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1 **A novel amidase signature family amidase from the**
2 **marine actinomycete *Salinispora arenicola* CNS-205**

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22 **Abstract**

23 We cloned a new gene from the amidase signature (AS) family,
24 designated *am*, from the marine actinomycete *Salinispora arenicola*
25 CNS-205. As indicated by bioinformatics analysis and site-directed
26 mutagenesis, the AM protein belonged to the AS family. AM was
27 expressed, purified, and characterised in *Escherichia coli* BL21 (DE3),
28 and the AM molecular mass was determined to be 51 kDa. The optimal
29 temperature and pH were 40 °C and pH 8.0, respectively. AM exhibited a
30 wide substrate spectrum and showed amidase, aryl acylamidase, and acyl
31 transferase activities. AM had high activity towards aromatic and
32 aliphatic amides. The AM substrate specificity for anilides was very
33 narrow; only propanil could be used as an effective substrate. The
34 extensive substrate range of AM indicates it may have broad potential
35 applications in biosynthetic processes and biodegradation.

36 **Keywords** AM·amidase signature (AS) family·Aryl acylamidase·Acyl
37 transferase activity·Propanil

38 **Introduction**

39 Carboxylic acid amides can be hydrolysed by amidases (EC 3.5.1.4),
40 forming carboxylic acids and ammonia. Most amidases also produce
41 hydroxamic acids through their acyltransferase activity (Asano et al.
42 [1982](#); Fournand et al. [1998](#)). Amidases are very important for chemical
43 industrial synthesis and for control of environmental pollution.

44 Amidases can be divided into two categories (Chebrou et al. 1996;
45 Fournand and Arnaud 2001). The first category is the nitrilase
46 superfamily, which is characterised by a cysteine residue and includes
47 aliphatic amidases. The second category is the amidase signature (AS)
48 family, which has a conserved GGSS signature in the amino acid
49 sequence (Mayaux et al. 1990; Chebrou et al. 1996). Amidases are
50 extensively present in bacteria, archaea and eukaryotes (d'Abusco et al.
51 2001; Galadari et al. 2006; Neu et al. 2007; Ohtaki et al. 2010; Politi et al.
52 2009).

53 The marine actinomycete *Salinispora arenicola* CNS-205 produces
54 many bioactive natural products, including saliniketals A and B, which
55 were originally isolated by Fenical and co-workers in 2006 (Fenical et al.
56 2006). Genome sequencing of the strain *S. arenicola* CNS-205 identified
57 a gene encoding a putative amidase, named AM, which belongs to the AS
58 family. The amidase activity of AM was confirmed, and its catalytic
59 parameters and optimal conditions were determined. This enzyme was
60 shown to have an abnormally wide substrate spectrum and activities.

61 **Materials and methods**

62 **Chemicals**

63 The chemicals used in this paper were graded as analytical reagents and
64 purchased from J&K Scientific Company (Beijing, China).

65 **Bacterial strains and culture conditions**

66 *S. arenicola* CNS-205 was cultured in liquid ISP2S (0.4% yeast extract,
67 1% malt extract, 0.4% glucose, and 7% sea salt; pH 7.3) in an incubator
68 with rotation at 28 °C and 220 rpm and harvested after 2–3 days to obtain
69 the genomic DNA.

70 **Cloning of the am gene**

71 The genomic DNA of *S. arenicola* CNS-205 was extracted. Genes
72 encoding potential AS family amidases were identified through BLASTP
73 analysis (<http://www.ncbi.nlm.nih.gov/blast>). The primers used for the
74 polymerase chain reaction of the *am* ORF were *am*-F (5'-
75 GGGCATATGGCGGTGCAGGACATCA-3') and *am*-R (5'-
76 CAGGAATTCCAGTTTCGTCATGCCC-3'). The *Nde*I and *Eco*RI sites
77 (underlined) were used to clone *am* into the protein expression vector
78 pET-28a(+) (Novagen).

79 **AM expression and purification**

80 *Escherichia coli* BL21 (DE3) carrying pET28a(+)-*am* was grown in
81 Luria-Bertani medium with 100 µg ml⁻¹ ampicillin at 37 °C (1 l medium
82 was inoculated with 1% inoculum from a 20 ml overnight culture). The
83 cultures were induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside
84 at OD₆₀₀=0.6 and incubated for 20 h at 16 °C. Then, the cultures were
85 centrifuged at 5,000 rpm for 10 min at 4 °C. The cell pellet was
86 resuspended in prechilled binding buffer (20 mM Tris-HCl, pH 8.0, 0.5
87 mM NaCl, and 5 mM imidazole) and lysed by sonication on ice (60%

88 amplitude, 4 s on and 10 s off). The supernatant was harvested after
89 centrifugation at 15,000 rpm for 30 min at 4 °C. Then, the supernatant
90 was loaded onto a His-Bind Ni resin column pre-equilibrated with
91 binding buffer (GE Healthcare). An imidazole step gradient was used,
92 and the His₆-AM protein (50, 100, 200, 400, and 800 mM) was recovered
93 in elution buffer (20 mM Tris-HCl, pH 8.0, and 0.5 mM NaCl). Then,
94 SDS-PAGE and HPLC-MS (Thermo) were used to analyse the fractions
95 with His₆-AM. The supernatant containing His₆-AM was further purified
96 with PD-10 desalting columns (GE Healthcare) after it was concentrated
97 through a VIVASPIN concentrator. The protein concentration was
98 quantified with a NANODROP 2000c (Thermo), and a Thermo Hypersil
99 GOLD C4 column (1.9 μ, 100×2.1 mm) was used. The recombinant
100 proteins were assessed with HPLC-ESI-HRMS and eluted with a gradient
101 of 0.1% formic acid (A) and CH₃CN-containing 0.1% formic acid (B).
102 The elution program was 2% B for 3 min, 2 to 20% B for 1 min, 20 to
103 70% B for 16 min, 70 to 90% B for 1 min, 90% B for 4 min, 90 to 2% B
104 for 1 min, and 2% B for 4 min at a flow rate of 0.2 ml min⁻¹. A Orbitrap
105 mass spectrometer (Thermo) was used in positive ion mode, with
106 scanning from m/z 300 to 2,000. Xcalibur software (v.1.1; Thermo
107 Finnigan) was used for analysis, and the data were processed and
108 deconvoluted.

109 **Amidase assays**

110 The purified AM was resuspended in buffer (pH 8.0, 20 mM Tris-HCl,
111 10% glycerol and 100 mM NaCl). The assays were performed with 1 μ g
112 purified AM in 20 mM Tris-HCl, pH 8.0, 100 mM NaCl at 35 °C. The
113 phenol-hypochlorite ammonia method (Weatherburn 1967) was used to
114 assess the amidase activity, which yielded ammonia. The amount of
115 enzyme catalysing the release of 1 μ mol NH₃/min was defined as one unit
116 of enzyme activity. The Hanes-Woolf method was used to estimate the
117 K_m and V_{max} , and the k_{cat} and k_{cat}/K_m values were determined, indicating a
118 molecular mass of 51 kDa. Control reactions were performed without
119 AM.

120 **Aryl acylamidase activity assay**

121 The aryl anilide pesticides propanil, butachlor and acetochlor were
122 assessed as substrates to determine the aryl acylamidase activity of AM.
123 The aryl acylamidase activity was verified following the method of Shen
124 et al. (2012) as follows: 1 μ g of His₆-AM was added to 0.2 mM anilide in
125 1 ml of 20 mM Tris-HCl, pH 8.0, 100 mM NaCl and incubated at 35 °C.
126 Addition of HCl changed the pH to 3, and the sample was extracted with
127 ethyl acetate, terminating the reaction. This organic layer was dried and
128 re-dissolved in methanol. Reverse-phase HPLC (Shimadzu LC-20 AD,
129 Waters 2998 photodiode array detector) with a Thermo C18 cartridge
130 (particle size 3 μ ; 2.1 \times 150 mm) and 250 nm detection wavelength was
131 used to recognise the reaction products, with 2:3 0.1% formic

132 acid/methanol (isocratic elution mode) for 20 min at a flow rate of 0.2 ml
133 min⁻¹.

134 **Hydroxylamine-acyl transferase activity assay**

135 The acyl transfer activity was detected as described by Fournand et al.
136 (1998). All experiments were performed at 35 °C for 10 min, and the
137 reaction system was as follows: 1 µg of AM, hydroxylamine
138 hydrochloride (100 mM, pH 7.0), and amide or anilide (1~25 mM) in
139 buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl). An acidic solution of
140 FeCl₃ (0.133 M in 0.68 M HCl) was adopted to terminate the reactions.
141 The supernatant was initially centrifuged at 12,000 rpm for 10 min and
142 subsequently collected, and the hydroxamate concentration was measured
143 at λ=500 nm. The blank control experiments were performed without
144 AM. The optical density of the experimental groups (marked A1) was
145 determined. Additionally, the control group (marked A2) was assessed to
146 determine the concentration (C) of hydroxamate $C=(A1-A2)/\epsilon L$ (A refers
147 to the optical density, ε denotes the coefficient of molar extinction, and L
148 indicates the layer thickness). Different substrates have different ε values:
149 propionamide, 1,029 M⁻¹ cm⁻¹; hydroxamate derivative of acetamide, 996
150 M⁻¹ cm⁻¹; propanil, 1,029 M⁻¹ cm⁻¹; isobutyramide, 1,016 M⁻¹ cm⁻¹. The
151 control groups did not have AM. One unit of enzyme activity was defined
152 as the amount of enzyme required to catalyse the formation or hydrolysis
153 of 1 µmol of substrate or product every minute.

154 **Effects of temperature and pH on enzyme activity**

155 For determination of the optimal temperature, the experiments were
156 performed in buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl) with 20
157 mM of the substrate benzamide. Thermo stability was detected by pre-
158 incubation of the protein for 1 h at different temperatures. Then, the
159 residual activity was tested at 35 °C. For determination of the optimal pH,
160 the experiments were performed in various buffers as follows: 0.1 M
161 sodium acetate buffer (pH 4.0, 4.5, 5.0, 5.5, and 6.0), 0.1 M potassium-
162 phosphate buffer (pH 6.0, 6.5, 7.0, 7.5, 7.8, and 8.5), and 0.1 M Tris-HCl
163 buffer (pH 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0). For the pH stability detection,
164 the experiments were performed in buffers with pH values that ranged
165 from 4.0 to 10.0 with incubation at 25 °C for 1 h; then, the residual
166 protein activity was tested at 35 °C.

167 **Impact of metal ions and other reagents**

168 The impact of metal ions (Ni^{2+} , Ba^{2+} , Zn^{2+} and Ca^{2+}) and chemical agents
169 (1,10-phenanthroline, EDTA, SDS and PMSF) on the amidase activity
170 was detected. The samples were preincubated for 10 min at 35 °C with
171 benzamide as a substrate, and then, the amidase activity was determined
172 as described previously.

173 **Site-directed mutagenesis of *am***

174 Site-directed mutagenesis primer pairs (Table 1) were designed to
175 produce mutated *am* with a QuikChange site-directed mutagenesis kit

176 (Stratagene). The recombinant plasmid pET-28a(+)-*am* served as a
177 template in the mutagenesis reactions. The PCR products were purified
178 by agarose gel electrophoresis, and the DNA bands with the appropriate
179 sizes were eluted from the gel pieces. Then, the plasmid DNA was
180 digested with *DpnI* and transformed into competent *E. coli* BL21 (DE3)
181 cells. Three mutant (K84A, S158A, and S182A) plasmids were
182 constructed with this technique and were verified through DNA
183 sequencing. The mutant proteins were expressed, purified, and analysed
184 as described above.

185 **Nucleotide sequence accession number**

186 The nucleotide sequence of *am* was stored in GenBank with a sequence
187 ID of CP000850.1.

188 **Results**

189 **Sequence analysis of the *am* gene**

190 The amino acid sequence of AM was compared to sequences of known
191 amidases accessible in the GenBank database. The comparisons showed
192 that AM shared 29–45% identity with several enzymes, including an
193 amidase from *Rhodococcus* sp. N771 (45% identity), an amidase from
194 *Thermus thermophilus* HB8 (31% identity), the aspartyl/glutamyl-tRNA
195 amidotransferase subunit A from *T. thermophilus* HB8 (33% identity),
196 and the aspartyl/glutamyl-tRNA amidotransferase subunit A from
197 *Thermotoga maritima* MSB8 (28% identity). Additionally, we confirmed

198 the presence of Ser-Ser-Lys, the highly conserved catalytic triad of the
199 AS family, in the enzyme amino acid sequence (Fig 1).

200

201 **Fig 1. Comparison of the amino acid sequences of the AM and homologous**
202 **proteins.** Sequence alignment of the amino acid sequence of AM showing the high
203 homology with the AS amidase (3A1K_A) from *Rhodococcus* Sp. N771, the *Thermus*
204 *thermophilus* HB8 amidase (YP_145063.1), the *T. thermophilus* HB8
205 aspartyl/glutamyl-tRNA amidotransferase subunit A (YP_143839.1), and the
206 *Thermotoga maritima* MSB8 aspartyl/glutamyl-tRNA amidotransferase subunit A
207 (NP_229077.1). The alignment was produced by ClustalW. Multiple alignments were
208 generated with BioEdit. The *dark grey* under the sequence indicates the residues of
209 the Ser-Ser-Lys catalytic triad.

210

211 We demonstrated that this motif was the catalytic site through site-
212 directed mutagenesis (Table 1). Thus, we showed that AM of *S. arenicola*
213 CNS-205 contains the highly conserved catalytic triad Ser-Ser-Lys and is
214 a member of the AS family.

215 **Table 1. Oligonucleotides used for site-directed mutagenesis**

AM gene allele	Mutagenic primer sets
K84A	GTG CCG GTG GCG GTC <u>GCG</u> GAG AAC ACC GCT GTG G (K)
S158A	C CAC AGC GGT GTT CTC CGC GAC CGC CAC CGG CAC CGT ACC CCC GGT GGC <u>GCG</u> TCC GGT GGA TCG GCC G (S)

C GGC CGA TCC ACC GGA CGC GCC ACC GGG GGT
ACG
AAC GAC GGT CTC GGA **GCG** ATC CGG ATC CCG GCA
G
S182A (S)
C TGC CGG GAT CCG GAT CGC TCC GAG ACC GTC GTT

216 The boldface type indicates the mutated amino acids.

217 **Expression and purification of AM**

218 The fusion protein His₆-AM was overexpressed in *E. coli* BL21 (DE3).

219 The purity of the purified fusion protein was greater than 90%. The SDS-

220 PAGE results indicated that the molecular mass of the major band was

221 51.2 kDa (Fig 2), which conformed to mass of the deduced protein

222 sequence.

223

224 **Fig 2. SDS-PAGE of AM-1 and its mutants.** Lanes 1, protein molecular weight

225 marker; 2, wild-type AM-1; 3, K84A; 4, S158A; and 5, S182A

226

227 To obtain the molecular mass of the proteins, we analysed the His₆-AM

228 fusion protein and three mutants (K84A, S158A, and S182A) by HPLC-

229 ESI-HRMS. The molecular weights of the AM wild-type, K84A, S158A,

230 and S182A were 51.037, 50.980, 51.021, and 51.021 kDa, which were

231 consistent with the predicted values (Fig 3).

232

233 **Fig 3. HPLC-ESI-HRMS analysis of AM.** WT (a), K84A (b), S158A (c) and S182A
234 (d). Extra minor peaks marked by asterisks denote glycosylation (+178 Da) of the N-
235 terminal His₆-tag added during the expression of the recombinant protein in *E. coli*
236 (Geoghegan et al.1999)

237

238 **Effects of temperature and pH on AM activity and stability**

239 To determine the optimal temperature, we assessed the amidase activity at
240 a temperature range from 15 to 65 °C with benzamide as the substrate.
241 The AM activity peaked at 40 °C, and it showed an excessively wide
242 peak (Fig 4a). More than 50% of the residual activity was observed at
243 temperatures from 30 to 50 °C. Thermo stability tests indicated moderate
244 loss of amidase activity within 1 h up to approximately 45 °C (Fig 4b).
245 Only 9% of the activity was observed at 55 °C after 1 h, and the amidase
246 activity was lost completely after 1 h at 60 °C.

247 The optimal pH for AM was determined in the buffers described in the
248 Materials and methods. Figure 4c indicates that AM was highly active
249 between pH 7.5 and 8.5. AM showed low activity below pH 4.5 or above
250 pH 10.0. For the pH stability test, AM was preincubated for 1 h at
251 different pH values, and the results indicated that more than 60% residual
252 activity was observed between pH 4.5 and 10.0 (Fig 4d).

253

254 **Fig 4. Effects of pH and temperature on AM activity and stability. a**
255 Determination of the optimal pH value. The reactions were performed at 35 °C for 10
256 min in buffers with varying pH values. **b** pH stability. The assays were performed in
257 20 mM Tris-HCl, 100 mM NaCl buffer (pH 8.0) at 35 °C for 10 min after pretreatment
258 of the purified enzyme at 25 °C for 1 h in 0.1 M buffer (pH 4.0-10.0). **c** Determination
259 of the optimal temperature. The activity was measured in 20 mM Tris-HCl, 100 mM
260 NaCl, pH 8.0, at 15-65 °C for 10 min. **d** Thermal stability. The reactions were
261 performed under optimal conditions after incubation of AM at the indicated
262 temperature for 1 h.

263

264 **Impact of metal ions and other reagents**

265 The majority of the metal ions, including Ba²⁺, Ca²⁺, Zn²⁺, and Ni²⁺, in the
266 assays exerted no noticeable effect on the amidase activity of AM (Table
267 2).

268 **Table 2. Effects of metal ions and inhibitors on the amidase activity of AM**

Reagent (1 mM)	Relative activity (%)	Reagent (1 mM)	Relative activity (%)
No addition	100	PMSF	10.13 ± 0.60
Ba²⁺	94.30 ± 2.59	1,10-Phenanthroline	82.46 ± 0.73
Ca²⁺	88.60 ± 2.13	EDTA	77.64 ± 0.92
Zn²⁺	85.09 ± 3.87	SDS	51.78 ± 0.27
Ni²⁺	96.93 ± 1.90		

269 The activity was strongly inactivated by PMSF, which is an inhibitor of

270 serine hydrolases. However, the chelating agents EDTA and 1,10-
271 phenanthroline (10 mM) resulted in only a 20–30% inhibition of AM
272 hydrolysis, demonstrating that these chemicals did not chelate a possible
273 divalent cation(s) required for the activity of the enzyme. The surfactant
274 SDS showed a 48.22% inhibition of AM activity.

275 **Substrate spectrum**

276 To determine the substrate specificity of AM, we assessed whether the
277 purified AM could hydrolyse different aromatic and aliphatic amides. The
278 results showed that AM had high activity towards the aromatic and
279 aliphatic amides, including acetamide, propanamide, propanil,
280 benzeneacetamide and benzamide (Table 3).

281 **Table 3. Substrate spectrum of AM**

Substrate	Relative activity^a (%)	Substrate	Relative activity^a (%)
Acetamide	103.79 ± 0.46	Propanil	95.91 ± 0.42
Benzamide	100	Propanamide	106.36 ± 0.38
Benzeneacetami d	107.75 ± 0.48	Isobutyrami de	18.02 ± 0.72
Nicotinamide	56.42 ± 1.29	Acetochlor	0
Pyrazinamide	43.01 ± 2.76	Butachlor	0

282 ^a The activity with benzamide was defined as 100%. All measurements were performed in triplicate

283 The aromatic amides, including nicotinamide and pyrazinamide, with
284 substitutions of one or two carbons in the ring by a nitrogen, had a
285 negative impact on the activity. The anilide substrate range of AM was

286 very narrow, and the protein could not hydrolyse butachlor and
287 acetochlor. Only propanil was a good substrate for AM.

288 Kinetic parameters for AM were estimated by the Hanes-Woolf method.

289 The K_m values for acetamide and propionamide were 3.36 ± 0.17 mM and
290 3.33 ± 0.08 mM (Table 4).

291 **Table 4. Kinetic parameters for AM amidase reaction**

Substrate	K_m (mM)	V_{max} (mM min ⁻¹)	k_{cat} (s ⁻¹)	k_{cat}/K_m (mM ⁻¹ s ⁻¹)
Acetamide	3.36 ± 0.17	0.491 ± 0.001	63.89 ± 0.20	19.06 ± 0.94
Nicotinamide	6.20 ± 0.04	0.293 ± 0.006	38.19 ± 0.75	6.16 ± 0.12
Propionamide	3.33 ± 0.08	0.503 ± 0.001	65.54 ± 0.08	19.69 ± 0.43
Benzamide	3.49 ± 0.12	0.508 ± 0.005	66.15 ± 0.66	18.95 ± 0.72

292 ^a All substrates were tested at 25-200 mM, except for benzamide, which was insoluble above 50 mM

293 The anilide substrate range of AM was very narrow, and the protein could
294 not hydrolyse acetochlor and butachlor. Only propanil was a good
295 substrate for AM, but the K_m value could not be determined due to the
296 low solubility of the compound.

297 **Site-directed mutagenesis**

298 The potential catalytic active site residues of AM were replaced by the
299 QuikChange site-directed mutagenesis kit. The mutants were
300 overexpressed in *E. coli* BL21 (DE3) cells and further purified as
301 described above. The results indicated that the AM K84, S158 and S182
302 mutants had no activity with benzamide as a substrate. These results
303 suggest that AM is a member of the AS family and utilises the highly

304 conserved catalytic triad Ser-Ser-Lys.

305 **Discussion**

306 An isolate of *S. arenicola* CNS-205 identified by Fenical (Fenical et
307 al. 2006) and co-workers in 2006 encodes a putative amidase. Sequence
308 alignments of the primary AM sequence indicated that AM had a high
309 similarity with the AS family and showed that AM contains the central
310 GGSS signature, which is a typical characteristic of the AS family. The
311 point mutation results also indicated that no hydrolytic activity could be
312 detected in the K84A, S158A, and S182A mutants. These findings
313 indicated that AM belongs to the AS family.

314 The effects of different metal ions and chemical reagents on AM
315 activity were different. AM activity was affected by reducing agents, such
316 as PMSF, and the results revealed that serine was the active site of the
317 amidase. This result was consistent with the crucial role of Ser¹⁸³, as
318 revealed by site-directed mutagenesis experiments. A metal chelating
319 agent (EDTA) did not impact the activity, indicating that a possible
320 divalent cation(s) required for enzyme activity was not chelated by these
321 chemicals.

322 Analysis of the substrate specificity of AM showed that the enzyme had
323 high activity against short-chain aliphatic amide substrates (acetamide,
324 isobutyramide and propanamide), which are typical substrates of the AS
325 family. Interestingly, AM also hydrolyses ring amide substrates, such as

326 aromatic and heterocyclic amides. The hydrolytic product of nicotinamide
327 is nicotinic acid, a water-soluble B-complex vitamin, which has been
328 extensively applied in treatment of schizophrenia, autoimmune diseases,
329 hypercholesterolemia, diabetes and osteoarthritis. Benzoic acid, the
330 hydrolytic product of benzamide, has antifungal activity and is
331 extensively used as a preservative in production of processed and
332 convenience foods.

333 AM also had aryl acylamidase activity against aniline substrates,
334 including propanil (a commercial amide-containing pesticide), which was
335 hydrolysed to produce 3,4-dichloroaniline. However, acetochlor and
336 butachlor, which are structurally analogous to propanil, were not
337 substrates for AM, indicating the anilide substrate range of AM was very
338 narrow. Propanil, an acyl anilide herbicide, can contaminate the soil
339 environment, and AM, by attacking the amide bonds in propanil, can
340 reduce its concentrations in soil. Thus, AM may have potential
341 applications in bioremediation.

342 In addition to the amidase and aryl acylamidase activities, acyl
343 transferase activity is an important characteristic as it produces
344 hydroxamic acids (Fournand et al. 1998). In this study, AM from *S.*
345 *arenicola* CNS-205 had acyl transferase activity on anilide substrates,
346 including propanil. The extensive substrate specificity range and acyl

347 transferase activity indicate that AM has broad potential applications in
348 biosynthesis processes and biodegradation.

349 Overall, a new amidase gene, AM, was cloned from *S. arenicola*
350 CNS-205, and the amidase, aryl acylamidase, and acyl transferase
351 activities of the enzyme were verified. These activities indicate that AM
352 has a broad substrate spectrum. AM may be a potential agent for
353 environmental remediation and for the biosynthesis of novel amides by
354 virtue of these characteristics, as well as the broad pH tolerance of the
355 enzyme.

356 **References**

- 357 Asano Y, Tachibana M, Tani Y, Yamada H, Purification and
358 characterization of amidase which participates in nitrile degradation.
359 Agric Biol Chem. 1982; 46:1175–1181.
- 360 Chebrou H, Bigey F, Arnaud A, Galzy P, Study of the amidase signature
361 group. Biochim Biophys Acta. 1996; 1298:285–293.
- 362 Fournand D, Arnaud A, Aliphatic and enantioselective amidases: from
363 hydrolysis to acyl transfer activity. J Appl Microbiol. 2001; 91:381–393.
- 364 Fournand D, Bigey F, Arnaud A, Acyl transfer activity of an amidase
365 from *Rhodococcus* sp. strain R312: formation of a wide range of
366 hydroxamic acids. Appl Environ Microbiol. 1998; 64: 2844-2852.
- 367 Geoghegan KF, Dixon HB, Rosner PJ, Hoth LR, Lanzetti AJ, Borzilleri
368 KA, Marr ES, Pezzullo LH, Martin LB, LeMotte PK, McColl AS,

369 Kamath AV, Stroh JG, Spontaneous alpha-N-6-phosphogluconoylation of
370 a "His tag" in *Escherichia coli*: the cause of extra mass of 258 or 178 Da
371 in fusion proteins. *Anal Biochem.* 1999; 267: 169-184.

372 Mayaux J, Cerebelaud E, Soubrier F, Faucher D, Petre D, Purification,
373 cloning, and primary structure of an enantiomeraselective amidase from
374 *Brevibacterium* sp. strain R312: structural evidence for genetic coupling
375 with nitrile hydratase. *J Bacteriol.* 1990; 172:6764–6773.

376 Neu D, Lehmann T, Elleuche S, Pollmann S, Arabidopsis amidase, a
377 member of the amidase signature family. *FEBS J.* 2007; 274(13):3440–
378 3451.

379 Ohtaki A, Murata K, Sato Y, Noguchi K, Miyatake H, Dohmae N,
380 Yamada K, Yohda M, Odaka M, Structure and characterization of
381 amidase from *Rhodococcus* sp. N-771: insight into the molecular
382 mechanism of substrate recognition. *Biochim Biophys Acta.* 2010;
383 1804(1):184–192.

384 Politi L, Chiancone E, Giangiacomo L, Cervoni L, d’Abusco AS,
385 Scorsino S, Scandurra R, pH-, temperature- and iondependent
386 oligomerization of *Sulfolobus solfataricus* recombinant amidase: a study
387 with site-specific mutants. *Archaea.* 2009; 2:221–231.

388 d’Abusco AS, Ammendola S, Scandurra R, Politi L, Molecular and
389 biochemical characterization of the amidase from hyperthermophilic
390 archaeon. *Sulfolobus solfataricus*. *Extremophiles.* 2001; 5:183–192.

391 Galadari S, Wu BX, Mao C, Roddy P, El Bawab S, Hannun YA,
392 Identification of a novel amidase motif in neutral ceramidase. *Biochem J.*
393 2006; 393(3):687–695.

394 Weatherburn MW, Phenol-hypochlorite reaction for determination of
395 ammonia. *Anal Chem.* 1967; 39:971–974.

396 Fenical W, Jensen PR, Developing a new resource for drug discovery:
397 marine actinomycete bacteria. *Nat Chem Biol.* 2006; 2: 666–673.

398 Shen W, Chen H, Jia K, Ni J, Yan X, Li S, Cloning and characterization
399 of a novel amidase from *Paracoccus* sp. M-1, showing aryl acylamidase
400 and acyl transferase activities. *Appl Microbiol Biotechnol.* 2012;
401 94:1007–1018.

<i>Salinispora arenicola</i> CNS-205	73	- - - - - L P L A G V P V A V K E N T A V A G L P T W N G S A A A R S P V A	108
<i>Rhodococcus</i> Sp. N771	79	S I P P T S D G V L T G R R V A I K D N V T V A G V P M M N G S R T V E G F T P	118
<i>Thermus thermophilus</i> HB8	63	- - - - - P L H G L P L T V K D L F P V K G M P T R A G T K A P L P P L P	95
<i>Thermus thermophilus</i> HB8	57	- - - - - L A G L V V A V K D N I A T R G L R T T A G S R L L E N F V P	88
<i>Thermotoga maritima</i> MSB8	57	- - - - - F W G I P V A I K D N I L T L G M R T T C A S R I L E N Y E S	88

<i>Salinispora arenicola</i> CNS-205	107	E A D H E V V R R L R G A G A V I L G V T R M P E L G L W G T T D D - A T A V T	145
<i>Rhodococcus</i> Sp. N771	119	S R D A T V V T R L L A A G A T V A G K A V C E D L C F S G S S F T P A S G P V	158
<i>Thermus thermophilus</i> HB8	95	- E E A R A V R R L R E A G A L L F A K T N M H E I A L G I T G E N P W T G P V	134
<i>Thermus thermophilus</i> HB8	89	P Y E A T A V A R L K A L G A L V L G K T N L D E F G M G S S T E H S A F F P T	128
<i>Thermotoga maritima</i> MSB8	89	V F D A T V V K K M K E A G F V V V G K A N L D E F A M G S S T E R S A F F P T	128

<i>Salinispora arenicola</i> CNS-205	146	R N P W E L G R T P G G S S G G S A A A V A A G L V P I A H A N D G L G S I R I	185
<i>Rhodococcus</i> Sp. N771	159	R N P W D R Q R E A G G S S G G S A A L V A N G D V D F A I G G D Q G G S I R I	198
<i>Thermus thermophilus</i> HB8	135	R N A V D P S R Q A G G S S G G S A V A V A L G I G L A S L G T D T G G S I R I	174
<i>Thermus thermophilus</i> HB8	129	K N P F D P D R V P G G S S G G S A A A L A A D L A P L A L G S D T G G S V R Q	168
<i>Thermotoga maritima</i> MSB8	129	R N P W D L E R V P G G S S G G S A A A V S A G M V V A A L G S D T G G S V R Q	168



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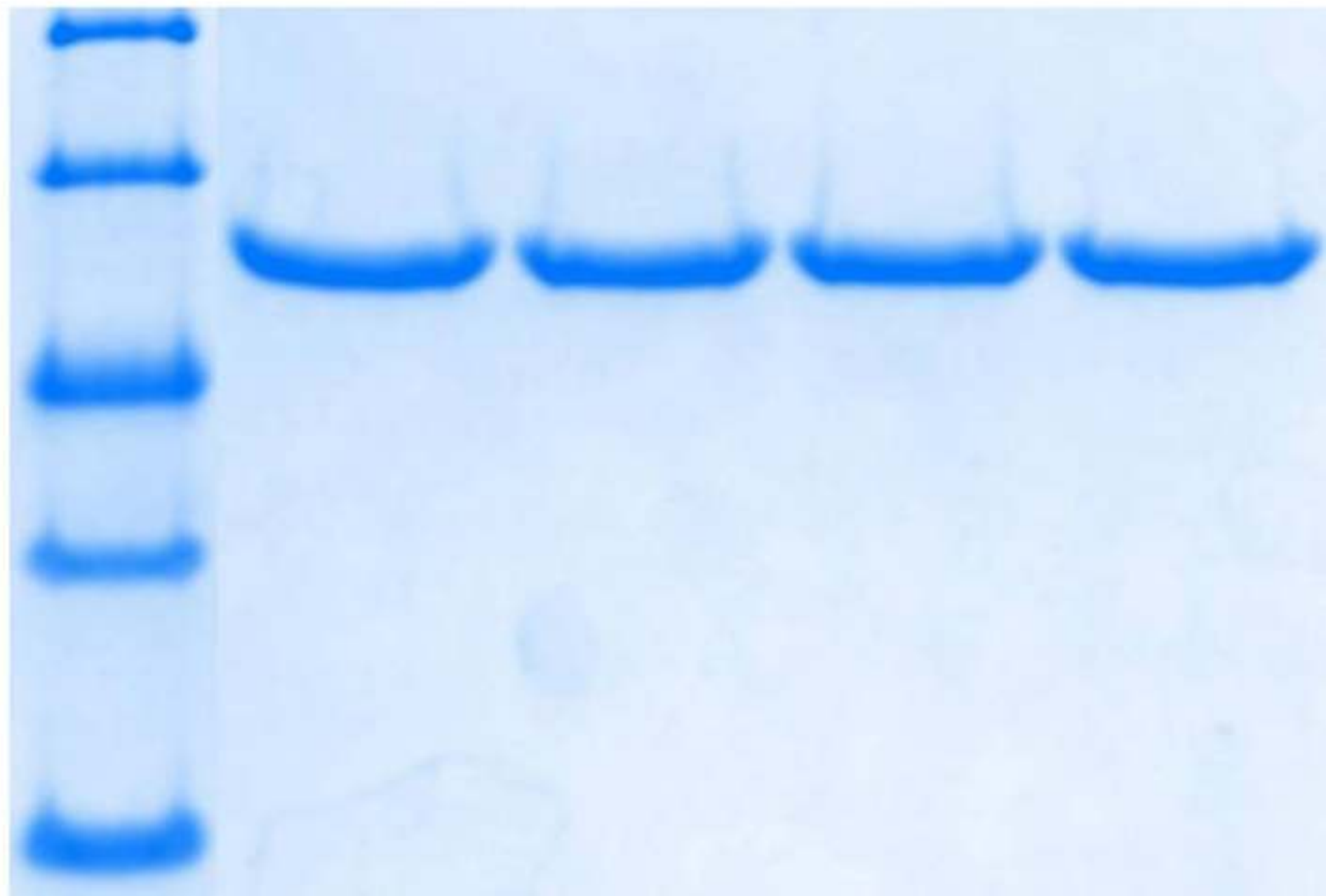
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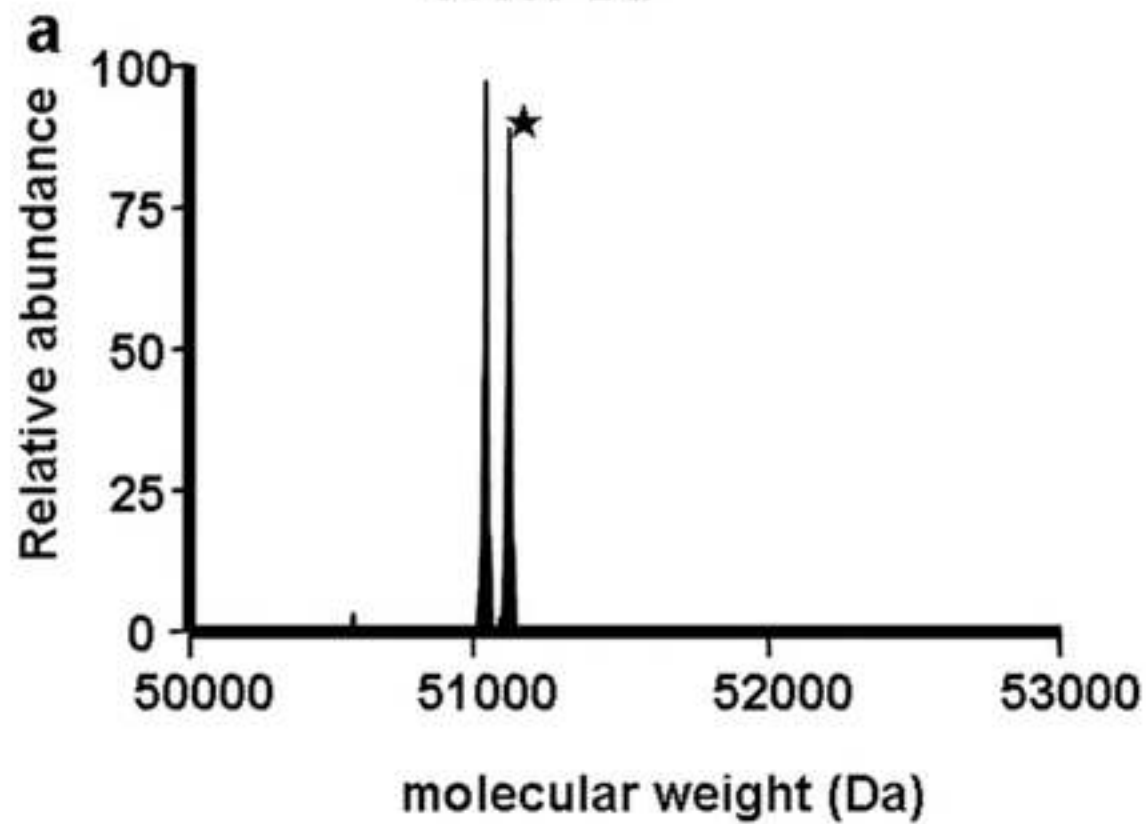
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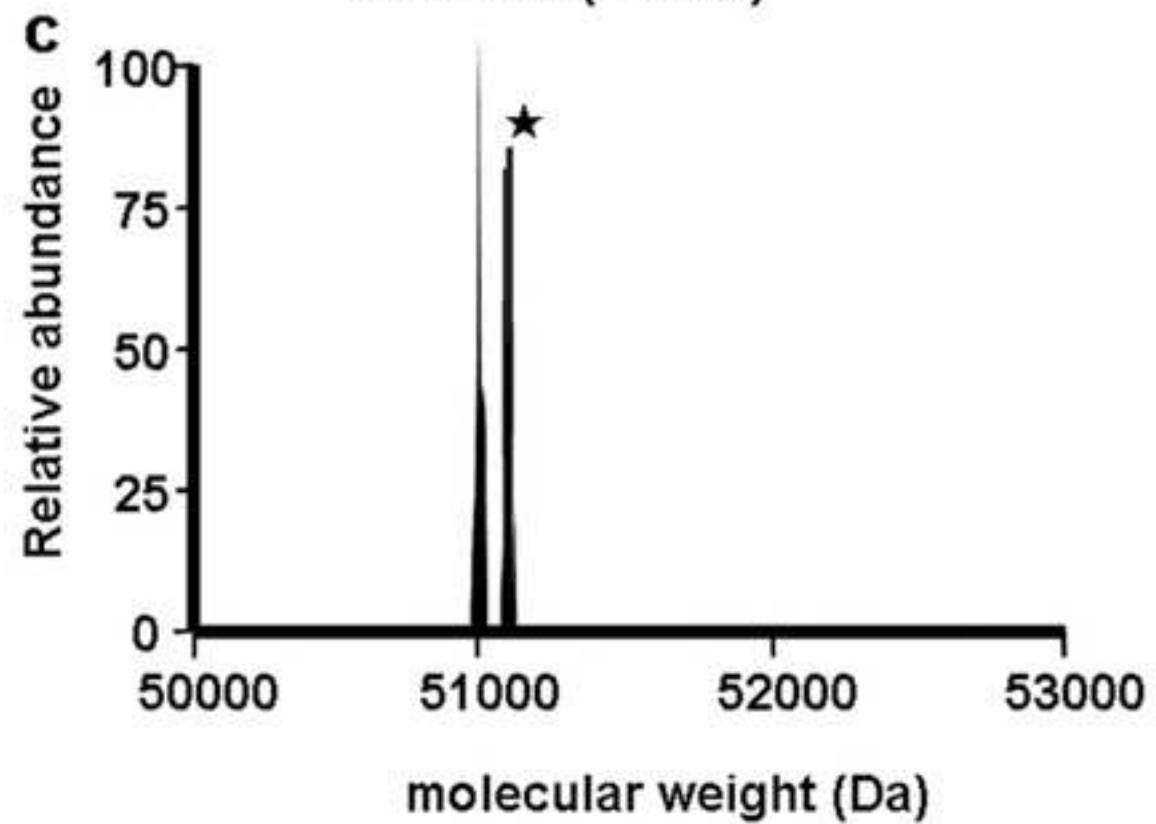
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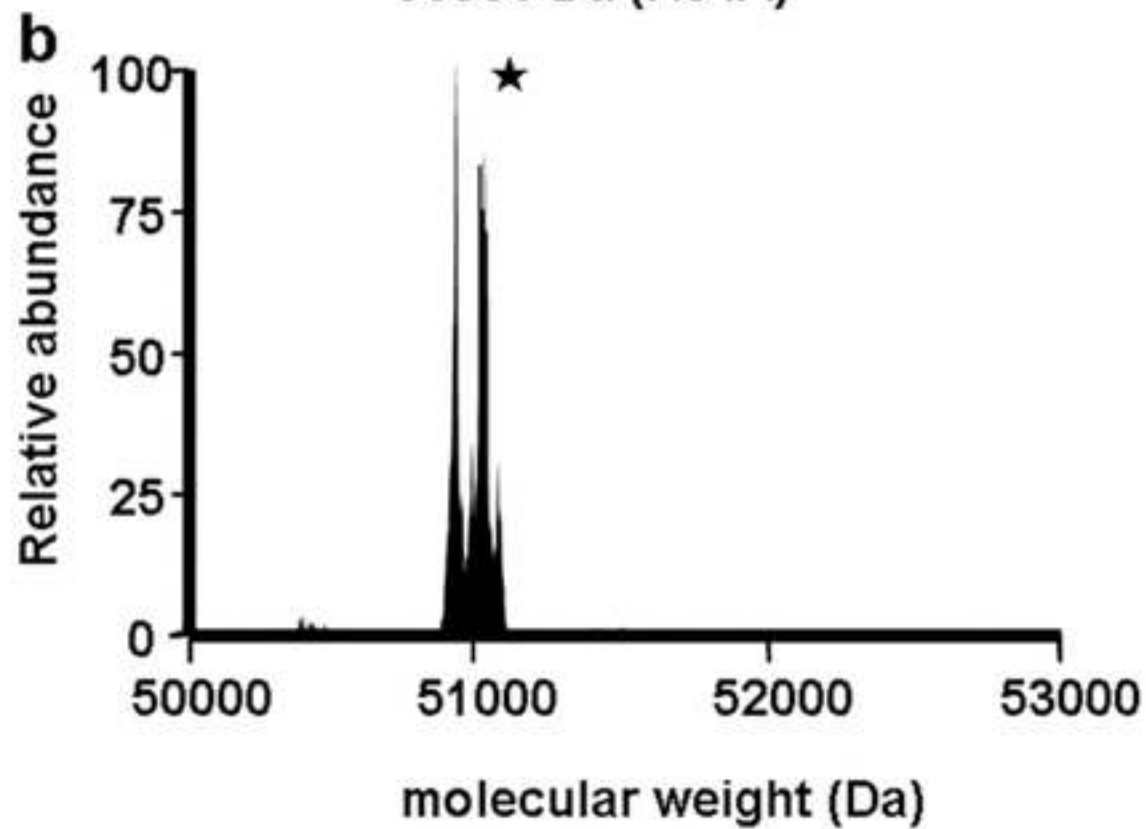
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