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ORIGINAL ARTICLE

Biosynthesis of antibiotic prodiginines in the marine bacterium *Hahella chejuensis* KCTC 2396

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Abstract

Aims: *Hahella chejuensis* KCTC 2396 produces red pigments, showing antibacterial and algicidal activities. The main red-coloured metabolite of the pigments was identified as antibiotic prodigiosin. With the expectation that the red pigments are a mixture of a series of close relatives, the aim of the present study is to detect new antibiotic prodigiosin analogues and to analyse the biosynthetic pattern for prodiginines in KCTC 2396.

Methods and Results: Except prodigiosin, the other constituents in the red pigments were confirmed as well-known dipyrrolyldipyrromethene prodigiosin, norprodigiosin, and undecylprodiginine. Additionally, four new prodigiosin analogues, each of which was distinguished from prodigiosin (C_5), according to differences in alkyl chain length (C_3–C_7), were detected in small quantities by liquid chromatography mass spectrometry/mass spectrometry spectroscopy. Owing to the presence of a cytotoxic methoxy group, it is expected that all the new prodigiosin analogues are bioactive.

Conclusions: Four characterized prodiginines, including prodigiosin and four new prodigiosin analogues are produced in different ratio in KCTC 2396. All of the prodiginines possess a common linear tripyrrolyl structure and a cytotoxic methoxy group.

Significance and Impact of the Study: This study shows for the first time that KCTC 2396 is able to produce antibiotic prodigiosin, undecylprodiginine and new prodigiosin analogues in a mixture of pigments. It is also shown that KCTC 2396 possesses a novel system for the simultaneous production of multiple prodiginines in a single micro-organism.

Introduction

Prodiginines (6-methoxyprodigiosenes) are a large family of pigmented tripyrrole antibiotics, having medical potential as immunosuppressants and antitumour agents (Han *et al.* 1998; Fürstner *et al.* 1999; Montaner and Pérez-Tomás 2003; Pérez-Tomás *et al.* 2003). They are produced by actinomycete and other eubacterial strains (Fürstner 2003). The red-coloured linear tripyrrolyl prodiginine, produced by *Serratia marcescens*, is called prodigiosin (2-methyl-3-amyl-6-methoxyprodigiosene). It has a series of close relatives bearing the same prodigiosene core with different alkyl substituents (Fig. 1). Norprodigiosin and dipyrrolyldipyrromethene prodigiosin are synthesized via the condensation of intermediates, accumulated by *S. marcescens* mutants, which are blocked in the prodigiosin pathway. Norprodigiosin is a byproduct, formed by the connection of 2-methyl-3-amylpyrrole (MAP) and 4-hydroxy-2,2′-bipyrrole-5-carboxaldehyde (HBC) [an intermediate in the 4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (MBC) biosynthetic pathway] (Williams 1973; Gerber 1975). Dipyrrolyldipyrromethene

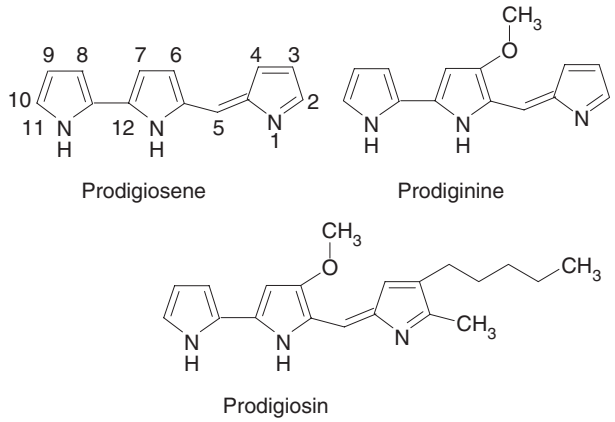

Figure 1 Chemical structure of the parent nucleus in a variety of linear oligopyrrolyl compounds. The common pyrrolylpyrromethene skeleton of prodigiosin and prodigiosin-like pigments was named prodigiosene (Hearn *et al.* 1970). Prodiginine was proposed as a trivial name for 6-methoxyprodigiosene (Gerber 1969). In accordance with the nomenclature, prodigiosin could be called 2-methyl-3-amyl-6-methoxyprodigiosene or 2-methyl-3-amylprodiginine.

prodigiosin is a blue tetrapyrrole pigment, and is derived from the condensation of two MBC molecules (Wasserman *et al.* 1968; Gerber 1975) (Fig. 2). A red antibiotic produced by actinomycete *Streptomyces coelicolor* A3(2) was shown to be a mixture of predominantly linear undecylprodiginine (C-25 prodigiosin analogue) and isomeric, cyclic derivative butyl-*meta*-cycloheptylprodiginine, in about a 2 : 1 ratio, and an unidentified purple pigment (Tsao *et al.* 1985). Generally, several other actinomycete strains are likely to be able to produce two analogous pigments in a single cell, one of which has an alternative cyclic side chain converted from a linear form by an oxidative cyclization reaction within the cell (Wasserman *et al.* 1966, 1969; Gerber 1969, 1970; Tsao *et al.* 1985). Williams (1973) rationalized that the ability to produce these cyclic compounds might be characteristic of actinomycetes.

Following intensive study on the biosynthesis of actinomycete prodiginines at the biochemical and molecular level, including undecylprodiginine in particular (Cerdeño *et al.* 2001; Thomas *et al.* 2002), the organization of the

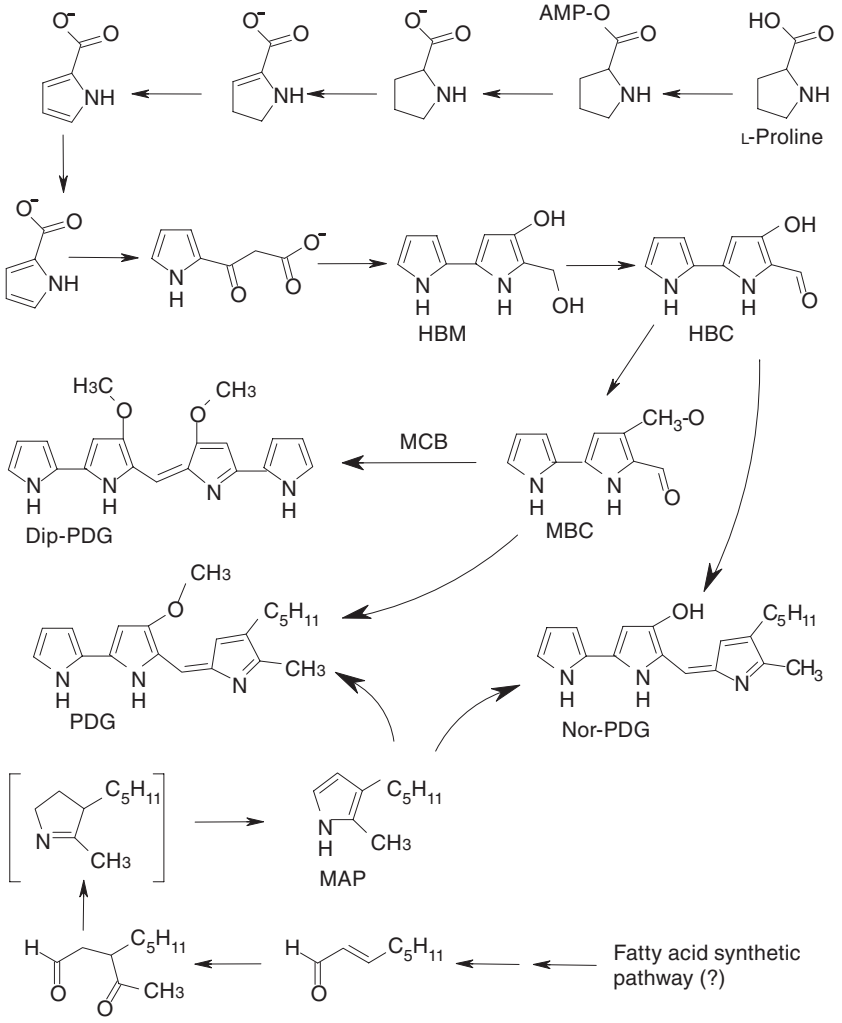

Figure 2 Proposed pathway for the bifurcated biosynthetic routes in the prodigiosin production by *Serratia marcescens* strains. The pathway was adapted from previously published papers (see the text for description and references) and modified by the authors. The names for the key intermediates and final products in this pathway are indicated as follows: HBM, 4-hydroxy-2,2′-bipyrrole-5-methylalcohol; HBC, 4-hydroxy-2,2′-bipyrrole-5-carboxaldehyde; MBC, 4-methoxy-2,2′-bipyrrole-5-carboxaldehyde; MAP, 2-methyl-3-amylpyrrole; PDG, prodigiosin; Nor-PDG, norprodigiosin; Dip-PDG, dipyrrolyldipyrromethene prodigiosin.

undecylprodiginine biosynthetic gene cluster has been clearly delineated. In addition, based on functional and comparative analyses on several key enzymes, the synthetic pathway was subsequently proposed. These studies showed that undecylprodiginine is derived from one unit of proline, one unit of glycine, one unit of serine and several units of acetate, via a convergent pathway involving condensation of MBC and 2-undecylpyrrole (a monopyrrolyl counterpart for MAP) at a later stage, with butyl-*meta*-cycloheptylprodiginine perhaps being derived from undecylprodiginine by oxidative cyclization.

Hahella chejuensis KCTC 2396, producing a large amount of extracellular polysaccharides and a red pigment showing algicidal activity (Jeong *et al.* 2005), was isolated from marine sediment collected from Marado, Cheju Island, Republic of Korea (Lee *et al.* 2001). A phylogenetic analysis indicated that KCTC 2396 is a member of the oceanic γ-*proteobacteria* – a prokaryotic group prevalent in marine environments (Cho and Giovannoni 2004).

Materials and methods

Bacterial strains and culture conditions

Hahella chejuensis KCTC 2396 was routinely grown on a Difco marine broth 2216 (MB) agar plate (Zhao *et al.* 2006) for 48 h at 30°C. *Serratia marcescens* wild-type Nima and *S. marcescens* mutants OF, WF and 9-3-3, which are blocked in the prodigiosin biosynthetic pathway, were cultured in PG broth (peptone, 0·5%; glycerol, 1·0%) at 30°C as a producer of the authentic compound used in the liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analysis: Nima for prodigiosin, OF for norprodigiosin, WF for MAP and 9-3-3 for MBC and dipyrrolyldipyrromethene prodigiosin (Wasserman *et al.* 1968; Gerber 1975; Ding and Williams 1983). *Streptomyces coelicolor* A3(2), producing antibiotic red undecylprodiginine, was grown on YM (yeast extract, 0·4%; malt extract, 1·0%; glucose, 0·4%; pH 7·3) at 30°C.

Structural identification of red pigment

KCTC 2396, grown on MB plate, was inoculated in 50 ml of MB liquid, and cultured for 24 h at 30°C with vigorous shaking. A portion of the culture (5 ml) was transferred to 200 ml of fresh MB liquid (OD_{700} nm = 0·04), further cultivated for 12–144 h, and centrifuged at 10 000 ***g*** for 20 min. The supernatant was extracted with an equal volume of ethyl acetate. The pigment from the cell pellet was extracted with 20 ml of 100% acetone. The extract was then centrifuged at 10 000 ***g*** for 20 min, and the white pellet was subsequently discarded. Both the ethyl acetate and acetone extracts were mixed and dehydrated with sodium sulfate, and then evaporated. The dried extract was dissolved in 50% methanol (to a final concentration of 5 mg ml^{-1}), and 10 μl of that was analysed by LC-MS/MS technique via comparison with reference compounds synthesized by *S. marcescens* wild-type or mutants, or *S. coelicolor* A3(2).

Prodigiosin production during growth

KCTC 2396, grown on MB plate, was inoculated in 50 ml of MB liquid, and cultured for 24 h at 30°C with vigorous shaking. Ten millilitre of the culture was transferred to 650 ml of fresh MB liquid (OD_{700} nm = 0·04), aliquoted into each 20 ml, and further cultivated. After culturing at the indicated times up to 162 h, the cell growth was determined by measuring the absorbance at OD_{700} nm. Prodigiosin was extracted by shaking the KCTC 2396 cell culture with 40 ml of methanol/1-N HCl (24 : 1) for 30 min at 30°C. After centrifugation (10 000 ***g*** for 20 min), the white cell pellet was discarded, and a supernatant containing prodigiosin was obtained. To quantify prodigiosin in the methanol extract using mass spectrometry, reference prodigiosin at different concentrations was used for plotting the standard graph versus peak area at *m/z* 324·2 ($[M + H]^+$) (prodigiosin's molecular weight, 323) in LC-electrospray ionization (ESI)-MS. The quantity of prodigiosin was determined in accordance with the standard graph.

LC-MS/MS analysis

LC-MS was performed using a Finnigan LCQ Advantage MAX ion trap mass spectrometer (Thermo Electron Co., Waltham, MA, USA), equipped with an ESI source. HPLC separation was performed on a Finnigan Surveyor^{TM} Modular HPLC System (Thermo Electron Co.), using a XTerra MS C18 (5 μm, 2·1 × 150 mm, Waters, Ireland) with BetaBasic-18 guard column (2·1 × 10 mm; Thermo Electron Co.). The system was operated under Xcalibur software (version 1.3 SP2; Thermo Electron Co.). Mobile phase A was water and mobile phase B was acetonitrile. Both phases contained 0·1% formic acid. Gradient elution, at a flow rate of 0·2 ml min^{-1}, was performed as follows: 0–30 min 10–100% B (linear gradient), 30–50 min 100% B (isocratic), and 50–60 min 10% B (isocratic). The spray needle voltage was 5 kV; ion transfer capillary temperature 220°C; nitrogen sheath gas flow rate 60 arbitrary units; and auxiliary gas flow rate setting five arbitrary units. The ion trap contained helium damping gas, introduced according to the manufacturer's recommendations. Full-scan mass spectra were obtained in a range of *m/z* 50–700, with three microscans and a maximum ion injection time of 200 ms. Utilizing the capacity

of the LCQ mass spectrometers to provide continuous polarity switching, data acquisition was conducted in two modes, positive and negative. Data-dependent tandem mass spectrometry (MS/MS) experiments were controlled by menu-driven software provided with the Xcalibur system. All experiments were performed under automatic gain control conditions.

Results

Structural identification of red pigment

Fifty micrograms of the metabolite mixture (123 mg) extracted from the KCTC 2396 48-h culture was analysed by LC-MS/MS, revealing a major peak at 24·5 min in the total ion chromatogram. The fragmentation pattern of the major peak, designated as **B**, having a molecular weight of *m/z* 324·2 ($[M + H]^+$) was identical to that of authentic prodigiosin from *S. marcescens* Nima (Fig. 3). The red metabolite was further confirmed to be prodigiosin by ^{1}H-NMR [6·94 (m, 1H), 6·71 (m, 1H), 6·66 (s, 1H), 6·39 (s, 1H), 6·21 (m, 1H), 6·01 (s, 1H), 3·89 (s, 3H), 2·37 (t, 2H), 2·27 (s, 3H), 1·53 (m, 2H), 1·33 (m, 4H), 0·90 (t, 3H)] and ^{13}C-NMR (169·6, 160·2, 141·0, 135·8, 129·9, 129·2, 125·1, 123·0, 120·6, 115·9, 113·1, 110·9, 95·9, 58·9, 32·7, 31·8, 26·6, 23·6, 14·4, 11·5) analysis (Jeong *et al.* 2005).

After separation of the red pigments by HPLC, three additional compounds other than prodigiosin were detected at different retention times under the same LC-MS conditions (Fig. 3). The detected compounds (peaks **A**, **C** and **D**) had respective molecular weights of *m/z* 335 ($[M + H]^+$), 310 ($[M + H]^+$) and 394 ($[M + H]^+$), respectively. Furthermore, they displayed identical fragmentation patterns and retention time with those of authentic dipyrrolyldipyrromethene prodigiosin, norprodigiosin and undecylprodiginine, respectively, which were produced from bacterial strains, as described in Materials and methods. In 48-h culture extracts, the relative peak area of each compound was very small compared with that of prodigiosin (100·0%): dipyrrolyldipyrromethene prodigiosin, 0·5%; norprodigiosin, 4·0%; and undecylprodiginine, 1·0%. However, the relative production ratio of these prodiginines was not maintained at a steady level during the culture. For example, undecylprodiginine ceased to be produced after 48 h, while the remaining prodiginines were detected up to 144 h (Table 1).

Additionally, in an initial LC-MS/MS analysis of the red pigment containing prodigiosin (*m/z* 324, $[M + H]^+$), other minor peaks were detected around the prodigiosin peak (Fig. 4). These appeared to be composed of at least four separate compounds, showing parent ions with *m/z* 296 ($[M + H - 2CH_2]^+$ from *m/z* 324 value of prodigiosin), *m/z* 310 ($[M + H - CH_2]^+$), *m/z* 338 ($[M + H + CH_2]^+$) and *m/z* 352 ($[M + H + 2CH_2]^+$) for each peak eluted at slightly different retention times than that of prodigiosin (22·5, 23·8, 26·9 and 28·2 min, respectively). Moreover, the *m/z* values and retention time shifts of these parent ions were in accordance with the sequential decrease or increase in prodigiosin alkyl chain length. This indicated the presence of novel prodigiosin

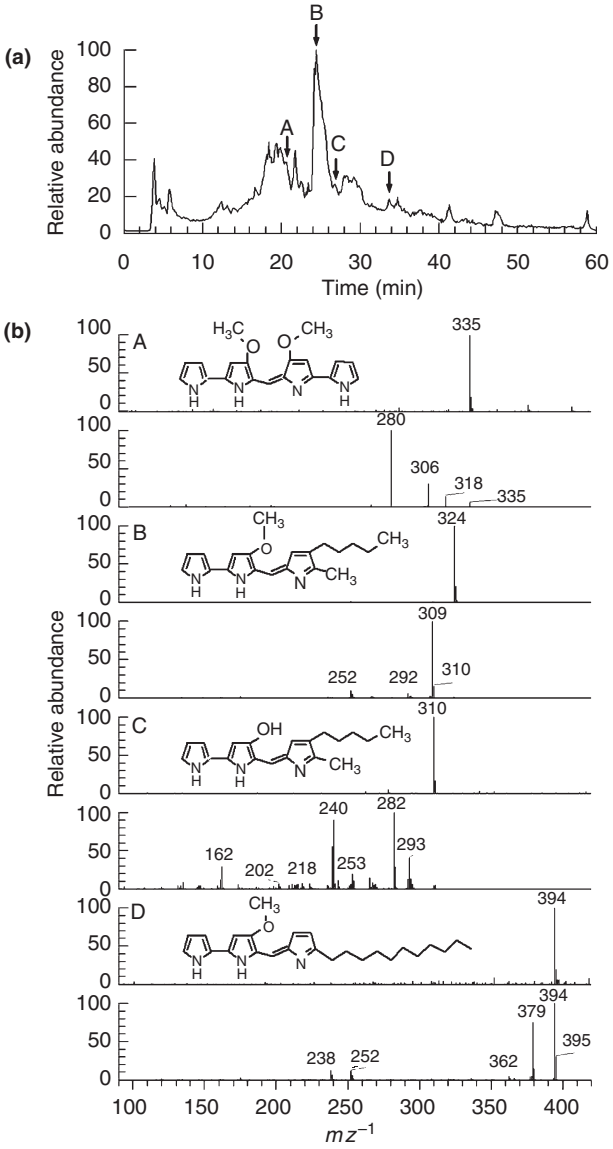

Figure 3 Mass spectrometry/mass spectrometry (MS/MS) spectral analysis of the red pigments extracted from KCTC 2396 48-h culture. (a) Total ion chromatogram of the red-pigment extract containing four prodigiosin-like compounds, which were separated by HPLC at the indicated retention time: A, dipyrrolyldipyrromethene prodigiosin at 20·8 min; B, prodigiosin at 24·5 min; C, norprodigiosin at 27·6 min; D, undecylprodiginine at 33·4 min. (b) MS/MS fragmentation patterns of prodigiosin-like compounds.

Table 1 Detection of prodiginines and intermediates in KCTC 2396 culture extracts

Compound	Culture time (h)					
	12	24	36	48	72	144
MBC	+*	+	+	+	–†	–
MAP	–	–	–	–	–	–
Prodigiosin	+	+	+	+	+	+
Dipyrrolyldipyrromethene prodigiosin	–	–	–	+	+	+
Norprodigiosin	–	–	+	+	+	+
Undecylprodiginine	–	+	+	+	–	–

Abbreviations: MBC, 4-methoxy-2,2′-bipyrrole-5-carboxaldehyde; MAP, 2-methyl-3-amylpyrrole.
*Detected by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) technique, but not determined quantitatively.
†Not detected.

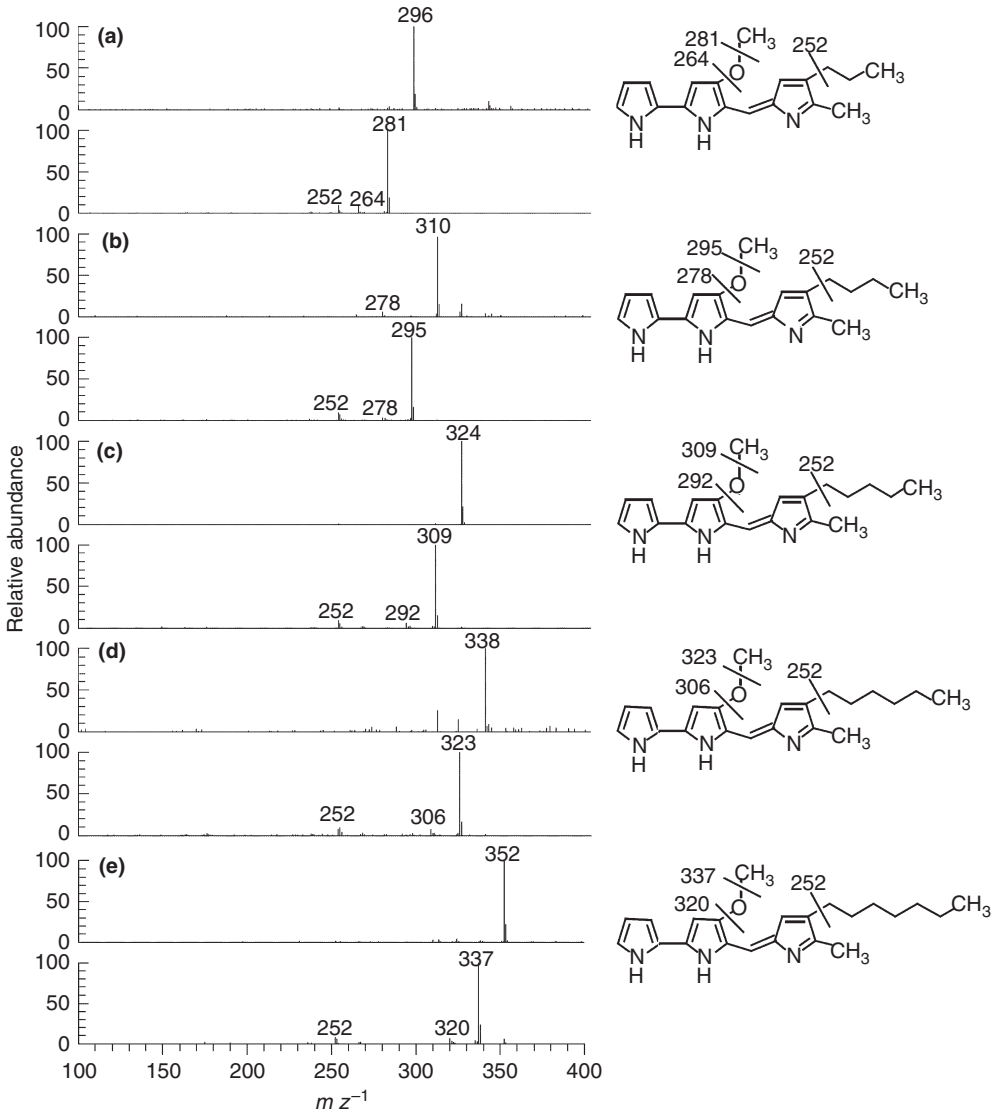

Figure 4 Mass spectrometry/mass spectrometry (MS/MS) spectral analysis of four new prodigiosin analogues in KCTC 2396 48-h culture. The MS/MS spectrum of each prodigiosin analogue (relative production) separated by HPLC at the indicated retention time: a, 2-methyl-3-propyl-prodiginine (1·6%) at 22·5 min; b, 2-methyl-3-butyl-prodiginine (2·0%) at 23·8 min; c, 2-methyl-3-pentyl-prodiginine (prodigiosin, 100·0%) at 24·5 min; d, 2-methyl-3-hexyl-prodiginine (1·3%) at 26·9 min; e, 2-methyl-3-heptyl-prodiginine (17·0%) at 28·2 min.

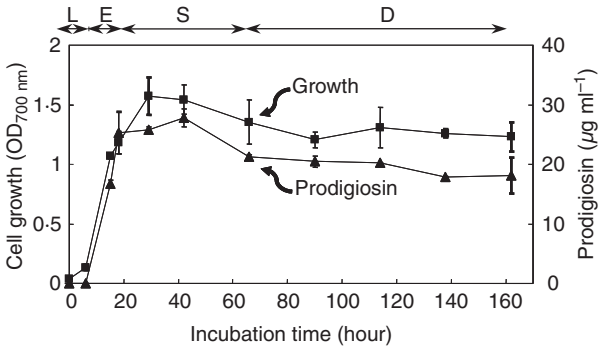

Figure 5 Prodigiosin production during the growth of KCTC 2396. Cell growth was measured as culture absorbance at 700 nm. The four phases of growth are labelled: lag (L), exponential (E), stationary (S) and death phase (D). Prodigiosin production was determined according to the prodigiosin-standard curve by liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS). Values shown are means of three determinations. Bars indicate standard deviation.

analogues, the structural identification of which was carried out by an MS/MS fragmentation study using Mass Frontier software v4.0 (HighChem Ltd., Bratislava, Slovakia). The four prodigiosin analogues had a common fragment ion at *m/z* 252, which was derived from the loss of alkyl chains, and showed the same dissociation fragment patterns, such as the loss of a methyl and methoxyl group. In 48-h culture extracts, these new compounds were detected in relatively small quantities (1·3–17·0% of prodigiosin peak area), and other prodigiosin analogues having parent ion *m/z* values of 268 ($[M + H - 4CH_2]^+$ from *m/z* 324 value of prodigiosin), 282 ($[M + H - 3CH_2]^+$), 366 ($[M + H + 3CH_2]^+$) and 380 ($[M + H + 4CH_2]^+$) were not detected.

The production profile of prodigiosin during growth

The production of prodigiosin was preliminarily determined by measuring the red-coloured methanol extract at 535 nm, spectrophotometrically. However, it has been reported that prodigiosin and its analogues have a similar visible absorption spectrum, originating from the characteristic chromophore structure (Goldschmidt and Williams 1968; Fürstner 2003). Thus, the LC-MS technique, which offers higher selectivity and resolution, was used. As shown in Fig. 5, KCTC 2396 produced only minimal amounts of prodigiosin during the early log-growth phase, when incubated in MB liquid at 30°C. The level was dramatically increased following the middle log-phase, and the maximal production of prodigiosin (28 mg l^{-1}) occurred after cellular multiplication ceased. The amounts of prodigiosin slowly decreased throughout the stationary and death phase (up to 7 days).

Discussion

The extract of KCTC 2396 culture contained mainly prodigiosin and three other prodigiosin-like metabolites (dipyrrolyldipyrromethene prodigiosin, norprodigiosin and undecylprodiginine). Interestingly, production of prodigiosin and undecylprodiginine in a single micro-organism has not been reported to date. Therefore, to the best of the authors' knowledge, this is the first report on the coproduction of these two prodiginines, each of which has been described to be synthesized through a specific pathway in different micro-organisms (Cerdeño *et al.* 2001; Williamson *et al.* 2005). Therefore, KCTC 2396 could be assumed to possess two separate pathways for prodiginine production (one for prodigiosin and another for undecylprodiginine), which would be strictly regulated by unknown regulatory elements. On the other hand, it is possible that KCTC 2396 has key enzymes with reduced substrate specificity. Owing to the characteristics of these enzymes, KCTC 2396 would thus be able to simultaneously produce prodigiosin and undecylprodiginine.

It is generally known that the terminal step in prodigiosin biosynthesis by *S. marcescens* Nima involves the enzymatic coupling of two central intermediates (a stable and diffusible MBC and a volatile MAP), which are synthesized via two separate bifurcated pathways (Ding and Williams 1983). In the case of *S. coelicolor* A3(2), undecylprodiginine is formed through the condensation of MBC and 2-undecylpyrrole (Cerdeño *et al.* 2001). As a matter of course, KCTC 2396 was expected to accumulate the common intermediate MBC to produce prodigiosin and several analogous prodiginines. Therefore, KCTC 2396 culture was extracted with ethyl acetate and acetone, as described in Materials and methods, and the extract was analysed. The fragmentation pattern of a major peak (*m/z* 191, $[M + H]^+$) was identical with that of authentic MBC. However, MAP was not detected in either the cell pellet or the supernatant of the KCTC 2396 culture, which was grown in MB liquid with several culture time intervals (Table 1).

In the KCTC 2396 extract, four new prodigiosin analogues were detected. These analogues contained shorter (C_3–C_4) or longer (C_6–C_7) alkyl chains compared with that (C_5) of prodigiosin. In a recent effort to produce new undecylprodiginine analogues, *S. coelicolor* M511 was engineered by the replacement of *redP*, which catalyses the initial biosynthesis of the 2-undecylpyrrole component, with *fabH*, which initiates fatty acid biosynthesis. Mo *et al.* (2005) produced two new branched-chain alkylprodiginines (methylundecylprodiginine and methyldodecylprodiginine) from the *redP*-deletion mutant. This indicates that even wild-type KCTC 2396 might produce prodigiosin analogues, each having a tripyrrolyl core

structure with alkyl groups that vary in chain length, via involvement of a catalytic enzyme(s) from the fatty acid synthase (FAS) system.

The maximal production of prodigiosin (28 mg l^{-1}) in KCTC 2396 occurred after cellular multiplication ceased, which corroborates the hypothesis that prodigiosin can be regarded as a secondary metabolite. Previously, Williams (1973) described prodigiosin, produced by *S. marcescens*, as a secondary metabolite by presenting several characteristics of the pigments commonly present among other secondary metabolites: prodigiosin, a small-molecular-weight substance, has no known metabolic function; biosynthesis of prodigiosin occurs at the stationary phase separated from the growth phase; and nonproliferating cells synthesize the pigment under alkaline conditions (Williams *et al.* 1971).

Prodigiosin and its analogues were studied as antibiotic and cytotoxic agent in the 1960s, but were not developed clinically owing to their high cell toxicity (Manderville 2001), which might be attributed to the presence of C-6 methoxy substituent in the 4-methoxy-2,2′-bipyrrolyl ring (Montaner and Pérez-Tomás 2003). Recently, the cytotoxic mechanism has been uncovered in relation to the interaction of prodiginines with target cells or molecule, such as DNA (Fürstner and Grabowski 2001; Melvin *et al.* 2002; Pérez-Tomás *et al.* 2003). However, during the past decade, some prodiginines and their analogues have shown potentially useful immunosuppressive and apoptotic activities when administrated at doses that are not toxic (Mortellaro *et al.* 1999; Manderville 2001). This potential of prodiginines as future prospective pharmaceutical agents has promoted researchers to study the biosynthesis of prodigiosin and its analogues (in particular, undecylprodiginine) from the viewpoint of the biosynthetic step and catalytic enzymes. KCTC 2396 can produce a mixture of at least eight prodiginines, which contain the common prodigiosene structure, with cytotoxic C-6 methoxy substituent. The biological activities of prodigiosin, undecylprodiginine, dipyrrolyldipyrromethene prodigiosin and norprodigiosin have been previously elucidated (Karuso and Scheuer 2002; Fürstner 2003; Pérez-Tomás *et al.* 2003). Accordingly, the other four prodiginines with alkyl substituents with varying chain length are expected to possess similar cytotoxic activity. Therefore, KCTC 2396 could provide several new strategies, both *in vivo* and *in vitro*, to develop novel prodigiosin derivatives with improved function and activity.

Acknowledgements

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References

Cerdeño, A.M., Bibb, M.J. and Challis, G.L. (2001) Analysis of the prodiginine biosynthesis gene cluster of *Streptomyces coelicolor* A3(2): new mechanisms for chain initiation and termination in modular multienzymes. *Chem Biol* **8**, 817–829.

Cho, J.C. and Giovannoni, S.J. (2004) Cultivation and growth characteristics of a diverse group of oligotrophic marine *Gammaproteobacteria*. *Appl Environ Microbiol* **70**, 432–440.

Ding, M.J. and Williams, R.P. (1983) Biosynthesis of prodigiosin by white strains of *Serratia marcescens* isolated from patients. *J Clin Microbiol* **17**, 476–480.

Fürstner, A. (2003) Chemistry and biology of roseophilin and the prodigiosin alkaloids: a survey of the last 2500 years. *Angew Chem Int Ed Engl* **42**, 3582–3603.

Fürstner, A. and Grabowski, E.J. (2001) Studies on DNA cleavage by cytotoxic pyrrole alkaloids reveal the distinctly different behavior of roseophilin and prodigiosin derivatives. *Chembiochemistry* **2**, 706–709.

Fürstner, A., Grabowski, J. and Lehmann, C.W. (1999) Total synthesis and structural refinement of the cyclic tripyrrole pigment nonylprodigiosin. *J Org Chem* **64**, 8275–8280.

Gerber, N.N. (1969) Prodigiosin-like pigments from *Actinomadura* (*Nocardia*) *pelletieri* and *Actinomadura madurae*. *Appl Microbiol* **18**, 1–3.

Gerber, N.N. (1970) A novel, cyclic, tripyrrole pigment from *Actinomadura* (*Nocardia*) *madurae*. *Tetrahedron Lett* **11**, 809–812.

Gerber, N.N. (1975) Prodigiosin-like pigments. *CRC Crit Rev Microbiol* **3**, 469–485.

Goldschmidt, M.C. and Williams, R.P. (1968) Thiamine-induced formation of the monopyrrole moiety of prodigiosin. *J Bacteriol* **96**, 609–616.

Han, S.B., Kim, H.M., Kim, Y.H., Lee, C.W., Jang, E.S., Son, K.H., Kim, S.U. and Kim, Y.K. (1998) T-cell specific immunosuppression by prodigiosin isolated from *Serratia marcescens*. *Int J Immunopharmacol* **20**, 1–13.

Hearn, W.R., Elson, M.K., Williams, R.H. and Medina-Castro, J. (1970) Prodigiosene [5-(2-pyrryl)-2,2′-dipyrrylmethene] and some substituted prodigiosenes. *J Org Chem* **35**, 142–146.

Jeong, H., Yim, J.H., Lee, C., Choi, S.H., Park, Y.K., Yoon, S.H., Hur, C.G., Kang, H.Y. *et al.* (2005) Genomic blueprint of *Hahella chejuensis*, a marine microbe producing an algicidal agent. *Nucleic Acids Res* **33**, 7066–7073.

Karuso, P. and Scheuer, P.J. (2002) Natural products from three nudibranchs: *Nembrotha kubaryana*, *Hypselodoris infucata* and *Chromodoris petechialis*. *Molecules* **7**, 1–6.

Lee, H.K., Chun, J., Moon, E.Y., Ko, S.H., Lee, D.S., Lee, H.S. and Bae, K.S. (2001) *Hahella chejuensis* gen. nov., sp. nov., an extracellular-polysaccharide-producing marine bacterium. *Int J Syst Evol Microbiol* **51**, 661–666.

Manderville, R.A. (2001) Synthesis, proton-affinity and anticancer properties of the prodigiosin-group natural products. *Curr Med Chem Anti-Cancer Agents* **1**, 195–218.

Melvin, M.S., Tomlinson, J.T., Park, G., Day, C.S., Saluta, G.R., Kucera, G.L. and Manderville, R.A. (2002) Influence of the A-ring on the proton affinity and anticancer properties of the prodigiosins. *Chem Res Toxicol* **15**, 734–741.

Mo, S., Kim, B.S. and Reynolds, K.A. (2005) Production of branched-chain alkylprodiginines in *S. coelicolor* by replacement of the 3-ketoacyl ACP synthase III initiation enzyme, RedP. *Chem Biol* **12**, 191–200.

Montaner, B. and Pérez-Tomás, R. (2003) The prodigiosins: a new family of anticancer drugs. *Curr Cancer Drug Targets* **3**, 57–65.

Mortellaro, A., Songia, S., Gnocchi, P., Ferrari, M., Fornasiero, C., D'alessio, R., Isetta, A., Colotta, F. *et al.* (1999) New immunosuppressive drug PNU156804 blocks IL-2-dependent proliferation and NF-kappa B and AP-1 activation. *J Immunol* **162**, 7102–7109.

Pérez-Tomás, R., Montaner, B., Llagostera, E. and Soto-Cerrato, V. (2003) The prodigiosins, proapoptotic drugs with anticancer properties. *Biochem Pharmacol* **66**, 1447–1452.

Thomas, M.G., Burkart, M.D. and Walsh, C.T. (2002) Conversion of L-proline to pyrrolyl-2-carboxyl-*S*-PCP during undecylprodigiosin and pyoluteorin biosynthesis. *Chem Biol* **9**, 171–184.

Tsao, S.W., Rudd, B.A., He, X.G., Chang, C.J. and Floss, H.G. (1985) Identification of a red pigment from *Streptomyces coelicolor* A3(2) as a mixture of prodigiosin derivatives. *J Antibiot* **38**, 128–131.

Wasserman, H.H., Friedland, D.J. and Morrison, D.A. (1968) A novel dipyrrolyldipyrromethene prodigiosin analog from *Serratia marcescens*. *Tetrahedron Lett* **6**, 641–644.

Wasserman, H.H., Rodgers, G.C. and Keith, D.D. (1966) The structure and synthesis of undecylprodigiosin. A prodigiosin analogue from *Streptomyces*. *Chem Commun* **22**, 825–826.

Wasserman, H.H., Rodgers, G.C. and Keith, D.D. (1969) Metacycloprodigiosin, a tripyrrole pigment from *Streptomyces longisporus ruber*. *J Am Chem Soc* **91**, 1263–1264.

Williams, R.P. (1973) Biosynthesis of prodigiosin, a secondary metabolite of *Serratia marcescens*. *Appl Microbiol* **25**, 396–402.

Williams, R.P., Gott, C.L., Qadri, S.M. and Scott, R.H. (1971) Influence of temperature of incubation and type of growth medium on pigmentation in *Serratia marcescens*. *J Bacteriol* **106**, 438–443.

Williamson, N.R., Simonsen, H.T., Ahmed, R.A., Goldet, G., Slater, H., Woodley, L., Leeper, F.J. and Salmond, G.P. (2005) Biosynthesis of the red antibiotic, prodigiosin, in *Serratia*: identification of a novel 2-methyl-3-*n*-amyl-pyrrole (MAP) assembly pathway, definition of the terminal condensing enzyme, and implications for undecylprodigiosin biosynthesis in *Streptomyces*. *Mol Microbiol* **56**, 971–989.

Zhao, J.S., Manno, D., Leggiadro, C., O'neil, D. and Hawari, J. (2006) *Shewanella halifaxensis* sp. nov., a novel obligately respiratory and denitrifying psychrophile. *Int J Syst Evol Microbiol* **56**, 205–512.