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TNO report

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Testing antimicrobial coating material for food application

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Samenvatting

Produsafe B.V. wil graag hun antimicrobiële product 'Produsafe QX coating' op de markt brengen als voedsel contact materiaal. Materialen die in contact komen met voedsel moeten hiervoor goedgekeurd zijn door de EFSA. Het indienen van een dossier ter goedkeuring bij de EFSA kan gedaan worden door TNO Triskelion BV. De microbiologische testen nodig voor dit dossier zijn door TNO uitgevoerd in nauwe samenwerking met TNO Triskelion. Deze microbiologische testen dienen als 1e stap ter indiening van het EFSA dossier.

De gegevens benodigd voor het dossier zijn in overleg met Triskelion vastgesteld als zijnde:

A. bepaling van het spectrum van de antimicrobiële activiteit,
B. bepaling van de Minimum Inhibitory Concentration (MIC),
C. bepaling van de antimicrobiële effectiviteit van het materiaal, ook na herhaaldelijk schoonmaken van het oppervlak,
D. aantonen dat het voedselcontactmateriaal geen effecten heeft op het voedsel zelf, met name dat de coating geen biocide-effect heeft op de microbiologische flora in en op het voedsel.

Wat betreft de punten A, B en C, werd de effectiviteit van de antimicrobiële coating aangetoond op alle 11 geteste micro-organismen. Daarnaast werd de MIC voor ieder micro-organisme afzonderlijk is bepaald. Schimmelsporen blijken resistent te zijn tegen het antimicrobiële effect van de coating, maar na ontkieming van deze sporen werden de schimmels alsnog afgedood. Het schoonmaken van de coating had geen effect op de effectiviteit ervan.
Aangaande punt D laat het resultaat van Test 4 een duidelijk biocide effect zien in een agar-gel op 0,5 cm afstand van het coating oppervlak. De oorzaak voor dit effect kon niet worden vastgesteld vanuit de resultaten. TNO Triskelion kan op basis van dit test resultaat meer informatie geven over de consequenties van het gebruik van de coating als voedsel contact materiaal.

Dit rapport vormt een basis voor TNO Triskelion om een EFSA dossier samen te stellen voor aanvragen van mogelijke goedkeuring voor het gebruik van de coating als voedsel contact materiaal.

Summary

Produsafe BV would like to market launch their antimicrobial product 'Produsafe QX coating' as a food contact material. For use as a food contact material, permission of the EFSA is required. To obtain such permission, a product performance dossier needs to be submitted to the EFSA. TNO Triskelion BV has the expertise to prepare and submit such a dossier for which information from microbiological tests are also required. Therefore TNO, in collaboration with Triskelion, was requested to perform the microbial tests for determining the antimicrobial effect of the nano-particles in coating as food contact material.

The microbiological data required for the dossier were described by Triskelion as:
A) Spectrum of antimicrobiological activity.
B) Level of antimicrobial activity: Minimum Inhibitory Concentration (MIC)
C) Demonstration antimicrobial efficacy, also upon repeated use, such as after cleaning.
D) Demonstration of the lack of biocidal activity against microbes in/on the food.

Regarding points A, B and C, the antimicrobial coating was shown to be effective towards all 11 organisms tested. In addition, the MIC values were determined for each organism. Fungal spores showed resistance to the biocidal effect of the coating, but once those spores germinated that resistance was lost. Cleaning had no influence on the efficacy of the antimicrobial coating.
Regarding point D the test results clearly show a biocidal effect in an agar-gel at 0.5 cm distance from the coated surface. The cause of this effect could not be deduced from the results. TNO Triskelion can indicate what the consequences of this result are regarding application of the coating as food contact material.

This report can be used as a basis by TNO Triskelion to prepare a food contact material product performance dossier for submission to the EFSA.

Contents

1 Introduction

Produsafe BV would like to launch to the market their antimicrobial product 'Produsafe QX coating'as a food contact material. The main antimicrobial ingredient of this coating is Titanium dioxide nano-particles. Also some silver is present in the coating to enhance its performance.
For use as a food contact material, permission of the EFSA is required. To obtain such permission, a product performance dossier needs to be submitted to the EFSA. TNO Triskelion BV has the expertise to prepare and submit such a dossier for which information from microbiological tests are also required. Therefore TNO, in collaboration with Triskelion, was requested to perform the microbial tests for determining the antimicrobial effect of the nano-particles in coating as food contact material.

The microbiological data required for the dossier was described by Triskelion as:

A) Spectrum of microbiological activity: data on the spectrum of activity against various food-associated microorganisms, including pathogens.
B) Level of activity: information on Minimum Inhibitory Concentration (MIC) of the pure biocidal substance or preferably its active component e.g. silver ions, for the microorganisms likely to be exposed to the substance.
   Possible consequences of the use of the biocide: Describe any possible encouragement to favour selective overgrowth of the flora on the surface of the food contact material containing the biocidal substance(s) by organisms that are insensitive to the biocidal substance(s)
C1) Efficacy: Provide data to demonstrate the efficacy under the intended conditions of use. When the biocide is to be used at low temperatures, e.g. in chill rooms, refrigerators, efficacy should be demonstrated at these temperatures.
C2) Efficacy upon repeated use: Information should be provided to describe the behaviour of the biocidal surface after, for example, repeated cleaning procedures.
D) Demonstration of the lack of biocidal activity against microbes in/on the food: Describe the evidence for absence of any effect on the microbiological flora in/on the food including comparison with data obtained from use of the same/comparable FCM not containing the biocidal substance(s).This should cover the worst case, e.g. the most sensitive micro-organism(s) should be used for this test.

This description was translated in four microbiological tests:

1. Test 1: Determine the MIC of the coating material (as fluid) on food relevant microorganisms being:
    a. Pathogenic bacteria
        i. *Listeria monocytogenes*
        ii. *Escherichia coli*
        iii. *Salmonella*
        iv. *Staphylococcus aureus*
        v. *Bacillus cereus*

   b. Spoilage bacteria
      i. Leuconostoc mesenteroides
      ii. Pseudomonas putida
      iii. Lactobacillus brevis

   c. Fungi and yeast
      i. Aspergillus niger
      ii. Penicillium discolor
      iii. Zygosaccharomyces bailii
2. Test 2: Determine the biocidal efficacy of the coating as a food contact surface on food relevant microorganisms by using the same collection of microorganisms as indicated at point 1.
3. Test 3: Determine the effects of surface cleaning on biocidal efficacy of the coating
4. Test 4: Determine possible migration of nano-particles displaying antimicrobial activity out of the coating into food-model agar.

The information collected in this study will be used by TNO Triskelion in a separate assignment from Produsafe for their product dossier as a basis for communication with EU and national authorities for product registration.

2 Materials and Methods

2.1 Microorganisms

In this project eleven food relevant microorganisms were used in tests. See Table 1 for overview of names and strain identification codes.

Table 1 Microorganisms used in this project.

Strain number	Name microorganism	Identification code
Pathogenic bacteria		
1	*Listeria monocytogenes*	ATCC 19114
2	*Escherichia coli*	ATCC 8739
3	*Salmonella typhimurium*	ATCC 14028
4	*Staphylococcus aureus*	ATCC 6538
5	*Bacillus cereus*	ATCC 11778
Spoilage bacteria		
6	*Leuconostoc mesenteroides*	DSM 20343
7	*Pseudomonas putida*	DSM 50198
8	*Lactobacillus brevis*	DSM 6235
Fungi and Yeast		
9	*Aspergillus niger*	DSM 16404
10	*Penicillium discolor*	CBS 22292
11	*Zygosaccharomyces bailii*	CBS 10063

2.2 Produsafe QX coating

Produsafe has supplied to TNO at the start of this project their Produsafe QX coating material, both in liquid form and applied as solid coating on the inside of petri dishes (Greiner Bio-One, article nr: 633185). No information was provided about concentration of the titanium dioxide nano-particles in the coating or about other chemicals present, except that also some silver is present in the coating. According to generally available information (http://www.titanshield.com/en/FAQ), a titanium dioxide coating is dust-dry after half an hour. After this drying period, contact with water will cause no harm. The coating is mechanical resilient after 12 to 14 days. The coating reaches the maximum durability after approx. 8 weeks. In this project the test with mechanical cleaning was done 8 weeks after receiving the coated petri dishes. Therefore, the test was done on a coating with maximum durability.
The antimicrobial effect of titanium dioxide particles is based on its photocatalyst properties. The method is known as PhotoCatalytic Oxidization (PCO) and is initiated by light, especially UV-irradiation. Silver doped titanium dioxide products are active even at low light intensity (http://www.titanshield.com/en/FAQ). According to the instructions of Produsafe their coating was exposed to normal lighting conditions in the lab (i.e. TL-tube light) during at least half an hour before use. In addition, tests were done in lighted environments where possible, details are given in the test descriptions.

2.3 Test 1: Minimal Inhibitory Concentration

This test was done with the coating in liquid form. Therefore the test results cannot be specifically ascribed to the titanium dioxide nanoparticles, but only to the coating formula as a whole.
The MIC of the coating was tested in a suspension assay setting. In a 96 well microtiter plate each microbe was separatedly exposed to 11 different concentrations of the liquid coating in steps of a factor 4, starting with the undiluted coating. Dilutions were made with water. Also growth control wells without any liquid coating were present on the plate. As inoculum the set of 11 microorganisms was cultured on rich non selective media. For most bacteria that was Tryptone Soy Agar, though Leuconostoc was cultured on Man Regosa Sharp Agar and Fungi on Yeast Glucose Agar. From these cultures, cell suspensions of the individual microorganisms were made at a level of approximately 10^6 colony forming units (cfu)/ml. For the bacteria and yeast this cell suspension consisted of living cells, while for the fungi spores were used. Small aliquots of inoculum were added to the microtiter plate in which the dilution series of liquid coating was present, resulting in a final concentration of 10^4 cfu/ml in each well. The microtiterplate was incubated for one hour at room temperature while exposed to light. Thereafter a multichannel pipet was used to transfer 2.5 µl from each well onto a agarplate with growthmedium. After incubation each spot was checked on whether growth of the microbe occurred. The MIC value is defined as the lowest concentration of liquid coating at which no growth occurred for that microbe.

2.4 Test 2: Film adherence test

The efficacy of the antimicrobial coating on a surface was tested by overlay testing in a film adherence set up in duplicate. The test is done according to the Japanese Industrial Standard method nr JIS Z 2801. For these tests, both coated and non-coated petri dishes were used. The individual 11 microorganisms used in the previous test are also used in this film adherence test. In the test a thin film of a 10^6 cfu/ml suspension of the microorganisms was kept in close contact with the coated surface during 24 hours at 10°C while exposed to light. At T=0 hours and T=24 hours the remaining cells were counted by using agar medium colony counting and these counts were compared with the counts of cells that have been in contact with the non-coated surface as well as to the initial cell concentration in the original cell suspension. Microbiological counting was conducted at the optimal growth temperatures of the different microorganisms at optimal medium conditions.

2.5 Test 3: Effects of cleaning of antimicrobial food contact surface

The effect of repeated use and thus surface cleaning on the efficacy of biocidal surface was tested in duplicate. For this test the antimicrobial surface was cleaned with a 1% house-hold soap solution and a cotton cloth. The surface was thoroughly rubbed with cloth wetted with the soap solution for in total 5 minutes with each minute an exchange of the used cloth for a new cloth wetted with soap solution. After this treatment a film adherence test was performed as described as test 2 with the two microorganisms representing a middle resistant organism (*Leuconostoc mesenteroides*) and the most sensitive microorganism (*Zygosaccharomyces bailii*) to assess the reduction of antimicrobial surface performance after cleaning.

2.6 Test 4: Possible migration of nano-particles into the food matrix

To demonstrate possible release or migration of biocidal activity against microbes from the coated surface into the food matrix an agar-layer migration test was performed. To that purpose, the biocidal surface was coated with a thin layer of agar nutrient medium in which the most sensitive microorganism (*Zygosaccharomyces bailii*) was seeded at a final concentration of 50 cfu/ml. The thickness of the agar was set on 0.5 cm. As control experiment the same setup was performed on a non-coated surface. After incubation at 25°C exposed to light for four days, the occurance or absence of growth was scored as + or – values. This setup is not suitable for cfu-counts.

3 Results

3.1 Test 1: Minimal Inhibitory Concentration

This test was done with the coating in liquid form. Therefore the test results cannot be specifically ascribed to the titanium dioxide nanoparticles, but only to the coating formula as a whole. The results of the test are summarized in Table 2. To simplify interpretation the undiluted liquid coating was set at a concentration of 1 AU = 1 arbitrary unit. The MIC value is defined as the lowest concentration of liquid coating at which no growth occurred for that microbe.

Table 2 MIC values of the 11 microbes towards the liquid coating material.

Strain number	Microorganism	MIC value (dilution factor)	MIC value (concentration in AU)
1	*Listeria monocytogenes*	1/16	0.0625
2	*Escherischia coli*	1/256	0.0039
3	*Salmonella typhimurium*	1/64	0.0156
4	*Staphylococcus aureus*	1/64	0.0156
5	*Bacillus cereus*	< 1/1048576#	< 9.54E-07#
6	*Leuconostoc mesenteroides*	1/1024	9.77E-04
7	*Pseudomonas putida*	1/64	0.0156
8	*Lactobacillus brevis*	1/1024	9.77E-04
9	*Aspergillus niger**	1/64	0.0156
10	*Penicillium discolor**	1/64	0.0156
11	*Zygosaccharomyces bailii*	1/65536	1.53E-05

* = spores used in test, not living cells
# = no growth, not even at lowest tested liquid coating concentration (growth did occur in control without coating)

The growth test was performed by pipetting 2.5µl out of each well onto solid growth medium. This includes a small amount of the liquid coating, which will therefore be present at the site and affect growth performance of the remaining viable cells. However, the concentration of antimicrobial components is anticipated to become lower at that application point due to diffusion of the components through the gel-like solid medium. Nevertheless, some antimicrobial activity might remain present at the application point and might influence the results.

3.2 Test 2: Film Adherence Test

The raw results of this test on the effectiveness of the anti-microbial coating are expressed as number of Colony Forming Units (CFU) and are given in Appendix A. From this raw data the decrease of CFU between measurements was calculated and is summarized in Table 3 as ^{10}log-units. A decrease of '1 log-unit' signifies a 10-fold decrease, while a decrease of '2 log units signifies a 100-fold decrease, etc. All measurements were done in duplicate and all results are given, without calculating the average results.

Table 3: Decrease of CFU per Film Adherence Test in ^{10}log-units.
A decrease <1 is regarded as not significant and is given in italics.

Strain No.	Name microorganism	Ino-culum (CFU)	Blanc		Test on coating	
			T=0 decrease from Inoculum	T=24 hour decrease from T=0 Blanc	T=0 decrease from Inoculum	T=24 hour decrease from T=0 Test
1	*Listeria monocytogenes*	$1{,}3x10^6$	*0.37*	*0.52*	*0.30*	5.31#
			0.34	*0.66*	*0.07*	5.54#
2	*Escherischia coli*	$7{,}5x10^6$	*0.17*	*0.05*	*0.16*	6.22#
			0.12	*-0.06*	*0.11*	6.26
3	*Salmonella typhimurium*	$7{,}1x10^6$	*0.29*	*0.38*	*0.36*	5.99
			0.33	*0.14*	*0.27*	6.08#
4	*Staphylococcus aureus*	$8{,}6x10^6$	*0.08*	*0.56*	*0.28*	6.15#
			0.13	*0.81*	*0.17*	6.26#
5	*Bacillus cereus*	$5{,}4x10^5$	*0.05*	0.99	*0.08*	5.15#
			0.10	0.96	*0.15*	5.08#
6	*Leuconostoc mesenteroides*	$1{,}1x10^7$	*0.10*	*0.19*	*0.32*	2.68
			0.09	*0.26*	*0.49*	1.66
7	*Pseudomonas putida*	$1{,}7x10^6$	*0.42*	*-0.06*	*0.15*	5.58
			0.29	*0.26*	*0.34*	5.39#
8	*Lactobacillus brevis*	$8{,}4x10^5$	*-0.03*	*0.40*	*-0.08*	5.50#
			-0.08	*0.48*	*0.07*	5.36#
9	*Aspergillus niger**	$5{,}5x10^4$	*0.38*	*0.00*	*0.13*	*0.82*
			0.24	*0.07*	*0.10*	*0.80*
10	*Penicillium discolor**	$3{,}9x10^5$	*0.23*	*0.41*	*0.02*	*0.63*
			0.18	*0.41*	*0.14*	*0.45*
11	*Zygosaccharomyces bailii*	$1{,}1x10^6$	*-0.07*	*0.56*	2.96	2.58#
			0.41	*0.19*	2.74	2.80#

* = fungal spores applied in test, not mycelium.

= no surviving CFU detected

The column 'Inoculum' represents the number of CFU introduced in each test.

Columns denoted T=0 represent the number of CFU counted after the film was applied to the surface and immediately removed again. In columns denoted T=24 the decrease of CFU is given after the film was in contact with the test-surface for 24 hours.

The 'Blanc' experiments are the control measurements, as no anti-microbial coating is present.

Interpretation of results:

- In the column 'Test on coating' T=24 hour a decrease in CFU indicates that the coating has indeed an antimicrobial effect. This is the case for the microorganisms: *Listeria monocytogenes*, *Escherischia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus cereus*, *Leuconostoc mesenteroides*, *Pseudomonas putida*, *Lactobacillus brevis* and *Zygosaccharomyces bailii*.

Moreover, most microorganisms show no surviving cells after exposure to the coating for 24 hours, except for three organisms:
  - No decrease in living cells is observed with *Aspergillus niger* en *Penicillium discolor*. For these organisms fungal spores were used in the test combined with incubation conditions unfavorable for germination. Apparently, the coating does not kill inert fungal spores, which is not surprising as spores are very resistant survival structures. Only at germination this resistance disappears.
  - In this test *Leuconostoc mesenteroides* only shows a ca. 100-fold reduction in living cells. Apparently, this organism has a good defense against oxidative stress.
- For most organisms T=0 in both the Blanc test and the Test on coating yields the same results, except for *Zygosaccharomyces bailii* where a 1000-fold decrease is detected. Apparently, this organism is very sensitive to the biocidical effect of the coating, as even a very short contact time results in a significant decrease in living cells.
- For *Bacillus cereus* the number of living cells has decreased with a factor 10 at T=24 hours in the Blanc test. This test is a control on the effects of the method itself, i.e. whether effects like evaporation might have an influence on the number of surviving CFU. Possibly this organism is the most sensitive to dehydration of all 11 tested organisms.
- No decrease in CFU is observed for any microorganism at T=0 in the Blanc test: As expected the application of the film to the surface and subsequent the transfer of the cells to the counting plates do not cause a loss of living cells.

3.3 Test 3: Effects of cleaning of antimicrobial food contact surface

To determine the effect of cleaning on antimicrobial activity, the antimicrobial surface was cleaned by rubbing the surface with a cloth wetted with soap solution. After this treatment the film adherence test was performed as described as test 2 with the two microorganisms representing a middle resistant organism (*Leuconostoc mesenteroides*) and the most sensitive microorganism (*Zygosaccharomyces bailii*).
Unfortunately, the inoculum of *Leuconostoc mesenteroides* turned out to be of bad quality (due to causes unknown), therefore the measurements with this organism were unreliable.
The results of the test with *Zygosaccharomyces bailii* are given in Table 4 as decreases in CFU expressed in ^{10}log-units. For comparison the results without cleaning are reiterated in this table just as they were presented in Table 3.

Interpretation of results:
The Film Adherence test results for *Z. bailii* are very similar with and without cleaning. Apparently, repeated cleaning of the coating does not decrease the antimicrobial efficacy of the coating. In Test 2 *Zygosaccharomyces bailii* was shown to be very sensitive to the biocidical effect of the coating, as even a very short contact time (T=0 on coating) resulted in a significant decrease in living cells. This same effect is again observed after cleaning of the coating, therefore cleaning does not remove whatever factor of the coating is responsible for the very rapid killing of *Z. bailii* cells.

Table 4: Results after surface cleaning: Decrease of CFU per Film Adherence Test in ^{10}log-units. A decrease <1 is regarded as not significant and is given in italics.

Strain No.	Name microorganism	Ino-culum (CFU)	Blanc		Test on coating	
			T=0 decrease from Inoculum	T=24 hour decrease from T=0 Blanc	T=0 decrease from Inoculum	T=24 hour decrease from T=0 Test
Results after cleaning coating						
11	*Zygosaccharomyces bailii*	$3,9x10^6$	*0.94*	*-0.04*	2.52	3.58#
			0.92	*-0.15*	2.60	3.49#
Previous results from Test2 without cleaning coating						
11	*Zygosaccharomyces bailii*	$1,1x10^8$	*-0.07*	*0.56*	2.96	2.58#
			0.41	*0.19*	2.74	2.80#

= no surviving CFU detected

3.4 Test 4: Possible migration of nano-particles into the food matrix

In this test both coated and non-coated surface were covered with a 0.5 cm thick layer of nutrient agar medium in which the most sensitive microorganism (*Zygosaccharomyces bailii*) was seeded throughout the whole layer thickness. After four days the layer was checked on outgrowth of the organism at any distance from the coated - and non-coated surface. No growth was observed at any distance from the coated surface, while growth was observed throughout the agar layer on the non-coated surface. Apparently, the biocidical effect of the coating does migrate through the agar medium to at least 0.5 cm distance from the coating itself.

4 Discussion and Conclusions

Produsafe BV would like to launch to the market their antimicrobial product 'Produsafe QX coating'as a food contact material. For use as a food contact material, permission of the EFSA is required. To obtain such permission, a product performance dossier needs to be submitted to the EFSA. TNO Triskelion BV has the expertise to prepare and submit such a dossier for which information from microbiological tests are also required. Therefore TNO, in collaboration with Triskelion, was requested to perform the microbial tests for determining the antimicrobial effect of the nano-particles in coating as food contact material.

The microbiological data needed for the dossier was described by Triskelion as:
A) Spectrum of microbiological activity.
B) Level of activity: information on Minimum Inhibitory Concentration (MICs) and possible consequences of the use of the biocide such as selective overgrowth by organisms that are insensitive to the biocidal substance(s)
C) Demonstrate the efficacy, also upon repeated use, such as after cleaning.
D) Demonstration of the lack of biocidal activity against microbes in/on the food

In regard to the spectrum of microbiological activity, the antimicrobial coating was shown to have a biocidal effect on all 11 tested microorganisms, although the effect on fungal spores was only shown with the product in liquid form. The most likely interpretation of the results with fungi is that the spores themselves are not sensitive to the coating material, but that this changes as soon as the spores germinate, because the emerging hyphal cells are then sensitive to the biocidal effect of the coating.

In regard to the level of antimicrobial activity, the MIC of fluid coating material for the different microbial species are given in Table 2. No selective overgrowth of insensitive organisms is expected, as all 11 tested organisms were sensitive and none will be able to grow on the coated surface.

The efficacy of the antimicrobial coating was demonstrated with the Film Adherence test. The results of this test are summarized in Table 3. No effect of cleaning was found on the efficacy as can be seen in Table 5. For the effect of cleaning only results with *Zygosaccharomyces bailii* are available, as due to failure of the inoculum no results with *L. mesenteroides* were obtained. However, the *Z. bailii* results are clearly interpretable and no significant influence on this interpretation is expected from the missing data.

Regarding the lack of biocidal activity against microbes in/on the food the results of the tests are more difficult to interpret. To check for a possible migration of biocidal activity from the coated surface into a food matrix an agar-layer migration test was performed. Triskelion indicated that for use as a food contact material the microbes inside the food material should not be affected by the coated surface. However, the results from Test 4 clearly show a biocidal effect at 0.5 cm distance from the coated surface. This might indicate that TiO2 nanoparticles migrate out of the coating into the agar layer. Also possible is that not coating-particles but oxygen-radicals produced at the coated surface migrate through the agar. However, this is less likely as radicals immediately react with any organic molecules they encounter. Even another possibility regarding the migrating biocidal effect is that the chemical reactions with oxygen radicals at the coated surface result in new molecules with antimicrobial properties and that these new molecules migrate through the agar and kill the *Z. bailii* cells at 0.5 cm distance from the coated surface.

The agar-layer test indicates that the biocidal effect of the coating does migrate through at least 0.5 cm agar-gel. Clarifying the exact consequenses of this finding in regard to a product performance dossier and any potentially needed subsequent tests to pinpoint the cause of the observed migration are not part of this specific project. This report was commisioned as the basis on which TNO Triskelion can expand towards submission of a product performance dossier for a food contact material towards the EFSA. If additional microbial tests are needed for such a dossier, then TNO's expertise can again be requested in a new project with a new setup.

5 Signature

Zeist, December 2014

TNO

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A CFU results of Test 2: Film Adherence Test

The raw results of Test 2: Film Adherence Test on the effectiveness of the anti-microbial coating are given in the Table below as number of Colony Forming Units (CFU).

Table 5: Results Film Adherence Test in number of Colony Forming Units (CFU)

Strain No.	Name microorganism	Ino-culum	Blanc		Test on coating	
			T=0	T=24 hour	T=0	T=24 hour
1	*Listeria monocytogenes*	$1,3x10^6$	$5,6x10^5$	$1,7x10^5$	$6,5x10^5$	<10
			$5,9x10^5$	$1,3x10^5$	$1,1x10^6$	<10
2	*Escherischia coli*	$7,5x10^6$	$5,1x10^6$	$4,5x10^6$	$5,2x10^6$	<10
			$5,7x10^6$	$6,5x10^6$	$5,8x10^6$	Ca. 30
3	*Salmonella typhimurium*	$7,1x10^6$	$3,6x10^6$	$1,5x10^6$	$3,1x10^6$	Ca. 10
			$3,3x10^6$	$2,4x10^6$	$3,8x10^6$	<10
4	*Staphylococcus aureus*	$8,6x10^6$	$7,2x10^6$	$2,0x10^6$	$4,5x10^6$	<10
			$6,4x10^6$	$1,0x10^6$	$5,8x10^6$	<10
5	*Bacillus cereus*	$5,4x10^5$	$4,8x10^5$	$4,9x10^4$	$4,5x10^5$	<10
			$4,3x10^5$	$4,7x10^4$	$3,8x10^5$	<10
6	*Leuconostoc mesenteroides*	$1,1x10^7$	$8,7x10^6$	$5,6x10^6$	$5,3x10^6$	$1,1x10^4$
			$9,0x10^6$	$5,0x10^6$	$3,6x10^6$	$7,8x10^4$
7	*Pseudomonas putida*	$1,7x10^6$	$6,5x10^5$	$7,5x10^5$	$1,2x10^6$	Ca. 20
			$8,8x10^5$	$4,8x10^5$	$7,7x10^5$	<10
8	*Lactobacillus brevis*	$8,4x10^5$	$9,1x10^5$	$3,6x10^5$	$1,0x10^6$	<10
			$1,0x10^6$	$3,3x10^5$	$7,2x10^5$	<10
9	*Aspergillus niger**	$5,5x10^4$	$2,3x10^4$	$2,3x10^4$	$4,1x10^4$	$6,2x10^3$
			$3,1x10^4$	$2,7x10^4$	$4,4x10^4$	$6,9x10^3$
10	*Penicillium discolor**	$3,9x10^5$	$2,3x10^5$	$8,9x10^4$	$3,7x10^5$	$8,7x10^4$
			$2,6x10^5$	$1,0x10^5$	$2,8x10^5$	$1,0x10^5$
11	*Zygosaccharomyces bailii*	$1,1x10^6$	$1,3x10^6$	$3,6x10^5$	$1,2x10^3$	<10
			$4,3x10^5$	$2,8x10^5$	$2,0x10^3$	<10

<10 = below detection limit

* = fungal spores applied in test, not mycelium.