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Purification methods for extracellular 28- and 42-kilodalton β-1,3-Glucanases from *Bacillus circulans* IAM 1165

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The strain Bacillus circulans IAM 1165 produces three major glucanases during its stationary phase of growth. These β-1,3-Glucanases are isolated because of their ability to lyse fungal cell walls based on the cleavage of glucans which are the main compounds of cell walls. β-Glucan has two different types of linkages, the β-1.4 (~70 %) and the β-1.3 (~30 %). By cleaving the β-1,3-linkage of these polysaccharides, the glucanase is able to form oligosaccharides out of polysaccharides.

In this study, different possibilities for purification of the 28- and 42-kDa β-1,3-glucanase from *Bacillus circulans* IAM 1165 with chromatographic methods are studied to find possible simplifications and improvements of the process of enzyme purification whereby the concentration and the specific activity should increase. The purification is performed with ammonium sulphate precipitation. As experimental result were compared to published results, the same tendencies are in general shown in the loss of enzyme amount during the purification process as well as for the distribution of both types of enzyme. In both cases the 42 kDa-Glucanase is the minor formed glucanase. After the hydrophobic interaction chromatography, which is for separating other proteins from the glucanases, the recovery is 30 fold smaller than in the forward performed experiments. This corresponds to the supposition that these conditions for the hydrophobic interaction chromatography are not good for the purification of small enzyme amount. Further, like discussed above, the bad separation of the chromatogram leads to the same conclusion.