Journal of Industrial Microbiology, 1 (1987) 303–310
Elsevier
SIM 00044

Analytical HPLC of the aridicin glycopeptide complex and its application to fermentation development

C.H. Pan, G. Chan, L.J. Nisbet, M.J. Polansky, S. Vogt-Speth and R.D. Sitrin

Research and Development Division, Smith Kline & French Laboratories, Swedeland, PA 19479, U.S.A.

Received 23 April 1986
Revised 8 October 1986
Accepted 8 October 1986

Key words: HPLC; Aridicin; Glycopeptide; Fermentation; Antibiotic; SepPAK; Titer

SUMMARY

A gradient analytical HPLC system was developed to assay titers of the three major components of the aridicin (Ardacin) complex produced by *Kibdelosporangium aridum* (SK&F AAD-216). The separation was performed on a Beckman Ultrasphere column using a gradient of acetonitrile (26–43%) in 0.1 M pH 3.2 phosphate buffer with UV detection at 220 nm. The gradient system was necessary to analyze all three major factors within a reasonable recycle time (14 min) without interference by front eluting impurities. The assay was linear from 12 to 200 μg/ml (multiple $R^2 = 0.998$) with a standard deviation for retention time of 1.4%. A SepPAK isolation scheme was developed to assay samples in complex matrices such as fermentation broths. Using this assay as a monitor, fermentation medium optimization increased the total titers of the three factors from approximately 5 μg/ml to over 200 μg/ml. The optimal medium contained glucose, beet molasses and methyl oleate. The latter substrate was particularly effective in enhancing production 10-fold, presumably by enhancing the supply of acetyl-CoA. This is a biosynthetic precursor of both dihydroxyphenylglycine, present in the nucleus, and the acyl side chains present on the amino-glucuronic acids.

INTRODUCTION

We recently described the isolation and characterization of the aridicins (USAN: Ardacin), a novel complex of glycopeptide antibiotics of the vancomycin-ristocetin class produced by *Kibdelosporangium aridum* (ATCC 39323, SKF AAD-216) [9,10,14,16]. Since initial fermentation of this organism gave minimal amounts of the desired complex, increased titers were required in order to obtain sufficient material for detailed structural and biological studies and for semi-synthetic modifications. The AAD-216 culture produces a complex of at least 25 antibiotics [4] structurally related to the major components, aridicins A, B and C (Fig. 1). Microbiologically based assays, typically used to monitor fermentations, are non-specific and cannot discriminate between various components having different potencies. Furthermore, for many glycopeptides such as the aridicins, aglycone derivatives

Correspondence: Dr. R.D. Sitrin, Research and Development Division, Smith, Kline and French Laboratories, 709 Swedeland Road, Swedeland, PA 19479, U.S.A.

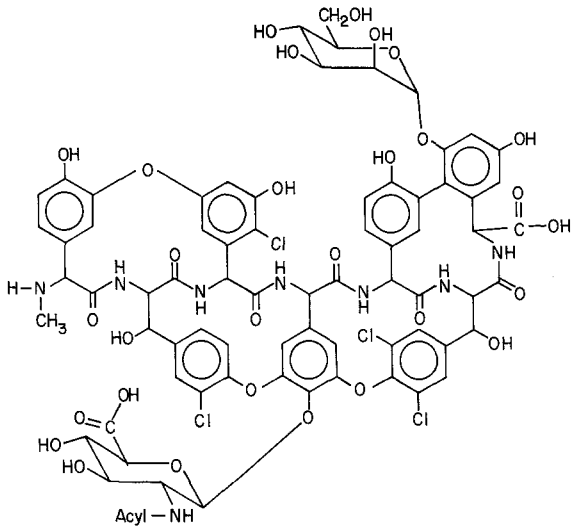

Fig. 1. Structures of aridicins: A, acyl = $C_9H_{19}CO$; B, acyl = $(CH_3)_2CH(CH_2)_7CO$; and C, acyl = $(CH_3)_2CH(CH_2)_8CO$.

have higher levels of activity than the parent antibiotics [15] and their inadvertent production [1] under different environmental conditions could yield misleading results in a biological assay. We therefore developed a gradient HPLC assay which simultaneously determined the levels of the three major components of interest. It was also useful for monitoring the subsequent purification of the antibiotic complex. In order to monitor levels in fermentation broths, a solid phase (SepPAK) isolation scheme was developed to prepare samples for analysis. This manuscript describes the development of a high performance liquid chromatography (HPLC) gradient analysis system and its subsequent use in medium modification studies to increase titers of antibiotic production 40-fold.

MATERIALS AND METHODS

HPLC procedures

Analytical HPLC was done on a Beckman 345 gradient HPLC system monitored at 220 nm with a Beckman Model 165 detector set at 0.5 AUFS with a time constant of 0.5 s using an Altex CRIB integrator with the following parameters for peak detection and area measurement: width, 5 s; slope, 1256 μV/min; drift, 0; doubling time, 1000 s; atten, 3; speed, 2 mm/min. Chromatography was run on a Beckman Ultrasphere ® ODS column (0.46 × 15 cm) equipped with a Brownlee guard cartridge containing C-18 packing (Catalog 18-GU) purchased from Rainin Instrument Co. (Woburn, MA) Precolumns were changed regularly on a biweekly schedule. Phosphoric acid buffer (0.1 M) was prepared from Fischer HPLC grade phosphoric acid, 85% and adjusted to pH 3.2 with KOH. Acetonitrile was HPLC grade, UV (Burdick and Jackson). Elution was run at a flow rate of 1.5 ml/min, initially at 26% acetonitrile in 0.1 M pH 3.2 phosphoric acid-potassium phosphate followed by a linear gradient to 43% and a reset to 26% (see Fig. 2B). Injections were performed with a WISP 710 B autoinjector with a reinjection programmed every 14 min (actual reinjection time, 15.6 min). The integrator was calibrated daily after two blank gradient runs with sequential triplicate injections of a standard solution of aridicins A, B and C (25 μg/ml each in H_2O). Injector volumes were 100 μl for the SepPAK products and 25 μl for non-SepPAK products. Standards were reinjected in duplicate after every tenth sample.

SepPAK procedure

SepPAK C_{18} cartridges (Waters Associates, Milford, MA) were activated by washing successively with 5 ml of acetonitrile, 5 ml of water containing 0.01% NaN_3 and 4 ml of 0.1 M pH 3.2 phosphoric acid-potassium phosphate buffer ("buffer"). All SepPAK operations were performed using a 5 ml glass syringe. Solvents were dispensed from Repippet Dispensers (Lab Industries, Berkeley, CA) using a 10 ml dispenser for the acetonitrile and water bottles and a 5 ml dispenser for the others. Fermentation broth was clarified by centrifugation (1500 rpm, 15 min, International Centrifuge TR-2) and adjusted to pH 6–6.5 (pH meter) with 0.3 M phosphoric acid. A broth sample of 1.0 ml (Eppendorf Pippette) was passed through the cartridge followed by successive washes with 2 ml of buffer and 2 ml of 20% (v/v) acetonitrile buffer. The complex was slowly eluted from the cartridge with 2.0 ml of 50% (v/v) acetonitrile buffer with

careful collection into a 4 ml Wisp® vial to minimize losses from foaming. To this solution was added 2.0 ml of buffer to give a final solution (in 25% acetonitrile) suitable for injection. For recycling, the used SepPAK cartridge was washed with 4 ml of 50% acetonitrile buffer, followed by 5 ml of acetonitrile, etc., as above. Cartridges were reused five times. Broths were extracted as soon as possible after centrifugation. SepPAK extracts were stable at 4°C for several weeks but deteriorated on freezing.

Culture media

All medium constituents are given as %w/v except where stated otherwise.

Agar medium consists of: glucose 1.0%, soluble starch 2.0%, yeast extract 0.5%, N-Z amine (Sheffield Chemicals, Norwich, NY) 0.5%, $CaCO_3$ 0.1% and agar 2.0%.

Seed medium consists of: starch 1.5%, sucrose 0.5%, glucose 0.5%, soy peptone 0.75%, corn steep liquor 0.5%, K_2HPO_4 0.15%, NaCl 0.05%, $CaCO_3$ 0.15% and mineral "S" solution 0.5% (v/v) at pH 7. Mineral "S" solution consists of $ZnSO_4 \cdot 7H_2O$ 0.28%, $Fe(NH_4)_2HC_6H_5O_7$ 0.27%, $CuSO_4 \cdot 5H_2O$ 0.0125%, $MnSO_4 \cdot H_2O$ 0.1%, $CoCl_2 \cdot 6H_2O$ 0.01%, $Na_2B_4O_7 \cdot H_2O$ 0.01% and $Na_2MoO_4 \cdot 2H_2O$ 0.005%.

Production medium P-1 consists of: glycerol 2.0%, glucose 0.5%, $(NH_4)_2SO_4$ 0.2%, lard water 2.0%, peptone 0.5%, KH_2PO_4 0.01%, NaCl 0.2%, $CaCO_3$ 0.2%, amber BYF 0.5%, corn steep liquor 0.1% and $CoCl_2 \cdot 6H_2O$ 0.0001% at pH 7.

Production medium P-2 consists of: soybean meal 1.5%, beet molasses 1.0%, glucose 2.0% and NaCl 0.03% at pH 7.

Production medium P-3 consists of: soybean meal 1.5%, beet molasses 1.0%, glucose 1.0%, NaCl 0.03% and Estrasan No. 4 1.0% (industrial methyl oleate, 55%, Reilly Whitman, Conshohocken, PA) at pH 7.0.

Antibiotic production

A culture of *K. aridum* ATCC 39323 was prepared by inoculation of agar slant medium followed by incubation at 28°C for 10–14 days. For a typical fermentation, a first seed was prepared by inoculating the contents of the agar slant culture into 500 ml of seed medium in a 4-liter aspirator bottle. The bottle was incubated at 28°C for 3–4 days on a shaker (New Brunswick rotary shaker Model G53) at 250 rpm and 2-inch stroke. The seed culture was then transferred to a New Brunswick 14-liter fermentor containing 10 liters of production medium P-1, P-2 or P-3, The fermentation was carried out at 28°C with aeration and agitation at 0.4 vvm and 400–500 rpm respectively.

When the 750-liter fermentor was used (ABEC INC., Allentown, PA), a second and third stage seed was required. This was prepared by transferring the first seed culture into 10 liters of the same seed medium in a 14-liter fermentor. The second seed was run for a further 3–4 days at 28°C with aeration at 0.4–0.5 vvm and agitation at 400 rpm following which it was further transferred to 50 liters of the same medium in a 75-liter fermentor (Chemapec Inc., Woodbury, NY). The third seed was propagated under similar conditions before being transferred to the final production medium (650 liters in the 750-liter vessel).

Biomass was measured by centrifuging 10 ml of the whole broth for 15 min at 2000 rpm in a graduated centrifuge tube. The ratio between the volume of the spun-down solid and the total volume was measured and expressed as biomass % (v/v).

pH control strategy

The fermentations were carried out in 14-liter fermentors in which pH was measured by an Ingold combination electrode and was controlled by a multiple three-step automated pH controller device (New Brunswick Scientific) using hydrochloric acid (0.5 N). The desired pH was programmed at the middle set point of the device while the first and the third points were set at a half pH unit on either side.

RESULTS AND DISCUSSION

HPLC gradient analysis

Since the aridicin components are highly

charged amphoteric molecules, a buffered system (0.1 M phosphate buffer, pH 3.2) was necessary to give reproducible retention times, sharp peaks and column stability. A detection wavelength of 220 nm was chosen to enhance sensitivity relative to colored products of the culture. Use of a 254 nm wavelength resulted in approximately 10-fold less sensitivity. Fig. 2A and B shows chromatograms of a standard solution containing 25 μg/ml of each component run in isocratic and gradient modes, respectively. Isocratic systems are normally used for analytical HPLC assays due to their ease of operation. Although an isocratic system could be developed to elute the A component away from the solvent front, this resulted in an extended retention time (15 min) for the C component (Fig. 2A). Attempts to use higher acetonitrile concentrations to elute the C component in a shorter retention time resulted in moving the A peak too close to the solvent front where interfering contaminants would be expected to elute in actual samples. By contrast, a gradient system (Fig. 2B) gave a good separation for all three antibiotics as equally sized sharp peaks well-

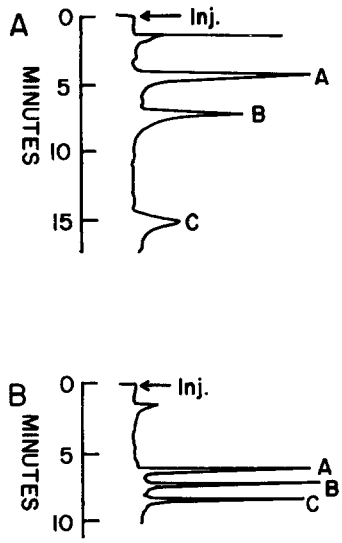

Fig. 2. Isocratic and gradient HPLC analyses of aridicins A, B and C. Sample: aridicins A, B and C (25 μg/ml each; 25 μl). Column: Beckman Ultrasphere ODS 4.6 × 150 mm. Solvent: (A) isocratic system – 30% acetonitrile in 0.1 M phosphoric acid, pH 3.2; (B) gradient system – 26% acetonitrile in 0.1 M phosphoric acid, pH 3.2; gradient to 36% acetonitrile over 5 min starting at 1 min; gradient (1 min) to 43% at 8 min. Flow: 1.5 ml/min. Detection: 220 nm at 0.5 AUFS.

resolved from the front with reasonable retention times. Use of the gradient also eliminated the substantial tailing observed in the isocratic system evident in Fig. 2A. As noted for other large peptides [7,8], retention times were particularly sensitive to small changes in acetonitrile concentration. However, in a 14-h run consisting of over 40 injections of standards and samples, retention times for the A, B and C components displayed 1% standard deviations and were insensitive to amounts injected up to 400 μg/ml. Suitable integration parameters were chosen by observation of the peak detection light on the integrator. Using these parameters, the area integration of the A component had a 1.5% standard deviation for nine sequential injections. Slightly larger (2–3%) standard deviations were observed for the B and C components because of the presence of minor shoulder peaks in the standards. However, the total area for the A, B, and C peaks (including the area from minor components) had a standard deviation of 1.4%.

To test linearity of the assay, a solution of 200 μg/ml each of aridicins A, B and C in water was prepared, serially diluted 2-fold in water and assayed by duplicate injections in the gradient system. Regression analysis using the Fit Function program of the VAX RS-1 system (BBN Software Products, Cambridge, MA) indicated linearity for aridicin A concentrations of between 12 and 200 μg/ml (multiple R^2 = 0.998). In other experiments, it was shown that above 200 μg/ml and especially over 400 μg/ml, response was nonlinear. Thus, the working range for the assay is between 10 and 200 μg/ml for each component. With samples outside this range, dilutions in water are required.

SepPAK isolation procedure

Where direct injection was not possible, such as for fermentation broth or samples in highly buffered media, a SepPAK isolation procedure was developed as described in the experimental section. The sample volumes were chosen to be convenient for easy operation with the WISP autosampler. Thus, 1 ml of sample was put onto the SepPAK and the product was eluted with 2 ml of 50% acetonitrile buffer. Since direct injection of the 50%

acetonitrile solution resulted in broadened peaks, it was diluted with 2 ml of plain buffer to give a final solution in 4 ml of 25% acetonitrile. This solution was suitable for injection since the gradient starts at 27% acetonitrile. To compensate for the 4-fold dilution occurring in the SepPAK procedure, the injection volume was increased to 100 μl. This did not result in peak broadening for either the isocratic or gradient system. Fig. 3A and B shows typical chromatograms for a sample of a typical production culture supernatant (aridicins A, B and C were 47, 30 and 25 μg/ml, respectively) run in isocratic and gradient modes. Note that the gradient is necessary to separate the A peak from early eluting contaminants. To test the linearity of the SepPAK procedure, sets of standard solutions containing aridicins A, B and C (each at concentrations of 100 μg/ml) and their serial 2-fold dilutions were put through the procedure with each SepPAK preparation being performed nine times and with injections run in duplicate. No breakthrough of aridicins was observed for the 100 μg/ml solution in the SepPAK cartridge eluate or 20% wash samples. In the final elution sample, there was a consistent recovery of 81% for aridicin A (multiple R^2 = 0.997). Similar recoveries were observed for aridicins B (78%) and C (79%) as well as the summation of peaks A, B and C (79%). For these multiple injections at concentrations of 50 and 100 μg/ml, the standard deviations for the areas of peaks A, B and total aridicins were 3%. As expected, C showed the largest variation (7%) because of the proximal peak from a structurally related factor. The approximately 80% recovery resulted from incomplete elution of antibiotic from the SepPAK cartridge. Although the remaining 20% could be recovered by a second 2 ml wash with 50% acetonitrile buffer, the final volume of eluate became inconveniently large. The incomplete SepPAK recovery only became significant in the latter stages of our work as fermentation titers increased to 50 μg/ml and the loss greatly exceeded the error of the assay. Although an internal standard would have corrected this problem, one was not available during these titer improvement studies. To correct for the incomplete recovery, results from the SepPak assay were multiplied by 1.25. In separate experiments, two production culture samples were spiked to raise the level of aridicin A by 25 μg/ml. In the SepPAK HPLC assay of these spiked samples, the measured levels of aridicin A increased by 20 and 22 μg/ml (25 and 27.5 μg/ml after correction).

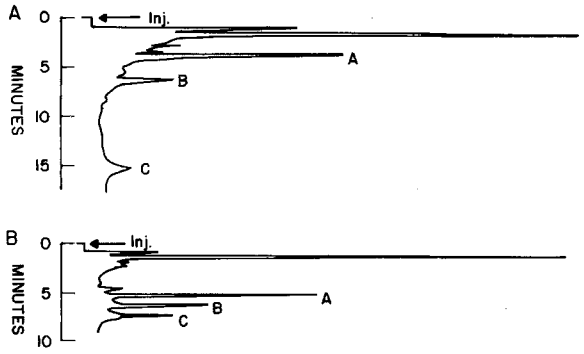

Fig. 3. Isocratic and gradient HPLC analyses of the aridicins production complex. Sample: SepPAK eluate of culture supernatant (100 μl). (A) Isocratic system, conditions as Fig. 2A. (B) Gradient system, conditions as Fig. 2B.

The gradient system and the SepPAK isolation procedure were necessary to measure low levels of antibiotics present in complex media present at the early stages of the fermentation titer improvement study. However, when production levels exceeded 60 μg/ml in each component, neither of these procedures were necessary and a direct isocratic assay was possible. Fig. 3A shows an isocratic assay for the same SepPAK sample as was run in Fig. 3B (aridicin A = 47 μg/ml) run at 30% acetonitrile. At levels of antibiotic above 60 μg/ml the peak for aridicin A becomes substantially larger than the early eluting contaminant peak – visible in this chromatogram as tailing into the A peak. Thus, the integrator can measure its area with reasonable accuracy. This allows the use of 33% acetonitrile as the isocratic eluant (not shown) and results in a reasonable elution time for aridicin C, less than 10 min. This is currently the assay of choice for further yield improvement studies on this complex.

Improvements in aridicin production

Antibiotic production was monitored in the initial production medium P-1 using 14-liter fermentors with 10 liters working volume. HPLC analysis

showed each of the glycopeptides A, B and C to be present at less than 3 μg/ml with a total concentration of approximately 5 μg/ml (Fig. 4). Aridicin production, in common with many antibiotics, appeared to increase markedly after the period of maximum growth. The pH of the medium remained between 7 and 8 units during the course of the fermentation. Examining the medium constituents of medium P-1, there are multiple factors that could be responsible for low antibiotic yields. Most significant amongst these are the readily assimilatable forms of phosphorus (KH_2PO_4), nitrogen (NH^+_4) and carbon (glucose, glycerol). Additional studies confirmed that aridicin production could be suppressed by the inclusion of inorganic phosphate and ammonium salts in the media, as will be discussed later in relation to medium P-3. In the design of an improved medium, P-2, soybean meal was included to provide a complexed and sole source of nitrogen and phosphate. Glucose was retained, since it did not appear to directly repress aridicin production, and beet molasses was included to provide a source of betaine, a potential methyl donor to the amino terminus of the aridicin nucleus. Aridicin production was markedly improved in the new medium. Thus aridicin A was increased in yield by a factor of six, to 17 μg/ml, compared to production in medium P-1 (Fig. 4).

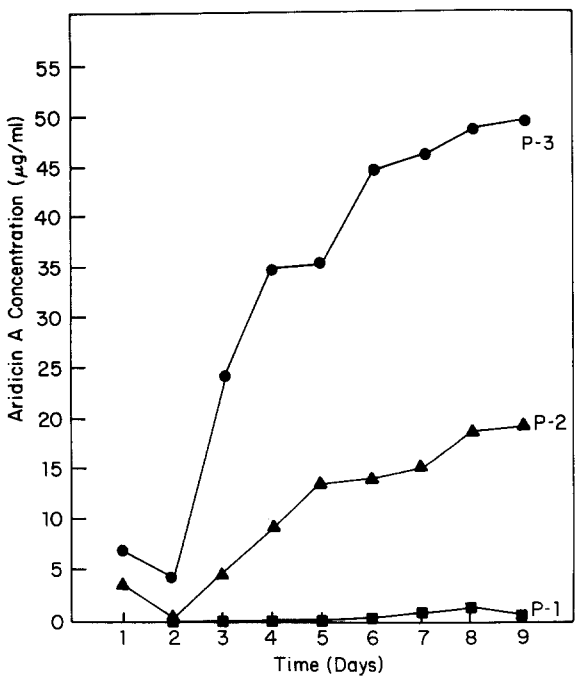

Fig. 4. Production of aridicin A in production media P-1, P-2 and P-3 in a 130-liter fermentor.

Further medium optimization was initiated on the basis of improving the pool of precursors to the aromatic amino acids in the biosynthetic pathway. Previous workers [6] have demonstrated that the dihydroxyphenylglycine portion of vancomycin is derived from acetate. The aridicins (Fig. 1) contain two of these structural components as well as a fatty acid (also derived from acetate) linked to an amino-glucuronic acid. Thus, a substrate was required that could yield an intracellular pool of acetyl-CoA.

Medium P-3 contained Estrasan No. 4, a rich source of methyl oleate that could undergo β-oxidation to provide acetyl-CoA, a precursor for the polyketide pathway. With this substrate, a substantial yield improvement was obtained with the aridicin A concentration reaching 50 μg/ml (Fig. 4). The methyl oleate may stimulate aridicin production by supplying precursors to both the dihydroxyphenylglycine and fatty acid constituents. Using this optimized medium at the 750-liter fermentor scale, the yield improvement of all the major components was high (A = 67 μg/ml, B = 57 μg/ml, C = 83 μg/ml) resulting in a total antibiotics concentration of over 200 μg/ml (Fig. 5).

In order to study the effects of pH on production, fermentation in medium P-3 was studied on the 10-liter scale using pH control. At lower pH values (6.5–7.0) production decreased markedly to 5–8 μg/ml from control levels, 18–31 μg/ml, achieved at higher pH values (7.5–8.5). During production in P-3 medium, the pH naturally rose to pH 8 and required no adjustment (Fig. 5).

Addition of ammonium chloride to medium P-3 markedly suppressed aridicin production. Partial suppression occurred at 10 mM and complete inhibition at 80 mM. Similarly, in the case of phosphate, supplementation with 10 mM or more resulted in marked inhibition of aridicin production. This supported the earlier supposition of the potential deleterious effects of these constituents in medium P-1.

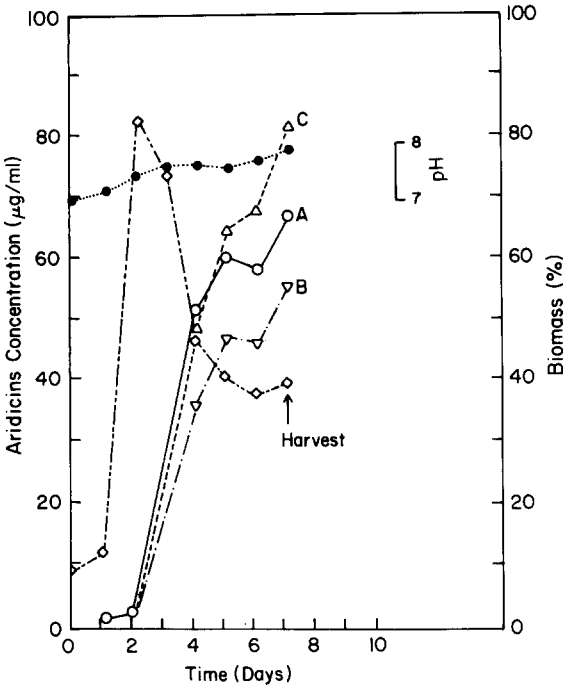

Fig. 5. Production of aridicins in medium P-3 in a 750-liter fermentor. Aridicin A (○), aridicin B (▽), aridicin C (△). Biomass (◇) is expressed in packed cell volume as a percentage of the total volume and pH (●).

CONCLUSIONS

The gradient HPLC analysis and SepPAK isolation scheme were crucial in monitoring the fermentation yield improvement of the aridicin complex and enabled the simultaneous measurement of the major aridicin components in a single 13 min assay. It has been successfully run on over 5000 fermentation samples, thus permitting the numerous medium modification studies to be undertaken, leading to the 40-fold yield improvement in antibiotic titers.

The observed production of aridicins by *K. aridum* in the presence of simple carbon sources is similar to the case of avoparcin [11] production by *Streptomyces candidus* (NRRL 3218) and that of ristocetin [5] by *Nocardia lurida*. Also, actaplanin [3] production by *Actinoplanes missouriensis* was equally supported by simple or complex carbon sources. However, the production of vancomycin [12,13] or *N*-demethyl vancomycin [2] by *Nocardia orientalis* and that of A-41030 [1] by *Streptomyces virginiae* (NRRL 15156) were markedly depressed by simple carbon sources and enhanced on complex carbohydrates. Thus, the effects of carbon sources on glycopeptide production seem highly host-specific even though the antibiotics are structurally closely related and share many of the same precursors. In the case of phosphate the deleterious effect observed on aridicin production agrees with reports for other glycopeptides, including vancomycin [12] thus suggesting a potential common repressive effect on the biosynthetic pathway. The most marked improvement in yield was obtained by the inclusion of methyl oleate, a potential precursor to the aridicin nucleus that has been found to enhance production of other glycopeptides in our laboratory.

ACKNOWLEDGEMENT

The authors wish to acknowledge G. Udowenko and R. Gerber for providing valuable technical assistance.

REFERENCES

1 Boeck, L.D., F. P. Mertz and G.M. Clem. 1985. A41030, a complex of novel glycopeptide antibiotics produced by a strain of *Streptomyces virginiae*. J. Antibiot. 38: 1–8.

2 Boeck, L.D., F.P. Mertz, R.K. Wolter and C.E. Higgins. 1984. N-demethyl vancomycin, a novel antibiotic produced by a strain of *Nocardia orientalis* taxonomy and fermentation. J. Antibiot. 37 (5): 446–453.

3 Boeck, L.D. and W.M. Stark. 1983. Actaplanin, a complex of new glycopeptide antibiotics-Fermentation. Dev. Ind. Microbiol. 25: 505–514.

4 DePhillips, P.A., A.J. Giovenella, S.F. Grappel, R.D. Hedde, P.W. Jeffs, L.B. Killmer, T.O. Lindsey, L.J. Nisbet, G.D. Roberts, K.M. Snader and R.D. Sitrin. 1985. Isolation, structural studies and biological evaluation of the major and minor components of the aridicin (AAD-216) complex. Abstr. 790, 25th Interscience Conference on Antimicrobial Agents and Chemotherapy, Minneapolis, MN, October 1985, American Society for Microbiology, Washington DC.

Sylvester. 1956–57. Ristocetin, microbiological properties. Antibiotic. Ann., 687–692.

6 Hammond, S.J., M.P. Williamson, D.H. Williams, L.D. Boeck and G.G. Marconi. 1982. On the biosynthesis of the antibiotic vancomycin. J. Chem. Soc. Chem. Commun., 344–345.

7 Hearn, M. 1983. High Performance Liquid Chromatography of Peptides. In: High-Performance Liquid Chromatography: Advances and Perspectives (Horvath, Cs., ed.), Vol. 3, p. 88, Academic Press, New York.

8 Hearn, M., F. Regnier, and T. Wehr (eds). 1983. High Performance Liquid Chromatography of Proteins and Peptides. Academic Press, New York.

9 Jeffs, P.W., G.W. Chan, R.D. Sitrin, N. Holder, G.D. Roberts, C. De Brosse. 1985. The structure of the glycolipid components of the aridicin antibiotic complex. J. Org. Chem. 50: 1726–1731.

10 Jeffs, P.W., L. Mueller, C. DeBrosse, S.L. Heald and R. Fisher. 1986. The structure of aridicin A. An integrated approach employing 2D NMR, energy minimization and distance contraints. J. Am. Chem. Soc. 108: 3063–3075.

11 Kunstmann, M.P., L.A. Mitscher, J.N. Porter, A.J. Shay and M.A. Darken. 1968. LL-AV290, A new antibiotic. Antimicrob. Agents Chemother., 242–245.

12 Mertz, F.P. and L.E. Doolin. 1973. The effect of inorganic phosphate on the biosynthesis of vancomycin. Can. J. Microbiol. 19: 263–270.

13 Pittenger, R.C. and R.B. Brigham. 1956. *Streptomyces orientalis,* n. sp., the source of Vancomycin. Antibiot. Chemother. 6: 642–647.

14 Shearer, M.C., P. Actor, B.A. Bowie, S.F. Grappel, C.H. Nash, D.J. Newman, Y.K. Oh, C.H. Pan and L.J. Nisbet. 1985. Aridicins, novel glycopeptide antibiotics. I. Taxonomy, production and biological activity. J. Antibiot. 38: 555–560.

15 Sitrin, R.D., G.W. Chan, F. Chapin, A.J. Giovenella, S.F. Grappel, P.W. Jeffs, L. Phillips, K.M. Snader and L.J. Nisbet. 1986. Aridicins, novel glycopeptide antibiotics: III. Preparation, characterization, and biological activities of aglycone derivatives. J. Antibiot. 39: 68–75.

16 Sitrin, R.D., G.W. Chan, J.J. Dingerdissen, W. Holl, J.R.E. Hoover, J.R. Valenta, L. Webb and K.M. Snader. 1985. Aridicins, novel glycopeptide antibiotics. II. Isolation and chemical characterization. J. Antibiot. 38: 561–571.