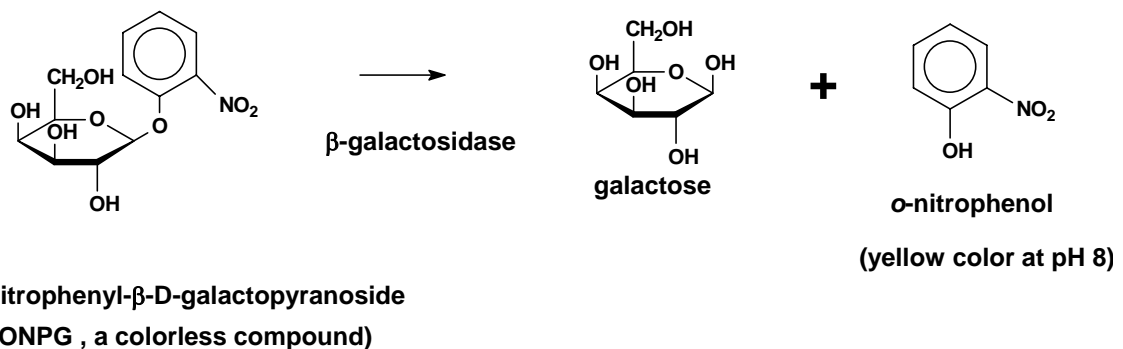
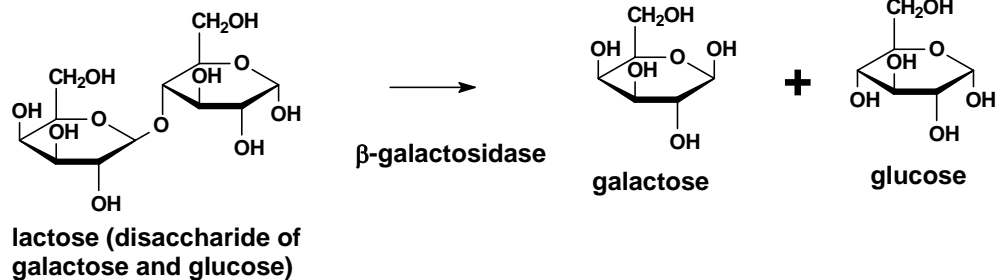


ONPG Biochemical Test for β -galactosidase activity

β -galactosidase is an enzyme found in most coliform bacteria. It catalyzes the hydrolysis of the disaccharide lactose into galactose and glucose. The presence of this enzyme can be easily detected with the water-soluble substrate analog ONPG (see below). The formation of yellow color following incubation in the presence of the ONPG substrate is indicative of β -galactosidase activity and presumptive for coliform bacteria (Fig. 6-70, 6-71, and 6-72 of the Atlas, p. 71).



ONPG is a substrate analog of the enzyme β -galactosidase

Procedure:

To demonstrate the presence or absence of β -galactosidase activity we will use *Escherichia coli* and *Proteus vulgaris* as the test organisms. Your instructor will demonstrate this test for you.

1. Suspend a heavy inoculum of each test organism individually in 1 ml sterile saline.
2. Add a disk containing the ONPG colorimetric substrate.
3. Incubate the tubes at 35 °C for a few hours. (It is best to start this right at the beginning of class, or even before.)
4. Observe for the formation of a yellow color which indicates the organism has β -galactosidase activity.
5. Record the results on the following page.

