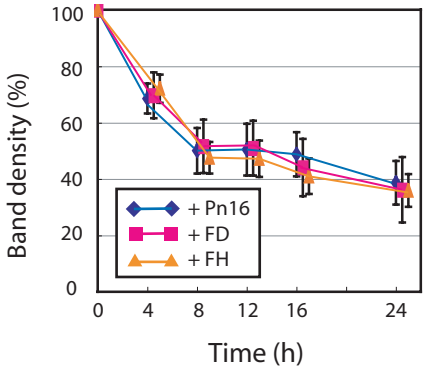
Supplemental Figure 4

Aoki et al. (2005)



Supplemental Figure 4. Effect of Pn16, FD, and FH on in vitro degradation rate of B-HisGFP in root extract.

Fresh crude extract of root was prepared at 4 ºC prior to the assay. Tissues were homogenized in 0.1 M sodium phosphate buffer (pH 7.4). Homogenate was clarified by filtration and centrifugation at 15000 x g for 20 min. Protein concentration in assay mixture was as follows; B-HisGFP 20 ng/µl, Pn16, FD, or FH 200 ng/µl, and root extract 2 µg/µl. Mixture was incubated at 25 ºC for indicated time. Assay mixtures were run on 12.5 % acrylamide gel and transferred onto PVDF membrane (Millipore). Biotinylated proteins were detected by using horseradish peroxidase-conjugated streptavidin (Vector Laboratories, Burlingame, CA). Chemiluminescence signal was captured by Typhoon 8600 (Amersham Biosciences), and then analyzed by ImageQuant (Molecular Dynamics). Values represent means ± SE of three independent measurements.

The degradation rate of B-HisGFP was nearly the same in Pn16, FD, and FH. This result demonstrated that neither FD nor FH affected the stability of B-HisGFP in vitro.