Calorimetric Characterization of Microorganisms

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Summary. A short survey is given on recent calorimetric studies of microbial growth, which raised the question whether calorimetrically obtained thermograms can be used for identification of different organisms. Advantages and the efficiency of the method are critically discussed.

1. *Introduction*

Although microcalorimetric studies on bacteria were introduced 20 years ago[1], research in this field was not very active until 1973, when a short communication on the microcalorimetric identification of bacteria was published[2]. It stimulated intensive research and discussion on this topic and its microbiological applications. It has been shown by several workers that interesting data on microbial metabolism may be obtained by calorimetry and that some theoretical problems in biological thermodynamics can be solved only by this method[3–5].

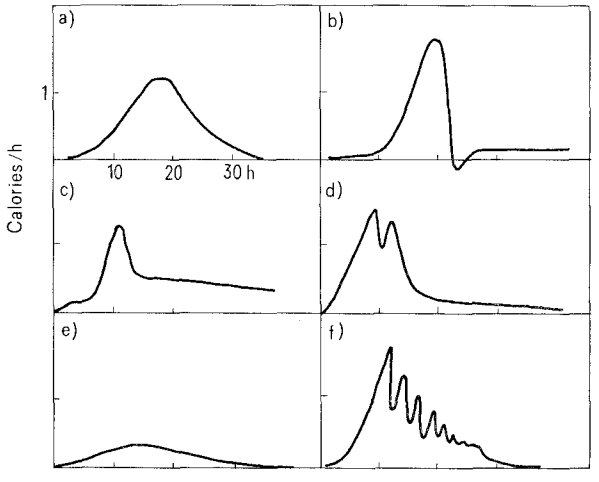

Fig. 1. Thermograms of *Saccharomyces cerevisiae* under different culturing conditions. a) liquid glucose-medium, unstirred; b) liquid glucose-medium, stirred; c) solid (agar) glucose-medium; d) liquid medium containing glucose and saccharose; e) liquid glucose-medium under 2 kp/cm² O_2-atmosphere; f) liquid glucose-buffer under O_2-atmosphere of 2 kp/cm².

The conduction type calorimeters normally used measure the heat transferred from the calorimeter vessel to the surroundings. In case of a growing culture, this represents the momentary heat production arizing from the catabolic and anabolic processes in the organisms. Recording the heat flux as a function of time yields a thermogram. The amplitude of the thermogram is proportional to the intensity or the rate of the reactions, and the integral value gives the sum of the heat produced, which is related to the extent of the processes. In view of the complexity and multiplicity of microbial metabolism and the variety in the chronological order of metabolic processes in the different kinds of microorganisms, one would expect the thermograms produced by the microbes under investigation to differ sufficiently for identification of individual species.

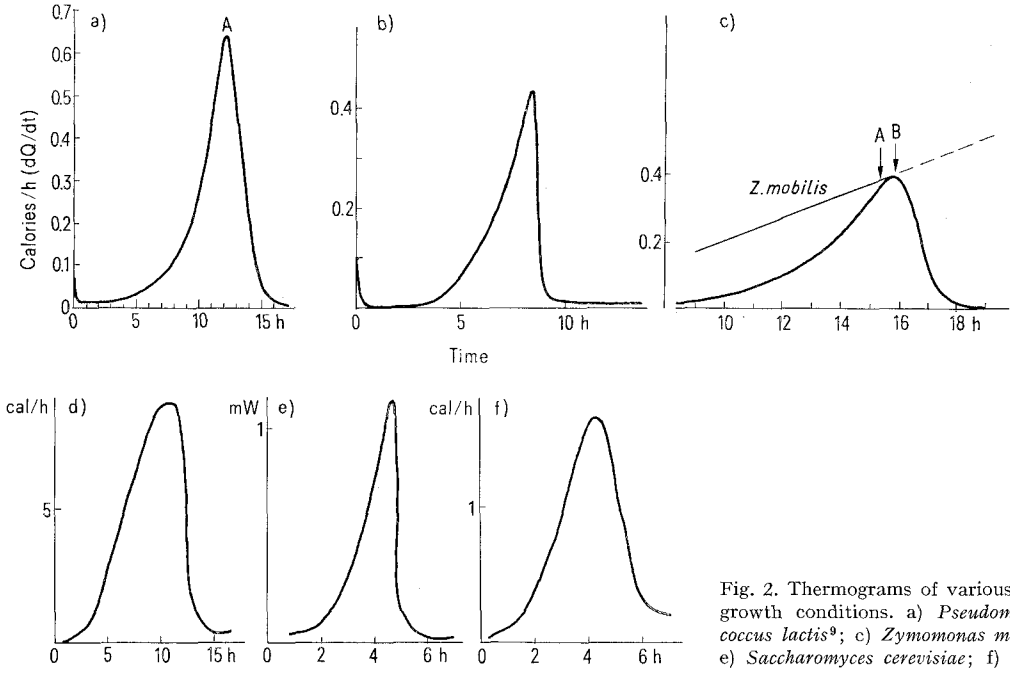

Fig. 2. Thermograms of various organisms under optimum growth conditions. a) *Pseudomonas lindneri*[8]; b) *Streptococcus lactis*[9]; c) *Zymomonas mobilis*[10]; d) *Escherichia coli*; e) *Saccharomyces cerevisiae*; f) *Bacillus stearothermophilus.*

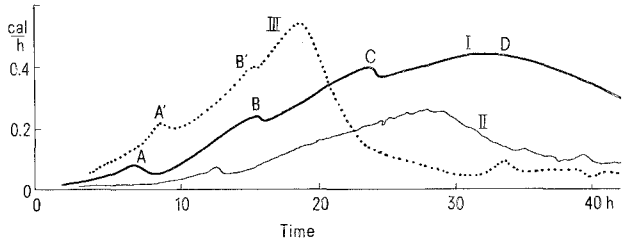

Fig. 3. Thermogram of 3 different types of typhi[1]: I, *Salmonella* (Eberthella) *typhi*; II, *S. paratyphi* A; III, *S. paratyphi* B (Schottmülleri).

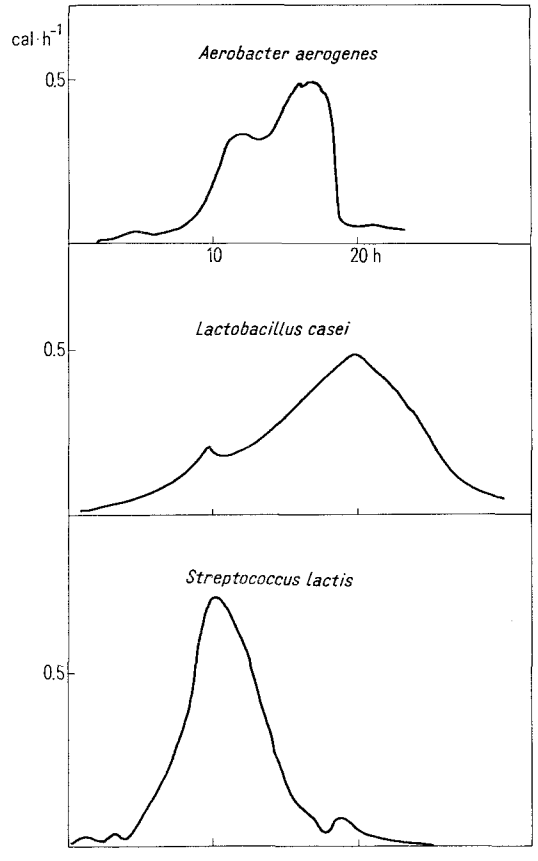

Fig. 4. Thermograms of 3 different milk bacteria[9].

2. *Instrumentation*

The different types of calorimeters used for biological experiments have been described in detail elsewhere[6,7]. Therefore, only a brief illustration of the action principle shall be given here.

Batch calorimeter. The calorimeters mainly used in biological and biochemical research up to the present were of the batch type, i.e. a closed calorimetric vessel of limited volume (1 to 100 ml), which simultaneously served as the culturing vessel. With this type it is difficult to perform stirring, aeration or other manipulations on the culture, and high thermal disturbances may be initiated by such operations.

Flow calorimeter. Calorimeters of the flow type have recently become available. A sample of the suspension is sucked through the calorimeter vessel and exchanges its heat continuously with the calorimeter. A microbial culture may be run outside the calorimeter in a culturing vessel and only a small part of it is pumped continuously through the calorimeter. Measurements, tests and manipulations are possible without any disturbance of the calorimetric recording device.

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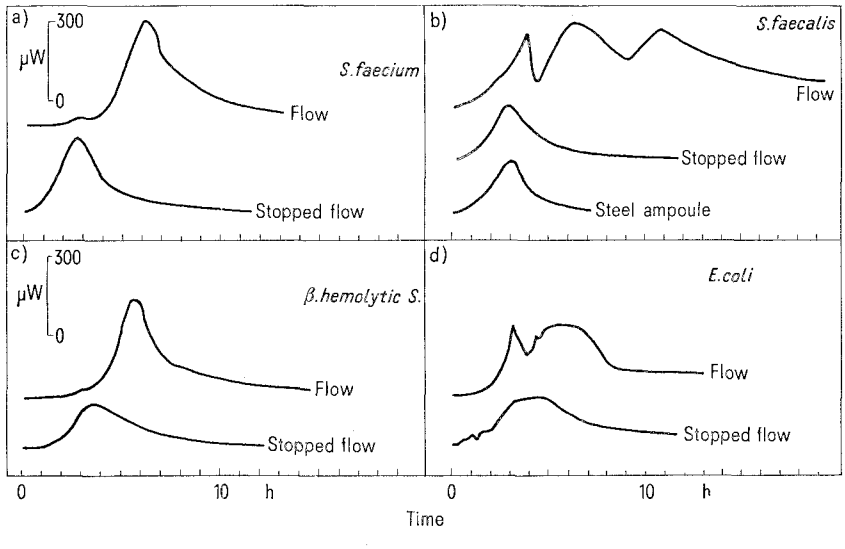

Fig. 5. Thermograms of 4 different intestinal bacteria under aerobic (flow) and anaerobic (stopped flow) conditions[11].

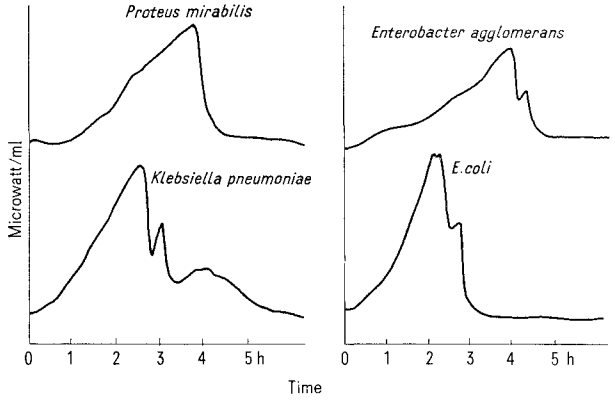

Fig. 6. Thermogram of 4 different intestinal bacteria[12].

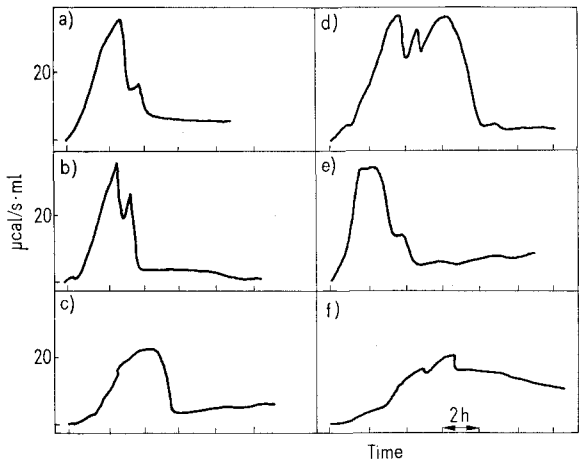

Fig. 7. Thermogram of 6 different enterobacteriaceae[2]. a) *Enterobacter aerogenes*; b) *Klebsiella*; c) *Proteus vulgaris*; d) *Enterobacter cloacae*; e) *Escherichia coli*; f) *Proteus rettgeri*.

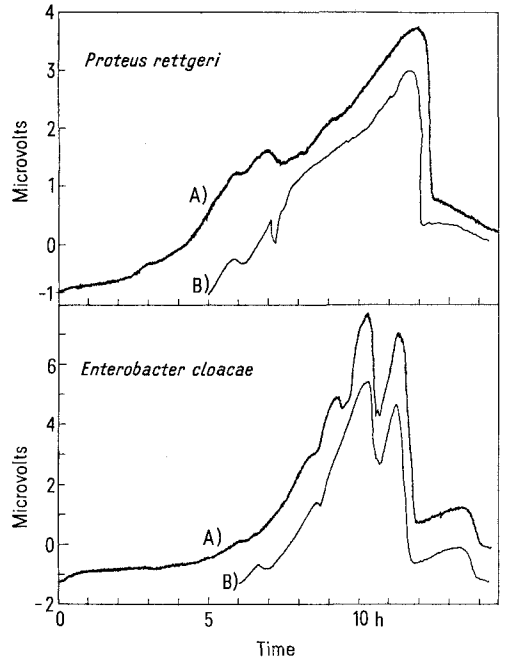

Fig. 8. Repeated growth curves of 2 different enterobacteriaceae[13]. The lower curves B have been displaced in time and in response to indicate similarities.

3. *Influence of culture conditions*

It is well known that the metabolism of an organism depends strongly on the culture conditions and the nutrient supply. Special methods have been prepared for shifting metabolism from one biochemical pathway to another.

Figure 1 demonstrates how different conditions of growth influence the heat production of a yeast culture. A change in just one parameter produces a distinct change in the thermogram. One can imagine the difficulty of distinguishing different species only on the basis of the thermograms, if a single organism can produce many different thermogram profiles.

On the other hand, different microbes can evolve similar thermograms during growth. Figure 2 illustrates this fact. The smooth, simple thermograms obtained in all cases are the expression of the same simple metabolism proceeding in the organisms. In all of these experiments, the culture grew up in a glucose-rich medium under nearly anaerobic conditions. Therefore, only a relatively simple catabolism took place in all the organisms, i.e. degradation of the glucose via glycolysis to ethanol, acetate or lactate.

Concerning the question of identification, two postulates on the experimental method can be based on the examples of Figures 1 and 2:1. The conditions

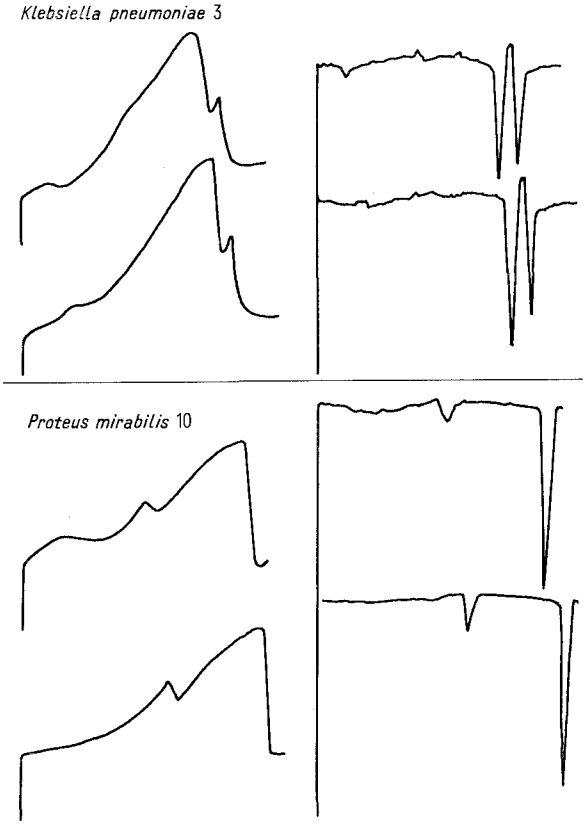

Fig. 9. Repeated growth curves of 2 species of enterobacteriaceae[14]. The right curves are the derivatives of the left thermograms to show the stronger characteristics of this type of recording.

of culturing and the growth medium must be accurately defined, so that reproducibility of the experiment is guaranteed. 2. Growth medium and culturing conditions must be chosen so that the metabolism is not simple. Instead, the individual metabolic traits of each kind of organism should be revealed.

These two postulates are in some ways contradictory to the normally used methods of culturing microorganisms, which are chosen for each organism to produce optimum growth. At present, there are no such conventions about the experimental conditions, but several working groups are looking intensively for conditions which are suitable in light of the above postulates. Postulate 1 causes no difficulty in principle, because it can be satisfied by detailed agreement. The second postulate cannot be fulfilled for all bacteria or microbes, but only more or less sufficiently for a smaller group. The following chapter reproduces examples given by different authors for calorimetric differentiation between microbes of similar ecology.

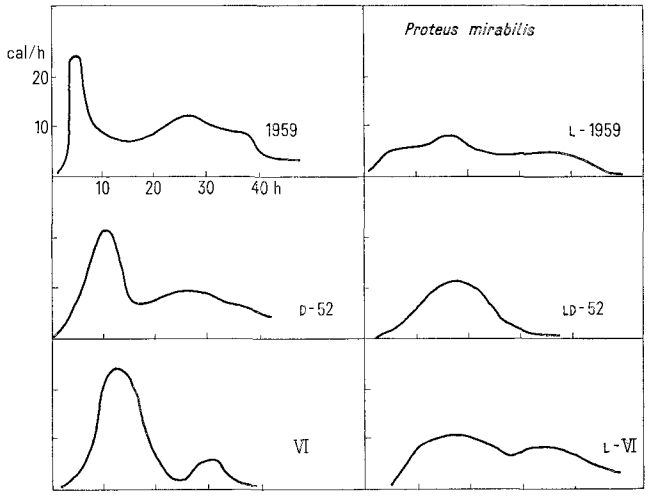

Fig. 10. Thermograms of 3 different strains of *Proteus mirabilis* and their related L-forms[15].

4. *Examples of thermogram profiles*

Figures 3 and 4 are taken from two investigations undertaken in the early phase of biological microcalorimetry. The thermograms were obtained with the early types of the Calvet batch calorimeter, but they demonstrate clearly that under simple experimental conditions, characteristic thermo-profiles were produced. The Figures 5–9 show the results from experiments dealing with the characterization of intestinal bacteria. Although the experimental conditions in the examples shown were not identical, one can recognize some characteristic parts of the thermogram of the same species in different figures. Just these repeating characteristics indicate a certain applicability of the method. Although the positions of the maxima and

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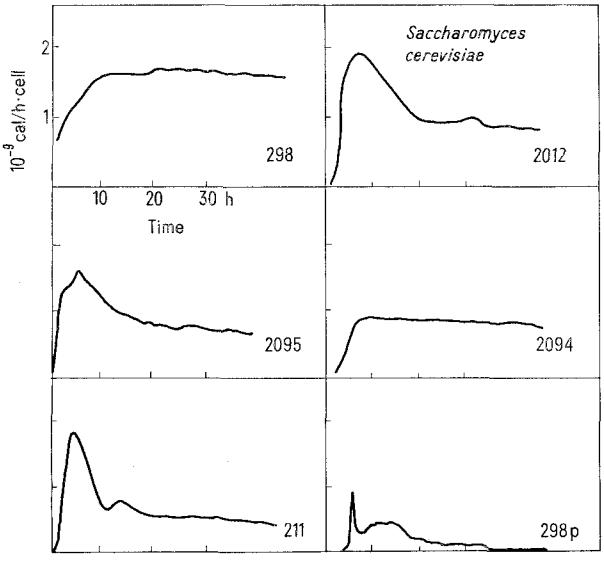

Fig. 11. Thermograms of 6 mutants of *Saccharomyces cerevisiae* differing in UV-sensibility.

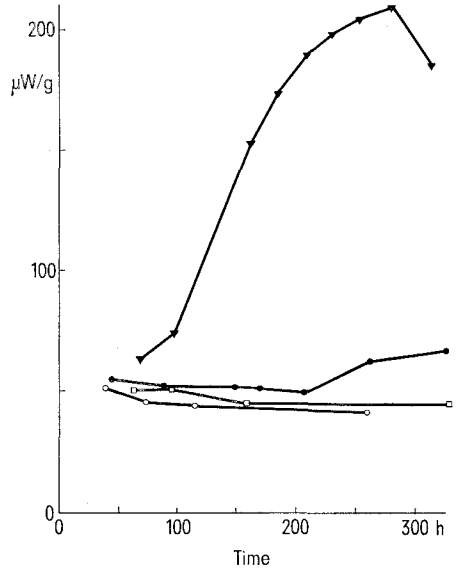

Fig. 12. Heat effect from variously fertilized soil samples[16]. ○, untreated; □, salt mixture added; ●, cellulose powder added; ▼, salts and cellulose added.

minima on the time axis and the absolute values of heat flow vary somewhat from experiment to experiment – due to the amount of inoculum and also to the length of the lag phase associated with the adjustment of the organism to the growth medium – the general characteristics of the curves are well reproducible. In each example, the thermograms of two independent experiments with the same organism are shown. In some cases, the calorimetric characterization could be improved for characterizing different strains or mutants of the same organisms. Two examples are shown in Figures 10 and 11.

5. *Mixed cultures*

Only a few calorimetric investigations on the growth of mixed populations have been undertaken. Figure 12 shows the thermogram of a soil sample with different salt additions, and Figure 13, thermograms of microbial growth in cattle manure. In both cases it is impossible to recognize any characteristic profiles of the growing organism. The smoothing of the curves is caused by the superposition of the heat profiles of the growing species, but also by the unknown change in metabolism caused by the mutual interference of the species.

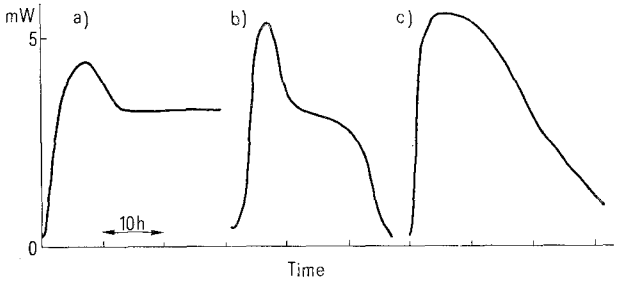

Fig. 13. Heat production from bacterial activity in cattle manure. a) solid manure at 40 °C; b) liquid manure at 45 °C; c) liquid manure at 60 °C.

6. *Conclusion*

It was shown in chapter 4 that, under exactly defined experimental conditions, a sufficient identification of some groups of bacteria is possible. In comparison with the usual microbiological methods, the calorimetric method has the great advantage that results are already at hand after 5 to 10 h. But, as with every other method, the organism under investigation has to be isolated before it can be identified. It is therefore not possible to carry out any thermic spectroscopy in a mixed culture. For clinical application, this question is of less interest, because contamination in blood or urine, for instance, is usually caused by just one species. At present, the growth profiles obtained are most sensitive to differences in experimental conditions, for example oxygen potential. Therefore, the calorimetric method is not yet suitable for practical use in clinical microbiology. However, with technical improvement and development of suitable culturing conditions, the microcalorimetric method may become a useful tool in the routine laboratory, even if it will never supersede any other conventional method[17].

[17] Acknowledgment. The kind permission of the cited authors and journals to reproduce their figures is gratefully acknowledged.

SPECIALIA

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Defensive Substances of Opilionids[1]

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Department of Chemistry and Section of Neurobiology and Behavior, Cornell University, Ithaca (New York 14853, USA), 6 April 1976.

Summary. The defensive secretions of 4 species of opilionids were analyzed. *Leiobunum ventricosum* and *Hadrobunus maculosus* produce 4-methylheptan-3-one, while *L. calcar* produces E-4,6-dimethyl-6-octen-3-one. *L. longipes* produces E-4,6-dimethyl-6-nonen-3-one, a new natural product.

Arachnids of the order Opiliones possess a pair of dischargeable defensive glands[3,4] that produce volatile odorous secretions. Chemical work has so far been done only on 2 species of the suborder Laniatores and 3 species of the suborder Palpatores. The Laniatores produce methylated 1,4-benzoquinones[5–7], while the Palpatores produce acyclic ketones, including 4-methylheptan-3-one (I) and E-4,6-dimethyl-6-octen-3-one (II)[8,9]. We here report on the chemistry of the secretions of 4 additional Palpatores. Two of these, *Leiobunum ventricosum* and *Hadrobunus maculosus*, produce compound I, and a third, *L. calcar*, produces compound II. The fourth, *L. longipes*, produces E-4,6-dimethyl-6-nonen-3-one (III), a previously undescribed natural product.