Validation of high throughput comet assay to study gut health effects of prebiotics

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Introduction

The single cell gel or comet assay is a method used to determine the genotoxicity of substances. It is however both time and material intensive. In order to overcome these limitations a high throughput method using 96-well multi chamber plates was examined as a method to analyse the effect of a prebiotic (inulin) on detoxification of 2-amino-1-methyl-6-phenylimidazo (4, 5-b) pyridine (PhIP) [1], a carcinogenic heterocyclic amine formed during cooking of meat, using an in vitro model of colonic fermentation with human faecal microbiota.

Methods

Incubation of PhIP with gut bacteria

Inulin at 1 g% was incubated with faecal bacteria, and 30µM PhIP was added at the end of 2 h and fermentation continued for 24 h. The fermentation was done at 37˚C with shaking at 80 rpm under anaerobic conditions.

Digesta	Faecal Slurry	*B. longum (BL)*	*B. thetaiotamicron (BT)*
Inulin			
	+		
		+	
	+	+	
			+
	+		+
Water			
	+		

Table 1: Fermentation treatment protocol

High throughput comet assay

Caco-2 cells were seeded in the 96 well multi chamber plates (MCP) and incubated for 4 h. The cells were then treated with fermenta and the cells incubated for another 2 h. Control treatment with 60 µM H_2O_2 was only for 15 min. After incubation the treatments and the well walls of the MCP were removed and the plate immersed in low-melting agarose. The plate was immersed in a lysis buffer for one h. The plate was placed in a horizontal electrophoresis unit and covered with alkaline buffer and incubated for 40 min, after which electrophoresis was performed. The plate was neutralized with a buffer before being stained Ethidium bromide and analysed by fluorescence microscopy [2].

Results

High throughput comet assay

The results of the high throughput method validation show comet formation in cells treated with 60µM H_2O_2 (Fig 1). PhIP treated cells showed extensive DNA damage (Fig 2). Cells present in the 100µM PhIP incubated with faecal bacteria appeared to show less DNA damage than the 100µM PhIP treated cells (Fig 3).

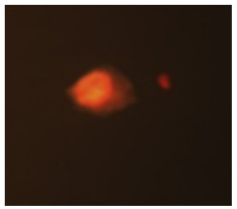

Figure 1: Comet observed in the 60µM H_2O_2 treated wells.

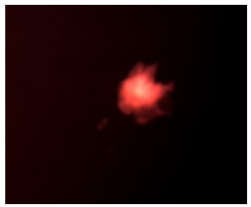

Figure 2: Extensively damaged cell present in the 100µM PhIP treated wells.

Figure 3: Damaged CaCo-2 cell observed in the 100µM PhIP in 12 hour water + faecal fermenta treated wells

Discussion

With use of the high through put comet assay comets and cell damage were observed, showing this assay does work. However the protocol needs more optimisation, as we found issues in maintaining cell numbers, due to leaking from the wells or improper adhesion of the cells to the bottom plate.

Conclusion

With further optimisation, the high throughput method using the 96 well multi chamber plate may provide an efficient method for studying DNA strand breaks in cells.

References

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