Recombinant expression of human adenosine 2a receptor in yeast; understanding the bioprocess factors affecting functional yield

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Over 50% of medicines target membrane proteins, especially G protein - coupled receptors (GPCRs). A major challenge for structural and functional studies is the production of high yields of stable, functional recombinant GPCRs.

In this study, we investigated the effect of bioprocess factors on functional yield using a statistical 'Design of Experiments' (DoE) approach for the Family A GPCR, human adenosine-2a receptor (hA2aR). Production of the hA2aR was assessed by measuring its Bmax with 20 nM [^{3}H]-ZM241385 to membranes (Fraser, 2006). Expression of the receptor and culture of yeast were as described previously (Ferndahl *et al*, 2010).

hA2aR was expressed in *Saccharomyces cerevisiae* wild-type (WT), yTHC*BMS1* (BMS1) and TM6* (TM6) strains. The effect of temperature (T), dissolved oxygen (dO), pH and dimethyl sulphoxide (DMSO) addition on hA2aR yield was studied using a DoE in a high-throughput format. Results showed that these input parameters did not significantly (ANOVA p> 0.05) affect the growth of the three strains. However, significant effects were observed on the production of the hA2aR receptor when subject to different culture conditions (Table 1). This is consistent with work in a second yeast species, *Pichia pastoris* for which T, dO, pH and antifoam addition affects yields (Holmes *et al*, 2009; Routledge *et al*, 2011,).

Table 1. B_{max} estimates for three *S. cerevisiae* strains producing hA2aR under different culture conditions.

				B_{max} estimate (fmol mg^{-1})		
Temperature (°C)	pH	dO (%)	2.5% DMSO	WT	BMS1	TM6
22	6	30	Absent	442.1 (20.9) ***	18.1 (7.4)	4.6 (3.1)
24	6	40	Absent	4.4 (2.7)	56.2 (8.9)***	5.8 (2.9)
24	5.5	50	Present	14.0 (1.5)	11.6 (5.8)	35.5 (5.5)**

Values represent means ± s.e.m. from 3 determinations. ***, **, P<0.001, 0.01. The data show the different culture conditions for different *S. cerevisiae* strains and the effect they have on the Bmax of hA2aR. Each strain favours a different combination of culture conditions to give the best Bmax.

P. pastoris has the ability to utilise methanol and therefore protein production is driven by a very strong, tightly regulated inducible alcohol oxidase (*AOXI*) promoter. However, when feeding with methanol, careful consideration must be given to the duration and rate of addition. We therefore investigated the exponential feeding rates of methanol by applying two different desired growth rates (μ = 0.01 and 0.03 h^{-1}) and determined whether cell biomass and/or hA2aR yield is affected. Results showed that biomass (71.82 ± 0.24 g L^{-1},) and (5.0 ± 0.2 pmol mg $^{-1}$) increased when a low set growth rate, slower feeding of methanol (μ= 0.01 h^{-1}) was applied to the *P.pastoris* cultivation compared to a high set growth rate (biomass = 37.64 ± 0.83 g L $^{-1}$, and Bmax = 3.1 ± 0.1 pmol mg $^{-1}$).

In conclusion, careful control of culture and induction conditions is required to optimise the yield of correctly-folder receptor.

References

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