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The final acylation step in aromatic dithiolopyrrolone biosyntheses: Identification and characterization of the first bacterium *N*-benzoyltransferase from *Saccharothrix algeriensis* NRRL B-24137

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A B S T R A C T

The last step in the biosynthesis of dithiolopyrrolone antibiotics was thought to involve the transfer of acyl group from acyl-CoA to pyrrothine/holothin core. In *Saccharothrix algeriensis* NRRL B-24137, two acyltransferases, an acetyltransferase and a benzoyltransferase were proposed to catalyze this step. We have previously identified, in *Sa. algeriensis* genome, two open read frames, *actA* and *actB* patiently encoded these enzymes. This study focuses primarily on the characterization of the protein encoded by *actA*. After cloning and expressing of *actA* in *Escherichia coli* BL21, the recombinant protein encoded by *actA* was purified. Selectivity of ActA for pyrrothine/holothin as substrate and different acyl-CoA as co-substrate was evaluated using two acyls-groups, linear and aromatic. The enzyme was shown to prefer aromatic groups over linear groups as donor group; further neither product nor transfer was observed for linear groups. Therefore ActA has been determined to be a pyrrothine/holothin *N*-benzoyltransferase which can either pyrrothine (K_m of 72 μM) or holothin (K_m of 129.5 μM) as substrates and benzoyl-CoA (K_m of 348.65 and 395.28 μM) as co-substrates for pyrrothine and holothin, respectively. The optimum pH and temperature has been shown to be 8, 40 °C, respectively. ActA is the first enzyme characterized as *N*-benzoyltransferase in bacteria.

1. Introduction

Dithiolopyrrolone (DTP) group antibiotics are characterized by an electronically unique bicyclic structure, which contains a compact disulfide bridge between two ene-thiols [1]. Points of diversity within the compound class occur outside of the bicyclic core, at the two amide nitrogens (Fig. 1a). An activity acyltransferase in the dithiolopyrrolones pathway was reported to be responsible of this diversity. This enzyme utilizes CoA substrates of different chain lengths to generate novel DTPs with various acyl groups [2]. *Saccharothrix algeriensis* NRRL B-24137 produces at least six pyrrothine derivatives characterized by their different *N*-acyl groups such as thiolutin (acetyl-pyrrothine), senecioyl-pyrrothine (SEP), tigloyl-pyrrothine (TIP), isobutyrylpyrrothine (ISP), butanoyl-pyrrothine (BUP) and benzoyl-pyrrothine (BEP) [3,4] (Fig. 1b). Precursors supply has been successfully applied to induce both the dithiolopyrrolones which have already been produced and new dithiolopyrrolones derivatives production for example: cystine and benzoic acid increase the production of thiolutin, benzoyl-pyrrothine (BEP) respectively, while valeric acid and sorbic acid induce valeryl-pyrrothin and sorbyl-pyrrothin, respectively [5–9] (Fig. 1c). Recently, different dithiolopyrrolones were also produced *in vitro* using cell-free extract of *Sa. algeriensis* which have never been produced *in vivo* [10] (Fig. 1d). A pyrrothine *N*-acyltransferase activity in *Sa. algeriensis* was proposed to be responsible for the transfer of the acyl groups from the acyl-CoA to pyrrothine core leading to different dithiolopyrrolone derivatives [11]. Similar acetyltransferase activity called holomycin synthase, in crude extract of *Streptomyces clavuligerus*, had already been reported to be responsible, *in vitro*, for holomycine formation [12]. Recently, three DTP gene clusters were identified in three different bacteria including *S. clavuligerus* [2], *Yersinia ruckeri* [13] and *Pseudoalteromonas* sp SANK 73390 [14]. In addition to the holomycine, *Pseudoalteromonas* produces several linear holothin derivatives, but neither aromatic holothin derivatives nor

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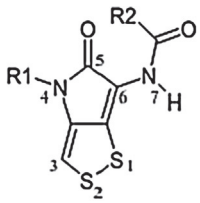

Fig. 1. General structures of dithiolopyrrolones produced *in vivo* and *in vitro*.

pyrrothine derivatives had been yet produced by these bacteria. The three clusters contain only one acetyltransferase, and sole the activity of acyltransferase HlmA for the holomycin pathway in *S. clavuligerus* was reconstituted *in vitro* [2]. This enzyme utilizes CoA substrates of different chain lengths to generate novel DTPs with various acyl groups including isovaleryl-, hexanoyl, octanoyl-, palmitoyl-, oleoyl-, and 12-hydroxy-stearoyl DTPs demonstrating the power of engineered biosynthesis with purified enzymes [2]. This enzyme displays somewhat relaxed substrate specificity and can accommodate branched, unsaturated and even hydroxylated acyl-CoAs with a preference for short chain CoAs. It does not seem to utilize any aromatic CoA variant such as benzoyl or sinapoyl-CoA [2]. Recently, the study of pyrrothine *N*-acyltransferase activities in the cell-free extract of *Sa. algeriensis* NRRL B-24137 in particular, the transfer of acetyl and benzoyl groups on pyrrothine core gave thiolutin and BEP, respectively suggested the presence of two different enzymes [10]. More recently, we have proposed two ORFs, *actA* and *actB* to be involved in dithiolopyrrolones biosynthesis in *Sa. algeriensis* [15]. Furthermore, we have tried to reveal the role of benzoic acid on the production of BEP. A hypothesis was based on the transcription analysis of *actA* and *actB* with and without benzoic acid. The effect of benzoic acid addition is to supply the precursor for BEP production, and less for gene induction.

Here, we characterized the protein encoded by *actA*. This characterization allows the confirmation of our hypotheses; firstly the involvement of ActA in the formation of aromatic dithiolopyrrolones and this in turn confirms the hypothesis of the presence of, at least, two *N*-acyltransferases in *Sa. algeriensis*. Secondly, it leads to clarify the role of benzoic acid as precursor supply for BEP production.

2. Materials and methods

2.1. Cloning

The sequences of the forward and reverse primers used to amplify *actA* (NCBI Accession No.: KF719091.1) were: 5′GGTATTGAGGGTCGCGTGA CCACGACGGACGTGAACC-3′ and 5′-AGAGGAGAGTTAGAGCCTCAGCCCAGCTCCAGG-3′ (The ligation-independent cloning (LIC) extension is underlined in each primer). Amplification was done using genomic DNA from *Sa. algeriensis* as template. The PCR was performed with Hot Star Hifidelity Polymerase kit (Qiagen), one cycle at 94 °C for 4 min for initial denaturation; 30 cycles at 94 °C, 45 s; 64 °C, 1 min; 72 °C, 1 min for amplification, and a final elongation cycle at 72 °C for 5 min. PCR products were purified from 0.8% agarose gels with QIAquick Gel Extraction kit from Qiagen. The purified fragment was cloned into pET30-Xa/LIC vector (Novagen). The PCR fidelity was verified by DNA sequencing. The obtained vector was introduced into *Escherichia coli* BL21(DE3) chemical competent cells (Novagen).

2.2. Optimization of over-expression conditions for soluble protein expression

We tested our construction system to optimize the expression condition for improving soluble expression with high production yield of recombinant protein. *E. coli* BL21(DE3) containing the plasmid pET-30Xa/LIC/*actA* was grown at 37 °C until OD_{600} reached 0.6. Protein expression was induced at different conditions. The conditions were a combination of three different temperatures (15, 25 and 37 °C) and three different IPTG concentrations (100, 500 and 1000 μM). For small scale, free-cell extract was prepared as follows, the biomass of 25 mL was obtained after centrifugation and resuspended in 1 mL of lysis buffer (Tris–HCl 50 mM pH 8), then it was transferred to a Fast Protein Blue tube (MP Biomedicals, Irvine, CA, USA). Two disruption cycles (30 s, 6 m s^{-1}) were carried out in a Fast Prep disruptor (MP Biomedicals, California, USA). The lysing matrix was discarded and then the sample was centrifuged at 10,000 × *g* for 10 min to remove the cell debris. The supernatant constituting the soluble cell-free extract was used immediately for the assay of pyrrothine N-acyltransferase activity. A sample was frozen at −80 °C for further protein assays. Samples were always kept in ice and centrifugations were carried out at 4 °C. The cell-free extract was evaluated using SDS–PAGE and acyltransferase activities to determine the effects of the temperature and IPTG concentration on both productivity and solubility of the protein. Enzyme assays were also carried out with protein identically prepared from empty vector controls.

2.3. Purification of the over-expressed protein

The over-expression involved inoculating a 1 L LB broth with 10–15 mL of preculture (OD_{600} 0.6) started from 200 μl of glycerol stocks and inducing protein over-expression at an OD_{600nm} of 0.6–0.8 with appropriate temperature and IPTG concentration. After, the cells were harvested by centrifugation; cell lysis was carried out in Tris–HCL 50 mM and pH 8 using a sonicator "BRANSON Sonifier Digital" with amplitude of 35%, a 10-s pulse and an interval of 20 s for 30 min. After clarification of the lysis mixture by centrifugation, the supernatant was ammonium sulfate precipitated as follows; the crude extract was successively saturated with ammonium sulfate, and three protein fractions were obtained: 0–40%, 40–60 and the supernatant. The precipitates were recovered in pellet form by centrifugation for 20 min at 5000 x *g* at 4 °C. The protein pellet was then dissolved in 10 mL of binding buffer (200 mM NaCl, 20 mM Tris–HCl, 5 mM imidazole, pH 7.9). Then the purification was continued using His·Bind Purification Kit from Novagen. Briefly, the fraction which presented the highest specific acyltransferase activity was incubated with 2–5 mL of Nickel-NTA agarose resin (Novagen) at 4 °C with gentle mixing for 1 h, and then loaded onto a column to drain the flowthrough. The resin bound protein was washed with 20 mL of binding buffer, followed by 12 mL of washing buffer (500 mM NaCl, 60 mM imidazole, 20 mM Tris–HCl, pH 7.9), and eluted with 10 mL of elution buffer (500 mM imidazole, 250 mM NaCl, 10 mM Tris–HCl, pH 7.9). The eluted protein was concentrated to 2.5–5 mL with a 15-mL Amicon (Millipore) with the appropriate molecular weight cut-off. Removal of the imidazole from concentrated protein was achieved by passing the protein through a PD-10 desalting column (GE Healthcare) twice. The desalted protein was flash frozen with liquid N_2, and stored at −80 °C.

2.4. Dithiolopyrrolones detection and quantification

Dithiolopyrrolones were quantified by HPLC as described by the published literature [10]. The identification of the formed products '*i.e.* dithiolopyrrolone derivatives' throughout enzymatic synthesis was determined as follows: 10 μL of reaction mixture was injected after filtration using 0.2 μm membrane filters (Advantec, Dublin, Ireland) in LC–MS instrument (Agilent system 1100). All analyzes are performed on a reverse phase column Prontosil 120-5 C18-SH, 150 mm × 4.6 mm (Bischoff Chromatography, Leonberg, Germany). The ionization is performed by electrospray. Then the mass spectrum is determined with a Q TRAP device (Applied Biosystems) with a triple quadrupole.

2.5. Enzyme essays

The enzymatic activities were quantified as described by the published literature [10]. Enzymatic activity was identified as benzoyltransferase activity. An enzyme unit is defined as the enzyme activity producing 1 μmol of BEP per minute. Specific enzymatic activity was expressed in mU mg^{-1} of protein. The proteins were determined by the method of Bradford.

For all essays, control reaction without enzyme was achieved in order to verify no transformation of pyrrothine or holothin to the related products whatever the chosen temperature for 10 min.

2.5.1. Substrate specificity and kinetic evaluation

Different acyl groups were used to establish the substrate selectivity of the enzyme. All acyl-CoA used in this study were purchased from Sigma Aldrich. The pyrrothine and holothin are chemically synthesized and verified in our laboratory by the published literature [11]. Two acyls-groups were tested, aromatic group (i.e. benzoyl-CoA and phenylacetyl-CoA) and linear groups with different chain length (i.e. acetyl, butyryl, crotonoyl, β-hydroxybutyryl, acetoacetyl, hexanoyl and lauroyl-CoA). The two groups were tested with pyrrothine and holothin as substrate; finally the formed products were analyzed by LC/MS. Activity assays were performed as described previously at the optimal pH and temperature of the enzyme.

Kinetic assays were performed under linear reaction conditions of protein concentration for 10 min at pH 8, by varying the concentration of pyrrothine and holothin (0–250 μM) with benzoyl-CoA held at saturation (500 μM). The reverse condition was also evaluated by varying the concentration of benzoyl-CoA (0–500 μM) with pyrrothine and holothin as co-substrate held at saturation (250 μM). Replicate kinetic assays, with three independent enzyme preparations, were then carried out. The resulting data sets were evaluated as double-reciprocal plots *via* the program Enzyme Kinetics Pro (ChemSW, Fairfield, CA), using direct linear plots for comparison, and the lines of best fit were determined. All data are reported as the averages of triplicate, independent assay sets, with an SE of less than ±11% of the mean.

2.5.2. Effect of pH and temperature on enzyme activity and stability

Optimum pH was determined in reaction mixture containing HCl–glycine for pH from 4 to 5, Tris–HCl buffer for pH from 5 to 8 and NaOH–glycine for pH from 8 to 10. All buffers were at 50 mM. The buffers are adjusted to the desired pH at room temperature. The assays were performed at 30 °C and buffers were pre-incubated at 30 °C. To determine the enzyme stability upon storage at different pH, aliquots of the enzyme were incubated for 24 h at 4 °C with 50 mM buffers ranging from pH 4 to 10. Residual activity was then measured at standard conditions (*T* 30 °C and pH 8) and compared to the reference sample to calculate relative activity. Optimal temperature was determined using pyrrothine as a substrate and at the optimal pH established for the enzymes. The assays were done at temperatures ranging from 20 to 60 °C. Buffers were pre-incubated for at least 5 min at the temperature of the assay before initiating the reaction. The stability of the enzymes upon storage at various temperatures at pH 8 for a period of 30 min was determined. The samples were allowed to temper to room temperature before enzyme activity was determined at standard conditions. Residual activity was then measured and compared to the reference sample to calculate relative activity.

3. Results and discussion

3.1. Optimization of over-expression conditions and enzyme purification

After cultivation of *E. coli* BL21 (DE3) (pET 30Xa-LIC/*actA*), the cell lysate was analyzed by SDS–PAGE with Coomassie brilliant blue staining. There was a protein band appeared on the gel with molecular weight in agreement with the expected size of 40 kD for ActA (including 2.5 kD of His-tag, 6 kD originating from the pET 30Xa-LIC vector) (Fig. 2). The optimization of over-expression conditions was carried out using acetyltransferase and benzoyltransferase specific activities. For all conditions, no acetyltransferase activity was detected, otherwise benzoyltransferase activity was detected in all conditions and notably at 15 °C and 100 μM IPTG. Although a band for the recombinant protein was also observed in the samples at 1000 μM, benzoyltransferase specific activity was lower than the samples obtained from different concentrations (Table 1). As judged by the expression efficiency, 15 °C and 100 μM were the best conditions for ActA expression. No detectable benzoyltransferase activity was observed for enzyme assays carried out with protein identically prepared from empty vector controls.

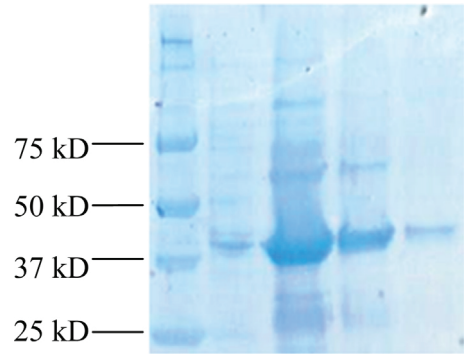

Fig. 2. SDS–PAGE of different steps of enzyme purification.

Table 1
Benzoyltransferase specific activity (mU mg^{-1}) for ActA according to IPTG concentration and incubation temperature.

Temperature (°C)	IPTG Concentration (mM)		
	100	500	1000
15	51.9	40.2	36.4
25	17.7	15	11.3
37	7.1	5.2	4.7

The recombinant enzyme was purified from the crude extract of transformed *E. coli* strain BL21 as an N-terminally His-tagged soluble protein to apparent homogeneity in two steps with a purification factor of 16.4 and yield of 46.5% (Table 2), and it was ~80% pure as estimated by SDS–PAGE.

3.2. Substrate specificity

In addition to BEP, *Sa. algeriensis* produces several closely related *N*-acylated pyrrothine and holothin derivatives (see Fig. 1). The crude extract of *Sa. algeriensis* had already shown the capacity to transfer the linear and aromatic acyl groups from acyl-CoA to the pyrrothine and the holothin core to produce the related products [10]. Therefore, it was of interest to determine whether one or more *N*-acyltransferases are responsible for the biosynthesis of these related products as previous results with the *N*-benzoyltransferase had eliminated only acetyl-CoA as a possible acyl donor with this enzyme. To realize this test, we incubated separately the previously mentioned acyl-CoAs, using pyrrothine and holothin as substrates under optimal, and saturating, conditions. Under these conditions, the recombinant enzyme did not detectably *N*-transfer from any linear group neither with pyrrothine nor with holothin. Otherwise, for aromatic groups, we have obtained, as expected, the benzoyl-pyrrothine (BEP) and the benzoyl-holothin (BEH). Their spectra and masses were confirmed by HPLC (Fig. 3) and by LC/MS analysis (Fig. 4). Furthermore, unknown compounds were produced using phenylacetyl-CoA either with pyrrothine and holothin, their HPLC retention times close to these of BEP and BEH respectively but not coincident with any known retention of all dithiolopyrrolone derivatives reported in the literature (data not show). These compounds might be the product of pyrrothine and holothin with phenylacetyl-CoA by the recombinant transferase, a reaction

Table 2
Summary of the results of the purification of recombinant ActA.

Purification step	Protein (mg)	Total activity (mU)	Specific activity (mU mg^{-1})	Yield (%)	Purification (fold)
Cell-free extract	160.32	10,547	65.78	100	1
40–60% ammonium sulfate	60.6	6538.6	107.8	62	1.63
Ni-NTA affinity chromatography	2.76	4907.52	1778	46.53	16.49

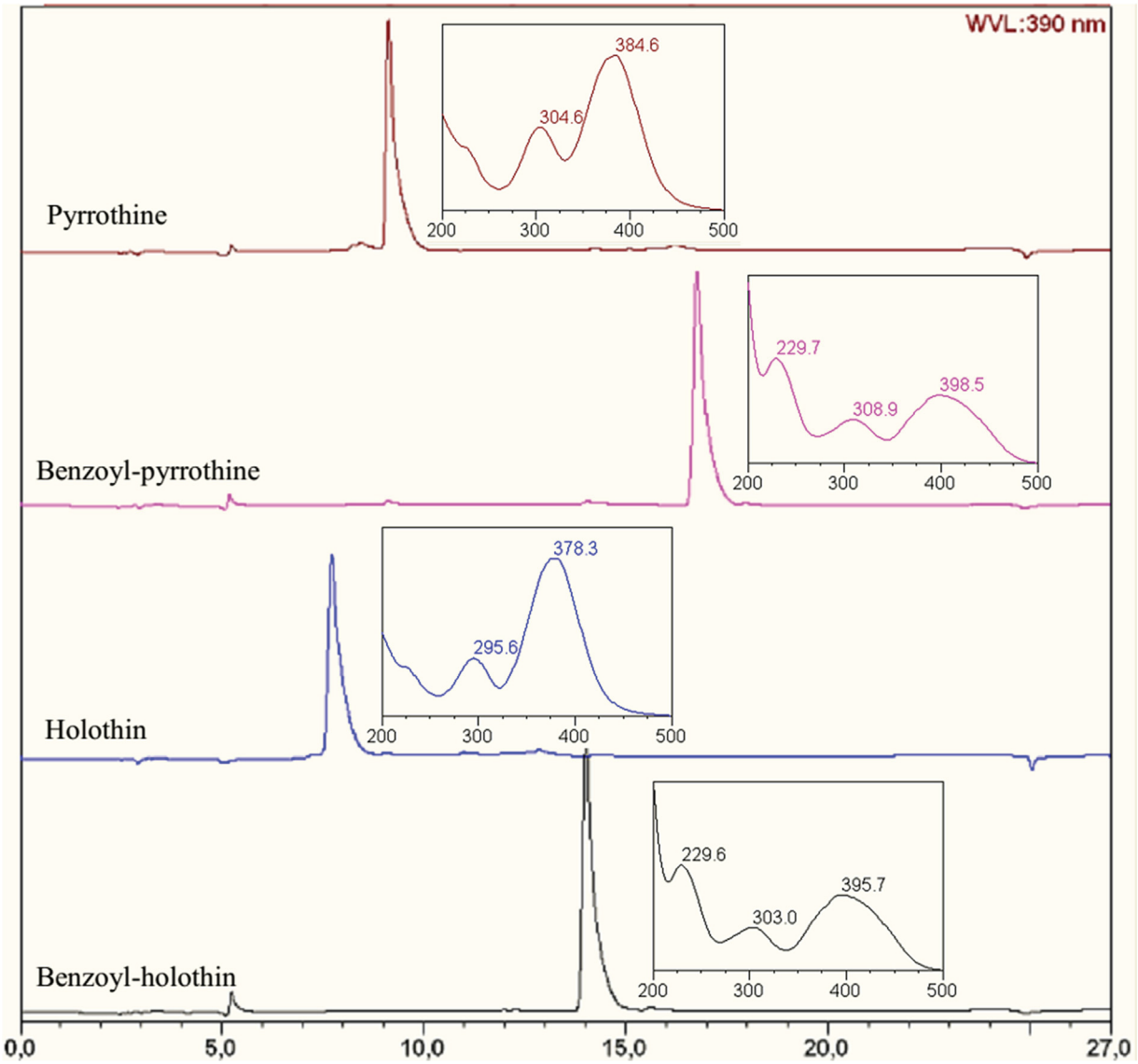

Fig. 3. Conversion of pyrrothine to benzoyl-pyrrothine and holothin to benzoyl-holothine by purified ActA with benzoyl-CoA as co-substrates.

seemingly not observed *in vivo*. The mass spectrometry analyses revealed that their calculated (cald.) and observed (obsd) masses for $[M+H]^+$ are as follows: phenylacetyl-pyrrothine $C_{14}H_{12}N_2O_2S_2$, cald. 304.38, absd. 305.30, phenylacetyl-holothin $C_{13}H_{10}N_2O_2S_2$, cald. 290.36 absd. 271.00 (Fig. 4c and d). These results confirm the proposed structure for these products. LC/MS analysis of these derivatives was suggestive of such a dithiolopyrrolones, but insufficient material was available to permit more identification.

Finally, if it is assumed that these *in vitro* results reflect the *in vivo* specificity of this enzyme, then these data indicate that the ActA which catalyzing the biosynthesis of aromatic dithiolopyrrolones is specific for aromatic-CoAs, and clearly cannot be responsible for the biosynthesis of the related *N*-acylated pyrrothine. The only *N*-benzoyltransferase reported in the litterateurs is TAX10 which proposed to be responsible for the last step of Taxol biosynthesis [16], it showed similar specificity toward its substrate, and it does not seem to utilize any linear CoA variant which confirm the high specificity of this family of enzyme.

3.3. Enzymatic kinetic parameters

The kinetic model of the global enzymatic activity could be expressed using the simple Lineweaver–Burk plot. The resulted plot has a slope equal K_m/V_{max} and an intercept equal $1/V_{max}$. With benzoyl-CoA as variable co-substrate 0–500 μM (pyrrothine and holothin at 250 μM), a K_m apparent value

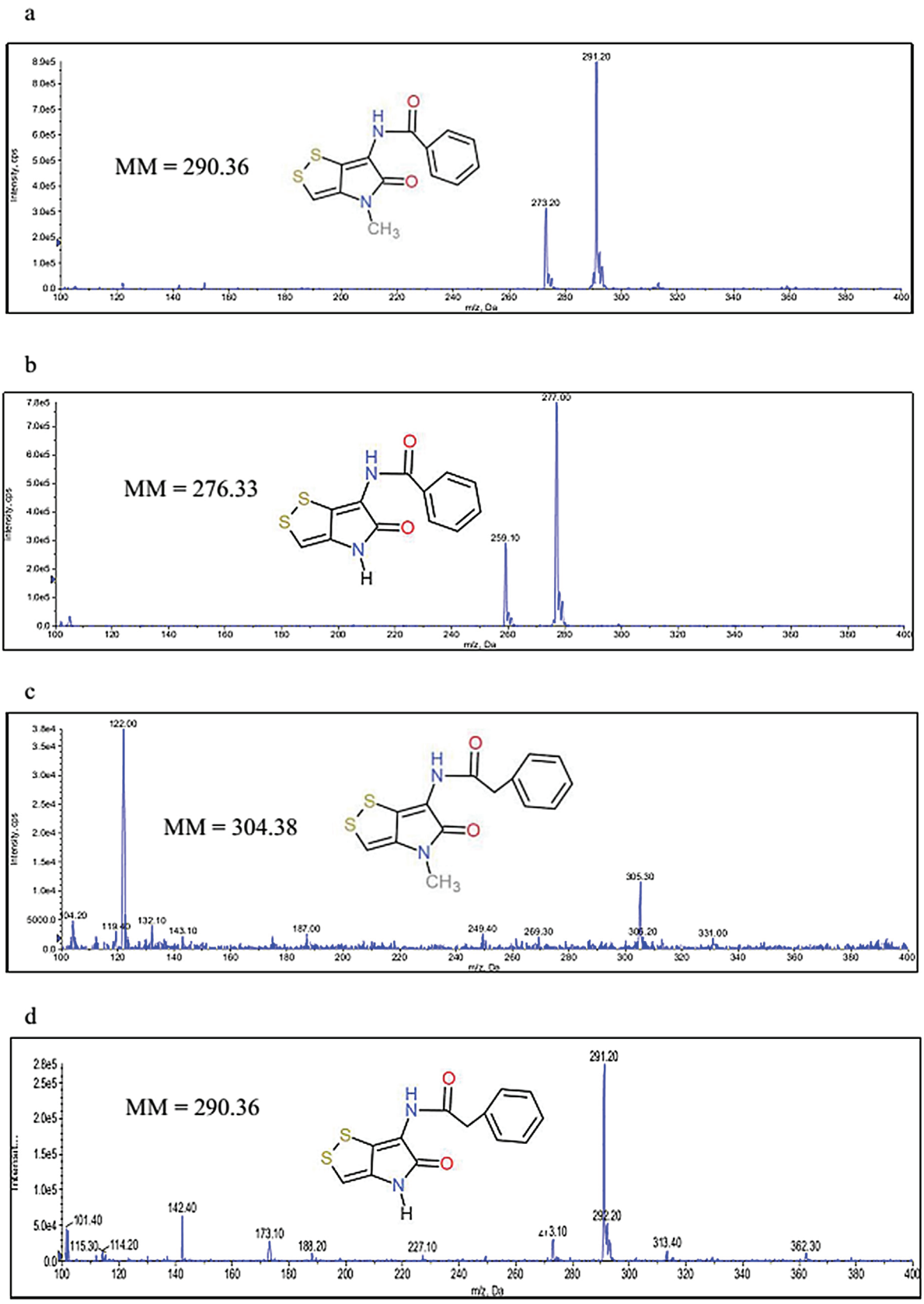

Fig. 4. Structures and mass spectra of dithiolopyrrolones derivatives synthesized with ActA.

Table 3
Enzymatic data of the recombinant ActA.

Limited substrate	Co-substrate	V_{max} (μml min^{-1})	K_m (μM)	K_{cat} (min^{-1})	K_{cat}/K_m (min^{-1} μM^{-1})
Pyrrothine	Benzoyl-CoA	4.65 ± 0.34	72 ± 6	232.5 ± 17	3.22
Holothin	Benzoyl-CoA	3.33 ± 0.19	129.5 ± 17	165.5 ± 9	1.27
Benzoyl-CoA	Pyrrothine	7.69 ± 0.89	348.65 ± 63	384.5 ± 44	1.1
Benzoyl-CoA	Holothin	8.4 ± 1.09	395.28 ± 47	420 ± 54	1.06

of 348.65 ± 63 μM and a V_{max} value of 7.69 ± 0.89 μmol min^{-1} (V/K_m = 0.022), and a K_m apparent value of 395.28 ± 47 and a V_{max} value of 8.4 ± 1.09 μmol min^{-1} (V/K_m = 0.021) were determined for pyrrothine and holothin, respectively (Table 3). K_m values are close to those previously reported for recombinant enzyme TAX10 (K_m = 529 ± 59 and 375 ± 67 using benzoyl-CoA as variable substrate and *N*-debenzoyl-2′-deoxytaxol *N*-debenzoyltaxol respectively as co-substrates [16]. These values are more important of those found for holomycin synthase in *S. clavuligerus* [2]. For example, at a 20 μM of holothin, an apparent K_m of 6, 15 and 30 μM were determined for acetyl, propionyl and octanoyl-CoA respectively.

In the case of pyrrothine as the variable substrate (benzoyl-CoA at 500 μM), a K_m value of 72 ± 6 μM and a V_{max} value of 4.65 ± 0.34 μmol min^{-1} (k_{cat}/K_m = 3.22 min^{-1} μM^{-1}). With holothin as variable substrate (benzoyl-CoA again at 500 μM), a K_m value of 129.5 ± 17 μM and a V_{max} value of 3.33 ± 0.19 μmol min^{-1} (k_{cat}/K_m = 1.27 min^{-1} μM^{-1}) were similarly determined (Table 3). ActA, therefore, has been shown to be a pyrrothine/holothin benzoyltransferase with pyrrothine being the preferred substrate. The difference in kinetic efficiencies for benzoyltransferase between the pyrrothine and the holothin is not great (about two-fold, reflected primarily in difference in k_{cat}/K_m values), but it appears significant given the very small difference in size and polarity between these two pyrroles, and is consistent with the fact that *Sa. algeriensis* produce BEP more than BEH. But this difference cannot explain the great difference in production of BEP comparing with BEH. We suppose that this difference due to the availability of holothin substrate comparing to pyrrothine one. This hypothesis is supported by the fact that *Sa. algeriensis* produces large specter of pyrrothine derivatives (around 20 products) comparing to just two holothin products. In all case our results showed that *Sa. algeriensis* processes an enzymatic system sufficient flexible and capable to produce the two families.

3.4. Effects of pH and temperature on enzyme activity and stability

Benzoyltransferase was active in the pH range of 4–10, with an optimum at pH 8 in Tris–HCl buffer (Fig. 5). The profile showed that the activity is affected by the change of pH. The loss of activity between the maximal and the minimal activity was of 40%. Furthermore, at the same pH value the activity was more important in HCl–glycine buffer comparing to Tris–HCl, this letter was best for activity comparing to NaOH–glycine at pH 8 (Fig. 5). Others acyltransferases seem to share this basic optimum pH, spermidine/spermine acetyltransferase from *Streptomyces* sp. 139, ornithine acetyltransferase from *S. clavuligerus*, and streptothricin acetyltransferase from *S. lividans* were maximal at pH of 7.5, 7.8, and 8.5, respectively [17–19]. Furthermore, in *S. murayamaensis* a kinamycin acetyltransferase I was more active in pH slightly acidic and its optimum pH was found to be 6.2 [20].

Benzoyltransferase activity for ActA was measured at temperatures ranging from 25 to 60 °C. The optimum activity was observed at 40 °C, and only about 3% and 10% of this activity was inactivated after exposure to 45 °C and 50 respectively for 10 min (Fig. 6). Up to these temperatures the relative activity decreased sharply (*i.e.*

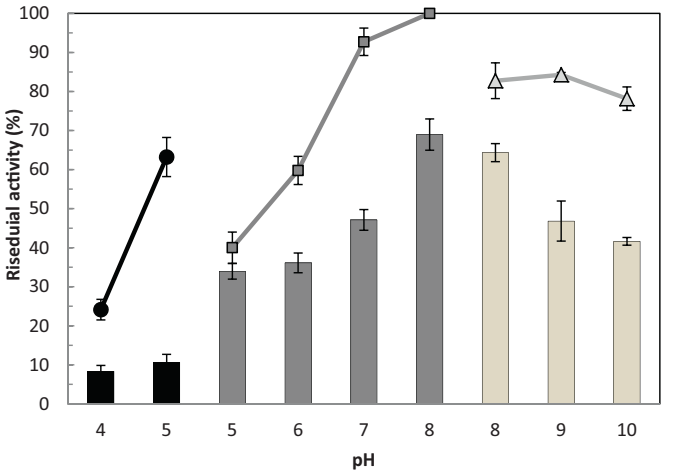

Fig. 5. pH optimum of *N*-benzoyltransferase, ●, HCl–glycine; ■, Tris–HCl; ▲, NaOH–glycine. Effect of pH on *N*-benzoyltransferase stabilities, HCl–glycine, black colum; Tris–HCl, dark-gray column; NaOH–glycine, light-gray column.

at 55 °C it was 26% lower than at 50 °C). This optimum temperature were near to these known for others acyltransferases, the optimum temperature measured in 0.1 mM Tris–HCl buffer (pH 7.5) was found to be 37 °C for spermidine/spermine acetyltransferase and ornithine acetyltransferase [17,18].

The thermostability was carried out at temperature ranging from 20 to 40 °C. The thermal stability profile showed that the residual activities decreased dramatically with the increasing of temperature up to 30 °C with only 9% of residual activity at 40 °C after 30 min (Fig. 7). Similar stabilities were already observed for other acetyltransferases under the same conditions as spermidine/spermine acetyltransferase which was stable at 35 °C, it become inactive at 50 °C [19]. BEBT which catalyze the formation of benzylbenzoate was 100% stable for 30 min at 30 °C and 80% stable for 30 min at 37 °C. After incubation at 50 °C for 30 min, the enzyme was completely inactivated [21].

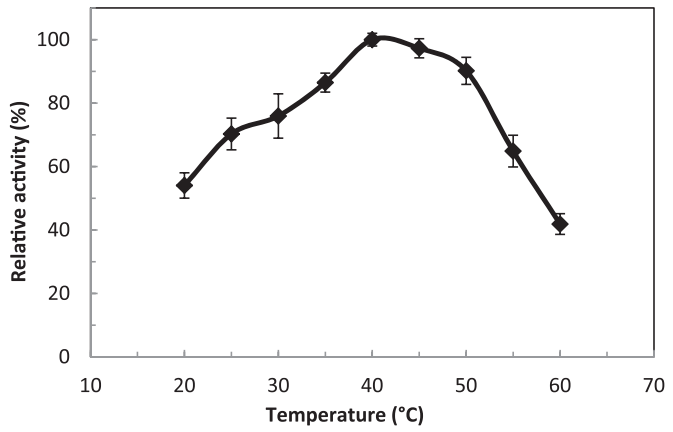

Fig. 6. Optimum temperature of benzoyltransferase.

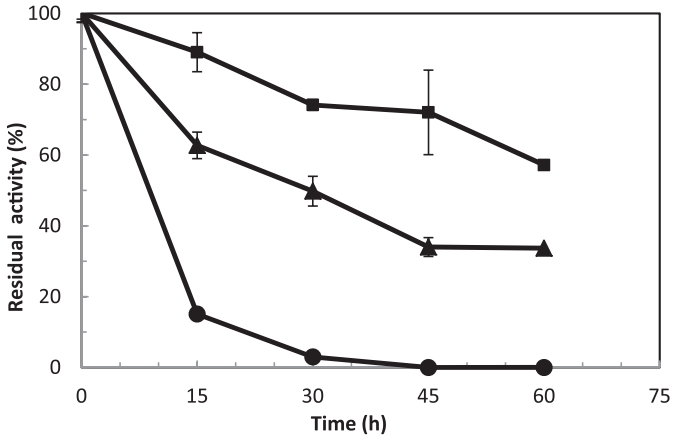

Fig. 7. Thermal stability of benzoyltransferase at 20 (■), 30 (▲) and 40 (●).

4. Conclusion

Till now, the presence of more than one acyltransferase involved in DTP biosyntheses in *Sa. algeriensis* has never been confirmed by purification of these enzymes. However, the temperature and pH profiles of these acyltransferases in the cell-free extract of *Sa. algeriensis* clearly indicated that the two enzymes have different kinetic constants, optimal pHs and temperatures [10]. The significant results from the present work are: firstly, the confirmation of the involvement of ActA in DTP biosyntheses. The second, ActA effectively does not catalyze the formation of the linear dithiolopyrrolones in *Sa. algeriensis*. Another transferase, or several transferases, must be responsible for converting pyrrothine/holothin to their related products. Finally, if we couple these results with our previous results concerning the transcriptional analyses of *actA* on the two media SSM and SSM supplemented by benzoic acid [15], it is clear now that the role of benzoic acid as well as the similar compounds seemed to be benzoyl-CoA precursor supply than their role as gene inducer.

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