Metabolites of *Pseudomonas* involved in the biocontrol of plant disease

David N. Dowling and Fergal O’Gara

There is increasing commercial and environmental interest in the use of microbe-based agents as alternatives to, or in combination with, chemicals for controlling the spread and severity of a range of crop diseases. The identification of specific microbial metabolites that are able to control certain plant diseases has led to the development of strategies for improving the performance and predictability of the microbial strains that produce these metabolites for application in the agricultural industry. This article focuses on antimicrobial metabolites produced by fluorescent pseudomonads, discusses their role in suppressing fungal diseases of important crops and reviews the prospects of genetically manipulating the producer organisms to improve the efficacy of these biocontrol agents.

The ability of some soils to suppress plant disease has been well documented. An important example is the development of soils suppressive to *Gaeumannomyces graminis* var. *tritici* (Ggt), the causative agent of 'take-all' of wheat. Such natural examples of biological control have been attributed to the indigenous beneficial rhizosphere microflora (see Glossary), especially fluorescent pseudomonads. Numerous mechanisms may account for these biocontrol properties (see Glossary), including the production of inhibitory compounds or metabolites. Microbial metabolites such as siderophores (see Glossary) and secondary metabolites with antimicrobial properties are considered to play a major role in disease suppression (see Table 1). Metabolites with biocontrol properties have been reported from diverse members of the beneficial rhizosphere flora; however, those produced by the fluorescent pseudomonads have received the most attention. This is probably due to the abundance of this diverse group of bacteria and their obvious importance in the rhizosphere, coupled with the relative ease with which they can be genetically manipulated.

Fluorescent pseudomonads produce a variety of metabolites (see Fig. 1), many of which are inhibitory to other microorganisms and some of which are implicated in the biological control of plant pathogens. The identification of a link between a metabolite(s) and the suppression of a particular disease is an on-going goal of many research groups, which the use of recombinant DNA (rDNA) techniques has facilitated. Some of the criteria used to indicate that a particular metabolite has a primary role in biological control are shown below.

- Mutants defective in metabolite(s) are unable to show inhibition of the pathogen in the laboratory.
- The biocontrol ability of the mutants is reduced in the field.
- Complementation of the mutant with wild-type DNA sequences restores biocontrol ability.
- The purified metabolite shows fungicidal or antimicrobial properties.
- The metabolite may be detected in situ (i.e. in the rhizosphere) when producing strains are present.

The ability to fulfil some or all of these points provides good evidence for the involvement of a particular metabolite in biocontrol. A selection of plant diseases controlled by specific metabolites are summarized in Table 1 and the structures of a range of these compounds are shown in Fig. 2.

Role of metabolites in biological control

Some of the metabolites implicated in biocontrol appear to be broad-ranging in their inhibitory action. For example, phloroglucinols and phenazines have
Table 1. Examples of specific microbial metabolites implicated in the control of crop diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Pathogen</th>
<th>Effective metabolite</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Take-all of wheat</td>
<td>Gaeumannomyces graminis var. tritici (Ggt)</td>
<td>Phenazines</td>
<td>9, 10, 11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c-Acetyl phloroglucinols</td>
<td></td>
</tr>
<tr>
<td>Tan spot of wheat</td>
<td>Pyrenophora triticirepentis</td>
<td>Pyrrolnitrin</td>
<td>12</td>
</tr>
<tr>
<td>Pre-emergent damping-off</td>
<td>Pythium spp.</td>
<td>Oomycin A</td>
<td>13</td>
</tr>
<tr>
<td>of:</td>
<td>Pythium ultimum</td>
<td>Pyoluteorin</td>
<td>14</td>
</tr>
<tr>
<td>cotton;</td>
<td></td>
<td>2,4-Diacetylphloroglucinol</td>
<td>15</td>
</tr>
<tr>
<td>sugarbeet</td>
<td></td>
<td>Hydrogen cyanide</td>
<td>16</td>
</tr>
<tr>
<td>Black root-rot of tobacco</td>
<td>Thielabiopsis basicola</td>
<td>2,4-Diacetylphloroglucinol</td>
<td>10</td>
</tr>
<tr>
<td>Crown gall of fruit trees</td>
<td>Agrobacterium tumefaciens</td>
<td>Agrocin 84</td>
<td>17-19</td>
</tr>
<tr>
<td>Flax wilt</td>
<td>Fusarium oxysporum</td>
<td>Pseudobactin B10</td>
<td>20</td>
</tr>
<tr>
<td>Damping-off</td>
<td>Pythium spp.</td>
<td>Ammonia</td>
<td>21</td>
</tr>
</tbody>
</table>

been shown to inhibit a wide range of fungal pathogens in the laboratory. Siderophores exhibit both fungicidal and bacteriostatic effects in the laboratory under conditions of low iron. In the field, these iron-chelating compounds are thought to deprive the pathogen of iron, a limiting essential nutrient.

Other metabolites are known to have very specific effects and to target particular pathogens; for example, agrocin 84, produced by *Agrobacterium radiobacter*, is specific for virulent strains of *Agrobacterium tumefaciens*. At the molecular level, agrocin 84 (a di-substituted nucleotide) is thought to act by chain termination of DNA synthesis. However, the precise mode of actions of many other metabolites is poorly understood.

Evidence is accumulating to support the theory that metabolite production has beneficial effects on the ecological competence of the producer strain. Production of these compounds is thought to provide the producing strain with a selective advantage in the highly competitive environment of the plant rhizosphere (see Ref. 25 for review). This idea has been substantiated by a recent report, which demonstrated that phenazine (Phz) antibiotics contributed to the persistence of the producer strains (*P. fluorescens* 2-79 and *P. aureofaciens* 30-84) compared with Phz-deficient mutants in a simulated wheat-rotation microcosm. Persistence may lead to improved competition and colonization of the producer strain on the plant surface, leading to niche-exclusion of the pathogen. The role of siderophores and other metabolite(s) in biocontrol may not be simply a direct antagonism of the pathogenic fungus, but may also involve more subtle ecological effects.

Alternative, more indirect, modes of action could involve stimulation of the plant's own defence mechanisms by induced systemic acquired resistance or, possibly, by direct uptake and translocation of the metabolite within the plant.
**Siderophores**
Peroxidin
Pyoverdine
Pseudobactin B10, M114, A214, 7SR1, A112, B117
Ferrichrome
Phyto-Phytochrome
Ferroxamine B
Alginate

**Antibiotic properties miscellaneous**
Cyanhydric acid
Aeruginoic acid
Magnesidin
Pseudomonac acid
Pseudomonac acid a
Pseudomonac acid b
Antibiotic P2563
P2563 a
P2563 b
Amino-2 acetophenone
Acetoclyic acid
Antibiotic DB-2073
Fluopsin C+F
Sorbidin A1+B
Salicylic acid

**Pterines**
Pterine
Riblopterine
Rilbyllumazine
Putidolumazine

**Pyroles**
Pyrole
Pyrole
Pyrole
Pyrole

**Indoles**
3-chloroindoled
Indole
Indole
Indole
Indole

**Lipids/Pyocompounds**
Pseudanes
Rhamnolipids
Pyolipids
Compound B
Jarvis rhamnolipid
Compound A

**Indole-3-acetic acid**

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**Some key metabolites implicated in biocontrol**

**Siderophores**
Siderophores are produced by many microorganisms as a means of sequestering limiting iron. Pseudomonads produce a range of iron-chelating compounds including salicylic acid, pyochelins and fluorescent pseudobactins and pyoverdines (see Fig. 2 for structures). Fluorescent siderophores are unique to pseudomonads - a trait that has implicated these organisms as PGPR. Fluorescent siderophores have been isolated from soil and there is considerable genetic and biochemical evidence that demonstrates their role in the promotion of plant growth and in biocontrol.

Fluorescent siderophores such as pseudobactins have a very high affinity for ferric iron (iron in the trivalent state). The molecules comprise a fluorescent chromophore and a quinoline moiety with two hydroxyl groups, which is involved in binding iron in conjunction with two hydroxyl groups from the peptide backbone of the molecule (for review see Ref. 35). The structures of two fluorescent siderophores, pseudobactin and pyoverdine, are shown in Fig. 2. Variations in the peptide moiety of the molecule are responsible for the strain specificity often observed with pseudobactins.

Uptake of the fluorescent iron–siderophore complex by an organism requires a specific outer membrane receptor. Although an individual *Pseudomonas* strain usually produces only one type of pseudobactin, strains that can utilize a range of pseudobactin siderophores by harbouring a number of different outer membrane siderophore receptors can be isolated.

Siderophore production and uptake of the iron–siderophore complex are considered to be significant for an organism as they may provide it with a competitive advantage under conditions of low iron, which would contribute directly or indirectly to preventing the establishment of a pathogen in the rhizosphere. Indeed, in laboratory experiments, the introduction of an additional receptor gene may improve the performance of the host strain.

**Antimicrobial metabolites**
Soil bacteria, in particular *Pseudomonas* spp., produce an array of low-molecular-weight metabolites, some of which are potent antifungal agents. Apart from the direct use of microbial metabolites as lead or precursor molecules by the chemical industry for production of new disease-control agents, metabolites are normally produced *in situ* (i.e. on the root surface) by the producing organism or biocontrol agent. Table 1 lists some of the metabolites involved in disease control in soil.

Important antimicrobial metabolites include the phenazines, such as phenazine-1-carboxylate (PCA) (Ref. 9) and the c-acetyl-phloroglucinol, which...
are effective against 'take-all' of wheat. Both of these compounds have been isolated from microcosm rhizospheres colonized by the producing strain, but not from roots colonized by mutants defective in the metabolite-synthesis pathway.\textsuperscript{10,42}

Phloroglucinol and pyoluteorin have been shown to be largely responsible for the prevention of 'damping-off' of sugar-beet\textsuperscript{15} (caused by \textit{Pythium ultimum}) and cotton\textsuperscript{13} (caused by \textit{Pythium spp.}). whereas phloroglucinol and hydrogen cyanide (HCN) are responsible for the control of black root-rot of tobacco (caused by \textit{Thielaviopsis basicola}) by \textit{P. fluorescens} CHA0 (Refs 10,16). Putative biochemical pathways for the synthesis of many of these compounds have been reviewed (see Ref. 22).

Volatile compounds such as ammonia\textsuperscript{21} and HCN are produced by many rhizosphere strains and have been implicated as important metabolites in biocontrol. For example, some species of \textit{Pseudomonas} can produce levels of HCN \textit{in vitro} that are toxic to certain pathogenic fungi, e.g. \textit{Thielaviopsis basicola}, and, thus, prevent black root-rot of tobacco\textsuperscript{15}. High concentrations of HCN are toxic to some plants and it has been suggested that fluorescent pseudomonads producing HCN may be responsible for a reduction in the yield of certain crops, e.g. potato\textsuperscript{34}.

\textbf{Figure 2}

Structures of some of the bacterial metabolites implicated in the biological control of plant disease in the field.
Plant hormones

Salicylic acid, a precursor of pyochelin and a siderophore in its own right, is also a plant hormone and is implicated in the induction of systemic acquired resistance in plants. Another plant hormone, indole acetic acid (IAA), is also produced by many strains that exhibit biocontrol properties. Although IAA has not been directly implicated as a metabolite in disease control, it is bioactive and stimulates root elongation (by stimulating the growth of root hairs). Indole acetic acid is produced by the nitrogen-fixing bacteria, *Azospirillum*, and is thought to play a key role in the plant-growth-promoting effect that these bacteria have on graminaceous plants.

Factors affecting metabolite production

Antimicrobial-metabolite production and its effect on pathogens is dependent on environmental factors such as soil chemistry, temperature and water potential – factors that will be beyond the control of the biotechnologist. In addition to active and aggressive colonization of the producing strain, the organism must synthesize the metabolite at the correct time and in the appropriate location. In the laboratory, adequate production of metabolites requires the producing strain to be grown in synthetic media containing a suitable carbon and nutrient source. In the field, these nutrients will be supplied in the form of root or seed exudates.

A number of key factors have been shown to influence the metabolite production of some biocontrol strains. These include:

- **Zinc**: increases the production of phenazine (PCA) (Ref. 49).
- **Temperature**: 12°C is optimum for the production of 2,4-diacetylphloroglucinol (DAPG) (Ref. 15).
- **Surface contact**: DAPG production by strain F113 is enhanced if the bacteria attach to solid surfaces.
- **Water potential**: oomycin A (Ref. 13).
- **Carbon sources**: glucose stimulates the production of oomycin A (Ref. 50), but represses the production of DAPG (Ref. 15). Sucrose stimulates the production of DAPG (Ref. 15).
- **Iron**: represses the production of siderophores and stimulates the production of HCN (Ref. 16).

Carbon sources usually have a major influence on the type and levels of antibiotics produced, and laboratory studies on the production of metabolites with respect to carbon sources are yielding interesting results. In combination with a knowledge of the relative amounts and types of sugars, amino acids, etc. present in the rhizosphere, it may be possible to extrapolate this information to the field in order to predict whether or not metabolites will be produced in situ.

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Modification</th>
<th>Effect</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered expression</td>
<td>Inserted alternative promoter (ptac) upstream of biosynthesis genes</td>
<td>Constitutive expression of oomycin A. Enhanced biocontrol</td>
<td>13</td>
</tr>
<tr>
<td>Gene dosage</td>
<td>Increased copy number of gene(s) in wild type</td>
<td>Over-production of 2,4-diacetylphloroglucinol and pyoluteorin. Enhanced biocontrol on cucumber, phytotoxic to cress</td>
<td>52</td>
</tr>
<tr>
<td>Heterologous expression</td>
<td>Introduced <em>phi</em>, <em>hcn</em>, <em>phz</em> genes into a non-producer strain</td>
<td>Non-producing strains converted to producing strains. Enhanced biocontrol ability in soil</td>
<td>7, 11, 16, 54</td>
</tr>
<tr>
<td>Deletion</td>
<td>Specifically deleted <em>tra</em> genes from plasmid encoding agrocin 84</td>
<td>Eliminates potential transfer of agrocin 84 resistance gene to target pathogen. Safer strain</td>
<td>18, 19</td>
</tr>
</tbody>
</table>

**Abbreviations**: *ptac*, a synthetic promoter made from the lac and trp promoters; *phi*, gene for the synthesis of phloroglucinols; *hcn*, gene for synthesis of hydrogen cyanide; *phz*, gene for synthesis of phenazines; *tra*, transfer gene.
Current strategies to improve biocontrol agents

Unmodified strains and consortia

Most of the commercial biocontrol strains that are currently available are not genetically manipulated, and the use of wild-type strains as a primary source of material for biological control is likely to continue. The use of more rational screening systems that simulate field conditions are likely to generate many new isolates. For example, in our laboratory, a screen of natural rhizosphere pseudomonads for strains that produced fluorescent siderophores under conditions of high levels of iron, produced one isolate that also showed good biocontrol ability (D. J. O’Sullivan and F. O’Gara, unpublished).

The use of mixtures of microbial strains as biocontrol agents is an important development in the use of unmodified inoculants. Many reports illustrate the synergistic effect of mixed cultures in the control of certain pathogens. This may be due to the production of an increased range of metabolites by the consortia and/or other mechanisms. By choosing strains with diverse mechanisms for biocontrol, e.g. siderophores and phloroglucinols, it may be possible to design consortia that have improved biocontrol properties in the field.

Much of the information on the role of metabolites in biocontrol has come from the use of rDNA-based technology. The use of such technology can also be a powerful tool for manipulating the production and range of metabolites produced by a single strain.

Genetically modified strains

Standard rDNA and genetic approaches are currently being used to manipulate strains for improved production (and uptake) of important metabolites. A summary of the different strategies, with examples, are shown in Table 2. Some examples are discussed in more detail below.

The genetics and biochemistry of siderophore biosynthesis is complex, and it is obvious that siderophores will only be effective in soils with low levels of iron. Engineering strains to produce siderophores at higher levels of soil iron may lead to improved performance of an inoculant strain. Studies on iron-mediated regulation of siderophores indicate that siderophore biosynthesis is under both positive and negative regulation, and that the generation of deregulated mutants with a constitutive phenotype may offer a possible approach to improving this important trait. As discussed previously, broadening the ferric iron–siderophore uptake potential of a strain may also increase its rhizosphere and biocontrol competence.

Engineering over-production of metabolites has been a strategy used by many researchers. The first antibiotic genes to be cloned and manipulated were from P. fluorescens HV37a, which produces oomycin A. This antibiotic is primarily responsible for biocontrol (70% of disease control) by this strain of Pythium induced root infection of cotton seedlings. The loci responsible for biosynthesis, AfuE, AfuR, AfuP, and regulation, AfuAB, were identified and cloned. Expression of the AfuE gene has been increased by cloning it downstream from the tac promoter (a synthetic promoter made from the lac and trp promoters). This led to increased production of oomycin A by the engineered strain. Preliminary experiments have suggested that over-production of oomycin leads to improved inhibition of Pythium ultimum.

Careful evaluation of the host plant, target pathogen and inoculant is required when over-producing metabolites in the absence of appropriate regulation. A derivative of the wild-type biocontrol strain CHA0 harbours a recombinant plasmid that confers the ability to over-produce both DAPG and pyoluteorin. This strain showed enhanced biocontrol activity with cucumber but was phytotoxic, compared with the unmodified parent strain, to cress. This indicates that metabolite production may have to be regulated for optimum disease control on susceptible host plants.

Another strategy is the introduction of a new trait into heterologous hosts with a view to improving their biocontrol potential. The production of DAPG by Pseudomonas sp. strain F113 is a key trait in the biocontrol of P. ultimum ‘damping-off’ of sugar-beet seedlings. The genes involved in the biosynthesis of DAPG have been cloned and the final step in the pathway, monoacetylphloroglucinol (MAPG) acetyltransferase activity, is encoded by a 6kb DNA fragment. This fragment was cloned upstream of a constitutive tetracycline resistance promoter (tet) in the cloning vector pSUP106 (see Fig. 3) and introduced into a wild-type strain (M114) that is unable to synthesize DAPG. The engineered strain expresses the enzymic activity that converts MAPG to DAPG and, thus, is able to synthesize DAPG. The presence of this new trait has enhanced the biocontrol ability of strain M114 against P. ultimum, both in the laboratory and in greenhouse experiments. Introduction of the DNA fragment into other pseudomonads and E. coli does not lead to DAPG production, suggesting that
these organisms may lack other components that are necessary for production of the antibiotic.

Other examples of this strategy include the heterologous expression of the genes for HCN biosynthesis, which leads to enhanced biocontrol of tobacco black root-rot in the recipient strain, and the introduction of the genes for the synthesis of DAPG (Ref. 11) and phenazine into other pseudomonads. This strategy may offer a simple way to construct strains that produce multiple bioactive metabolites.

Antimicrobial compounds can be considered as secondary metabolites that preferentially accumulate late in the growth cycle (i.e. in stationary phase) in laboratory media. This is consistent with the notion that antimicrobial compounds have evolved as microbial defence mechanisms to inhibit competitors under stress situations such as nutrient depletions.

The regulation of the synthesis of important antimicrobials from fluorescent pseudomonads is summarized in Fig. 4. Antibiotics are regulated by a global regulator, GacA (Ref. 60). The environmental stimulus that triggers this response is not yet known; however, there is physiological and genetic evidence that a cell-density-dependent autoinduction mechanism, analogous to the prototype luxR/I system [the regulatory genes for bacterial (Vibrio sp.) light-production enzymes], may also be involved. The lux genes are regulated in a cell-density-dependent manner that requires the accumulation of a small-molecular-weight autoinducing metabolite, N-(3-oxohexanoyl)homoserine lactone (see Fig. 2 for structure), in combination with the luxR activator.

Recently, the phzA gene, a homologue of luxR, was shown to be required for the regulation of phenazine biosynthesis (Fig. 4). The production of phenazines is also enhanced by the presence of a small-molecular-weight compound isolated from culture supernatants of the producing strain. Such a cell-density-dependent requirement for efficient production of antimicrobial metabolites may explain the apparent necessity for efficient root- or seed-colonization by the producer strain for efficient and reproducible biocontrol in the field.

The luxR/I type of regulatory system has been implicated in the control of a range of important microbial processes, including plasmid transfer, antibiotic resistance, and exoenzyme production. Exoenzymes such as chitinases may be useful factors in the biological control of pests, including some chitin-containing fungi, nematodes and insects. It will be of interest to determine if such enzymes are also under the control of autoinducing metabolites.

The presence of autoinducing metabolites and pheromone-mediated regulation is a new and exciting development that may prove to be of major importance in understanding and manipulating mechanisms of biocontrol.

Future research directions

The major bottle-neck to the commercial use of microbe-based biocontrol agents is their unpre-

dictability in the field, relative to conventional chemical treatments. This may be due to a variety of reasons, including abiotic factors, poor and inconsistent colonization, and the failure to produce the metabolite(s) at the appropriate time or levels. Laboratory studies are increasing our knowledge of the environmental factors that may influence the production of metabolite(s) at the appropriate microsites in the rhizosphere; however, a large gap remains in our understanding of the interactions between rhizosphere exudates and microbial metabolite production. The appropriate temporal and spatial regulation of metabolite(s) synthesis by the
biocontrol agent is essential to improve the effectiveness of biological control. Recently, sensor bacteria with reporter genes fused to the promoters of antibiotic synthesis genes have been used to measure transcription of these genes in situ on the seed coat. The extensive use of appropriate reporter bacteria with suitable reporter genes (e.g., lux, luc, ice, lacZ) fused to the promoters of genes included in the synthesis of antimicrobial metabolites will allow researchers to monitor precisely the expression of these genes in situ, and will complement the direct measurement of metabolites in soils and rhizospheres. However, the final goal will be to regulate and fine-tune metabolic synthesis such that the appropriate biocontrol genes are expressed at the required level by a predetermined environmental signal. This signal could be derived from some appropriate environmental conditions during crop cultivation, such as soil-nutrient level, water potential or temperature, which are known to favour the pathogen or disease. A more elegant 'trigger' for the expression of biocontrol genes in an introduced inoculant strain would be for the genes to be activated directly, or indirectly, by the presence of the specific pathogen in the rhizosphere.

Conclusions

The evidence that some microbial metabolites play a key role in the biological control of certain key plant pathogens provides a strong incentive to develop such material as biocontrol agents for commercial use. The use of microbial strains producing antibiotic metabolites in controlled environments, such as horticulture in greenhouses, offers the best possibility for success in the short term. The major challenges to be resolved prior to widespread commercial exploitation of biocontrol strains lies in the ability to predict more confidently the behaviour of such strains in the field. Fortunately, as interest in these organisms grows, more information on the genetics, physiology and ecology of metabolite production is becoming available. Such data are of immense importance for the selection of wild-type strains with desirable traits from nature and to provide a more rational framework for the choice of strains for use in inoculant consortia. Recombinant DNA methods have enabled genetic manipulation of metabolite production with promising results. In this context, research is also directed towards the evaluation of possible risks associated with the large-scale release of these genetically modified organisms (GMOs). Based on the knowledge that is now being accumulated, particularly in the area of molecular microbial ecology of the rhizosphere and metabolite regulation, improved or manipulated biocontrol agents should become more predictable and reliable for use under field conditions.

Acknowledgements

We should like to thank the members of the *Pseudomonas* biocontrol group at UCC, especially Anne Fenton, Ray Sexton and Paul Gill, Jr for the inclusion of unpublished data. Work in the authors’ laboratory was supported, in part, by contracts from the European Community (ECLAIR AGRE 0019-C; BRIDGE BIOT-CT90-0166-C, BIOT-CT91-0293, BIOT-CT91-0283; FLAIRE AGRE-0019-C; BIOTECH BIO2-CT93-0053, BIO2-CT93-0196, BIO2-CT92-0084).

References

Errata

We apologize to the authors, and to the readers who may have been misled by the publication errors which appeared in the article “Retinal–protein complexes as optoelectronic components” by N. N. Vsevolodov and T. V. Dyukova, published in the March 1994 issue of TIBTECH. The corrected information appears below:

Table 2. The correct structure and information for 4-keto retinal is:

<table>
<thead>
<tr>
<th>Retinal and its analogs</th>
<th>Structural formula of retinals</th>
<th>Absorption spectra for ground (-) and photoinduced (-) BR forms</th>
<th>Half life time for photoinduced forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Keto retinal</td>
<td><img src="image" alt="Structure of 4-Keto retinal" /></td>
<td><img src="image" alt="Absorption spectra" /></td>
<td>From minutes to tens of minutes</td>
</tr>
</tbody>
</table>

Table 3. The correct reference for sensory rhodopsins SR-1 and SR-2 should have been Ref. 26 [Späthich, J. L. and Bogomolni, R. A. (1992) J. Bioenergetics Bioenerg. 24, 193–200].