APPENDIX 1

CELLULAR WIDTH, LENGTH AND VOLUME MEASUREMENTS FOR *Pseudomonas aeruginosa* PAO (DSM 1707) AND *Pseudomonas aeruginosa* TRANSFORMED WITH THE pALacZsd REPORTER VECTOR

Table 7.1: Cell length, cell width and cell volume for *P. aeruginosa* planktonic, SIP and biofilm cells grown for 16 h in the presence of glass wool in LB broth

	Planktonic Culture			SIP Culture			Biofilm Culture		
P. aeruginosa PAO (DSM 1707)	Length	Width	Volume	Length	Width	Volume	Length	Width	Volume
	3.423	0.952	2.383	3.624	0.949	2.494	4.103	1.028	3.385
	3.526	0.795	1.595	3.923	1.044	3.101	3.693	0.997	2.639
	3.457	0.789	1.591	3.703	0.929	2.354	4.291	1.063	3.609
Average	3.468	0.846	1.856	3.750	0.974	2.650	4.029	1.029	3.211
SD	0.712	0.145	0.858	0.619	0.152	1.033	0.612	0.235	1.674
P. aeruginosa pALacZsd	Length	Width	Volume	Length	Width	Volume	Length	Width	Volume
	4.138	1.024	3.152	3.257	0.962	2.162	3.224	0.850	2.259
	3.902	0.849	2.045	3.810	0.890	2.209	3.247	0.791	1.765
	4.041	0.785	1.853	3.919	0.896	2.299	3.476	0.883	1.954
Average	4.027	0.886	2.350	3.662	0.916	2.223	3.316	0.841	1.993
SD	0.714	0.148	0.903	0.635	0.105	0.592	0.588	0.123	0.917

Table 7.2: Cell length, cell width and cell volume for *P. aeruginosa* planktonic, SIP and biofilm cells grown for 16 h in the presence of glass wool in LB broth with 0.7 M NaCl (LB + NaCl)

	Planktonic Culture			SIP Culture			Biofilm Culture		
P. aeruginosa PAO (DSM 1707)	Length	Width	Volume	Length	Width	Volume	Length	Width	Volume
	3.234	0.836	1.596	3.070	1.067	2.456	3.032	0.926	1.813
	3.484	0.885	1.992	3.390	0.974	2,377	2.694	0.822	1.288
	4.325	0.864	2.568	3.552	0.954	2.348	3.078	0.844	1.554
Average	3.681	0.861	2.052	3.337	0.998	2.394	2.935	0.864	1.552
SD	1.137	0.108	1.095	0.768	0.154	0.909	0.537	0.110	0.432
P. aeruginosa pALacZsd	Length	Width	Volume	Length	Width	Volume	Length	Width	Volume
	3.418	0.761	1.404	4.985	0.823	2.573	3.547	0.754	1.540
	3.705	0.798	1.746	5.417	0.873	3.356	3.657	0.747	1.592
	3.071	0.865	1.653	4.256	0.857	2.294	3.550	0.811	1.695
Average	3.398	0.808	1.601	4.904	0.851	2.741	3.585	0.771	1.609
SD	0.923	0.101	0.544	2.181	0.159	1.818	1.101	0.112	0.738

Table 7.3: Cell length, cell width and cell volume for *P. aeruginosa* planktonic, SIP and biofilm cells grown for 16 h in the presence of glass wool in LB broth with 2.5% (v/v) ethanol (LB + EtOH)

	Planktonic Culture			SIP Culture			Biofilm Culture		
P. aeruginosa PAO (DSM 1707)	Length	Width	Volume	Length	Width	Volume	Length	Width	Volume
	4.534	0.950	3.050	4.556	0.966	3.146	4.157	0.933	2.700
	5.804	0.881	3.346	4.937	0.948	3.263	3.818	0.889	2.214
	4.177	0.867	2.497	4.692	0.928	2.960	4.005	0.881	2.372
Average	4.838	0.899	2.964	4.728	0.947	3.123	3.993	0.901	2.429
SD	1.457	0.142	1.205	1.354	0.130	1.185	0.844	0.124	0.924
P. aeruginosa pALacZsd	Length	Width	Volume	Length	Width	Volume	Length	Width	Volume
	5.422	0.739	2.259	4.078	0.700	1.503	4.053	0.997	2.868
	4.183	0.746	1.765	5.092	0.802	2.426	3.377	0.814	1.608
	3.747	0.826	1.594	4.371	0.830	2.280	3.544	0.785	1.587
Average	4.451	0.771	1.993	4.514	0.777	2.070	3.658	0.865	2.021
SD	1.512	0.124	0.917	1.097	0.113	0.822	0.699	0.106	0.836

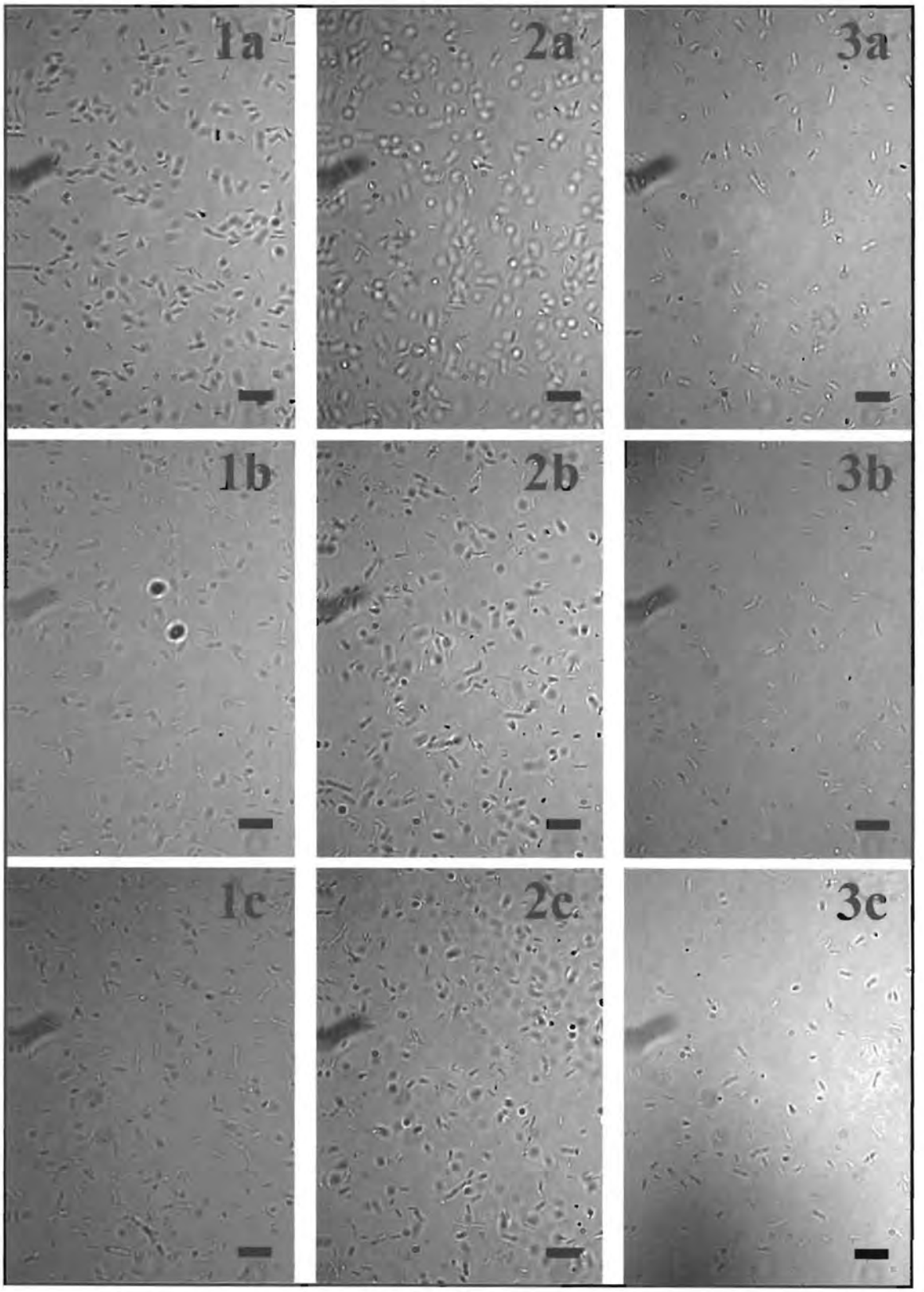

Fig. 7.1: Triplicate photomicrographs (a – c) showing *P. aeruginosa* PAO (DSM 1707) planktonic cells after 16 h of incubation in LB-S. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 µm.

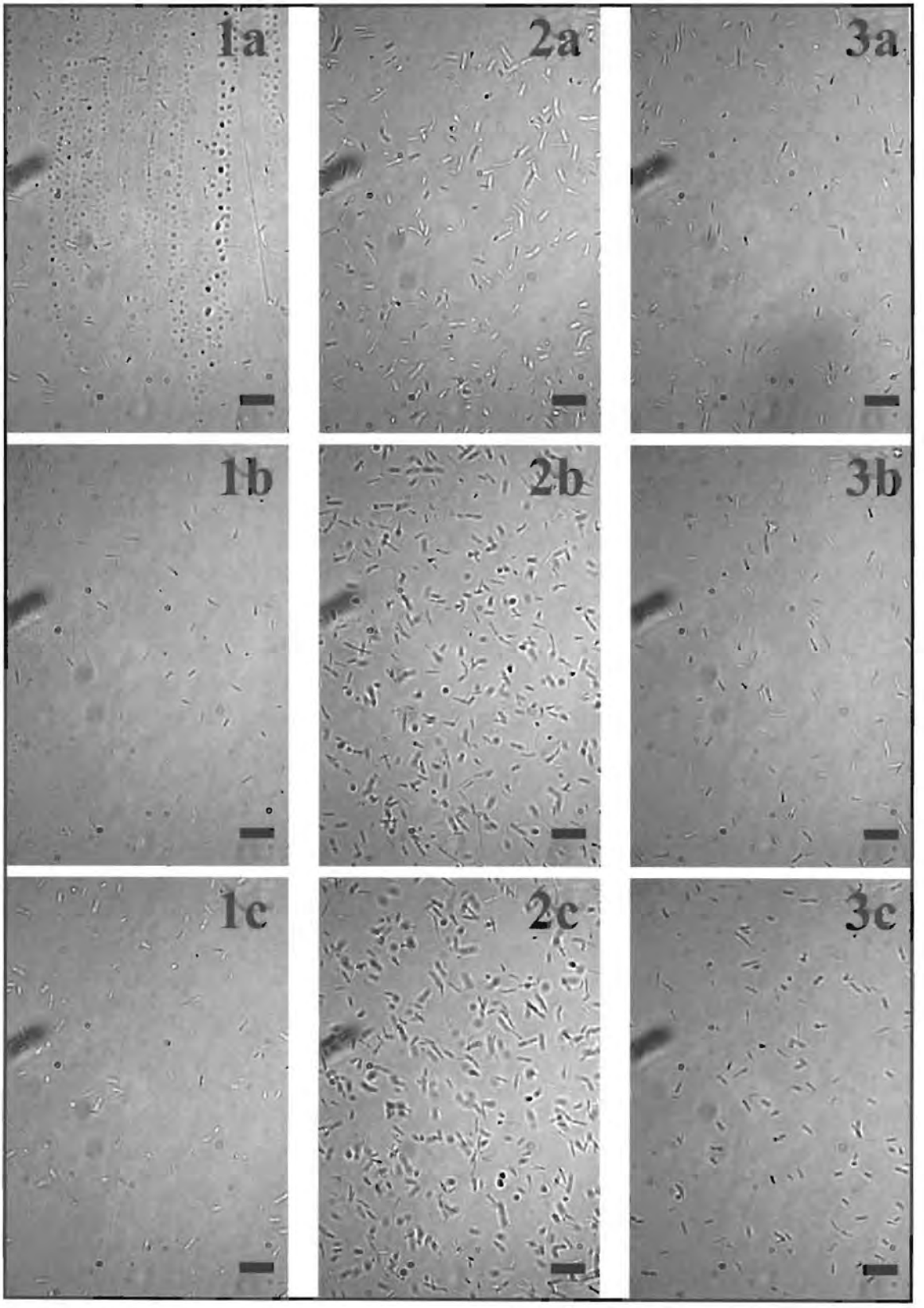

Fig. 7.2: Triplicate photomicrographs (a – c) showing *P. aeruginosa* PAO (DSM 1707) SIP cells after 16 h of incubation in LB-S. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 μm.

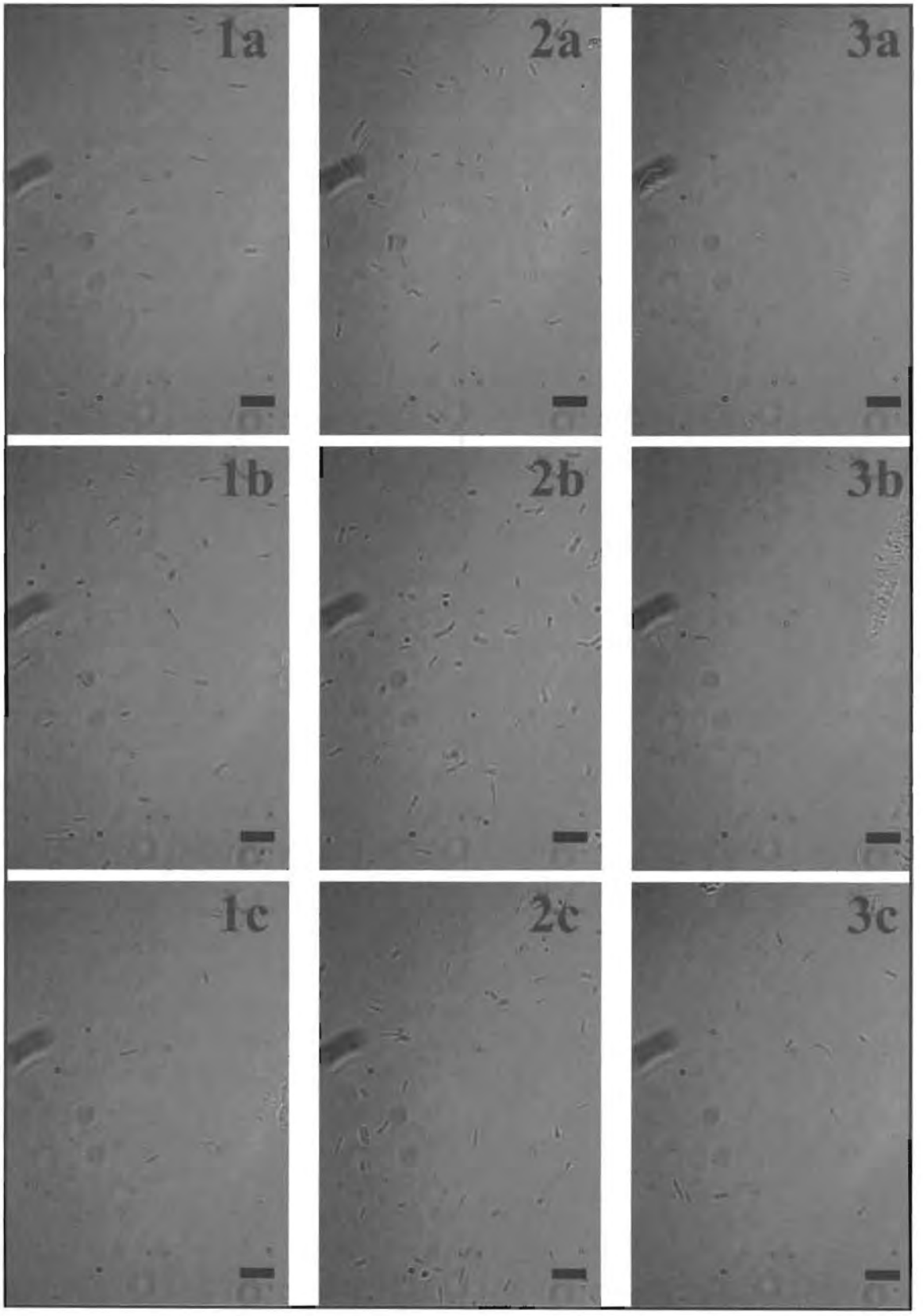

Fig. 7.3: Triplicate photomicrographs (a – c) showing *P. aeruginosa* PAO (DSM 1707) biofilm cells after 16 h of incubation in LB-S. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 µm.

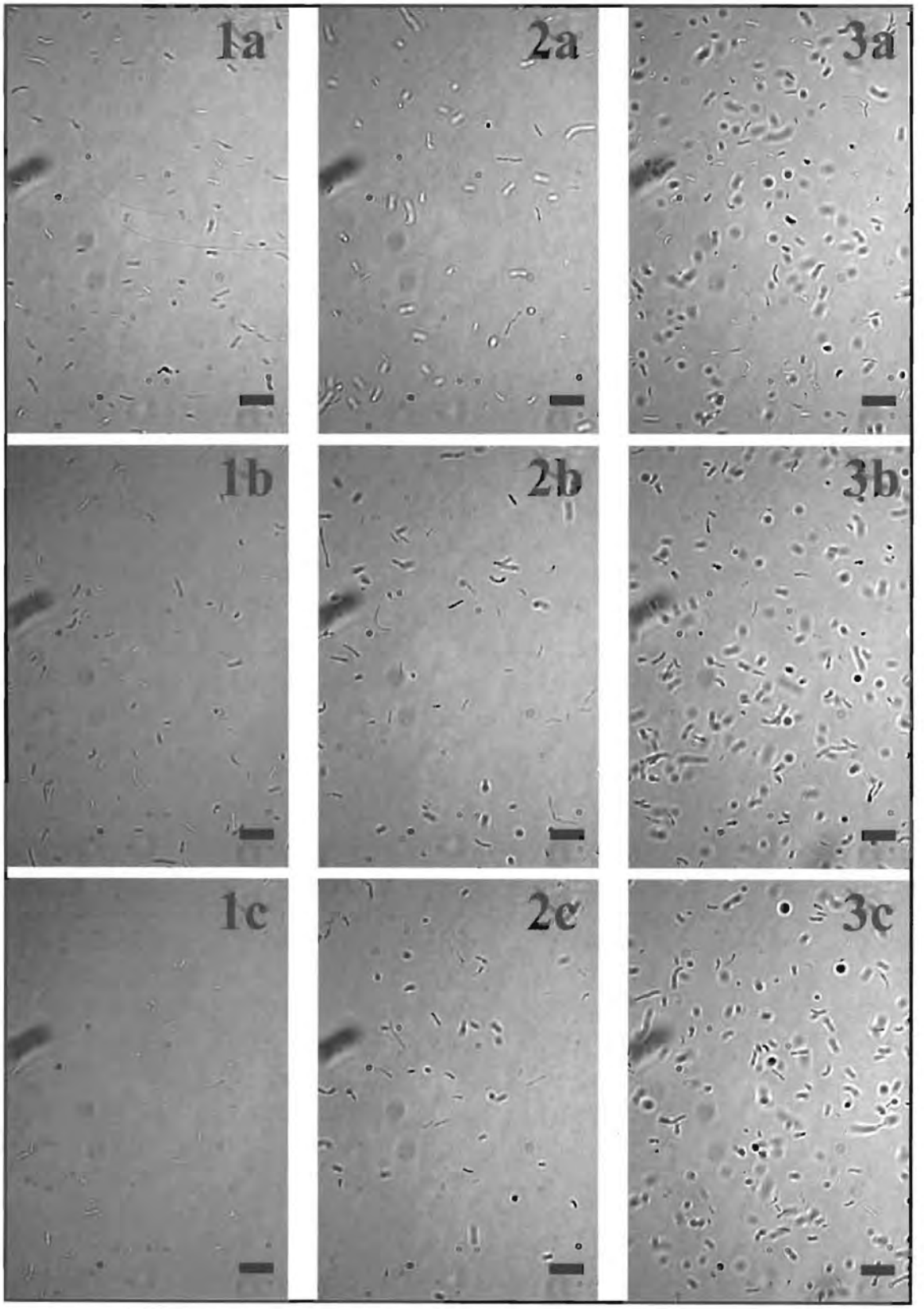

Fig. 7.4: Triplicate photomicrographs (a – c) showing *P. aeruginosa* PAO (DSM 1707) planktonic cells after 16 h of incubation in LB + NaCl. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 µm.

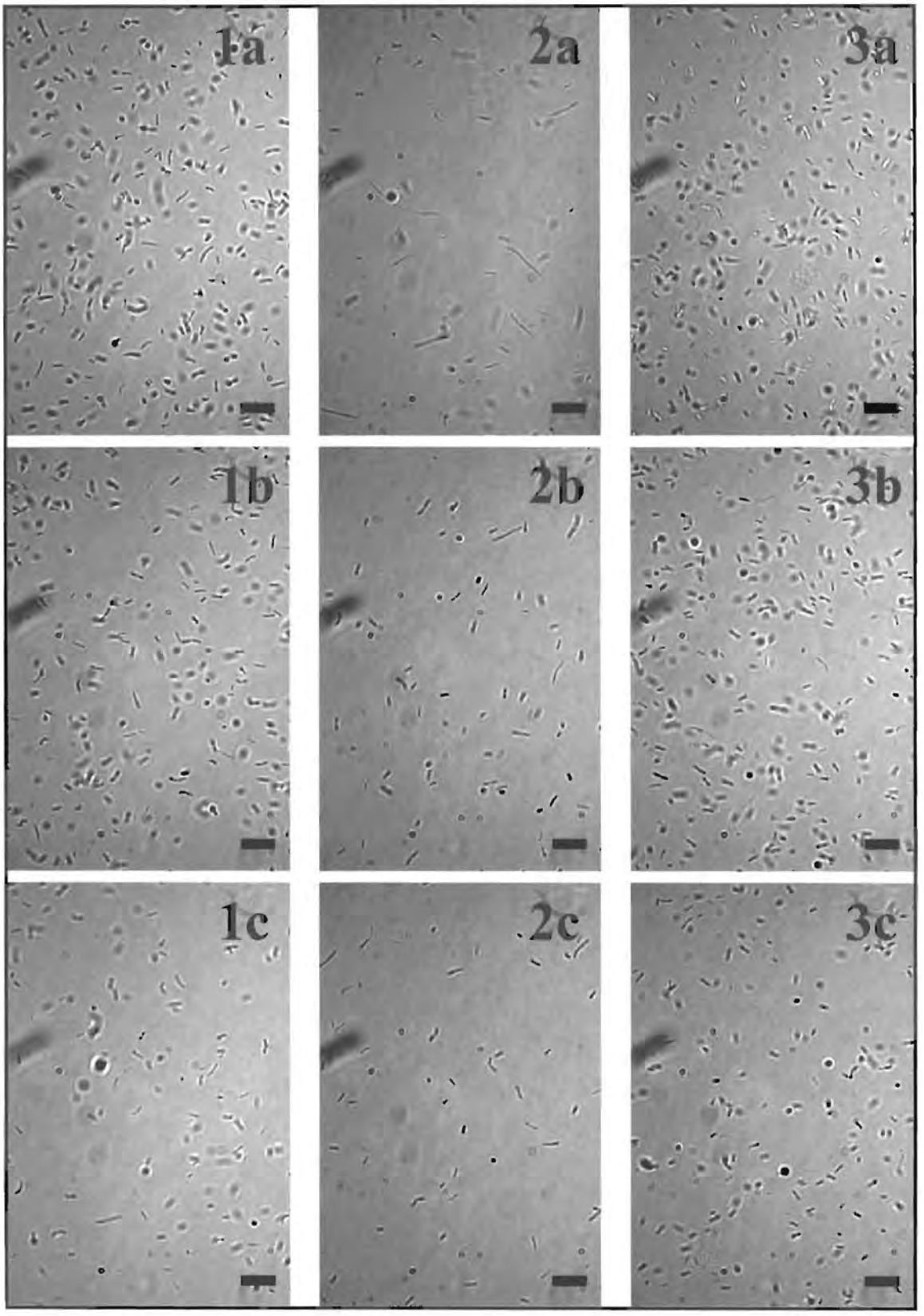

Fig. 7.5: Triplicate photomicrographs (a – c) showing *P. aeruginosa* PAO (DSM 1707) SIP cells after 16 h of incubation in LB + NaCl. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 μm.

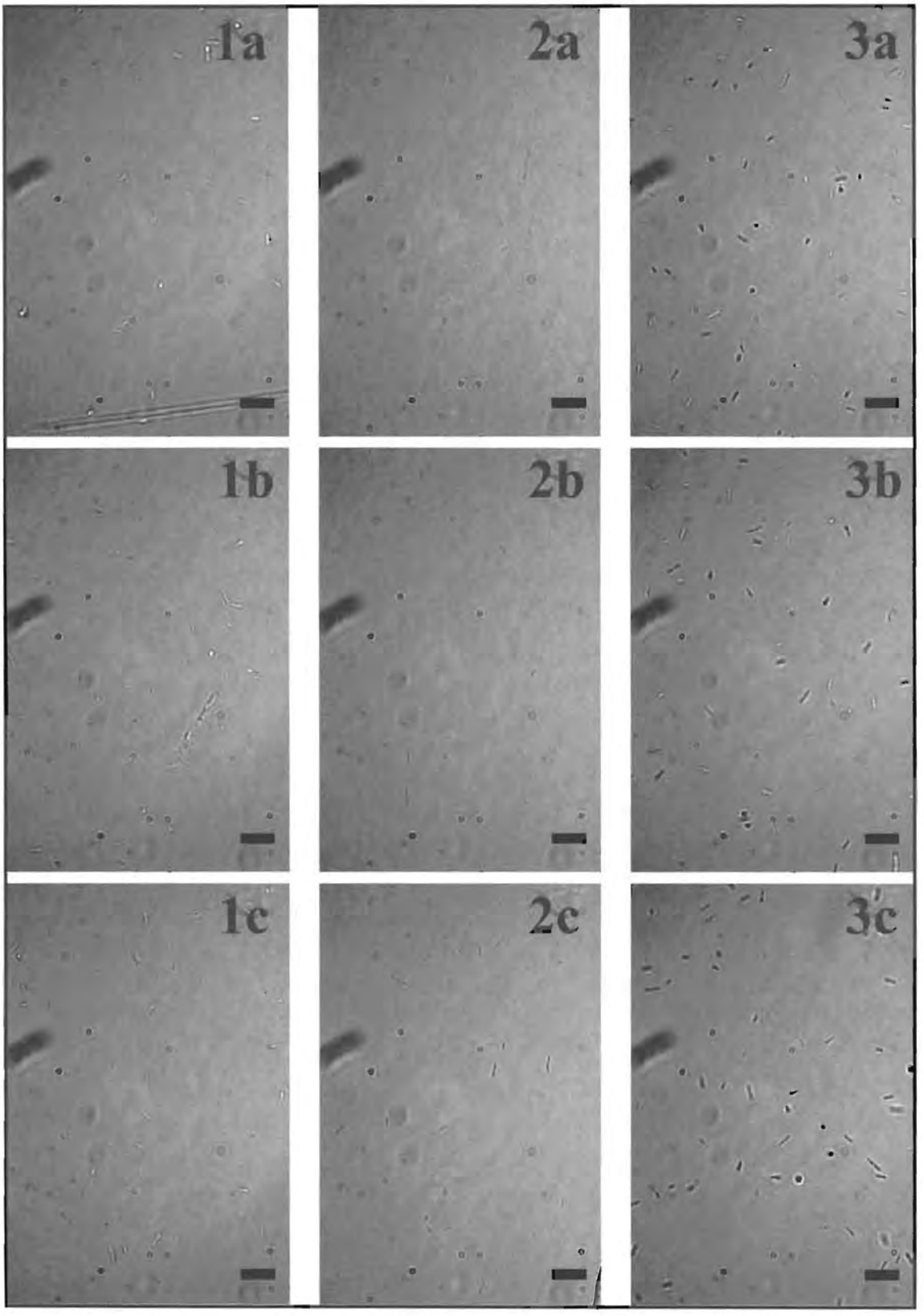

Fig. 7.6: Triplicate photomicrographs (a – c) showing *P. aeruginosa* PAO (DSM 1707) biofilm cells after 16 h of incubation in LB + NaCl. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 µm.

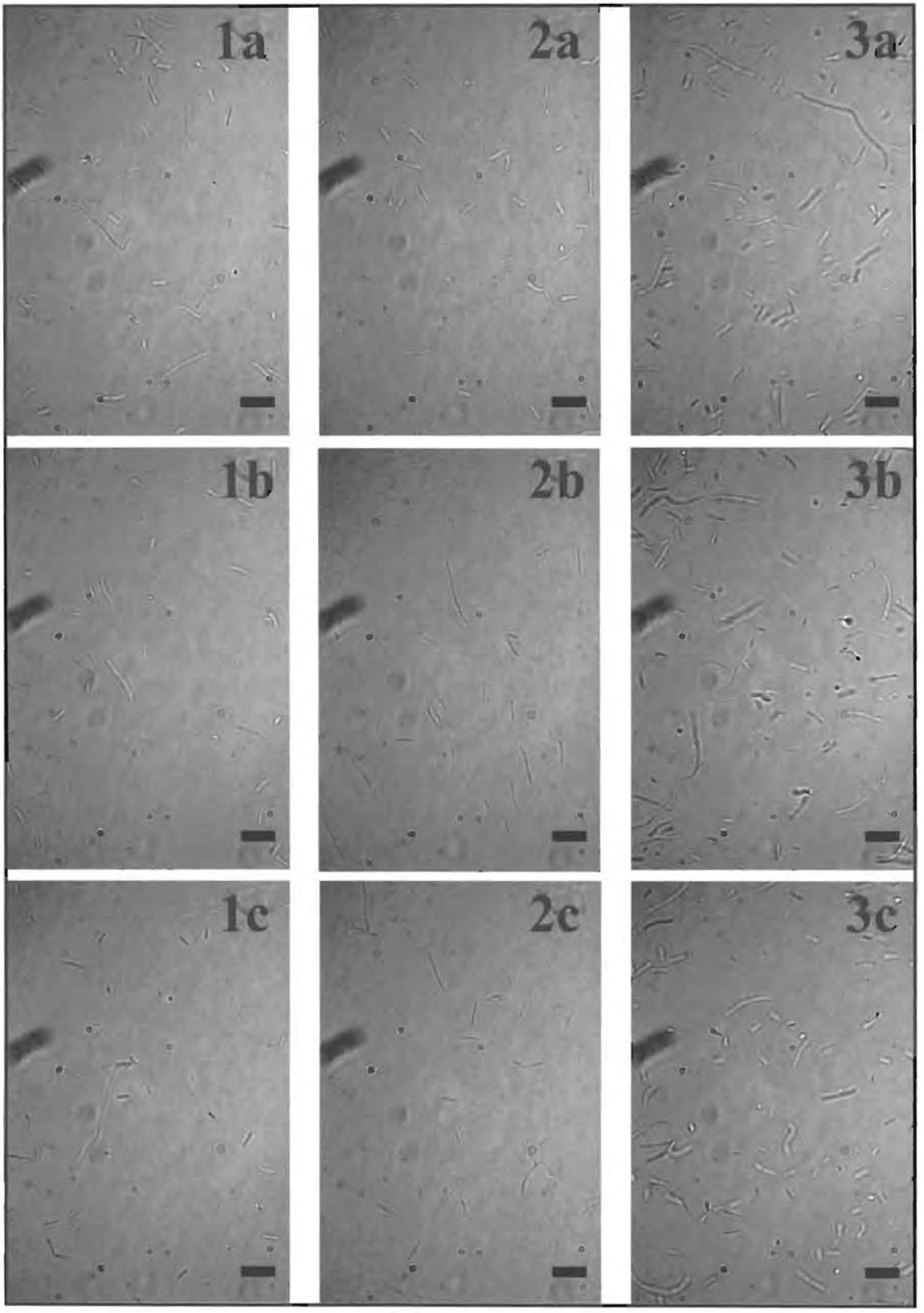

Fig. 7.7: Triplicate photomicrographs (a – c) showing *P. aeruginosa* PAO (DSM 1707) planktonic cells after 16 h of incubation in LB + EtOH. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 µm.

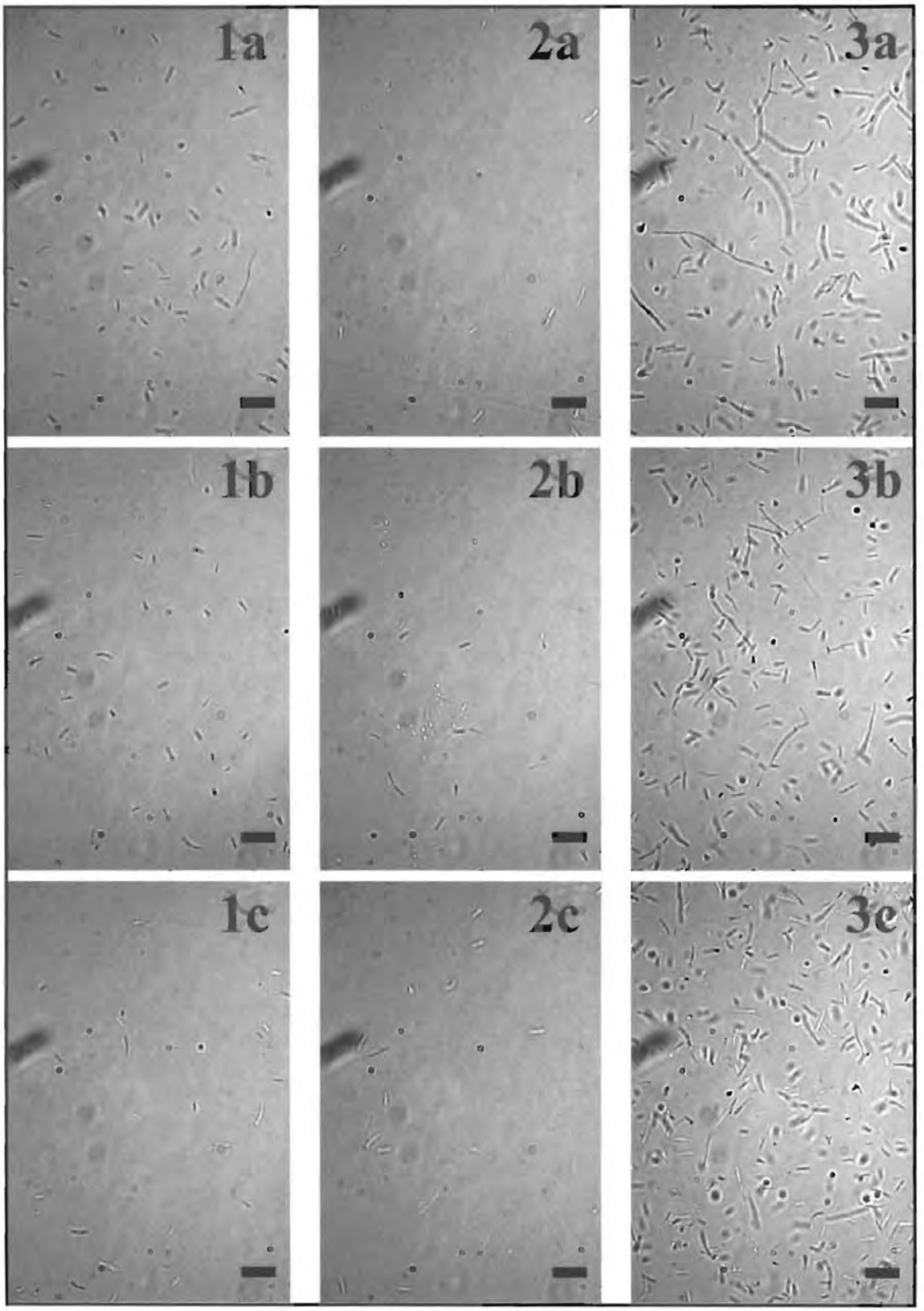

Fig. 7.8: Triplicate photomicrographs (a – c) showing *P. aeruginosa* PAO (DSM 1707) SIP cells after 16 h of incubation in LB + EtOH. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 µm.

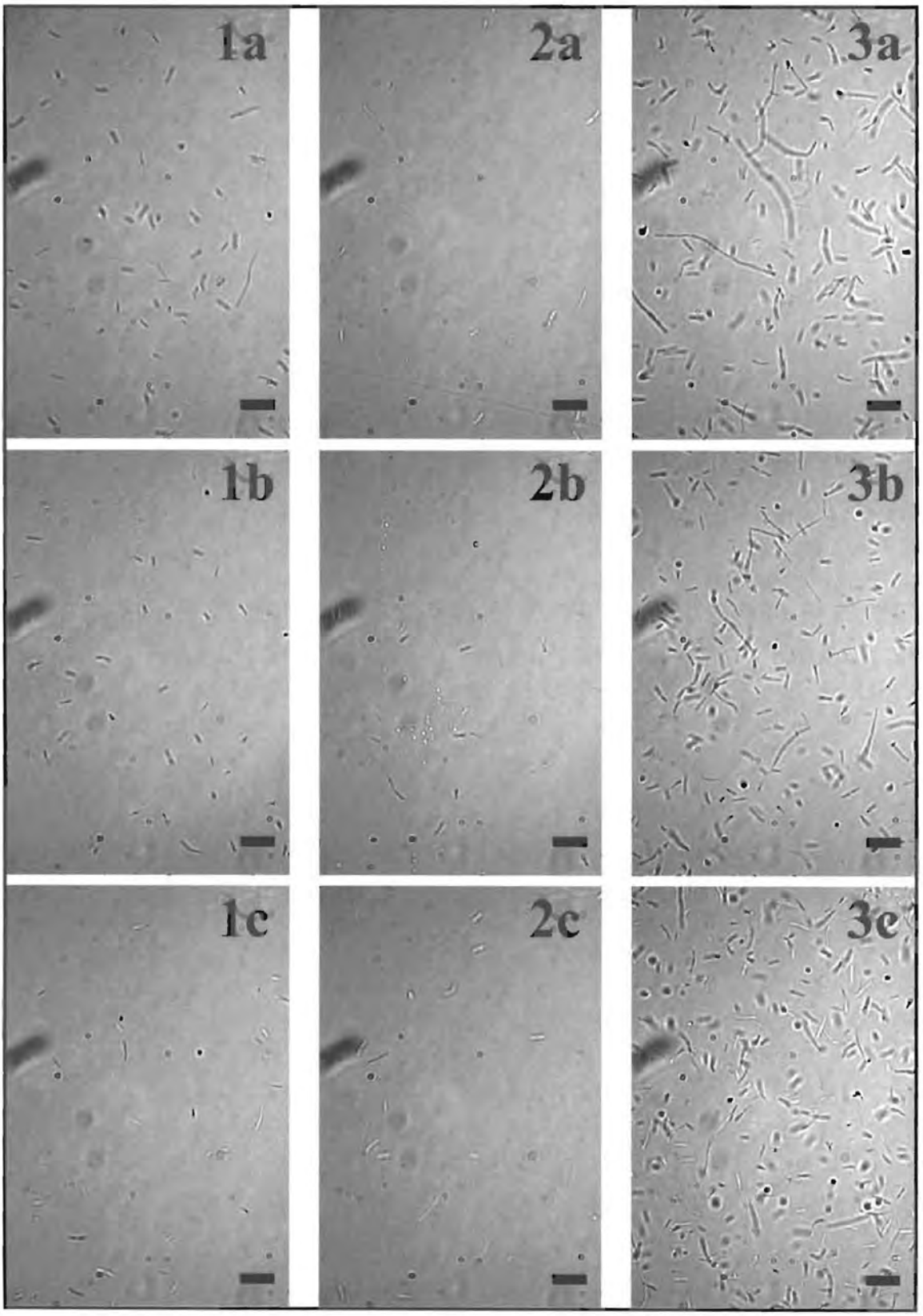

Fig. 7.9: Triplicate photomicrographs (a – c) showing *P. aeruginosa* PAO (DSM 1707) biofilm cells after 16 h of incubation in LB + EtOH. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 µm.

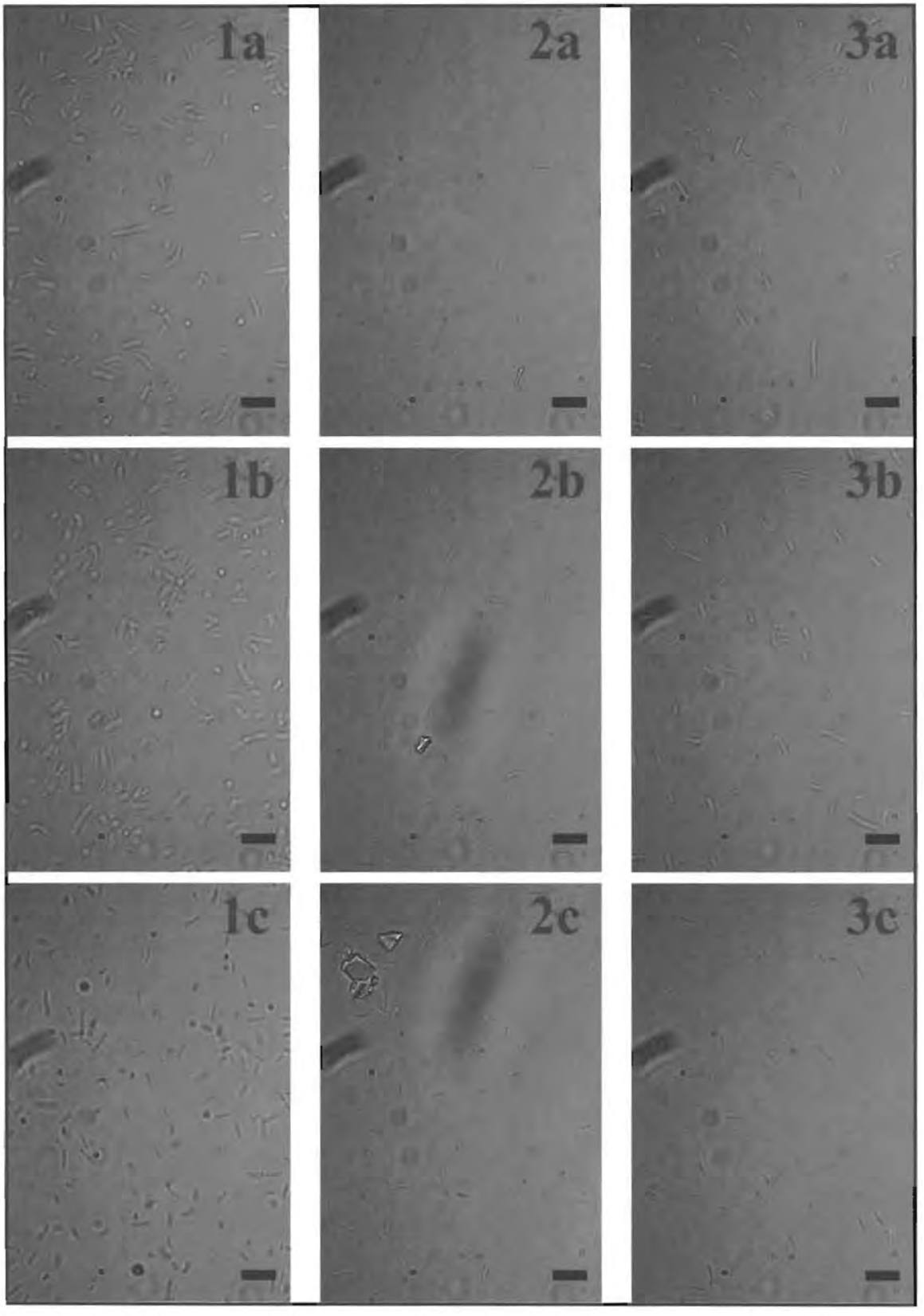

Fig. 7.10: Triplicate photomicrographs (a – c) showing *P. aeruginosa* pALacZsd planktonic cells after 16 h of incubation in LB-S. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 μm.

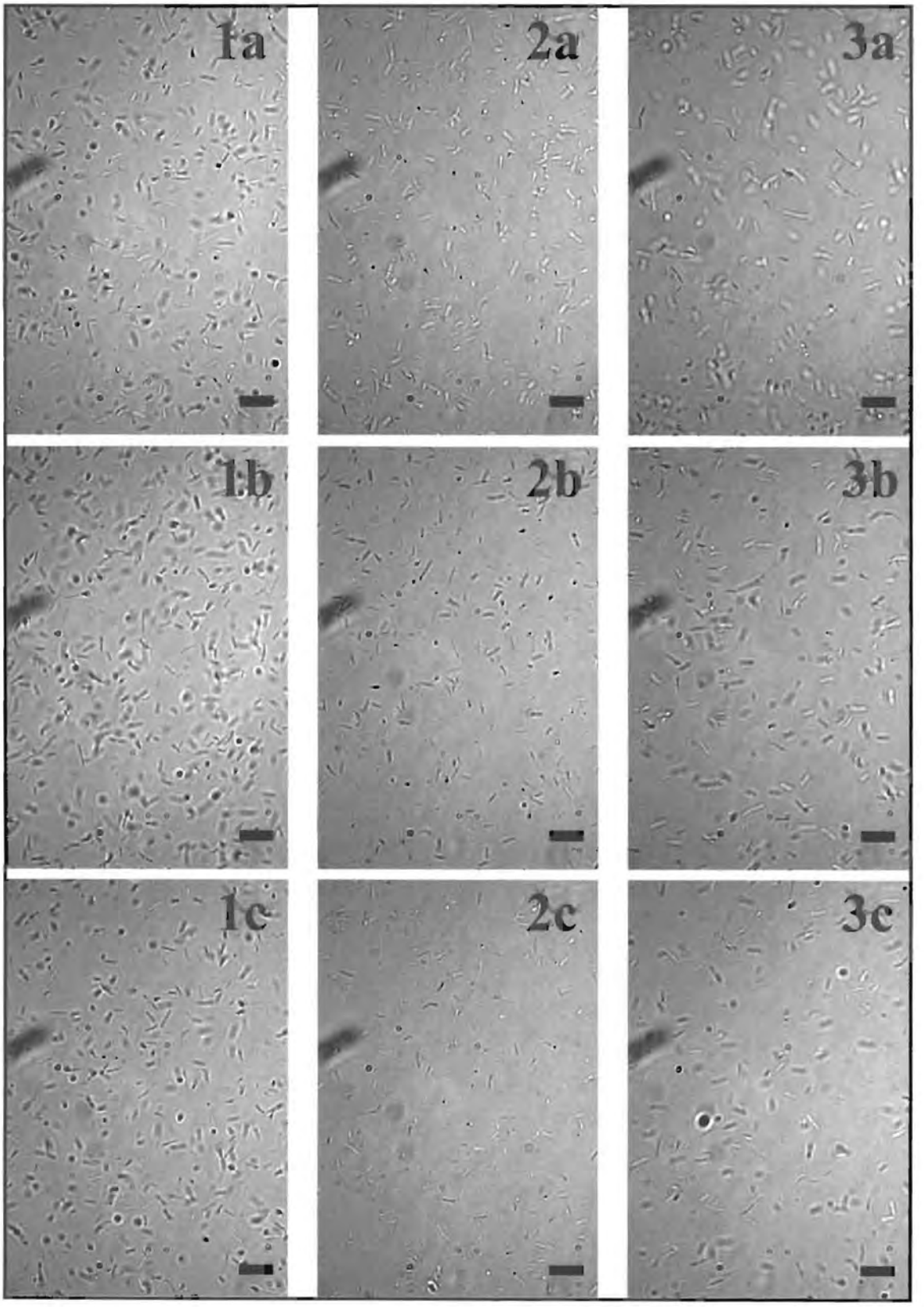

Fig. 7.11: Triplicate photomicrographs (a – c) showing *P. aeruginosa* pALacZsd SIP cells after 16 h of incubation in LB-S. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 μm.

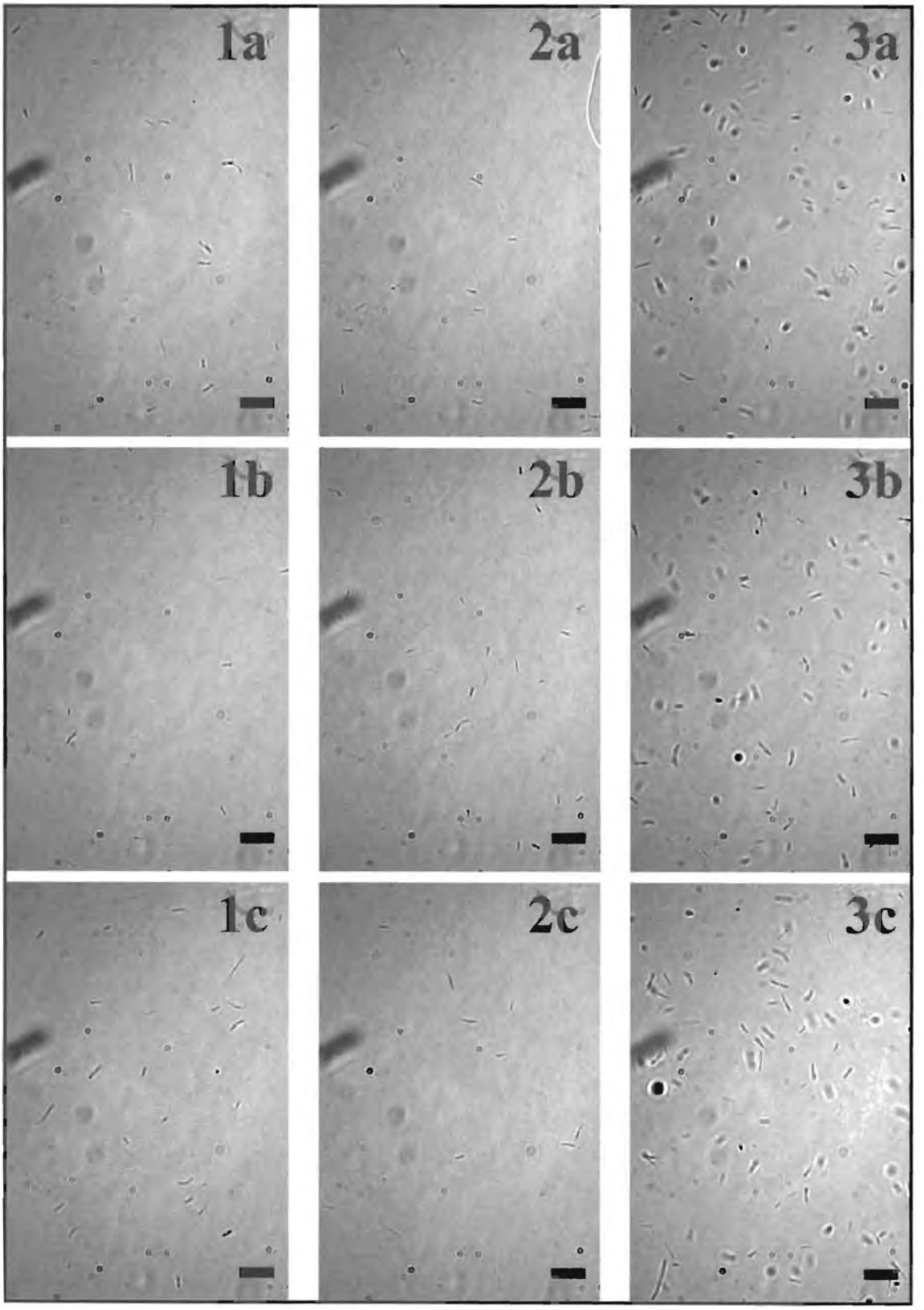

Fig. 7.12: Triplicate photomicrographs (a – c) showing *P. aeruginosa* pALacZsd biofilm cells after 16 h of incubation in LB-S. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 µm.

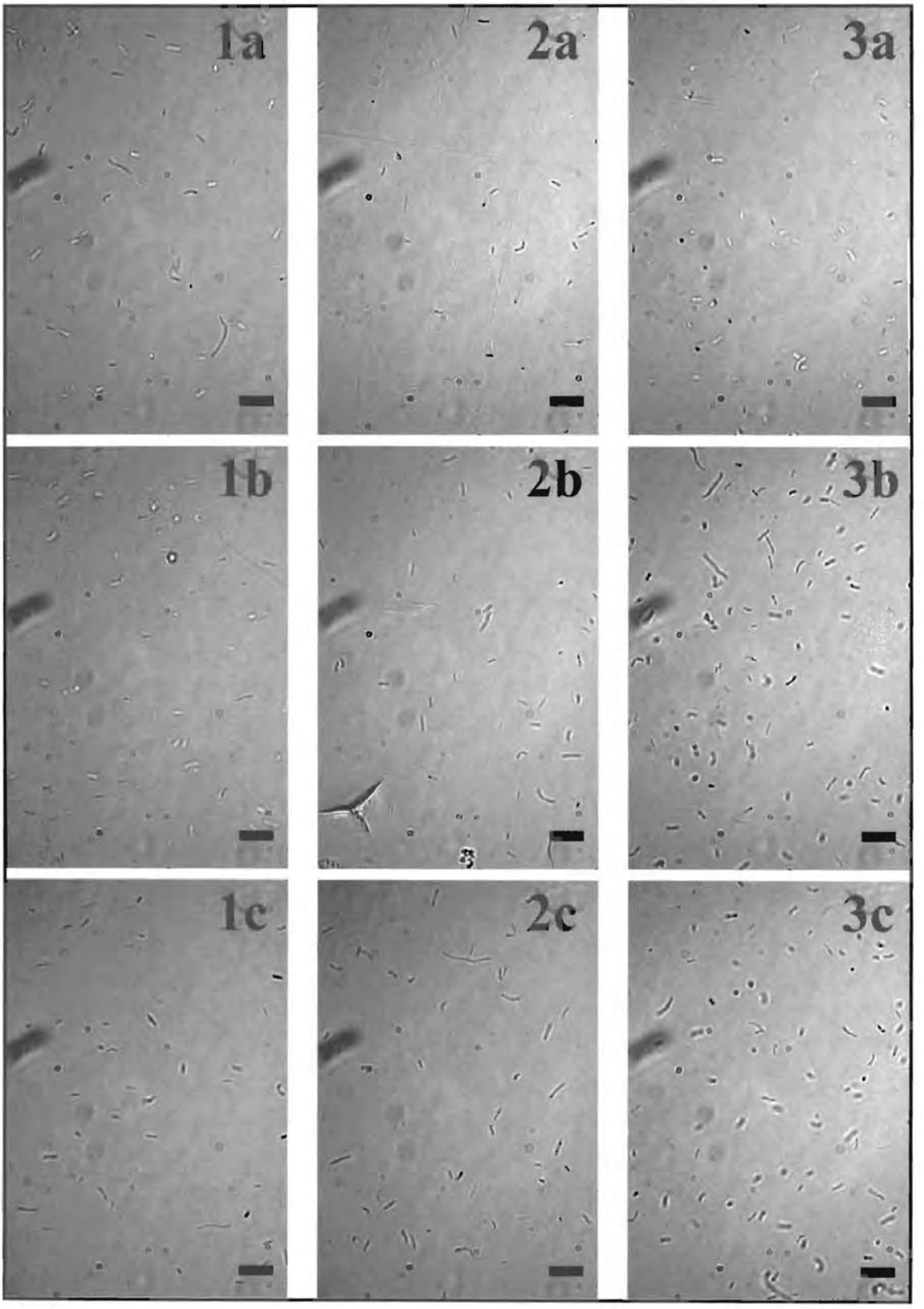

Fig. 7.13: Triplicate photomicrographs (a – c) showing *P. aeruginosa* pALacZsd planktonic cells after 16 h of incubation in LB + NaCl. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 μm.

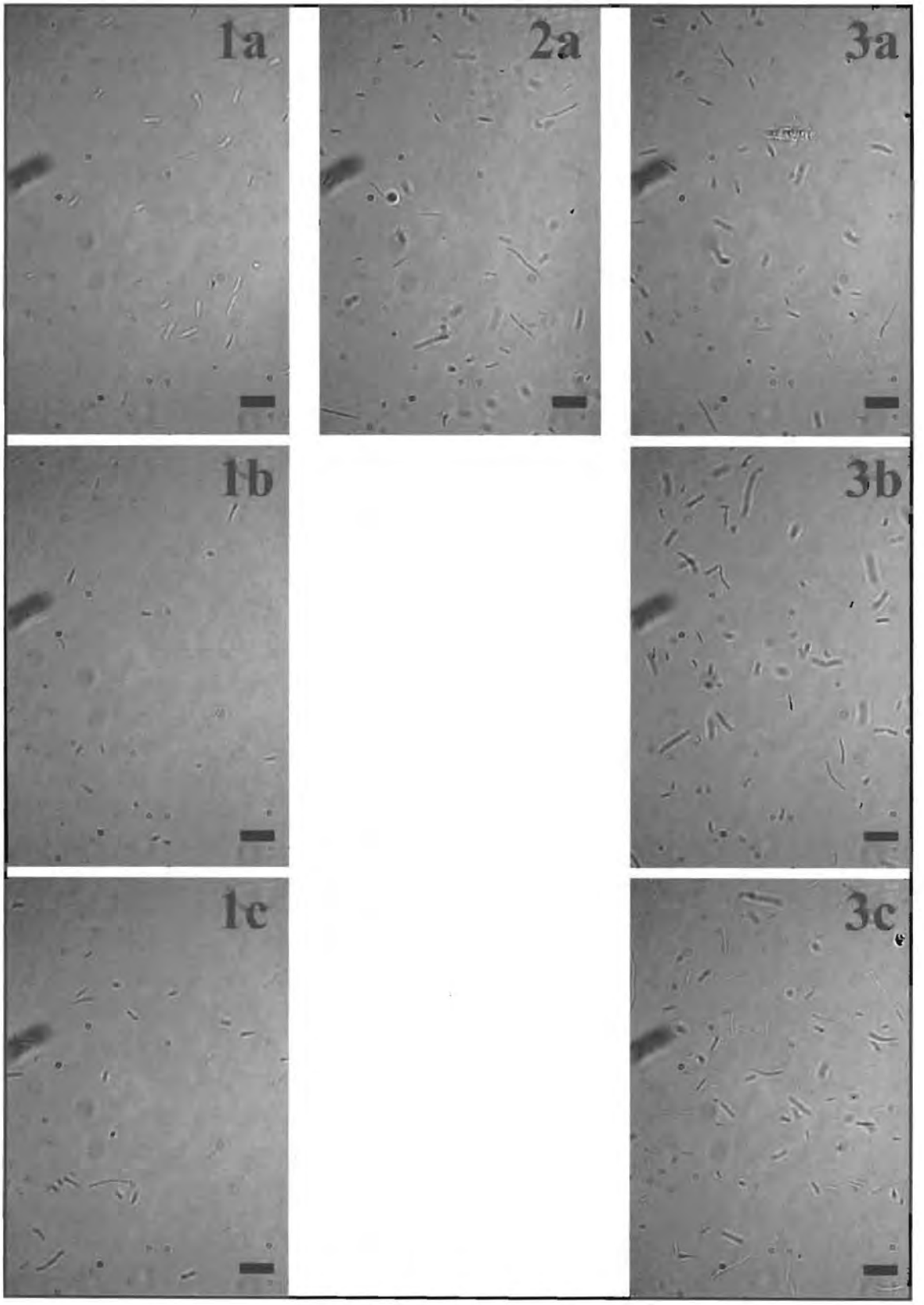

Fig. 7.14: Triplicate photomicrographs (a – c) showing *P. aeruginosa* pALacZsd SIP cells after 16 h of incubation in LB + NaCl. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 μm.

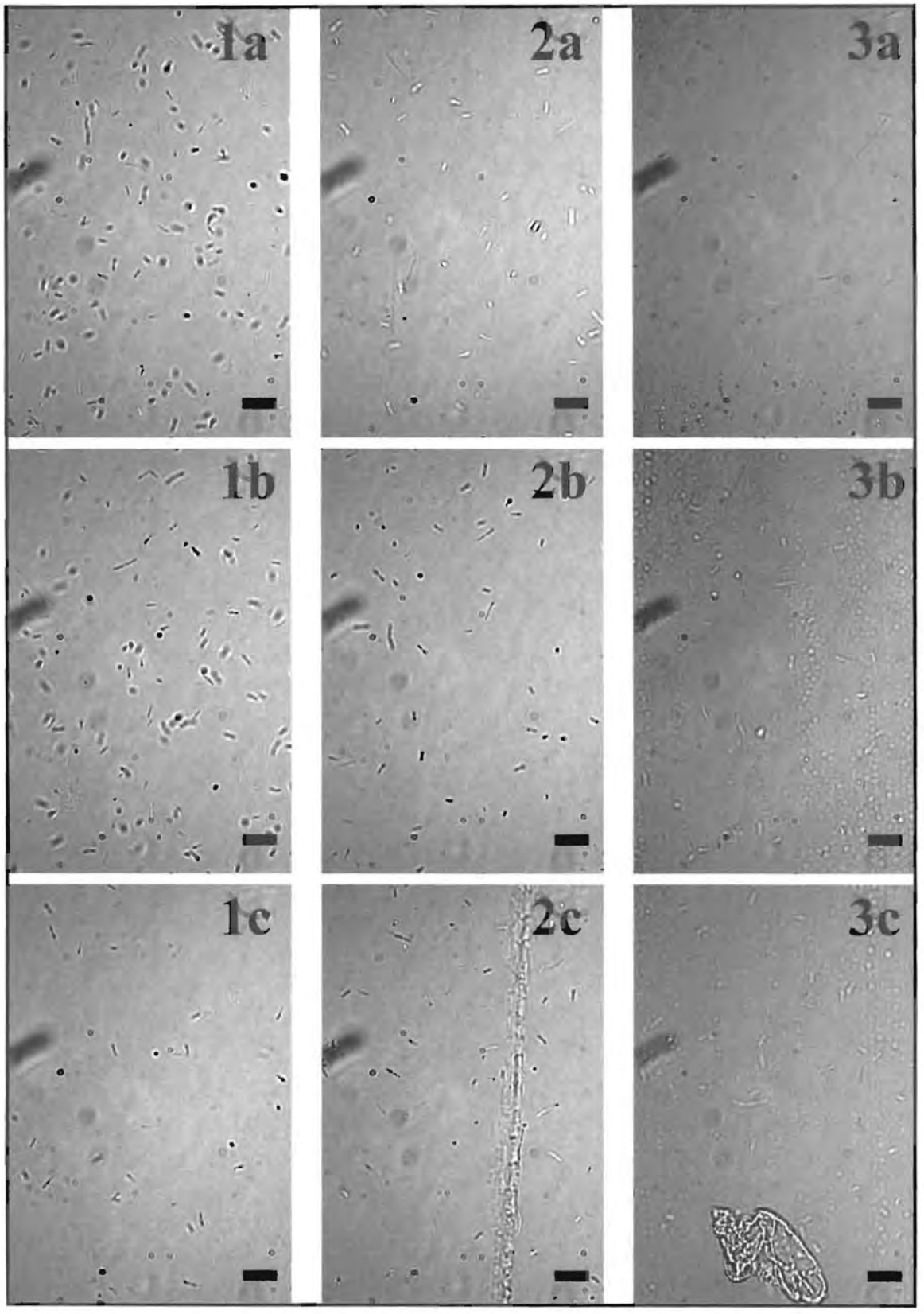

Fig. 7.15: Triplicate photomicrographs (a – c) showing *P. aeruginosa* pALacZsd biofilm cells after 16 h of incubation in LB + NaCl. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 μm.

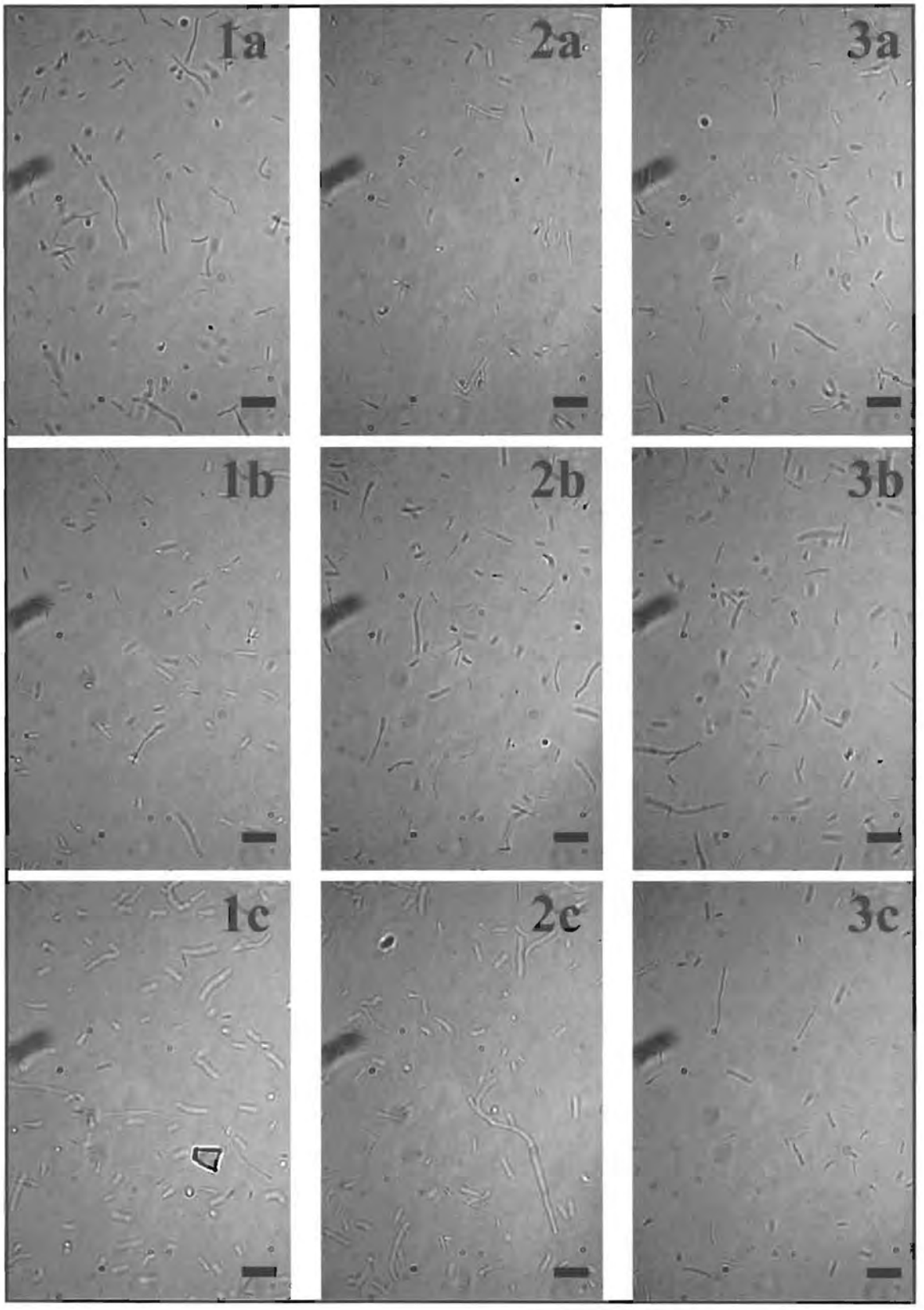

Fig. 7.16: Triplicate photomicrographs (a – c) showing *P. aeruginosa* pALacZsd planktonic cells after 16 h of incubation in LB + EtOH. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 μm.

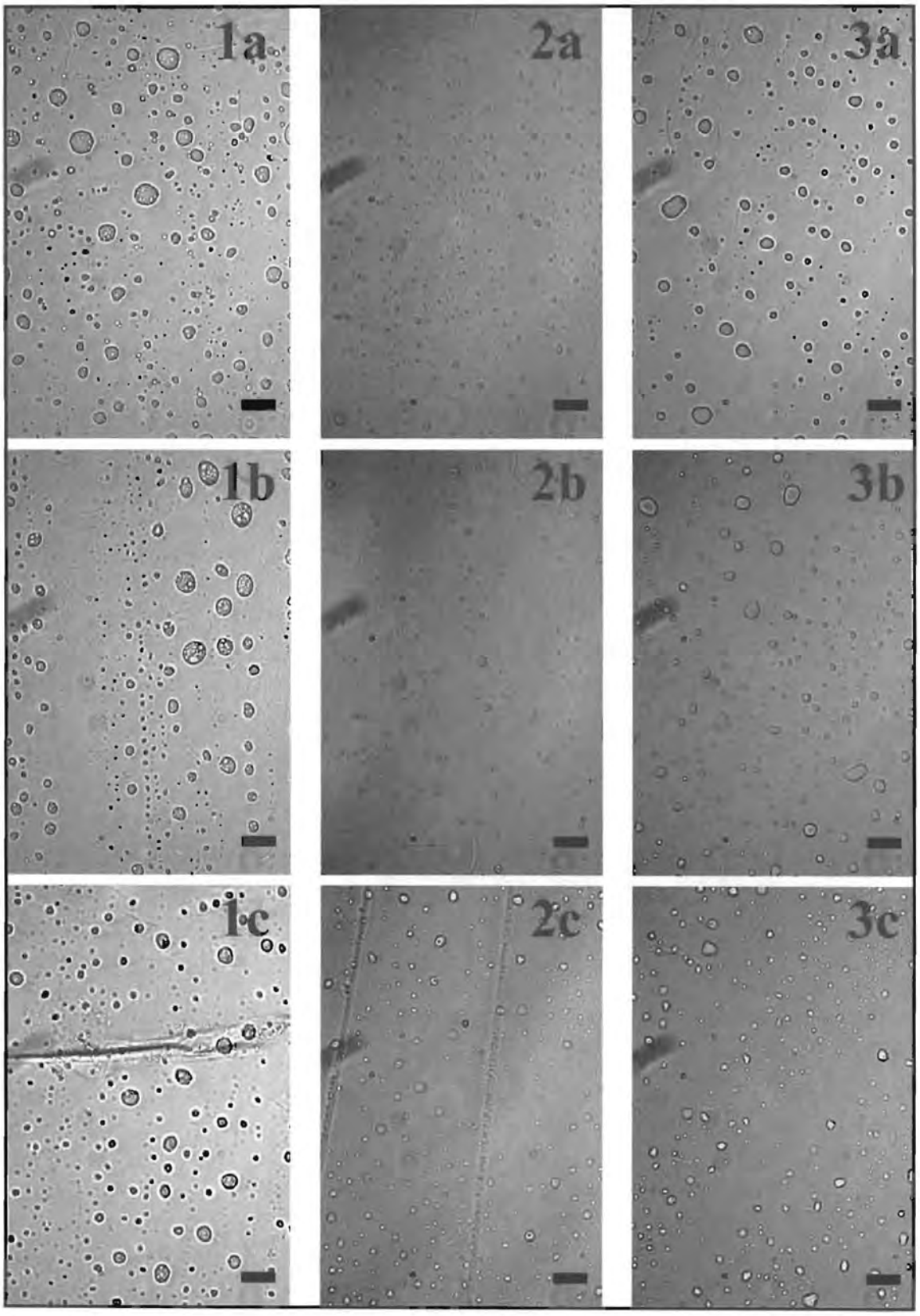

Fig. 7.17: Triplicate photomicrographs (a – c) showing *P. aeruginosa* pALacZsd SIP cells after 16 h of incubation in LB + EtOH. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 μm.

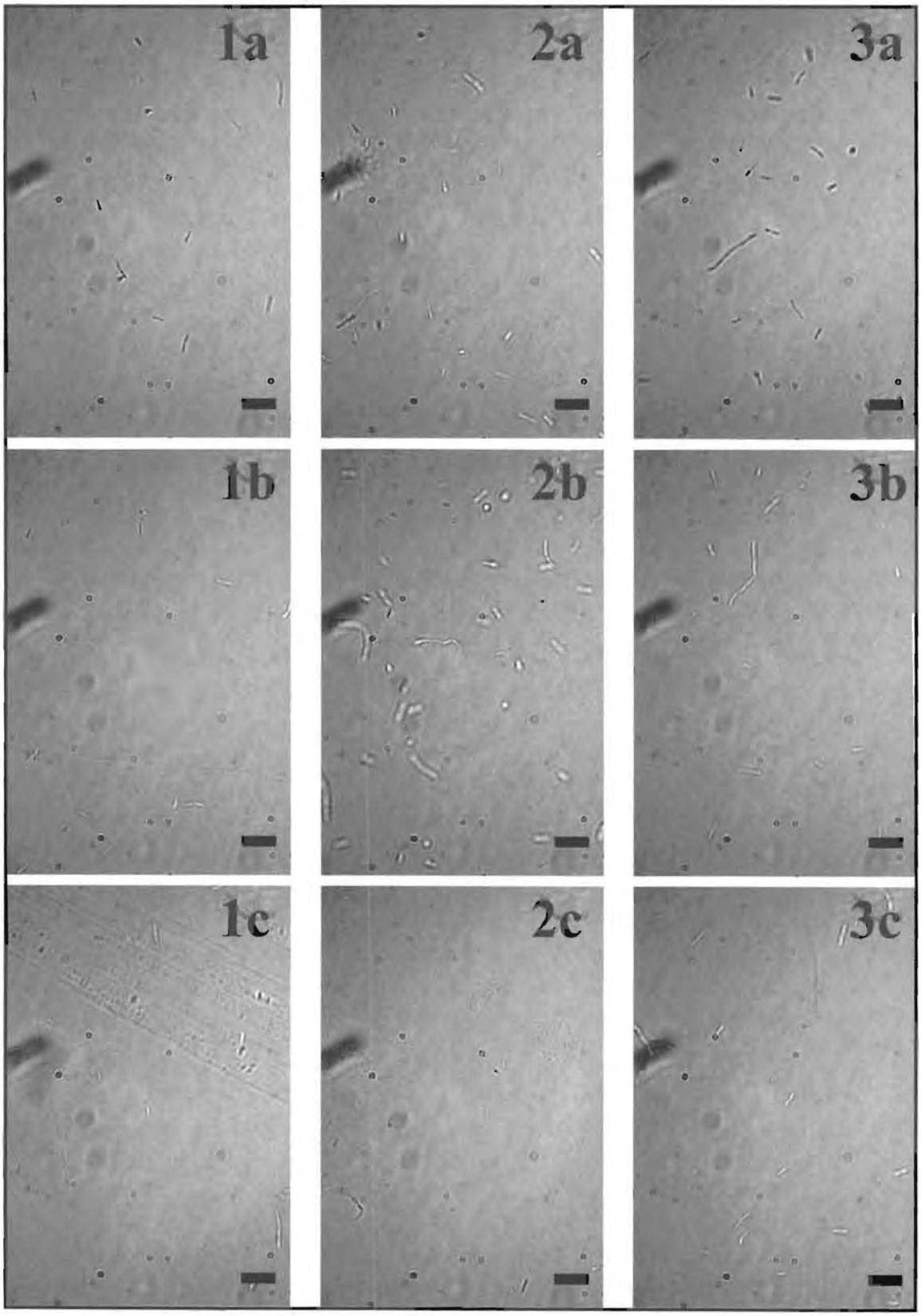

Fig. 7.18: Triplicate photomicrographs (a – c) showing *P. aeruginosa* pALacZsd biofilm cells after 16 h of incubation in LB + EtOH. All photomicrographs were taken from triplicate cultures (1 – 3). Bars = 10 μm.

APPENDIX 2

FLUORESCENCE MICROSCOPY OF *Pseudomonas aeruginosa* PAO (DSM 1707) AFTER STAINING OF CELLS WITH 23S rRNA GAMMA PROTEOBACTERIAL OR *Pseudomonas* GROUP 1 SPECIFIC PROBES

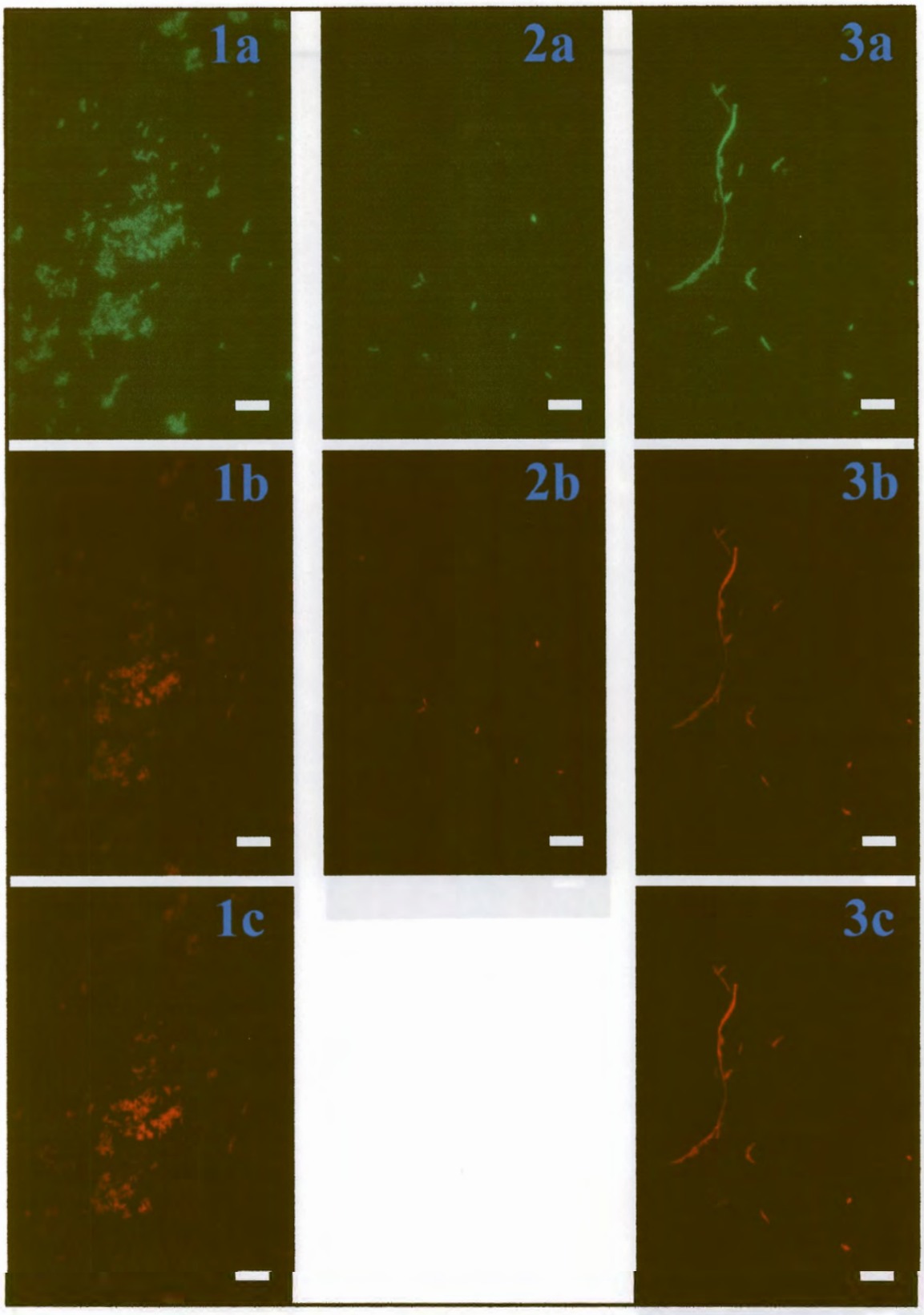

Fig. 8.1: Photomicrographs showing *P. aeruginosa* (1) planktonic cells grown in LB-S, (2) total attached cells grown in LB + NaCl and (3) total attached cells grown in LB + EtOH after 16 h incubation. Cells have been probed with a Gamma proteobacterial (green) or a *Pseudomonas* group 1 specific (red) probe. Bars = 10 µm.

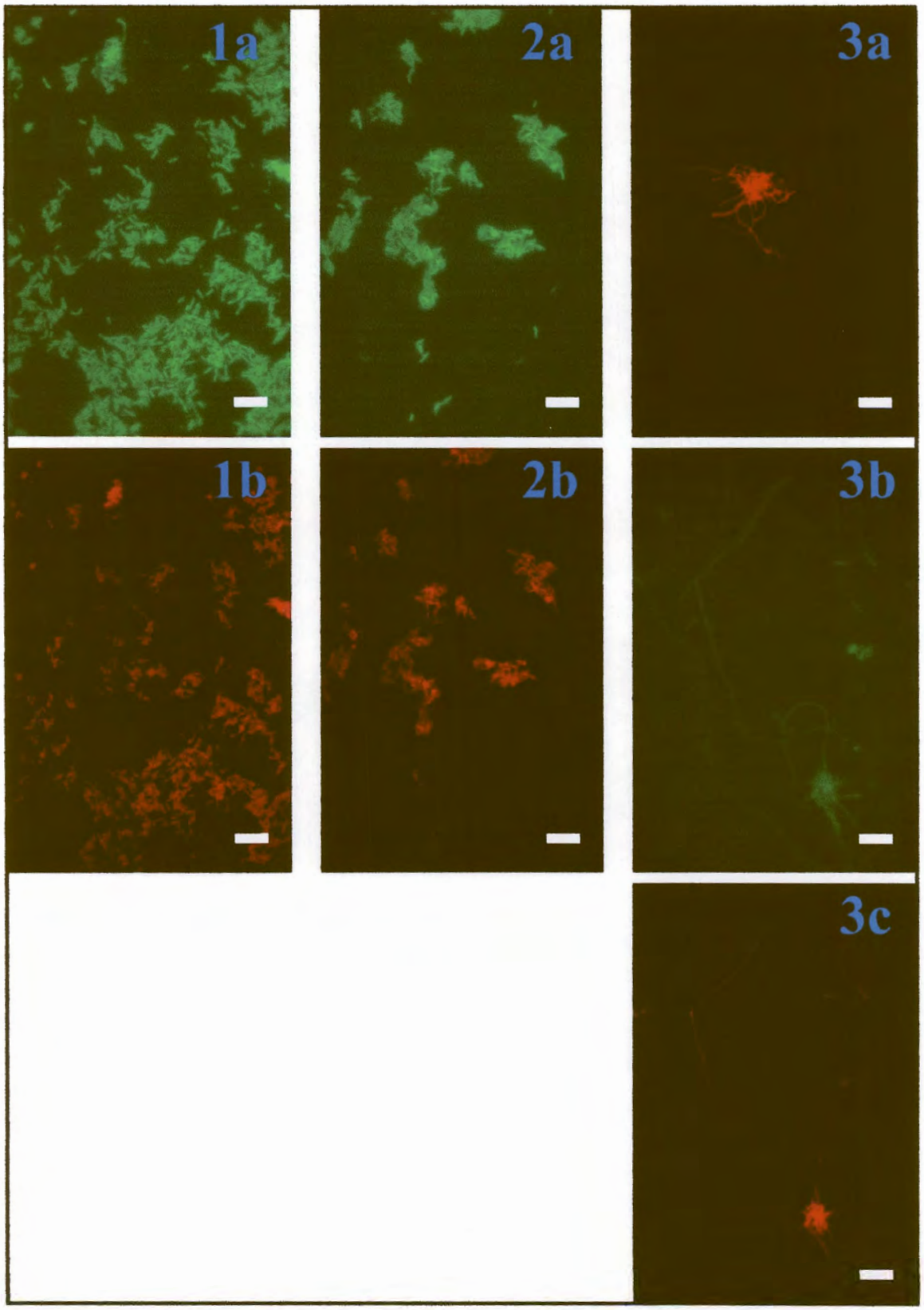

Fig. 8.2: Photomicrographs showing *P. aeruginosa* pALacZsd (1) planktonic cells grown in LB-S, (2) total attached cells grown in LB + NaCl and (3) total attached cells grown in LB + EtOH after 16 h incubation. Cells have been probed with a Gamma proteobacterial (green) or a *Pseudomonas* group 1 specific (red) probe. Bars = 10 µm.

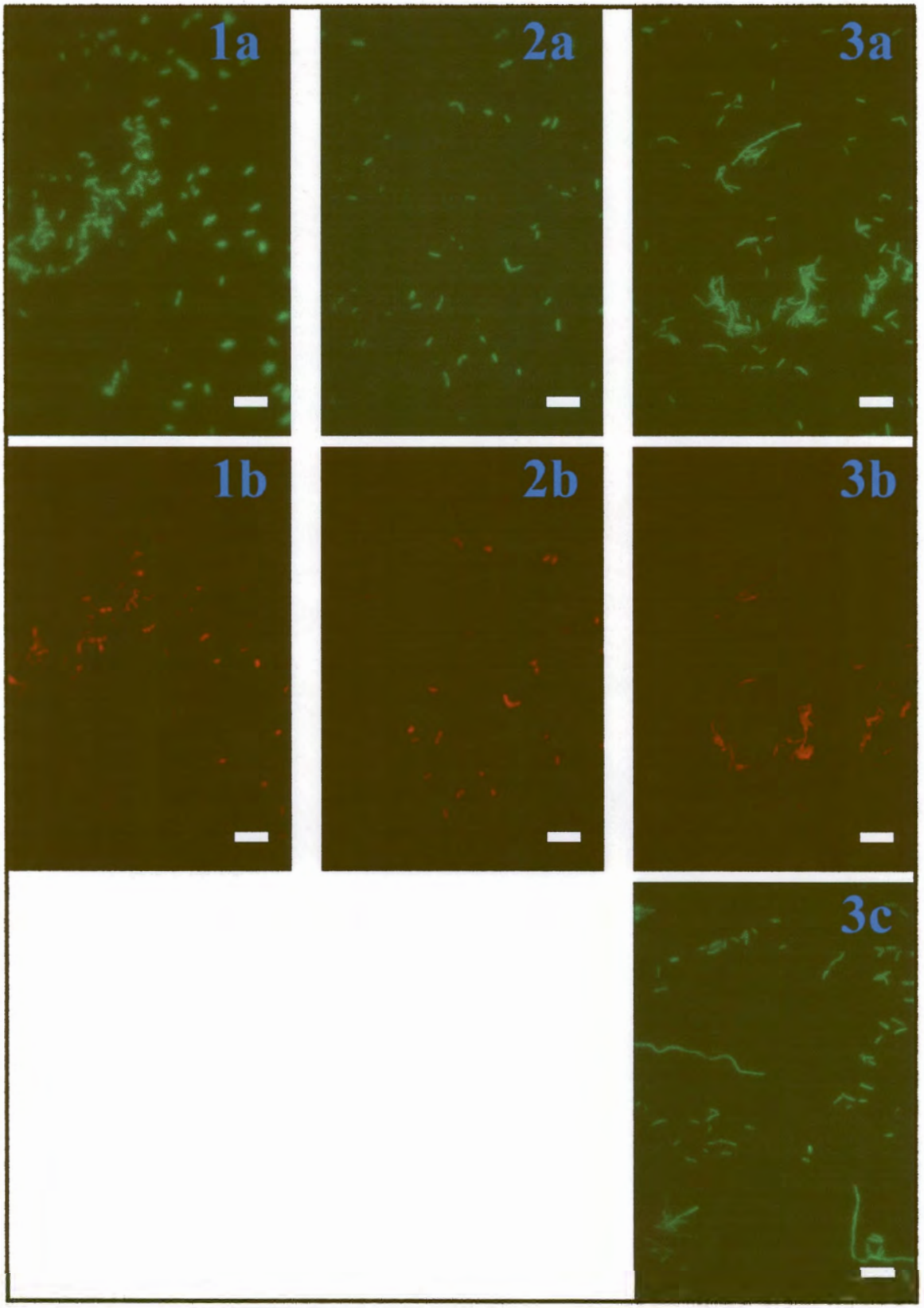

Fig. 8.3: Photomicrographs showing *P. aeruginosa* pALacZsdAg (1) planktonic cells grown in LB-S, (2) total attached cells grown in LB + NaCl and (3) total attached cells grown in LB + EtOH after 16 h incubation. Cells have been probed with a Gamma proteobacterial (green) or a *Pseudomonas* group 1 specific (red) probe. Bars = 10 μm.

REFERENCES

Allison, D. G. & Gilbert, P. (1995). Modification of surface association of antimicrobial susceptibility of bacterial populations. *J of Indus Micro* **15**, 311-317.

Allison, D. G. & Sutherland, I. W. (1987). The role of exopolysaccharides in adhesion of freshwater bacteria. *J Gen Microbiol* **133**, 1319-1327.

Amman, R. I., Ludwig, W. & Schleifer, K. H. (1995). Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol Rev* **59**, 143-169.

Amman, R. I., Ludwig, W. Schulze, R., Spring, S., Moore, E. & Schleifer, K. H. (1996). rRNA-targeted oligonucleotide probes for the identification of genuine and former Pseudomonads. *Syst Appl Microbiol* **19**, 501-509.

Andersen, J. B., Sternberg, C., Poulsen, L. K., Bjørn, S. P., Givskov, M. & Molin, S. (1998). New unstable variants of green fluorescent protein for studies of transient gene expression in bacteria. *Appl Env Microbiol* **64**, 2240-2246.

Benson, S. A. (1984). Rapid procedure of isolation of DNA fragments from agarose gel. *BioTechniques* **2**, 66-67.

Berry, A., DeVault, J. D. & Chakrabarty, A. M. (1989). High osmolarity is a signal for enhanced *algD* transcription in mucoid and non-mucoid *Pseudomonas aeruginosa* strains. *J Bacteriol* **171**, 2312-2317.

Biesterfeld, S & Figueroa, LLL. (2002). Nitrifying biofilm development with time

Black, T., Alderson, G. & Thomas, T. H. (1988). Refinement of an adherence assay for the measurement of bacterial attachment to silica beads. *J Microbiol Meth* **7**, 265-276.

Blatny, J. M., Brautaset, T., Winther-Larsen, H. C., Haugan, K. & Valla, S. (1997). Construction and use of a versatile set of broad-host-range cloning and expression vectors based on the RK2 replicon. *Appl Environ Microbiol* **63**, 370-379.

Boronin, A. M. (1992). Diversity of *Pseudomonas* plasmids: to what extent? *FEMS Microbiol Lett* **100**, 461-468.

Boucher, J. C., Martinez-Salazar, J., Schurr, M. J., Mudd, M. H., Yu, H. & Deretic, V. (1996). Two distinct loci affecting the conversion to mucoidy in *Pseudomonas aeruginosa* in cystic fibrosis encode homologs of the serine protease HtrA. *J Bacteriol* **178**, 511-523.

Boucher, J. C., Yu, H., Mudd, M. H. & Deretic, V. (1997). Mucoid *Pseudomonas aeruginosa* in cystic fibrosis: characterization of *muc* mutations in clinical isolates and analysis of clearance in a mouse model of respiratory infection. *Infec Immun* **65**, 3838-3846.

Boucher, J. C., Schurr, M. J. & Deretic, V. (2000). Dual regulation of mucoidy in *Pseudomonas aeruginosa* and sigma factor antagonism. *Mol Microbiol* **36**, 341-351.

Boyd, A. & Chakrabarty, A. M. (1994). Role of alginate lyase in cell detachment of *Pseudomonas aeruginosa*. *Appl Environ Microbiol* **60**, 2355-2359.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248-254.

Broach, J. R., Li, Y. Y., Wu, L. C. C. & Jayaram, M. (1983). Vectors for high-level, inducible expression of cloned genes in yeast, p.83-117. In M. Inouye (ed.), Experimental Manipulation of Gene Expression, Academic Press, New York.

Brown, M. L., Aldrich, H. C. & Gauthier, J. J. (1995). Relationship between glycocalyx and povidone-iodine resistance in *Pseudomonas aeruginosa* (ATCC 27853) biofilms. *Appl Environ Microbiol* **61**, 187-193.

Caldwell, D. E., Korber, D. R. & Lawrence, J. R. (1992). Confocal laser microscopy and computer image analysis. In *Advances in Microbial Ecology*, ed. K. C. Marshall, **12**, 1-67. New York: Plenum

Caldwell, D. E., Korber, D. R. & Lawrence, J. R. (1993). Analysis of biofilm formation using 2-D versus 3-D digital imaging. In *Microbial Cell Envelopes: Interactions and Biofilms*, eds. Quesnel, L. B., Gilbert, P. & Handley, P. S. Oxford: Blackwell Sci Publ.

Costerton, J. W., Cheng, K. J., Geesey, G. G., Ladd, T. I., Nickel, J. C., Dasgupta, M. & Marrie, T. J. (1987). Bacterial biofilms in nature and disease. *Annu Rev Microbiol* **41**, 435-464.

Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R. & Lappin-Scott, H. M. (1995). Microbial biofilms. *Annu Rev Microbiol* **49**, 711-745.

Costerton, J. W., Lewandowski, Z., DeBeer, D., Caldwell, D. E., Korber, D. R. & James, G. A. (1994). Biofilms: the customised microniche. *J Bacteriol* **176**, 2137-2142.

Dagostino, L., Goodman, A. E. & Marshall K. C. (1991). Physiological responses induced in bacteria adhering to surfaces. *Biofouling* **40**, 113-119.

Dalton, H. M., Poulsen, L. K., Halasz, P., Angles, M. L., Goodman, A. E. & Marshall, K. C. (1994). Substratum-induced morphological changes in a marine bacterium and their relevance to biofilm structure. *J Bacteriol* **176**, 6900-6909.

Dalton, H. M. & March, P. E. (1998). Molecular genetics of bacterial attachment and biofouling. *Curr Opp Biotech* **9**, 252-255.

Davey, M. E. & O' Toole, G. A. (2000). Microbial biofilms: From ecology to molecular genetics. *Microbiol Mol Biol* Rev **64**, 847-867.

Davies, D. G., Chakrabarty, A. M. & Geesey, G. G. (1993). Exopolysaccharide production in biofilms: substratum activation of alginate gene expression by *Pseudomonas aeruginosa*. *Appl Environ Microbiol* **59**, 1181-1186.

Davies, D. G. & Geesey G. G. (1995). Regulation of the alginate biosynthesis gene *algC* in *Pseudomonas aeruginosa* during biofilm development in continuous culture. *Appl Environ Microbiol* **61**, 860-867.

Davies, D. G., Parsek, M. R., Pearson, J. P., Iglewski, B. H., Costerton, J. W. & Greenberg, E. P. (1998). The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* **280**, 295-298.

De Beer, D., Stoodley, P., Roe, F. & Lewandowski, Z. (1994). Effects of biofilm structure on oxygen distribution and mass transport. *Biotech Bioeng* **43**, 1131-1138.

Degiorgi, C. F., Fernandez, R. O. & Pizarro, R. A. (1996). Ultraviolet-B lethal damage on *Pseudomonas aeruginosa*. *Curr Microbiol* **33**, 141-146.

De Kievit, T. R., Gillis, R., Marx, S., Brown, C. & Iglewski, B. H. (2001). Quorum-sensing genes in *Pseudomonas aeruginosa* biofilms: their role and expression patterns. *Appl Environ Microbiol* **67**, 1865-1873.

Deog-Hwan, O. H. & Marshall, D. L. (1995). Destruction of *Listeria monocytogenes* biofilms on stainless steel using monolaurin and heat. *J Food Protec* **57**, 251-255.

Deretic, V., Gill, J. F. & Chakrabarty, A. M. (1987a). Gene *algD* coding for the GDPmannose dehydrogenase is transcriptionally activated in mucoid *Pseudomonas aeruginosa*. *J Bacteriol* **169**, 351-358.

Deretic, V., Gill, J. F. & Chakrabarty, A. M. (1987b). *Pseudomonas aeruginosa* infection in cystic fibrosis: nucleotide sequence and transcriptional regulation of the *algD* gene. *Nucl Acids Res* **15**, 4567-4581.

Deretic, V., Dikshit, R., Konyecsni, W. M., Chakrabarty, A. M. & Misra, T. K. (1989). The *algR* gene, which regulates mucoidy in *Pseudomonas aeruginosa*, belongs to a class of environmentally responsive genes. *J Bacteriol* **171**, 1278-1283.

DeVault, J. D., Hendrickson, W., Kato, J. & Chakrabarty, A. M. (1991). Environmentally regulated *algD* promoter is responsive to the cAMP receptor protein in *Escherichia coli*. *Mol Microbiol* **5**, 2503-2509.

DeVault, J. D., Kimbara, K. & Chakrabarty, A. M. (1990). Pulmonary dehydration and infection in cystic fibrosis: evidence that ethanol activates alginate gene expression and induction of mucoidy in *Pseudomonas aeruginosa*. *Mol Microbiol* **4**, 737-745.

DeVries, C. A. & Ohman, D. E. (1994). Mucoid-to-nonmucoid conversion in alginate producing *Pseudomonas aeruginosa* often results from spontaneous mutations in *algT*, encoding a putative alternate sigma factor, and shows evidence of autoregulation. *J Bacteriol* **176**, 6677-6687.

Drummond, D. W., Hirst, E. L. & Persival, E. E. (1962). The constitution of alginic acid. *J Chem Soc*, 1208-1206.

Edwards, K. J. & Saunders, N. A. (2001). Real-time PCR used to measure stress-induced changes in the expression of the genes of the alginate pathway of *Pseudomonas aeruginosa*. *J Appl Microbiol* **19**, 29-37.

Fernandes, R. A., Zaniqueeelli, O. & Paranhos, Hde F. (2002). Analysis of the point-counting and planimetric methods in the quantification of denture biofilm – a methodological validation study. *Pesqui Odontol Bras* **16**, 63-68.

Fletcher, M. (1987). How do bacteria attach to solid surfaces? *Microbiol Sci* **4**, 133-136.

Fletcher, M. (1991). The physiological activity of bacteria attached to solid surfaces. *Adv Micro Phys* **32**, 52-85.

Fletcher, M. & Loeb, G. I. (1979). Influence of substratum characteristics on the attachment of a marine Pseudomonad to solid surfaces. *Appl Environ Microbiol* **37**, 67-72.

Gacesa, P. (1998). Bacterial alginate biosynthesis – recent progress and future prospects. *Microbiology* **144**, 1133-1143.

Gallardo-Moreno, A. M., van der Mei, H. C., Busscher, H. J., González-Martin, M. L., Bruque, J. M. & Pérez-Giraldo, C. (2001). Adhesion of *Enterococcus faecalis* 1131 grown under subinhibitory concentrations of ampicillin and vancomycin to a hydrophilic and a hydrophobic substratum. *FEMS Microbiol Lett* **10095**, 1-5.

Gorin, P. A. & Spencer, J. F. T. (1966). Exocellular alginic acid from *Azotobacter vinelandii*. *Can J Chem* **44**, 993-998.

Greener, A., Lehmen, S. M. & Helinski, D. R. (1992). Promoters of the broad host range plasmid RK2: analysis of transcription (initiation) in five species of gram-negative bacteria. *Genetics* **130**, 27-36.

Gristina, A. G., Hobgood, C. D., Webb, L. X. & Myrvik, O. N. (1987). Adhesive colonisation of biomaterials and antibiotic resistance. *Biomaterials* **8**, 423-426.

Hager, P. W., Calfee, M. W. & Phibbs, P. V. (2000). The *Pseudomonas aeruginosa devB/SOL* homologue, *pgl*, is a member of the *hex* regulon and encodes 6-phosphogluconolactonase. *J Bacteriol* **182**, 3934-3941.

Hancock, I. C. (1991). Microbial cell surface architecture. In *Microbial cell surface architecture*, eds. Mozes, P. S., Handely, H. J., Busscher, H. J. & Rouxhet, P. G. VCH Weinheim, Weinheim, Germany, 21-59.

Hassett, D. J. (1996). Anaerobic production of alginate by *Pseudomonas aeruginosa*: Alginate restricts diffusion of oxygen. *J Bacteriol* **178**, 7322-7325.

Hester, K. L., Lehman, J., Najar, F., Song, L., Roe, B., MacGregor, C. H., Hager, P. W., Phibbs, P. V. & Sokatch, J. R. (2000). Crc is involved in catabolite repression control of the *bdk* operons of *Pseudomonas putida* and *Pseudomonas aeruginosa*. *J Bacteriol* **182**, 1144-1149.

Heydorn, A., Ersbøll, B., Kato, J., Hentzer, M., Parsek, M. R., Tolker-Nielsen, T., Givskov, M. & Molin, S. (2002). Statistical analysis of *Pseudomonas aeruginosa* biofilm development: Impact of mutations in genes involved in twitching motility, cell-to-cell signalling, and stationary-phase sigma factor expression. *Appl Environ Microbiol* **68**, 2008-2017.

Hoyle, B. D., Williams, L. J. & Costerton, J. W. (1993). Production of mucoid exopolysaccharide during development of *Pseudomonas aeruginosa* biofilms. *Infect Immun* **61**, 777-780.

Hunt, J. C. & Phibbs, P. V. (1983). Regulation of alternative peripheral pathways of glucose catabolism during aerobic growth of *Pseudomonas aeruginosa*. *J Bacteriol* **154**, 793-802.

Hussain, K., Begg, K. J., Salmond, G. P. & Donachie, W. D. (1987). *ParD*: a new gene coding for a protein required for chromosome partitioning and septum localization in *Escherichia coli*. *Mol Microbiol* **1**, 73-81.

Iriberri, J., Unanue, M., Ayo, B., Barcina, I. & Egea, L. (1990). Bacterial production and growth rate estimation from [^{3}H] thymidine incorporation for attached and free-living bacteria in aquatic systems. *Appl Environ Microbiol* **56**, 483-487.

James, A. M. (1991). Charge properties of microbial cell surfaces. In *Microbial cell surface analysis*, eds. Mozes, N., Handely, P. S., Busscher, H. J. & Rouxhet, P. G. VCH Weinheim, Weinheim, Germany, 221-263.

Jansen, K (ed). (1995). Current protocols in molecular biology. John Willey & Son Inc., USA.

Jouenne, T., Tresse, O. & Junter, G. A. (1994). Agar entrapped bacteria as an in vitro model of biofilms and their susceptibility to antibiotics. *FEMS Microbiol Lett* **119**, 237-242.

Jucker, B. A., Harms, H. & Zehnder, A. J. B. (1996). Adhesion of the positively charged bacterium *Stenotrophomonas* (*Xanthomonas*) *maltophilia* 70401 to glass and Teflon. *J Bacteriol* **178**, 5472-5479.

Jürgen, B., Schweder, T. & Hecker, M. (1998). The stability of mRNA from the *gsiB* gene of *Bacillus subtilis* is dependant on the presence of a strong ribosome binding site. *Mol Gen Genet* **258**, 538-545.

Kato, J. & Chakrabarty, A. M. (1991). Purification of the regulatory protein AlgR1 and its binding in the far upstream region of the *algD* promoter in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* **88**, 1760-1764.

Kellner, R. (2000). Proteomics. Concepts and perspectives. *Fresenius J Anal Chem* **366**, 517-524.

Kinniment, S. L. & Wimpenny, W. T. (1992). Measurements of the distribution of Adenylate concentrations and Adenylate energy charge across *Pseudomonas aeruginosa* biofilms. *Appl Environ Microbiol* **58**, 1629-1635.

Kjelleberg, S. Personal communication. School of Biotechnology and Biomolecular Sciences, University of New South Wales, Randwick, Sydney, NSW 2052, Australia.

Knutson, C. A. & Jeanes, A. (1968). A new modification of the carbazole reaction: application to heteropolysaccharides. *Anal Biochem* **24**, 470-481.

Kolter, R. & Losick, R. (1998). One for all and all for one. *Science* **280**, 226-227.

Kuchma, S. L. & O'Toole, G. (2000). Surface-induced and biofilm-induced changes in gene expression. *Curr Opin Biotech* **11**, 429-433.

Lawrence, J. R., Korber, D. R., Hoyle, B. D., Costerton, J. W. & Caldwell, D. E. (1991). Optical sectioning of microbial biofilms. *J Bacteriol* **173**, 6558-6567.

Lawrence, J. R. & Neu, T. R. (1999). Confocal scanning laser microscopy for analysis of microbial biofilms. *Methods Enzymol* **310**, 131-144.

Lawrence, J. R., Wolfaardt, G. M. & Korber, D. R. (1994). Monitoring diffusion in biofilm matrices using confocal laser microscopy. *Appl Environ Microbiol* **60,** 1166-1173.

Lee, H. S., Berger, D. K. & Kutsu (1993). Activity of purified NIFA, a transcriptional activator of nitrogen fixation genes. *Proc Natl Acad Sci USA* **90,** 2266-2270.

Leitão, J. H., Fialho, A. M. & Sá-Correia, I. (1992). Effects of growth temperature on alginate synthesis and enzymes in *Pseudomonas aeruginosa* variants. *J Gen Microbiol* **138**, 605-610.

Leitão, J. H. & Sá-Correia, I. (1993). Manipulation of the *Pseudomonas aeruginosa* alginate pathway by varying the level of biosynthetic enzymes and growth temperature. *J Appl Bacteriol* **74,** 452-459.

Leitão, J. H. & Sá-Correia, I. (1995). Growth-phase-dependent alginate synthesis, activity of biosynthetic enzymes and transcription of alginate genes in *Pseudomonas aeruginosa*. *Arch Microbiol* **163,** 217-222.

Leitão, J. H. & Sá-Correia, I. (1997). Oxygen dependant upregulation of transcription of alginate genes *algA*, *algC* and *algD* in *Pseudomonas aeruginosa*. *Res Microbiol* **148**, 37-43.

Leslie, T. G. & Phibbs, P. V. (1984). Alternative pathway of carbohydrate utilization in Pseudomonads. *Annu Rev Microbiol* **38**, 359-387.

Ma, J., Hager, P. W., Howell, M. L., Phibbs, P. V. & Hassett, D. (1998). Cloning and characterization of the *Pseudomonas aeruginosa zwf* gene encoding glucose-6-phosphate dehydrogenase, an enzyme important in the resistance to methyl viologen (paraquat). *J Bacteriol* **180**, 1741-1749.

Ma, J., Phibbs, P. V. & Hassett, D. J. (1997). Glucose stimulates alginate production and *algD* transcription in *Pseudomonas aeruginosa*. *FEMS Microbiol Lett* **148,** 217-221.

Mac Donald, R. Department of Microbiology and Plant Pathology. University of Pretoria. Pretoria. 0002. South Africa.

MacGregor, C. H., Arora, S. K., Hager, P. W., Dail, M. B. & Phibbs, P. V. (1996). The nucleotide sequence of the *Pseudomonas aeruginosa pyrE-crc-rph* region and the purification of the *crc* gene product. *J Bacteriol* **178**, 5627-5635.

Mah, T. & O'Toole, G. (2001). Mechanisms of biofilm resistance to antimicrobial agents. *Trends in Microbiol* **9,** 34-39.

Manz, W., Eisenbrecher, M., Neu, T. & Szewzyk, U. (1998). Abundance and spatial organisation of Gram-negative sulphate-reducing bacteria in activated sludge investigated by *in situ* probing with specific 16S rRNA targeted oligonucleotides. *FEMS Microb Ecol* **25,** 43-61.

Margolin, W. (1999). The bacterial cell division machine. Fluorescent techniques for examining intact cells and mutant studies provide insights into bacterial cell division. *ASM News* **65**, 137-143.

Marshall, A. & Hodgson, J. (1998). DNA chips; An array of possibilities. *Nature Biotech* **16,** 27-31.

Marshall, K. C. (1986). Adsorption and adhesion process in microbial growth at interfaces. *Adv Colloid Interface Sci* **25,** 59-86.

Marshall, K. C. (1992). Biofilms: an overview of bacterial adhesion, activity, and control at surfaces. *ASM News* **58**, 202-207.

Marshall, K. C., Stout, R. & Mitchell, R. (1971). Mechanisms of the initial events in the sorption of marine bacteria to surfaces. *J Gen Microbiol* **68,** 337-348.

Martin, D. W., Schurr, M. J., Mudd, M. H. & Deretic, V. (1993a). Differentiation of *Pseudomonas aeruginosa* into the alginate-producing form: inactivation of *mucB* causes conversion to mucoidy. *Mol Microbiol* **9**, 497-506.

Martin, D. W., Schurr, M. J., Mudd, M. H., Govan, J. R. W., Holloway, B. W. & Deretic, V. (1993b). Mechanism of conversion to mucoidy in *Pseudomonas aeruginosa* infecting cystic fibrosis patients. *Proc Natl Acad Sci USA* **90**, 8377-8381.

Martin, D. W., Schurr, M. J., Yu, H. & Deretic, V. (1994). Analysis of promoters controlled by the putative sigma-factor AlgU regulating conversion to mucoidy in *Pseudomonas aeruginosa* – relationship to sigma(e) and stress-response. *J Bacteriol* **176,** 6688-6696.

Mathee, K., McPherson, C. J. & Ohman, D. E. (1997). Posttranslational control of the *algT* (*algU*)-encoded σ^{22} for expression of the alginate regulon in *Pseudomonas aeruginosa* and localization of its antagonist proteins MucA and MucB (AlgN). *J Bacteriol* **179**, 3711-3720.

May, T. B. & Chakrabarty, A. M. (1994). Isolation and assay of *Pseudomonas aeruginosa* alginate. *Methods Enzymol* **235**, 295-304.

McLeod, F. A., Guiot, S. R. & Costerton, J. W. (1995). Layered structure of bacterial aggregates produced in an upflow anaerobic sludge bed and filter reactor. *Appl Environ Microbiol* **56,** 1598-1607.

Miller, J. H. (1972). Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, USA.

Miller, J. H. (1992). A short course in bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, USA.

Møller, S., Korber, D., Wolfaardt, G., Molin, S. & Caldwell, D. (1997). Impact of nutrient composition on a degradative biofilm community. *Appl Environ Microbiol* **63,** 2433-2438.

Møller, S., Kristensen, C. S., Poulsen, L. K., Carstensen, J. M. & Mollin, S. (1995). Bacterial growth on surfaces: automated image analysis for quantification of growth-rate related parameters. *Appl Environ Microbiol* **61**, 741-748.

Møller, S., Pederson, A. R., Poulsen, L. K., Arvin, E. & Molin, S. (1996). Activity and three-dimensional distribution of toluene-degrading *Pseudomonas putida* in a multispecies biofilm assessed by quantitative *in situ* hybridisation and scanning confocal laser microscopy. *Appl Environ Microbiol* **62,** 4632-4640.

Monday, S. R. & Schiller, N. L. (1996). Alginate synthesis in *Pseudomonas aeruginosa*: the role of *algL* (alginate lyase) and *algX*. *J Bacteriol* **178**, 625-632.

Neu, T. R. & Marshall, K. C. (1991). Microbial "footprints" – a new approach to adhesive polymers. *Biofouling* **3,** 101-112.

Nickel, J. C., Ruseska, I., Wright, J. B. & Costerton, J. W. (1985). Tobramycin resistance of cells of *Pseudomonas aeruginosa* growing as a biofilm on urinary catheter material. *Antimicrob Agents Chemother* **27,** 619-624.

Nifa, A. J., Atkinson, M. R., Kamberov, E. S., Feng, J. & Nifa, E. G. (1995). Control of nitrogen assimilation by the NRI-NRII two-component system of enteric bacteria. In *Two Component Signal Transduction.* Eds. Hoch, J. A. & Silhavy, T. J.. Washington: ASM Press, 67-88.

Norde, W. & Lyklema, J. (1989). Protein adsorption and bacterial adhesion to solid surfaces: a colloid chemical approach. *Colloids Surf* **38,** 1-13.

O'Toole, G. A., Gibbs, K. A., Hager, P. W., Phibbs, P. V. & Kolter, R. (2000). The global carbon metabolism regulator Crc is a component of a signal transduction pathway required for biofilm development by *Pseudomonas aeruginosa*. *J Bacteriol* **182**, 425-431.

O'Toole, G. A. & Kolter, R. (1998). Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol Microbiol* **30**, 295-304.

O'Toole, G. A., Pratt, L. A., Watnick, P. I., Newman, D. K., Weaver, V. B. & Kolter, R. (1999) Genetic approaches to study biofilms. *Methods Enzymol* **310**, 91-109.

Olsen, R. H., DeBusscher, G. & McCombie, C. (1982). Development of broad host range vectors and gene banks: self-cloning of the *Pseudomonas aeruginosa* PAOI chromosome. *J Bacteriol* **150**, 60-69.

Olsen, R. H. & Shipley, P. (1973). Host range properties of the *Pseudomonas aeruginosa* R factor R1822. *J Bacteriol* **113**, 772-780.

Otto, K., Norbeck, J., Larsson, T., Karlsson, K. A. & Hermansson, M. (2001) Adhesion of type 1-fimbriated *Escherichia coli* to abiotic surfaces leads to altered composition of outer membrane proteins. *J Bacteriol* **183**, 2445-2453.

Palmer, R. (1999). Microscopy flow cells: perfusion chambers for real-time study of biofilms. In *Biofilms*, ed. Doyle, R. Orlando, Academic Press.

Palmer, R. J. & Sternberg, C. (1999). Modern microscopy in biofilm research: confocal microscopy and other approaches. *Curr Opp in Biotech* **10,** 263-268.

Perez-Martin, J. & de Lorenzo, V. (1996). ATP binding to the sigma 54-dependent activator XylR triggers a protein multimerization cycle catalyzed by UAS DNA. *Cell* **86,** 331-339.

Rees, D. J. G. (1986). Studies on the gene coding for the anti-haemophiliac factor IX. D Phil Thesis. Oxford University. United Kingdom.

Rehm, B. H. A. & Valla, S. (1997). Bacterial alginates: biosynthesis and applications. *Appl Microbiol Biotechnol* **48**, 281-288.

Rice, J. F., Fowler, R. F., Arage, A. A., White, D. C. & Sayler, G. S. (1995). Effects of external stimuli on environmental bacterial strains harbouring an *algD-lux* bioluminescent reporter plasmid for the study of corrosive biofilms. *J Ind Microbiol.* **15**, 318-328.

Rice, A. R., Hamilton, M. A. & Camper, A. K. (2000). Apparent surface associated lag time in growth of primary biofilm cells. *Microbiol Ecol* **41**, 8-15.

Rijnaarts, H. H. M., Norde, W., Lyklema, J. & Zehnder, A. J. B. (1995). The isoelectric point of bacteria as an indicator for the presence of cell surface polymers that inhibit adhesion. *Colloids Surf B: Biointerfaces* **4,** 191-197.

Rutter, P. R. & Vincent, B. (1980). The adhesion of microorganisms to surfaces: physico-chemical aspects. In *Microbial adhesion to surfaces,* eds. Berkeley, R. C. W., Lynch, R. M., Melling, J., Rutter, P. R. & Vincent, B. E. Horwood Ltd., Chichester, United Kingdom, 79-93.

Sage, A. E. & Vasil, M. L. (1997). Osmoprotectant-dependent expression of *plcH*, encoding the hemolytic phospholipase C, is subject to novel catabolite repression control in *Pseudomonas aeruginosa* PAO1. *J Bacteriol* **179**, 4874-4881.

Sambrook, J., Fritsch, E. F. & Maniatis. (1989). *Molecular Cloning: A Laboratory Manual,* 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sauer, K., Camper, A. K., Ehrlich, G. D., Costerton, J. W. & Davies, D. G. (2002). *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. *J Bacteriol* **184**, 1140-1154.

Schurr, M. J., Yu, H., Martinez-Salazar, J. M., Hibler, N. S. & Deretic, V. (1995). Biochemical characterisation and posttranslational modification of AlgU, a regulator of stress response in *Pseudomonas aeruginosa. Biochem Biophys Res Comm* **216,** 874-880.

Selvaraj, M. S., Ohman, D. E., Quarless, R., Hassett, D. J. & Wozniak, D. J. (1998). Phosphorylation-independent activity of the response regulators AlgB and AlgR in promoting alginate biosynthesis in mucoid *Pseudomonas aeruginosa. J Bacteriol* **180,** 956-968.

Shapiro, J. A. & Dworkin, M. (1997). Bacteria as multicellular organisms. Oxford University Press, Inc., New York, N.Y.

Singleton, S., Treolar, R., Warren, R., Watson, G., Hodgson, R. & Allison, C. (1997). Methods for characterisation of oral biofilms: analysis of colonisation, microstructure, and molecular transport phenomena. *Adv Dent Res* **11,** 133-149.

Sissons, C. H., Wong, L. & Cutress, T. W. (1996). Inhibition by ethanol of the growth of biofilm and dispersed microcosm dental plaques. *Arch Oral Biol* **41**, 27-34.

Sternberg, C., Christensen, B. B., Johansen, T., Nielsen, A. T., Andersen, J. B., Givskov, M. & Molin, S. (1999). Distribution in bacterial growth activity in flow-chamber biofilms. *Appl Environ Microbiol* **65,** 4108-4117.

Stewart, P. S. (1996). Theoretical aspects of antibiotic diffusion into microbial biofilms. *Antimicrob Agents Chemother* **40**, 2517-2522.

Steyn, B., Oosthuizen, M. C., MacDonald, R., Theron, J. & Brözel, V. S. (2001). The use of glass wool as an attachment surface for studying phenotypic changes in *Pseudomonas aeruginosa* biofilms by two dimensional gel electrophoresis. *Proteomics* **1**, 871-879.

Stock, J. B., Surette, M. G., Levit, M. & Park, P. (1995). Two-component signal transduction systems: structure-function relationships and mechanisms of catalysis. In *Two Component Signal Transduction*. Eds. Hoch, J. A. & Silhavy, T. J.. Washington: ASM Press, 25-52.

Stoodley, P., de Beer, D. & Lewandowski, Z. (1994). Liquid flow in biofilm systems. *Appl Environ Microbiol* **60**, 2711-2716.

Stoodley, P., Boyle, J. D., Dodds, I. & Lappin-Scott, H. M. (1998). Influence of hydrodynamics and nutrients on biofilm structure. *J Appl Microbiol Symp Ser* **85**, 19-28.

Suh, S., Silo-Suh, L., Woods, D. E., Hassett, D. J., West, S. E. H. & Ohman, D. E. (1999). Effect of *rpoS* mutation on the stress response and expression of virulence factors in *Pseudomonas aeruginosa*. *J Bacteriol* **181**, 3890-3897.

Syed, A. & Gralla, J. D. (1998). Identification of an N-terminal region of sigma 54 required for enhancer responsiveness. *J Bacteriol* **180**, 5619-5625.

Tresse, O., Jouenne, T. & Junter, G. A. (1997). Underexpression of porin protein OmpF in agar entrapped, sessile-like *Escherichia coli*. *Antonie Van Leeuwenhoek* **72**, 271-274.

Van Loosdrecht, M. C. W., Lyklema, J., Norde, W., Schraa, G. & Zehnder, A. J. B. (1987). The role of bacterial cell wall hydrophobicity in adhesion. *Appl Environ Microbiol* **53**, 1893-1897.

Van Loosdrecht, M. C. W., Lyklema, J., Norde, W. & Zehnder, A. J. B. (1990). Influence of interfaces on microbial activity. *Microbiol Rev* **54**, 73-87.

Velasco, R., Burgoa, R., Flores, E., Hernandez, E., Villa, A. & Vaca, S. (1995). Osmoregulation in *Pseudomonas aeruginosa* under hyperosmotic shock. *Rev Latinoam Microbiol* **37**, 209-216.

Wainwright, M., Canham, L. T., Al-Wajeeh, K. & Reeves, C. L. (1999). Morphological changes (including filamentation) in *Escherichia coli* grown under starvation conditions on silicon wafers and other surfaces. *Lett Appl Microbiol* **29**, 224-227.

Wang, J. T. & Gralla, J. D. (1996). The transcription initiation pathway of sigma 54 mutants that bypass the enhancer protein requirement. Implications for the mechanism of activation. *J Biol Chem* **271**, 32707-32713.

Wang, J. T., Syed, A., Hsieh, M. & Gralla, J. D. (1995). Converting *Escherichia coli* RNA polymerase into an enhancer-responsive enzyme: role of an NH2-terminal leucine patch in sigma 54. *Science* **270,** 992-994.

Warner, J. B. & Lolkema, J. S. (2002). *LacZ*-promoter fusions: the effect of growth. *Microbiology* **148**, 1241-1243.

Watnick, P. I., Fulner, K. J. & Kolter, R. (1999). A role for the mannose-sensitive hemagglutinin in biofilm formation by *Vibrio cholera* El Tor. *J Bacteriol* **181**, 3606-3609.

Watnick, P. & Kolter, R. (2000). Biofilm, city of microbes. *J Bacteriol* **182**, 2675-2679.

Weiner, R., Langille, S. & Quintero, E. (1995). Structure, function and immunochemistry of bacterial exopolysaccharides. *J Ind Microbiol* **15,** 339-346.

Weyers, J. B. (1999). Isolation and characterization of attachment-induced regulatory elements in *Pseudomonas aeruginosa* PAO1. MSc Thesis. University of the Western Cape, Belville. South Africa.

Whiteley, M., Bangera, M. G., Bumgarner, R. E., Parsek, M. R., Teitzek, G. M., Lory, S. & Greenberg, E. P. (2001). Gene expression in *Pseudomonas aeruginosa* biofilms. *Nature* **413**, 860-864.