

Enzyme Specificity - or is it?

We have been receiving reports that the Intermediate 2 Biology LO3 experiment "Determination of the Specificity of Enzymes" is causing some difficulties for students. To recap the experiment, pupils examine the activity of four different enzymes (trypsin, amylase, urease, pectinase) with the substrate casein. The casein is mixed with agar and poured into a Petri dish to produce an opaque, white jelly. Wells are cut into the agar to hold the different enzyme solutions.



Figure 1 - A typical problematic result after 2-3 hours incubation at 37°C followed by storage at 4°C for 24 hours. Wells contain (clockwise from the top): 2% pectinase solution, water as control, 2% liquid alpha amylase (Philip Harris), 2% trypsin solution, 2% urease solution (Philip Harris)

When casein is broken down, a clear zone appears in the milk agar around the well. The diameter of the clear zone is a measure of the activity of the enzyme. The intended outcome is that 2% trypsin solution will be the only enzyme that produces a clear area around the well, showing that this enzyme is specific for casein.

According to reports we have received, the well containing amylase also seems



Figure 2 - The effect of different amylase solutions on casein breakdown. Clockwise from top: 2% NCBE bacterial alpha amylase (liquid), 2% bacterial alpha amylase (Philip Harris liquid), 2% bacterial alpha amylase (Philip Harris powder), 2% fungal alpha amylase (Philip Harris powder)

to be causing the casein to break down (Fig 1). The reason for this may be that some amylase preparations can be contaminated with proteases. This would then produce a similar result to the trypsin, thus making the experimental outcome somewhat confusing to students.

Here at SSERC the experiment was tried with a variety of amylases at different concentrations. We also found it to be the case that certain amylases caused the casein to break down (Fig 2). However, by using 2% amylase provided by NCBE, casein breakdown was negligible if not altogether absent, allowing the well containing trypsin to be shown as the only one producing the desired effect (Fig 3).



Figure 3 - Experiment set up as for Figure 1 with NCBE amylase replacing Philip Harris amylase. No digestion evident.

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