

Excreta and Secreta of medicinal maggots *Lucilia serica* and Biofilm of chronic wounds

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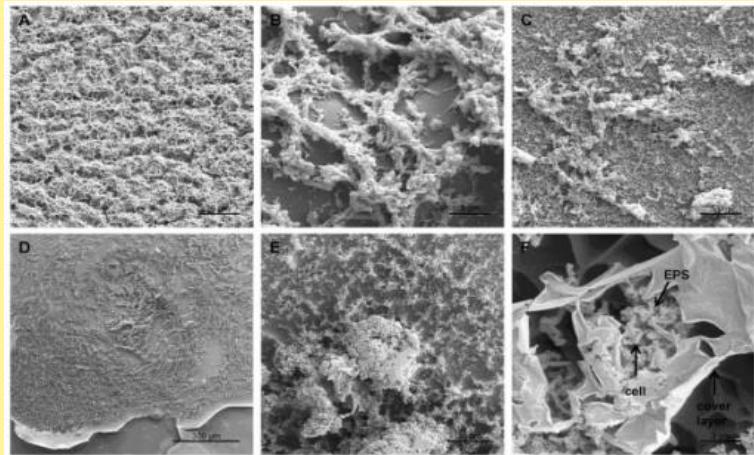
L. Sericata larvae and maggot therapy:

Stimmulattion of healing procecces of nonhealing wounds in first phases (antibiofilm, antibacterial, immunomodulating properties)

- Isolation, identification of antibacterial and ANTIBIOFILM compounds in *L. sericata* maggots
- Effect of particular secreted compounds on human keratinocytes

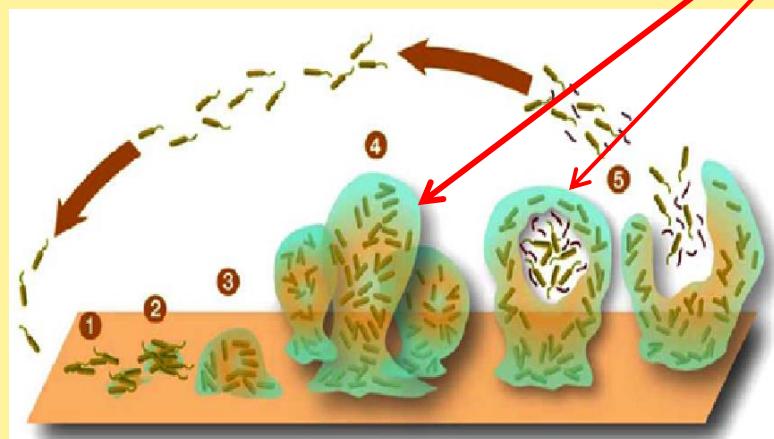


Biofilm



Matured biofilm of chronic wounds bed
(Rändler *et al*, 2010)

- Strongly adherent complex of microorganisms (bacteria, algae, protists,)
- Biofilm structure
 - 1) Cells in biofilm
 - 2) **Extracellular matrix** (exopolysaccharids, proteins, lipids and nucleic acids)



Biofilm formation (Monroe, 2007)

- › PROTECTION
- › NUTRIENTS
- › CELL TO CELL COMMUNICATION
- → Complication for healing proces (ECM = protects microorganisms within the biofilm, factors of immune system and ATB treatment are disabled to penetrate biofilm structure)
› CHRONICKÉ RANY

What is known about Antibiofilm activity of *L. sericata* maggots

- Inhibition of forming *P. aeruginosa* biofilm(Cazander et al, 2009)
- *Partial degradation of formed biofilm of chronic wound bed bacteria S. aureus, S. epidermidis, E. coli a P. aeruginosa* (van der Plas *et al*, 2008; Jaklic et al, 2008; Harris *et al*, 2009)
- Excreta and secreta (ES) partially degrade biofilm, kombination of antibiotics and ES treatment completely degrade *S. aureus* (van der Plas *et al*, 2010; Brown *et al*, 2012)
- Supposed antibiofilm compounds in ES: PROTEINASES(Harris *et al*, 2009,2013; Brown *et al*, 2012)

Antibiofilm activity of maggots *Lucilia sericata*: Methods

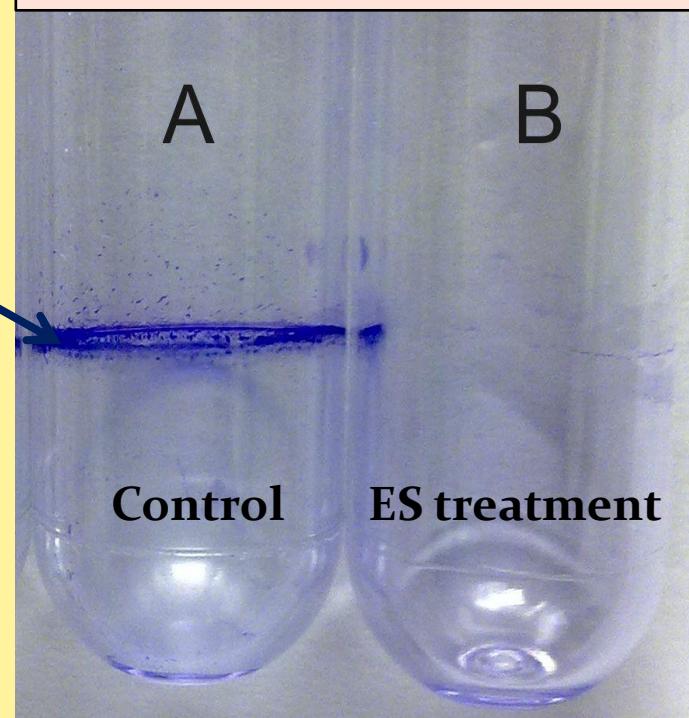
- Inhibition of forming biofilm
- Degradation of fully matured biofilm

→ *Staining biofilm structure with Crystal violet, Absorbance 570nm*

- Cell viability within the biofilm

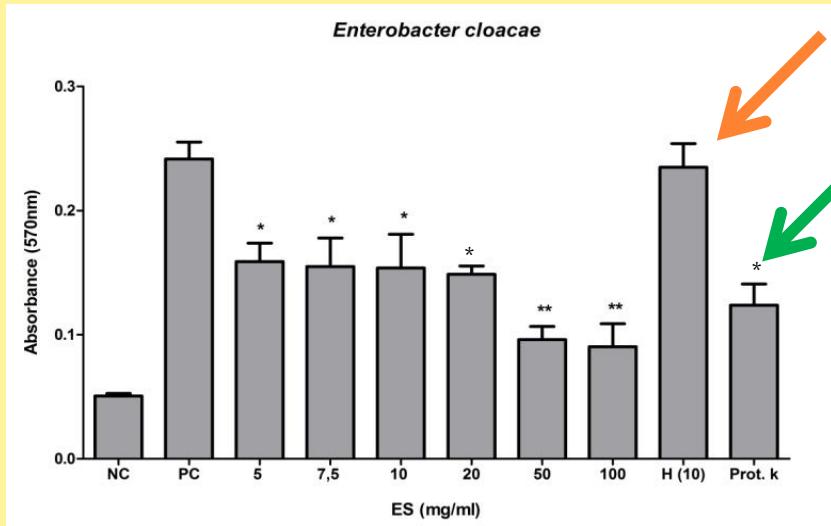
→ *CFU (colony forming units) number*

- › *Staphylococcus aureus*
- › *Enterobacter cloacae*
- › *Proteus mirabilis*

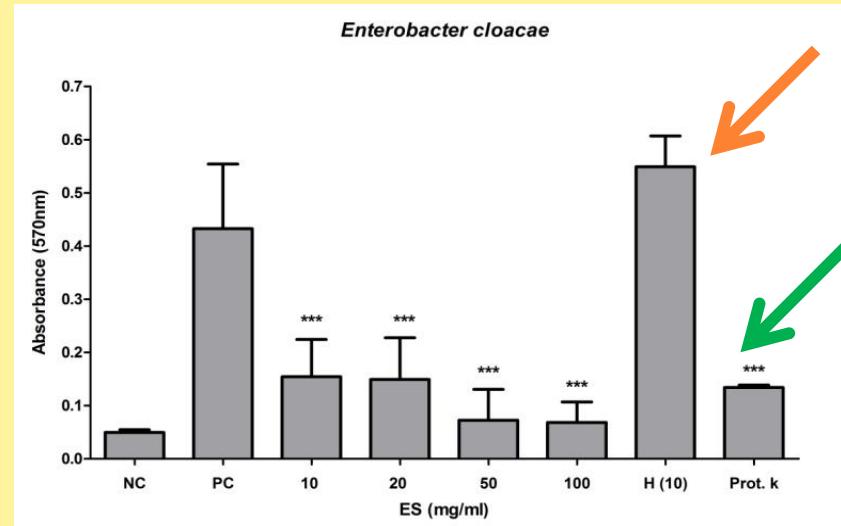


Enterobacter cloacae

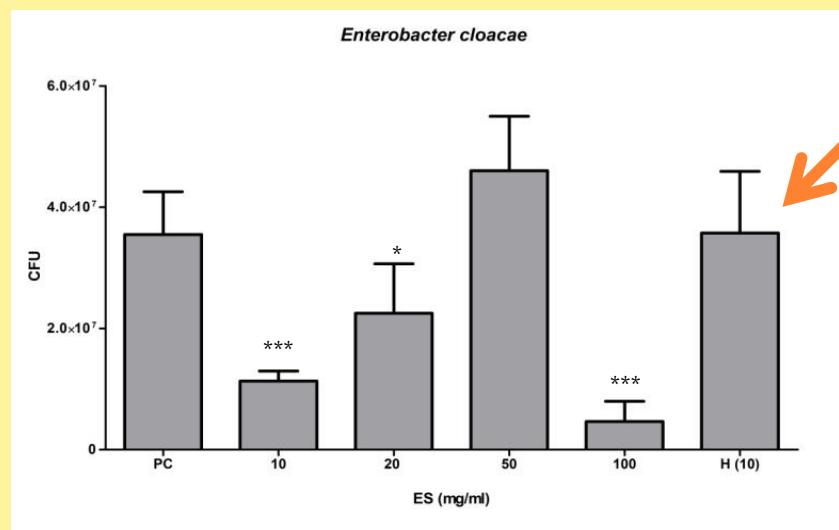
ES and biofilm forming



ES and degradation of matured biofilm

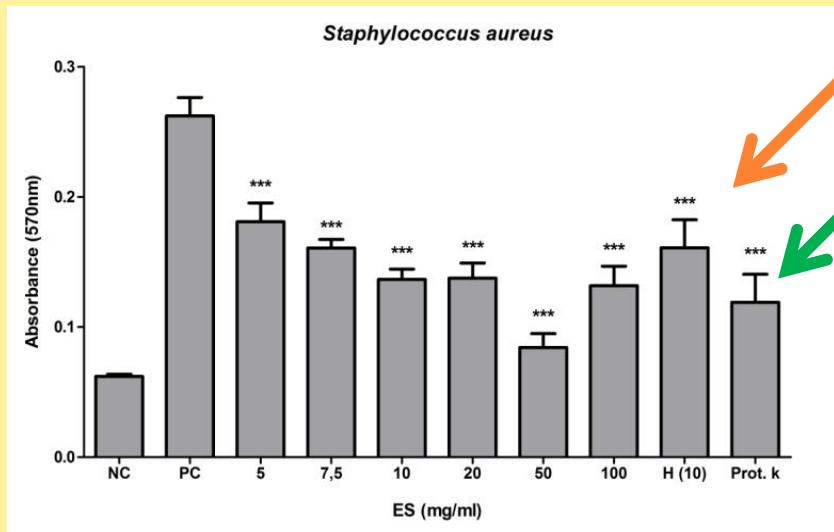


ES and cell viability

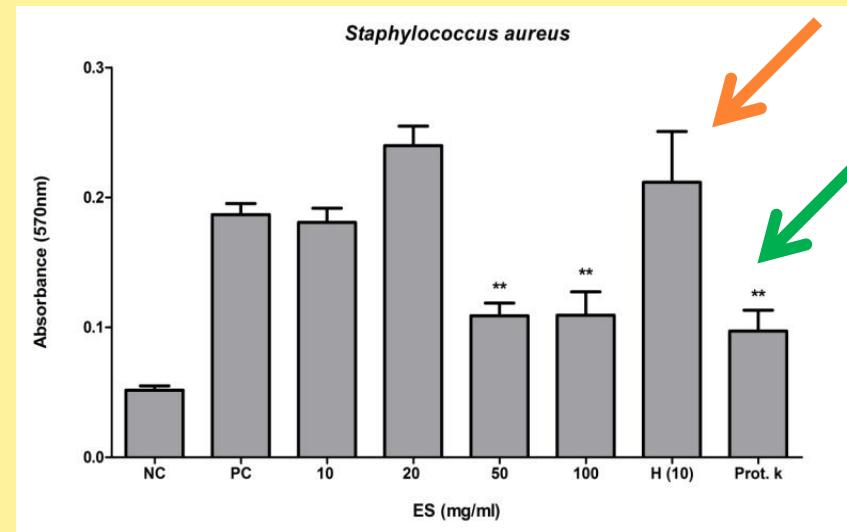


Staphylococcus aureus

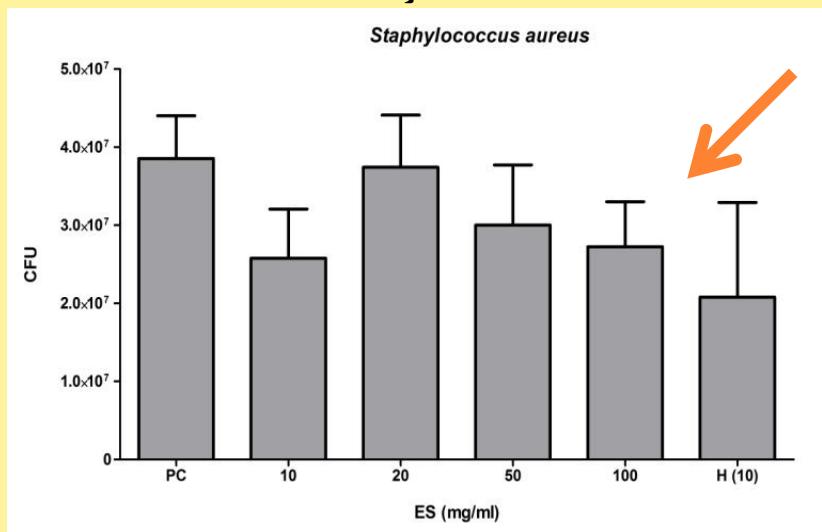
ES and biofilm forming



ES and degradation of matured biofilm

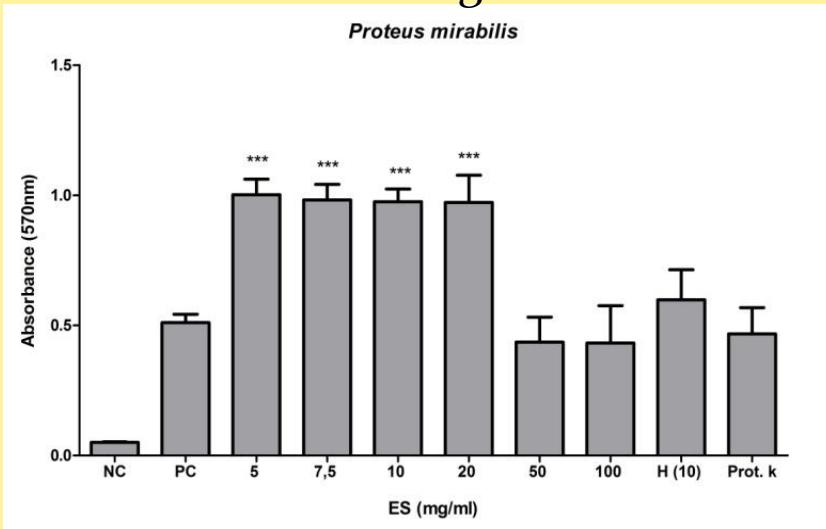


ES and cell viability

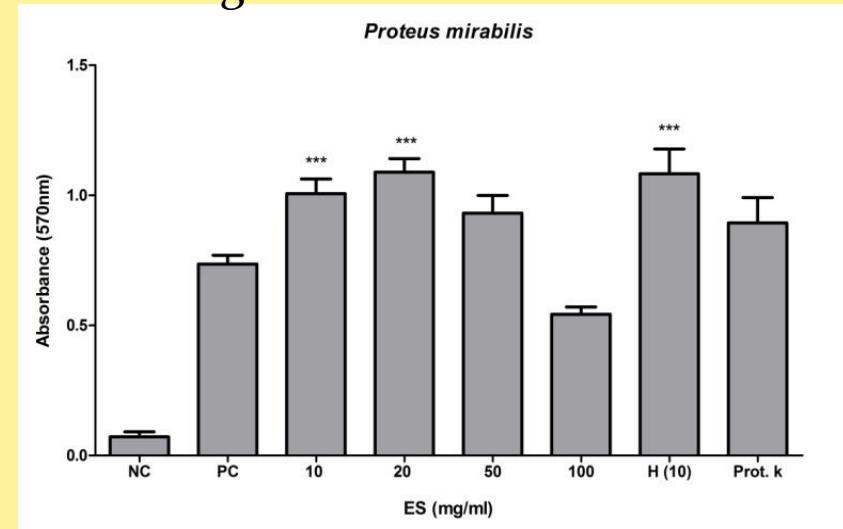


Proteus mirabilis

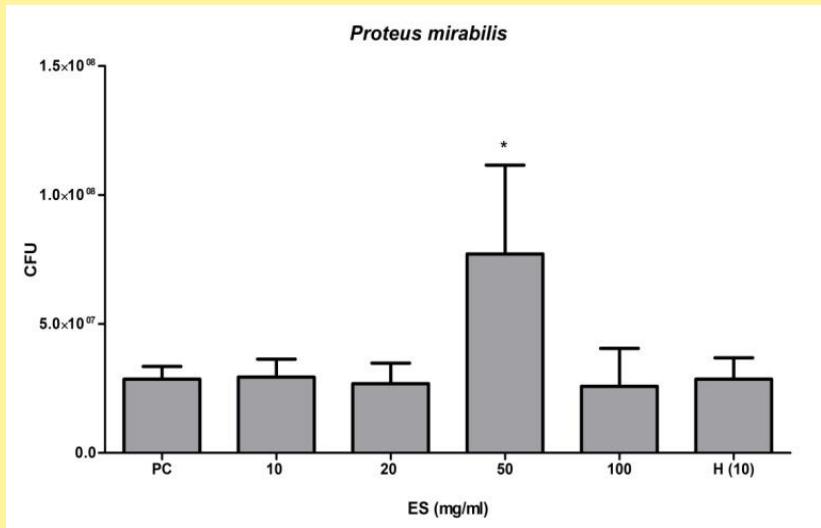
ES and biofilm forming



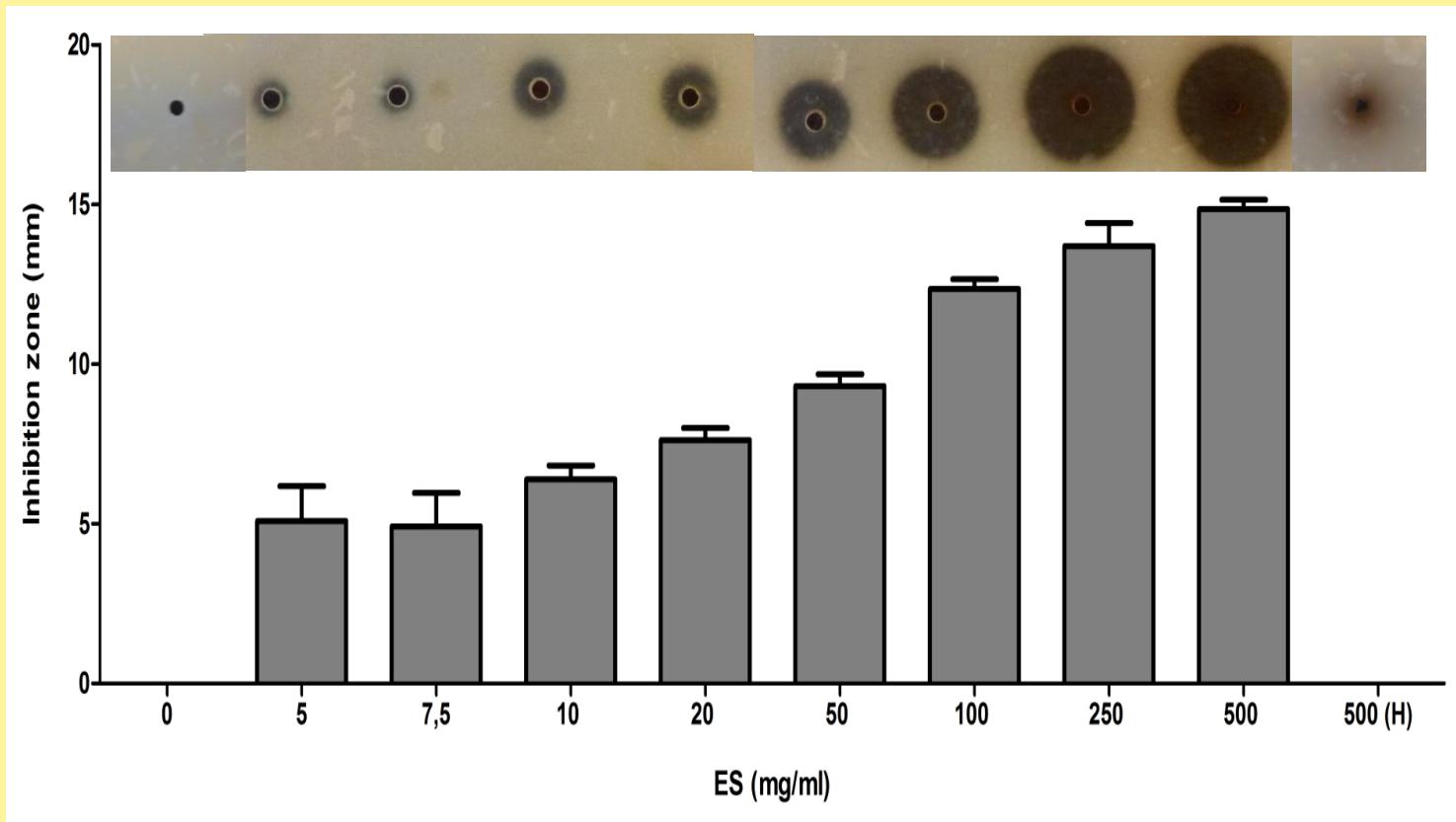
ES and degradation of matured biofilm



ES and cell viability



Nonspecificic proteolytic activity of maggots ES



Compounds within the maggots ES with potential antibiofil activity

GenBank Acc. #	Best BLAST hits	E-value	Score	Amino acid identity
JN215468	AAA17382.1 serine proteinase [Lucilia cuprina]	6.32 e-50	511	101/108 (93%)
	AAA68986.1 chymotrypsinogen [Lucilia cuprina]	1.78 e-44	464	91/157 (57%)
JN215469	ADD18566.1 large serine protease [Glossina morsitans]	3.82 e-161	1474	274/424 (64%)
	ADD18568.1 salivary trypsin [Glossina morsitans]	3.46 e-154	1474	267/420 (63%)
JN215470	EDS29955.1 CAAX prenyl protease 1 [Culex quinquefasciatus]	2.79 e-153	1407	266/435 (61%)
	ABF18495.1 prenyl-dependent CAAX metalloprotease [Aedes aegypti]	1.06 e-152	1402	269/437 (61%)
JN215471	ADD19121.1 signal peptide protease [Glossina morsitans]	4.43 e-172	1568	301/355 (84%)
	AAF51486.1 signal peptide protease [Drosophila melanogaster]	2.52 e-167	1527	297/358 (82%)
JN215472	ADD20181.1 salivary serine protease [Glossina morsitans]	2.49 e-131	1217	226/381 (59%)
	ACN69171.1 salivary trypsin [Stomyx calcitrans]	2.61 e-80	777	151/264 (57%)

Conclusions

- *L. sericata* maggots secrete mainly proteolytic compounds which are able to degrade strongly resistent biofilm of chronic wounds pathogens → wound bed cleaning (debridement)
- These particular compounds differently affect biofilm stadium, ECM and cell viability in species selective manner
- *L. sericata* maggots secrete also compounds stimulating growth of *P.mirabilis* biofilm (*P. mirabilis* – symbiont in larvae gut flora) → contraindication of maggot therapy
- *L.sericata* maggots are huge source of antibiofilm agents

Thank you for attention



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