Downstream Processing - Protein Secretion from Yeast

ONPS2116 Industrial Microbiology

**AIM**

The aim of this practical is to induce production and secretion of a protein, insignificant to the host organism, through the introduction and expression of plasmids. In this particular practical, yeast is transformed using plasmid pYEX-S1 and pYEX-S1 βla.

**METHOD**

Refer to Practical Manual

**RESULTS**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Transformant Number | | | | | | | | | |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| **MEDIA** |  |  |  |  |  |  |  |  |  |  |
| Complete | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| -Uracil | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| -Leucine | + | + | + | + | + | ++ | ++ | ++ | ++ | ++ |
| -Tryptophan | + | - | - | - | - | - | - | - | - | - |
| -Adenine | - | - | - | - | - | - | - | - | - | - |
| -Histidine | - | - | - | - | - | - | - | - | - | - |

Figure 1. Phenotype Scoring after growing on selective media

+++ = excellent growth; ++ = good growth; + = some growth; - = no growth

Figure 2. Enzyme assay reaction between sample supernatant and nitrocefin.

Well 1: Transformed with pYEX-S1 βla in rich LB

Well 2: Transformed in pYEX-S1 in rich LB

Well 3: Transformed in pYEX-S1 βla in minimal media

Well 4: Transformed in pYEX-S1 in minimal media

Figure 3. SDS Page gel electrophoresis, stained in Coomasie instant blue

**DISCUSSION**

Yeast transformants were obtained by using commercially available EZ-transformation mix containing the desired plasmid and then incubated at 42oC to induce heat shock, allowing the uptake of the plasmids into the yeast.

This method is insufficient to ensure transformants, however, so the resulting cells are cultured on YEPD Rich media(complete) for guaranteed growth and then selective media; Rich - Uracil and Rich - Leucine; for selective growth.

Growth of transformants of both plasmids is expected on -Uracil media as pYEX-S1 plasmid contains the URA3 gene, allowing the host cell to produce its own Uracil. Growth is also expected on -Leucine media from Yeast transformed with pYEX-S1 βla as it has the LEU2 gene. The standard pYEX-S1 plasmid however has leu2-d gene, a defective gene meaning it does not allow the host to produce Leucine. Referring to Figure 1, some growth was still seen from pYEX-S1 transformants but were not as good as pYEX-S1 βla transformants.

There was a notable difference between pYEX-S1 transformants growing on -Leucine and -Uracil plates. The main difference is -Uracil growers appear red/pink in colour. This is due to the lack of Uracil utilization.

This difference is not seen on pYEX-S1 βla transformants as both -Uracil and -Leucine growers are both red in appearance. Reason behind it is unknown.

β-lactamase was expressed in yeast transformed using pYEX-S1 βla plasmid, visible by the Enzyme Assay