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# A novel secreted metzincin metalloproteinase from *Bacillus intermedius*

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ABSTRACT

**The *mprBi* gene from *Bacillus intermedius* 3–19 encoding a novel secreted metalloproteinase was identified. The *mpriBi* gene was expressed in an extracellular proteinase-deficient *Bacillus subtilis BG 2036* strain and the corresponding protein was characterized biochemically. The 19 kDa MprBi protein was purified to homogeneity and sequenced by mass spectroscopy and Edman degradation methods. Amino acid sequence analysis of MprBi identified an active site motif HEYGHNFGLPHD and a conserved structural component Met-turn, both of which are unique features of the metzincin clan. Furthermore, MprBi harbors a number of distinct sequence elements characteristic of proteinase domains in eukaryotic adamalysins. We conclude that MprBi and similar proteins from other *Bacillus* species form a novel group of metzincin metalloproteinases in prokaryotes.**


## 1. Introduction

Proteinases, which hydrolyze proteins into short peptides or amino acids, represent one of the most commercially important groups of industrial enzymes and comprise the majority of the total enzyme sales worldwide. *Bacillus* species synthesize several groups of extracellular proteinases, such as serine and metal proteinases, which are classified based on their mechanism of action and catalytic amino acids [1].

Zinc-dependent endo-metalloproteinases are a subset of metalloproteinases found in all kingdoms of life. These enzymes are characterized by a consensus amino acid sequence HExxH, where the histidines are zinc ligands and the glutamic acid functions as a catalytic base. A third zinc ligand is provided by the side-chain of His, Glu or Asp, usually located downstream of this motif [2]. In the MEROPS proteinase database, the HExxH motif-containing enzymes are grouped in the MA clan (http://merops.sanger.ac.uk).

The metzincin subclan of zinc-dependent endo-metalloproteinases includes several extracellular and membrane-bound proteinase families containing an extended consensus sequence, HExxHxxGxxHx, which comprises three zinc ligands (underlined) and the general base glutamic acid [3,4]. Many typical metzincins display very limited amino acid conservation overall and often share less than 20% sequence identity. Despite this fact, the tertiary structure of their catalytic domains and their active site sequences are remarkably conserved [5–7]. The name of this subclan originates from a conserved methionine located in a 1,4-β-turn (so called Met-turn) of the protein. Metzincins have been recognized as key players in a variety of biological systems, where they regulate the activity of other biological molecules (cytokines, growth factors, other proteinases) by limited proteolysis [4,6].

The metzincin subclan contains several proteinase families, including astacins (BMP-1, tolloids, meprins) and adamalysins or ADAMs (a disintegrin and metalloproteinase-like). Over 120 astacins have been described in a variety of organisms from bacteria to mammals, but interestingly not in plants, fungi or in Bacilli. Astacins are produced as zymogenes, with a propeptide and a signal peptide at the N-terminus of the mature form, and typically require activation via proteolytic processing, in many cases after a basic residue (autocatalytically in the case of astacin) [7–10]. The ADAMs (adamalysins/reprolysins) are multi-domain proteins found in mammalian reproductive tissues and in snake venom. The ADAMs are secreted as pro-enzymes with the N-terminal signal sequence and propeptide and are activated upon propeptide removal either by other proteinases or autocatalytically [11,12].

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