x co-ordinated with the Director of the Institute / Head of Department

Institute of Ecological Chemistry (IÖC)
Independent Department Microbe-Plant Interactions (AMP)
Research Group Kremmer, Inst. of Molecular Immunology (IMI)

PSP-Element: G-505100-006; G-504600-001; G-504600-001; G-501700-003

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Title of the Highlight:
Development and Characterisation of new rat Monoclonal Antibodies for the Determination of \(N\)-Acyl-Homoserine Lactones and \(N\)-Acyl-Homoserines

Keywords:
Quorum sensing; Monoclonal antibodies, \(N\)-acyl homoserine lactone; \(N\)-acyl-homoserine; Enzyme-linked immunosorbent assay (ELISA)

Central statement of the Highlight in one sentence:
A new set of monoclonal antibodies was developed for the immunochemical detection of \(N\)-acyl-homoserine lactones (AHLs or HSLs) and \(N\)-acyl-homoserines (HS) in different immunochemical formats and for different applications.

Text of the Highlight:
Quorum sensing (QS) is a process that enables bacteria to communicate via chemical signaling molecules. It was confirmed that many Gram-negative bacteria use \(N\)-acylated homoserine lactones (AHLs or HSLs) as autoinducers. HSL analysis has gained interest due to the broad biological functions of HSLs in bacterial biofilms in medicine, biotechnology and agriculture. HSL molecules consist of a homoserine lactone ring and a side chain; they differ in the length of their \(N\)-linked side chain (4–18 carbon atoms) and the nature of the substitution at the 3-carbon (C3) position. Conventional analytical methods such as chromatography, mass spectroscopy, and NMR have been very successfully used for identification of HSLs. However, these conventional methods have higher instrumental detection limits (than immunoassays), and require time-consuming sample preparation, including purification and preconcentration. Plenty of sensitive bioassays have been developed by using different LuxR-based bioreporters, which contain LuxR family functional proteins, but do not produce HSLs. A combination of different bioassays is strongly recommended, since no bioreporter is sensitive to all HSLs. As a new and complementary and/or alternative analytical technology anti-HSL antibody-based immunochemical detection methods have been developed. According to the possible variety of molecular structure, four different HSL haptens, named HSL1, HSL2, HSL3, and HSL4, have been designed for antibody and assay development. HSL1 and HSL3 contain a long side chain (C10-COOH), while HSL1 has an oxo group, and HSL3 has an OH group in the C3 position. HSL2 (C4-COOH) and HSL4 (C6-COOH) have a shorter side chain and contain hydrogen at the C3 position. Rat anti-HSL monoclonal antibodies (mAbs) have been developed and characterized with enzyme-linked immunosorbent assays (ELISA). Eight mAbs
have been selected from about 200 mAbs and characterized by using coating antigen and/or enzyme-tracer formats. The antibodies showed distinguished selectivities toward HSLs corresponding to their hapten structures and have detection ranges for HSLs in microgram per litre concentrations, which are similar to the concentrations of HSLs present in bacterial culture supernatants. Interestingly, anti-HSLs mAbs have at least a 20 times higher sensitivity against hydrolyzed HSLs (homoserines, HSs) than original HSLs. This implies that our HSL mAb-based immunoassays will also detect HSs when they are in samples. When the concentrations of the sum of HSLs and of HSs have to be distinguished in a sample, samples will have to be measured before and after hydrolysis. The results will be expressed as ‘HSL equivalents’ of the main analyte of this particular immunoassay. As an alternative and/or complementary to conventional analysis and bioreporter assays, immunoassays will offer additional analytical techniques for quorum sensing (or quorum quenching) detection. The developed antibodies can also be broadly applied for many other immunochemical techniques, such as immunosensors and in-situ test systems.

Publications:

Taking account of the HMGU mission:
How does the Highlight described relate to the HMGU mission?
(1-2 sentences)
HMGU mission: We developed immunochemical detection methods for the investigation of bacterial communication. Bacterial communication plays an important role in the formation of biofilms. With these analytical tools one can investigate bacterial-bacterial, bacterial-plant cells, and bacterial-human cells interactions.

The internal HMGU co-operation partners with whom the Highlight was compiled, if appropriate:
Anti-HSL monoclonal antibodies were developed by Dr. E. Kremmer, IMI PSP G-501700-003 (see above).