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Report on:

Assessment of BioDtex unit instrument for detection of food industry biofilms

Work performed by Campden BRI (Chipping Campden) Limited
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1 SUMMARY

A study was conducted on a BioDtex biofilm detection instrument as provided by BioDtex, to assess the ability of the unit to detect biofilms within food industry settings. The unit was compared against two commercially available units.

Stage 1 of the study focused on the detection of single species biofilms. Single species biofilms were generated for microorganisms in 10 categories representing important pathogens in the food, medical and veterinary industries on stainless steel discs. Biofilms were assessed using the BioDtex unit, 2 competitor instruments and the presence and extent of biofilm formation was confirmed by fluorescent microscopy.

The BioDtex unit performed the best at detection of single species biofilms, with all biofilms detected strongly. The presence of biofilms was visually evident as defined areas of turquoise fluorescence when using the BioDtex unit, whereas the other instruments returned a more diffuse blue fluorescence, which was more difficult to distinguish from reflected light/other debris. The BioDtex unit was also able to pick up weaker biofilms as direct turquoise light.

The presence of bacteria or biofilms are visually evident as a turquoise fluorescence on the surfaces using the BioDtex unit. Determination of the presence of biofilm in practice within the Campden BRI microbiology process hall showed it to be highly effective at locating areas of contamination compared against other selected UV torches.

Stage 2 of the study used a strong biofilm forming strain, *Aeromonas hydrophilia* RA6046. This organism was grown under conditions of high shear in a CDC bioreactor system to achieve dense, compact biofilm growth. Samples of the biofilm were taken at 5 stages during formation and analysed using the 3 instruments. The BioDtex unit could detect laboratory biofilms at an early stage of development. By comparison, the competitor UV torches could only detect the stronger biofilms. The BioDtex unit was also able to detect biofilm after hot water immersion at 60°C for 5 minutes, PAA immersion at 200ppm for 20 minutes and sterilising at 121°C for 3 minutes. Total viable counts and direct live/dead fluorescent microscopy confirmed the presence of live bacteria after each of these treatments.

It can be concluded that the BioDtex unit is able to detect biofilms from species typical of pathogens and other target microorganisms present in a multitude of environments from across many industry areas including food, medical and veterinary areas.

2 BACKGROUND

The customer approached Campden BRI to independently assess their BioDtex unit instrument as a tool to detect biofilms within a food industry setting, against two UV detection products currently available on the market.

The study comprised the following stages:

- Stage 1. Detection of single species biofilms of relevant food industry microorganisms
- Stage 2: Detection of biofilms at different stages of development

3 SAMPLES/MATERIALS

The client provided Campden BRI with a BioDtex unit for use within the study. Campden BRI provided use of two other units for use within the study- these were both two standard UV torches.

All microorganisms as used within the study were provided by Campden BRI.

4 METHODS

Culture of microorganisms

Cultures of the following organisms were obtained from the Campden BRI culture collection (Table 1).

Table 1. Microorganisms used in the study

Organism Category	Microorganism strain	Rationale for inclusion/areas of importance
Listeria monocytogenes	*Listeria monocytogenes* ATCC 11994	Food industry (all areas, particularly RTE) Medical industry
	Listeria monocytogenes Scott A strain	
	Listeria monocytogenes RA4377	
Gram positive cocci	Toxin producing *Staphylococcus aureus* RA 1224	Food industry (all areas) Medical industry Veterinary industry Hotel industry Public transport Cosmetics/homecare industries
	Methicillin-resistant *Staphylococcus aureus* ATCC 13143 (MRSA)	
	Vancomycin resistant *Enterococcus faecalis* (VRE) NCTC 12202	
Salmonella enterica	S. Typhimurium ATCC 14028	Food industry (all areas) Veterinary industry Food service industry Hotel industry Public transport
	S. Enteritidis PT30 ATCC BAA-1045	
	S. Montevideo FH/158	
Escherichia coli	*E. coli ATCC 25922*	Food industry (all areas) Veterinary industry Food service industry Hotel industry Public transport Water industry Cosmetics/homecare industries
	E. coli K12 ATCC 12435	
	Escherichia coli O157:H7 (non-toxigenic) ATCC 12900	
Cronobacter sakazakii	Food and outbreak strain RA16909	Food Industry
Pseudomonads	*Pseudomonas aeruginosa* PA01	Food Industry Medical industry Veterinary industry
	Acinetobacter baumanii NCTC 12156	

	Aeromonas hydrophila RA6046	Water industry Cosmetics/homecare industries
Gram positive spoilage organisms	*Brocothrix thermosphacta* NCTC 10822	Food industry (meats/fish, ready meals, dairy)
	Micrococcus luteus H143	
	Carnobacterium divergens RA 16623	
Lactic acid bacteria	*Lactobacillus plantarum* RA 16658	Food industry (dairy, meats, ready meals, sauces)
	Lactobacillus paracasei RA 16659	
	Leuconostoc mesenteroides RA 5732	
Yeasts	*Candida albicans* NCTC 3179	Food industry (soft drinks, dairy, dessert sauces, fruit preparations, bakery) Medical industry (*C. albicans*) Veterinary industry (*C. albicans*) Cosmetics/homecare industries (*C. albicans*) Hotel industry (*C. albicans*) Public transport (*C. albicans*)
	Brettanomyces Bruxellensis (Dekkera) RA 16012	
	Zygosaccharomyces rouxii RA 16630	
Spore-forming bacteria	*Bacillus licheniformis* RA 16684	Food industry (dairies, commercially sterile products, pH-preserved ambient stable products) Medical industry (*C. difficile*)
	Clostridioides difficile RA 16629	
	Geobacillus stearothermophilus RA 16533	

Stage 1: Detection of single species biofilms of relevant food industry microorganisms

A culture of the organisms for the study were taken from frozen cryobead storage at -80°C, streaked onto either pre-poured Tryptone Soya Agar (TSA), Malt extract agar (MEA) or De Man, Rogosa & Sharpe (MRSA) agar plates and incubated at the optimum growth temperatures overnight. Plates were examined to confirm purity and colony morphology, and individual, well-isolated colonies were then transferred into a 100ml aliquot of the applicable nutrient media for that organism:

- *Listeria monocytogenes, Salmonella* spp, *Pseudomonas* spp*, Escherichia coli, Cronobacter sakazakii,* gram positive spore spoilage bacteria and spore forming bacteria – Tryptone soya broth (TSB*)*
- *Lactobacillus* spp- De Man, Rogosa & Sharpe broth (MRSB)
- Yeasts- Malt extract broth (MEB)

The nutrient broths were incubated for between 24 - 48 hours dependent on organism. For each microorganism, triplicate 304b stainless steel surfaces (2cm diameter, ~1.5mm thickness) were placed into the bottom of 3 x100ml containers, immersed in broth culture and left for 1 hour at ambient temperature to allow initial attachment of microorganisms to steel surfaces.

After 1 hour, the inoculum was drained from each container and the stainless-steel surfaces were rinsed with sterile distilled water to remove non-attached cells and then immersed in dilute growth medium (1 :100 tryptone soya broth). Samples were then incubated at the optimum temperature for each organism for 4 hours. Post incubation, the surfaces were removed from the growth medium, rinsed in SDW, transferred to a petri dish and left to dry at ambient temperature for 2 hours.

Biofilms on each coupon were assessed visually using the BioDtex unit and 2 competitor UV torches and microscopically by fluorescent staining with acridine orange. Each instrument under assessment was shined onto each surface from ~20cm, minimising the distortion of the image as a result of background light.

The presence of microorganisms elicited a turquoise fluorescence on surfaces when assessed using the BioDtex unit system. The 2 UV light instruments would show up the bacteria if present as a light blue UV hue.

The strength of response to the presence of biofilm observed with each system was assessed according to a points system:

- **Excellent** bacterial detection/visual. Strong light fluorescence observed **Score of 3**
- **Good** bacterial detection. Weaker light response observed **Score of 2**
- **Poor** bacterial detection. Very little light response observed. **Score of 1**.
- **No bacterial biofilm detection**- no visual seen. **Score of 0.**

Once the coupon biofilms had been visually inspected, they were subjected to an acridine orange staining procedure. The coupon was immersed in acridine orange solution using a Pasteur pipette and left for 10 minutes, dried in a flame for 2-3 seconds to fix the stain, then assessed using a fluorescent microscope with equipped with a mercury vapour lamp.

Negative control coupons were also assessed using the 3 instruments, to determine whether food debris or other material present within a factory would be detected by each system. 5 different substances were used as negative controls:

- Bovine serum albumen (protein soil used as standard for food, domestic, industrial, institutional, medical and veterinary areas)
- Skimmed milk (dairy residue)
- Sugar solution (sugar refineries)
- Pureed sterilised food
- Sheep erythrocytes (Used to simulate blood in meat processing facilities/medical/veterinary areas)

These substances were also placed into 100ml containers, and the stainless-steel coupons immersed for 1 hour to attach in each solution and rinsed using sterile distilled water. Coupons were dried and then assessed.

Stage 2: Detection of biofilms at different stages of development

A model microorganism was selected based on data from stage 1- *Aeromonas hydrophilia* was chosen as a strong biofilm former and used to seed a CDC bioreactor.

Biofilms of the *Aeromonas hydrophilia* were grown on stainless steel coupons during batch and continuous phases within a bioreactor under conditions of high shear. 24 coupons were grown in total during the process (1 full bioreactor).

Triplicate samples were taken at 5 timed intervals during biofilm formation:

- Batch phase + 4 hours

- Batch phase + 24 hours
- Continuous phase + 2 hours
- Continuous phase + 6 hours
- Continuous phase + 24 hours

Following the bioreactor growth above, the coupons at each of the 5 times stages were tested as follows:

- 1 coupon was assessed using the BioDtex unit + 2 UV light products
- 1 coupon was assessed by plating to determine viable TVC count
- 1 coupon was assessed using fluorescent microscopy

For the 9 remaining coupons left following the full growth protocol, the biofilms were heat or chemical treated as follows:

- Immersed in water at 60°C for 5 minutes using test tubes pre- heated to 60°C
- Treatment with 200ppm peroxyacetic acid for 20 minutes
- Autoclaved (sterilised) at 121°C for 3 minutes.

After each treatment, coupons were again assessed using the BioDtex unit + the 2 competitor products against the score system described above, fluorescent microscopy staining and TVC aerobic count plating. Results were compared and used to determine the extent to which damaged or dead biofilm is detected using BioDtex unit.

5 RESULTS

Stage 1

Results- Detection of single species biofilms of relevant food industry microorganisms

Results obtained for single species biofilms and food residues on steel surfaces tested with BioDtex unit and competitor systems are shown in table 2, below. A summary of the overall scores obtained for each detection system is shown in table 3.

Results showed that BioDtex unit provided a much clearer indication of the presence of biofilm.

All results displayed are using the BioDtex “standard” light beam setting – the unit also has a “boost” function which can be used to enhance the UV-C light amplified for detection. The standard setting displayed units of between 35-37C at a 20cm distance from the test pieces, with ECO displayed at 20%.

Table 2- Microorganism biofilm visual detection using 3 systems- BioDtex unit UV handheld torch 1 and UV handheld torch 2:

Organism Category	Microorganism strain/control	BioDtex unit detection score	UV torch 1 detection score	UV torch 2 detection score	Biofilm coverage observed with fluorescent microscopy
Listeria monocytogenes	*Listeria monocytogenes* ATCC 11994	3	2	2	Strong
	Listeria monocytogenes Scott A strain	2	1	1	Strong
	Listeria monocytogenes RA4377	2	1	1	Medium
Gram positive cocci	Toxin producing *Staphylococcus aureus* RA 1224	1	0	0	Weak
	Methicillin-resistant *Staphylococcus aureus* ATCC 13143 (MRSA)	3	2	2	Strong
	Vancomycin resistant *Enterococcus faecalis* (VRE) NCTC 12202	2	1	1	Strong
Salmonella enterica	S. Typhimurium ATCC 14028	3	1	2	Medium
	S. Enteritidis PT30 ATCC BAA-1045	2	1	1	Weak
	S. Montevideo FH/158	2	2	1	Medium
Escherichia coli	*E. coli* ATCC 25922	3	1	1	Strong
	E. coli K12 ATCC 12435	2	2	1	Medium
	Escherichia coli O157:H7 (non-toxigenic) ATCC 12900	3	1	1	Strong
Cronobacter Sakazakii	Food and outbreak strain RA16909	3	2	2	Medium
Pseudomonads	*Pseudomonas aeruginosa* H257	3	1	2	Very strong
	Acinetobacter baumanii NCTC 12156	3	2	2	Very strong
	Aeromonas hydrophila	3	2	2	Very strong
Gram positive spoilage organisms	*Brocothrix thermosphacta* NCTC 10822	3	1	2	Medium
	Micrococcus luteus H143	2	1	2	Medium
	Carnobacterium divergens RA 16623	3	2	2	Very strong

Lactic acid bacteria	*Lactobacillus plantarum* RA 16658	2	1	1	Strong
	Lactobacillus paracasei RA 16659	2	1	2	Strong
	Leuconostoc mesenteroides RA 5732	2	1	1	Strong
Yeasts	*Candida albicans* NCTC 3179	3	1	2	Very strong
	Brettanomyces Bruxellensis (Dekkera) RA 16012	3	2	2	strong
	Zygosaccharomyces rouxii RA 16630	2	2	2	strong
Spore-forming bacteria	*Bacillus licheniformis* RA 16684	3	2	1	Strong
	Clostridioides difficile RA 16629	2	0	1	Medium
	Geobacillus stearothermophilus RA 16533	2	1	2	Medium
Negative controls	Bovine serum albumen	0*	1**	1**	Ok- no biofilm growth
	Skimmed milk	0*	1**	1**	Ok- no biofilm growth
	Sugar solution	0*	1**	1**	Ok- no biofilm growth
	Pureed sterilised food	0*	1**	1**	Ok- no biofilm growth
	Sheep erythrocytes	0*	1**	1**	Ok- no biofilm growth

* No fluorescent response emitted
** Light blue, fluorescent response emitted

Table 3- Microorganism biofilm visual detection using 3 systems - score summary

Organism group	Overall score- BioDtex unit detection	Overall score- UV torch 1 detection	Overall score- UV torch 2 detection
Listeria monocytogenes	7	4	4
Gram positive cocci	6	3	3
Salmonella enterica	7	4	4
Escherichia coli	8	4	3
Pseudomonads	9	5	6

Cronobacter sakazakii	3	2	2
Gram positive spoilage organisms	8	4	6
Lactic acid bacteria	6	3	4
Yeasts	8	5	6
Spore-forming bacteria	7	3	4
Overall score/**84**	**69**	**37**	**42**

- From the visual scoring of biofilm detection, the BioDtex unit scored 69/81, which indicates that it could detect biofilms on all bacterial groups well, including some weaker biofilm formers e.g *S.Enteritidis* PT30.

- The UV torches performed very similarly with scores of 37 and 42 respectively. The scored well on the strong biofilm formers, but responses were not as clear as seen with the BioDtex unit- the light was more a light blue hue around the edge of the disc with some flecks of blue light visible on the surfaces, which also picked up some other debris such as dust. Overall, the UV torch systems appeared much less selective than BioDtex unit.

- For the negative control samples (sterile food residues), the BioDtex unit did not emit a fluorescent response for all 5 components. On the staining images, there was no biofilm growth detected. Therefore, it can be concluded that the BioDtex unit can differentiate between biofilm and typical residues often found on environmental surfaces in a range of applications.

- The other two UV torch systems did not pick up any noticeable differences on the negative samples to the bacterial samples. Both showed a lower intensity light blue, fluorescent response similar to the response seen for bacterial contamination.

Example of **Excellent** (score of 3) biofilm visual- *Aeromonas hydrophila* picked up using BioDtex unit (**Figure 1**) and UV torch (**Figure 2)**

Figure 1

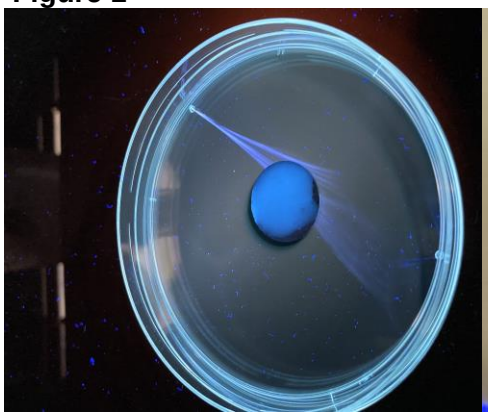
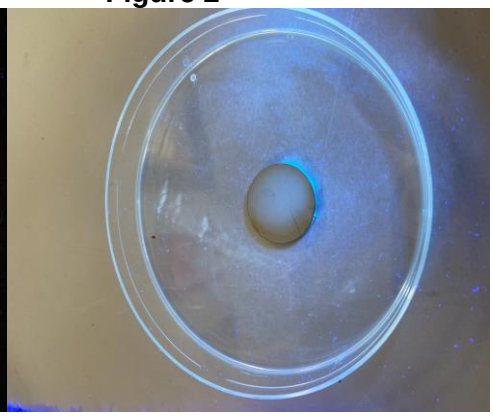


Figure 2



- Note using the UV torch, there was a shadow cast by the torch itself next to the discs as shown in **figure 3**, however the biofilm could be picked out on the disc as a lighted blue.

Figures 3 & 4 below illustrate the BioDtex unit & UV torch 1 respectively on *Brocothrix thermosphacta* NCTC 10822 the BioDtex unit image in figure 5 showing a more turquoise hue, the UV torch picked up the light as blue around the edges only and was quite difficult to locate.

Figure 3

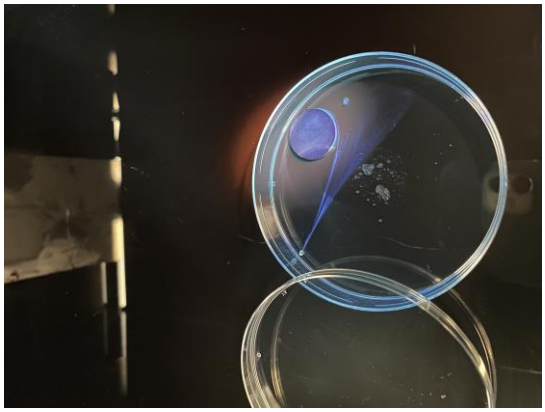
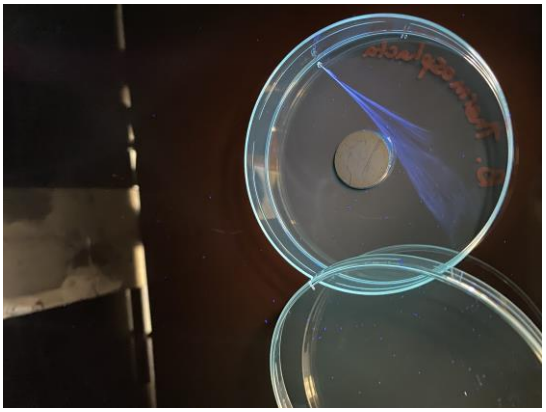


Figure 4



Figures 5 & 6 illustrate the BioDtex unit & UV torch respectively on *Brettanomyces Bruxellensis* RA 16012 the BioDtex unit image in figure 5 showing a strong turquoise hue, the UV torch picked up the light as blue but not so intense and also detected other debris as blue in the dish.

Figure 5

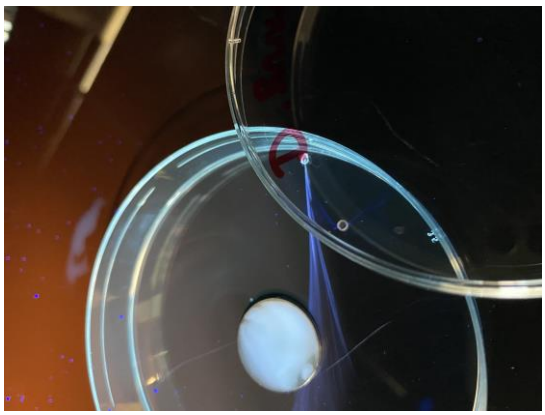
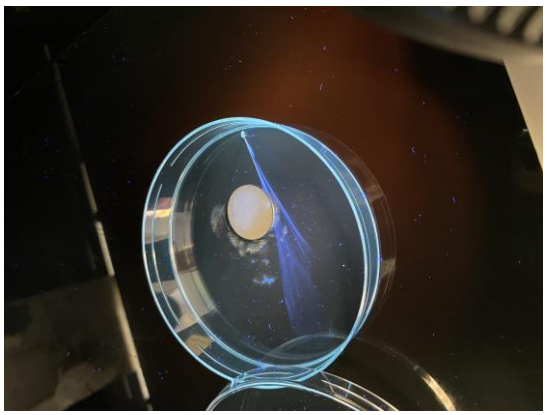


Figure 6



Figures 7 & 8 illustrate the BioDtex unit & UV torch respectively on *Cronobacter sakazakii* RA16909 the BioDtex unit image in figure 5 showing a strong turquoise hue

Figure 7

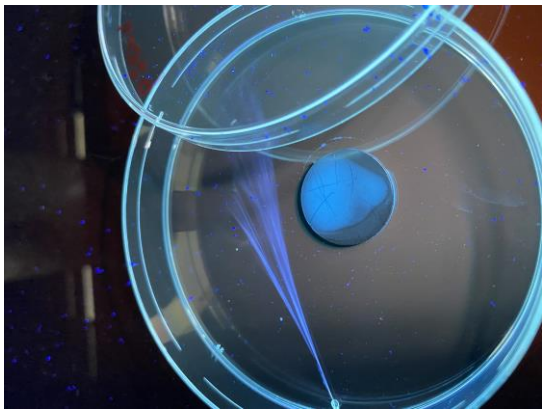
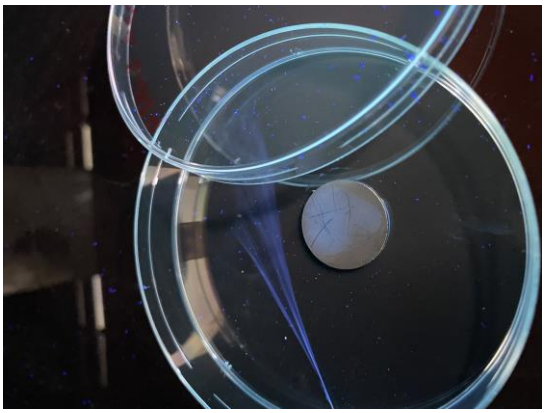


Figure 8



The fluorescent staining images of all the biofilms can be found under **appendix 1**

The images show that the BioDtex unit could detect all biofilms assessed, including those formed by less profuse biofilm formers.

Determination of the presence of biofilm within the IPM Campden BRI process hall facility

To further illustrate the relative detection capabilities of BioDtex unit and the other UV devices, the BioDtex unit was used to detect the presence of biofilm at two locations within the IPM laboratory at Campden BRI. Swabs were taken from each area and analysed to determine total viable count.

Figures 9 & 10 show the hand soap dispenser visualised with BioDtex unit and a UV torch. The BioDtex unit (figure 11) showed the presence of biofilm as well-defined areas of turquoise fluorescence, whereas the UV torch (figure 12) showed only a diffuse blue light which did not indicate the presence of bacteria. The TVC result for this swab area was 5,100 cfu/g.

Figure 9

Figure 10

Figures 11 & 12 show the underside of the handwash sink visualised using BioDtex unit and a UV torch. The BioDtex unit (figure 13) showed the presence of biofilm as well-defined areas of turquoise fluorescence, whereas the UV torch (figure 14) showed only diffuse blue light. The TVC result for this swab area was 150 cfu/g, indicating the BioDtex unit more reliably indicates the presence of biofilms, even at relatively low levels of culturable organisms.

Figure 11

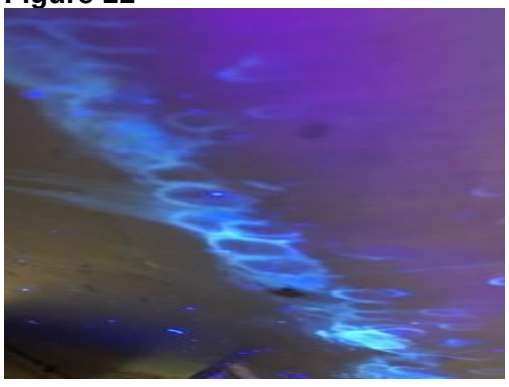


Figure 12

These results show that in practice, the BioDtex unit clearly indicated the presence of microorganisms at low medium and high levels of contamination, allowing easy identification of areas in which cleaning had not been effective. The cheaper competitor torch did not indicate contamination on any of these surfaces, despite the high levels of viable organisms on the sink overflow and safety shower spray head.

Stage 2 results- Detection of biofilms at different stages of development

Aeromonas hydrophila was selected for the stage 2 experiment, due to its known strong biofilm forming characteristics and the results shown for this organism in stage 1.

Table 4 shows the total viable count achieved on stainless steel CDC bioreactor coupons before and after various sublethal treatments, the fluorescent responses observed with BioDtex unit and two competitor UV torches, and the fluorescent staining results.

Table 4. BioDtex unit results on CDC bioreactor coupons at different stages of growth

Sample ID/condition	TVC Log cfu/g	BioDtex unit detection score	UV torch 1 detection score	UV torch 2 detection score	Fluorescent staining image
Batch phase + 4 hours	3.52	2	1	1	Medium
Batch phase + 24 hours	7.04	3	1	1	Strong
Continuous phase + 2 hours	7.52	3	1	2	Strong
Continuous phase + 6 hours	7.66	3	1	2	Strong
Continuous phase + 24 hours	7.98	3	1	2	Very strong
Hot water immersion 60°C - 5 minutes	4.58	2	1	1	Medium (live/dead)
200ppm PAA 20 minutes	5.22	2	1	2	Strong (live/dead)
Autoclaving at 121°C 3 minutes	3.98	2	1	1	Medium (live dead)
Overall detection score/27	N/A	22	8	12	N/A

- The BioDtex unit picked up the biofilm growth at all stages of the CDC bioreactor growth process, starting at +4 hours into the batch phase, with scores of either 2 or 3.
- The UV torches did detect all stages of biofilm growth, but the visual wasn't as clear as with the BioDtex unit with a light blue hue detected.
- The BioDtex unit scored 22/27 for these tests against 18/22 and 12/22 for the UV torches.
- The BioDtex unit could also still detect the biofilm post-PAA, hot water and sterilising treatments, this matches up well to the live/dead & fluorescent staining images from the post treatment biofilm discs, which indicated that there was still viable biofilm growth on these discs. **See figures 13-20.**

Figure 13

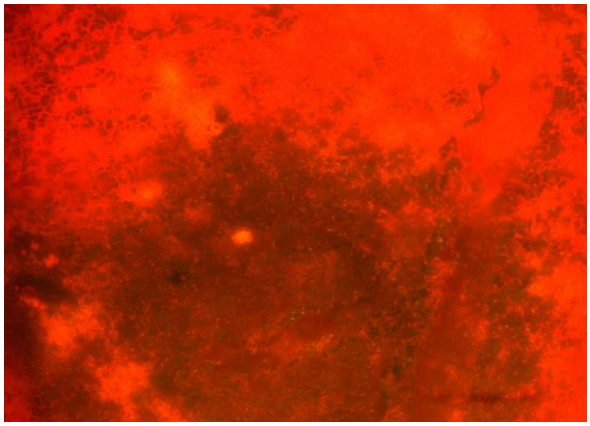
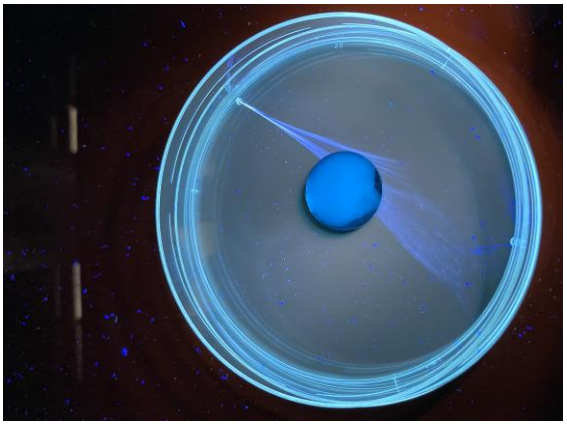


Figure 14



***Aeromonas hydrophila* fluorescent stain & BioDtex image at Batch phase + 24 hours**

Figure 15

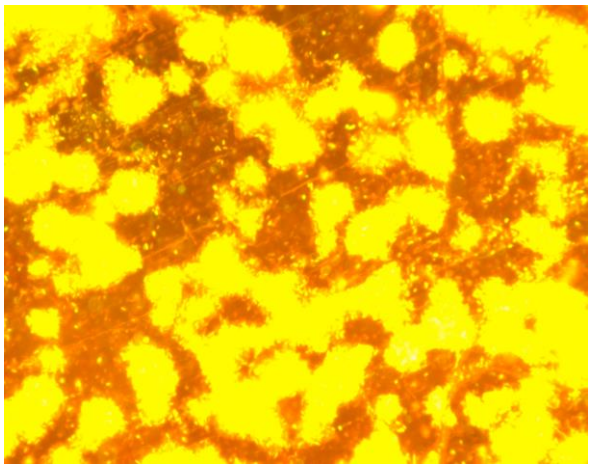
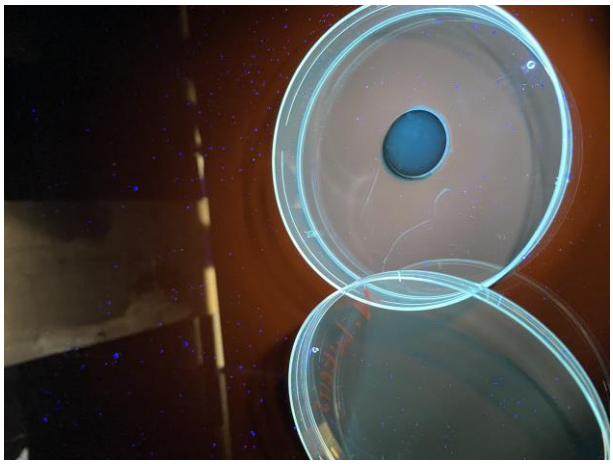


Figure 16



***Aeromonas hydrophila* fluorescent stain & BioDtex image – Post hot water treatment**

Figure 17

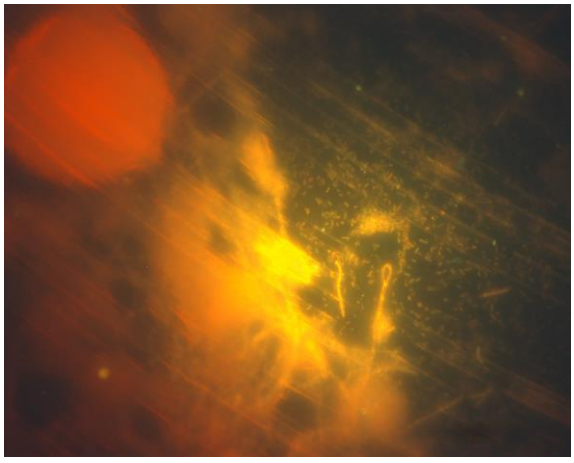
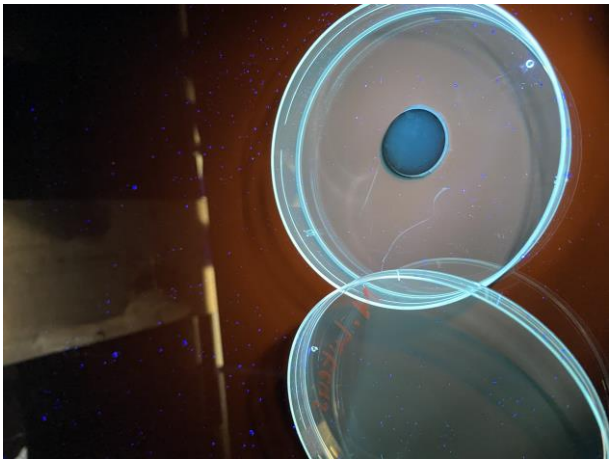
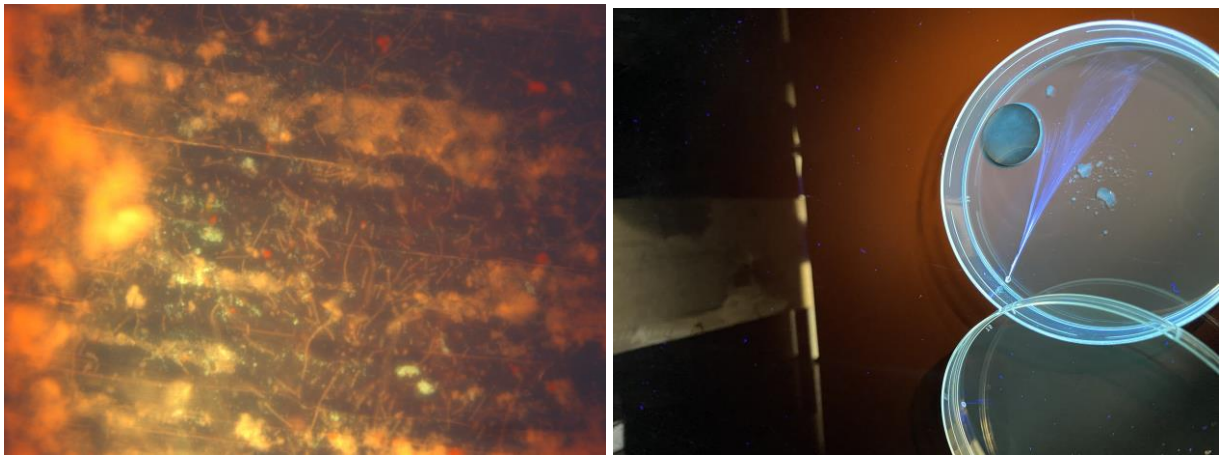


Figure 18



***Aeromonas hydrophilia* post 200ppm PAA treatment – BioDtex unit detection**

Live/Dead Stain
Figure 19 **Figure 20**



***Aeromonas hydrophilia* post autoclave sterilisation at 121°C treatment – BioDtex unit detection Live/Dead Stain**

6. CONCLUSION/DISCUSSION

A study was conducted on a BioDtex biofilm detection instrument as provided by BioDtex, to assess the ability of the unit to detect biofilms within food industry settings. The unit was compared against two commercially available units.

Stage 1 of the study focused on the detection of single species biofilms. Single species biofilms were generated for microorganisms in 10 categories representing important pathogens in the food, medical and veterinary industries on stainless steel discs. Biofilms were assessed using the BioDtex unit, 2 competitor instruments and the presence and extent of biofilm formation was confirmed by fluorescent microscopy.

The BioDtex unit performed the best at detection of single species biofilms, with all biofilms detected strongly. The presence of biofilms was visually evident as defined areas of turquoise fluorescence when using the BioDtex unit, whereas the other instruments returned a more diffuse blue fluorescence, which was more difficult to distinguish from reflected light/other debris. The BioDtex unit was also able to pick up weaker biofilms as direct turquoise light.

The presence of bacteria or biofilms are visually evident as a turquoise fluorescence on the surfaces using the BioDtex unit. Determination of the presence of biofilm in practice within the Campden BRI microbiology process hall showed it to be highly effective at locating areas of contamination compared against other selected UV torches.

Stage 2 of the study used a strong biofilm forming strain, *Aeromonas hydrophilia* RA6046. This organism was grown under conditions of high shear in a CDC bioreactor system to achieve dense, compact biofilm growth. Samples of the biofilm were taken at 5 stages during formation and analysed using the 3 instruments. The BioDtex unit could detect laboratory biofilms at an early stage of development. By comparison, the competitor UV torches could only detect the stronger biofilms. The BioDtex unit was also able to detect biofilm after hot water immersion at 60°C for 5 minutes, PAA immersion at 200ppm for 20 minutes and sterilising at 121°C for 3 minutes. Total viable counts and direct live/dead fluorescent microscopy confirmed the presence of live bacteria after each of these treatments.

It can be concluded that the BioDtex unit is able to detect biofilms from species typical of pathogens and other target microorganisms present in a multitude of environments from across many industry areas including food, medical and veterinary areas.

7 STATEMENTS

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8. Appendix 1- Fluorescent staining images from organisms assessed in Stage 1, Figures A- Y

Figure A: Acinetobacter baumanii

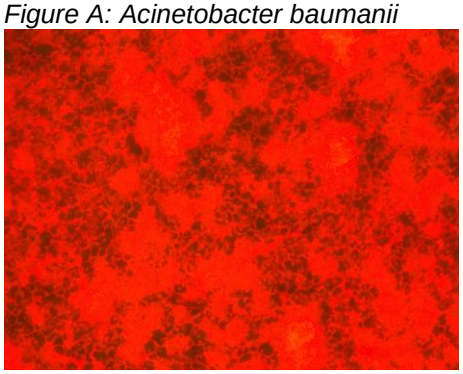

Figure B: Aeromonas hydrophila

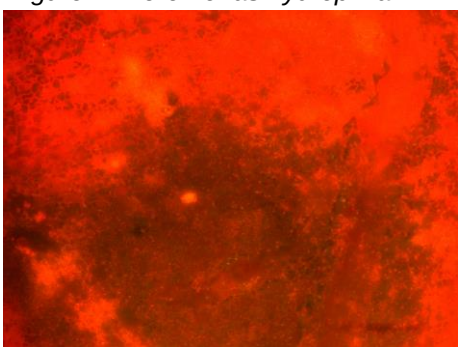

Figure C:Brocothrix.thermosphacta

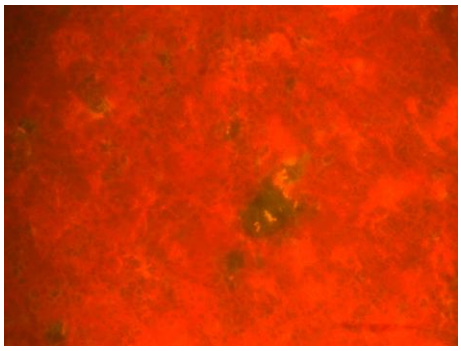

Figure D: Bacillus licheniformis

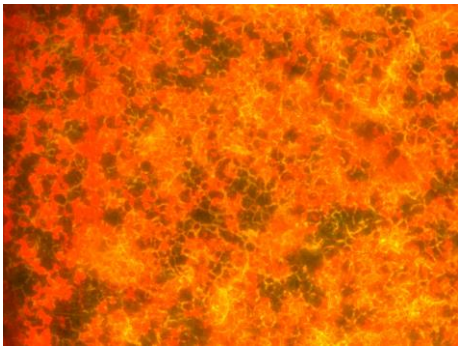

Figure E: Candida albicans

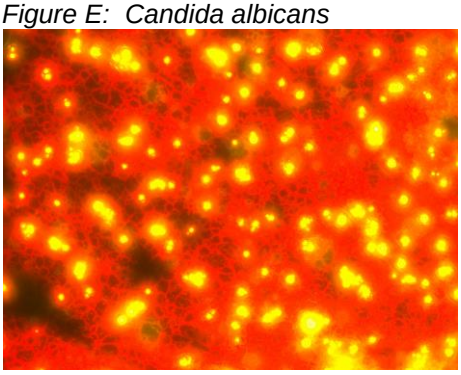

Figure F: *Clostridium dificile*

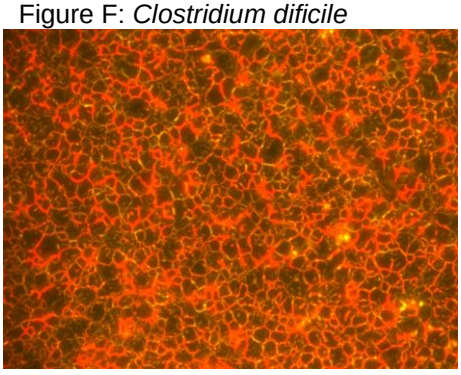

Figure G: Carnobacterium divergens

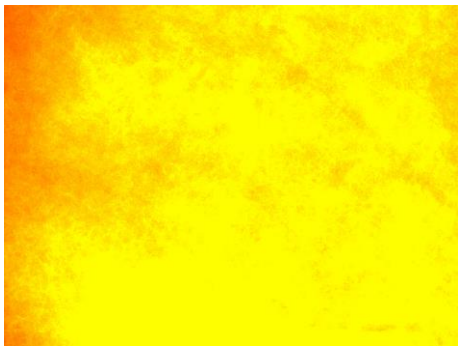

Figure H: E.coli ATCC 25922

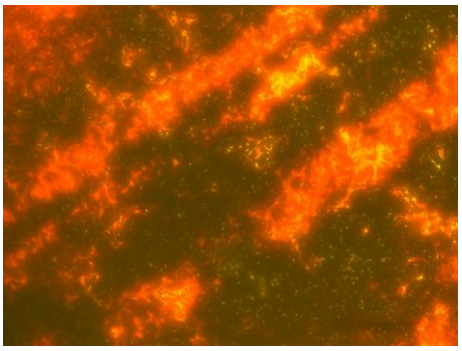

Figure I: Leuconostoc mesenteroides

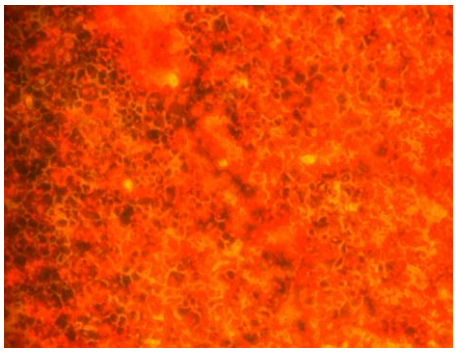

Figure J: Listeria monocytogenes 4374

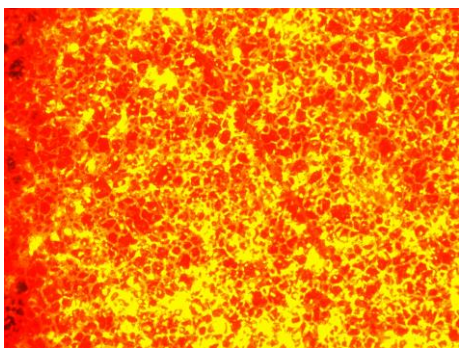

Figure K: Listeria monocytogenes NCTC 11994

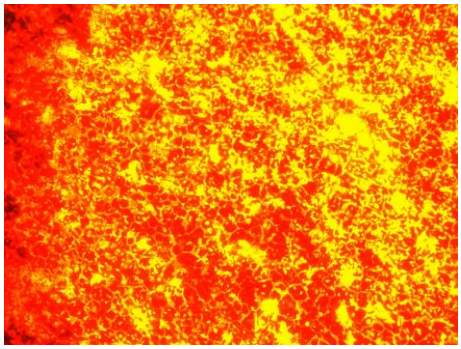

Figure L: Listeria monocytogenes NCTC 11994

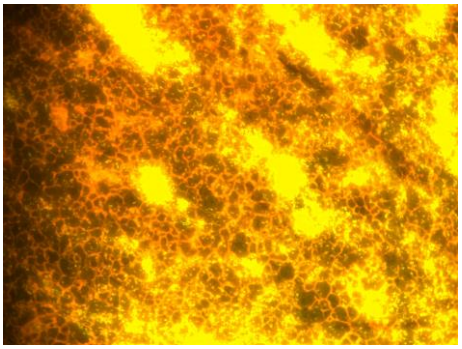

Figure M: E.coli O157:H7

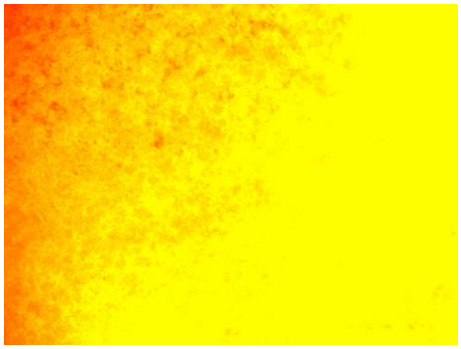

Figure N: Micrococcus luteus

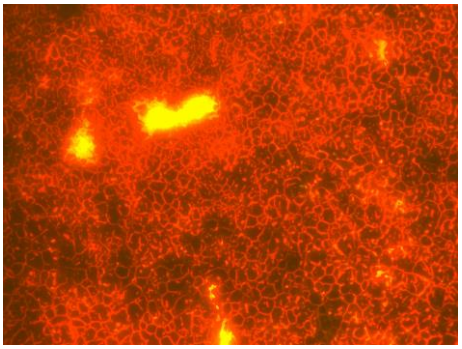

Figure O: Cronobacter sakazakii

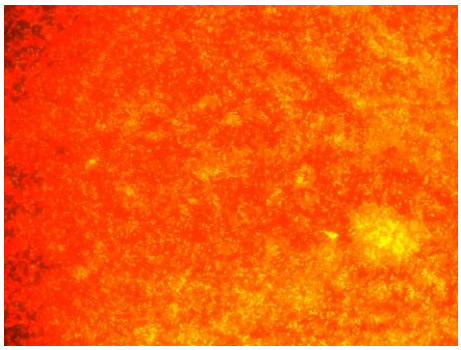

Figure P: Salmonella Enteritidis

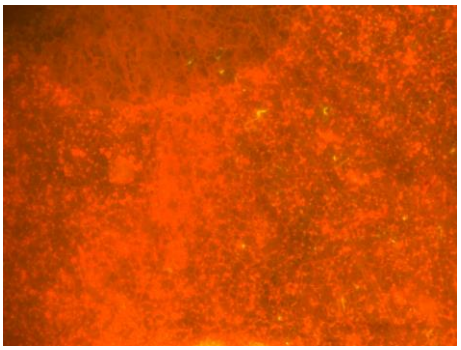

Figure Q: Salmonella Typhimurium

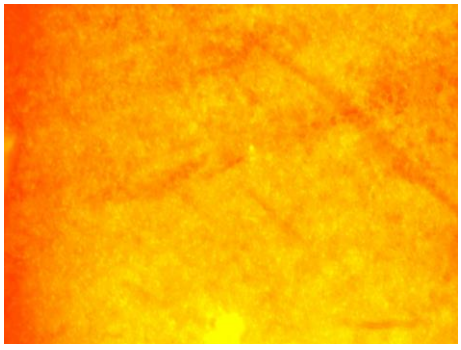

Figure R: Salmonella Montevideo

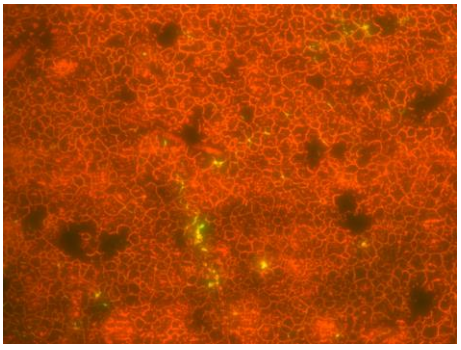

Figure S: Pseudomonas aeruginosa

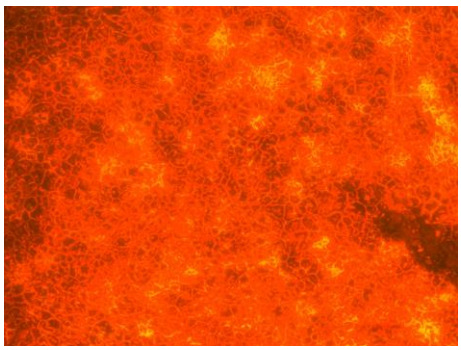

Figure T: Enterococcus faecalis (VRE)

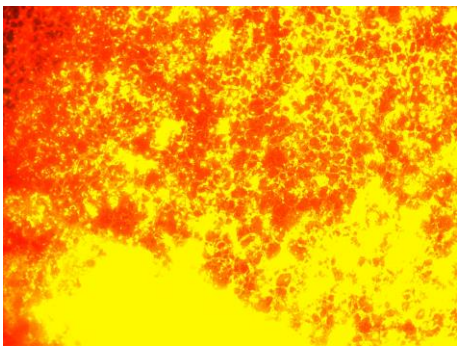

Figure U: Brettanomyces Bruxellensis

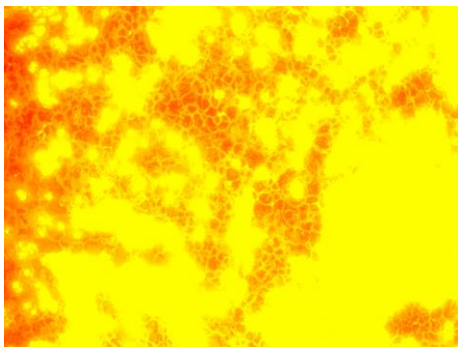

Figure V: Geobacillus stearothermophilus

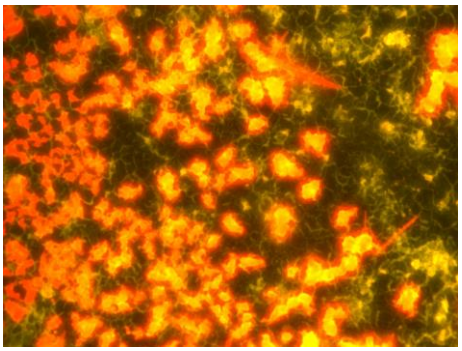

Figure W: Lactobacillus paracasei

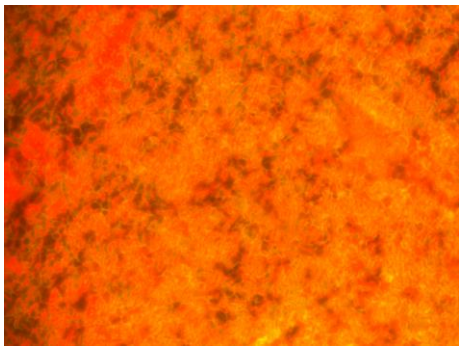

Figure X: Lactobacillus plantarum