms of yield, quality, and purity is also an imperative step that ratiocinates different genes and gene combinations, or expression constructs containing various regulatory elements and targeting signals to finetune the product accumulation. Currently, several hosts ranging from prokaryotic, *E. coli* to higher eukaryotic organisms or their cell cultures are potentially employed to express and produce recombinant proteins of interest. However, each system has its own advantage and disadvantage over the application. Among all, conditions for the production system, authentic posttranslational modifications of recombinant protein, endotoxins production, degree of downstream processing, and ethical concerns are major deciding factors supporting the choice of expression host.

Plant or plant cell suspension as expression and screening platform

Plant or plant cell suspension expression systems are cost-effective, self-contained bioreactors. Hence, they are inherently presumed to be safe as it doesn't act as a host for human pathogens. Further, scale-up in them can be achievable at a low cost by localizing proteins to different organs at different stages. But the unavailability of functional assays and lower yields due to target-dependent transient expression limits its application as an expression system. Therefore, the development of a high throughput screening of recombinant protein expression is desirable during early process development as it is opportune for the identification of optimal expression constructs as well as process conditions.

Micro-titre plate-based screening platforms are already available for optimizing different genes and gene combinations, or expression constructs having regulatory elements and targeting signals in microbes and animal cells' expression systems, but this was not possible for plants. Because of major limitations associated with whole plant platforms and cell suspension cultures. whole plant platforms are prone to data variation due to changes in cultivation conditions and biological heterogeneity over long growth periods and whereas in cell suspension cultures, though they are grown under controlled conditions, they are not scalable as whole plant and processes may not be transferable to plants. In this context, a new plant biotechnology platform, the plant cell pack can resolve the problem by creating a bridge between the convenience of plant cells and the scalability of whole plants.

Plant Cell Pack (PCP)

Plant cell packs (PCPs), also known as "cookies," provide a versatile and scalable screening tool for recombinant protein production. PCPs are prepared from plant cell suspension cultures by removing the medium and molding the biomass in the form of 3-dimensional medium-deprived porous plant cell aggregates. This approach is compatible with different plant species such as Nicotiana tabacum BY2, Nicotiana benthamiana, or Daucus carota and is 10-times more effective than transient expression in liquid plant cell culture. PCPs can be cast into different formats depending on purpose either for initial functionality tests or to facilitate scale-up of products as

flat discs, microtiter plates, or column formats respectively. Presently it permits a manual handling high throughput screening of up to 500 samples per day.

Methodology

1. Preparation of plant cell cultures using different plant species such as *Nicotiana tabacum* BY2, *Nicotiana benthamiana,* or *Daucus carota* and weekly subculturing of respective species into the fresh medium was done with inoculums of 15%, 4%, or 50% (v/v).
2. Culturing of Agrobacterium tumefaciens containing TDNA constructs with a gene of interest
3. Further porous semidry plant cell packs were prepared by dispensing 4 to 11day old suspension cultures into desired format shape and size followed by removal of the excess medium by vacuum filtration (500 millibars for 1 min).
4. The transient expression of recombinant proteins in PCPs done by dropwise infiltration of Agrobacterium suspension.
5. After 5 days of infiltration, recombinant proteins or metabolites are extracted from PCP and analyzed for their suitability as a scalable platform for recombinant proteins and metabolic engineering through monitoring the fluorescence of scorable genes such as DsRed or tryptamine

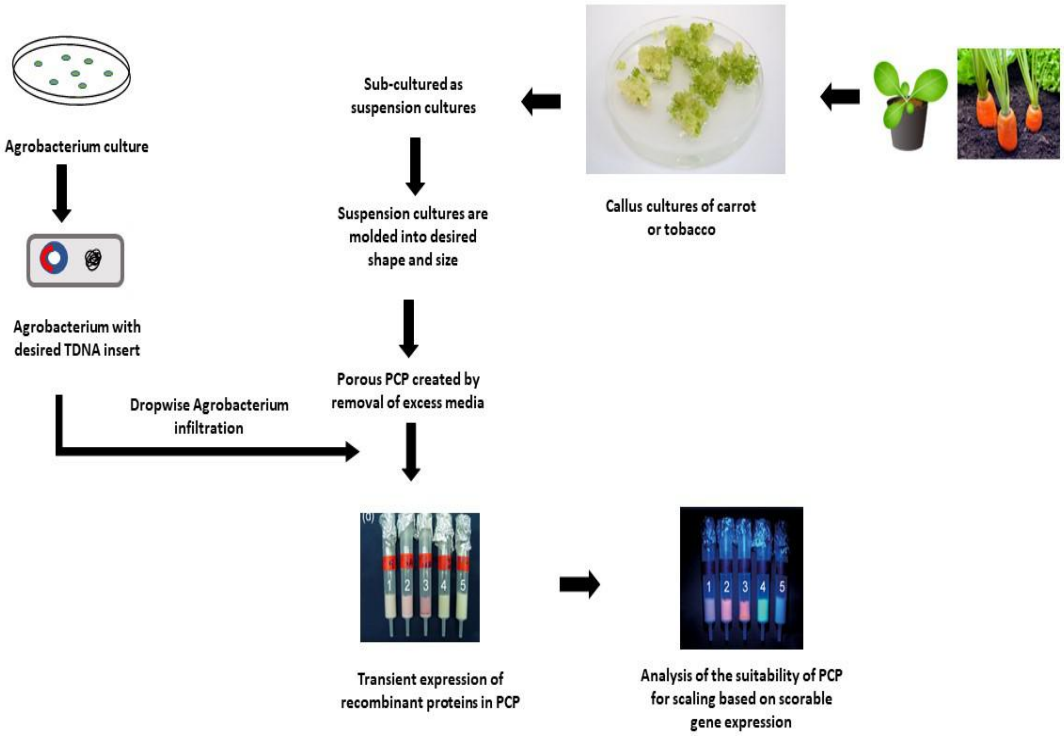

Figure: Schematic illustration of PCP methodology

Advantages and limitations of PCP:

PCP infusion with agrobacterium is easy and more straightforward in terms of apparatus and space requirements whereas expression in plants is influenced by plant

age and physiological state. Further, the lack of chlorophyll in the PCP, makes the macroscopic detection of reporter genes (such as DsRed) easier compared to intact plants where autofluorescence of chlorophyll interferes with the fluorescence of reporter gene. High throughput screening platform that reduces cost and time for expression of recombinant proteins. PCP supports the rapid and cost-effective scale-up. The implication of appropriate signal sequences (KDEL, Zein) or tags (His) efficiently directs the recombinant proteins to specific subcellular compartments such as plastid, endoplasmic reticulum, Apo-plastic secretion, and cytosol respectively. However, this technique also has a few limitations viz., 1) it demands humid but aerated condition maintenance to ensure the viability of PCP. 2) Incomplete removal of media causes browning within 24 to 48 hr and later, reduces yield. 3) Heterogeneous cell packaging in larger columns results in poor aeration.

Conclusion

PCP technology is a simple new plant biotechnology platform that allows the rapid identification of optimal expression constructs and process conditions for recombinant protein production. Further, the selected conditions can be transferred to whole plants. The platform itself can be scaled up to a range of 50–100 mg/kg, even without silencing inhibitors. Protein expression in PCPs can be achieved within 5 days through Agrobacterium tumefaciens infiltration, which either directly yields the desired recombinant product or endows the cells with the ability to synthesize specific metabolites. Currently, robotic cookies are developed that integrated the PCP method with a fully automated laboratory liquid-handling station that further increases high throughput.

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