SECTION 1 - PUBLISHABLE EXECUTIVE SUMMARY

Summary description of the project objectives

The Quorum approach has four objectives:

- Investigation of fungal **quorum sensing** for better understanding of biosynthesis of the industrially important target products.
- Study of quorum sensing and signal transduction in relation to fungal physiology and morphology in agitated systems.
- Comparative analyses of fungal cell communication in small and large scale submerged fermentations.
- Exploration of the relevance and interrelation of traditional scale-up factors to quorum sensing in fungal cultures.

The above-mentioned objectives were achieved through joint functional genomics (fungal genomics/transcriptomics/proteomics) and molecular biology, fungal physiology and biochemical engineering investigations.

Contractors involved

After three years, the consortium is the same as in the project proposal.
Coordinator, partners, scientific officer, and management team contacts details have been constantly updated during the whole duration of the project and are hereafter listed:

Function	Responsible Institution	Name and e-mail	Phone
Coordinator	**DCOB** - Department of Organic Chemistry and Biochemistry, University of Naples Federico II, Complesso Universitario di Monte S. Angelo, via Cynthia 4 – I-80126 – Naples - ITALY Responsible of the Unit: Prof. **Giovanni Sannia**	**Prof. Giovanni Sannia** sannia@unina.it	+39081674310 +39081674420
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Financial Manager	**WET** - Wetlands engineering SPRL Biotech Department Rue du Laid Burniat, 5 – 1348 Louvain-la-Neuve – BELGIUM	**Corine Deben** c.deben@wetlands.be	+3210861525
Financial Manager	**DCOB** - Department of Organic Chemistry and Biochemistry, University of Naples Federico II, Complesso Universitario di Monte S. Angelo, via Cynthia 4 – I-80126 – Naples - ITALY Responsible of the Unit: Prof. **Giovanni Sannia**	**Giovanna Marrone** marrone@unina.it	+39081674395
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Logistic Manager	**DCOB** - Department of Organic Chemistry and Biochemistry, University of Naples Federico II, Complesso Universitario di Monte S. Angelo, via Cynthia 4 – I-80126 – Naples - ITALY Responsible of the Unit: Prof. **Giovanni Sannia**	**Giacoma Fodale** quorum@unina.it	+39081674498
Scientific Leader	UOW – University of Westminster Molecular and Applied Biosciences Department, New Cavendish Street 115, W1W 6UW - London – ENGLAND	**Tajalli Keshavarz** T.Keshavarz@westminster.ac.uk	+442079115000
IT Manager	WET - Wetlands engineering SPRL Biotech Department Rue du Laid Burniat, 5 – 1348 Louvain-la-Neuve – BELGIUM	**Alban Gobin** a.gobin@wetlands.be	+3210861525

List of QUORUM participants:

Participant Role	Participant Number	Participant name	Participant short name	Country	Date enter project	Date exit project
CO	1	Dept. Organic Chemistry and Biochemistry, University of Naples Federico II	DCOB	IT	1	36
CR	2	University of Westminster	UOW	UK	1	36
CR	3	Institute of Chemical Technology, Prague Department of Chemical Engineering	ICTP	CZ	1	36
CR	4	Wetlands engineering SPRL	WET	BE	1	36
CR	5	Centre National de la Recherche Scientifique, CNRS/ FRE 3005 biosciences	CNRS/FRE 3005	FR	1	36
CR	6	University of Pécs	PECS	HU	1	36
CR	7	Turku Centre for Biotechnology, University of Turku	U. TURKU	FI	1	36
CR	8	Université catholique de Louvain	UCL	BE	1	36

Publishable Summary of the 5 Workpackages

The work has been divided into 5 work packages (WPs): the first one (WP1) deals with the management of the project, while Workpackages from 2 to 5 are involved in scientific activities.

WP1 Summary

D1 - Web based platform for internal and external communication

Internal and external communication have been performed in different ways: the Quorum project has its own website, available at the following address: www.quorumsensing.eu, where contractors may find information, reports, documents, forms and template etc. in the private zone, while a public zone has been provided for general information to the public; the skype and e-mails exchange have shown to be excellent forms of internal communication. For what concerns dissemination, several authorised publications have already been published and many others are planned for the next few months, which have been authorised thanks to the internal approval procedure. For what concerns exploitation, a very useful strategy has been developed in order to motivate partners in identifying the intellectual assets developed through the project and avoid misunderstanding at the end of the project. Moreover, in May 2008 project partners attended an Explotation Strategy Seminar which proved to be extremely useful. After a pre-selection of exploitable knowledge as potential patents and secrets, two patents have been elected where different exploitable knowledge has been included. It was decided to avoid secrets through an inclusion of strategic knowledge in the patents. Other knowledge has been, is being or will be published.

D6 - Report on management issues

The main activities deal with the administrative and managing tasks that have been performed internally and externally within the Consortium, with the public and the EU services.

Communication with the European Commission has been performed for what concerns project evaluation and approval, Form A delivery to the Commission, contact and communication with EU project officer and project PTA and then for what concerns the reception of funding from the Commission after reports approval. Funds were then divided among partners, according to what had been previously decided by the Consortium and from the EC's calculations. For what concerns internal communication, circulation and signature of the Consortium Agreement among partners, supervision of project meetings and redaction, circulation and approval of the agendas and minutes of all project's meetings among partners were performed. Moreover, the Management Team sent partners reminders of project decisions and deadlines and managed problem solving for what concerns different aspects of the project, such as administrative and financial issues. The Management Team provided,

organized, customised, distributed among partners, collected, checked templates and forms and then sent to the Commission all documents concerning first, second, third reporting periods and the 11 Deliverables of the Quorum Project.

WP2 Summary

From preliminary screenings, both commercial compounds already known as QS molecules and new endogenous or exogenous QSM were selected. In particular, **3-HAA**, **tryptophol**, **farnesol**, **benzyl ethanol** and **Isobutyric acid** from *Saccharomyces cerevisiae* spent medium, Benzoic acid and derivates from *Pleorotus ostreatus* spent medium proved to induce a modification of the canonical behaviour of the filamentous fungi analysed and could be considered as potential QS molecules. Cultivation media (complex samples) analysis by GC-MS and HPLC/MS allowed the detection of: butyrolactone I in *Aspergillus terreus* cultures , ^{1}-butyrolactone in *Penicillum* sp cultures, tyrosol, ,tryptophol and farnesol in *Pleorotus ostreatus* cultures. MS based metabolomics on *P. ostreatus* spent medium (time course analysis) revealed endogenous secreted compounds **benzaldhyde**, **benzoic acid**, **1-octen-3-one** and **1-octen-3-ol** and allowed to link them to effects on *P. ostreatus* mycelium development. Exo-metabolites added to *P. ostreatus* cultures can regulate hyphal length, branching and spore germination. The phenotype of *Pycnoporus sanguineus* in culture was affected by **phenyl ethanol** and **butyric acid**, two QS-like molecules detectable in the *S.cerevisiae* spent medium by GC-MS. These molecules, among 22 potential "chemical signals" were formally characterized from the fungus conditioned medium. Phenylethanol was detected in higher amounts in the *Pycnoporus sanguineus* conditioned culture than in the conditioning medium. **Benzaldhyde, phenyl ethanol** and **tryptophol** were characterized from *Coriolopsis polyzona* cultures.

WP3 Summary

D7- Report on gene coding for QS molecules

A combination of genomics, transcriptomics and proteomics tools were applied to three of the fungal systems studied in Workpackage 2 (i.e. *Aspergillus terreus, Pleurotus ostreatus,* and *Pycnoporus sanguineus*), with the aim of identifying the specific genes (and their respective encoded product) involved in the QS molecule biosynthesis. Our research activity resulted in the identification, for the first time, of candidate *A. terreus* gene loci responsible for the biosynthesis of a QS molecule. Furthermore, *P. ostreatus* genes, likely to be involved in biosynthetic pathway of a QS molecule, were identified *in silico*. The role of the identified QS molecule and its derivates in *P. sanguineus* QS signaling was also investigated, although this research activity was limited by the very recent availability of its genome sequence.

D8 - Report on functional studies

Differential transcriptomics and proteomics analyse

the sensors and of their electronics parts. At the end the sensors fully applicable in mini- and micro-scale bioreactors were fabricated and tested.

Milestones M6 - *Fabrication of the sensors and bioreactor for fermentation and quorum sensing studies* and M7 - *Validation of the bioreactor for fermentation* have been reached.

The deliverables D3 - *Report on a novel sensor for micro bioreactor*, D4 - *Report on a novel micro-scale bioreactors* and D9 - *Report on a novel bioreactor integrating novel sensor* have been delivered in due time.

WP5 Summary

D10 - Report on an active QS nano-process in submerged fungal cultures.

The objectives of this workpackage have been to investigate the relation between the physiological state of the fungal cultures in presence of quorum sensing molecules and the levels of the productivity of the target product at different scales of fermentation. To this end we have successfully demonstrated the effect of γ-butyrolactones, oxylipins and benzaldehyde in *Aspergillus terreus*, *Aspergillus nidulans* and *Pleurotus ostreatus* respectively on production of secondary metabolites and growth. We have demonstrated that oxylipins isolated from *A. nidulans* cultures regulate sporulation both in *A. nidulans* and *A. terreus* cultures and have a role in lipid based signal transduction. In *A. terreus* we have established the role of a potential quorum sensing molecule that affects the production of lovastatin as well as autoinduces its own production. In *Pleurotus ostreatus* we report that benzaldehyde inhibits radial growth and has an effect on many secondary metabolites.

D11 - Report on strategy based on QS for potential industrial application.

The objective of this workpackage has also been to establish a novel strategy for production of secondary metabolites at industrial scale employing quorum sensing molecules. Efforts were made in setting up a continuous culture with *Aspergillus terreus* to establish a strategy for enhanced production of secondary metabolites which will have commercial importance and industrial application. The exogenous addition of known quorum sensing molecules also unravels a potential industrial application whereby the levels of productivity of commercially important metabolites (laccases) were improved in *Coriolopsis polyzona*. We also reported that exogenous addition of spent medium from *Coriolopsis polyzona* also has a positive effect on laccase production. Laccase productivity was also improved by supplementing the cultures of *Pleurotus ostreatus* with the identified QS molecule at 10 L bioreactor level. Butyrolactone I from *A. terreus* was also investigated for its potential in enhancing lovastatin production in shaken flask, mid scale bioreactors as well as in continuous culture conditions.