Bakers Yeast (*Saccharomyces cerevisiae*) in drug and pesticide delivery.

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Abstract

The technology for encapsulating active ingredients within micro-organisms has developed over the past 30 years. Although other fungi and bacteria are amenable *Saccharomyces cerevisiae* has been the primary focus of research. Initial successes were achieved protecting volatile components from premature evaporation in the flavour and fragrance industry. Now, trials are underway to demonstrate the principle of using the technology to deliver principle components effectively for crop protection and drug delivery applications. In crop protection, microencapsulation may offer the prospect of reduced application rates where efficacy is improved. This will have an added attraction where environmental and food-residue advantages may be delivered. Yeast encapsulated fungicides have proven effective in controlling crop pathogens *in situ* and it has been demonstrated that yeast has been effective at promoting the transport of both lipophilic and hydrophilic drugs *in vitro*.

Introduction

For many years, yeast has been used as a carrier for the delivery of principle components. In the food flavour industry, the main aim was initially to convert volatile flavour oils into easy to handle flowable powders. Bishop *et al.* (1998) reported how a range of lipophilic volatile chemicals, including orange peel and peppermint essential oils, could be absorbed into yeast at high concentrations, over 400 mg/g product in some cases. The impact of temperature and hydrocarbon chain length on encapsulation potential was used to propose a mechanism whereby diffusion across the cell membrane was the key selective barrier to encapsulation. Addressing the impact of cell viability on the uptake process, it was reported that active bakers yeast (*S. cerevisiae)* was killed early in the process of encapsulation and that encapsulation continued. This indicated that a passive diffusion process was indeed the primary mechanism for the accumulation of material within the yeast cells. Initially appearing as small droplets within the yeast, the droplets then appeared to coalesce to form a single zone occupying the majority of the cell volume.

Recently, the mechanisms of yeast based encapsulation have been revealed in more details by Normand *et al.* (2005). Using the model of limonene, the principal component of orange peel oil,, these authors demonstrated how, by being encapsulated within yeast cells, limonene became protected against evaporation at high temperatures. The basis of the exceptional combination of thermal stability and the hydration facilitated release of encapsulated flavours was clearly demonstrated. The properties of the flavour compounds most suited to this form of encapsulation are principally based on their octanol-water partition coefficients (log P) (Dardelle *et al.* 2007). Practical examples of the use of this technology included the pre-processing flavour addition to French fries, and fry-dry noodles.

Those studies support the principles of encapsulation of lipophilic materials in yeast cells propounded in patents by Pannell (1986, 1987). Applications for yeast based encapsulated materials have ranged from applying fragrances, biocides and insecticides to textiles (Sager *et al*, 1991; Nelson, 2002), to the use of microencapsulated nicotine in smoking cessation products (McNeight 2000) and to their use in carbonless copy paper (Pannell 1986).

These applications have been expanded to include insect and weed control in crop and household environments (Mooney 1991, Duckham *et al*. 2005) and for targeted drug delivery (Nelson *et al* 2006). One of the surprising findings of recent investigations has been the effect of yeast on model cell systems used for measuring drug delivery across epithelia.

Recent developments in yeast mediated delivery of active ingredients

Methods

Epithelial cells form a selective barrier between the external environment and the circulatory system, a barrier that must be breached for effective drug delivery to occur. The administration of drugs using nasal and oral/buccal administration via epithelial mucosa offers alternatives to the parenteral route. The barrier to adsorption of active ingredients via these routes is maintained through the formation of protein complex based tight junctions which form a seal between neighbouring cells. These complexes provide a mechanism for regulating the passage of small molecules and electrolytes through the inter-cellular passage *via* what is known as the paracellular route (Ward *et al*. 2000). What we have found is that exposure to common bakers yeast (*S. cerevisiae*) triggers the opening of tight junctions between cells in a model epithelia system in a controlled manner (Fuller *et al*, 2007). A model consisting of a confluent cell monolayer based on human colon cancer carcinoma cells (Caco-2) was used to test enhanced drug delivery, initially using yeast alone, prior to trials with encapsulated actives. Subsequent studies used methods of encapsulating active ingredients inside yeast microcapsules by simply dissolving the active ingredient in a suitable solvent and mixing with an aqueous yeast suspension at an elevated temperature, followed by spray drying the slurry (see Bishop *et al* 1998 and Nelson *et al* 2006 for further details). A combination of scanning electron microscopy and florescence microscopy was used to observe the mechanisms of how the yeast can modulate drug delivery and the concentrations of active ingredients were quantified by high performance liquid chromatography, mass spectrometry and ELISA.

Results and Discussion

Electron micrographs showed that gaps appeared between Caco-2 cells on exposure to non-viable yeast cells (Figure 1). In an untreated control monolayer (Figure 1A), there were no gaps present between cells. After applying dead yeast cells, continuous spaces formed at the cell edges, indicated in figure 1B. Yeast cells can be seen attached to the Caco-2 cell surface in figure 1B (indicated by an arrow).

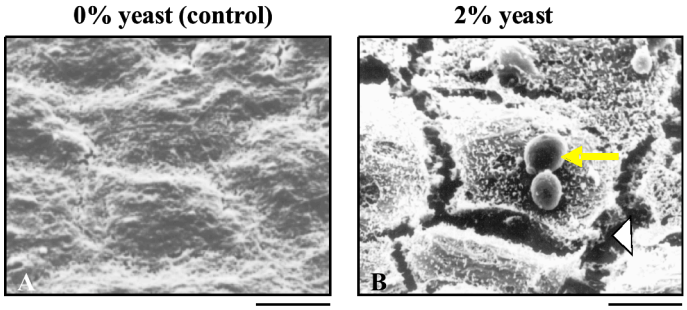

Figure 1. Scanning Electron Microscopy of control and yeast-treated Caco-2 cell monolayers. (Scale: Bar = 10 µm).

The opening of the tight junctions was quantified by the measuring the trans-epithelial electrical resistance (TEER) generated when an electrical current is applied between the upper and lower

surface of the monolayer. When closed, resistance is high as electrolytes are prevented from migrating in solution between the electrodes. The only means of transport for drugs in this state is through the body of the cells via the transcellular route. This is the case for both water soluble and poorly soluble drugs.

The potential for increasing the delivery of drugs using yeast as a permeability enhancer has been demonstrated using horseradish peroxidase (HRP) as a model protein (Fuller *et al*, 2007) and with fluorescently labelled insulin initially and subsequently with insulin at therapeutic concentrations. In the presence of yeast, over 20% of insulin applied was transported across the membrane (Figure 2); in some cases, this phenomenon increased to over 40% in 24 hours (Fuller 2007).

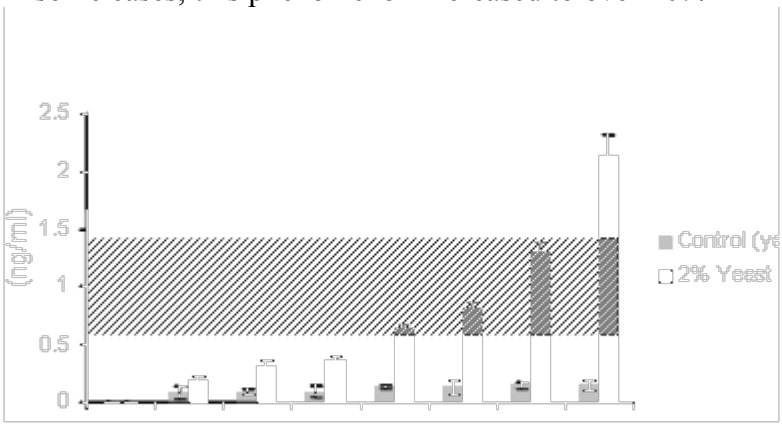

Figure 2. Penetration enhancement of insulin with 2% yeast application through a Caco-2 cells monolayer. 10 ng/ml insulin was applied at the upper side of Caco-2 cells and was incubated with 2% yeast suspensions, or without (control), for 24 h. Samples were removed from the basal layer and assayed for insulin content using ELISA. The hatched area represents a range of concentration of insulin representative of physiological levels. (Error bars are ± S.E.M, n=3).

Subsequent studies with yeast encapsulated ibuprofen and propranolol have also demonstrated an increase in drug transport in terms of doubling the permeability coefficient of the transport of free drug in solution (Fuller 2007). A more lipophilic drug, fenofibrate, was encapsulated and administered as a crude formulation *in vivo*. Uptake into dogs bloodstream was facilitated, producing a sustained release profile that was at least as effective at delivering a payload as a commercially available formulation (Tricor®, Abbot, US) (Nelson *et al*. 2006). Following promising results from the delivery of antifungals, including econazole nitrate, used for trials in topical healthcare applications (Nelson *et al.* 2006), fungicide formulations were tested for crop protection purposes.

Tebuconazole, a common agrochemical fungicide, was used as a model compound. It was encapsulated in yeast for foliar spray application with no additional adjuvents and was applied at commercial rates. Micap tebuconazole was compared with an industry standard product, (Folicur®, Bayer Cropscience, Netherlands) for preventative and curative treatment of Fusarium ear blight in wheat plants. Fusarium disease was controlled well and grain was protected most effectively by the yeast based formulation in terms of incidence, yield and mycotoxin contamination. When yeast encapsulated foliar formulations of fungicides prochloraz and flusilazole were tested for efficacy against the stem based disease eyespot in winter wheat, performance was improved when compared to commercial equivalents (Rossall, *pers comm*.).

Conclusions

There is an increasing body of evidence that yeast can not only be used as a carrier for the encapsulation of lipophilic small molecules but can act to promote the absorption of both encapsulated and unencapsulated active ingredients. The sustained release properties of the natural microcapsules predispose the material for applications in pesticide delivery in crop protection. Fungicide delivery for some classes of active ingredients was particularly advantageous.

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