Chapter 5 Changes in the PL profile of *S. coelicolor* M145 during batch and fed-batch fermentations

5.1 Introduction to Chapter 5

Most secondary metabolite industrial production processes by streptomycetes are carried out in liquid cultures. Therefore, physiological and morphological studies are required for liquid cultures with the aim of developing more efficient ways to grow this mycelial bacterium in an industrial setting. Many reports on *Streptomyces* in submerged cultures have focused on the relationships between culture media and microbial morphology and antibiotic production (Elibol, 2004, Jonsbu *et al.*, 2002). Physiological studies have indicated that the filamentous growth habit of *Streptomyces* has been the major difficulty in large scale fermentations by members of this genus (Hobbs *et al.*, 1989). Members of the genus *Streptomyces* often grow in liquid cultures in pellet form (Williams & Entwistle, 1974). Moreover, *Streptomyces* pellets contain cells in a variety of physiological states and a proportion of the cells are nutrient-limited, even during the period of rapid growth (Hobbs *et al*., 1989). *Streptomyces* strains usually do not sporulate under submerged culture (Manteca *et al.*, 2008), however streptomycetes can be induced to sporulate in submerged culture (Glazebrook *et al*., 1990) although the sporulation process under these conditions does not involve aerial mycelium formation. Additionally, sporulation process in submerged culture is different from solid media (Karandikar *et al.*, 1997). It is difficult to compare between developmental cycles on solid substrates and in liquid culture when the patterns of morphological development are dissimilar. However, so some degree, genetic studies on development that have been carried out using solid media may be applicable for physiological studies under similar growth conditions (Kalakoutskii & Agre, 1976). Therefore, changes in the membrane components of

streptomycetes during batch fermentations may affect the morphology of the mycelium produced during that fermentation (Shahab *et al.*, 1996). This approach allows the definition of nutrient conditions that support the complex differentiation process of the organism (Karandikar *et al*., 1996). Dispersed growth of *Streptomyces* is particularly difficult to achieve in chemically defined media and previous attempts have involved either prolonged selection in chemostat culture or a complicated batch culture approach (Hobbs *et al*., 1989). The batch technique (Hodgson, 1982) is time-consuming and involves a pre-germination step in rich medium which may itself cause problems for studies involving nutrient limitation (Hobbs *et al*., 1989). Work carried out in Chapter 3 suggested that membrane PLs changed during the development of *S. coelicolor* in plate grown cultures, which caused us to wonder whether membrane PLs might change during liquid growth as well. The fermentative production of antibiotics and other secondary metabolites has used glucose as a substrate (Escalante *et al.*, 1999) and as this is the carbohydrate on which much microbial metabolism is based we decided to use this sugar as a carbon source for liquid fermentations of *S. coelicolor*.

Aims of this chapter

In this chapter, we extended our previous investigations of changes in the PL profile of *S. coelicolor* during growth on agar and submerged cultures supplemented with different concentrations of glucose. In addition to the determination of changes in the PL profile of *S. coelicolor* during the change from exponential phase to stationary phase, we also investigated the effects of a glucose pulse in simulation of a fed batch culture to gain an insight in changes in the membrane PLs that might take place during antibiotic fermentations.

5.2 Changes in the PL profile of *S. coelicolor* M145 on solid cultures at different glucose concentrations

In order to investigate whether supplementation by glucose of a commonly used-medium for growth of *S. coelicolor* had an effect on the PL profile of *S. coelicolor* we first examined the effect of glucose supplementation on solid grown cultures. A pre-germinated *S. coelicolor* M145 spore suspension (50 μl) was inoculated on cellophane discs on 20 ml YEME plates with 0.5% glycine and supplemented with no glucose (No glu), 1 g glucose l^{-1} (1 g l^{-1} glu), 10 g glucose l^{-1} (10 g l^{-1} glu), 20 g glucose l^{-1} (20 g l^{-1} glu) and with 340 g sucrose l^{-1}+10 g glucose l^{-1} (suc+10 g l^{-1} glu). Plates were incubated at 30^{o}C and harvested with a sterile razor blade. 100 mg of wet mass, equivalent to 20 mg dry weight of mycelium (after incubated wet mass at 60^{o}C over night), was isolated at 36 and 48 h in order to compare the PLs present during vegetative and aerial growth. PLs were developed by TLC to resolve the PLs under the different glucose treatments (see Materials and Methods, section 2.11) (Fig. 5.1).

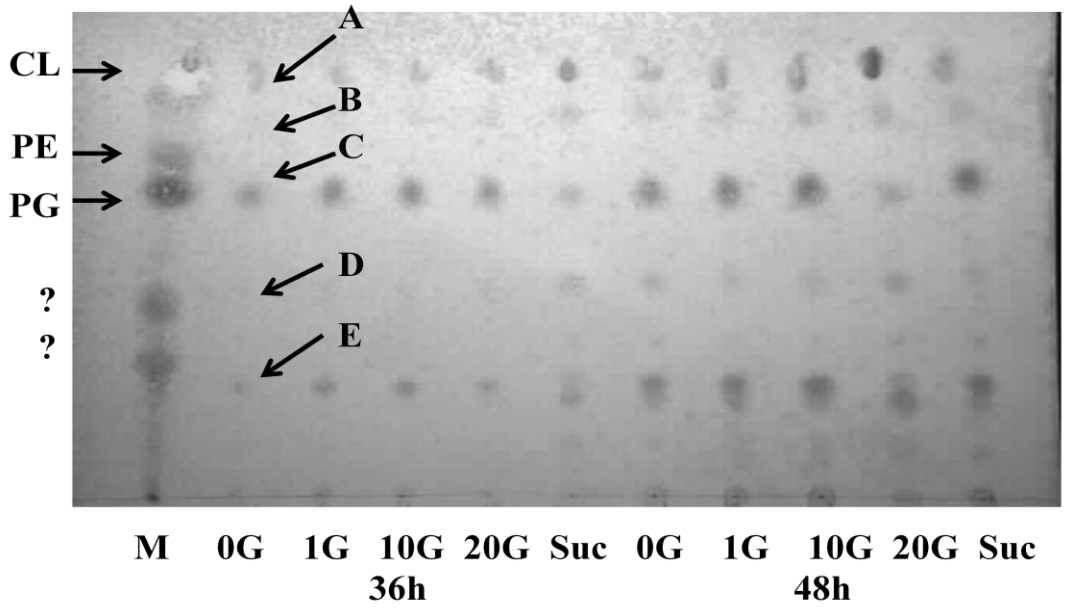

Fig. 5.1 Separation of PLs extracted from 100 mg wet mass of YEME agar-grown *S. coelicolor* M145 at different concentrations of glucose at 36 and 48 h. PLs were analyzed on silica gel TLC plate and developed in chloroform/methanol/acetic acid/water (80:12:15:4 vol/vol/vol/vol). Staining was with molybdenum blue spray. *S. coelicolor* M145 was grown on YEME agar supplemented with no glucose (0G), 1 g glucose l^{-1} (1G), 10 g glucose l^{-1} (10G), 20 g glucose l^{-1} (20G) and with 340 g sucrose l^{-1}+10 g glucose l^{-1} (Suc). Spot A is assumed to be CL; spot B, PE and spot C, PG, respectively. Two unidentified spots (D and E) were also visualised.

Sucrose is often used as a supplement for YEME in order to encourage dispersed growth of *S. coelicolor* (Kieser *et al*., 2000). However addition of 34% (w/v) sucrose is likely to present an osmotic challenge to the organism (Bishop *et al.*, 2004) that might affect membrane composition. Consequently we decided to carry out this experiment by omitting sucrose from the plates that were supplemented with glucose. We also set up a control plate that was supplemented with 34% (w/v) sucrose in addition to 10g l^{-1} glucose. PLs were extracted at 36 h and 48 of growth in order to determine the PL composition during vegetative and aerial growth respectively. After the development of

the PL profile of *S. coelicolor* M145 on solid cultures grown at different concentrations of glucose, a number of PL spots were visible (Fig. 5.1, A-E). On the basis of their similarity to the PL markers, we assumed that spots A, B and C were CL, PE and PG respectively. We were unable to predict the identity of spots D and E. Interestingly spot A, corresponding to CL, was more intense in the presence of sucrose (Fig. 5.1; 36h, Suc) with respect to PG at 36 h than in the equivalent lane grown without sucrose (Fig. 5.1 36h, 10g). This position was reversed at 48h and might reflect changes in membrane composition due to osmotic stress. There appeared to be few changes in the intensity of most spots, although spot A (CL) increased in intensity in line with glucose concentration at 48h, but remained unchanged at 36 h.

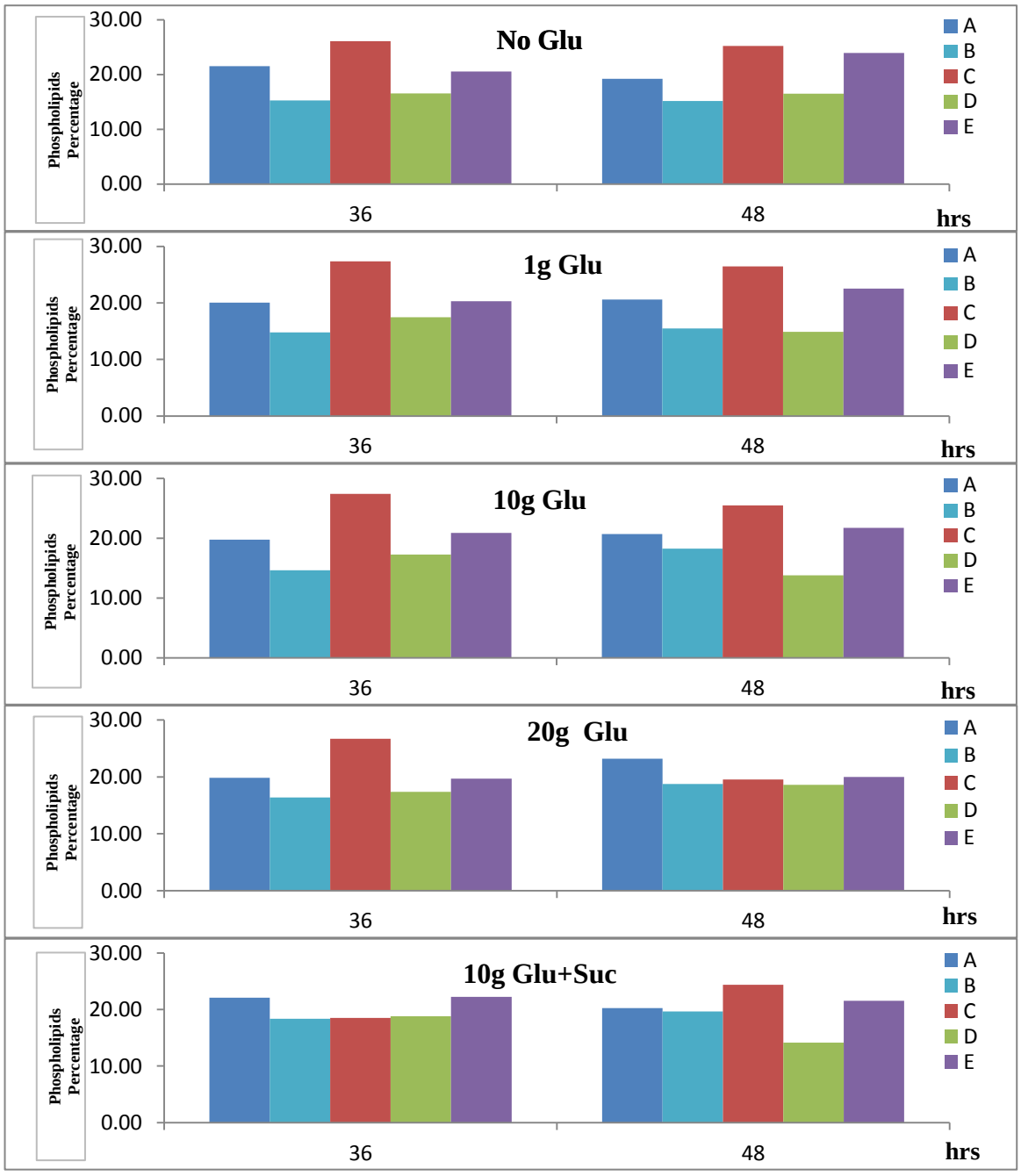

Fig. 5.2 The percentage PL composition of *S. coelicolor* grown on YEME plates. PLs were visualised by TLC (Fig. 5.1) and spots quantified by densitometry after growth at different concentration of glucose gradients: no glucose (No glu), glucose 1 g l^{-1} (1g Glu), glucose 10 g l^{-1} (10g Glu), glucose 20 g l^{-1} (20g Glu) and with sucrose 340 g l^{-1} $^{+}$ glucose 10 g l^{-1} (10g Glu+Suc). Spot A is assumed to be CL; spot B, PE and spot C, PG, respectively. Two unidentified spots (D and E) were also visualised. Spot quantification was calculated according to the following formula.

$$\text{Percentage of each PL spot} = \frac{\text{Spot intensity (pixels) X 100}}{\text{Total spot intensity for all PLs (pixels)}}$$

In order to more accurately determine changes in PL composition in response to glucose concentration, we quantified the five PL spots that were visible on the TLC plates by densitometry (Materials and Methods). In general the percentage composition of the PL profile did not change in response to changes in glucose concentration either at 36 h or 48 h (Fig. 5.2) with spot A (CL), spot C (PG) and spot D (unidentified) being the most abundant PLs. The most noticeable change however was a depression of spot C and E levels in the presence of 20g glucose at 48 h with respect to spot A (CL). This might reflect requirement of CL for development under these conditions and is consistent with genetic evidence for the requirement of *SCO1389* for aerial development (Jyothikumar *et al*., 2012). The addition of sucrose to YEME agar supplemented with sucrose and designed to simulate osmotic stress exhibited an apparent increase in the proportion of spot A (CL) at the expense of spot C (PG) at 36 h and might reflect a requirement for CL under hyperosmotic conditions.

5.3 Effect of glucose supplementation on the *S. coelicolor* PL profile during growth in liquid culture.

5.3.1 Growth and Development of PL profile of *S. coelicolor* M145 in liquid cultures at different concentrations of glucose

After investigating changes in the *S. coelicolor* PL profile during growth on YEME agar we then carried out an experiment where we analysed the PL content of liquid grown *S. coelicolor* cultured in the same medium (YEME) also supplemented with glucose (Olukoshi & Packter, 1994). The cultures *S. coelicolor* were inoculated with 50 µl of pre-germinated spore suspension inoculated into 500 ml of YEME medium in sterile baffled 2 L flasks to enhance dispersed growth with 0.5% glycine added to each flask, without sucrose and incubated at 30^{o}C with shaking (220 rpm) (Fig. 5.3). Flasks were supplemented with no glucose (No glu), glucose 1 g l^{-1} (1 g l^{-1} glu), glucose 10 g l^{-1} (10 g l^{-1} glu), glucose 20 g l^{-1} (20 g l^{-1} glu) and with sucrose 340 g l^{-1} + 10 g glucose l^{-1} (10 g l^{-1} + glu suc) in an analogous experiment to that carried out on agar described in the previous section. Three 5ml samples were collected every 3 h till the stationary phase (from 9 h until 33 h). The cells were filtered through pre weighed Whatman filter paper, the filtrate was washed with sterile distilled water three times and the filter paper was dried at 60°C for 14h and subsequently weighed. The data were processed and using the average dry cell weight as a measure of biomass, the growth curve was plotted against time (hrs). In parallel, 20 ml of the culture was also collected at the time indicated, centrifuged at 4,000 rpm for 10 minute at room temperature and PLs extracted and developed according to the Materials and Methods (section 2.11).

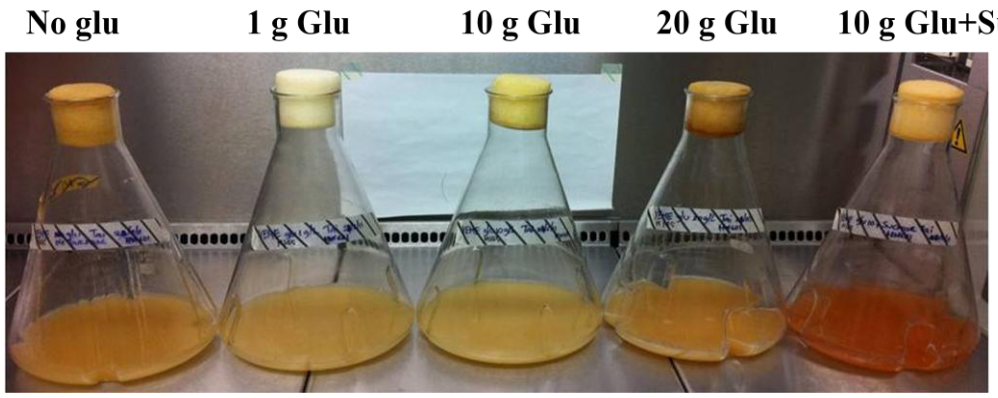

Fig. 5.3 *S. coelicolor* M145 growth in YEME grown at different concentrations of glucose. No glucose (No glu), 1 glucose g l^{-1} (1g Glu), glucose 10 g l^{-1} (10g Glu), glucose 20 g l^{-1} (20g Glu) and with sucrose 340 g l^{-1}+ glucose 10 g l^{-1} (10g Glu+Suc).

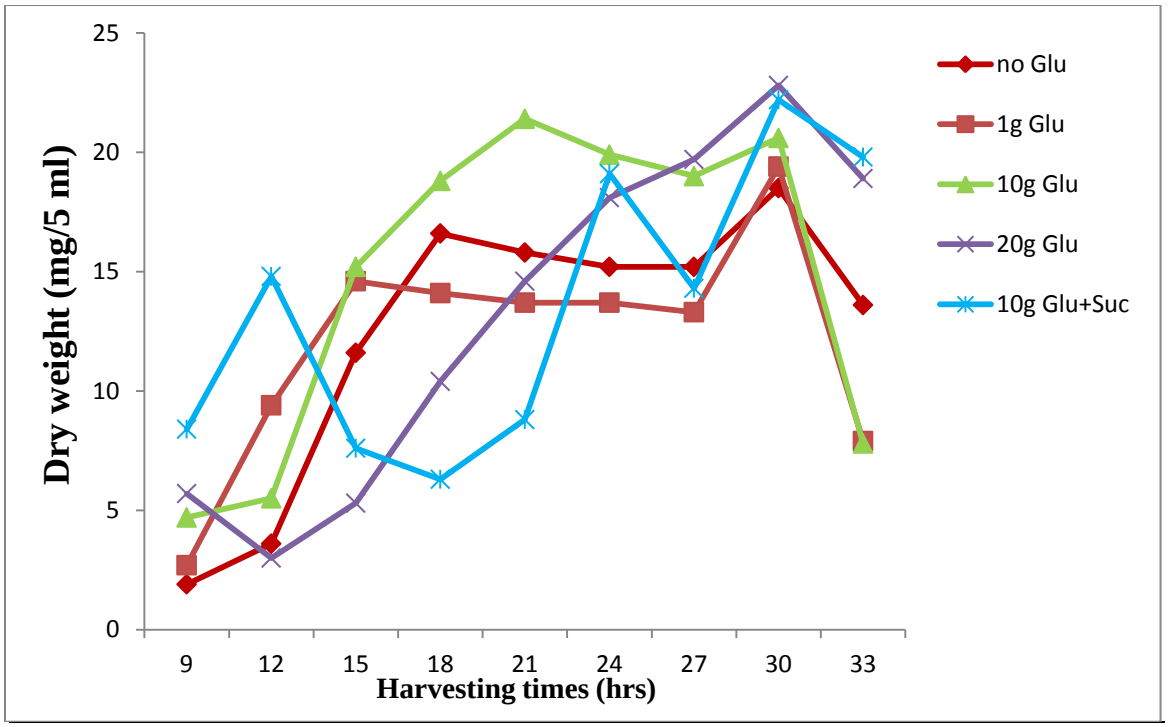

Fig. 5.4 Growth curve of *S. coelicolor* M145 in YEME medium from single sample measured every 3 h at from 9 h until 33 h. No glucose (No glu), 1 glucose g l^{-1} (1g Glu), glucose 10 g l^{-1} (10g Glu), glucose 20 g l^{-1} (20g Glu) and with sucrose 340 g l^{-1}+ glucose 10 g l^{-1} (10g Glu+Suc).

The growth curve (Fig. 5.4) was measured by cell dry weight and it was found that the culture of *S. coelicolor* supplemented with glucose 10 g l^{-1} gave the best growth. This is the recommended glucose content used in YEME broth (Kieser *et al*., 2000). Biomass increased during exponential growth (from 12-21 h) and reached a maximal level around entry to the transition phase (after 21 h), then decreased in stationary phase (24-33 h). Supplementation with no glucose and glucose 1 g l^{-1} gave a similar pattern of growth curve as glucose 10 g l^{-1} where biomass increased during exponential growth from 12-18 h and reached a maximal level around entry to the transition phase after 18 h before decreasing in stationary phase (21-33 h), presumably due to autolysis. This is consistent with exhaustion of the lower glucose content at an earlier time point in these flasks. Similarly 20 g glucose l^{-1} prolonged exponential growth from 12-30 h since this culture was supplemented with a higher concentration of glucose to extend exponential phase through the higher levels of glucose in this flask. However, the flask with 340 sucrose g l^{-1} + 10 glucose g l^{-1} gave large fluctuations in biomass due to the sticky texture of the medium; despite this, it is worth noting that the highest biomass was achieved at a consistent level with the maximal biomass value reached when *S. coelicolor* was grown without sucrose, but with the same glucose content.

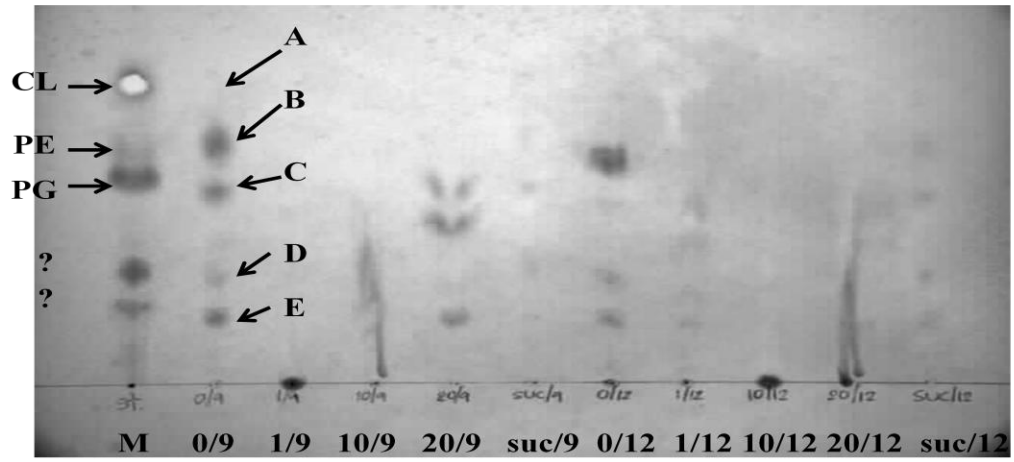

Fig. 5.5 Separation of PLs extracted from 100 mg wet mass of YEME liquid-grown *S. coelicolor* M145 at different concentrations of glucose at 9 and 12 h. PLs were analyzed on silica gel TLC plate and developed in chloroform/methanol/acetic acid/water (80:12:15:4 vol/vol/vol/vol). Staining was with molybdenum blue spray. *S. coelicolor* M145 was grown in YEME broth supplemented with no glucose (0), glucose 1 g l^{-1} (1), glucose 10 g l^{-1} (10), glucose 20 g l^{-1} (20) and with 340 sucrose g l^{-1} + glucose 10 g l^{-1} (Suc). Spot A is assumed to be CL; spot B, PE and spot C, PG, respectively. Two unidentified spots (D and E) were also visualised.

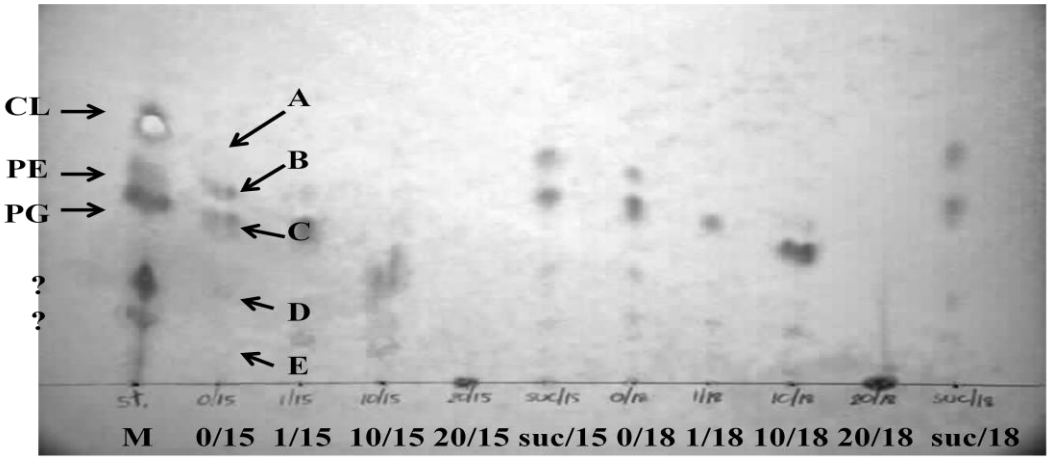

Fig. 5.6 Separation of PLs extracted from 100 mg wet mass of YEME liquid-grown *S. coelicolor* M145 at different concentrations of glucose at 15 and 18 h. PLs were analyzed on silica gel TLC plate and developed in chloroform/methanol/acetic acid/water (80:12:15:4 vol/vol/vol/vol). Staining was with molybdenum blue spray. *S. coelicolor* M145 was grown in YEME broth supplemented with no glucose (0), glucose 1 g l^{-1} (1), glucose 10 g l^{-1} (10), glucose 20 g l^{-1} (20) and with 340 sucrose g l^{-1} + glucose 10 g l^{-1} (Suc). Spot A is assumed to be CL; spot B, PE and spot C, PG, respectively. Two unidentified spots (D and E) were also visualised.

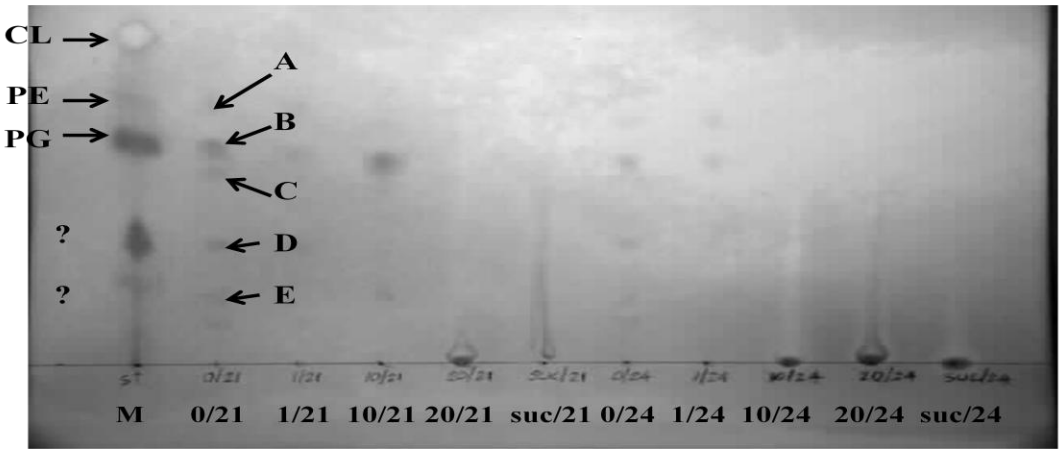

Fig. 5.7 Separation of PLs extracted from 100 mg wet mass of YEME liquid-grown *S. coelicolor* M145 at different concentrations of glucose at 21 and 24 h. PLs were analyzed on silica gel TLC plate and developed in chloroform/methanol/acetic acid/water (80:12:15:4 vol/vol/vol/vol). Staining was with molybdenum blue spray. *S. coelicolor* M145 was grown in YEME broth supplemented with no glucose (0), glucose 1 g l^{-1} (1), glucose 10 g l^{-1} (10), glucose 20 g l^{-1} (20) and with 340 sucrose g l^{-1} + glucose 10 g l^{-1} (Suc). Spot A is assumed to be CL; spot B, PE and spot C, PG, respectively. Two unidentified spots (D and E) were also visualised.

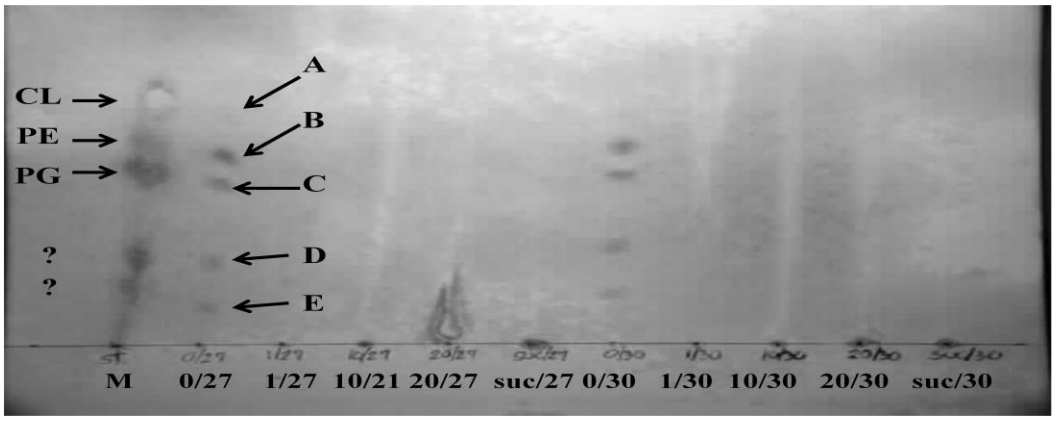

Fig. 5.8 Separation of PLs extracted from 100 mg wet mass of YEME liquid-grown *S. coelicolor* M145 at different concentrations of glucose at 27 and 30 h. PLs were analyzed on silica gel TLC plate and developed in chloroform/methanol/acetic acid/water (80:12:15:4 vol/vol/vol/vol). Staining was with molybdenum blue spray. *S. coelicolor* M145 was grown in YEME broth supplemented with no glucose (0), glucose 1 g l^{-1} (1), glucose 10 g l^{-1} (10), glucose 20 g l^{-1} (20) and with 340 sucrose g l^{-1} + glucose 10 g l^{-1} (Suc). Spot A is assumed to be CL; spot B, PE and spot C, PG, respectively. Two unidentified spots (D and E) were also visualised.

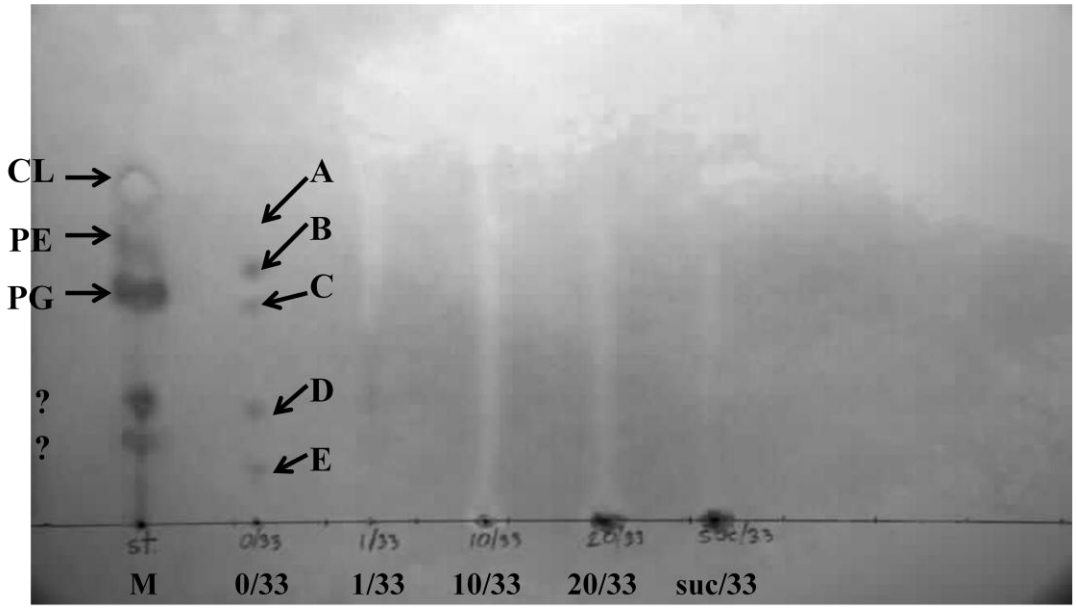

Fig. 5.9 Separation of PLs extracted from 100 mg wet mass of YEME liquid-grown *S. coelicolor* M145 at different concentrations of glucose at 33 h. PLs were analyzed on silica gel TLC plate and developed in chloroform/methanol/acetic acid/water (80:12:15:4 vol/vol/vol/vol). Staining was with molybdenum blue spray. *S. coelicolor* M145 was grown in YEME broth supplemented with no glucose (0), glucose 1 g l^{-1} (1), glucose 10 g l^{-1} (10), glucose 20 g l^{-1} (20) and with 340 sucrose g l^{-1} + glucose 10 g l^{-1} (Suc). Spot A is assumed to be CL; spot B, PE and spot C, PG, respectively. Two unidentified spots (D and E) were also visualised.

In parallel to the determination of the *S. coelicolor* growth curve in YEME supplemented with different concentrations of glucose, we also analysed the PL content of the organism at each sample point (every 3 h) (Fig. 5.5-5.9). It was impossible to resolve PL spots in all samples with the exception of cultures grown in the absence of glucose. The reason for this is unclear, but we believe the appearance of the red pigment (undecylprodigiosin) from the antibiotic affected the migration of PL on the TLC plate. Although spots B, C, D and E could be resolved in lanes loaded with extracts from YEME containing no glucose it was impossible to visualise a spot that equated to CL

(spot A). As it was impossible to visualise PL spots on TLC plates form any extracts except those that were taken from the culture grown in the absence of glucose, it was only possible to analyse changes in the PL profile over time from this culture. Spot A, assumed to be CL, could not be visualised in this experiment and indeed the proportion of all PL spots did not change a great deal. Following spot quantification by densitometry (data not shown). The major change that could be visualised over the course of this experiment was that the proportion of spot D increased during the growth of *S. coelicolor* in batch culture. Although it was not possible to identify this PL spot, the fact that increases in relation to time with respect to the major membrane PLs, PE (spot B) and PG (spot C) suggests that this spot maybe a precursor molecule such as PA that might be expected to increase as exponential shifts to stationary phase and consequently growth slows. As mycelial growth slows, membrane synthesis will also slow, presumably resulting in a reduced requirement for PG or PE. It might be expected therefore that this would result in a precursor PL such as PA.

5.4 Changes in the PL profile of *S. coelicolor* M145 in liquid cultures grown as batch and fed-batch cultures (Olukoshi & Packter, 1994)

To further study the effect of glucose concentration on the PL profile of *S. coelicolor* liquid cultures, we also investigated the effect of adding a pulse of glucose during late exponential phase in simulation of a fed batch culture. Cultures of *S. coelicolor* were inoculated as 50 µl of pre-germinated spores in 500 ml of YEME medium in sterile baffled 2 L flasks grown with 0.5% glycine in each flask, without sucrose and containing either 10 g l^{-1} (two flasks) and 20 g l^{-1}glucose (two flasks). These flasks incubated at 30^{o}C with shaking (220 rpm). When the cultures reached stationary phase, after 36 h incubation, a feed of glucose 10 g l^{-1} was added to one 10 g l^{-1} flask and one 20 g l^{-1} flasks and all four flasks incubated further. This resulted in four flasks, one batch culture supplemented with 10 g l^{-1} glucose (10gB) and one batch culture supplemented with 20 g l^{-1} glucose (20gB). The third flask was a fed batch culture initially containing 10 g l^{-1} glucose, but fed with 10 g l^{-1} glucose after 36 h (10gFB). The fourth flask was also a fed batch culture initially containing 20 g l^{-1}, but fed with 10 g l^{-1} glucose after 36 h (20gFB).

Three, 5ml samples were collected at 9, 16, 24, 36, 44, 48, 64, 72, 84 and 90 h that were then filtered through pre weighed Whatman filter paper and the filtrate was washed with sterile distilled water three times. The filter paper was dried at 60°C for 14h and subsequently weighed (Fig. 5.10). The data were processed and using the average dry cell biomass, the growth curve was plotted against time (hrs). In parallel, 20 ml of the culture was also collected at the time indicated, centrifuged at 4000 rpm for 10 min at

room temperature and PLs extracted and developed according to the Materials and Methods (section 2.11).

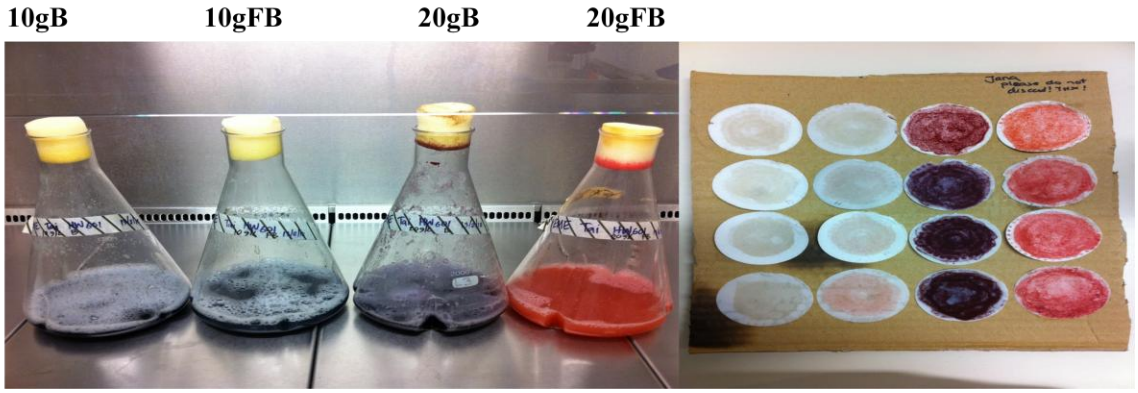

Fig. 5.10 Growth of *S. coelicolor* in YEME when grown as batch and fed batch cultures. Four flasks were inoculated, one batch culture supplemented with 10 g l^{-1} glucose (10gB) and one batch culture supplemented with 20 g l^{-1} glucose (20gB). The third flask was a fed batch culture initially containing10 g l^{-1} glucose, but fed with 10 g l^{-1} glucose after 36 h (10gFB). The fourth flask was also a fed batch culture initially containing 20 g l^{-1}, but fed with 10 g l^{-1} glucose after 36 h (20gFB).

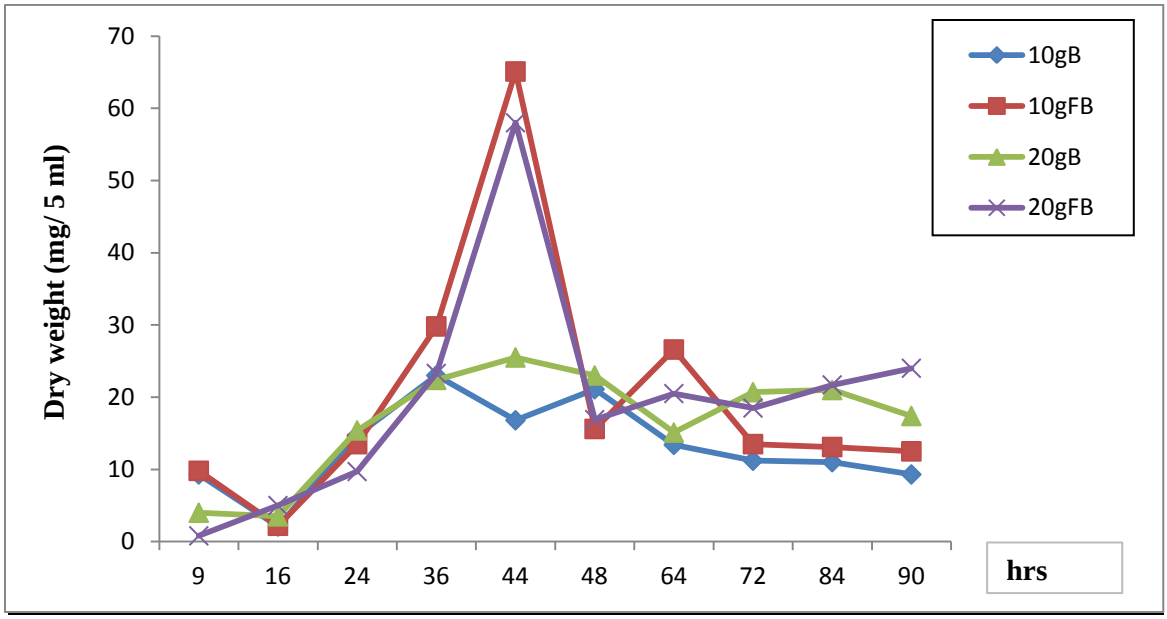

Fig. 5.11 Growth curve of *S. coelicolor* M145 in YEME medium from single sample and measured at 9, 16, 24, 36, 44, 48, 64, 72, 84 and 90 h. Four flasks were inoculated, one batch culture supplemented with 10 g l^{-1} glucose (10gB) and one batch culture supplemented with 20 g l^{-1} glucose (20gB). The third flask was a fed batch culture initially containing10 g l^{-1} glucose, but fed with 10 g l^{-1} glucose after 36 h (10gFB). The fourth flask was also a fed batch culture initially containing 20 g l^{-1}, but fed with 10 g l^{-1} glucose after 36 h (20gFB).

The growth curve (Fig. 5.11) was measured by cell dry weight and it was found that all cultures of *S. coelicolor* grew similarly for the first part of the experiment irrespective of glucose levels from 16-36 h. However after feeding with glucose, flasks 10gFB and 20gFB showed an increase in biomass at 44 h, before the amount of biomass decreased for the remainder of the experiment; presumably this decrease in biomass was due to autolysis brought about by nutrient depletion. In contrast, the batch cultures 10gB and 20gB gave the expected pattern of growth with biomass increasing during the exponential phase (16-36 h), after which it reached a maximal level around entry to the transition phase (after 44 h) before decreasing in stationary phase (48-90 h).

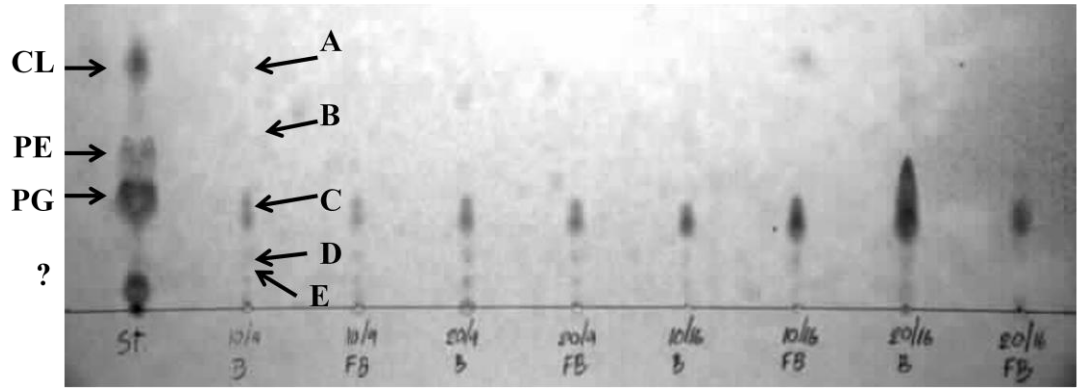

Fig. 5.12 Separation of PLs extracted from 100 mg wet mass of YEME liquid-grown *S. coelicolor* grown as batch and fed-batch cultures at 9 and 16 h. Four flasks were inoculated, one batch culture supplemented with 10 g l^{-1} glucose (10gB) and one batch culture supplemented with 20 g l^{-1} glucose (20gB). The third flask was a fed batch culture initially containing10 g l^{-1} glucose, but fed with 10 g l^{-1} glucose after 36 h (10gFB). The fourth flask was also a fed batch culture initially containing 20 g l^{-1}, but fed with 10 g l^{-1} glucose after 36 h (20gFB).

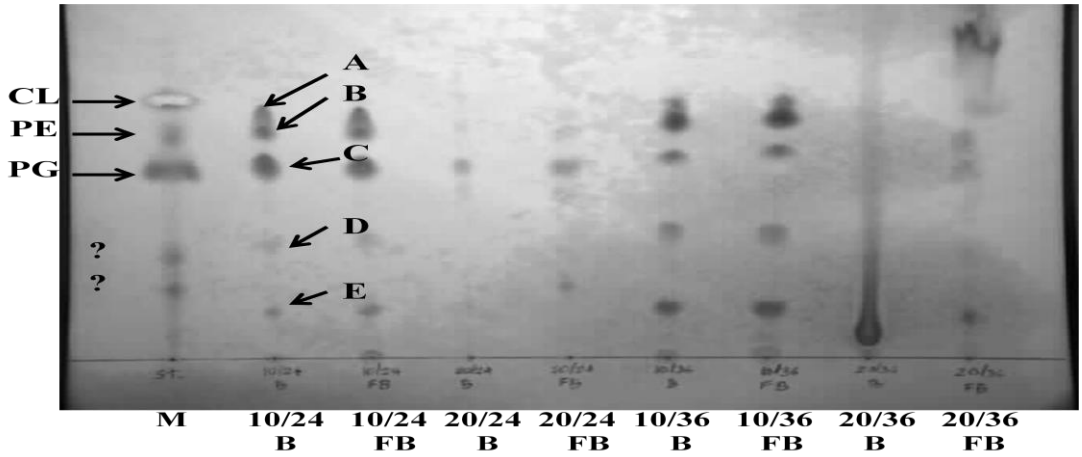

Fig. 5.13 Separation of PLs extracted from 100 mg wet mass h of YEME liquid-grown *S. coelicolor* grown as batch and fed-batch cultures at 24 and 36. Four flasks were inoculated, one batch culture supplemented with 10 g l^{-1} glucose (10gB) and one batch culture supplemented with 20 g l^{-1} glucose (20gB). The third flask was a fed batch culture initially containing10 g l^{-1} glucose, but fed with 10 g l^{-1} glucose after 36 h (10gFB). The fourth flask was also a fed batch culture initially containing 20 g l^{-1}, but fed with 10 g l^{-1} glucose after 36 h (20gFB).

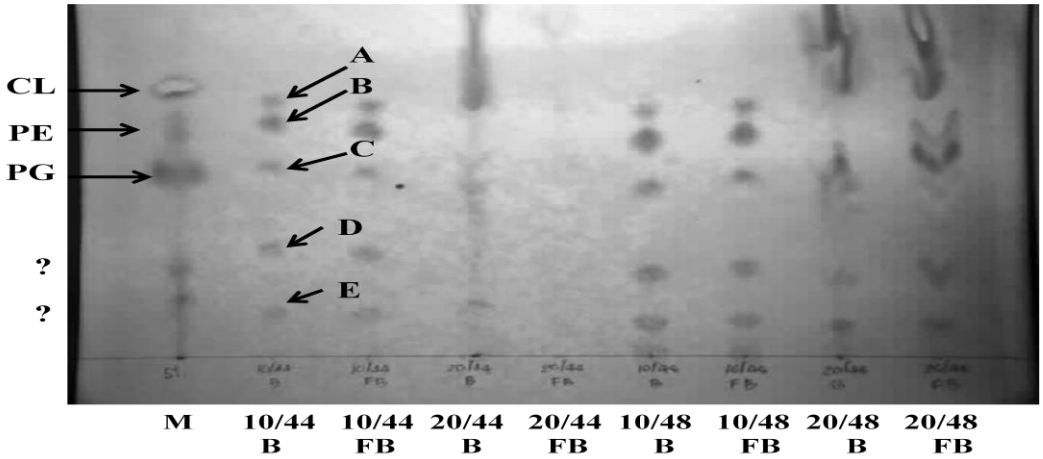

Fig. 5.14 Separation of PLs extracted from 100 mg wet mass of YEME liquid-grown *S. coelicolor* grown as batch and fed-batch cultures at 44 and 48 h. Four flasks were inoculated, one batch culture supplemented with 10 g l^{-1} glucose (10gB) and one batch culture supplemented with 20 g l^{-1} glucose (20gB). The third flask was a fed batch culture initially containing10 g l^{-1} glucose, but fed with 10 g l^{-1} glucose after 36 h (10gFB). The fourth flask was also a fed batch culture initially containing 20 g l^{-1}, but fed with 10 g l^{-1} glucose after 36 h (20gFB).

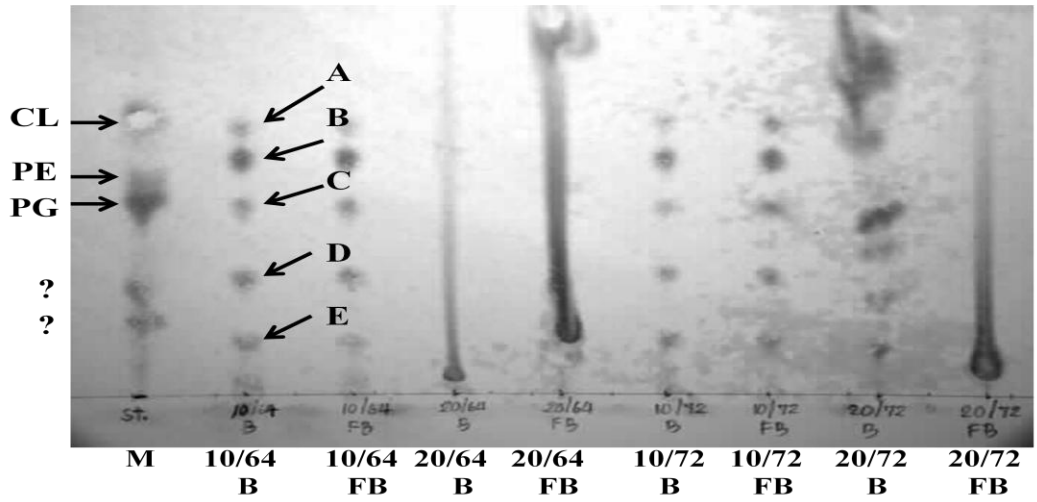

Fig. 5.15 Separation of PLs extracted from 100 mg wet mass of YEME liquid-grown *S. coelicolor* grown as batch and fed-batch cultures at 64 and 72 h. Four flasks were inoculated, one batch culture supplemented with 10 g l^{-1} glucose (10gB) and one batch culture supplemented with 20 g l^{-1} glucose (20gB). The third flask was a fed batch culture initially containing10 g l^{-1} glucose, but fed with 10 g l^{-1} glucose after 36 h (10gFB). The fourth flask was also a fed batch culture initially containing 20 g l^{-1}, but fed with 10 g l^{-1} glucose after 36 h (20gFB).

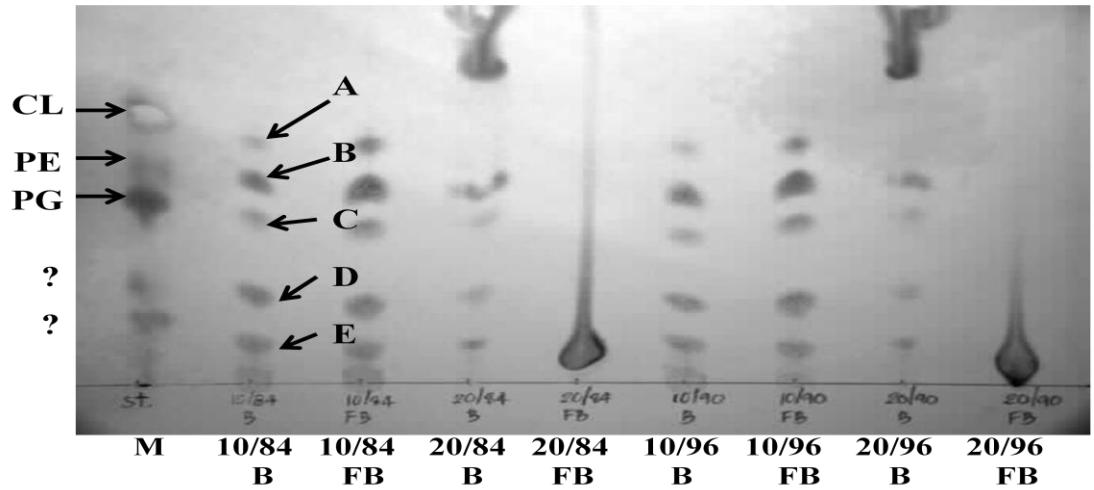

Fig. 5.16 Separation of PLs extracted from 100 mg wet mass h of YEME liquid-grown *S. coelicolor* grown as batch and fed-batch cultures at 84 and 96. Four flasks were inoculated, one batch culture supplemented with 10 g l^{-1} glucose (10gB) and one batch culture supplemented with 20 g l^{-1} glucose (20gB). The third flask was a fed batch culture initially containing10 g l^{-1} glucose, but fed with 10 g l^{-1} glucose after 36 h (10gFB). The fourth flask was also a fed batch culture initially containing 20 g l^{-1}, but fed with 10 g l^{-1} glucose after 36 h (20gFB).

PLs were extracted and developed by TLC, as shown in Fig. 5.12-5.16 and, as was seen in previous experiments, five spots could be visualised (A-E). Spot A was assumed to be CL, spot B, PE and spot PG on the basis of their similarity with the PL markers resolved on the TLC plate. We were unable to identify spots D and E. It was not possible to identify spots grow in the presence of 20 g l^{-1} due to the poor PL resolution of extracts taken from these cultures. This was especially true of the later time points where high levels of undecylprodigiosin in the PL extracts interfered with PL migration.

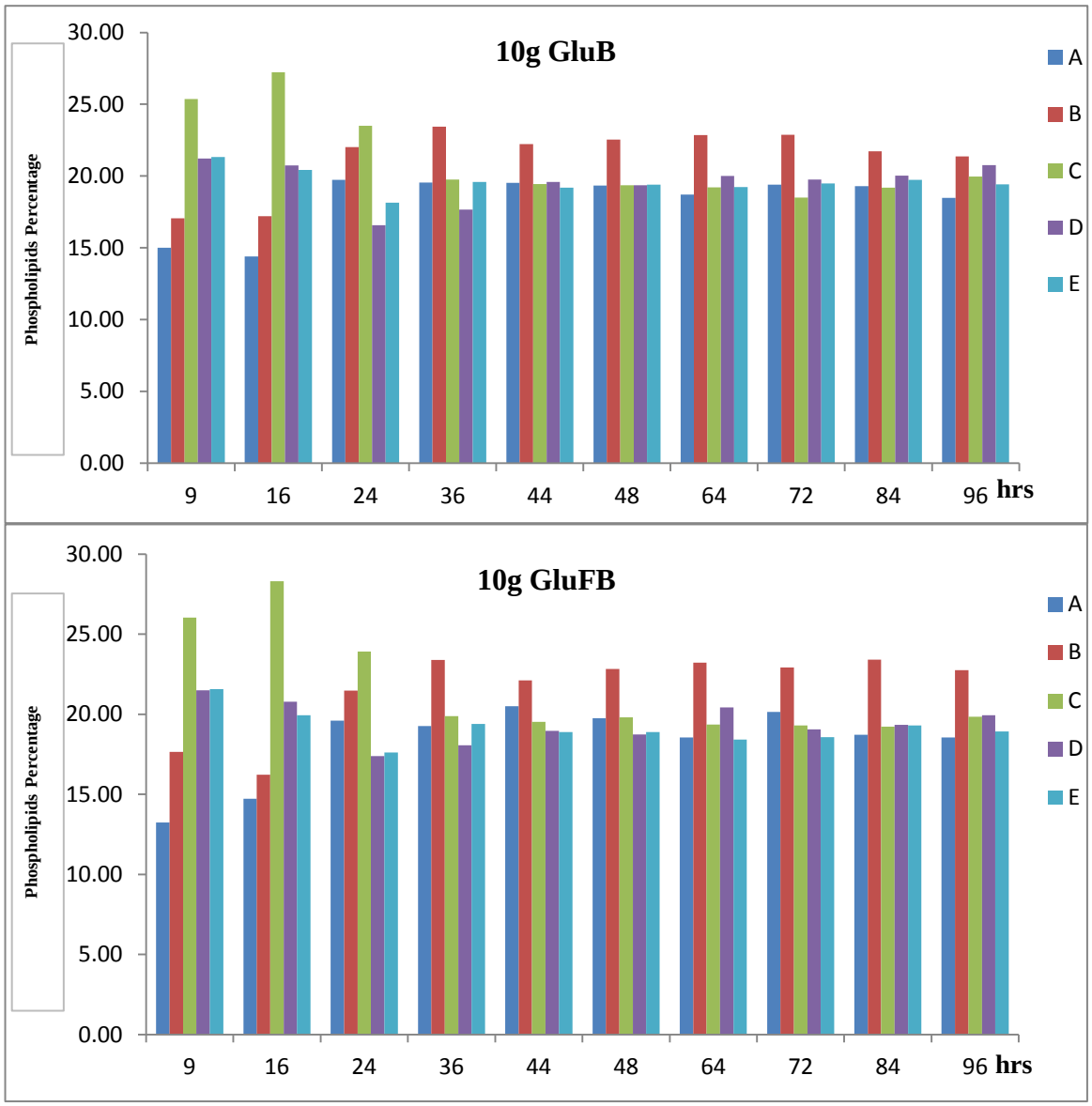

Fig. 5.17 Percentage of PLs extracted from 100 mg wet mass of *S. coelicolor* in YEME liquid-grown from single sample as batch and fed-batch cultures. Four flasks were inoculated, one batch culture supplemented with 10 g l^{-1} glucose (10gB) and one batch culture supplemented with 20 g l^{-1} glucose (20gB) (data not shown since it was not possible to identify spots grow). The third flask was a fed batch culture initially containing10 g l^{-1} glucose, but fed with 10 g l^{-1} glucose after 36 h (10gFB). The fourth flask was also a fed batch culture initially containing 20 g l^{-1}, but fed with 10 g l^{-1} glucose after 36 h (20gFB) (data not shown since it was not possible to identify spots grow).

In parallel to the determination of the *S. coelicolor* growth curve in batch and fed batch YEME cultures supplemented with glucose, we also analysed the PL content of the organism at each sample point (every 3 h) (Figs. 5.12-5.16). It was impossible to visualise spots on TLC plates from cultures grown at a glucose concentration of 20g l^{-1}, so it was only possible to quantify spots by densitometry for the cultures supplemented with 10g l^{-1} glucose (Fig. 5.17). During the early part of the experiments spot C, assumed to be PG was the dominant PL, but as time increased, spot B, that was assumed to be PE, replaced the former spot as the dominant PL. Spot A, assumed to be CL, also increased as a proportion of total PLs during the course of the experiment. Interestingly the changes in PL abundance took place during exponential phase and there were no significant changes after 36h. This is important as the culture 10gFB was fed with glucose at 36h; this pulse of glucose did not therefore cause any changes in the PL content of the cell. This suggests that glucose is not the determining factor in controlling the relative PL abundance in the cell membrane of *S. coelicolor*.

5.5 Conclusions to Chapter 5

For the purpose of extending our earlier studies on the changes in the PL profile of *S. coelicolor* during development, we moved from 3MA or minimal medium plus mannitol. This medium is a good vehicle for the study of morphological development but, despite this, is a poor medium for the growth of this organism in liquid culture (Kieser *et al*., 2000). The reason for this is that it is very difficult to obtain dispersed growth of *S. coelicolor* based on minimal medium. As a result, we moved to YEME based medium; this medium, when supplemented with agar, supports morphological development of *S. coelicolor*. In addition, when used in its liquid form, YEME can support dispersed growth of *S. coelicolor* and has been routinely used for preparation of DNA and antibiotics from this organism. As a result, we first investigated the changes in the *S. coelicolor* PL profile on YEME agar by extracting PLs at time points designed to coincide with vegetative and aerial growth. In this experiment we also investigated the effect of glucose concentration in order to discover if this important nutrient source played a role in the determination of the PL content of the membrane. In general the percentage composition of the PL profile did not change in response to changes in glucose concentration (Fig 5.1), although CL levels were evaluated at 48 h in the presence of 20 g ml^{-1} glucose and might reflect a requirement of CL for development under these conditions. This is consistent with genetic evidence for the requirement of *SCO1389* for aerial development (Jyothikumar *et al*., 2012). Sucrose is often used as a supplement for YEME in order to encourage dispersed growth of *S. coelicolor* in liquid (Kieser *et al*., 2000). However addition of 34% (w/v) sucrose is likely to present an

osmotic challenge to the organism (Bishop *et al*., 2004) that might affect membrane composition. Interestingly a spot, corresponding to CL, was more intense in the presence of sucrose with respect to PG at 36 h than in the equivalent lane grown without sucrose (Fig. 5.1). This position was reversed at 48h and might reflect changes in membrane composition due to osmotic stress. In *E. coli* CL is known to control the response of the cell to osmotic stress through its role in the localization of ProP (Romantsov *et al.*, 2007, Romantsov *et al.*, 2008). The fact that the CL content of *S. coelicolor* is enhanced by the presence of high levels of sucrose on YEME agar suggests that the CL content of this organism may also be involved to in adaptation to osmotic stress.

We attempted to recreate this experiment in liquid culture for the purpose of comparing the PL profiles of *S. coelicolor* in both solid and liquid grown YEME cultures. It was impossible to resolve PL spots in all samples with the exception of cultures grown in the absence of glucose. The reason for this is unclear, but we believe the appearance of the red pigment (undecylprodigiosin) affected the migration of PL on the TLC plate. However an unidentified PL spot increased in relation to the major membrane PLs, PE and PG with time suggesting that this spot maybe a precursor molecule such as PA that might be expected to increase as exponential shifts to stationary phase and consequently growth slows. As mycelial growth slows, membrane synthesis will also slow, presumably resulting in a reduced requirement for PG or PE. It might be expected therefore that this would result in a precursor PL such as PA.

As antibiotic fermentations are often carried out as fed-batch fermentations we were interested in discovering whether the PL profile of *S. coelicolor* changed after

feeding with a glucose nutrient source. During this experiment, it was not possible to identify spots grow in the presence of 20 g l^{-1} due to the poor PL resolution of extracts taken from these cultures. This was especially true of the later time points where high levels of undecylprodigiosin in the PL extracts interfered with PL migration and it was impossible to visualise spots on TLC plates from cultures grown at a glucose concentration of 20 g l^{-1}, so it was only possible to quantify spots by densitometry for the cultures supplemented with 10 g l^{-1} glucose (Fig. 5.17). During the early part of the experiment PG was the dominant PL, but as time increased, PE replaced the former spot as the dominant PL. CL also increased as a proportion of total PLs during the course of the experiment. Few changes in PL abundance took place after the glucose feed at 36 h suggesting that glucose is not the determining factor in controlling the relative PL abundance in the cell membrane during liquid fermentations of *S. coelicolor*. At least on the basis of the results presented here, it seems unlikely that feeding with glucose is likely to affect membrane CL content, the mycelial architecture and, as a result, the behaviour in industrial fermentations through reduced pellet formation (Manteca *et al*., 2008, van Wezel *et al.*, 2005).