Strain Development Using Atmospheric Room Temperature Plasma (ARTP) and Microbial Microdroplet Culture (MMC) Technology

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Introduction

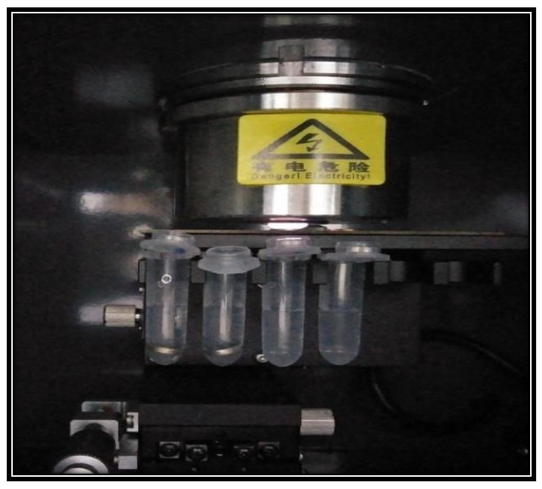
The expression of active and high-quality proteins is a crucial step in biopharmaceutical drug development where the activity and level of expression of proteins largely depend on the strains and culture condition. Therefore, there is an inherent need to screen as many variables as rapidly as feasible to find optimal strain and fermentation conditions for industrial production. Leveraging the market demand for rapid biologics development, Esco Aster offers strain development platform using state-of-the-art technology of random mutagenesis using Atmospheric Room Temperature Plasma (ARTP) technology and high-throughput and parallel screening of strains using Microbial Microdroplet Culture system (MMC) system. Compared to other mutagenesis approaches like UV light, α-rays, β-rays, γ-rays, X-rays and chemical mutagenesis, Atmospheric Room Temperature Plasma (ARTP) uses low-pressure plasma to randomly mutate the bases in DNA which offers the advantage of high mutation rates, shorter processing time and environmental friendliness. On other hand, Microbial Microdroplet Culture system (MMC) is a platform designed for rapid screening of thousands of strains to identify the optimal production strain with improved phenotypes. This microfluidic-based technology eliminates the limitations of conventional screening and saves enormous time, labour and cost. In addition, MMC offers more options for strain engineering such as adaptive evolution, growth optimization and one-factor optimization. Combining the technologies of ARTP and MMC, Esco Aster offers a platform for fast and efficient optimization of microbial strains for production in biopharmaceuticals industry.

Workflow

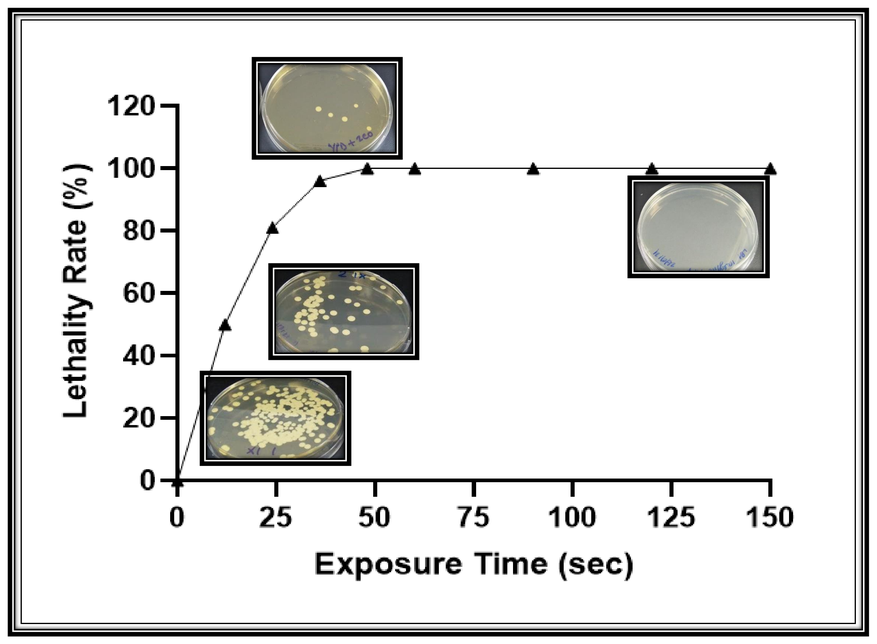
1. Original Strain
2. Integration of Desired/ Reporter Gene (Optional) — Recombinant vector
3. Exposure to ARTP
4. High Throughput Screening in MMC
5. Product Specific Screening (Optional)

Mutation of Microbial Cells Using ARTP

- ARTP offers high mutation rate
- Large number of mutated strain
- Shorter operation time



** ARTP machine in operation*



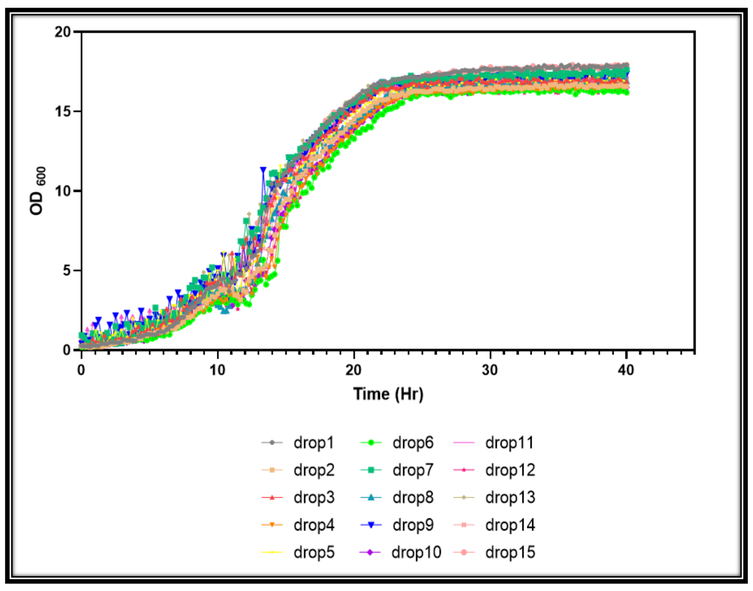
** Pichia pastoris cells treated with ARTP at 120W for various time period. Lethal rate of >90% was obtained at 36 sec exposure.*

High Throughput Screening Using MMC

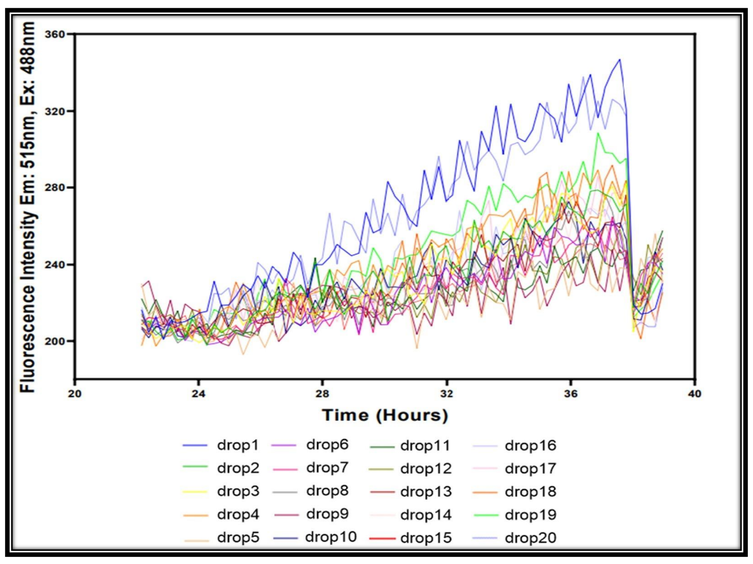
Screening of Strains by Biomass and Expression Yield

- *Pichia pastoris* GS115 growth screening in MMC system

Growth Curve of Pichia pastoris GS115



- Screening the expression level of secondary metabolite by measuring fluorescence intensity of recombinant GFP as reporter gene

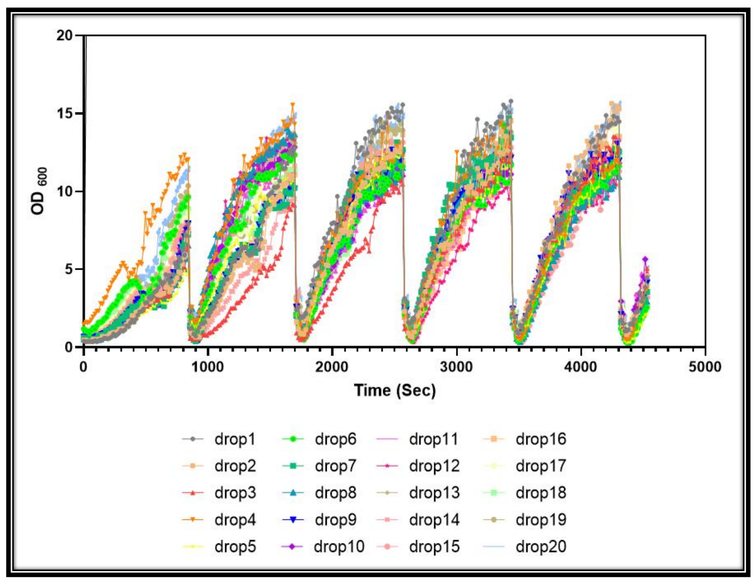


Adaptive Evolution

- Adaptive Evolution is an approach that allows the development of desired characteristics in the microbial strains by applying selective pressure over a prolonged period.

MMC offers strain engineering to produce strains with desired properties through the continuous subcultivation of the microorganisms under specified growth conditions. The microorganism adapted to modified conditions after growing over a few generations.

Screening of mutated Pichia pastoris GS115 strains



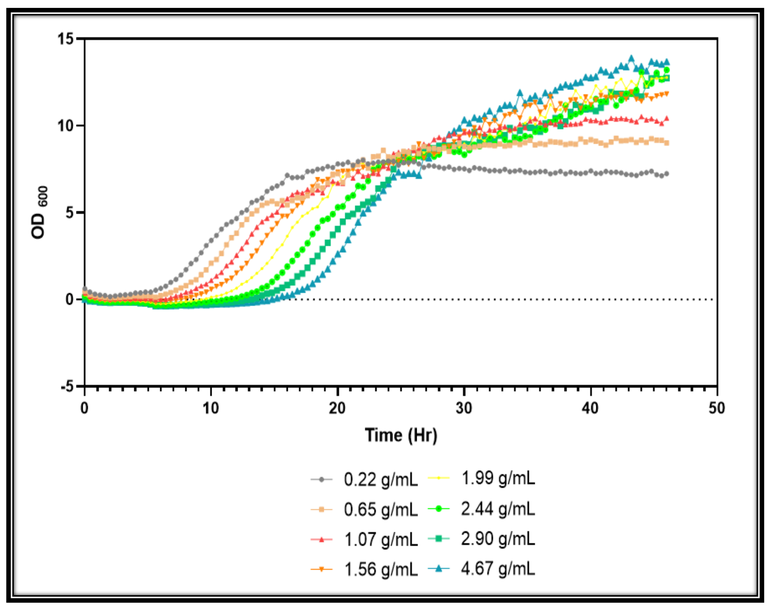
Applications:

- ✓ Adapting microorganisms to grow in desired condition (e.g., high/low osmolarity, high/low pH and high/low temperatures)
- ✓ Determine strain stability and robustness

Single Factor Multi-Level (One-Factor)

- MMC offers to optimize single factor at multi-level configuration by culturing the strains in the various concentration of one chemical factor, e.g., carbon-source, antibiotic concentration, and inducer concentration.

Growth of Pichia pastoris GS115 at different glucose concentration (g/ml)



Applications:

- ✓ Determine optimal antibiotic concentration
- ✓ Determine optimal inoculum concentration
- ✓ Determine optimum concentration of inducer or other vitamins
- ✓ Growth media optimization

INDUSTRIAL STRAIN DEVELOPMENT PLATFORM AT ESCO ASTER