

# Effects of Masson Pine Pollen Extracts on the Gene Expression Profile of Porcine Ileal Cell Cultures

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## Introduction

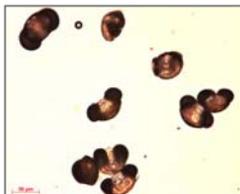


Fig. 1: *Pinus massoniana* pollen

Pollen of *Pinus massoniana* has been used in the traditional Chinese medicine for several hundred years e.g. for the treatment of digestive disorders or rash. Up until now only little research has been done on pine pollen in general and masson pine pollen in special to verify possible beneficial properties. One of these studies performed on mice found anti-nociceptive and anti-inflammatory activities of *Pinus densiflora* pollen extracts (Choi, 2007). A feeding experiment with weaning piglets fed whole masson pine pollen resulted in higher daily weight gains and changes in the mRNA expression levels of inflammatory, cell cycle and growth associated genes in intestinal tissues and mesenteric lymph nodes (Schedle et al., 2006 and 2008).

In the present study the effects of *Pinus massoniana* pollen extracts on epithelial cell proliferation and on mRNA expression patterns of some genes involved in proliferation, cell death and inflammation should be elucidated.

## Material and Methods

Ground masson pine pollen was extracted with the following solvents: 100% water, 50% ethanol, 100% ethanol, 100% hexane, 80% methanol. Dried residues were dissolved in PBS containing 20% (2-hydroxypropyl)- $\beta$ -cyclodextrin. For control treatments residues of evaporated solvents were used.

Cell proliferation of the porcine ileal cell line IPI-21 was investigated with an electric cell-substrate impedance sensing device (ECIS™ Model 1600, Applied Biophysics, Troy, New York). Extracts were added to reach a final concentration equivalent to 1% pollen matter in the medium. Impedance results were normalized to the first value obtained immediately after addition of cells.

After 48 h of cultivation total RNA was extracted and qRT-PCR reactions were performed with Rotor-Gene 3000 (Corbett Live Science, Sydney, Australia) and the SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR kit (Invitrogen). Statistical analysis was done with REST 2008 V.2.0.1 (Technische Universität München, Germany and Corbett Life Sciences, Sydney, Australia).

## Cell Proliferation Results

100% water (fig. 2) and 50% ethanol (fig. 3) pollen extracts significantly decreased cell densities 20 h after seeding. The 100% ethanol pollen extract transiently decreased normalized impedance values during hours 10 to 25 leading to the assumption that adhesion of the cells was altered (fig. 4). 80% methanol or 100% hexane pollen extracts had no significant effects on cell proliferation (data not shown). Results are presented as mean  $\pm$  standard deviation cloud (blue for control, red for pollen extract treatment, n=4). Dark lines show results of the statistical analysis, red border marks significance ( $P = 0.05$ ).

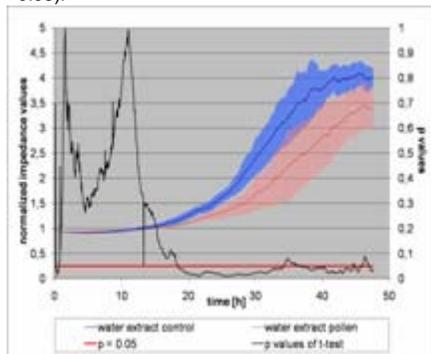


Fig.2: Treatment with 100% water extracts

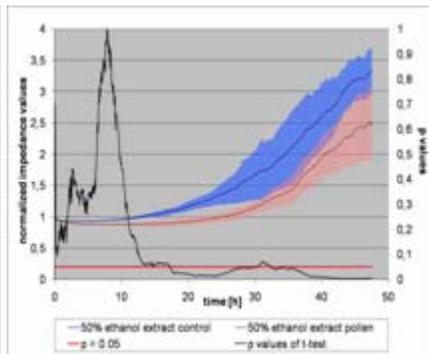


Fig.3: Treatment with 50% ethanol extracts

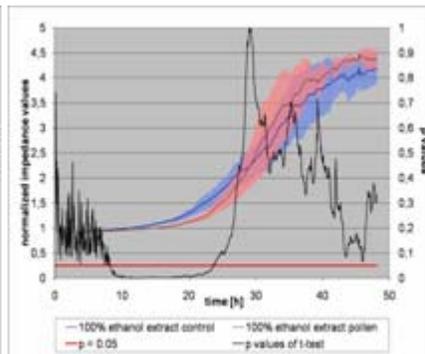
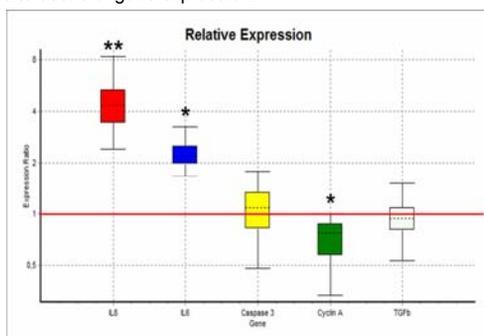


Fig.4: Treatment with 100% ethanol extracts

## Gene Expression Results

Only the 50% ethanol extract of *Pinus massoniana* pollen led to significant up-regulations of IL-6 and IL-8 ( $P=0.012$  and  $P=0.024$  respectively) while cyclin A was significantly decreased ( $P=0.039$ ). Expression of caspase 3 and TGF $\beta$  was not altered ( $P=0.943$   $P=0.662$  respectively, fig. 5). The other pollen extracts did not lead to significant alterations of gene expression.

Fig. 5: Influence of 50% ethanol pine pollen extract on gene expression patterns. Results are shown normalized to three housekeeping genes ubiquitin, GAPDH and histon H3 and to treatment with control extracts (n = 4). (REST 2008)



## Discussion

The results of the present study clearly show an influence of masson pine pollen compounds on cell proliferation and gene expression patterns. The decrease of cell proliferation after treatment with 50% ethanol pollen extracts can be partly explained by a decrease of cyclin A expression. But since cell numbers were also lowered by the water extract which did not lead to alterations of cyclin A mRNA levels, additional effects have to be expected. A decrease of cell proliferation as was found in the present study allows the assumption that ingested pine pollen may have effects on cell proliferation or tumor growth in the intestine.

Alterations of expression levels of inflammation mediators such as IL-6 and IL-8 by pollen substances soluble in 50% ethanol hint to possible influences of pine pollen compounds on pro-inflammatory processes.

A first attempt to identify effective compounds in *Pinus massoniana* pollen extracts by LC-ESI-ToF-MS lead to a number of distinct mass signals in 50% ethanol extracts that were absent or strongly reduced in other pollen extracts. Unfortunately identification was not yet possible with the exception of fisetin and naringenin. Further research is in progress.